A Record of Research directed towards

ENHANCEMENT OF THE SAFETY OF CLINICAL ANAESTHESIA.

by

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- DEDICATION -

To Mary

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PREFACE

Clinical Anaesthesia is an indispensable adjunct to the surgical treatment of disease. It directly affects the lives of a great number of people since every year no fewer than one in every twenty-five persons in the population is required to undergo a procedure which necessistates it. Of itself non-therapeutic, clinical anaesthesia must, above all else, be safe for the patient. Any adverse outcome to clinical anaesthesia and factors relevant to its administration results, at best, in postoperative morbidity for the patient and, at worst, his demise.

Identification and examination of the factors and circumstances which have a material influence on the safety of anaesthesia for the patient, provided the motivation for and is the central theme of this collection of research publications which was submitted to the University of Cape Town in fulfilment of the requirements for the award of the Degree of Doctor of Science (Medicine).

The publications submitted report the results of forty years of endeavour. In terms of their subject matter, these publications may be broadly grouped into the following five fields of interest:-

1) The Epidemiology of Death Attributable to Anaesthesia.
2) Pharmacogenetic States of concern to the Anaesthetist -
    a) The Malignant Hyperthermia Myopathy.
    b) The Acute Porphyrias.
3) The Effects of Anaesthetics on the Liver-
    a) Studies of Hepatic Drug Metabolism of relevance to post-Halothane Hepatitis and the hepatotoxicity of anaesthetic agents.
    b) The treatment of Fulminant Liver Failure.
4) Heat Homeostasis during Anaesthesia-
    a) Inadvertent Hypothermia during anaesthesia.
    b) Induced Hypothermia during anaesthesia.
5) Miscellaneous.

Within these fields of interest, papers have been grouped in terms of related aspects of the main topic they cover. Editorial comment is included where appropriate. The nature and scope of many of the above investigations was such as to require, for their satisfactory conclusion, collaborative interdisciplinary research combining the endeavours of other clinicians and paramedical scientists. Appropriate recognition of such collaboration has resulted in the multiple authorship registered for many of the publications in this collection.

1) THE EPIDEMIOLOGY OF DEATH ATTRIBUTABLE TO ANAESTHESIA.

The most fundamental index of the safety of anaesthesia for the patient is the incidence with which factors relevant to the anaesthetic and its administration cause or are significantly contributory to the death of a patient. Based on this premise, I commenced in 1956 a surveillance study of all peri-operative deaths associated with anaesthesia at Groote Schuur Hospital.

The primary objectives of this study were:-

1) to establish the incidence of Death Attributable to Anaesthesia.
2) to identify the mechanisms responsible and the hazard situations from which they arose.
3) to formulate appropriate strategies for their prevention.

When finally concluded, this surveillance study had acquired data from a population of three quarters of a million anaesthetics and covered a time span of thirty years. It had become the longest running and largest descriptive epidemiological study of Anaesthetic Associated Mortality conducted in a single academic institution under the same individual direction yet reported. In a field of interest in which lack of uniformity - in taxonomy, criteria for case inclusion and assessment as well as peri-operative time period studied - invalidates any precise 'between study' comparisons, the characteristics of this study do serve to validate 'within study' inferences and conclusions.

During its course, major analyses of the data captured by this ongoing study were published every ten years (Papers 1.1,1.5,1.7) - the first earning the award of the Hamilton-Maynard medal of the Medical Association of South Africa. All enjoyed wide international recognition as providing authoritative estimates of the incidence of the commoner causes of Death Attributable to Anaesthesia. Subject to journal editorial comment and republication in the Year Books of Anesthesiology and some others, data and conclusions from these publications have been widely cited internationally by authors in this field.

Broad reviews of this topic are provided in the two book chapters which conclude this group of publications (Papers 1.8,1.9).

Considerable changes occurred in the clinical practice of anaesthesia during the thirty year period of this study - changes consequent on advances in knowledge, the development of a more versatile drug armamentarium, improved professional training standards and, in particular, the advent and ready availability of sophisticated monitors of vital functions, including biochemical parameters. The enhancement of the safety of anaesthesia for the patient brought about by these improvements in practice is reflected in the publications presented by the six fold decrease in the incidence of Death Attributable to Anaesthesia reported over the duration of this study. This incidence today is less than one death per ten thousand anaesthetics - a `rare event' level that has important logistic implications for the statistical design of future surveillance studies. The need for the latter remains, for as improvements in anaesthetic technique and safety permit surgical advance, this itself presents an ever greater challenge to the anaesthetist in the type of patient presented for anaesthesia for operations of increasing scope and complexity.

2) PHARMACOGENETIC STATES OF CONCERN TO THE ANAESTHETIST.

Of the pharmacogenetic states of relevance in clinical anaesthesia, two are of particular concern because the abnormal drug responses that characterise them may be immediately life threatening. Further, both are covert, their presence revealed only by family history backed by sophisticated diagnostic tests. These conditions are a) the Malignant Hyperthermia Myopathy and b) the Acute Porphyrias.

Motivating research directed towards the enhancement of the Safety of Anaesthesia for those genetically susceptible to these states has been the concept that prevention of acute crises would follow:-

1) the identification of the genetically susceptible individuals,
2) the identification of those drugs which provoke the abnormal reaction and their avoidance in the above subjects,
3) the corollary, identification of those drugs which were safe to use in the susceptible individual.

The research publications presented in this section are largely a product of my long association with two MRC sponsored research units established at the University of Cape Town - the Liver Research Unit and the Porphyria Research Unit. Of recent years these units have been combined and upgraded to MRC Research Centre status as the MRC/UCT Liver Research Centre. In addition much of the early investigation of Malignant Hyperthermia was made possible by assistance from the UCT Department of Chemical Pathology, in which was later established the MRC/UCT Biomembrane Research Unit.

CONTENTS

PART 1

The Epidemiology of Death Attributable to Anaesthesia

PART 2

Pharmacogenetic States of Concern to the Anaesthetist -

a) The Malignant Hyperthermia Myopathy
b) The Acute Porphyrias

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PART 3

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b) The Treatment of Fulminant Liver Failure

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PART 4

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a) Inadvertent Hypothermia
b) Induced Hypothermia

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5.3 The Effect of Cardiac Lesions on the Action of Suxamethonium. Anaesthesia. 1966; 21:28-36.

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a) The Malignant Hyperthermia Myopathy.

First recognised in 1960, the syndrome of Malignant Hyperthermia soon came to confront the clinical anaesthetist as something of a nightmare. Though rare, its occurence was unpredictable, its pathogenesis an enigma and its course fulminant. There was no effective treatment and the acute syndrome was, almost invariably, fatal. Since that time, knowledge of the syndrome and its underlying myopathy has progressed to the stage that today its pathogenesis and characteristic biochemical disturbance is well recognised; the site of the responsible genetic functional lesion in skeletal muscle - the sarcotubular ryanodine receptor calcium channel - is known, as is its DNA sequencing in MH susceptible swine; the factors which trigger the acute attack in susceptible individuals are recognised; pharmacological control of the established syndrome and its prophylaxis is available and reliable methods for the diagnostic screening of those suspected of possessing the MH gene(s) are established.

The contributions to this exicting story which emanated from the publications presented here were, in summary:-

1) The identification of Stress Susceptible Swine as a valid animal experimental model of the Human MH syndrome and myopathy. (Papers 2.1, 2.2, 2.7)
2) Localisation of the basic functional lesion responsible to skeletal muscle and formulation of the earliest diagnostic method for identifying swine susceptible to MH (Papers 2.2, 2.6, 2.8)
3) Characterisation of the fundamental biochemical reactions underlying the acute crisis (Paper 2.5)
4) The identification of agents which triggered the MH reaction and the consequent establishment of anaesthetic techniques safe to use in MH susceptible individuals (Papers 2.2, 2.21 - 2.27)
5) The establishment of effective therapy and prophylaxis for the acute MH syndrome:-
    a) Earliest attempts (Paper 2.2)
    b) Procaine (Papers 2.8, 2.9)
    c) Dantrolene (Papers 2.14 - 2.20)
    Dantrolene proved to offer specific and complete control of the syndrome as well as prophylaxis. The very specificity of its action served to assist in the further elucidation of the myopathy's underlying sarcotubular dysfunction. Consequent on its introduction into clinical practice, the mortality from the acute MH syndrome has been reduced from its rate of 70 - 80% during the first decade after it was described to a level of virtually zero today in cases treated timeously.
    d) Beta adrenergic blocking agents (Paper 2.29) For observations of the action of alpha adrenergic blocking agents - see Paper 2.2.
6) Identification and investigation of dysfunctional `unit membrane' other than sarcoplasmic reticulum (Papers 2.12, 2.28, 2.29)

The original serendipitous observations which led to the identification of Stress Susceptible Swine as a valid animal experimental model of the MH syndrome and myopathy (Papers 2.1, 2.2) were to prove seminal in its investigation internationally. They were made during a program of surgical research in swine directed towards liver transplantation - a program which involved an interdisciplinary team of surgeons, anaesthetists, physicians and technicians. I was primarily responsible for the material in these two papers but in wishing to give credit to all who were part of the original discovery and whose ideas and services had contributed to the final outcome, all active members of the team were included in the list of authors. Paper 2.2 was subsequently designated a 'Citation Classic' by Current Contents (Paper 2.3).

The characterisation of the biochemical reactions underlying the fulminant MH syndrome (Paper 2.5) must still stand as one of the most fundamental contributions to an understanding of the pathogenesis of this condition. Though I was indeed the `mover', it was the biochemical expertise of M. Berman that provided the authority behind this investigation. We extended our collaboration in this work to the investigation described in Paper 2.6. In this, knowledge gained in the first investigation was applied 'in vivo' to identify skeletal muscle as the source of the biochemical changes observed and the putative site of the functional

lesion underlying the syndrome. J.Terblanche and R.Hickman were responsible for provision of the isolated perfused preparation of skeletal muscle on which the observations were made.

All the subsequent publications in this section, Papers 2.7 - 2.30, report the outcome of investigations that were motivated, planned and undertaken by myself or by postgraduate students or junior colleagues working under my direction. Finally, the whole subject of the Porcine and Human Malignant Hyperthermia myopathy is reviewed in the five book chapters (Papers 2.31 - 2.34a & b) which conclude this group of publications.

b) The Acute Porphyrias.

Although not encountered commonly, the Acute Porphyrias are of particular concern to the anaesthetist since anaesthetic agents - in particular intravenous induction agents - have featured among those drugs most strongly incriminated in initiating acute attacks.

South Africa boasts the highest incidence in the world of one of the acute porphyrias, Variegate Porphyria (VP). Here the average anaesthetist can anticipate being presented with about two such cases per year to anaesthetise.

The first three papers (2.35 - 2.37) in this group report the screening for porphyrinogenicity, in the DDC primed rat model of VP, of various intravenous anaesthetic agents introduced into clinical practice over the last three decades. These investigations which provided information of importance to the safe practice of anaesthesia, were essentially collaborative projects. I was responsible for the `in vivo' aspects of the experiments, the paramedical scientists for the biochemical assays and their evaluation. The manuscripts, though managed in each case by the first author, were ultimately joint affairs.

The fourth paper in this group (2.38) if of importance for it reports the first ever prospective controlled clinical and biochemical drug trial conducted in a group of porphyric individuals. In this instance the trial was of the new intravenous anaesthetic agent Propofol. Our trial confirmed the lack of porphyrinogenicity of this drug, first displayed in the experimental animal model, and its potential for safe use in susceptible patients.

As with previous sections, this group of publications is rounded off with a review of the porphyrinogenic potential of drugs currently commonly used in anaesthetic practice (Paper 2.39).

3) <u>THE EFFECTS OF ANAESTHETICS ON THE LIVER.</u>

a) Studies of Hepatic Drug Metabolism of relevance to post-Halothane hepatitis and the hepatotoxicity of anaesthetic agents.

Halothane, introduced into clinical practice in 1956, was soon to replace in usage all the inhalational agents that preceded it. However, spearheaded by a letter to the Lancet from J. Barton of Pietermaritzburg (1), widespread reports of post-Halothane juandice - many of which proved fatal - soon cast serious doubts on the safety of what otherwise appeared to be an ideal anaesthetic. The need to monitor our own practice for this problem motivated the epidemiological survey of postoperative/anaesthetic jaundice which introduces this group of publications (Paper 3.1) This showed the entity of post-Halothane hepatitis to be rare, as did other more comprehensive contemporaneous and later studies. (2).

An important question that emerged from these surveys was the role of multiple exposures to Halothane in the pathogenesis of this new syndrome. In order to isolate the effects of drug exposure alone from all the many other potential causes of liver dysfunction associated with the clinical situation, we chose to investigate this problem in a preparation of isolated perfused rat liver. This study, the first to be so

documented, is reported in Paper 3.2 - a collaborative project of which J.F. Biebuyck was the chief executor. And indeed, this study did show a depression of liver function to follow multiple exposures to Halothane that was not apparent after single exposure, nor after multiple exposures to diethyl ether. This theme was pursued further eighteen years later in studies documented in publications 3.13, 3.14.

The fundamental discovery, reported by Van Dyke and associates in 1964 (3,4), that all volatile anaesthetic agents are biotransformed to a greater or lesser extent, directed attention to the possibility that the mechanism of anaesthetic hepatotoxicity might have a biochemical basis not previously considered. On this premise I planned the extension of the above investigation to studying `in vivo' the effects of multiple anaesthetic exposure on liver function and histology as affected by enhancement of drug biotransformation induced by microsomal enzyme induction. These experiments, reported in Paper 3.3, produced the surprise finding that Fluroxene anaesthesia, in the presence of microsomal enzyme induction, caused a predictable, repeatable, fulminant and lethal necrosis of the liver in experimental animals. Even in the absence of enzyme induction, histological evidence of liver damage was apparent. At this time, Fluroxene was considered to be so innocuous in terms of its effects on the liver as to merit its recommendation as the anaesthetic of choice for liver transplantation (5). Our further investigation (publications 3.4, 3.5, 3.6) demonstrated this effect to be the direct outcome of the generation of highly reactive toxic intermediates during the hepatic microsomal Cytochrome P450 metabolism of Fluroxene. These observations together with those reported contemporaneously by Cascorbi and Singh-Amaranath (6) later confirmed by others, and the report by Reynolds and associates (7) of a human fatality following the Fluroxene anaesthesia of a patient on enzyme inducing medication, led to the withdrawal of Fluroxene from clinical practice after twenty years of use.

This series of investigations heralded a long and fruitful collaboration with K. Ivanetich and the Department of Medical Biochemistry. The main thrust of this research was into aspects of the hepatic microsomal metabolism of volatile anaesthetics that were considered to have relevance to an understanding of post-anasethetic liver dysfunction or the possible protection against such. In most instances the studies reported in these publications (3.7 - 3.13) featured as thesis topics for postgraduate M Sc and Ph D students supervised by K. Ivanetich with myself as co-supervisor in two instances. In general, I was responsible for maintaining the clinical relevance of the studies, for the performance and/or supervision of `in vivo' aspects of the experiments and for the histology data where relevant. Concerning the manuscripts, I was entirely responsible for those in which I am listed as first author and for the aspects recorded above in the remainder. Though elucidation of the pathogenesis of post-Halothane hepatitis - now thought to be based on an immunological response to a nontoxic intermediate species of the cytochrome P450 oxydative metabolism of Halothane - eluded us, these studies contributed much new and original knowledge to the detail of the microsomal biotransformation of the volatile anaesthetics and related xenobiotics. A general review of matters relevant to the safe anaesthesia of patients suffering liver disease and for surgery of the liver (Paper 3.15) concludes this section of publications.

b) The Treatment of Fulminant Failure.

Three papers in this group, 3.16 - 3.18, report the outcome of inovative and radical attempts at treating end-stage fulminant liver failure by cross circulation between patient and animal liver `in vivo' and `in vitro'. This project was conceived and directed by S.J. Saunders. The patients treated did manifest some short term beneficial effect from the cross circulation and biochemical improvement was observed. However, the fear of patient infection with an endemic encephalitic virus from the baboons and the regular occurrence of xenogenic thrombocytopoenia when use was made of isolated perfused pig liver, forced our abandonment of this treatment.

4) HEAT HOMEOSTASIS DURING ANAESTHESIA.

a) Inadvertent Hypothermia.

General Anaesthesia renders the patient poikilothermic. Control of the patient's body temperature is, therefore, an important factor in the maintenance of his general physiological homeostasis while anaesthetised. The introduction into clinical practice of Halothane coincided with that of the air conditioning of operating theatres. This coincidence of the introduction of cooler operating theatre temperatures with an anaesthetic that is characterised by its vasodilatory properties combined to produce the circumstances that could result in inadvertent hypothermia in the anaesthetised infant. Paper 4.1 documents the first published study of these effects on children.

Massive transfusion of the anaesthetised patient with cold stored blood is another common circumstance which can lead to dangerous levels of inadvertent hypothermia. This may be avoided by pretransfusion warming of blood. Paper 4.2 describes an apparatus for the rapid warming of stored blood by radio frequency emission and evaluates the degree of resultant red cell damage. The method used to assay red cell survival 'in vivo' was novel.

b) Induced Hypothermia during Anaesthesia - Anaesthetic poblems posed by radical tumour radiosensitisation techniques.

The remaining papers (4.3 - 4.6) describe attempts at enhancing the radio seensitivity of malignant tumours by the reduction of the hypoxic cell populations of tumour cores. Increased tumour sensitivity to radiation and consequent increase in cure or control rates had been demonstrated to follow irradiation of certain malignant tumours under conditions of hyperbaric oxygenation (3 AtA) (8). This response was presumed to follow from an increase in tumour radiosensitivity secondary to a reduction in the hypoxic cell populations at tumour cores. The achievement of further reduction in hypoxic cell populations by the exposure of the patient to higher pressures of oxygen is barred by the convulsive threshold of 4 - 5 AtA oxygen. We sought to circumvent this problem by the addition of total body cooling (hypothermia) to hyperbaric oxygen exposure of the patient in order to reduce tissue oxygen utilisation. The target patient core temperature chosen was 30°C. This brought with it the need to subject the patient to general anaesthesia with neuromuscular blockade and intermittent positive pressure respiration together with the hazards which accompany hypothermia. Further, all was subject to the solution of the grave logistic difficulties posed by having to achieve these conditions safely within the confines of a single patient pressure vessel. These publications describe our preliminary developmental studies using pigs and thereafter their application to humans.

While some improvement in patient life expectancy did follow irradiation of squamous carcinomas of the mouth in these circumstances, the risk of hypothermic ventricular fibrillation and the grave difficulties of its effective management when it occured with the patient in the pressure vessel, led us to seek a safer method of applying these same principles to enhance tumour radiosensitivity. In this attempt we abandoned the pressure vessel, cooled the patient to 16°C. while on femoral artery - femoral vein cardiopulmonary bypass and irradiated the tumours while the patient was exposed to oxygen at normal ambient pressure i.e. 1 AtA. This project was ultimately abandoned when the initial therapeutic results produced by this method failed to match those of the original. Further, both methods had proved to be unacceptably costly in terms both of staff and time requirements.

5) MISCELLANEOUS.

This final group of publications is diverse and contains some papers that are now largely of historical interest. The first (5.1) documents the first clinical experience of the use of Halothane as the anaesthetic for cardiopulmonary bypass surgery. Paper 5.2 records another situation in which the particular properties

of Halothane - in this case its depression of pharyngo-laryngeal reflexes and quick recovery time - were applied to solve problems peculiar to paediatric radiotherapy.

Papers 5.3 and 5.4 describe the effects that certain circumstances associated with open heart surgery have on the pharmacokinetic behaviour of drugs. These are Valvular Heart disease (5.3) and Extracorporeal Circulation itself (5.4).

Of the following two papers (5.5 and 5.6) which concern Obstetrical Anaesthesia and factors affecting the establishment of sustained foetal respiration after Caesarean section, the first has a degree of historical interest. It reports the first investigation to demonstrate the lack of correlation between the duration of anaesthesia (per se) and the time to sustained foetal respiration after delivery. At the time, the mistaken concept that there was such a correlation had led to the adoption of the so called `smash and grab' technique of anaesthesia for Caesarean section.

The next two papers are concerned with aspects of Anaesthetic circuits. Paper 5.7 is of historical interest in that it documents the first direct observations of the performance of the classical Boyle semi-open circuit relevant to the elimination of Carbon Dioxide from the circuit.

The final publication, 5.9, was written at a time when Respiratory Intensive Care, as we know it today, was in its infancy. Based on an investigation conducted on patients in the immediate recovery phase after open heart surgery and experience in the clinical postoperative management of other patients with respiratory impairment, it defined the indications for postoperative ventilatory support. Its recommendations are still valid.

The publications in this collection report four decades of investigations of increasing complexity and sophistication as the range of research techniques available was advanced. At one extreme they demonstrate that research which can contribute significantly to knowledge can be performed with the minimum of technical equipment and resources. For example, a stop-watch alone was all that was needed for the investigation which examined the "Effect of duration of Anaesthesia on Apnoea Neonatorum after Caesarean Secion" (Paper 5.5), while a simple clinical thermometer sufficed for the study of "Temperature Changes in Children during Gegneral Anaesthesia" (Paper 4.1) At the other extreme they illustrate how the advances in physico-chemical technology now applied to biological research have rendered the collaboration of the paramedical scientist in clinical research highly desirable, often indispensable. In this regard many of these publications are a tribute to the high degree of cooperative interdisciplinary collaboration that has characterised research at this medical school. At the same time they highlight one of the great shortcomings of our university departments of Anaesthesiology and, by contrast, the prophetic insight displayed by Sir Robert Macintosh, the inaugural professor of Anaesthetics at Oxford University. As far back as 1940, Macintosh insisted on the appointment to the staff of the nascent Nuffield Department of Anaesthetics at Oxford of a paramedical scientist (Dr. H.G. Epstein - a physicist), together with a support technician (R.Salt)(9). His example was later followed by other academic departments then developing in the United Kingdon and North America. Within twenty years the inclusion of posts for paramedical scientists on the staff establishment of university departments of Anaesthesia had become the norm in these countries. Regrettably this practice, so essential to the sound and balanced development of research in our discipline, has not been followed in South Africa. Though comment on this defiency is pertinent, discussion of its causes and possible resolution have no place here.

Thirty years ago the Lancet stated in an editorial that "... the most obvious risks of operation are those associated with Anaesthesia" (10). Data presented in the first group of these publications show convincingly that this statement can no longer be supported. In fact, the major factors affecting perioperative death now are related to the patient's disease and surgery itself - these factors being responsible for approximately 90% of all perioperative deaths (11). Over the last three decades the incidence

of Death Attributable to Anaesthesia itself at Groote Schuur Hospital has declined progressively to approximate 1 or less per 10000 anaesthetic administrations. Parallel reductions have occurred at similar institutions in Australia and England. This is a level which represents a six fold increase in the safety of anaesthesia for the patient. It is my belief that the data and conclusions that have emanated from this collection of publications have, in their way, all made some contribution to this "Enchamcement of the Safety of Anaesthesia".

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PART 1.

THE EPIDEMIOLOGY OF DEATH ATTRIBUTABLE TO ANAESTHESIA.

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ANAESTHETIC CONTRIBUTORY DEATH—ITS INCIDENCE AND CAUSES*

PART I. INCIDENCE

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A learned judge once said, 'It is a fact that to anaesthetize a human being, to deprive him of consciousness outright, is to take a considerable step along the road to killing him'.[1] Yet, in South Africa, in any one year, at least one in every 30 persons of the total population requires to be anaesthetized for some surgical operation.[2] In the past, official disquiet has been voiced because of the impression that the incidence of death due to anaesthetics was high. A commission appointed by the Minister of the Interior 'to investigate the causes of the high death rate in the Union from anaesthetics . . .' published its report in 1936.[3] The report of the National Health Services Commission, published in 1944,[4] contains in the section on 'Recommendations regarding the administration of anaesthetics' the statement, 'We are impressed by the evidence showing that there is an unnecessary mortality from this cause (anaesthetics)', though what this evidence was or what the incidence of death due to anaesthesia was, is not quoted.

The most fundamental index we have of the safety of anaesthesia is the incidence with which factors related to the anaesthetic cause, or are contributory to, the death of a patient. To ascertain this incidence, about which there existed little relevant modern information, especially in South Africa, I commenced a contemporary survey in 1956 of all mortality associated with anaesthetics at Groote Schuur Hospital, the principal teaching hospital of the University of Cape Town Medical School. This paper is a report of the findings of this survey from its inception until the end of 1966. Though this survey has proceeded for 11 years, I report on the data for 9 years only, namely 1956 - 1960 inclusive and 1963 - 1966 inclusive. Data for the years 1961 and 1962 were incomplete and are omitted.

THE SURVEY

The detailed survey was confined to mortality occurring during the period of anaesthesia and the 24 hours immediately thereafter. I have called this 'immediate operative mortality'. This 24-hour period was chosen arbitrarily as being the period most likely to reveal those cases relevant to the anaesthetic, without the study becoming unmanageably diffuse. Clinical information was obtained from the anaesthetist concerned on all patients who (*a*) died during the administration of an anaesthetic, (*b*) died within 24 hours of receiving an anaesthetic, and (*c*) having been conscious before anaesthesia, died without regaining consciousness thereafter, regardless of the postanaesthetic time lapse.

Where possible, clinical documentation was completed by the findings at autopsy.

The results were then examined, assessed and classified. In this assessment, though the final opinion was my own, the opinions of members of the Department of Anaesthetics of this Hospital were sought, a regular weekly departmental meeting providing the forum for discussion. Where relevant, the opinion of the surgeon concerned was also sought.

In any biological study where the observations are compounded from the interaction of many variables, the classification of data into meaningful groups from which valid inferences can be made, is frequently a most difficult problem. As Treloar rather despairingly remarks, 'The causation of a given death is always multiple, so that the necessity of selecting a single cause for purposes of tabulation has become a distressing problem'.[5]

Deaths associated with anaesthesia result ultimately from a combination of circumstances which may be broadly grouped as follows:

1. The patient's disease, which is often coincidental and not directly associated with the lesion necessitating surgery. Such disease, even if not itself directly responsible for the death of the patient, will, by its deleterious effects on the patient's physiological homeostatic mechanisms, render the administration of an anaesthetic and the immediate pre-operative and postoperative surgical care more critical.
2. Factors associated with the surgical lesion and the operation itself, including the magnitude of the operation and mishaps or errors in the surgical procedure.
3. The conduct of the anaesthetic.

For the data from this survey I adopted a very simple classification and, in so doing, did not allow for the combinations and permutations of the above factors which are a feature of the classifications adopted by many workers in this field — a feature which I feel does nothing for clarity.[3,6-22]

*Partly based on a thesis submitted for the degree of Doctor of Medicine, University of Cape Town. Date received: 15 December 1967.

1. Anaesthetic Contributory Death

In this group I classified all deaths to which the administration of the anaesthetic itself, or factors within the ambit of the anaesthetist's responsibility, were considered to have been a significant contributory factor.

2. Deaths Due to Other Causes

In this group I classified those deaths which resulted primarily from the patient's disease or factors directly related to the operation. Though, from its very nature, there are few deaths which occur in association with anaesthesia to which it can be said the anaesthetic made no contribution, there are such deaths where the conduct of the anaesthetic appears to have been faultless, where any contribution appears to have been minor and where other circumstances appear to have provided adequate reason for death.

3. Inevitable Deaths (Last-Ditch Surgery)

This is a group of not uncommon cases which require separate identification. They are deaths from which the anaesthetic and its management, though apparently clinically faultless, cannot be excluded as a significant contributory factor. This may be because death occurred during anaesthesia, or it may be that the final collapse of circulatory homeostasis in a patient, moribund before anaesthesia, appears to have been unavoidably precipitated by the known effects of anaesthetic drugs, e.g. vasodilatation. These deaths may be regarded as those to which anaesthesia was unavoidably or necessarily contributory. Precision of assessment of clinical cause is often impossible. All deaths which followed cardiac arrest during anaesthesia, where the contributory role of the anaesthetic appeared to be minor and where the conduct of the anaesthetic appeared to be faultless, were also classified in this group, even though death may have occurred in the postoperative period after termination of the anaesthetic.

The phrases 'death associated with anaesthesia' and 'death to which anaesthesia was considered a significant contributory factor' are cumbersome, and will hereafter be abbreviated to 'anaesthetic associated death' and 'anaesthetic contributory death', respectively. The former term has no implication as to the precise cause of the death and includes all deaths associated with an anaesthetic, be they due to the patient's disease, the operation or the anaesthetic *per se*. The second term refers only to those deaths occurring in association with an anaesthetic, which have been caused in large measure by factors for which the anaesthetic and its management were responsible.

Ideally, all deaths specifically due to anaesthesia are preventable. Macintosh states, 'I hold there should be no deaths due to anaesthesia'.[3] All anaesthetic contributory deaths in this survey were further examined and classified as 'probably preventable', 'possibly preventable', or 'no verdict'. The first 2 subdivisions include those cases best described (as suggested by Morton)[4] as 'those deaths for which a reasonably satisfactory explanation can be provided and for which countermeasures are practicable'. The last subdivision includes those cases 'which cannot at present be fully explained and for which countermeasures are either lacking or largely empirical'.

THE INCIDENCE OF ANAESTHETIC CONTRIBUTORY DEATH

During the 9 years covered by this survey, 177,928 anaesthetics were administered at Groote Schuur Hospital. Of the 2,026 patients who died following surgery, 414 (20%) constituted the group defined here as 'immediate operative mortality' (Table I).

TABLE I. OPERATIVE MORTALITY INCIDENCE IN 177,928 OPERATIONS AND ANAESTHETICS, GROOTE SCHUUR HOSPITAL (1956–60, 1963–66)

	No. of deaths	*Deaths per 1,000 anaes.*	
Total operative mortality	2,026	11·38	
Immediate operative mortality	414	2·33	20% of total op. mortality
Group 1 (anaes.)	58	0·33	14% of immed. op. mortality
Group 2	261	1·47	
Group 3	95	0·53	

To 58 of these deaths the anaesthetic and its administration were considered to have been significantly contributory. This results in an over-all incidence of anaesthetic contributory death of 0·33 deaths per 1,000 anaesthetics. The majority of these deaths (54—93%) were considered to have been either probably or possibly preventable.

Considered in the perspective of the total care of the surgical patient, it can be said that the anaesthetic and its management were responsible, in a way that was possibly preventable, for 2·1% of the total operative surgical mortality.

Such an estimate of the incidence of anaesthetic contributory death immediately poses two questions: How do our standards of clinical anaesthesia, judged on the basis of mortality, compare with other medical centres in the world? Have the advances claimed for clinical anaesthesia over the years resulted in a greater safety for the patient?

For purposes of comparison, surveys were examined of anaesthetic contributory mortality published in the 25 years preceding the commencement of the present study, i.e. surveys published since 1930. Such examination showed that a great number of variables influenced the estimates of incidence of anaesthetic contributory death, to an extent that rendered all but the very broadest comparisons invalid. These factors were:

1. The criteria by which data were assessed.
2. Differences in the classification of data used.
3. Differences in the peri-operative period included in the surveys.
4. Differences in the type and scope of surgery covered.
5. Limitation of the techniques of anaesthesia included in the survey.
6. Failure to enumerate the parent surgical population.

For purposes of comparison, two groups of surveys are presented here. These are grouped in terms of the peri-operative period:

(*a*) Those which included the period of operation and an arbitrary postoperative period (usually 24 hours), together with the inclusion of those patients who failed to regain consciousness after anaesthesia. The

present study belongs to this group. (Authors as in Fig. 1.)

(*b*) Those which included the period of operation only —so-called 'operating-room deaths'. (Authors as in Fig. 2.)

Estimates from these surveys of the incidence of anaesthetic contributory death are presented in the form of histograms in Figs. 1 and 2. They are arranged in chronological order.

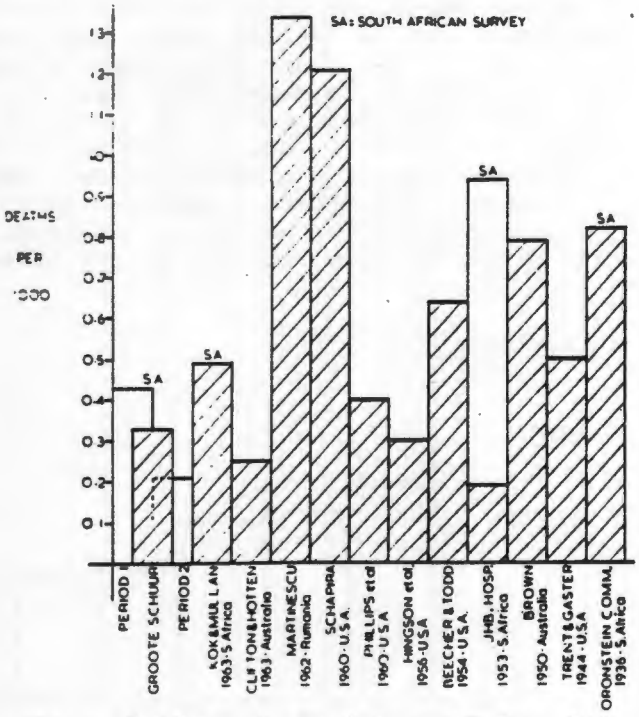

Fig. 1. Incidence of anaesthetic contributory death. Complete surveys.

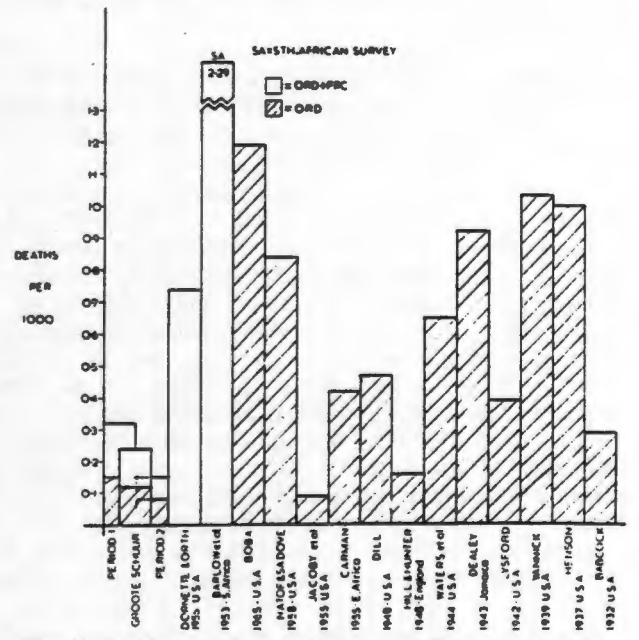

Fig. 2. Incidence of anaesthetic contributory operating-room death. ORD = operating-room death, FRC = failed to regain consciousness.

It should be noted that the incidence of anaesthetic contributory mortality that emerges from the Groote Schuur Hospital survey, when selected on the basis of 'operating-room deaths' only, is less than one-half of that which results when the 24-hour postoperative period is included in the period surveyed. This demonstrates the fallacy inherent in the common method of surveying 'operating-room deaths' only, when attempting to estimate the incidence of anaesthetic contributory mortality.

A wide disparity is evident in these estimates of the incidence of anaesthetic contributory death. No general trend is apparent. Of the 12 surveys presented in the first group, all but 2 estimate the incidence of anaesthetic contributory death as 1 death or less/1,000 anaesthetics, while one-half (including this present Groote Schuur Hospital survey) give an estimate of 0·5 or less/1,000 anaesthetics. In the second group of 15 surveys of 'operating-room deaths', all but 3 estimate an incidence of anaesthetic contributory death that does not exceed 1 death/1,000 anaesthetics, while from half of the surveys (including this survey) this estimate of anaesthetic contributory death does not exceed 0·5 deaths/1,000 anaesthetics.

Though only the broadest comparisons are possible, it would appear that, judged on the basis of mortality, the standard of clinical anaesthesia practised at Groote Schuur Hospital compares more than favourably with that of other centres.

In attempting to answer the second question on evidence of progress towards greater safety for the patient of anaesthesia and operation, I have confined my attention to conditions in South Africa. Besides the present study at Groote Schuur Hospital, 5 other surveys of anaesthetic associated mortality have been undertaken in this country within the last 30 years. (Of these, only 3 have been published.) Details of these are presented in Table II and are graphically illustrated in Fig. 3. It will be seen that there is no real difference between the incidence of anaesthetic contributory death reflected by the findings of the Orenstein Committee of 1936[3] (0·8 deaths/1,000 anaesthetics), and that computed from a survey of the Johannesburg Teaching Hospitals Group[17] conducted 15 years later (0·94 deaths/1,000 anaesthetics). At the same time the incidence of anaesthetic associated death is shown to have increased slightly.

TABLE II. INCIDENCE OF ANAESTHETIC ASSOCIATED AND ANAESTHETIC CONTRIBUTORY DEATHS IN SOUTH AFRICA

Author	Years included in survey	Incidence per 1,000 anaesthetics: Anaesthetic associated deaths		Anaesthetic contributory deaths	
Orenstein Committee[3]	1931–1935	1·57		0·82	
Melzer[16]	1941–1945	2·85	White 1·48 Non-White 6·7		
Barlow *et al.*[18]	1945–1951	6·23		2·29	
Johannesburg Hospital[17]	1951–1952	1·87	White 1·11 Non-White 2·70	0·94	
Kok and Mullan[15]	1956–1962	0·94		0·49	
Groote Schuur Hospital	1956–1960	2·49	2·32	0·43	0·33
	and 1963–1966	2·18		0·23	

A survey conducted by Melzer[25] during the war years in the time between these 2 previous surveys, showed a marked increase in the number of anaesthetic associated deaths, that among non-Whites being disturbingly high, 6·7 deaths/1,000 anaesthetics. Melzer dealt only with the broad category of anaesthetic associated deaths and did not attempt to identify anaesthetic contributory deaths.

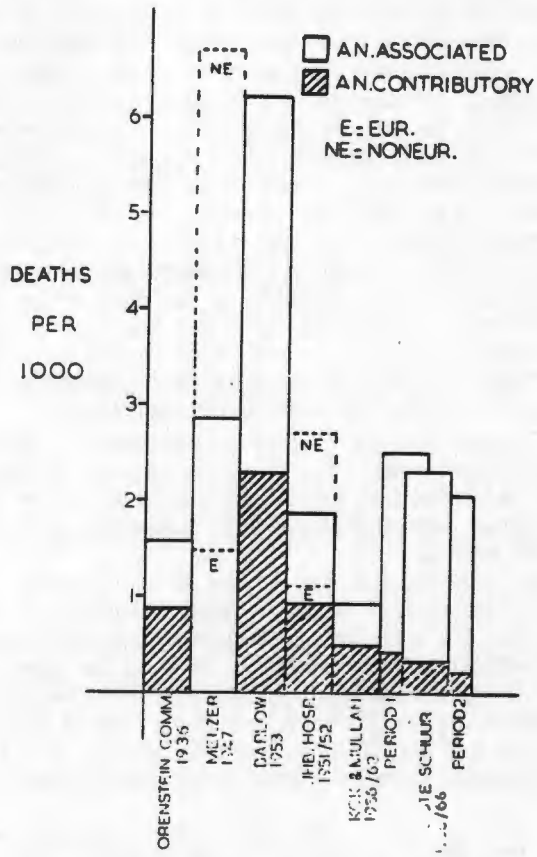

Fig. 3. Anaesthetic associated and anaesthetic contributory mortality in South Africa.

His findings were confirmed by the survey of the years 1945 - 1951 conducted by Barlow, Ginsberg and Gottlich at the Coronation non-White Hospital in Johannesburg.[26] Anaesthetic contributory deaths taken over the whole period of the survey had an incidence well over twice as high as that computed for South Africa by the Orenstein Committee. This fact must be taken to indicate a distinct inadequacy in anaesthetic services available at this hospital at that time. As the position ameliorated during the period of the survey, both anaesthetic associated and anaesthetic contributory mortality rates dropped. The subsequent survey in the Johannesburg Teaching Hospitals Group 1951 - 1952[27] showed a decrease in the deaths associated with anaesthesia from that found by Melzer, both over-all (1·87/1,000 anaesthetics as against 2·85/1,000) and in the incidence for the individual racial groups (White 1·1/1,000 anaesthetics and non-White 2·7/1,000 anaesthetics as against White 1·48/1,000 and non-White 6·7/1,000). This decrease in the anaesthetic associated mortality rate in the non-Whites is particularly striking. However, the incidence of both anaesthetic associated death and anaesthetic contributory death at this time showed no improvement over that computed for South Africa in 1936.

This disappointing and static picture is relieved by the facts that emerge from the 2 most recent surveys of anaesthetic associated mortality quoted. These contemporary surveys, that of the CSIR Anaesthetic Deaths Research Unit[28] and the present Groote Schuur Hospital survey, do present evidence of an improvement in the safety of anaesthesia. A general analysis by the CSIR Unit of anaesthetic associated deaths at 150 South African hospitals shows a progressive decrease in incidence between the years 1956 - 62[28] (see Fig. 4). The incidence of anaesthetic contributory death disclosed by the more

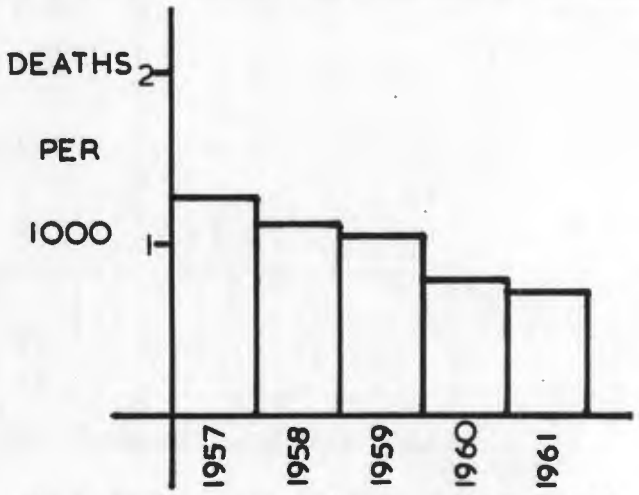

Fig. 4. Anaesthetic associated deaths at 150 South African hospitals.

detailed examination of the data from the survey of Kok and Mullan[28] shows an incidence (0·49 deaths/1,000 anaesthetics), less than two-thirds of that found for South Africa by the Orenstein Committee in 1936.[3]

At the same time, this indication of a measurable increase in the safety of anaesthesia is further borne out by the incidence of anaesthetic contributory death that emerges from my study (0·33 deaths/1,000 anaesthetics), an incidence of two-fifths of the 1936 figure. A slight difference in the method of selection of cases in the Groote Schuur Hospital survey from that of the other South African surveys quoted, accounts for the slightly higher incidence of anaesthetic associated death shown in this study—2·3 deaths/1,000 anaesthetics, as compared with Kok and Mullan's 0·94/1,000 anaesthetics. Whereas all cases dying within an arbitrary 24-hour period or who failed to regain consciousness after operation were included in the Groote Schuur Hospital survey, the other surveys used Section 86 of the Medical, Dental and Pharmacy Act of 1928 as their criterion for the selection of cases. When examined on an annual basis, the incidence of anaesthetic associated death at Groote Schuur Hospital shows no marked trend (Fig. 5). The incidence of anaesthetic contributory death, however, shows a progressive

diminution, the figure for the last year reviewed being approximately one-half of that of the first year.

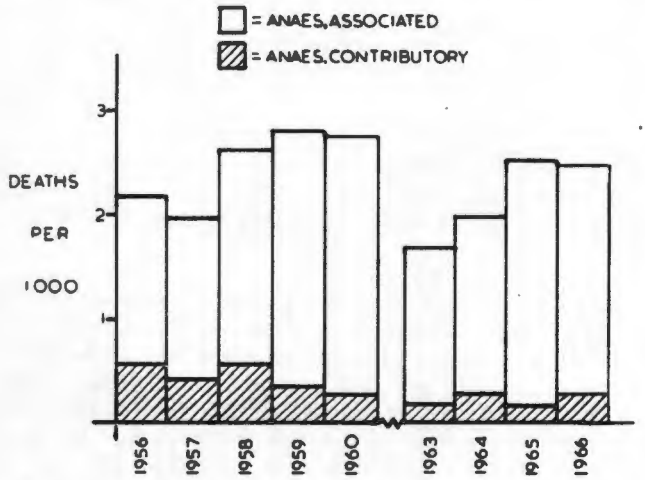

Fig. 5. Anaesthetic associated and anaesthetic contributory deaths at Groote Schuur Hospital.

Such advance towards the goal expressed by Macintosh that 'there should be no anaesthetic deaths'[23] is gratifying, but the fact that anaesthetic contributory deaths do still occur and that of these the majority (94% in the Groote Schuur Hospital survey) are preventable, must keep us from any self-satisfied complacency. The figures quoted here reflect but a segment of the total population at risk throughout the country. It must be our goal that the patent benefits of advances in clinical anaesthesia in safety for the patient are further spread by the continued supply of more and better-trained clinical anaesthetists. In outlying areas away from the larger centres which are now becoming specialist-orientated, it becomes important to increase the training and skill of those practitioners who administer anaesthetics. It must be borne in mind that the major proportion of anaesthetics given for routine surgery in this country is administered by general practitioners.[2] Possible practical measures to increase their skill are:

1. Provision for short-term attachment as a 'supernumerary staff' member to recognized teaching departments of anaesthetics. Such a scheme does exist at this Medical School, but it is not widely known.

2. The provision by teaching departments of what may be called 'visiting demonstrators' who would visit selected country hospitals for periods of up to a week, giving practical demonstrations of accepted techniques of safe anaesthesia for routine surgery.

Fifty years ago Paul Wood said, 'given a properly trained and equipped anaesthetist, mortality and morbidity will fall regardless of the agent, the technique, or the patient's condition'.[20] We must provide such men.

PART II. CAUSES

'I have at the same time and with even more earnest efforts labored to impress on the minds of those as yet unfamiliar with their use, the lessons of caution and watchfulness without which, I know, these agents (anaesthetics) are, and must be, ministers of death' (Gilman 1851).[21]

Though undertaken primarily to establish the incidence of anaesthetic contributory death,† an important corollary to this survey of immediate operative mortality was the accumulation of clinical data on the mechanisms and causes of anaesthetic contributory death and the clinical errors and pitfalls from which it may result.

In this communication I shall report on the cause of the 58 deaths that were classed as anaesthetic contributory deaths. Although anaesthesia was not considered contributory to the 95 deaths in group 3, I shall comment briefly on aetiological aspects of some of these.

Fundamental Causes

In general, the fundamental causes of death during anaesthesia may be thought of in terms of the following concept (Fig. 6): Death is the ultimate result of anoxic,

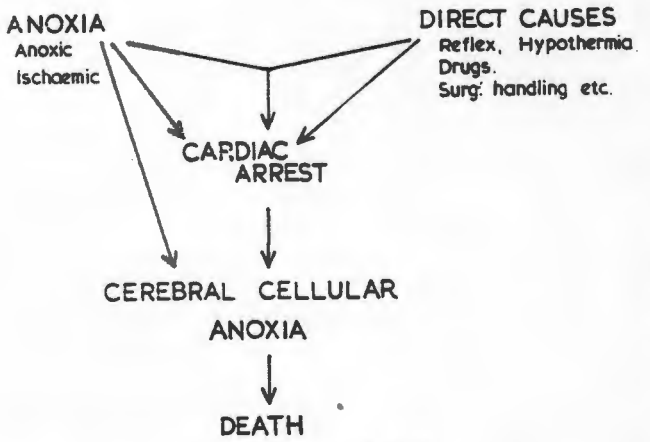

Fig. 6. Cause of death.

cerebral cellular damage. This may have resulted directly from a general state of anoxia but more usually follows cardiac arrest. Cardiac arrest itself may result from anoxia or from certain direct causes such as drugs, hypothermia, etc. Many of these causes have a greater tendency to cause cardiac arrest in an environment of anoxia. The state of cellular anoxia which arises when the cellular utilization of oxygen exceeds the amount available, may result from a decrease in arterial oxygen saturation—anoxic anoxia—or from poor tissue perfusion for some or other reason—ischaemic anoxia.

Based on this concept, the following classification of causes of anaesthetic contributory death was used.

1. Anoxia:
   (*a*) Anoxic
   (*b*) Ischaemic
2. Cardiac arrest:
   (*a*) Direct causes other than anoxia
   (*b*) Uncertain aetiology
3. Miscellaneous

Cases of 'cardiac arrest' were classified in relation to the type of anoxia considered causal. Those cases for which some direct cause other than anoxia was responsible, or where the aetiology was uncertain, were classified under the separate heading 'cardiac arrest'. Though the state of anoxia is stressed, it must be remembered that almost invariably there must have been associated acidosis (respiratory in the case of anoxic anoxia, and metabolic in the case of ischaemic anoxia) which contributed towards the death.

Within the framework of this general classification, the deaths were then further classified with regard to the more precise clinical fault or 'departure from accepted practice'[22] which was considered causal. The causes of the 58 anaesthetic contributory deaths are presented in Table III.

DEATHS DUE TO ANOXIC ANOXIA

Not surprisingly, deaths due to anoxic anoxia constitute the greatest proportion (47%) of this group of anaesthetic contributory deaths. Of these, more than half were due to respiratory obstruction, that most basic and obvious cause of anaesthetic contributory death.

Complications of Endotracheal Intubation

An unpleasant surprise for the clinical anaesthetist is the fact that half of this group (8 cases) followed the acute complications of endotracheal intubation. These complications are simple and mechanical. Yet they may be rapidly catastrophic before the anaesthetist, lulled into a false sense of security of the airway by the very presence of an endotracheal tube, has realized that anything is amiss. These cases may be briefly enumerated as follows:

(*a*) Respiratory obstruction in curarized patients due to kinking of the endotracheal tube in the pharynx,[23] not observed initially because of the inexperience of the anaesthetist involved (2 cases).

(*b*) Obstruction of the airway by herniation of the tube cuff. This form of respiratory obstruction, of which the mechanism is well documented,[24] is particularly dangerous. It may occur some time after the original intubation, or may cause initially incomplete, but insidiously increasing, respiratory obstruction which is difficult to spot clinically until it is too late (3 cases).

(*c*) Obstruction of a Carlen double lumen tube in a patient with gross bronchiectasis and bronchial distortion (1 case).

(*d*) Endotracheal intubation performed in the absence of sufficiently deep general anaesthesia, or complete muscular relaxation, or adequate topical analgesia of the larynx

*Partly based on a thesis submitted for the degree Doctor of Medicine, University of Cape Town.
†See part I for terminology.

TABLE III. ANAESTHETIC CONTRIBUTORY DEATHS, GROOTE SCHUUR HOSPITAL (1956–60, 1963–66)

Mechanism	*Cause of death*		*No. of deaths*	*% of anaes. contrib. deaths*	
I. Anoxia	Atmospheric		1	1·7	
Anoxic anoxia (*cardiac arrest, 13)	Tidal	*Respiratory obstruction*	16	27·6	(47%)
		Vomiting and regurg. 3			
		Secretional bronch. obstruction 2			
		Compl. of intubation 8			
		Inad. postop. supervision 3			
		Relaxant-contrib. death	9	15·5	
		Resp. abnormality 8			
		Neostig. cardiac arrest 1			
	Alveolar†	Pulmonary oedema (overtransfusion)	1	1·7	
Ischaemic anoxia (*cardiac arrest, 8)		*Prolonged hypotension*	13	22·4	(26%)
		Circulatory failure 9			
		Cerebral damage 4			
		Hypertension (Urovert)	2	3·4	
II. Cardiac arrest (*13)		†Direct causes, uncertain aetiology	5	8·6	
		Ventric. tachycardia (postop. death)	2	3·5	
III. Miscellaneous		Incompatible blood transfusion	1	1·7	
		Total	58	99·9	

*No. of cases that suffered cardiac arrest during anaes. and operation.
†Types of anoxia – after Saklad.[24]

or trachea. In these circumstances such intubation may be followed by bronchospasm and, more important, spasm of the respiratory muscles in expiration so severe as to render ventilation of the patient impossible. The effects of the gross anoxic anoxia that follows are compounded by simultaneous profound vagal stimulation. In such circumstances, only the rapid administration of a paralysing dose of muscle relaxant will permit adequate passive ventilation of the lungs (2 cases).

Inhalation of Vomitus

Deaths due to inhalation of vomitus or regurgitated stomach-content during anaesthesia, feature in most surveys of deaths due to anaesthesia. In this survey the number was small. One of the 3 cases recorded was an example of silent gastric regurgitation and aspiration.[25,26]

Secretional Bronchial Obstruction

The 2 patients who suffered anoxic cardiac arrest from secretional bronchial obstruction were children, a circumstance where the smallness of the bronchial anatomy is a factor which markedly worsens the degree of obstruction which may result from secretions. One of these little patients had bronchiectasis, while the other had inhaled a foreign body.

Inadequate Postoperative Supervision

It is fundamental to safe anaesthetic practice that a patient's emergence from the anaesthetic state be well supervised.[27] The number of deaths due to inadequate postoperative supervision still reported is surprising.[28–30] All 3 deaths attributed to inadequate postoperative supervision were associated with the use of muscle relaxants. After the use of muscle relaxants, though respiration may appear to be adequate in depth following pharmacological reversal of neuromuscular block, residual muscle weakness may remain.[31,32] This may result in the inability of the patient to cough strongly to expel bronchial secretions, or in the inability of the patient to overcome minor degrees of respiratory obstruction by an extra respiratory effort. Mild residual curarization was probably a factor in each of these cases, together with respiratory obstruction. In each case adequate supervision would have prevented the tragedy.

Relaxant-Associated Deaths

One-third of the group of deaths considered to be the result of anoxic anoxia (15·5% of all anaesthetic contributory deaths) resulted from causes related to the use of muscle relaxants. I choose to identify 'relaxant-associated deaths' as deaths which followed the use of a relaxant drug with an IPPR technique during anaesthesia: (*i*) after which anaesthetic the patient failed to respire spontaneously with a normal respiratory pattern and adequate minute volume of ventilation; (*ii*) for which respiratory abnormality there was no other obvious explanation; (*iii*) for which respiratory inadequacy no efficient pulmonary ventilation was provided postoperatively by the anaesthetist; (*iv*) which may have been associated with neostigmine, the pharmacological antidote to non-depolarizing relaxants.

While all 9 of these cases involved abdominal operations, 7 were acute abdominal emergencies, suffered marked abdominal distension and were of poor physical status pre-operatively. Seven of the 9 had lesions which would produce rather similar patterns of fluid and electrolyte depletion. Acute pancreatitis was present in 3. I feel that this disease warrants separate emphasis, as it features in a similar context in several recorded cases of anaesthetic dilemmas which did not result in death.

Non-depolarizing relaxants had been used in all these patients, and in each case the respiratory inadequacy appeared to be 'neostigmine resistant'.[33] Though not investigated at the time, it is probable by clinical hindsight that 7 of these 9 patients had severe metabolic acidosis—a now well-documented cause of persistent curarization.[34] Now that the means for the rapid diagnosis and treatment of

acidosis are widely available, this cause of persistent curarization should be eliminated. It is significant that more than half of these cases occurred before 1961 and that none occurred in 1966. A point of some clinical importance is that in none of these cases was the volume of ventilation adequately monitored postoperatively, the anaesthetist contenting himself with clinical assessment of its adequacy and the absence of clinical cyanosis before return of the patient to the ward.

Though listed here as occurring in only 1 case, fatal cardiac arrest followed, and appeared to be precipitated by, the injection of neostigmine in 2 cases. Though an adequate dose of atropine had been administered in each case, hypoventilation had been permitted for some time before the administration of neostigmine, circumstances shown by Riding and Robinson[25] to render the administration of neostigmine dangerous. (As hypoventilation in one of these cases was due to respiratory obstruction, the result of herniation of the endotracheal tube cuff, this case is listed under this cause in Table III).

Atmospheric and Alveolar Anoxia

The remaining 2 deaths in this group, those listed as atmospheric anoxia and alveolar anoxia,[26] require little comment. The one due to atmospheric anoxia resulted from the incorrect coupling of the anaesthetic machine. As a result, no fresh gas flow reached the patient, who consequently re-breathed only his own exhalations for several minutes. The one listed as alveolar anoxia developed pulmonary oedema, resulting from overtransfusion of blood by the anaesthetist due to an error of judgement. The patient had a ruptured ectopic pregnancy of 48 hours' duration.

DEATHS DUE TO ISCHAEMIC ANOXIA

Hypotension During Anaesthesia

A mode of anaesthetic contributory death that is prominent in my survey is that which follows progressive, prolonged and intractable postinduction hypotension, or, less commonly, profound intractable hypotension occurring during anaesthesia. This was responsible for 22% of the anaesthetic contributory deaths. In some cases death followed precipitation of cardiac arrest by this phenomenon. In the remainder, though cardiac arrest did not ensue during operation, death followed postoperatively from what appeared to be irreversible cerebral damage.

When hypotension is observed to follow the induction of anaesthesia, it is often impossible to identify the cause precisely. This is because of the simultaneous or sequential use, during anaesthesia, of a number of drugs, any of which may itself cause hypotension. In addition, other factors such as the effects of IPPR and induced respiratory alkalosis must be considered. It is pertinent to draw attention to some clinical factors that were commonly associated with the onset of intractable hypotension:

1. More than half of these patients were over 65 years of age and the majority had evidence of degenerative vascular disease.

2. Pre-operative hypovolaemia, a factor strongly stressed by Dinnick[29] in his survey, was present in one-third of the cases.

3. Inadequate replacement of blood during the operation was considered an important factor in one-third of the cases.

4. The onset of hypotension followed closely on, and appeared to have been precipitated by, the use of thiopentone sodium in 7 of these 13 deaths. In only 1 of these was the dose of thiopentone considered excessive. As clinical anaesthesia today is induced with the use of an intravenous barbiturate almost as a routine, attention must be drawn to these deaths. Both the major reports emanating from the Committee on Deaths Associated with Anaesthesia of the Association of Anaesthetists of Great Britain and Ireland strongly emphasized this entity of circulatory failure following the use of an intravenous barbiturate for the induction of anaesthesia.[22,23] Clifton and Hotten,[22] in their survey of deaths associated with anaesthesia in Australia, attributed 17% of anaesthetic contributory deaths to circulatory collapse after the administration of thiopentone. In South Africa, Kok and Mullan[28] attributed 19·5% of the anaesthetic contributory deaths in their survey to this mechanism.

Hypertension During Anaesthesia

In this group of deaths due to ischaemic anoxia it is convenient to include 2 cases in which death resulted from the effects of massive subarachnoid haemorrhage, including probable cerebral ischaemia. In each case massive subarachnoid haemorrhage was due to rupture of an intracranial berry-aneurysm, following an episode of hypertension during anaesthesia involving the use of intravenous urea (Urovert). The use of intravenous urea as an agent to reduce brain bulk and tension has become a standard ancillary to anaesthetic techniques for neurosurgery. It is known that before the diuresis commences, following administration of the urea, the blood pressure may rise.[27] It is also recognized that intracranial berry-aneurysms that have previously leaked, may rupture following a rise in blood pressure. While anaesthetizing a patient for the operation of ligation of such a berry aneurysm, care must be taken to avoid circumstances that might provoke a sudden rise in blood pressure. For these reasons it is thought that, following the use of intravenous urea in the anaesthetic technique for such an operation, a rise in blood pressure should be anticipated and prevented if necessary. This may be done by the use of drugs such as halothane or ganglioplegic agents. One death, listed in Table IV, resulted from a pontine haemorrhage which followed an episode of severe hypertension occurring at the time the aorta was clamped during aortic resection. Again the same lesson applies. Such hypertension, if anticipated, should be cushioned by some artifice of the anaesthetist.

CARDIAC ARREST DURING ANAESTHESIA

Of the 58 anaesthetic contributory deaths, 34 were associated with the occurrence of cardiac arrest during anaesthesia. Twenty-one of these were attributed to anoxia, either anoxic or ischaemic. The causes of these have already been discussed. In the remaining 13 cases the cardiac arrest was due to identifiable direct causes (the responsibility of the anaesthetist) in 5, while in 8 the final aetiology was not identifiable with any certainty, though

anoxic and ischaemic anoxia were the most probable associated factors. The direct causes identified in 5 cases were as follows:

1. Reflex vagal arrest (plus anoxic anoxia) (1 case). This occurred at the time of bronchial clamping during a pneumonectomy.

2. Inadvertent hypothermia (1 case). Cardiac arrest followed the too rapid transfusion of cold blood in a child.

3. Drug induced (3 cases): (*a*) overdosage of ether—1 case; (*b*) thiopentone sodium—1 case—cardiac arrest immediately followed injection of thiopentone down a cardiac catheter in a baby with pulmonary stenosis; (*c*) halothane—1 case—ventricular fibrillation occurred during the delirium stage which followed attempted induction of anaesthesia with 4% halothane in a fit, healthy adult male. The stormy delirium, with struggling and breath-holding, doubtless led to hypoxia, hypercarbia and a high level of endogenous catecholamines.

The 2 patients listed as having had ventricular tachycardia did not develop cardiac arrest during anaesthesia. They are listed here because the ultimately fatal arrhythmia commenced during anaesthesia. In one the arrhythmia was precipitated by the intravenous administration of Digoxin, while in the other, anoxia and hypercarbia were probably precipitating factors in a digitalized patient who suffered cardiac failure from gross ischaemic heart disease.

INCOMPATIBLE BLOOD TRANSFUSION

During this survey 2 deaths associated with anaesthesia occurred as a direct result of the transfusion of incompatible blood. One, listed in Table II, was the responsibility of the anaesthetist, while the other, listed in Table III, was due to an error in the ward in identification of blood specimens for cross-matching.

INEVITABLE DEATHS (GROUP 3)

Though anaesthesia was not considered contributory, other than unavoidably so, to the 95 deaths in group 3 (see Table IV), the majority of these occurred during anaesthesia and I therefore wish to comment on aetiological aspects of some of them. A little over one-half of these cases involved cardiac or major vascular surgery. Neurosurgery, the next commonest group, was involved in 11% of these cases.

TABLE IV. CAUSE OF DEATH, GROUP 3: IMMEDIATE OPERATIVE MORTALITY

Cause of death	*No. o deaths*	*% of group*	*Cardiac arrest during operation*
Massive haemorrhage	44	46·3	41
(inadvertent hypothermia)	15		10
Cardiac surgery (other than haemorrhage)	19	20·0	17
Prolonged hypotension (refractory shock)	15	15·8	8
Coronary thrombosis	3	3·2	2
Pulmonary embolism	2	2·1	2
Air embolism	1	1·1	1
Cerebral embolism (mitral valvotomy)	1	1·1	0
Cerebral ischaemia (carotid stenosis/thromb.)	2	2·1	1
Pontine haemorrhage (clamping aorta)	1	1·1	0
Brainstem oedema/neuronal inj.	5	5·3	2
Vena caval obst. prone position	1	1·1	1
Incompatible blood transfusion	1	1·1	1
Total	95	100·3	76

Occurring in nearly one-half of the cases in this group, massive haemorrhage was the factor most commonly associated with death. The extent to which cardiac arrest and the ultimate death of the patient were due to the hypovolaemia, or to the known deleterious effects of the concomitant massive transfusion of stored blood, was impossible to assess. One of the more easily monitored effects of massive transfusion—inadvertent hypothermia—was known to be present in one-third of these cases of cardiac arrest associated with gross haemorrhage and massive transfusion. It may have been present in more. In those in which the oesophageal temperature was monitored, this ranged from 28° to 32°C at the time of cardiac arrest. Until fairly recently, the inadvertent hypothermia that followed massive transfusion of stored blood was in large measure unavoidable. The means of warming such blood with the speed required and the necessary safety were not available. However, many devices for this purpose have now been described.[28] In future, deaths associated with gross haemorrhage which appear to have resulted from the hypothermia of massive transfusion, rather than from the hypovolaemia of blood loss, should be regarded as preventable.

One other aspect of the deaths I have listed here associated with gross haemorrhage concerns those cases which occurred during resection and grafting of aortic aneurysms—predominantly ruptured aortic aneurysms. During the period of this survey 8 patients died during aortic resection. The majority of these were emergency resections for rupture of the aneurysm. Though all of these were associated with gross haemorrhage and massive transfusion, it is significant that 3 of these patients died at the time the aortic clamp was released, following completion of the aortic graft. It is pertinent to note that at this time of maximum aortic run-off, severe hypotension will inevitably result if hypovolaemia, masked by the previous application of the aortic clamp, has been allowed to persist. And it must be remembered that this sudden hypotension occurs in a patient who suffers from generalized vascular disease with a great likelihood of coronary atherosclerosis.

Ischaemic Heart Disease

Coronary thrombosis as a cause of death during anaesthesia—certainly coronary thrombosis with recent myocardial infarction demonstrable at autopsy—is relatively rare. During the 9 years of this survey, 3 such cases occurred. What is perhaps more important is the profound effect ischaemic heart disease has on postoperative mortality. A recent survey by Topkins and Artusio[29] showed that of patients with a pre-operative history of infarction, 6·5% sustained a postoperative infarct, whereas the incidence of postoperative myocardial infarction in patients with no such history was 0·66%. Of those whose pre-operative history of infarction was shorter than 6 months, no less than 55% had fresh infarcts postoperatively. Arkins and Hicks,[30] who report a similar incidence of postoperative infarction, report a mortality of 69% in those who suffered postoperative re-infarction. In this survey, of the 261 patients who died postoperatively from causes unrelated to the anaesthetic (those in group 2), 23, or approximately 9% of the group, died of myocardial infarction. It must be noted that these are deaths occurring in the first 24 hours postoperatively, and do not include deaths from this cause that may have occurred later in the postoperative period.

Pulmonary Embolism

Two patients died during anaesthesia from pulmonary embolism. It is perhaps worthy of comment that in both cases pulmonary embolism followed movement and manipulation of the patient some days after fractures of the leg.

Air Embolism

The death due to air embolism, listed here, occurred during insufflation of the fallopian tubes, in spite of the fact that carbon dioxide gas and not air was used for the insufflation.

Refractory Shock

Next in size to those involving cardiac and major vascular surgery is a group of cases where death followed prolonged and intractable hypotension, not the immediate result of massive haemorrhage. The condition manifested by this group of 15 cases can perhaps be best described as 'refractory shock'. In the majority, this state was obviously present before anaesthesia. Surgical operation was undertaken as a measure of desperation.

It must be realized that in this group as a whole, involving those patients who, if not moribund, were gravely ill, the assessment of the extent to which the anaesthetic and its management may have been contributory to the patient's death and the extent to which this was possibly avoidable, is extremely difficult.

Cardiac Arrest During Operation

Finally, it is relevant to summarize the causes of ultimately fatal cardiac arrest during operation and so to evaluate the relative importance of anaesthetic factors in its causation (see Table V). During the 9 years of this survey, 110 ultimately fatal cardiac arrests occurred during anaesthesia (0·93/1,000 operations). Factors directly related to the anaesthetic and its management were responsible for 31%. The commonest aetiological factors were the combined effects of gross haemorrhage and massive transfusion. Together these were responsible for 37% of this group. Factors other than haemorrhage related to direct cardiac surgery were responsible for the only other individual group of cases of any size (15%).

TABLE V. FATAL CARDIAC ARREST DURING OPERATION

	Causes	*Number of cases*	*% of group*
Anaesthetic contributory (30·9%)	Anoxic anoxia	13	11·8
	Ischaemic anoxia	8	7·3
	Reflex	1	0·9
	Drug (induced)	3	2·7
	Inadvertent hypothermia	1	0·9
	Uncertain aetiology	8	7·3
		34	30·9
Other causes (69%)	Gross haemorrhage/massive transfusion	41	37·2
	Cardiac surgery (other than haemorrhage)	17	15·5
	Prolonged hypotension	8	7·3
	Pulmonary embolism	2	1·8
	Air embolism	1	0·9
	Coronary thrombosis	2	1·8
	Carotid thrombosis (traumatic)	1	0·9
	Vena caval prone obstr.	1	0·9
	Brainstem oedema	2	1·8
		76	69·0
	Total	110	99·9

CONCLUSION

The actual incidence of anaesthetic death is low, yet the anaesthetic and its management were considered a significant contributory factor to 14% of the 'immediate operative mortality' associated with 177,928 operations at Groote Schuur Hospital. Clinical evaluation revealed that almost all of this anaesthetic contributory mortality was either probably or possibly preventable. Though the fundamental causes of anaesthetic contributory death remain unaltered, continued collection on a wide scale and study of clinical data are necessary to reveal their precise mechanisms and the errors and pitfalls from which they result. That these change with advancing surgical initiative and changing anaesthetic practice necessitates constant review, so that when they arise the necessary corrective action may be taken, or that, by anticipation, they may be avoided altogether.

Though such continuing reviews of all anaesthetic associated mortality are so obviously necessary, they are by and large neglected. Yet mortality, the crudest measure, is but the tip of the iceberg of clinical anaesthetic mistakes. To be of more value in the understanding and elimination of anaesthetic contributory deaths, these studies ought to include a constant review of all anaesthetic morbidity. 'The tolerance of the human body is phenomenal and if a few die every year, consider how many have been subjected to abusive practices. How many have almost died?'[9]

Such surveys, consistently and conscientiously carried out, provide the only means by which we may monitor our standards of clinical anaesthesia. Further, they provide a vital means of measuring and evaluating the safety of anaesthesia for the patient and consequently progress in anaesthesia.

SUMMARY

A survey of the immediate operative mortality, undertaken at Groote Schuur Hospital, is described, covering the 9 years 1956 - 1960 and 1963 - 1966. During this period 177,928 anaesthetics were administered.

Of 2,026 patients who died following surgery, 414 died during operation or within the immediate postoperative 24 hours, or failed to regain consciousness after anaesthesia, and constitute the group of 'immediate operative mortality'. To 58 of these deaths the anaesthetic and its administration were considered to have been significantly contributory, resulting in an over-all incidence of anaesthetic contributory death of 0·33 deaths/1,000 anaesthetics. Of these anaesthetic contributory deaths, 93% were probably or possibly preventable.

Evidence is presented from which we may infer that this mortality rate compares more than favourably with other world centres, and that in South Africa the incidence of anaesthetic contributory death has decreased over the last 30 years.

Anoxic anoxia was the commonest fundamental cause of death, being responsible for 47% of all anaesthetic contributory deaths. In over half of these, respiratory obstruction was the causal mechanism. Special attention is drawn to the acute complications of endotracheal intubation in this context.

Anaesthetic contributory death resulting from progressive, prolonged and intractable hypotension following induction of anaesthesia—a failure of circulatory homeostasis—is discussed.

Hypertension resulting from factors in the anaesthetic technique is also noted as being responsible for anaesthetic contributory deaths.

The aetiological aspects of some of 95 other deaths, classed as inevitable deaths, which occurred during anaesthesia and to which anaesthesia could be considered necessarily and unavoidably contributory, are discussed. In this group, gross haemorrhage and massive transfusion were the commonest causes of death.

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"I would have everie man write what he knowes and no more."—MONTAIGNE

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EDITORIAL

MORTALITY IN ANAESTHESIA

In a remarkably short period of years a situation has been reached in which the number of patients regarded as being unfit for general anaesthesia has become extremely small. Prominent amongst possible explanations for this trend is the improved understanding of the effects of anaesthesia and surgery in healthy and diseased patients; this understanding has led to corresponding improvement in management before, during and after surgery. Common experience confirms that a successful outcome may be expected in countless patients who might formerly have been considered unfit to withstand the proposed procedure and its aftermath.

Nevertheless deaths associated with anaesthesia due to causes that might be considered preventable (particularly with the aid of hindsight) continue to occur from time to time. There is no doubt that the series of reports on deaths associated with anaesthesia published under the auspices of the Association of Anaesthetists of Great Britain and Ireland has had a profound and beneficial influence in alerting anaesthetists to the predisposing factors and causes of these fatalities. These reports were based on information submitted voluntarily and the incidence of deaths could not, therefore, be readily related to the total numbers of anaesthetics administered in different circumstances. Reports of the incidence of deaths are uncommon and the survey of 9 years' experience, published by Harrison (1968) is accordingly of considerable importance. The period covered was 1956–60 inclusive and 1963–66 inclusive and was concerned with approximately 178,000 anaesthetics administered at Groote Schuur Hospital, Cape Town. Of the 414 patients included in the group defined as "immediate operative mortality" 58 were considered by Harrison to have died in circumstances in which ". . . the anaesthetic and its administration were considered to have been significantly contributory". This represents an incidence of 0.33 per 1000 anaesthetics, and 14 per cent of the deaths at operation and in the 24 hours following. He believed that the majority of these deaths (54–93 per cent) were probably or possibly preventable. The author discusses the problems inherent in making comparisons with reports from other medical centres and indicates those fallacies rendering invalid superficial direct comparison.

Examination of the data concerning the group of "anaesthetic contributory deaths" prompts reflection on the extent of progress towards safer anaesthesia. Respiratory obstruction accounted for 16 deaths, of which 8 were related to complications of endotracheal intubation, and only 3 to the inhalation of vomitus. Considering the probable total number of patients at risk the numbers are not large. They emphasize again that the passage of an endotracheal tube is not of itself a guarantee of safety. On the other hand, it would appear that repeated stress on the dangers of vomiting and regurgitation has not been in vain.

Harrison found that of deaths occurring as a result of anoxic anoxia one-third (9) were "relaxant associated" and notes that metabolic acidosis was probably a feature in 7 of these. It is heartening to read that none of these occurred in 1966 and it may be expected that the frequency of deaths from this cause is likely to decline substantially with intelligent use of facilities for acid-base studies and ventilation monitoring.

Points of significance emerge also from the group entitled "Inevitable Deaths" (95 patients), so termed because anaesthesia, although apparently clinically faultless, could not be excluded as a contributory factor. In about one-half gross haemorrhage was the factor most commonly associated with death. In several inadvertent hypothermia following massive transfusion of stored blood was thought responsible for cardiac arrest. In Harrison's view this cause of death should now be regarded as preventable.

Ischaemic heart disease is an increasingly common pre-operative finding, and in this survey it appears that of 261 patients who died postoperatively from causes unrelated to the anaesthetic 23 (9 per cent) succumbed to myocardial infarction in the first 24 hours. The risks of surgery and anaesthesia in patients with a history of myocardial infarction (especially if recent) are known to be considerable, if perhaps not always appreciated.

Surveys of this kind repay close study because they indicate the changing pattern of factors implicated in deaths following surgery and the range of pitfalls to be negotiated if "preventable deaths" are to be prevented.

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A Classification for Deaths Associated With Anaesthesia and

An Evaluation of Autopsy in Their Assessment*

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Section 86 of Act 13 of 1928, Union of South Africa, reads: "The death of a person whilst under the influence of a general anaesthetic or local anaesthesia, or to which the administration of an anaesthetic has been a contributory cause, shall not be deemed to be a death from natural causes within the meaning of the Inquest Act of 1919† or the Births, Marriages and Deaths Registration Act of 1923‡ or any amendment of these Acts'.

In many countries legislation such as this quoted from South Africa ordains the need for autopsy and inquest in all cases of death associated with anaesthesia. Its motivation is not the scientific investigation of the cause, incidence or prevention of death due to or associated with anaesthesia, but the investigation of possible medical negligence, i.e. the protection of the public. In such proceedings the forensic pathologist (in smaller centres, the district surgeon) is often called on to perform the triple task[18] of:

i. Performing the autopsy;
ii. Inferring therefrom the cause of death; and
iii. Guiding the Magistrate or, in some countries, the Coroner, in his findings.

It is important, therefore, that he have a simple and clear classification of deaths associated with anaesthesia; criteria for assessing them; and a clear concept of the ways in which the anaesthetic and its administration may cause or contribute to death.

Death associated with anaesthesia is the ultimate result of a combination of circumstances which may be broadly grouped as:

1. *The Environment of the Anaesthetic.*

(*a*) *The Patient's Disease.* This is often coincidental and not directly associated with the lesion necessitating surgery. Even if not itself responsible directly for the death of a patient, such disease (by its deleterious effects on the patient's physiological, homeostatic mechanisms) will render the administration of an anaesthetic and the immediate pre- and post-operative course more critical.

(*b*) *The Operation.* This includes the effects of the surgical lesion itself, the

*Based in part on a Thesis *Death due to Anaesthesia*, submitted to the University of Cape Town for the degree of M.D.

† Now the Inquest Act, Act 58 of 1959.

‡ Now the Births, Marriages and Deaths Registration Act, Act 81 of 1963.

magnitude of the surgical procedure and the effects of possible surgical mishaps and errors.

2. *The Conduct of the Anaesthetic Itself.* These factors and their possible combinations and permutations have resulted in elaborate classifications:[12, 35]

1. Death due to the operation.
2. Death due to the patient's disease.
3. Death due to the operation and the patient's disease.
4. Death due to the anaesthesia.
5. Death due to the anaesthesia and the patient's disease.
6. Death due to the anaesthesia, the patient's disease and the operation.

The classification used by many workers is a variation of this type.[2, 8, 9, 16, 19, 28, 30, 31, 36] An example of a slightly different classification is that adopted by a Committee on Deaths Associated with Anaesthesia of the Association of Anaesthetists of Great Britain and Ireland.[11, 13] This classification is also used by the South African Council of Scientific and Industrial Research (CSIR) Anaesthetic Deaths Research Unit,[20] and a similar investigating body in New South Wales, Australia.[25]

1. Those deaths where it is reasonably certain that death was caused by the anaesthetic agent, or technique, or in other ways within the province of the anaesthetist.
2. Similar cases in which there is some element of doubt whether agent or technique is entirely responsible.
3. Those deaths due to surgical and anaesthetic factors.
4. Surgical deaths.
5. Inevitable, e.g. cases of severe peritonitis but in which the surgical and anaesthetic technique is satisfactory.
6. Fortuitous deaths, e.g. pulmonary embolism.
7. Cases that could not be assessed.
8. Cases in which the data presented were inadequate. No opinion.

As such classifications have gained in detail, so they have lost in simplicity and clarity. For the forensic pathologist or, for that matter, the anaesthetic statistician, a far simpler classification is necessary.

1. *Death due to Anaesthesia.* From their very nature there are few, if any, deaths that occur in association with anaesthesia to which it can be said that the anaesthetic has made no contribution. The anaesthetic and its administration as a cause of death should be identified in a separate category only when it can be seen to have been the sole cause of, or a significant contributory factor to, a death. In this category the culpability of the anaesthetic administration should be considered not only in the context of the fit patient, but also equally in that of the more seriously ill and aged patient, nowadays an everyday surgical problem. This group may also be referred to as *Anaesthetic Contributory Death.*

2. *Death due to Other Causes.* When the conduct of the anaesthetic appears faultless, where other circumstances appear to have provided adequate reason for death and a contributory role of the anaesthetic, if present at all, is minor, cases are identified in a second group *Death due to Other Causes,* a group of less medico-legal importance.

3. *Inevitable Deaths.* Anaesthesia is necessarily and unavoidably contributory.

Lastly, there is another group of not uncommon cases which requires separate identification. These are deaths from which the anaesthetic and its management, even though clinically faultless, cannot be excluded as a contributory factor. This may be because death has occurred during anaesthesia; or it may be that the final collapse of circulatory homeostasis in a patient moribund before anaesthesia was induced, appears to have been finally precipitated unavoidably by the known effects of anaesthetic drugs and anaesthesia, e.g. vasodilation. Such may be regarded as deaths to which anaesthesia was 'necessarily and unavoidably contributory' or perhaps as 'inevitable' in the terms of the classification of the Association of Anaesthetists of Great Britain and Ireland.

Such deaths are more often than not associated with the surgery of desperation. In these circumstances precise assessment of the exact contributory role of the anaesthetic is impossible and of little clinical importance. All cases in which death has occurred following a cardiac arrest during anaesthesia, but to which the contributory role of the anaesthetic administration was minor, should also be classified in this group, even though death may have occurred in the post-operative period after the termination of the anaesthetic.

Cases classed as *anaesthetic contributory deaths* must now be further examined for their more precise cause.

Anaesthetic Contributory Deaths: The Cause of Death

The causes of anaesthetic contributory death may be thought of in terms of a simple concept on which a simple classification is based (Fig. 1). Death is ultimately the result of irreversible, anoxic, cerebral cellular damage. This may have resulted directly and finally from a general state of anoxia but more commonly follows at the end of the common pathway of cardiac arrest. Such cardiac arrest itself may result from a state of anoxia or from certain direct causes (e.g. hypothermia, acidosis, effects of certain drugs, changes in the intra- or extra-cellular potassium ratio, autonomic reflexes, direct handling of the heart, etc.

Many of these direct causes have a greater potentiality to cause cardiac arrest in the presence of anoxia. The fundamental state of anoxia which arises when the cellular oxygen demand and utilization exceed that available [26, 27] may arise as a result of a decreased arterial oxygen saturation (anoxic anoxia) or from decreased tissue perfusion for one or other reason (ischaemic anoxia). Though the state of anoxia is stressed in this classification, it must be remembered that acidosis (respiratory in the case of anoxic, and metabolic in the case of ischaemic anoxia) is an almost

invariable accompaniment and is synergistic with anoxia in the genesis of catastrophe.

The greater proportion of deaths to which anaesthesia was a significantly contributory factor present as cardiac arrest during an operation. In some cases the etiological factors that result in the cardiac arrest are apparent before the event occurs. More often these escape notice until the dramatic arrest of the heart. In these circumstances the causal factors, though they may be elucidated on occasion in retrospect, are often obscure. Beyond the fact that the conduct of the anaesthetic may appear in general to be contributory in the absence of more obvious causes, there may be no certainty about the precise cause. In this classification cases of cardiac arrest are classified in relation to the type of anoxia that was considered causal.

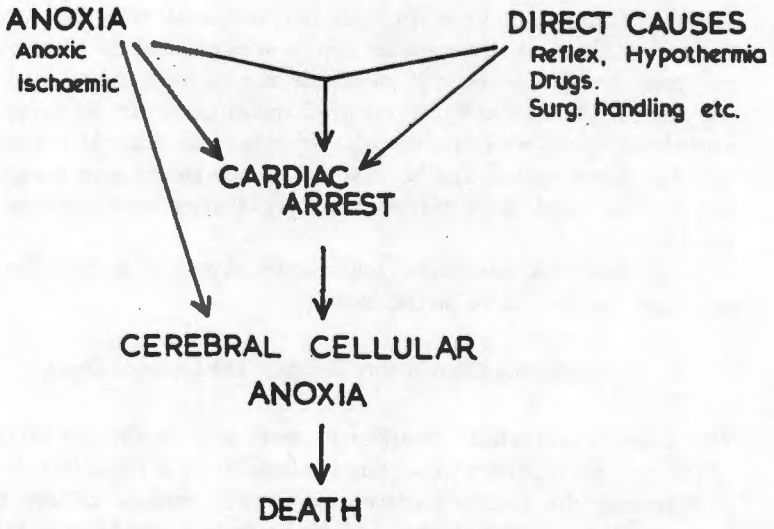

Fig 1. The cause of death.

Where some direct cause other than anoxia is responsible, or where the etiology is uncertain, they are classified in a separate category headed *Cardiac Arrest.*

Lastly, a category is provided for those few cases that do not fall into any of the foregoing categories, e.g. incompatible blood transfusions. This category is headed *Miscellaneous.*

In summary, the classification of causes of death is as follows:

1. *Anoxia* (*a*) Anoxic;
   (*b*) Ischaemic.
2. *Cardiac Arrest* (*a*) Direct causes;
   (*b*) Uncertain etiology.
3. *Miscellaneous.*

Within the framework of this classification, anaesthetic contributory

deaths must be further examined with regard to the more precise clinical fault or 'departure from accepted practice'.[13]

Preventability

Looked at broadly, deaths due to anaesthesia are a function of 3 factors:[24]

i. The skill of the anaesthetist;
ii. The condition of the patient;
iii. The agent used.

Of these, the skill of the anaesthetist is the most important—skill which is compounded of many parts, e.g. training, clinical experience and ability. The condition of the patient, which will respond to some extent to skilful pre-operative treatment, may often be something over which the anaesthetist will have no final control.

As regards the agent used, the anaesthetist should have sufficient knowledge of the drugs he uses clinically, of the actions, side actions and methods of combating these, that any death that may be said to result from so-called inherent toxicity of the drug used becomes death due to lack of skill. If we accept that lack of skill on the part of the anaesthetist is the most important factor in the cause of death due to anaesthesia, it follows that many, if not most, deaths due solely to anaesthesia are preventable. This aspect of preventability must be stressed in any consideration of a death due to anaesthesia.

In the assessment of any particular death that is considered due to anaesthesia, it is often difficult to say accurately that such a death was definitely preventable. Clinical anaesthesia is not an exact science. We seek always to identify 'departures from accepted practice'.[13] But this in itself must change with the passing of time and accumulation of further knowledge and experience.

On examination of the circumstances of any death in the light of present knowledge, we may be able to say that such a death was 'probably preventable'. More frequently the circumstances may allow only the conjecture that such a death was 'possibly preventable'. In some circumstances, though we may conclude (virtually by exclusion) that a particular death was in large measure due to the administration of an anaesthetic, examination of the conduct of the anaesthetic may fail to reveal any obviously correctable fault. We are then able to give 'no verdict' as to its preventability. This does not detract from the premise that the majority of deaths actually due to anaesthesia are preventable.

Following from this, those cases classed as anaesthetic contributory deaths should be subdivided into the 3 following accessory categories:

1. Probably preventable.
2. Possibly preventable.
3. No verdict.

Categories 1 and 2 include those cases described by Morton[24] as 'those deaths for which a reasonably satisfactory explanation can be provided and for which countermeasures are practicable'. While the last category includes those cases 'which cannot at present be fully explained and for which countermeasures are either lacking or largely empirical'. The possibility of medical negligence can only be considered in those cases regarded as *Probably Preventable* and, having been considered, it may then be very difficult, if not impossible, to distinguish between an error of judgement and frank medical negligence.

In summary, the classification of deaths associated with anaesthesia that would be of medico-legal use is as follows:

1. *Anaesthetic Contributory Death.*

Those deaths to which anaesthesia is considered to have been contributory to a significant degree.

Cause of Death.

1. *Anoxia*
   (*a*) Anoxic anoxia;
   (*b*) Ischaemic anoxia.
2. *Cardiac Arrest*
   (*a*) Direct causes other than anoxia;
   (*b*) Uncertain etiology.
3. *Miscellaneous.*

Preventability.

1. Probably preventable. (Category of medico-legal importance).
2. Possibly preventable.
3. No verdict.

2. *Deaths Due to Other Causes.*

Those deaths occurring in association with anaesthesia which are due to a patient's disease or factors relating to the surgical operation and to which the anaesthetic was considered either non-contributory or at most a minor contributory factor.

3. *Inevitable Deaths.*

Those deaths associated with the surgery of desperation to which the administration of an anaesthetic, though clinically faultless may be considered to have been necessarily and unavoidably contributory.

The Value of Autopsy in the Assessment of Death Associated with Anaesthesia

The positive value of autopsy in the assessment of the contributory role an anaesthetic and its management may have played in an associated death is disappointing.[7, 10, 17, 23, 24] Little has changed since a committee of the Boston Society of Medical Improvement reported in 1861 that

'No conclusive light can be thrown on the subject by post-mortem examination. It can only demonstrate a cause exculpating the anaesthetic, there being no pathognomonic signs of death from these agents'.[4]

Being acute, the factors that may lead to anaesthetic contributory death leave no pathognomonic change discernible at autopsy.[1, 29] That most basic

of mechanisms of death due to anaesthesia, gross anoxic anoxia, may result in no histological change if death is rapid.[3, 21] The events that may precede a cardiac arrest, e.g. hypotension, cardiac arrhythmias, autonomic reflexes, leave no trace.[32] Even as gross a finding as vomitus in the respiratory tract may require careful interpretation.[13, 17]

Essential to the correct assessment of a death associated with anaesthesia is the clinical account of the administration of the anaesthetic with relevant records of changes in the vital parameters.[36] Consideration must be given not only to the ways in which the known actions of anaesthetic drugs and errors or misjudgement in their administration may have lethal sequelae, but also to the ways in which errors of commission or omission in the wider field of responsibility that has by practice become that of the anaesthetist, may cause death, e.g. failure to replace adequately by transfusion blood lost during the operation, or the administration by the anaesthetist of incompatible blood.

Examination of this aspect involves clarification of what may be considered the extent of the anaesthetist's responsibility. This is something that is difficult to define precisely as the compass of the anaesthetist's responsibility must vary with time and place, depending on contemporary practice. It is submitted that the ambit of the anaesthetist's responsibility extends to involve attention to all matters that have a bearing on the maintenance of the patient's general physiological homeostasis during and immediately after the performance of a surgical operation under any form of anaesthesia.

Though disappointing in a positive sense, autopsy may have value in an important negative sense. Occasionally it may reveal the presence of a morbid process sufficient of itself to account for death in circumstances where an anaesthetic etiology may have been suspected,[6, 34, 36] e.g. massive pulmonary embolus. More often autopsy may reveal a state of general systemic disease which may have reduced the general circulatory, respiratory and other homeostatic mechanisms of the patient, so rendering him more susceptible to the effects of anaesthetic drugs, a state we may regard as reducing the margin of clinical error permitted the anaesthetist. Such findings will reveal the 'defective soil' on which the 'seed' of anaesthesia has been sown.

A contemporary survey of the mortality associated with 177,928 anaesthetics administered over a 9-year period at the Groote Schuur Hospital, Cape Town, was recently completed.[15] Criteria for inclusion of cases in this survey were wider than those of Section 86 of the Medical, Dental and Pharmacy Act quoted in the introduction to this paper. Clinical documentation of cases was completed by the findings at autopsy, where possible. When deaths associated with anaesthesia fell within the provisions of the Inquests Act of 1919 (and later 1959) or Section 86 of the Medical, Dental and Pharmacy Act, autopsy was performed by the State Pathologist.

When this was not the case, the necessary legal permission for autopsy was sought from the patients' relatives. Where this was obtained autopsy was performed by the Department of Pathology of the University of Cape Town.

Of the 414 patients who constituted the group classed as 'immediate operative mortality', 293 (71%) were submitted to autopsy. The overall value of the findings of these autopsies is presented here.

Immediate operative morality was defined as including those patients who died:

1. During operation and anaesthesia;
2. Within 24 hours of anaesthesia;
3. Without regaining consciousness after an anaesthetic, having been conscious before.

Assessed in terms of the classification described in this paper, the findings are reflected in Table 1.

Table 1: Operative Mortality Incidence in 177,928 Operations and Anaesethetics *Groote Schuur Hospital:* 1956–60, 1963–66 (*Inclusive 9 years*)

	No. of Deaths	*Deaths per* 1,000 *Anaesthetics*	
Total operative mortality	2026	11·38	
'mmediate operative mortality	414	2·33	20% of total operative mortality
Group 1 (Anaesthetics)	58	0·33	14% of immediate operative mortality
Group 2	261	1·47	
Group 3	95	0·53	

The autopsy findings may be grouped in general terms as showing:

1. Pathological lesions adequate to account for death, including the surgical lesion itself.
2. No adequate immediate cause for death.
3. Incidental pathology sufficient to seriously increase the hazard of anaesthesia for the patient. In the case of cardiac and vascular surgery the surgical lesion itself was often of a nature that affected the patient's circulatory, physiological homeostasis directly.
4. Pathological lesions sufficient of themselves to account for death where an anaesthetic etiology was suspected.

An evaluation of the autopsy findings in these terms is reflected in Table 2.

In very few instances of anaesthetic contributory death (Group 1) were the autopsy findings of any positive help in the assessment of the cause of death. Whereas pathological lesions of a severity adequate to account for death were shown in more than two thirds of the deaths to which anaesthesia was not regarded as contributory (Groups 2 and 3), such lesions

Table 2: General Evaluation of Autopsy Findings
Percentage of individual autopsy group in brackets

Group	*No. in Group*	*No. of Autopsies*	*Lesion Adequate to Account for Death*	*No immediate Cause for Death*	*Grave Incidental Pathology*	*Lesion Exculpating Anaesthesia*
1	58	52	18 (34%)	34 (65%)	11 (21%)	0
2	261	154	128 (83%)	26 (17%)	42 (27%)	2
3	95	87	59 (68%)	28 (32%)	37 (43%)	13
Total	414	293	202	91	103	15

could only be demonstrated in one-third of the anaesthetic contributory deaths (Group 1). In only 7 cases was it easy to relate a demonstrable acute cause of death with an incident in the anaesthetic administration (Table 3).

Table 3: Relation of Incident in Anaesthesia to Autopsy Finding

Cause of Death Clinical Diagnosis		*Incident in Anaesthetic Administration*	*Lesion Demonstrated at Autopsy*
Anoxic Anoxia	Vomiting and aspiration	Signs of anoxic anoxia	Stomach content soiling bronchi
	Bronchial obstruction	Open ether anaesthesia without atropine premedication	Frothy material obstructing bronchi and trachea
	Pulmonary oedema	Overtransfusion	Pulmonary oedema
Ischaemic Anoxia	Ischaemia Cerebral damage	Hypotensive anaesthesia Haemorrhage Profound hypotension	Areas of cerebral softening
	Ischaemia Cerebral damage	Profound hypotension Patient in sitting position	Areas of cerebral softening
	Cerebral compression and ischaemia	Episode of hypertension during induction of anaesthesia	Rupture of berry aneurysm. Subarachnoid haemorrhage
	Cerebral compression and ischaemia	Episode of hypertension during induction of anaesthesia	Rupture of berry aneurysm. Subarachnoid haemorrhage

In a further 11 cases of anaesthetic contributory death, gross incidental pathology of the cardiovascular and respiratory systems was demonstrated. But in 34 of the 52 anaesthetic contributory deaths, the autopsy showed no immediate cause of death. In all cases of anaesthetic contributory death, the correct evaluation of the contributory role of the anaesthetic and its

administration depended entirely on the clinical account of the conduct of the anaesthetic.

Lest, however, the importance of autopsy be underestimated, attention must be drawn to 15 cases in which the findings at autopsy virtually exculpated the anaesthetic administration from a contributory role in the patient's death, though death had occurred during the anaesthetic administration or very shortly afterwards. In each case, though the anaesthetic administration appeared in general adequate, aspects of each were open to criticism. Little other concrete cause of death was immediately apparent at the time it occurred. In each case, autopsy revealed gross lesions which quite adequately accounted for death (Table 4).

Table 4: Autopsy Findings exculpating Anaesthesia as a Cause of Death.

Surgical Procedure	*Autopsy Finding*	*No. of cases*
Craniectomy: Head injury	Brain stem oedema	2
Excision of haemangioma (hypothermia)	Herniation of uncus	1
Aortico-renal endarectomy	Pontine haemorrhage	1
Repair post-aortic graft wound dehiscence	Gross ischaemic heart disease	1
Oophorectomy Transurethral resection of the prostate Laparotomy	Fresh myocardial infarct	3
Manipulation of fractured hip Repair of wound dehiscence	Pulmonary embolus	2
Tubal insufflation	Air embolus	1
Laparotomy: Intestinal obstruction	Ruptured thoracic aortic aneurysm	1
Laminectomy: Spinal tumour	Secondaries in liver and kidney, obstructing inferior vena cava	1
Oesophagectomy	Trauma to right atrium and atrio-septal defect	1
Repair of lacerations of neck	Right carotid thrombosis	1

Fresh myocardial infarction, demonstrable at autopsy and occurring during anaesthesia, is uncommon. In these 293 autopsies, 3 were demonstrated. The relationship of various anaesthetic drugs and techniques to such infarction is not clear but does not appear at this time to be more than coincidental.[33] More difficult to interpret and evaluate in relation to deaths associated with anaesthesia is the much more common finding of gross coronary atherosclerosis. This was noted in 50 of these autopsies. Myocardial perfusion depends *inter alia* on the aortic diastolic pressure and the length of diastole. Fluctuations in the blood pressure and heart rate are common during the course of any anaesthetic. It is easy to envisage that in the face of marked narrowing of the coronary ostia, episodes of hypotension, even of short duration, may result in poor myocardial perfusion severe enough to cause ischaemic arrest of the heart or ventricular

fibrillation. But how is this to be assessed at autopsy in the individual case when it is known that patients with equally severe lesions have survived unscathed long periods of hypotension during anaesthesia and operation?

Conclusion. In view of the limited positive value autopsy has in the assessment of the cause of a death associated with anaesthesia, especially in those cases in which the anaesthetic itself is causal, one may with justice question the value of legislation requiring compulsory autopsy and inquest in such cases. Where it exists, such legislation has been criticized as imposing an unfair burden on the clinical anaesthetist besides being of little scientific value.[5, 10, 14, 20, 22, 26, 36] But is dispensing with such medico-legal autopsies and inquiries the answer? Two factors alone should make us hesitate. Throughout the world the demand for anaesthetic services exceeds the supply of trained clinical anaesthetists. To fill the demand–supply gap, there is often pressure for the less adequately trained person to administer anaesthetics. This is a circumstance which surely makes it desirable that some form of review of anaesthetic mortality be retained. One must remember, too, the number of cases in which an autopsy is of material practical value in the negative sense of excluding the anaesthetic as a cause of death.

What is needed is an increase in the clinical and scientific value of such proceedings. It would be of value if independent regional review bodies consisting of specialist anaesthetists and surgeons in addition to the forensic pathologists were appointed to advise and guide the magistrate or the coroner in these cases. More important, such a body of experts could evaluate and propagate the clinical and scientific lessons that would emerge from a deeper consideration of this mortality associated with anaesthesia. In this fashion, from the necessity of providing legal protection of the public from possible medical negligence, could be derived the virtue of a means towards the promotion of greater safety in anaesthesia.

Summary

A simple classification of deaths associated with anaesthesia is described and the general etiology of anaesthetic contributory death is briefly reviewed.

From a series of 293 autopsies, the value of this investigation in the assessment of the cause of death associated with anaesthesia is evaluated.

I wish to acknowledge my gratitude to the State Pathologist, Prof. L. S. Smith, the Professor of Pathology of the University of Cape Town, Prof. J. G. Thomson, and their respective departments, who were responsible for the autopsies on these patients.

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Anaesthetic-Associated Mortality

G. G. HARRISON

SUMMARY

The most important measure of the safety of anaesthesia for the patient is the incidence of anaesthetic-contributory death. Evidence is presented from an ongoing survey of anaesthetic-associated mortality at Groote Schuur Hospital, that the incidence of anaesthetic-contributory death has decreased from 0,33 to 0,22 per 1 000 anaesthetics. Reasons for this improvement in the safety of anaesthesia and some of the clinical lessons that emerge from the survey are discussed. The incidence of cardiac arrest in the operating theatre (0,63‰ anaesthetics) and the results of resuscitation (19% survival) are presented.

S. Afr. Med. J., **48**, 550 (1974).

Anaesthesia is a highly important adjunct to the care of the patient. Anaesthesia is not therapy which corrects a deformity, which restores health, or stays death; it simply makes possible the acts which accomplish these things. So above all it must be safe. In no field of medical practice does the precept 'to do no harm' have more cogency than in anaesthesia. Whatever advances are claimed for anaesthesia, they can only be regarded as real advances if they result in increased safety for the patient. However, to measure this safety is difficult. Though one may postulate the most fundamental index of the safety of anaesthesia to be the incidence with which factors related to the administration of an anaesthetic cause or are contributory to a patient's death, such a postulate immediately poses the problem of apportioning blame. Patients who die under anaesthesia do so more often from the disease or the surgery undertaken to cure it. than from the anaesthesia. To measure this incidence we must separate as best we can, anaesthetic-contributory deaths from their parent population of anaesthetic-associated mortality.[2]

At Groote Schuur Hospital I have conducted a contemporary survey of mortality associated with anaesthesia since 1956. Data on the anaesthetic-associated mortality of 15 years in over 300 000 anaesthetics are now available. Findings during the first 9 years of this survey were reported in 1968.[1] The data of the last 6 years (1967 - 1972 inclusive) are reported here and compared, where relevant, with the former study.

DEFINITIONS AND METHOD

For the purposes of this article anaesthetic-associated death is defined as a death occurring during or within 24 hours of anaesthesia, or a failure to regain consciousness after anaesthesia in a patient conscious before anaesthesia. The choice of a period of 24 hours after anaesthesia is arbitrary. It embraces a period adequate to permit identification of anaesthetic-contributory death, without becoming unmanageable. Extension of this study to a surveillance of the whole postoperative period, although desirable in some respects, would add considerably to its difficulties. It is acknowledged that, in these circumstances, a small number of deaths to which anaesthesia was a major contributory factor, such as some late deaths from aspiration pneumonia, might have been missed.

For each case of anaesthetic-associated mortality, the clinical records and an account of the conduct of the anaesthesia were obtained from the anaesthetist concerned. This information and the autopsy report were examined from 3 points of view:

1. Was the administration of the anaesthetic the cause of, or a major contributory factor to, the patient's death? Or was the death primarily due to the patient's disease or the surgical procedure being undertaken?

2. If it was considered to be an anaesthetic-contributory death, what was the precise clinical cause—what departure was there from accepted clinical practice?

3. Was the death preventable? Was there a clinical lesson to be learnt?

With regard to the first question, besides the two groups of cases implied: (*i*) anaesthetic-contributory deaths, and (*ii*) death due to other causes, there emerged a need for a third group: deaths associated with the surgery of desperation, operations on the moribund, for which, though an anaesthetic aetiology may not have seemed directly responsible, it could often not be entirely excluded; circumstances in which anaesthesia might be regarded as being necessarily, but unavoidably, contributory, were classified as 'inevitable death'.

GENERAL INCIDENCE

This 6-year survey covers about 141 000 anaesthetics. This is a crude total and includes all anaesthetics, from simple casualty procedures to open-heart surgery. An obvious refinement of a study such as this would be an analysis of mortality associated with specific operations or anaesthetic procedures. This has not been done. For comparison, relevant figures from the previous survey are included in parentheses in Table I.

This survey yielded an anaesthetic-associated mortality of 253 cases, a rate of 1,8/1 000 anaesthetics. Anaesthesia was considered to be the sole or major contributory factor in 12% (31 deaths) of this anaesthetic-associated mortality—an anaesthetic-contributory death-rate of 0,22/1 000 anaesthetics.

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TABLE I. GROOTE SCHUUR HOSPITAL: ANAESTHETIC-ASSOCIATED MORTALITY IN 140 653 ANAESTHETICS
(1967 - 1972 (incl.), 1956 - 1966 in parenthesis)

	No. of deaths	Deaths/ 10^3 anaesth.
Total operative mortality	1 315 (2 026)	9,35 (11,38)
Anaesthetic-associated mortality	253 (414)	1,80 (2,33)
Anaesthetic-contributory deaths	31 (58)	0,22 (0,33)
Other causes	147 (261)	1,04 (1,47)
Inevitable	75 (95)	0,53 (0,53)

$ACD/_{AAD} \times 100 = 12\%$ (14%)

$ACD/_{TOM} \times 100 = 2,3\%$ (2,8%)

AAD = Anaesthetic-associated death
ACD = Anaesthetic-contributory death
TOM = Total operative mortality

Looked at against the background of the total care of the surgical patient, anaesthesia and its mismanagement can be said to have been responsible, by means possibly preventable, for 2,3% of the total surgical mortality.

In the sense that the rate of anaesthetic-contributory death has decreased over the 6 years from the previous 9-years' report (0,22/1 000 from 0,33/1 000) anaesthesia may be said to have become a little safer for our patients. This increased safety may be a reflection of a fundamental, though gradual and undramatic, change that has occurred in anaesthetic practice during the last decade. This change follows the gradual acceptance of the routine use in all clinical anaesthesia of the elementary monitoring of vital signs, over and above the old-fashioned recording of systolic blood pressure, such as ECG, central venous pressure and blood gas monitoring.

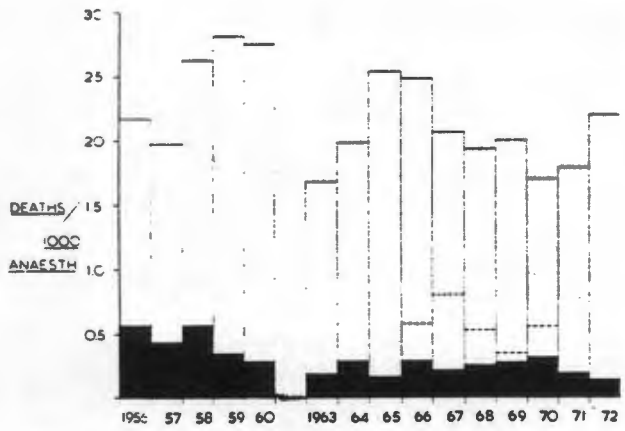

Fig. 1. Anaesthetic-associated mortality and anaesthetic-contributory death at Groote Schuur Hospital 1956 - 1972. (Open columns = anaesthetic-associated deaths; black columns = anaesthetic-contributory deaths.)

When anaesthetic-associated and contributory death rates are graphically represented on an annual basis (Fig. 1), although the initial downward trend in the latter is obvious, it appears that in this institution an anaesthetic-contributory death rate of 0,2/1 000 anaesthetics, regrettably constitutes an irreducible minimum. This is depressing when one considers that anaesthetic-contributory death is essentially preventable. Perhaps, in mitigation, it may be said that this is a teaching institution with a large, constantly changing staff, which results in a dilution of experience. It seems as though every trainee is educated through the same mistakes. Every trainee must be taught to acquire the right clinical reflexes. Constant and adequate supervision of trainees by experienced, specialist staff is only part of the answer, for the most enduring lessons for the trainee come only from the final acceptance of total clinical responsibility.

MECHANISMS OF ANAESTHETIC-CONTRIBUTORY DEATH

The causes and mechanisms of anaesthetic-contributory death were examined in terms of the following simple concepts (Fig. 2). The basic requirement for life is perfusion of tissue with oxygenated blood. Failure to perfuse tissue (and more particularly the brain) with an adequate

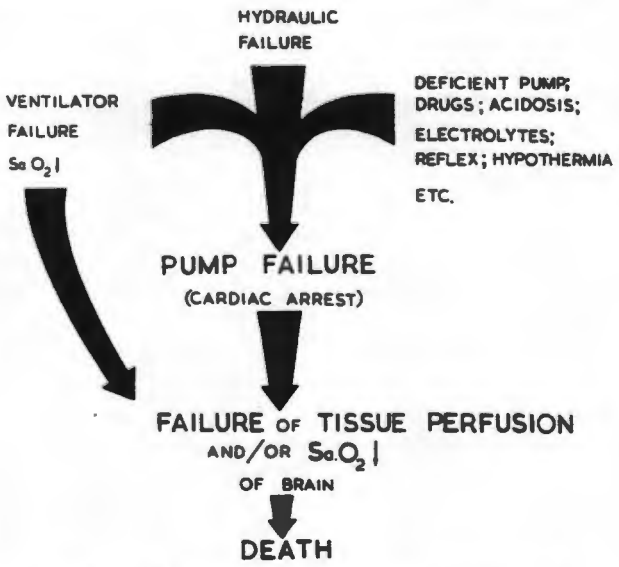

Fig. 2. Mechanisms of anaesthetic-contributory death.

supply of oxygenated blood, is the basic cause of death. In broad terms, this occurs when there is a failure of either the ventilator or the pump or both. While the former may directly cause cerebral death, the final common pathway is more usually pump failure and cardiac arrest. Cardiac arrest, although often caused by anoxia, has a wider aetiology, the most prominent factor of which, in a surgical sense, is a failure of the hydraulic system—hypovolaemia, compounded by the vasodilation of anaesthesia and its sequelae of concomitant poor circulatory homeostasis, acidosis, and electrolyte changes. It must be realised that the greater proportion of anaesthetic-contributory deaths present as cardiac arrest during operation. In some cases the reasons may be apparent before the event occurs, but more often they may escape notice until the dramatic arrest of the heart. At this stage it may be difficult to elucidate the cause in retrospect.

Analysed in these terms, the 31 deaths in this study are presented in Table II. This study reveals, like its predecessor, the same two clinical situations most frequently causally associated with anaesthetic-contributory death. These 2 situations between them are responsible for just under one-half of all anaesthetic-contributory deaths, or for the same number of anaesthetic-contributory deaths as all the other listed causes put together. These two situations are complications of intubation and hypovolaemia.

TABLE II. GROOTE SCHUUR HOSPITAL: CAUSES OF ANAESTHETIC-CONTRIBUTORY DEATHS IN 140 653 ANAESTHETICS
(1967 - 1972 (incl.), 1956 - 1966 in parenthesis)

	No. of deaths	Deaths/ 10^5 anaesth.
Ventilator failure		
$SaO_2\downarrow$		
F_IO_2 technical mishap	0 (1)	0 (0,5)
Vom. regurg. inhal.	2 (3)	1,4 (1,7)
Sec. bronchial obstruct.	2 (2)	1,4 (1,1)
Bronchospasm	1 (0)	0,7 (0)
Comp. intubation	7 (8)	4,9 (4,4)
Relax. related	3 (9)	2,1 (5,1)
Inadequate postop. care	3 (3)	2,1 (1,6)
Pump failure		
(circulatory haemostasis)		
Hypovolaemia	8 (12)	5,7 (6,7)
Hypervolaemia—overhyd.	2 (1)	1,4 (0,5)
urovert	0 (2)	0 (1)
Cardiac arrest—direct causes	2 (10)	1,4 (5,6)
uncertain	1 (6)	0,7 (3,3)
Miscellaneous		
Incompatible blood	0 (1)	0 (0,5)
	31 (58)	22 (33)

F_IO_2 = Inspired oxygen concentration.
Cardiac arrest—direct causes = Cardiac arrest due to factors listed at upper right of Fig. 2.
Overhyd. = Overhydration.

Complications of Intubation

Endotracheal intubation is indeed a necessary technique for the anaesthetist. Complications are simple and mechanical, but lethal, unless diagnosed and corrected immediately. The reason for failure is often a false sense of security engendered by the very presence of the endotracheal tube, together with, as often as not, a lack of experience. A brief look at these 7 cases is instructive, as one lesson in particular emerges strongly. Over half of these deaths followed the technical failure of intubation in the presence of anatomical difficulties. All could have been easily anticipated before anaesthesia by adequate clinical examination, yet in all instances succinylcholine was given before the anaesthetist had assured himself that he could intubate or ventilate the patient. The anatomical difficulties were caused by carcinoma of the larynx and oesophagus, cervical tuberculous spondylitis, and torticollis.

One case was an example of that ever-lurking trap—the overinflated, herniated cuff—a lesson that needs constant repetition, as there often is, as in this case, a period of many minutes between the inflation and the herniation of the cuff, with subsequent respiratory obstruction.

One death followed kinking of the endotracheal tube when the patient was turned prone. The last, less culpable than the others, followed the obstruction of the right side of a Carlen's tube by distortion caused by an aneurysm of the ascending aorta.

Inadequate Postoperative Supervision

Disappointing is the appearance in this study of 3 cases due entirely to inadequate postoperative supervision. One death was caused by the direct attachment of a line from an oxygen cylinder to an endotracheal tube left *in situ* by the anaesthetist for postoperative IPPV. A second was due to the booby-trap of the endotracheal tube catheter-mount of a nasal endotracheal tube becoming detached unobserved inside the nose in a patient requiring postoperative IPPV.

I should like to draw particular attention to the third case, for it is an example of a condition to which the now widespread use of respiratory-depressant, narcotic, analgesic drugs, such as Fentanyl, exposes patients—a condition for which the name 'Ondine's curse' is appropriate. As with the nymph, the patient, while recovering from anaesthesia, though apparently awake, seems to have forgotten how to breathe, unless he is stimulated. This particular patient, having initially regained consciousness after uncomplicated anaesthesia for a herniorrhaphy, relapsed into unconsciousness and apnoea while in the care of a nurse in a busy theatre corridor. This event was not reacted to until cardiac arrest with some anoxic jactitation occurred. Although cardiac massage and resuscitation were successful, the brain damage sustained was irreparable.

Vomiting and Regurgitation

More encouraging is the very low incidence of death due to vomiting, regurgitation, and inhalation. Such deaths often feature in studies of anaesthetic-associated mortality.

Relaxant-Related Deaths

Noteworthy too, is the great reduction in relaxant-related deaths compared with the previous study—deaths due to mismanagement of prolonged curarisation. There is really little excuse for such deaths today.

Hypovolaemia

Under this heading are included those cases in whom a frank failure of circulatory homeostasis has followed induction of anaesthesia, a failure that appears to have resulted from inadequate venous return. These circumstances proved to be the most common, single cause of anaesthetic-contributory death. Once circulatory failure had ensued, metabolic acidosis and concomitant electrolyte disturbance may have been important in the causation of the cardiac arrest. But I have chosen to call attention in my classification to the initial hypovolaemia, as this situation is eminently correctable. Some workers stress this by classifying these deaths as due to 'inadequate preparation of the patient'.[2]

These cases, who also feature prominently in other surveys,[2,3,4] present most commonly, as did half of the 8 listed in Table II, as postinduction hypotension proceeding to cardiac arrest. The precipitating factor is often the exhibition of thiopentone to the sick, old, and arteriosclerotic who have evidence of ischaemic heart disease, or the administration of thiopentone to the shocked. A typical example is the old man with diabetic gangrene, presenting for amputation of a leg. Whether it be the vasodilatation, or the direct depressant effect of thiopentone on the myocardium, the situation is readily preventable by judicious hydration to normal CVP levels before induction of anaesthesia and avoidance of thiopentone.

Two of these deaths followed sudden blood loss in the presence of vasodilatation, the result of sympathetic paralysis: 1 from an epidural anaesthetic, and 1 from the use of a uterine antispasmodic, isoxuprine.

I wish to stress that in none of these 8 patients was the central venous pressure monitored, and in 4, the failure of circulatory homeostasis was precipitated by thiopentone.

Hypervolaemia

The reverse situation is hypervolaemia from over-hydration. This usually results in pulmonary oedema when spontaneous respiration commences again at the conclusion of the anaesthesia. Two such cases are recorded here of a situation essentially avoidable by the substitution of fluid replacement guesswork, with CVP monitoring.

Cardiac Arrest in the Operating Theatre

This subject is exhaustively covered in the literature. I intend to summarise only the causes and the outcome of treatment in the cases occurring in Groote Schuur Hospital during the period of this survey. I have excluded from consideration all cardiac surgery in which so often cardiac arrest and its reversal are almost part of the operative technique. I have, however, included 5 cases of cardiac trauma from stab and gunshot wounds. The causes and results of treatment are set out in Table III.

TABLE III. GROOTE SCHUUR HOSPITAL 1967 - 1972: OPERATING ROOM CARDIAC ARRESTS IN 140 653 ANAESTHETICS

Causes				Resuscitation		
				Failed	Initial success	Ultimate success
Anaesthetic $SaO_2\downarrow$	Bronch. obstruc. spasm	2				
	Comp. intubation	8				
	Inhal. vom. regurg.	2				
	Underventilation	3	44%			
$Q\downarrow$	Hypovolaemia	13				
	Hypervolaemia	2				
	Cardiac arrest—direct causes	7				
	uncertain	2	39 (0,27‰)	21 (54%)	18 (46%)	12 (31%)
Other						
Haemorrhage + massive transf.		33				
Toxaemia refract. shock		8				
Cardiac trauma + tamponade		5	56%			
Ischaemic heart disease		2				
Diffuse neuronal injury		1				
Embolus (pulmonary)		1	50			
	Total		89 (0,63‰)	60 (68%)	29 (32%)	17 (19%)

$SaO_2\downarrow$ = Reduced arterial oxygen saturation.
$Q\downarrow$ = Decreased circulation/perfusion.

During the period under study, 89 patients suffered cardiac arrest during anaesthesia and operation, an incidence of 0,63/1 000 operations. Various anaesthetic problems were the sole or major contributory factor in the causation of 44% of all cardiac arrests.

As in the causation of anaesthetic-contributory death, the two principal faults were failure to maintain normovolaemia and complications of endotracheal intubation.

Worthy of comment in that group of anaesthetic-contributory cardiac arrests thought to be drug-induced, was 1 case in whom the cardiac arrest appeared to follow the administration of halothane (with adequate ventilation) to a parkinsonian patient being treated with L-dopa. Although this combination has been suggested as a potential cause of arrest because of th emobilisation of tissue catecholamine stores by L-dopa,[4] we are not aware of its having been so recorded.

Other drug-induced arrests followed the administration of succinylcholine, halothane in the presence of underventilation, and the hypotensive drug, trimetaphan, in the presence of a recent myocardial infarct—all well-known anaesthetic hazards.

The commonest single cause of cardiac arrest in the operating room, and responsible for 30% of cardiac arrests in this study, was gross haemorrhage with the deleterious effects of the concomitant massive transfusion. While many of the problems which follow the latter have been solved by monitoring of acid-base and electrolyte status, and the warming of transfused blood,[6] the basic problem is surgical and circumstantial.

With the present-day accepted use of vital-function monitoring in clinical anaesthesia, and the training of anaesthetists in prompt and informed resuscitative procedures, one could hope for a reasonable salvage from cardiac arrest occurring during surgery right under the eye of the anaesthetist, but the commonest cause—uncontrollable haemorrhage—precludes this.

In Table III the results of resuscitation from cardiac arrest are expressed as: 'failed'—death in operating theatre; 'initially successful'—patient survives to return to ward; 'ultimately successful'—patient survives to be discharged from hospital.

It can be seen that about one-third of all patients suffering from cardiac arrest survived to leave the operating theatre and one-fifth eventually left hospital. The prognosis for those which were the result of anaesthetic mismanagement (0,27/1 000) was slightly better. While about one-half survived to return to the ward, resuscitation was wholly successful for about one-third in that the patient was ultimately discharged alive from the hospital.

CONCLUSION

Whatever advances we claim for our speciality, anaesthesia, it must be safe for the patient. Surveys and studies such as this are somewhat pedestrian and tedious in the extreme to conduct, but if consistently and conscientiously carried out, they provide not only the clinical lessons by which we can improve our practice, but also the only means of measuring and evaluating the safety of anaesthesia.

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DEATH ATTRIBUTABLE TO ANAESTHESIA

A 10-year survey (1967–1976)

G. G. HARRISON

SUMMARY

The mortality associated with 240 483 anaesthetics administered over 10 years at Groote Schuur Hospital, Cape Town, is reported. The frequency of death to which anaesthesia contributed was 0.22 per 1000 anaesthetics (compared with 0.33 per 1000 in the previous 10 years). These deaths were responsible for 2.2% of the total mortality from surgery. Two-thirds of the "anaesthetic" deaths were attributable to (in order of frequency): (a) hypovolaemia; (b) respiratory inadequacy following myoneural blockade; (c) complications of tracheal intubation; (d) inadequate postoperative care and supervision.

Although anaesthesia *per se* does not correct deformity, restore health or stay death, it is perhaps the most important adjunct to the care of the surgical patient, making possible activities which do accomplish these things. Of itself non-therapeutic, above all it must be safe for the patient. In no field of medical endeavour does the precept "to do no harm" have more cogency than in clinical anaesthesia. Whatever advances are claimed for anaesthesia, they can be regarded as real advances only if they result in an increase in safety for the patient. A precise measure of this safety is difficult. We postulate that the most fundamental index of the safety of anaesthesia for the patient is the frequency with which factors related to the administration of an anaesthetic cause or are contributory to a patient's death. This in itself is not something that can be estimated with scientific precision, but if we can be content with a clinical assessment of the relevant data, a useful measure of the "safety of anaesthesia" does emerge. Of importance are the clinical lessons yielded by such assessment which are essential to the improvement of the service to the patient.

A prospective survey and assessment of all mortality associated with anaesthesia at Groote Schuur Hospital, Cape Town, since 1956 has been undertaken. Groote Schuur Hospital is the 1300-bedded principal teaching hospital of the University of Cape Town Medical School. The data from the past 10 years, 1967–1976 are reported here, that is the mortality associated with 240 483 anaesthetics.

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DEFINITIONS

Death associated with anaesthesia is defined as a death occurring during or within 24 h of anaesthesia or after the failure of a patient, conscious before, to regain consciousness after anaesthesia. The choice of a period of 24 h after anaesthesia is arbitrary. It embraces a period adequate to permit identification of death attributed to anaesthesia without the study becoming unmanageably large. Extension of this study to a surveillance of the whole period after operation, although desirable in some respects, would have added considerably to its difficulties and complexities. It is acknowledged that in these circumstances a very small number of deaths to which anaesthesia was a major contributory factor, such as late deaths from aspiration and pneumonia, might have been missed.

For each death associated with anaesthesia, the clinical records, together with an account of the conduct of the anaesthetic, were obtained from the anaesthetist concerned. This information, together with the postmortem report, was examined in the light of three questions:

(1) Was the administration of the anaesthetic or other factors within the ambit of the anaesthetist's responsibility the cause of or a major contributory factor in the patient's death? Or was the death primarily a result of the patient's disease or the surgical procedure being undertaken? The former were classed as "deaths contributed to by anaesthesia", the latter "deaths from other causes".

(2) If it was considered to be death to which anaesthesia contributed, what was the precise cause?

(3) Was the death preventable? What departure was there from accepted clinical practice? Was there a clinical lesson to be learned?

With regard to question (1), besides the two groups defined, deaths to which anaesthesia contributed and deaths from other causes, there emerged a need for a third group for deaths associated with the surgery of desperation, operations on the moribund, deaths for which although an anaesthetic aetiology may not have seemed directly responsible, often it could not be excluded entirely—circumstances in which anaesthesia might be regarded as being necessarily but unavoidably contributory. Such deaths were classified as a separate group, "inevitable deaths".

In assessing the contributory role of anaesthesia, harsh criteria were adopted. This was done in order to avoid any bias that might encourage complacency.

The causes of deaths attributable to anaesthesia were classified in terms of the simple concept of identification of the mechanism primarily responsible for the ultimate failure of perfusion of the brain with oxygenated blood which were, fundamentally, a failure of (a) respiratory homeostasis, or (b) circulatory homeostasis.

Although there may be a prominent feature in the circumstances of a particular death, from the clinical aspect the causes of deaths attributable to anaesthesia are often multiple, compounded from the interaction of many variables. This creates difficulties in terms of a precise classification, but in a study of this nature it is the aspect of preventability—the clinical lesson that may be learnt—that is important. This I have endeavoured to accentuate at the expense of some overlap and imprecision in the classification of causes of death presented.

GENERAL FREQUENCY

The general statistical data are presented in table I. For comparison relevant data from the first 10 years of this survey (1957–1966) are included in parentheses (Harrison, 1968a, b).

The figure for the number of anaesthetics administered is a crude total which includes all anaesthetics administered in the hospital, from those for minor surgery to those for cardiopulmonary bypass procedures. Though this begs the future refinement of this study to an analysis of mortality associated with anaesthesia in relation to specific surgical operations, pilot studies have not shown this to be really valuable in the present context.

During the period of this survey, 531 deaths occurred in association with anaesthesia (2.2 deaths per 1000 anaesthetics) of which 10% (53) were finally assessed as those to which anaesthesia contributed. This frequency (0.22 per 1000 anaesthetics) is an improvement on that reported from the previous 10-year period surveyed at this hospital, 0.33 per 1000 anaesthetics.

TABLE I. *Deaths associated with anaesthesia (AAD) at Groote Schuur Hospital* 1967–1976 (*number of anaesthetics* = 240 483). *Figures for* 1956–1966 *in parentheses* (*number of anaesthetics* = 177 928)

	No. of deaths	% of AAD	Frequency per 1000 anaesthetics
Total surgical mortality	2442 (2026)		10.15 (11.38)
Deaths associated with anaesthesia	531 (414)		2.20 (2.33)
Group			
1. Deaths to which anaesthesia contributed	53 (58)	10	0.22 (0.33)
2. Other causes	343 (261)	65	1.42 (1.43)
3. Inevitable deaths	135 (95)	25	0.56 (0.53)

As anaesthesia is but an adjunct to the total surgical care of the patient, a wider relevance emerges if the frequency of those deaths to which anaesthesia contributed is viewed against the background of the total surgical mortality, that is all deaths following operation, before discharge of the patient from hospital. Looked at in this way, we may say that, in our hospital, anaesthesia and its mismanagement were responsible in a preventable manner for 2.2% of the total surgical mortality.

An examination of the frequency of the deaths attributable to anaesthesia from 1956 on an annual basis (fig. 1) reveals that, following an initial improvement, the frequency decreased to what appears to be an irreducible minimum around 0.15 deaths per 1000 anaesthetics. This is perhaps a reflection of the sad fact that despite the preventability of such deaths and the repetitiveness of the errors that cause it, all trainees must be educated clinically through the same mistakes. While a constant and adequate supervision of trainees by experienced specialist staff does improve matters, it is only part of the answer, for the most enduring lessons for the trainee come ultimately from the final acceptance of total clinical responsibility.

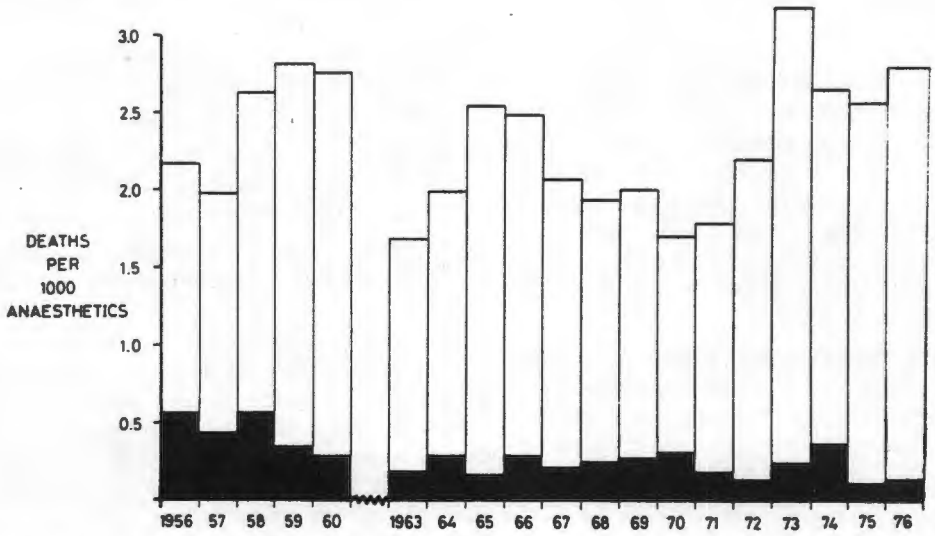

FIG. 1. Anaesthetic-associated mortality 1956–1976. Clear columns = Mortality associated with anaesthesia; Solid columns = Mortality attributable to anaesthesia.

TABLE II. *Causes of death to which anaesthesia contributed (ACD). *Clinical situation responsible for more than two-thirds of ACD* (68%)

Failure of	Cause of death	No. of deaths	% of ACD
Respiratory homeostasis	Technical failure	1	1.9
	Vomiting, regurgitation, inhalation	2	3.8
	Bronchial obstruction	3	5.7
	*Complications of tracheal intubation	9	17.0
	*Respiratory inadequacy following myoneural blockade	10	19.0
	*Inadequate postoperative care	6	11.3
Circulatory homeostasis	*Hypovolaemia (hypotension after induction)	11	20.8
	Overhydration	1	1.9
	Cardiac arrest		
	Drug induced 3		
	Heart block 1	7	13.2
	Air embolism 1		
	Uncertain 2		
Complications of regional anaesthesia	Spinal/extradural + haemorrhage 2		
	Extradural/massive spinal 1	3	5.7
		53	100.3

Status of the patient and urgency of operation

Of the 53 patients to whose deaths anaesthesia was considered contributory, 27 were in good to fair condition (ASA status I and II), while 26 were in poor condition (ASA status III and IV). With regards to the urgency of operation, 32 were subject to emergency operation while 19 underwent elective procedures.

MECHANISMS OF DEATHS TO WHICH ANAESTHESIA CONTRIBUTED

Four clinical situations were responsible for two-thirds of these deaths (table II). These were (in the order of the table):

(1) Complications of tracheal intubation.
(2) Respiratory inadequacy following myoneural blockade.

(3) Inadequate care and observation after operation.
(4) Hypovolaemia.

Complications of trachael intubation (nine patients)

Over one-half of these deaths (five) followed the technical failure of endotracheal intubation in the presence of anatomical abnormality. All could have been anticipated by adequate clinical examination before anaesthesia, yet, in each instance a neuromuscular blocking drug was given by the anaesthetist before he had assured himself that he could intubate the trachea and ventilate the lungs.

Other deaths followed the complications of kinking of the endotracheal tube (two), respiratory obstruction following herniation of the tube cuff (one) and inadvertent bronchial intubation of a patient undergoing an emergency operation while suffering from pneumonia.

Respiratory inadequacy following myoneural blockade (10 patients)

Seven of these deaths involved patients with severe chronic obstructive lung disease plus abdominal distension as a result of intestinal obstruction. The anaesthetist concerned had in each case hesitated in providing artificial ventilation in the period after operation.

The deaths of another three patients were in fact a result of "over-hydration", although the patients presented as respiratory inadequacy following myoneural blockade. The patient was in each case an overweight, middle-aged female who suffered intestinal obstruction as a result of an incarcerated para-umbilical hernia, with resultant gross abdominal distension, presenting at the hospital at night. In each case fluid replacement, both before and during operation, had been over-enthusiastic, misjudged and unmonitored by measurement of central venous pressure. The resultant interstitial pulmonary oedema presented as "respiratory inadequacy following myoneural blockade" following the establishment of spontaneous breathing at the end of operation.

Inadequate postoperative care (six patients)

Two further deaths from overhydration resulting in frank pulmonary oedema in the period after operation followed the inadvertent administration of excess fluid because of "inadequate postoperative care and supervision".

Other deaths in this category resulted from:

(a) direct connection of an oxygen cylinder to an endotracheal tube;
(b) unobserved intra-nasal disconnection of a catheter mount from a nasotracheal tube in a patient requiring ventilation;
(c) inhalation in the period after operation of a massive post-turbinectomy haemorrhage in a patient who was over-sedated with morphine;
(d) "Ondine's curse"—post-arousal respiratory depression following administration of morphine.

Hypovolaemia (11 patients)

This group might be sub-titled "post-induction hypotension" because this is how the majority presented, and it constituted the commonest single clinical situation leading to death attributable to anaesthesia. I have included under this heading all those patients in whom frank failure of circulatory homeostasis followed the induction of anaesthesia, a failure that appears to have resulted from inadequate venous return.

I have chosen to call attention in this classification to the initial hypovolaemia, as this is eminently correctable. The precipitating factor was frequently the exhibition of thiopentone to the sick, old and arteriosclerotic who have evidence of ischaemic heart disease. Although some might regard many of these deaths as "drug-induced" cardiac arrest because of their direct association with thiopentone, I have chosen to highlight the functional hypovolaemic state, as often this was the basic correctable fault.

Miscellaneous

The remaining patients, while not constituting such homogenous groups as the above, are worthy of brief comment.

Cardiac arrest (7 patients). Included in this group are:

(a) Three patients in whom cardiac arrest appeared to have been directly precipitated by the anaesthetic or adjuvant drugs (other than thiopentone). Two were associated with repeat doses of suxamethonium and one followed the administration of halothane to a patient with Parkinson's disease who was being treated with L-dopa.

(b) In one patient, suffering from Mobitz Type II heart block, anaesthesia and surgery precipitated complete heart block, followed by cardiac arrest on three occasions, resuscitation failing finally. This was deemed to have been preventable by the pre-anaesthetic insertion of temporary ventricular pacing.

(c) A death which resulted from "air embolism" following an attempt by the anaesthetist to expedite transfusion of blood to a patient bleeding from a gastric ulcer by the inflation of air from a sphygmomanometer cuff directly into the transfusion bottle.
(d) In two further instances the cause of cardiac arrest could not be established convincingly.

Overhydration (one patient). Although six patients died because of "overhydration", only one is listed here, the other five having been included above under "respiratory inadequacy following myoneural blockade" and "inadequate postoperative care". This remaining death was from pulmonary oedema following inadequately monitored resuscitation of a patient, who had a previous myocardial infarction, undergoing gastrectomy for haematemesis.

Deaths from failure of respiratory homeostasis other than those already described included:

Technical failure (one patient). Incorrect assembly of a self-inflating bellows caused the anoxic death of patient during transfer from the operating theatre to the intensive care unit.

Vomiting, regurgitation and inhalation (two patients). These followed (a) Caesarean section; (b) intestinal obstruction.

Bronchial obstruction (three patients). The following circumstances caused fatal bronchial obstruction during anaesthesia:

(a) Blood from oesophageal varices passing an insufficiently inflated endotracheal tube cuff.
(b) Lack of adequate bronchial toilette of a patient anaesthetized while suffering acute purulent bronchitis and undergoing laparotomy for an ectopic pregnancy.
(c) Total bronchospasm following trachael intubation in a patient known to have asthma.

Complications of major local anaesthetic techniques (three patients). Three patients died following spinal or extradural anaesthesia. In two instances major bleeding during operation in the face of sympathetic paralysis as a result of the technique caused profound and uncontrollable hypotension. In the remaining instance the mismanagement of total spinal anaesthesia complicating attempted extradural block caused the death of the patient.

INEVITABLE DEATHS

"Inevitable deaths" constituted 25% of those associated with anaesthesia (see table III). Three broad groups of surgical procedures and conditions were responsible for three-quarters of these deaths. These were:

TABLE III. *"Inevitable deaths"*

Circumstances of death	No. of deaths	% of group 3
Cardiac surgery	45	33.3
Ruptured abdominal aortic aneurysm	21	15.6
Other massive haemorrhage	23	17.0
Multiple injuries	15	11.1
Septicaemia/peritonitis	19	14.1
Neuro trauma	8	5.9
Pulmonary embolus	1	0.7
Myocardial infarction before operation	1	0.7
Other	2	1.5
	135	99.9

(a) Cardiac surgery with cardiopulmonary bypass.
(b) The surgery of major vascular catastrophes, such as a ruptured abdominal aortic aneurysm.
(c) Multiple injury and other conditions associated with massive haemorrhage.

CONCLUSION

Because of the great number of variables which influence various author's estimates of the frequency of those deaths to which anaesthesia contributed—differences in the peri-operative period and type of surgery, in the assessment criteria and classifications adopted and in the computation of the background surgical population—no statistical comparisons between surveys are valid. Comparison of this survey has been confined to that by the same author for the previous 10-year period at the same institution. There has been improvement. Four fundamental changes have occurred *pari passu*:

(1) A continuing improvement in routine monitoring of vital functions during anaesthesia.
(2) An increase in the consultant (full time specialist) registrar (trainee) ratio, now approximating 1 : 1.
(3) A decrease in the case load per anaesthetist.
(4) The introduction of recovery rooms within the theatre area and adjacent intensive care units.

Although the actual frequency of death attributable to anaesthesia was small (0.22 deaths per 1000 anaesthetics), it is salutory to reflect that the 10% of immediate operative mortality for which anaesthesia and its mismanagement was responsible was basically preventable. It is sad to reflect that the causes of anaesthetic deaths are, by and large, simple and usually

follow the lack of observation of simple precautions, and the lack of clinical alertness. What is more, in general, these causes recur. It is also sobering to reflect that these patients reported here are probably the tip of an iceberg of clinical mismanagement. How many more have nearly died?

Surveys and studies such as this are somewhat pedestrian and are tedious in the extreme to conduct, but if they are carried out consistently and conscientiously, they will provide us not only with the clinical lessons by which we can improve our practice, but also the only means by which we may measure and evaluate the safety of anaesthesia for the patient.

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DECES ATTRIBUABLES A L'ANESTHESIE

Etude portant sur 10 *ans* (1967–76)

RESUME

La mortalité associée à 240 483 cas d'anesthésie administrée pendant 10 ans à l'hôpital de Groote Schuur à Capetown fait l'objet de ce rapport. La fréquence des décès auxquels l'anesthésie a contribué a été de 0,22‰ (par rapport à 0,33‰ au cours des 10 années précédentes). Ces décès ont compté pour 2,2% du total de la mortalité résultant des interventions chirurgicales. Les deux-tiers des décès par suite d'anesthésie ont été attribuables (dans l'ordre de fréquence): (a) à l'hypovolémie; (b) à l'insuffisance respiratoire après blocage myoneural; (c) aux complications résultant de l'intubation trachéale; (d) à des soins et surveillance postopératoires inadéquats.

TOD DURCH ANÄSTHESIE

Ein Überblick auf die 10 *Jahre von* 1967–1976

ZUSAMMENFASSUNG

Berichtet wurde die Sterblichkeitsziffer in Verbindung mit 240 483 Narkosen, verabreicht während 10 Jahren im Groote Schuur Hospital in Cape Town. Die Sterblichkeitsrate, die auf Narkose zurückzuführen war, war 0,22 pro 1000 Narkosen (verglichen mit 0,33 pro 1000 in den vorausgegangenen 10 Jahren). Diese Tode machten 2,2% der gesamten Sterblichkeitsziffer der Chirurgie aus. Zwei Drittel der Narkosetode waren verursacht durch (in Reihenfolge der Häufigkeit): (a) Hypovolämie; (b) Atmungsversagen nach neuromuskulärer Blockade; (c) Komplikationen der trachealen Intubation; (d) unzulängliche, postoperative Pflege und Beufsichtigung.

MUERTE ATRIBUIBLE A ANESTESIA

Un estudio que abarcò 10 *años* (1967–1976)

SUMARIO

Se informa sobre la mortalidad asociada con 240 483 anestésicos administrados durante un perídodo de 10 años en el hospital de Groote Schuur, Ciudad del Cabo. La frecuencia de muerte atribuida a anestesia fue de 0,22 por 1000 anestesias (en comparación con un 0,33 por 1000 en los 10 años anteriores). Estas muertes corresponden a un 2,2% de la mortalidad total por cirugía. Dos tercios de la muertes "anestésicas" fueron imputables a (en orden de frecuencia): (a) hipovolemia; (b) insuficiencia respiratoria siguiendo un bloqueo mioneural; (c) complicaciones de intubación traqueal; (d) falta de ciudado y supervisión postoperatorios.

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Anaesthetic deaths

Death as a result of anaesthesia is rare; but when this does occur not uncommonly the patient has been apparently fit, and sometimes there seem to have been failures to maintain proper anaesthetic practice. We need, therefore, to examine the causes of these deaths if we are to avoid similar tragedies in the future.

Surveys over the past 20 years have identified the common problems. The Association of Anaesthetists of Great Britain and Ireland set up a voluntary reporting system to examine deaths resulting from anaesthesia. Two papers, in 1956[1] and 1964,[2] surveyed 1600 reports. Of these, 989 were thought to implicate the anaesthetic. Death was most often attributable to failure to maintain the airway, failure to maintain ventilation, and circulatory failure. Hypovolaemia was a common cause of the circulatory difficulties and was often exacerbated by a (relative) overdose of an intravenous injection of thiopentone. A few deaths were due to failure of anaesthetic apparatus.

In South Australia an anaesthetic mortality committee surveys all deaths associated with anaesthesia. A report of its findings for the years 1969-74[3] suggests that the incidence of death during or after anaesthesia is about 1 in 15 000. One problem it highlighted was cardiac arrest, and the suggested remedy was that more frequent blood pressure monitoring might detect such patients when hypotension preceded the arrest. Wylie[4] commented on the disastrous consequences of delay in recognising cardiac arrest: in those circumstances permanent neurological damage may be expected in those who survive.

The report of the confidential inquiries into maternal deaths[5] found that 37 deaths were due to anaesthesia in 1970-2. Obstetrics is known to present special risks, the inhalation of stomach contents being the most important. The other causes included hypoxic cardiac arrest. The important finding is that in three-quarters of the cases there were some avoidable factors. The additional risk of anaesthesia in caesarean section seems to give a death rate of about 1 in 3000 patients.

One of the virtues of the system of reporting maternal deaths is that it does not depend solely on voluntary reporting; the aim is to assess every maternal death. Harrison[6] has recently described a similar comprehensive survey of all patients anaesthetised in Groote Schuur Hospital, South Africa. In the decade studied 240 483 anaesthetics were given and 2442 patients died. Of these deaths, 531 were classified as associated with anaesthesia in that death occurred during or within 24 hours of anaesthesia or before full recovery of consciousness. But in only 53 of the cases was anaesthesia thought to have contributed; the rest were classified as inevitable (135) or a result of other causes (343). The death rate from anaesthesia was 1 in 4537. The list of causes is depressingly familiar: hypovolaemia, ventilatory failure, failure to maintain airway, cardiac arrest due to drugs or air embolism, and so on. Respiratory failure associated with the use of relaxants was common. The usual combination was severe obstructive airway disease, intestinal obstruction, and the use of neuromuscular blocking drugs.

The striking features in all these series are the preponderance of airway, ventilatory, or circulatory problems. Jaundice after halothane is absent,[7] but the definition of death from anaesthesia as used by Harrison[6] would miss that cause: jaundice develops some days after anaesthesia, and the patient would be conscious between the anaesthetic and the development of liver failure.

What are the lessons to be learned? Death due to anaesthesia is no respecter of persons—it may happen to any patient and in any anaesthetist's practice. Each anaesthetist should determine before, during, and after each anaesthetic that there are no problems with the airway, that ventilation is adequate, and that blood volume and cardiac function are sufficient for the patient's needs. Further training should emphasise fall-back procedures. How should the anaesthetist cope if he fails to secure endotracheal intubation? Tunstall[8] described one such drill for obstetric anaesthesia. Better monitoring must give earlier warning of cardiac arrest and minimise delay in treating it. Is it now time for all anaesthetised patients in Britain to have their electrocardiogram or pulse continuously displayed?

Further surveys should identify what progress is being made in reducing the death rate of anaesthesia. The Association of Anaesthetists is undertaking a new survey in selected areas in Britain. All deaths during and after operation will be examined to determine the role of anaesthesia. Sadly, the survey will not be extended to look at all the causes of death. The close collaboration of all concerned in obstetrics has made a major contribution to improved maternal care. Was there not a possibility of doing the same for surgery? In Harrison's series[6]

other causes not considered inevitable led to six times as many deaths as anaesthesia.

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SOUTH AFRICAN MEDICAL JOURNAL.Vol.55:192:1979.

NUUS EN KOMMENTAAR — *NEWS AND COMMENT*

STERFTE TE WYTE AAN NARKOSE

In 'n 10-jaar oorsig deur professor Harrison (*Brit. J. Anaesth.*, 1978, **50**, 1041) kom hy tot die gevolgtrekking dat die insidensie van sterfte na narkose by Groote Schuur-hospitaal 0,22 per 1 000 was, wat goed vergelyk met die 0,33 per 1 000 van die vorige 10 jaar. Daar is oor die 10-jaar tydperk 1967 - 1976 240 483 narkoses toegedien. Die sterftes was verantwoordelik vir 2,2% van die totale chirurgie-mortaliteit. Twee derdes van die narkose-sterftes, in volgorde van frekwensie, is toegeskryf aan hipovolemie, respiratoriese ontoereikendheid na mioneurale blokkade, komplikasies wat gepaard gaan met trageale intubasie en onvoldoende na-operatiewe sorg.

Die fundamentele veranderinge in die 10-jaar tydperk was die volgende: (*i*) verdere verbetering in die roetinebewaking van die pasiënt onder narkose; (*ii*) verbetering in die senior/junior personeelverhouding (tans byna 1:1); (*iii*) minder werk per narkotiseur; en (*iv*) die daarstelling van herstelkamers by die operasiesaal en intensiewe sorgeenhede in die nabye omgewing.

Death due to anaesthesia at Groote Schuur Hospital, Cape Town — 1956 - 1987

Part I. Incidence

G. G. HARRISON

Summary

The data from a 30-year surveillance study of anaesthetic mortality associated with 0,75 million anaesthetics administered at Groote Schuur Hospital, Cape Town, from 1956 to 1987 are presented. Behind an overall mortality rate of 0,19 deaths per 1 000 anaesthetics attributable to anaesthesia, lies a 6-fold decrease in the incidence, computed quinquennially, from 0,43 per 1 000 anaesthetics in the first quinquennium to 0,07 per 1 000 anaesthetics in the last. The latter figure portrays a standard of safety in anaesthesia for the patient equal to anywhere in the world.

S Afr Med J 1990; 77: 412-415.

> I hold that there should be no deaths due to anaesthetics. . .'
>
> — Sir Robert McIntosh, 1948[1]

Clinical anaesthesia is an indispensable adjunct to the surgical management of disease. Of itself non-therapeutic, above all else it must be safe for the patients. An adverse outcome to anaesthesia, and factors relevant to its administration, results, at best, in postoperative morbidity for the patient and at worst, in his death (Fig. 1). The incidence of the latter event provides us with the most fundamental measure of the safety of anaesthesia for the patient.

To audit the performance in this regard of the Department of Anaesthetics at Groote Schuur Hospital, an ongoing contemporaneous surveillance study of all deaths associated with anaesthesia was started in 1956 and has been continued to date. The surgery for which these anaesthetics were administered has increased from 16 000 to 40 000 operations annually and has included the whole spectrum of modern surgical practice from the simplest to the most complex. Although most of the anaesthetics were given for standard surgical procedures, the advancing edges of surgery were always in evidence. The years covered by this survey saw the birth and development of cardiopulmonary bypass surgery, major vascular surgery and organ transplantation. It also witnessed the increase, to almost epidemic proportions, of major trauma requiring management — in particular the dragon's teeth harvest of major multiple injuries spawned by motor vehicle accidents.

While the findings of this study have been reported over the years in various publications,[2-7] the completion of its 30th year provides an appropriate moment to report an overview of the study as a whole, with an examination of trends in the incidence and aetiological patterns of death attributable to anaesthesia.

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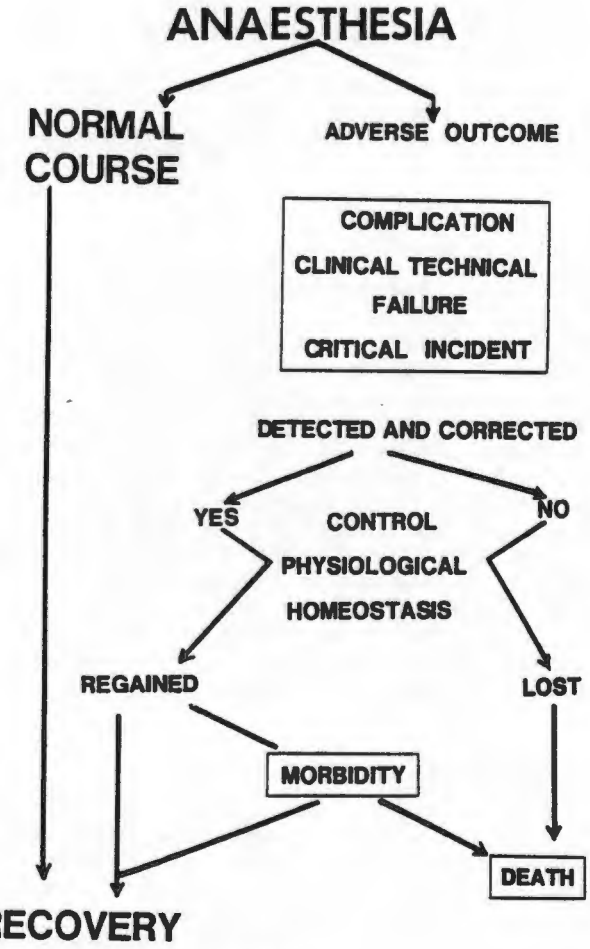

Fig. 1. The outcomes of anaesthesia.

Definitions

The objectives of this surveillance study have been: (*i*) to establish the incidence of death attributable to anaesthesia; (*ii*) to identify the mechanisms that caused death and the hazard situations from which they result; and (*iii*) to formulate strategies for prevention.

The starting point in this process was the examination of detailed records of the clinical course, management and autopsy of all patients whose death was chronologically closely associated with anaesthesia — anaesthetic-associated death (AAD) — in order to identify that approximate 10% subset whose deaths were considered due to anaesthesia and its mismanagement — anaesthetic contributory death (ACD).

An AAD was defined as the death of a patient occurring during or within 24 hours of anaesthesia or after failure of the patient, conscious before, to regain consciousness after anaesthesia.

The choice of a 24-hour period after anaesthesia was arbitrary. It was considered to embrace a time interval adequate to permit identification of those deaths attributable to anaesthesia without the study becoming unmanageably large. Patients suffering irreversible anoxic or anoxic/ischaemic brain damage from anaesthetic-induced crises, yet surviving after resuscitation, would be identifiable by their failure to regain consciousness. While today, for complete capture of relevant data, extension of the surveillance period to the 6th day after anaesthetic, is recommended,[8] such demand could not be met at the time of this study's inception. In these circumstances, it is acknowledged that a very small number of late deaths, which may have been attributable to anaesthesia, e.g. aspiration, may have been missed.

The background population of all anaesthetic administrations from which the above arose, was recorded as also the total postoperative hospital stay mortality — total operative mortality.

Identification

All surveillance studies of anaesthetic-associated mortality are bedevilled by difficulties inherent in the assessment of the responsibility of anaesthetic drugs and/or techniques for adverse outcome. These difficulties arise from the circumstance that the conduct of an anaesthetic is inseparable from its environment, which is a function of two complex variables: (*i*) the patient's disease, both surgical and intercurrent; and (*ii*) the surgical operation itself and its effects, including complications and errors of the surgical technique.

Interaction of these two variables is responsible for approximately 90% of peri-operative deaths.[2,5,8] But in circumstances in which this environment of the anaesthetic does not of itself provide sufficient explanation for adverse outcome, the conduct of the anaesthetic itself must be called in question. In this case we are faced primarily with an assessment of that difficult-to-define quality, the skill of the anaesthetist.

Preventability

If it is accepted that lack of skill or human error on the part of the anaesthetist is the major factor in causation of death attributable to anaesthesia, it follows that such deaths are preventable. To say this is not to attribute blame. To quote William Osler: 'Errors in judgement must occur in the practice of an art which consists largely in balancing probablities.' The implication is simply that on review, albeit with the benefit of hindsight, a reasonably satisfactory explanation of the circumstances can be found and countermeasures that could prevent the recurrence of the situation are practicable.

The unavoidable

Not all deaths attributable to anaesthesia are preventable. Besides those for which sufficient information for assessment is not available, there will be those which involve mechanisms not adequately understood, covert pharmacogenetic states or, perhaps, unforeseen technical or apparatus failure over which the anaesthetist has no control. A difficulty here is that, with advances in knowledge and accumulation of experience, complications that may be judged unavoidable today come to be regarded tomorrow as due to preventable error.

The unassessable

In this type of study it must be noted that it is only the more obvious and conventional errors in clinical anaesthetic management that can be identified as being contributory to a patient's death. The more subtle role that certain errors in clinical anaesthetic management may play in the genesis of postoperative morbid conditions that may lead to death, such as myocardial infarction, adult respiratory distress syndrome and renal and hepatic failure, is not precisely assessable. And, in view of their multifactorial aetiology, is it ever likely to be.

Classification

In terms of the above, records of the conduct of the anaesthetic and the autopsy report of each AAD were examined in the light of three questions:

1. Was the administration of the anaesthetic or other factors within the ambit of the anaesthetist's responsibility the cause of, or a major contributory factor in, the patient's death? Or was the death primarily a result of the patient's disease and/or the surgical procedure undertaken? The former were classed as ACD, the latter as 'death due to other causes'.
2. If considered an ACD, what was the precise cause?
3. Was the death preventable? What departures were there from accepted clinical practice? Was there a clinical lesson to be learnt?

Besides the two broad calssificatory classes of death consequent on the first question, viz: (*i*) ACD; and (*ii*) death due to other causes, a need arose from the circumstances of clinical practice for a third category for those deaths associated with the surgery of desperation; operations on the moribund — and the like — deaths for which, although the anaesthetic aetiology was not obvious it could not (from the very nature of the process) be entirely excluded. For such deaths a category of 'inevitable death' was allocated (Table I).

In assessing the contributory role of anaesthesia, an attitude was adopted that is best expressed by Dripps *et al.*:[9] 'There is nothing to be gained in a mortality study by omitting a particular death merely to lower a statistical death rate. Avoiding responsibility or taking refuge in the fact that the patient was desperately ill prior to anaesthesia and operation, may improve one's mortality figures, but it will not advance general knowledge or change one's own practice. On the other hand one should not resort to self-flagellation, assuming responsibility for a fatality merely because an anaesthetic was administered and death occurred.'

While it must be admitted that there is a considerable subjective element in the final assessment of the culpability of the three variables that bear on anaesthetic associated mortality — the patient's disease, surgery, and the conduct of the anaesthetic — this type of review is the only means by which some measure of the safety of anaesthesia and an audit of the efficacy of our service to the patient is gained.

Incidence of ACD

The general statistical data revealed by this study are presented in Table I. Of approximately 0,75 million patients anaesthetised for surgery, 7033 died before discharge from hospital (total operative mortality 8,99 per 1000 operations). Of these deaths 1427 (1,83 per 1000 anaesthetics) occurred in immediate association with anaesthesia (AAD) and the records and clinical treatment of these patients were examined and assessed in the manner described. The anaesthetic, or its mismanagement, was adjudged the sole cause or a major contributory factor to 145 of these deaths, yielding an overall incidence of death attributable to anaesthesia (ACD) of 0,19 per 1000 anaesthetics. In this reveiw, comment is confined to the latter. Of these, the deaths of 124 patients (86%) were assessed as probably or possibly preventable.

TABLE I. ANAESTHETIC-ASSOCIATED MORTALITY AT GROOTE SCHUUR HOSPITAL 1956 - 1987

	No. of deaths	Incidence/1 000 anaesthetics
AAD (< 24 h)	**1 427**	**1,83**
ACD	**145**	**0,19**
Preventable (86%)		
Physical status (ASA 4,5) (52%)		
Emergency operations (52%)		
Contribution to surgical mortality (2,06%)		
Inevitable	**354**	**0,45**
Other causes	**928**	**1,19**

Total anaesthetics = 782 182; total operative mortality = 7 033 (8,99 deaths/1 000 operations).

Over the 30-year period of this study, there have been considerable changes in clinical anaesthetic practice, stemming from advances in knowledge, a more versatile drug armamentarium, improvements in training, and the immediacy, sophistication, acceptance and availability of monitors of vital functions, including the biochemical aspects. That these changes have produced a beneficial increase in the safety of anaesthesia for the patient becomes readily apparent when the incidence of ACD is computed in serial 5-year periods (Fig. 2). This reveals a 6-fold decrease in the incidence of ACD over the period of this study, from 0,43 to 0,07/1 000 anaesthetics. This is accompanied by an approximately 4-fold decrease (3,3% - 0,9%) in anaesthetic contribution to total surgical mortality, which itself has fallen.

A major weakness of this type of study is that besides the element of subjectivity, lack of uniformity in the criteria of case assessment and classification as well as the peri-operative time period included by various investigators, render any but the broadest statistical comparisons between studies from different centres meaningless.

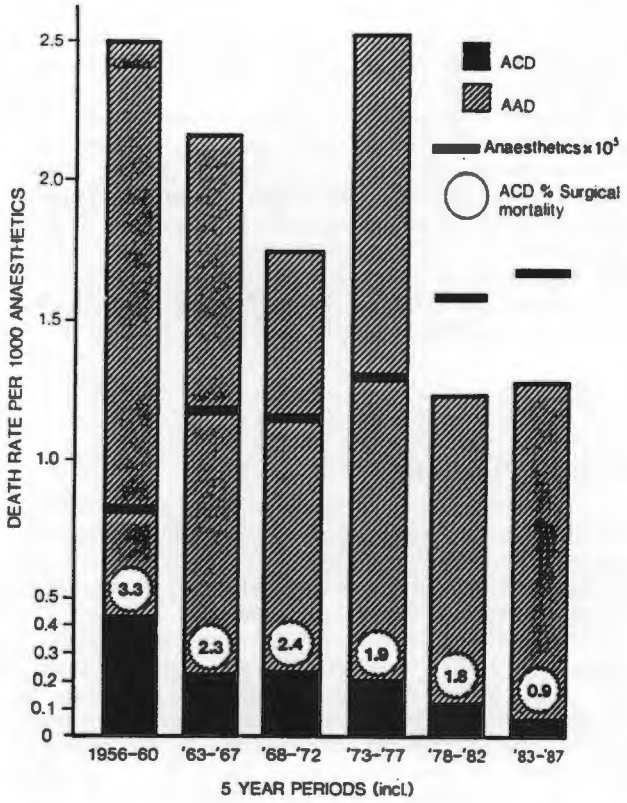

Fig. 2. The incidence of ACD and AAD in quinquennia, 1956 - 1987.

With this caveat, a context for this study is provided by the documented incidence of ACD computed at various other centres world-wide, as presented in Table II. Citations are

TABLE II. ESTIMATES OF INCIDENCE OF ACD FROM AROUND THE WORLD

Country, authors, year of publication	Years studied, No. of anaesthetics	Peri-operative period (d)	Incidence of ACD (rate/1 000 anaesthetics)
South Africa			
Kok and Mullan[10] (1963)	**1956-1962 (1 002 712)**	**≤ 1**	**0,49**
Kok and Mullan[11] (1969)	**1956-1965 (1 760 066)**	**≤ 1**	**0,42**
Harrison[21] (1966)	**1956-1966 (177 928)**	**≤ 1**	**0,33**
Harrison[8] (1968)	**1967-1976 (240 483)**	**≤ 1**	**0,22**
Australia			
Clifton and Hotten[12] (1963)	**1956-1962 (205 640)**	**≤ 1**	**0,17**
Bodlander[13] (1975)	**1963-1972 (211 130)**	**≤ 1**	**0,07**
Anaesthetic Mortality Committee S Australia[14] (1975)	**1969-1974 (465 000)**	**≤ 1**	**0,08**
Anaesthetic Mortality Committee S Australia[15] (1985)	**1974-1983 (900 000)**	**≤ 1**	**0,05**
New Zealand			
Gibbs[16] (1986)	**1979-1984 (1 100 000)**	**≤ 1**	**0,10**
Finland			
Hovi-Viander[17] (1980)	**1975 (338 934)**	**≤ 3**	**0,20**
Canada			
Turnbull *et al.*[18] (1980)	**1973-1977 (195 232)**	**≤ 2**	**0,18**
UK			
Lunn and Mushin[8] (1982)	**1979-1980 (1 147 362)**	**≤ 6**	**0,10**

confined to those documented over the last 15 years, except when serial figures over time are available from the same centre, allowing some validity to inferences from 'within study' chronological trends.

In the above context the incidence at Groote Schuur Hospital of death attributable to anaesthesia *per se* correlates well with that computed at the other centres cited. In spite of the statistical caveats this allows us to draw two firm conclusions: (*i*) of every 10 000 patients undergoing anaesthesia and surgery only 1 dies *because* of the anaesthetic; and (*ii*) the incidence of death attributable to anaesthesia has decreased greatly with time; our experience of a decrease over the last three decades reflects the general trend.

Some implications

One implication of such incidence (1 ACD per 10 000 anaesthetics) is that the average anaesthetist would be expected to encounter only 1 such death every 5 - 10 years of his working life. In terms of human frailty, it must be wondered whether it is possible for such a rate to be reduced further.

On the other hand, although the rate is acceptably low and perhaps at an irreducible minimum, it does mean that (based on the statistic that every year 1 in every 20 - 25 persons has to undergo a procedure which involves anaesthesia) in South Africa at least 100 patients die every year *because* they are anaesthetised. Of these 100 or more deaths, 90% would be considered preventable. Looked at in this light, death solely attributable to anaesthesia must continue to be a matter of concern. Furthermore, surveillance studies of death attributable to anaesthesia monitor only the extreme endpoint of the adverse outcome process — only the tip of the iceberg of clinical mismanagement. To quote Davis:[19] 'The tolerance of the human body is phenomenal and if a few die each year consider how many have been subjected to abusive practices — how many have almost died?'

It must be remembered, too, that these estimates of death attributable to anaesthesia were computed in First-World mainstream hospitals, usually teaching institutions. How well they represent the situation in the vast rural practice of surgery in those countries, and our own, let alone the circumstances in Third-World countries, is completely unknown. *A priori*, these are likely to be considerably higher.

Prediction of risk

While these studies audit how we *have* performed, as a predictive measure of the risk of anaesthesia they are of very little use. In this regard I can do no better than to quote Goldstein and Keats:[20] 'The poor predictability of anaesthetic mortality should be expected, since a significant proportion of this mortality is due to human error, which cannot be predicted, and to other factors which have never been quantified. To a small degree, physical status and the operation contemplated provide some predictive basis. To a large degree unknown factors related to the skill of the personnel and the environment of the therapy contribute to anaesthetic risk. Estimates of the anaesthetic risk for individual patients remain, therefore, almost entirely intuitive.'

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Death due to anaesthesia at Groote Schuur Hospital, Cape Town — 1956 - 1987

Part II. Causes and changes in aetiological pattern of anaesthetic-contributory death

G. G. HARRISON

Summary

A general analysis of the clinical failures that were responsible for deaths attributable to anaesthesia over a 30-year period, 1956 - 1987, is presented. Four particular general failures in clinical management were responsible for 80% of anaesthetic-contributory deaths (ACD). These were in descending order of frequency: (*i*) failures in airway management, of which the majority were associated with the complications of endotracheal intubation (27% of ACD); (*ii*) failures in pulmonary ventilation management (20% of ACD); (*iii*) failures in blood volume control (19% of ACD); and (*iv*) failures in arrhythmia control (17% of ACD).

Computation of these groups of causes by the decade reveals a distinct and progressive change in the aetiological pattern of these deaths with time. While the incidence of ACD over the period decreased 6-fold from 0,43 to 0,07/1 000 anaesthetics, that proportion due to failures in airway management, in general, and complications of intubation, in particular, has progressively increased. This has been accompanied by a reciprocal decrease in deaths due to circulatory factors. It is postulated that this change arises from the fact that the physical skills, manual dexterity and clinical judgement demanded by the former have not changed with time, whereas the latter depend on intellectual responses to information derived from ever-improving vital function monitoring.

S Afr Med J 1990. 77: 416-421.

'Experience is the best of school masters, only the school fees are heavy.'

— Thomas Carlyle, 1795-1881

Mechanisms of death

Death as a complication of anaesthesia is usually an acute event, which follows loss of clinical control of the patient's physiological homeostasis and the functional failure of those interrelated systems — respiratory and circulatory (Fig. 1). In assessing these, consideration must be given not only to the ways in which the known actions of anaesthetic drugs or errors and misjudgements in their administration may have lethal consequences, but also to the ways in which errors of commission or omissions in the wider field of responsibility, which by practice has become that of the anaesthetist, may cause death.

Based on Fig. 1, the following broad classification of mechanisms/causes of death attributable to anaesthesia was adopted: (*i*) failure to control respiratory homeostasis; (*ii*) failure to control circulatory homeostasis; (*iii*) complications of regional anaesthesia; and (*iv*) miscellaneous other causes.

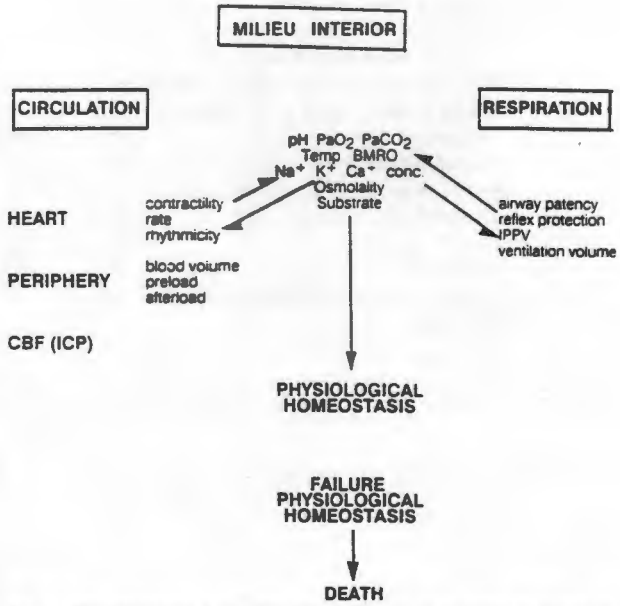

Fig. 1. The interrelated system failure of the ACD (CBF = cerebral blood flow; ICP = intracranial pressure; Temp = temperature; BMRO = basal metabolic rate for oxygen).

Those causes of death allocated to failures in control of respiratory and circulatory homeostasis, the major groups, were in turn subdivided in terms of the mechanisms or clinical failures responsible for them as listed in Table I.

Classificatory groups are broad with no allowance for the clinical minutiae which distinguish individual cases in order that broad practical inferences might emerge clearly.

The 'process of injury' (Fig. 2), which starts with some critical incident and ends in death, is a dynamic process that could be averted by correct intervention at any stage. This implies that failure to do so usually involves multiple errors, which in turn activate the composite mechanisms that ultimately result in death. For example, Holland,[1] in reporting findings of the New South Wales Special Committee on Deaths under Anaesthesia, computed an average of 4,3 errors per death. To which of the multiple errors the death is finally attributed for classificatory purposes, poses a problem. For the purposes of this study, the proximate identifiable critical incident, error or mechanism activated was chosen for classification.

General contributory circumstances

Before proceeding to the particular causes/mechanisms of death attributable to anaesthesia, let us briefly consider the general impact on anaesthetic-contributory death (ACD) of

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TABLE I. CAUSES OR MECHANISMS OF ANAESTHETIC-CONTRIBUTORY DEATHS

Causes/mechanisms of ACD			No. of deaths		% ACD	
1. Failures in control of respiratory homeostasis				**83**		**57**
Failures in airway management			**39**		**27**	
Vomiting/regurgitation	**5**	**(3%)**				
Complications, ET intubation	**26**	**(18%)**				
Bronchial obst.	**8**	**(6%)**				
Failures in ventilation management			**29**		**20**	
During operation	**7**	**(5%)**				
Postoperative hypoventilation						
Relaxant	**20**	**(14%)**				
Narcotic	**2**	**(1%)**				
Deficient post-op. nursing			**7**		**5**	
Tension pneumothorax			**3**		**2**	
Technical/equipment failure			**5**		**3**	
2. Failures in control of circulatory homeostasis				**51**		**35**
Failures in blood volume management			**27**		**19**	
Hypovolaemia/hypotension	**23**	**(15%)**				
Hypervolaemia/over-transfusion	**4**	**(3%)**				
Failures in arrhythmia control			**24**		**17**	
Drug related	**10**	**(7%)**				
Vagal and other	**11**	**(8%)**				
Uncertain	**3**	**(2%)**				
3. Complications of regional anaesthesia				**6**		**4**
4. Miscellaneous				**5**		**3**
Total				**145**		**100**

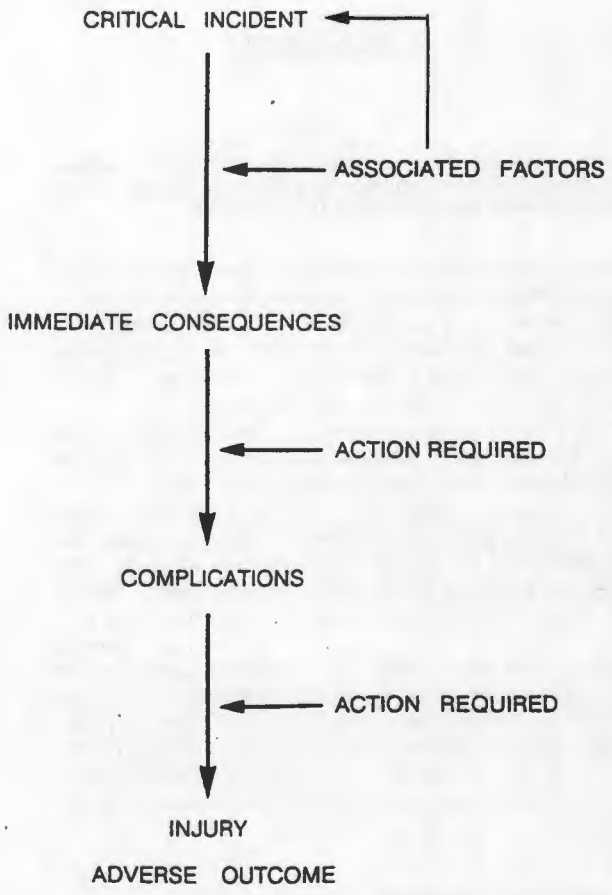

Fig. 2. The 'process of injury' leading to 'adverse outcome'.

the environment in which the anaesthetic is administered. Clinical anaesthesia has been described as a state of 'physiological trespass'.[2] The more disease has trespassed on the body's homeostatic mechanisms, the less anaesthetic trespass, *per se*, can be tolerated. The clinical anaesthetist's margin of error shrinks accordingly. The influence of this 'environment of the anaesthetic' on ACD is clearly attested by the observation in this survey that whereas patients in poor or critical physical status (American Society of Anesthesiologists 3, 4, 5) constitute but 5% of the general surgical population in this hospital,[3] such physical status is associated with 52% of the deaths attributable to anaesthesia (see Table I, part I). From these observations it can be computed that the incidence of ACD in patients in poor physical status was 1,19/1 000 anaesthetics, 13-fold greater than the 0,09/1 000 of those in good/fair physical status before anaesthesia.

The mechanisms/causes of the 145 deaths attributable to anaesthesia are classified in Table I. From the general causes, 4 particular groups of clinical failures stand out as being almost equally responsible for 80% of deaths attributable to anaesthesia. These failures were in: (*i*) airway management — 27%; (*ii*) pulmonary ventilation management — 20%; (*iii*) blood volume management — 19%; and (*iv*) arrhythmia control (or cardiac arrest due to causes other than anoxia and ischaemia) — 17%.

While the circumstances of each death are individual, a brief review of the general clinical failures responsible for them is presented as a guide to the formulation of strategies of prevention and to focus the direction of clinical training.

Failures in control of respiratory homeostasis

Failures in airway management

Failures in this most basic of anaesthetic clinical skills were the most common general cause of ACD (27% of ACD, 39

patients) and of these two-thirds resulted from complications of endotracheal intubation (18% of ACD, 26 patients).

Endotracheal intubation

Most prominent of the endotracheal tube mishaps (8 patients) was the anaesthetist's nightmare — a technical *failure of intubation* after the administration of a muscle relaxant, followed thereafter by an inability to ventilate the patient. In all cases, this situation was due to some anatomical or pathological abnormality that could have been anticipated by adequate pre-anaesthetic examination, but was not. In each instance the anaesthetist had failed to assure himself that he could intubate and/or ventilate the patient before administering a neuromuscular blocking drug.

Nine patients died as a result of anoxia that arose from *endotracheal tube obstruction* due to kinking of the tube or cuff herniation. The latter complication is particularly treacherous in that it can arise some time after the intubation with few or no warning signs. Four patients died from anoxia (plus vagal-induced cardiac arrest) when *acute massive bronchospasm* and expiratory muscle spasm — induced by attempts at intubation when anaesthesia was too light, laryngeal topical analgesia insufficient and muscle relaxation inadequate — rendered pulmonary ventilation impossible. *Disconnection and dislodgement* of the endotracheal tube that was not detected or corrected timeously caused the death of 5 patients. That 4 of these occurred postoperatively in patients being treated with intermittent positive pressure ventilation (IPPV) for various indications, reflects inadequacies in nursing care that have subsequently been largely corrected.

Closely related to these endotracheal tube complications, is a group of 8 deaths, which followed anoxia consequent on a *failure to clear obstruction of the lower airway* (the bronchi) by pus, blood or secretions due, in all but 1 case, to pulmonary or oesophageal disease. The latter case was a child who, having inhaled a foreign body, developed profuse frothy bronchial secretions when anaesthesia was induced with open ether (an historic remnant!). Of the remaining cases, 4 involved the investigation or surgery of bronchiectasis, a circumstance that highlights the grave difficulties inherent in the anaesthetic management of this condition, and 1 patient had bronchopneumonia.

Vomiting, regurgitation and aspiration

Anaesthesia in the presence of a 'full stomach', with its consequent risk of vomiting, regurgitation and aspiration, has been recognised traditionally as commonly posing an extremely hazardous situation for the patient. However, only 5 deaths (3% of ACD) resulted from this situation — 2 each during induction of anaesthesia and accompanying extubation, with 1 featuring silent regurgitation with aspiration.

Failures in pulmonary ventilation management

The second most common cause of death attributable to anaesthesia was a failure to ensure adequate pulmonary ventilation. This was the cause of 29 deaths (20% of ACD). While in the early years of this study, a few deaths (5) occurred during operation and anaesthesia — the outcome of permitting patients with various respiratory impediments to breathe spontaneously while anaesthetised with ether or halothane, instead of using IPPV — the majority (24 deaths) occurred at the termination of the anaesthetic or in the immediate or short-term post-anaesthetic recovery period. Of the latter, 22 cases were problems of *post-anaesthetic muscle relaxant reversal* with consequent hypoventilation that was mismanaged. Most commonly contributory to this situation were abdominal distention from intestinal obstruction, chronic obstructive airways disease or other pulmonary disease and obesity — often all combined.

The most common fault in clinical management was the adoption for too long after attempted reversal of neuromuscular block of a 'wait and see' policy, allowing the patient to breathe spontaneously, coupled with a reluctance to recognise or accept the indications for a return to IPPV — as if such action could be regarded as a professional failure. The lesson is obvious — when in doubt ventilate. Happily, over the course of this study the incidence of such deaths has decreased greatly.

In the remaining 2 incidents, the post-anaesthetic respiratory depression was due to *narcotic analgesics*. One was an example of 'Ondine's curse' (post-arousal respiratory depression after fentanyl administration), the other the outcome of a misdiagnosis of the postoperative restlessness of anoxia as being due to pain, so attracting the administration of more narcotic.

In two-thirds of these deaths, deficiencies in postoperative nursing care and observation were also a contributory factor.

Deficient postoperative nursing care

Attention has been drawn in the above groups of cases to the contributory role played in several deaths by deficient postoperative nursing care and/or observation. This was regarded as the sole cause for the death of a further 7 patients (5% of ACD). The particular errors involved were as follows: (*i*) failure to maintain a clear oropharyngeal airway when supervising the recovery to full consciousness of patients after anaesthesia (3 cases); (*ii*) inadvertent fluid overload due to failure to note and react to excessive intravenous infusion drip rates in the immediate post-anaesthetic recovery phase, resulting in pulmonary oedema (2 cases); (*iii*) connection of a pipeline from oxygen cylinder directly to the endotracheal tube left *in situ*, leading to tension pneumothorax (1 case); and (*iv*) failure to note the emptying of an oxygen cylinder, so causing the patient to breathe an anoxic gas mixture (1 case).

Although these tragic errors are laid at the door of the nursing attendants, their occurrence carries the definite implication of a culpable lack of adequate instruction of the nursing staff by the attending anaesthetists, for which omission they must bear a grave responsibility.

Deaths due to tension pneumothorax

Three deaths followed tension pneumothorax that developed during surgery and for which clinical recognition and treatment was tardy. Two were associated with pulmonary surgery and 1 — a neurosurgical case — involved the mechanism of alveolar rupture with peribronchial mediastinal air tracking.[4,5] These occurrences are of an order of rarity that blunts the clinician's awareness.

Death due to equipment misuse or technical failure

Five deaths (3% of ACD) resulted from equipment misuse or technical failure. Today modern alarm systems would have drawn attention to these hazardous situations before they became clinically irretrievable. Further, such events usually provoke changes in equipment design, so that these particular errors are unlikely to be repeated. They were: (*i*) an overdose of ether due to the incorporation of an ether vaporiser in the design of the now obsolete Coxeter Mushin CO_2 absorption unit — the anaesthetist confused the control taps; (*ii*) total rebreathing without oxygen due to faulty assembly of a closed circuit; (*iii*) the same due to assembly of a Puritan self-

inflating bellows without the inclusion of its expiratory valve; (*iv*) a rotameter fault leading to inhalation of an anoxic gas mixture; and (*v*) disconnection of the inspiratory valve-inflating mechanism of a Bear ventilator preventing inspiratory inflation of the patient although the ventilator itself appeared to be functioning normally.

Failures in the control of circulatory homeostasis

Responsible, as a group, for 35% of ACD, death due to the anaesthetist's failure to control the patient's circulatory homeostasis can be classified into two broad functional groups, simplistically perhaps, but in a manner that draws attention to the perceived major correctable faults: (*i*) those that followed from failure to *maintain normality of the patient's circulatory blood volume*, with resultant hypo- or hypervolaemia — in the former case cardiac arrest would have been preceded by, *inter alia*, functional myocardial ischaemia and in the latter cardiac overload together with anoxaemia from pulmonary oedema; and (*ii*) cardiac arrest from causes other than anoxia or ischaemia — *failure of arrhythmia control*.

To this category have been assigned those deaths that have followed cardiac arrest seemingly precipitated by circumstances such as the administration of a drug, vagal stimulation, gross acidosis, hypothermia or other circumstances, the correction of which was deemed to be, to a great extent, under the control of the anaesthetist.

Important general contributory factors to most deaths in both the above groups were: (*i*) tardiness in diagnosis; and (*ii*) inefficiencies in the application of cardiopulmonary resuscitation (CPR).

Even when CPR was apparently successful, many patients died from irreversible brain damage.

Failures in blood volume control (27 deaths, 19% ACD)

It must be noted that no use was made of central venous pressure monitoring in the clinical management of any of these cases, although such was readily available for the last two decades of this study. Again, the lesson is obvious.

Hypovolaemia (22 deaths, 15% ACD)

The feature common to all of these deaths was cardiac arrest preceded by profound and intractable post-induction hypotension of varying duration, the apparent result of inadequate cardiac venous return.

Clinical factors associated with this situation were: (*i*) in more than two-thirds of the cases there was evidence of uncorrected pre-operative hypovolaemia, coupled in 11 with inadequate blood and/or fluid replacement during operation; (*ii*) 75% of these patients were elderly and showed evidence of degenerative vascular disease and ischaemic heart disease; (*iii*) in 50% of cases, the profound hypotension followed closely on the administration of thiopentone for the induction of anaesthesia; (*iv*) a subset of 9 patients (6% ACD) warrants special mention as it highlights the grave risk anaesthesia poses to a common group of surgical patients — the elderly, those with arteriosclerosis and diabetes mellitus, possibly suffering from ischaemic heart disease, who present for lower limb amputation because of diabetic gangrene — an operation so often left to the anaesthetic and surgical registrars to deal with at the end of the day's operating list; and (*v*) the special circumstances in 4 deaths were: aortic clamp release (2); haemorrhage occurring during the technique of induced hypotension (1); and inferior vena caval compression resulting from the turning prone of a paraplegic with a full bladder (1).

Of the above patients, 7 were resuscitated only to die postoperatively from irreversible brain damage, having failed to regain consciousness.

Hypervolaemia (4 deaths, 3% ACD)

In contrast with the above, 4 patients died after pulmonary oedema induced by misjudged over-transfusion of blood and/or electrolyte solutions during surgery. In all, the manifestation of pulmonary oedema, masked or prevented during operation by the use of IPPV, was unmasked by relaxant reversal and the re-establishment of spontaneous respiration at the conclusion of operation. All of these patients died postoperatively.

Failures in arrhythmia control — cardiac arrest from causes other than anoxia and ischaemia

The combination of clinical circumstances and mechanisms other than anoxia and ischaemia that were adjudged to have caused cardiac arrest in this group of 24 patients (17% of ACD) were many and varied. The most common single associated factor (10 patients) was the administration of some *drug*. The drugs concerned and the arrhythmia precipitated were as follows: (*i*) succinylcholine, which was followed by severe bradycardia proceeding to asystole (4 patients) — in 2 patients this event was preceded by severe acidosis and endotoxaemia, 1 person had a crush injury and 1 exemplified the 'second dose succinylcholine' effect; (*ii*) halothane, which caused ventricular fibrillation in 2 patients — in one death was associated with the stormy struggling induction of an unpremedicated outpatient and the other was a patient with Parkinson's disease who was being treated with L-dopa; (*iii*) neostigmine, which was administered to a marginally anoxic patient causing bradycardia going on to asystole; (*iv*) intravenous digoxin, which led to ventricular tachycardia; and (*v*) isoxsuprine and droperidol, which both led to profound hypotension preceding ventricular fibrillation.

In 4 patients the cardiac arrest was directly associated with *severe vagal stimulation*, and in a further 3, all suffering *ischaemic heart disease*, ventricular tachycardia preceded intractable ventricular fibrillation.

Inadvertent *transfusion hypothermia* was associated with the onset of ventricular fibrillation in 2 patients.

A patient suffering *Mobitz second degree heart block* was submitted to operation unpaced and developed a complete heart block with later ventricular fibrillation.

Air embolism from a preventable transfusion mishap caused the death of the last patient in this group.

In addition, *no precise cause* could be assigned to cardiac arrest in 3 other patients, although all were attributed to anaesthetic mismanagement.

Complications of regional anaesthesia (6 deaths, 4% ACD)

This small group of cases illustrates the difficulties, already alluded to, inherent in anaesthetic mortality epidemiological studies in the formulation of cogent classifications. In terms of mechanisms, all could have been classified in the previous section. However, I have chosen to class them as a separate group to highlight adverse consequences that do occasionally follow regional techniques, *viś à viś*, the more commonly used general anaesthesia. It is pertinent here only to list briefly the complications that led to the death of these patients. These

were: (*i*) inadvertent massive spinal anaesthesia following epidural anaesthesia (2 cases — 1 caudal, 1 lumbar); (*ii*) profound intractable hypotension following epidural (2 cases) and spinal (1) anaesthesia — haemorrhage was the precipitating factor in 1 patient; and (*iii*) vagal cardiac arrest complicating the performance of retro-orbital block in a patient suffering very severe ischaemic heart disease.

Miscellaneous (5 deaths, 3% ACD)

Two patients suffered fatal subarachnoid haemorrhage when a rise in blood pressure after the administration of urovert, administered to reduce brain bulk, caused rupture of intracranial aneurysms. Use of this drug is now obsolete.

Two patients died from *acute anaphylaxis* due to administration of antibiotic during operation.

One patient died after an *incompatible blood* transfusion because the anaesthetist failed to apply accepted identity checking procedures.

Significant omissions

During the three decades covered by this study, 3 new syndromes (2 of which carry extremely high mortality rates), which result from exposure to anaesthetic agents, *per se*, have been described. These are the syndromes of post-anaesthetic hepatitis, anaesthetic-induced nephropathy and malignant hyperthermia.

All could once have been regarded as unavoidable in the sense that they were unpredictable and subject to no specific treatment. All have been the subject of intense interest and research and are topics of literally thousands of scientific communications, yet the incidence of their appearance in anaesthetic mortality surveillance studies as causes of death seems to be the inverse of this interest. In common with all the studies listed in Table II in Part I, with the exception of that of Lunn and Mushin,[6] none of these 3 conditions featured as a cause of any of the deaths in our study.

Changes in aetiological pattern

The gratifying 6-fold reduction in death attributable to anaesthesia was reported in part I of this review. Of interest here is the observation that computation of the proportional distribution of the general groups of causes of ACDs by the decade reveals this reduction in incidence to have been accompanied by a change in aetiological pattern. This involves a progressive increase in the proportion of deaths due to *failures in the control of respiratory homeostasis* accompanied by a reciprocal decrease in those due to *failures in the control of circulatory homeostasis* (Fig. 3). More detailed examination by a similar computation from the above of the proportional representation of subsets of the 4 most common clinical causes of death reported, i.e. failures in the *management of airway, ventilation, blood volume* and *arrhythmia* (Fig. 4), shows clearly that it is the failures in *airway management* — of which the majority involved complications of *endotracheal intubation* — that are responsible for the increased proportion of respiratory deaths.

Although it may be argued that failures in airway management are the more easily identified, an explanation for this state of affairs may lie in the fact that whereas endotracheal intubation and mismanagement of the airway involves age-old physical skills, manual dexterity and a degree of informed clinical judgement, control of the circulatory homeostasis depends to a large extent on the clinician's intelligent interpretation of, and prompt reaction to, information derived from

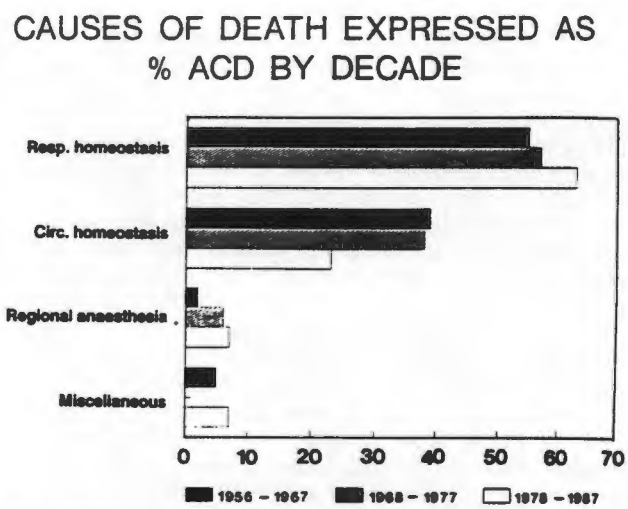

Fig. 3. Proportional distribution of causes of ACD by decade.

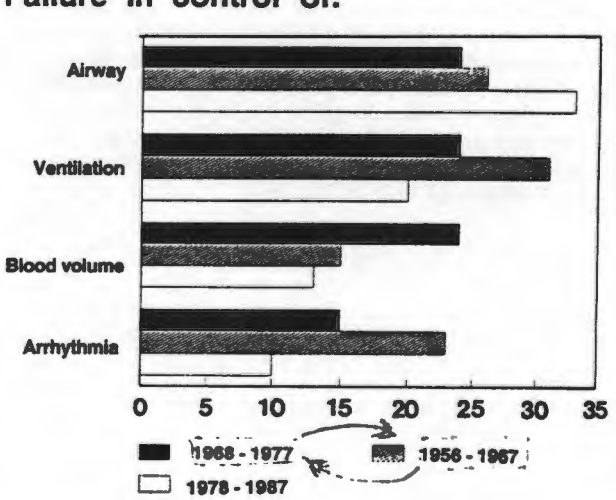

Fig. 4. Proportional distribution of the most common clinical management failures responsible for ACD, by decade.

the vital function monitoring that has improved so in sophistication, acceptability and availability in recent years. Such reaction requires intellectual rather than manual skills.

Conclusions

Thirty years ago *The Lancet* stated in an editorial that: 'The most obvious risks of an operation are those associated with anaesthesia.'[7] The truth of this statement must now be seriously questioned in the light of observations reported here, which evidence a decrease in the incidence of death attributable to anaesthesia to a level (0,07/1 000 anaesthetics) which, having regard for the human frailty is perhaps at an irreducible minimum. Indeed, the safety of anaesthesia for the patient has increased to the point that '. . . the most insidious hazard of anaesthesia today is its relative safety',[8] for this carries the inherent risk of generating boredom and in consequence the danger of complacency and inattention on the part of the anaesthetist. While the increasing rarity of death attributable to anaesthesia may lead one to question the continued value of conventional epidemiological studies such as this, in alerting the clinician to the above dangers they still serve a purpose.

They are of import, too, in providing the means for 'in-house' audit, so necessary for the maintenance of our professional standards. However, the fact that 'death' is the criterion for entry into the study, limits its focus to the tip of the iceberg of anaesthetic clinical mismanagement and information on those many 'who have almost died'[9] goes abegging. Yet the causes of a near brush with death and of death itself, are no different.

The further improvement in the safety of anaesthesia for the patient requires that the process of hazard identification and prevention be refined by extension of surveillance studies antegrade, to the stages of 'morbidity' and 'critical incident' (see Fig. 1, part I). In the past the manpower and resource needs for monitoring these entities have been prohibitive, but with today's computer technology they have become feasible.

Such refinement of the study would allow also of an evaluation of the more subtle role perceived substandard anaesthetic management may play in the genesis of such post-operative morbid conditions as myocardial infarction, adult respiratory distress syndrome, renal and hepatic failure, which may result in death.

Our wider objective is the facilitation of further improvement in the delivery and safety of surgical care. To achieve this, anaesthesia — an adjunct only to the whole — should no longer be viewed in isolation but holistically together with the surgeons, in the context of its environment, i.e. the patient's disease both specifically surgical and intercurrent, together with the implications and complications of the surgical procedure. Quality assurance of the management of all these phases jointly, in some manner similar to that pioneered by the British Confidential Enquiry into Peri-operative Deaths,[10,11] must surely lead to further and continued improvement in surgical outcome for the patient.

I acknowledge with gratitude the willing co-operation of my many colleagues in the Department of Anaesthetics for providing the clinical information upon which this analysis is based. A very special word of thanks is due to Mrs Maureen Scholtz, Secretary of the Department of Anaesthetics, and her predecessors for identifying the relevant cases and thereafter ensuring that the appropriate documentation was obtained.

This article is published with the permission of the Chief Medical Superintendent of Groote Schuur Hospital, Dr J. Kane-Berman.

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INHALATION ANAESTHESIOLOGY

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Anaesthetic Accidents

GAISFORD G. HARRISON

'It is the hand that gives the Anaesthetic and the eye that watches its effects that matter more than the choice of agent, dosage and method.'

[Keith Simpson, 1953]

Anaesthesia per se does not correct deformity, restore health nor stay death, but by making possible procedures which do accomplish these things it is an indispensable adjunct to the treatment of the surgical patient or any patient who needs to undergo, as part of the diagnosis and treatment of the malady, procedures which may be distressful or painful. Anaesthesia directly affects the lives of an appreciable section of the population; every year one in every 20 to 25 people is required to undergo a procedure which necessitates his being anaesthetized. For each of the millions who submits to a general anaesthetic, be it short or long, there is the realization—and in many cases perhaps the fear— that for the duration of the procedure he is surrendering total control of his consciousness and his physical well-being to the anaesthetist. Therefore, above all else, anaesthesia must be safe—devoid of accident.

A MEASURE OF SAFETY

A precise measure of safety (or its converse, the risk factor) of anaesthesia is difficult to establish. Safety means 'absence of danger' and, paradoxically, we measure it by establishing its reciprocal, the rate of death. Perhaps the most fundamental index of the safety of anaesthesia is the incidence with which factors, relevant to the administration of an anaesthetic, cause (or are contributory in a major way to) the death of a patient. This in itself is not something that can be estimated with precision. In the case of death associated with anaesthesia, autopsy is of little value in establishing the cause of death, let alone the manner in which it arose (Harrison, 1968a; Lunn and Mushin, 1982). Essential to the direct assessment of a death associated with anaesthesia is the account of the clinical conduct of the anaesthetic with relevant records of events and changes in vital physiological parameters. In judging these, consideration must be given not only to the ways in which the known actions of anaesthetic drugs or errors and misjudgements in their administration may have lethal sequelae but also to the ways in which errors of commission or

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omission in the wider field of responsibility, that has by practice become that of the anaesthetist, may cause death. Reversible depression of pain perception and creation of adequate conditions for surgery must be accompanied by control and preservation of the patient's physiological homeostasis. While this is controlled all is well; when control fails all is lost.

This control defines the ambit of the anaesthetist's responsibility. Published as long ago as 1916, Bellamy Gardiner's statement of the ambit of responsibility of the anaesthetist is still worth repeating. He wrote: 'the services of a practised administrator should relieve the operator of all responsibility with regard to the patient's general condition during operation. The anaesthetist therefore has in many instances to undertake duties of considerable gravity and should be thoroughly equipped not only by individual qualification but physically by possessing perfect senses of sight, hearing, keen scent and gentleness of touch. To his share fall the provision and accurate manipulation of the best drugs and apparatus for the administration of the different vapours; the detection of symptoms and physical signs of disease which will affect the subsequent anaesthesia; the choice of the particular anaesthetic or sequence of anaesthetics most suitable to the patient and operation in hand; the protection of the body from external harm; the regulation of atmospheric temperature; the resort to stimulants and methods of resuscitation in cases of failing vitality; the safe transference to bed and the supervision during recovery from insensibility.' (Bellamy Gardiner, 1916).

ACCIDENT, HUMAN ERROR AND PREVENTABILITY

Death occurring in association with anaesthesia and surgery is a function of three variables: the patient's pathology for which the operation is indicated; the surgical operation and its effects; and the conduct of the anaesthetic. These three are inseparable. In judging and assessing the contribution to the final outcome of the conduct of the anaesthetic it must be borne in mind that not only the patient's pathology but also surgical errors themselves may have precipitated the circumstances which subsequently involved the conduct of the anaesthetic as a contributory cause—even a major one—of the patient's death. Beecher and Todd (1954) in their classical survey of death associated with anaesthesia found that errors in surgical technique, as distinct from errors in surgical judgement and/or diagnosis, were three times as frequent as anaesthesia as a primary cause of death and five times as frequent as a contributory cause.

Looked at broadly, those in the third category—deaths attributable mainly to anaesthesia—are themselves a function of three variables: the skill of the anaesthetist; the agent used; and the environment of the anaesthetic (i.e., the condition of the patient). Of these, the skill of the anaesthetist is the most important. This skill is composed of many parts: training, relevant knowledge, experience and ability to deduce the correct inference from a set of observations and to react to such inference decisively and expeditiously.

As regards the agents used, the anaesthetist should have sufficient knowledge of these, of their actions, side-effects and methods of counteracting

these so that any death that may result from so-called 'functional toxicity' (Bruce, 1980) becomes basically a death due to lack of skill. Clinical anaesthesia has been likened to an iatrogenic illness. It behoves the 'iatros' therefore to be well versed in the recognition and control of its signs and symptoms. Much the same may be said, though perhaps with less cogency, of the environment of the anaesthetic—the condition of the patient. Although this may respond to and be improved by skilful preoperative treatment, it may often be something over which the anaesthetist will have no final control.

If the lack of skill on the part of the anaesthetist as the major factor in the causation of death attributable to anaesthesia is to be accepted, it follows that such deaths are preventable. To say this is not to attribute blame, the implication being that on review (albeit with the benefit of hindsight) a reasonably satisfactory explanation of the circumstances can be found and countermeasures that could prevent recurrence of the situation are practicable (Morton, 1958).

What then of the 'accidents' of our alliterative title? Accident is defined as an event without apparent cause, a chance mishap, the unexpected. Such events do happen in clinical anaesthesia but must be regarded as uncommon. One of the many difficulties posed in the assessment of death associated with anaesthesia is the fact that advance in knowledge and accumulation of experience with time often renders what may be judged an anaesthetic accident today a preventable error tomorrow. Of these there are many examples but one of particular relevance would be the occurrence of postanaesthetic kidney failure. Whereas in the light of present knowledge the occurrence of this condition after the administration of enflurane may still be regarded as an accident (fortunately rare) the same event following administration of methoxyflurane to a patient receiving contemporaneous medication with dilantin and phenobarbitone (or any microsomal enzyme inducing drug) could no longer be so regarded.

SOURCES OF ERROR OR ACCIDENT DURING INHALATION ANAESTHESIA

If we consider a diagram (Figure 1) of the interactive pattern of the anaesthetist, his patient and apparatus, the sources of possible error or accident are apparent. Each interactive step in this diagram may be a point of failure, a source of error.

Basic to correct action and reaction on the part of the anaesthetist is the availability of accurate information on vital physiological parameters. Electronic technology has made these readily available to the clinician. Euphemistically referred to in the past as an 'art', anaesthesia has now acquired the trappings of a science—the facilities for observation, measurement, inference and reaction. It is no longer necessary, nor should it be permissible, to guess. In these circumstances the failure to use adequate monitoring when facilities are available should surely be regarded in the same light as the driving of a motor vehicle blindfolded (i.e., negligent). Nevertheless, it happens (Lunn and Mushin, 1982).

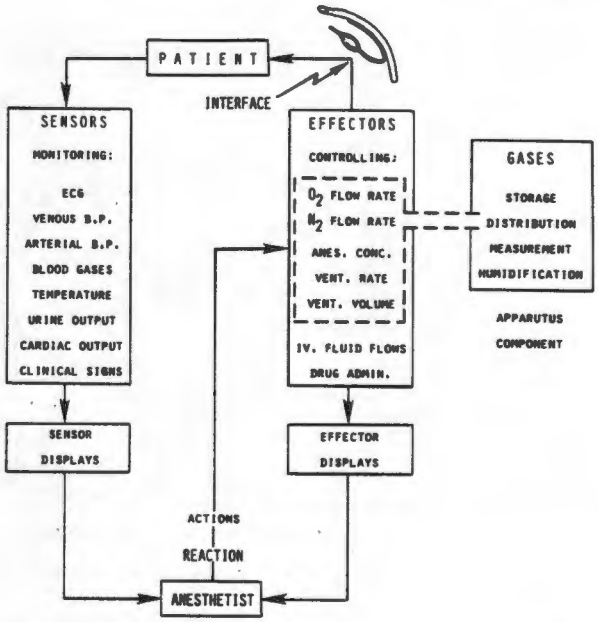

Figure 1. Pattern of interaction of anaesthetist, patient and apparatus. Modified from Cooper et al (1978a).

The second factor in the process of decision-making and reaction by the anaesthetist is clinical experience and relevant knowledge. These should be acquired in the vocational training process. Interpretative skills and appropriate reactions hopefully improve with experience. It is this aspect of clinical anaesthesia which is perhaps the most vulnerable to human error. Man is not a machine and there are many factors from day to day which affect his performance.

A third essential factor in the anaesthetist/patient interaction is the anaesthetic machine and breathing circuit which can be thought of as interfacing with the patient at the endotracheal tube (or face mask). This system (Figure 2) embraces: all the components necessary for the storage, distribution and measurement of flow of respirable and anaesthetic gases; the means of accurate dispensing of anaesthetic vapour to the patient and the means of providing and measuring adequate pulmonary ventilation. This is the system most subject to technical failure and in which development and elaboration have multiplied the chances of accident through machine failure and operator error.

It is not the intention here to review exhaustively the possible malfunctions in the system that may result in patient demise. These have been adequately reported (Dinnick, 1973; Dorsch and Dorsch, 1975; Feeley and Hedley-Whyte, 1976; Ward, 1975; Wyant, 1978). Let it suffice here to remind the reader that there are at least 12 sites in the total system at which technical failures have occurred (see Figure 2). These have resulted in: the wrong gas or

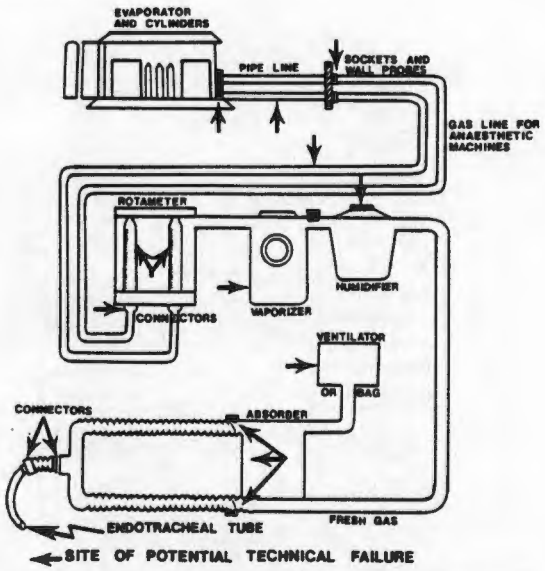

Figure 2. Sites of potential technical failure and accident.

contaminated gas being delivered to the patient; excessive pressure in the breathing system; major leaks in the system; obstruction to the system; excessive concentration of anaesthetic vapour; excessive concentration of CO_2 and overheated humidification. The implication of this formidable, yet incomplete, list of hazards is clear. If technical failures are to be recognized and their consequences averted the anaesthetist must not only fully understand the function of the breathing and anaesthetic system he is about to use but must also personally check all his apparatus before he uses it (Ward, 1975; Dripps, Eckenhoff and Van Dam, 1977).

HOW SAFE IS ANAESTHESIA?

While it is to be admitted that there must be a considerable subjective element in the final assessment of the culpability of the three variables that bear on anaesthesia associated mortality—the patient's disease, surgery and conduct of anaesthesia—this type of review is the only means by which some measure of the safety of anaesthesia and an audit of the efficacy of our service to the patient is gained. However, the lack of uniformity in anaesthesia mortality surveillance studies, in classification and assessment criteria used (perioperative period and type of surgery included) renders meaningless any but the very broadest statistical comparisons between studies (Harrison, 1968b; Goldstein and Keats, 1970).

For this reason the recent publication of two studies of anaesthesia-associated mortality of similar structure, which individually allow some between-study comparisons over a number of years, is of interest. These are the studies

Table 1. *Incidence of death attributable to anaesthesia*

Study	Years studied (number of anaesthetics)	ACD[a] Incidence per 1000 anaesthetics
Sydney		
Clifton and Hotton, 1963	1952–1962 (205 640)	0.17
Bodlander, 1975	1963–1972 (211 130)	0.07
Cape Town		
Harrison, 1968b	1956–1966 (177 928)	0.33
Harrison, 1978	1967–1976 (24 0483)	0.22
Harrison unpublished	1977–1980 (128 401)	0.14

Studies included deaths occurring during or ≤ 24 hours of anaesthetic or failure to regain consciousness.
[a]ACD = Anaesthetic contributory death—anaesthesia sole or major contributory cause.

of Bodlander (1975) from Sydney, Australia and Harrison (1978) from Cape Town, South Africa. Bodlander reviewed the anaesthetic-associated mortality at the Prince Alfred Hospital, Sydney, for ten years (1963 to 1972) as a follow-up to the study by Clifton and Hotten (1963) at the same hospital of the previous ten-year period (1952 to 1962). Harrison reviewed the anaesthetic associated mortality at Groote Schuur Hospital, Cape Town, for the ten years 1967 to 1976 as a follow-up of his own review of the previous ten years at the same hospital (Harrison, 1968b). For further comparison, as yet unpublished data from the Groote Schuur Hospital study for the subsequent four-year period (1977 to 1980) is also given (Table 1). These studies estimated the incidence of deaths attributable solely to anaesthesia to range from 0.07 to 0.22 deaths per 1000 anaesthetics. Not only is the computed rate low but encouragingly there is evidence from between-study comparisons that it has also fallen with time.

Four other recently published surveys support a similar conclusion (Table 2). In a class of its own, not least for size and breadth of coverage, is the comprehensive study in England by Lunn and Mushin (1982) sponsored by the Association of Anaesthetists of Great Britain and Ireland (AAGBI). Deriving from a background surgical population of 1.1 million, this is the largest study of anaesthetic-associated mortality ever reported other than that by Kok and Mullan (1969) in South Africa which covered 1.7 million anaesthetics. The other studies are those in Finland (Hovi-Viander, 1980), Canada (Turnbull, Fancourt-Smith and Banting, 1980) and Australia

Table 2. *Incidence of death attributable to anaesthesia*

Study	Years studied (number of anaesthetics)	Perioperative period included	ACD[a] Incidence per 1000 anaesthetics
Great Britain			
Lunn and Mushin, 1982	1979–1980 (1 147 362)	≤ 6 days	0.1
Finland			
Hovi-Viander, 1980	1975 (338 934)	≤ 3 days	0.2
Canada			
Turnbull, Fancourt-Smith and Banting, 1980	1973–1977 (195 232)	≤ 2 days	0.18
Australia			
Adelaide Committee, 1976	1969–1974 (465 000)	≤ 1 day	0.08

[a]ACD = Anaesthetic contributory death—anaesthesia sole or major contributory cause.

(Adelaide Committee, 1976). The incidence of deaths attributable to anaesthesia computed from these studies matches that reported by Bodlander and Harrison, ranging from 0.08 to 0.2 deaths per 1000 anaesthetics.

One implication of this sort of rate (one to two deaths attributable to anaesthesia per 10 000 anaesthetics) is that the average anaesthetist would be expected to encounter only one such death every five to ten years of his working life. In terms of human frailty it may be wondered whether it is possible for this rate to be further reduced. On the other hand, although this rate of anaesthetic-contributory death may be considered not only acceptably low but also perhaps at an irreducible minimum, it also means that every year in a country the size of the United States something of the order of 2000 patients die because they are anaesthetized (Epstein, 1978). Looked at in this light, death attributable solely to anaesthesia and anaesthetic accidents must continue to be a matter of concern.

As anaesthesia is an adjunct to the total care of the surgical patient, a relevant context in which to consider deaths attributable to anaesthesia would be the total background surgical mortality (i.e., the operative and postoperative hospital stay mortality). Although not presented in most anaesthesia mortality surveillance studies, such data is recorded in the studies from Groote Schuur Hospital (Harrison, 1968b; 1978). From 1956 to the present, anaesthesia and its mismanagement has been held responsible for 2.7 reducing to 2.0 per cent of total surgical mortality. Lunn and Mushin (1982) included in their study in Great Britain data and deaths up to six days postoperatively. In this study anaesthesia and its mismanagement were considered responsible for 1.5 per cent of the total surgical mortality and contributory in some way to almost one in 20 (4.5 per cent).

PATTERNS OF ANAESTHETIC MORTALITY

The most beneficial spin-off of anaesthetic mortality surveillance studies is that they serve to identify patterns of anaesthetic mortality and so provide the clinical lessons from which we may improve our practice. The lack of comparability between published mortality studies which frustrates attempts at establishing, except in very broad terms, a rate of death attributable to anaesthesia applies with equal, if not greater, force to attempts to evaluate common patterns of anaesthetic related deaths, their clinical environment and the risk situations that engender them.

To illustrate the immense variability that exists in the literature in classification of causes of anaesthetic-related deaths, two examples must suffice. As a first example, consider this actual case report.

The patient was an obese female aged 52 years who presented at hospital after 20 hours of symptoms with bowel obstruction following incarceration of colon in a paraumbilical hernia. Rehydration and electrolyte correction was undertaken and the patient presented for anaesthesia after replacement of three litres of fluid. Other than for her surgical disease, abdominal distention and obesity, the patient was considered generally fit. Following preoxygenation, anaesthesia was induced with thiopentone, paralysis obtained with succinylcholine and endotracheal intubation performed while cricoid pressure was applied. No regurgitation of gastric content or tracheal soiling was observed. Anaesthesia was maintained with an IPPV technique using N_2O and O_2 with 0.5 to 1 per cent halothane together with alloferin for muscular relaxation. The ECG and blood pressure were monitored but not central venous pressure. Surgery took three hours during which the anaesthetist transfused a further 3.5 litres of balanced salt solution. Anaesthesia was otherwise untoward other than that the anaesthetist noticed some decrease in pulmonary compliance as surgery progressed. At the conclusion of surgery, neuromuscular blockade was reversed with neostigmine and atropine, the patient was allowed to breathe spontaneously and extubated. Subsequently, when the patient was waking, some difficulty with respiration appeared and the colour of the patient became slightly dusky. The anaesthetist contented himself with giving additional neostigmine and sent the patient, breathing spontaneously with a 40 per cent O_2 entrainment mask, to the recovery ward. Shortly thereafter the anaesthetist was called into the recovery ward to find the patient in grave respiratory distress, cyanosed and sweating, her pulse bounding. While he attempted reintubation of the patient, cardiac arrest followed a brief period of extreme bradycardia. Subsequent attempts at CPR failed. Autopsy revealed firm stiff lungs with interstitial oedema and some frothy bronchial and tracheal fluid.

While many would find little difficulty in deciding that sufficient preventable errors of management were evident to attribute the patient's death largely to mismanagement of the anaesthetic, the cause of death might well be classified by different authors as one of:

1. post relaxant respiratory inadequacy;
2. inadequate ventilation—hypoxia from abdominal distention and obesity;
3. premature extubation;
4. covert regurgitation and inhalation of fluid gastric content;
5. inadequate monitoring—no central venous pressure;
6. inadequate postoperative care;
7. pulmonary oedema;
8. overhydration;
9. cardiac arrest;

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5. inadequate monitoring—no central venous pressure;
6. inadequate postoperative care;
7. pulmonary oedema;
8. overhydration;
9. cardiac arrest;

10. arrhythmia;
11. cardiac failure.

As a second example, let us consider the circumstance during an anaesthetic when respiratory obstruction develops suddenly because herniation of the endotracheal tube cuff totally obstructs its outlet. Death ensues from hypoxic cardiac arrest before the anaesthetist diagnoses the situation. In various surveys such has appeared under the following categories: technical failure; respiratory obstruction; complications of intubation; and cardiac arrest.

Reporting the findings of the New South Wales Special Committee for investigation of death under anaesthesia, Holland (1970) noted an average of 4.3 errors per anaesthetic contributory death. Yet when classified for study and publication, most authors will record cases under a single diagnosis or error only. In spite of these difficulties, certain common patterns of anaesthetic mortality and the risk situations from which they were engendered can be discerned in data presented in the mortality surveys quoted, as well as others.

Useful for this purpose, as again some between-study comparison is possible, is the AAGBI study in which Dinnick (1964) reviewed 600 anaesthetic-associated deaths. This report was a follow-up of the original 1000 case study, published 8 years previously (Edwards et al, 1956). A slightly different facet of the problem was shown by Utting, Gray and Shelley (1979) in their analysis of anaesthetic mortality patterns operative in cases reported to the Medical Defence Union in the United Kingdom (i.e., cases that were the subject of litigation or official enquiry).

A drawback manifest in all these studies is death being the criterion for case entry into the study; an endpoint is studied and little information is derived on the risk or hazard situation that preceded the crisis. Additionally, only the tip of the iceberg of clinical mismanagement is revealed. If these have died, what of those who nearly died? Complementary to the more conventional studies of death attributable to anaesthesia are two recent reports of what one might call the prodrome of anaesthetic-induced fatalities. Cooper et al (1978b) and Craig and Wilson (1981) analysed information submitted voluntarily by anaesthetists on 'critical incidents' occurring during clinical anaesthesia of which few, if any, led to the death of the patient but all of which, uncorrected, had the potential so to do. Cooper et al took note of incidents that:

1. involved a recognizable error by the anaesthetist or a failure of anaesthetic equipment;
2. occurred at a time when the patient was under the care of an anaesthetist;
3. could be described in clear detail by the person who either observed or was involved in the incident;
4. were clearly preventable.

These studies—the retrospective, as we may regard mortality studies, and the prospective, the 'critical incident' analysis—complement each other well, each highlighting a different aspect of the problem of anaesthetic accidents. In spite of tremendous variability in the presentation of data certain broad areas and

clinical circumstances can be delineated which seem to constitute the major hazard or accident situations.

The endotracheal tube

Common to both mortality studies and 'critical incident' analyses are problems at the anaesthetic equipment/patient interface—the endotracheal tube (Figure 1). Complications related to placement, misplacement or failure to place an endotracheal tube, obstructions due to kinking, cuff hernation, dislodgements and disconnections, feature as a very frequent (in many studies the most frequent) cause of death and, indeed, critical incident. A technique, peculiarly the stock-in-trade of the anaesthetist, has become his unconscious patient's Achilles' heel. The persistent reappearance of the endotracheal tube hazard in mortality studies must indicate where the major accent in 'education for safe practice' must fall (Dripps, Eckenhoff and Van Dam, 1977).

Relaxants

The other stock-in-trade of today's anaesthetist, the neuromuscular blocking agent, is still held responsible for many deaths attributable to anaesthesia. These deaths feature as post relaxant respiratory inadequacy, recurization, respiratory insufficiency or inadequate postoperative care. Considering the now standard provision of postanaesthesia recovery facilities, not to mention intensive care units with the concomitant availability of expertise in prolonged ventilation, it is hard to understand why this should still be. This recalls the study of Beecher and Todd (1954). In many cases the patient suffers from chronic obstructive airway disease and the anaesthetist, who is inexperienced and feels that putting the patient 'on a ventilator' postoperatively is a confession of failure, persists hopefully in permitting the patient to breathe spontaneously to the ultimate detriment of the patient. The lesson is obvious—when in doubt 'ventilate'.

Inadequate postoperative supervision

That 'inadequate postoperative supervision' is still held accountable for many anaesthetic related deaths surely needs little comment. While the provision of high intensity nursing care recovery areas within operating room precincts with ready access for the anaesthesiologist has now become standard in hospital design, the safety of the patient is still entirely dependent upon the quality of nursing care and vigilance.

Vomiting and regurgitation—the full stomach

The full stomach with its consequent risk of vomiting, regurgitation and aspiration has long been recognized as posing an extremely hazardous situation, especially in anaesthesia for obstetrical surgical procedures. It is gratifying to note that, in contrast to so many other causes of death attributable to anaesthesia, some recent mortality surveys show a decreased

incidence due to this cause (Dinnick, 1964; Harrison, 1978). While this may be due to increased awareness of the problem, better training and improved techniques, some would attribute it to the increased use of epidural anaesthesia and other regional techniques in obstetrics (Bodlander, 1975).

Circulatory homeostasis

In the management of the patient's circulatory homeostasis, not the least problematical variables for the anaesthetist are the patient's coincidental cardiac disease and abnormal circulatory status (i.e., the manner in which these conditions affect the reaction to anaesthetic drugs and the conditions imposed by surgery). Most important in this regard is ischaemic heart disease. Lunn and Mushin's study (1982) revealed that this was regarded as significant in 39 per cent of patients submitted to autopsy. It is not pertinent to detail here the many and varied mechanisms and combinations of circumstances by which anaesthetic-related factors might precipitate cardiac arrest and possibly death, but it is relevant to draw attention to the most common general circumstance or hazard situation associated with cardiovascular deaths attributable to anaesthesia. This is the state of preanaesthetic hypovolaemia which is often covert. Presenting often as profound postinduction hypotension (a lethal circumstance in the presence of grave ischaemic heart disease) it may be listed as 'drug-induced cardiac arrest' for the simple reason that it is so often directly associated with the administration of thiopentone. The situation may also be regarded as 'inadequate preparation for surgery' (Holland, 1970). Under whatever guise this general situation of preanaesthetic hypovolaemia presents, it is prominent in all recent mortality surveys as the situation with the highest anaesthetic accident potential. Less common, but not infrequently featured, is the reverse situation in which liberal fluid replacement, unmonitored by central venous pressure measurement, results in over-hydration which is manifested as pulmonary oedema with the re-establishment of spontaneous respiration on termination of anaesthesia. Bearing in mind the virtual universality of cardiopulmonary bypass surgery and the attendant growth in expertise in circulatory control, a diminution in this risk factor would be expected.

Apparatus/technical failure

Considering the number of possible sources of hazard and accident in the system concerned with the supply of O_2 and inhalational anaesthetic to the patient (see Figure 2) it is perhaps not surprising that in their 'critical incident' analyses both Cooper et al (1978b) and Craig and Wilson (1981) reported equipment failure and technical mishap as the major threat to the patient's safety. The most common failure was the 'disconnect'. The 'slip-on' becomes the 'slip-off' easily. When the multiplicity of connections that has evolved in the breathing circuit of today's anaesthetic machine is considered (many of them being unnecessary), it is obvious that systematic redesign is needed (Cooper et al, 1978a). Redesign and incorporation of safety devices can help in reducing the hazard, as evidenced by Cooper's (1978b) report; in 4 out of 9

incidents of failure of O_2 supply in which the anaesthetic machine was equipped with a pressure fail-safe system, this was known to have been activated before the absence of O_2 flow was noticed by the anaesthetist. Surely the time has come when the incorporation of such a fail-safe pressure alarm device, together with an O_2 analyser with low-limit alarm in the patient breathing circuit, should be mandatory in anaesthetic machine design (Mazze, 1972).

In spite of the above, it is somewhat surprising that equipment and technical failures feature so rarely as causes of death in mortality surveillance studies (Dinnick, 1973). Of the recently published surveys, only in the British Medical Defence Union cases do equipment and technical failures feature prominently (Utting, Gray and Shelley, 1979). That the cases submitted to the Medical Defence Union were basically the subject of an official enquiry or litigation may well have biased their selection.

Factors affecting performance

While the title of this chapter is 'Anaesthetic Accidents', the stress has been that, on the whole, deaths attributable to anaesthesia do not result from accident but from human error. Cooper et al (1978b) and Craig and Wilson (1981) have produced information on an important aspect of these errors that has been completely lacking in previous conventional mortality studies. This concerns their specific enquiry into the factors associated with the error situation. Hurry, inattention, carelessness and fatigue were high up on their lists. All of these could doubtless lead to the failure to perform normal checks and failure to assess the situation preoperatively which were also listed. 'Inadequate total experience' and 'inadequate familiarity with equipment, technique or surgical procedure', which also rated high in the study by Cooper et al must carry an admonition for the chairmen of departments and their academic programme directors.

THE REAL ACCIDENTS

The preoccupation with human error and frailty in the causation of anaesthetic related death and mortality should not detract from the fact that, over the last two decades, three new syndromes of mortality and morbidity that may follow exposure of a patient to inhalation anaesthetics have become apparent (i.e., anaesthetic-induced hepatitis, nephropathy and malignant hyperthermia). All of these may be regarded as anaesthetic accidents in that they are unpredictable and to a large extent unavoidable and must be treated simply on their merits when they arise. These three syndromes have been the subject of major anaesthetic research for the last 20 years and indeed two are the subject of chapters in this Issue. From these, especially the latter two, much of the mystery has now been removed. Yet in spite of all the attention paid to these syndromes in the literature they find scant mention in mortality surveillance studies. Halothane hepatic failure features only in the Medical Defence Union case studies, being held responsible for 3.4 per cent of deaths.

Malignant hyperthermia, also reported in this study, is reported in only one other, that of Lunn and Mushin (1982), in which three out of 58 deaths are attributed to it.

SUMMARY

Safe, accident-free anaesthesia is a prerequisite for the application of surgical treatment. While it is conceded that accidents do occur in clinical anaesthesia, it is arguable that deaths, when attributable to anaesthesia, are usually the result of error or lack of skill on the part of the anaesthetist and, as such, are basically preventable.

The sources of accident and error during inhalation anaesthesia are examined in terms of the pattern of interaction between anaesthetist, patient and apparatus. The latter subset of the system which includes the means of storage, distribution and measurement of flow of respirable and anaesthetic gases and vapours to the patient, together with the means of providing pulmonary ventilation, are highlighted for the number of sites of potential technical failure and accident they embrace.

An answer to the question 'How safe is anaesthesia today?' is sought in a brief review of the most recent surveillance studies of anaesthesia associated mortality. While differences between studies in structure, case inclusion, classification and assessment criteria render invalid any but the very broadest comparisons and inferences, evidence from these studies seems to suggest that, associated with surgery, there is an incidence of death attributable to anaesthesia of between 0.07 to 0.2 per 1000 anaesthetics. This represents a primary anaesthetic responsibility for approximately 2 per cent of total surgical operative mortality. Examination of the patterns of anaesthetic mortality in the studies reviewed reveals much agreement in the identification of the commoner accident and risk situations. These are:

1. complications of endotracheal intubation;
2. relaxant related postoperative problems;
3. inadequate postoperative supervision;
4. the full stomach;
5. failure to secure circulatory homeostasis;
6. apparatus and technical accident/failure which is the commonest cause of 'critical incident' but which seldom causes death;
7. hurry, inattentiveness, carelessness and fatigue, which are the factors that most commonly adversely affect the anaesthetist's performance.

In short the causes of death attributable to anaesthesia have changed little with time. They are basically simple and follow failure to observe elementary precautions together with a lack of clinical alertness.

CONCLUSIONS

Some evidence that the safety of anaesthesia for patients has increased is presented. The actual frequency of death attributable to anaesthesia seems in

many places, to have decreased to a level which, having regard for human frailty, is perhaps at an irreducible minimum. Indeed the diversity of modern surgery itself bears witness to the relative safety of contemporary anaesthetic practice. In spite of this, it is sad to reflect that the actual numbers of patients who die every year because they were anaesthetized is appreciable. Generally, the basic causes have not changed but simply recur. The causes of these deaths are mostly simple and usually follow the lack of observation of simple precautions and lack of clinical alertness. Because of this, in writing of anaesthetic safety one finds oneself uttering repeated trite aphorisms, so trite indeed that they are ignored and forgotten until too late. To quote Thompson (1981) 'The road to anaesthetic safety is paved with pious platitudes!'

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LECTURES IN ANAESTHESIOLOGY

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DEATH ATTRIBUTABLE TO ANAESTHESIA: ITS INCIDENCE AND THE COMMONER CAUSES

G.G. Harrison

'Anaesthesia is a Iatrogenic Illness' (Bruce, 1980).

The commonest outcome of anaesthesia—the achievement of the treatment goal—is a course without complication, permitting surgery, followed by rapid recovery of the patient to the pre-anaesthetic state (see Fig. 1).

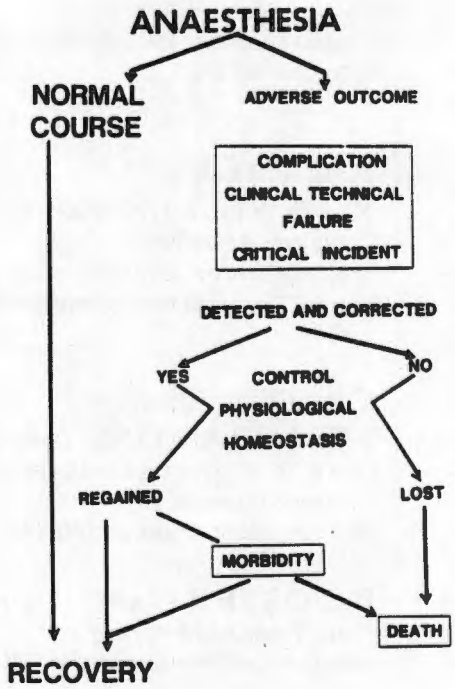


Fig. 1 The outcomes of anaesthesia.

Adverse outcome is less common. At worst, some complication of anaesthesia and/or the accompanying surgery—a critical incident—will not have been recognized, recognized too late and/or treated inappropriately, resulting in the patient's death. As will be discussed, this extreme is fortunately rare.

More commonly, detection, recognition and correct management of the complication/critical incident will result in re-establishment of control of the patient's physiological homeostasis and thereafter the recovery of the patient. Such recovery may be in the normal anaesthetic time-course or may be delayed by the development of a morbid process consequent

on the original complication or critical incident. Usually, the patient will recover from this completely, but the delayed time-course and increased hospital stay with added consumption of resources imply some failure in goal achievement.

The final outcome may become less favourable in that the patient, though he or she survives, suffers chronic loss of function of some major organ such as the brain, lung, liver or kidney as a result of the initial critical incident or subsequent morbid process. Finally, the morbid process itself may end in the death of the patient.

Prevention of adverse outcome and anaesthesia practice audit require evaluation of the role of anaesthesia in each of these circumstances—mortality, morbidity and critical incident.

While certain centres are now developing methods of data acquisition for the assessment of morbidity and critical incident (Cooper *et al*, 1978a; Flood, 1980), monitoring of these entities requires resources beyond the means of many anaesthetic services, and therefore only the extreme end-point of the adverse outcome process—anaesthesia-associated mortality—has been the subject of most surveillance studies to date. A drawback that manifests itself in studies of anaesthetic-associated mortality is that, death being the criterion of entry into the study, an end-point is studied; insufficient information may be derived on the risk or hazard situation which preceded or engendered the crisis. In addition, the event studied is but the tip of the iceberg of clinical malpractice. 'The tolerance of the human body for abuse is phenomenal and if a few patients die every year, consider how many more have been subjected to abusive practices. How many have almost died?' (Davis, 1957).

Anaesthesia-associated mortality surveillance studies should:

- identify deaths to which anaesthesia mismanagement or untoward reaction is considered to have been contributory in a material measure
- identify, with a view to future prevention, points of breakdown in the anaesthetist's control of the patient's physiological homeostasis and their cause
- include surveillance of an adequate time-span post-anaesthetic to permit detection of any morbid process induced by mismanagement of anaesthetic drugs or by inefficient technique
- include acquisition of background population data to allow for the rating of phenomena and the use of surveillance studies as a measure of the safety (or conversely the risk) of anaesthesia or as a practice audit to monitor maintenance of professional standards.

ANAESTHETIC CONTRIBUTORY DEATH

Identification

All surveillance studies of anaesthetic-associated mortality are bedevilled by difficulties inherent in the assessment of the responsibility of anaesthetic drugs or techniques for adverse outcome. These difficulties arise because anaesthesia is inseparable from:

- the patient's pathology, both surgical and incidental
- the surgical operation and its effects including surgical complications which may subsequently involve the conduct of the anaesthetic as a contributory cause of the patient's death (see Fig. 2).

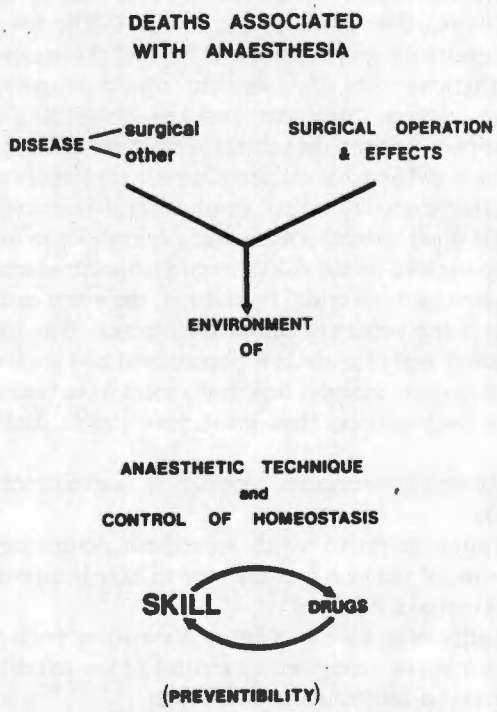


Fig. 2 Variables inherently inseparable from deaths associated with anaesthesia.

In circumstances in which the relation of the anaesthetic to the patient's pathology and the surgical operation does not provide sufficient explanation for the adverse outcome and the conduct of anaesthesia in question, we are faced primarily with an assessment of the difficult-to-define quality of *skill* of the anaesthetist. This skill is composed of many parts: training, relevant knowledge, experience and the ability to make correct deductions from a set of observations and to react decisively and timely to the deduction.

As regards the agents used, the anaesthetist should have sufficient knowledge of these, of their actions, side-effects and methods of counteracting these that any death which may result from so-called 'functional toxicity' (Bruce, 1980) must be judged a death basically due to lack of skill. Much the same may be said, though perhaps with less cogency, of the environment of the anaesthetic—the condition of the patient. Although this may respond to and be improved by skilful preoperative treatment, it may be something over which the anaesthetist will often have no final control.

Preventability

If it is accepted that lack of skill on the part of the anaesthetist is the major cause of death attributable to anaesthesia, it follows that such deaths are *preventable*. To say this is not to attribute blame; the implication is simply that on review (albeit with the benefit of hindsight) a reasonably satisfactory explanation of the circumstances can be found and countermeasures that could prevent recurrence of the situation are practicable (Morton, 1958).

The unavoidable

Not all deaths attributable to anaesthesia are preventable. There will always be those which involve mechanisms not adequately understood, covert pharmacogenetic states or perhaps unforseen technical or apparatus failure over which the anaesthetist has no control. A difficulty here is that, with advance in knowledge and accumulation of experience, complications that may be judged unavoidable today come to be regarded as due to preventable error tomorrow, e.g. the death from fulminant liver failure following anaesthesia with fluroxene of a patient on microsomal enzyme inducing medication or renal failure following methoxyflurane administration.

HOW SAFE IS ANAESTHESIA?

While it is to be admitted that there must be a considerable subjective element in the final assessment of the culpability of the three variables that bear on anaesthesia-associated mortality—the patient's disease, surgery and conduct of anaesthesia—this type of review is the only means by which some measure of the safety of anaesthesia and an audit of the efficacy of our service to the patient is gained. Sadly, any but the very broadest of statistical comparisons between studies is rendered meaningless by the lack of uniformity in the classification and

assessment criteria used and perioperative period and type of surgery included in anaesthesia mortality surveillance studies published to date (Harrison, 1968; Goldstein & Keats, 1970).

It is to be hoped that in the future greater uniformity and compatibility between studies will emerge following the work of the 1984 Royal Society of Medicine Foundation/Harvard University Symposium on 'Preventable Anaesthesia Mortality and Morbidity' which sought to construct a universal thesaurus of definitions and classifications for anaesthetic-associated mortality and morbidity.

Because of the many difficulties involved, relatively few epidemiological studies of anaesthetic-associated mortality of any magnitude have been published during the last four decades and the relevance of any but those of the last decade is seriously called into question by the constantly changing patterns of practice in response to continued technological advance.

Two recently published studies of anaesthetic-associated mortality of similar structure and which individually do allow some comparisons within and between studies over a number of years are of interest. These are the studies of Bodlander (1975) and Harrison (1978). Bodlander reviewed the anaesthetic-associated mortality at the Prince Alfred Hospital, Sydney for 10 years (1963–1972) as a follow-up to the study by Clifton & Hotten (1963) of the previous 10-year period (1952–1962) at the same hospital. Harrison reviewed the anaesthetic-associated mortality at Groote Schuur Hospital, Cape Town for the 10 years from 1967–1976 as a follow-up of his own reivew of the previous 10 years at the same hospital (Harrison, 1968). For further comparison, as yet unpublished data from

Table 1 Incidence of death attributable to anaesthesia

Place Author Year of publication	Years studied and No. of anaesthetics	*ACD incidence per 1000 anaesthetics
Sydney		
Clifton & Hotton (1963)	1952–62 205640	0.17
Bodlander (1975)	1963–72 211130	0.07
Cape Town		
Harrison (1968)	1956–66 177928	0.33
Harrison (1978)	1967–76 240483	0.22
Harrison (unpublished)	1977–82 195164	0.12

Studies included deaths occurring during or within 24 hours of anaesthetic or failure to regain consciousness
*ACD = Anaesthetic contributory death
Anaesthesia sole or major contributory cause

the Groote Schuur Hospital study for the subsequent 6-year period (1977–1982) is also given (Table 1).

These studies estimated the incidence of death attributable solely to anaesthesia to range from 0.07 to 0.22 deaths per 1000 anaesthetics. Not only is the computed rate low but, encouragingly, there is evidence from comparison between and within studies that this rate has fallen with time.

Four other recently published studies, although they lack the uniformity of criteria and classification of the former two, support a similar conclusion (Table 2).

Table 2 Incidence of death attributable to anaesthesia.

Place Author Year of publication	Years studied and No. of anaesthetics	*ACD incidence per 1000 anaesthetics	Perioperative period included
Great Britain			
Lunn & Mushin (1982)	1979–80 1147362	0.1	≷ 6 days
Finland			
Hovi-Viander (1980)	1975 338934	0.2	≷ 3 days
Canada			
Turnbull *et al.* (1980)	1973–77 195232	0.18	≷ 2 days
Australia			
Anaesthetic Mortality Committee (1976)	1969–74 465000	0.08	≷ 1 day

*ACD = Anaesthetic contributory death
anaesthesia sole or major contributory cause

In a class of its own, not least for size and breadth of coverage, is the comprehensive study in England by Lunn & Mushin (1982) sponsored by the Association of Anaesthetists of Great Britain and Ireland. Deriving from a background surgical population of 1.1 million, this is the largest study of anaesthetic-associated mortality ever reported other than that by Kok & Mullan (1969) in South Africa which covered 1.7 million anaesthetics. The other studies are those of the Anaesthetic Mortality Committee (1976) in Australia, Hovi-Viander (1980) in Finland and Turnbull *et al* (1980) in Canada. The incidence of death attributable to anaesthesia computed from these studies matches that reported by Bodlander (1975) and Harrison (1978) (Table 1), ranging from 0.08 to 0.2 deaths per 1000 anaesthetics.

It must be remembered that these estimates of the incidence of death attributable to anaesthesia were computed in mainstream first world hospitals. How well they represent the situation in the vast rural practice of medicine in those countries let alone the circumstances in third world countries is completely unknown. *A priori*, it is probable that the rates

computed represent what must be the minimum of the global range.

Some implications

One implication of such a rate (1–2 deaths attributable to anaesthesia per 10 000 anaesthetics) is that the average anaesthetist would be expected to encounter only one such death every 5–10 years of his working life. In terms of human frailty, it may be wondered whether it is possible for such a rate to be further reduced. On the other hand, although the rate of anaesthetic contributory death may be considered not only acceptably low but perhaps at an irreducible minimum, it means also that every year, in a country the size of the United States, something of the order of 2000 patients die *because* they are anaesthetized (Epstein, 1978). (One in every 20–25 persons in a population is submitted to anaesthesia every year). Looked at in this light, death attributable solely to anaesthesia and anaesthetic misadventure must continue to be a matter of concern.

A further implication to be considered is the anaesthetic contribution to surgical mortality. As anaesthesia is an adjunct to the total care of the surgical patient, a relevant context in which to consider deaths attributable to anaesthesia is the total background surgical mortality i.e. the operative and post-operative hospital stay mortality. Although not presented in most anaesthesia mortality surveillance studies, such data are recorded in the studies from Groote Schuur Hospital (Harrison, 1968, 1978). From 1956 to the present, anaesthesia and its mismanagement was held responsible for 2.9 reducing to 1.8% of total surgical mortality. Lunn & Mushin (1982) in their study in Great Britain included data and deaths up to 6 days postoperatively. In this study, anaesthesia and its mismanagement were considered responsible for 1.5 per cent of the total surgical mortality and contributory in some way to almost one in 20 (4.5%).

Prediction of risk

It must be remembered that, while these studies audit how one *has* performed, as a predictive measure of risk they are of little use. In this regard one can do no better than to quote Goldstein & Keats (1970) that—'The poor predictability of anaesthetic mortality should be expected since a significant portion of this mortality is due to human error which cannot be predicted and to other factors which have never been quantified. To a small degree, physical status and the operation contemplated provide some predictive basis. To a large degree unknown factors related to the *skill of the personnel* and the *environment*

of the therapy contribute to anaesthetic risk. Estimates of anaesthetic risk for individual patients remain therefore almost entirely intuitive and one cannot deny an anaesthetic to any patient who requires operation'.

POINTS OF BREAKDOWN— IDENTIFICATION AND PREVENTION

Having identified the deaths attributable to anaesthesia it is necessary to establish the point of breakdown in the anaesthetist's control of the patient's physiological homeostasis and its pathogenesis and thereafter to assess the aspect of preventability. In this way patterns of anaesthetic mortality and the hazard situations that spawn them come to be recognized. Death as a complication of anaesthesia follows functional failure of the respiratory and circulatory systems, which are interrelated, and against this background (Fig. 3) it is necessary

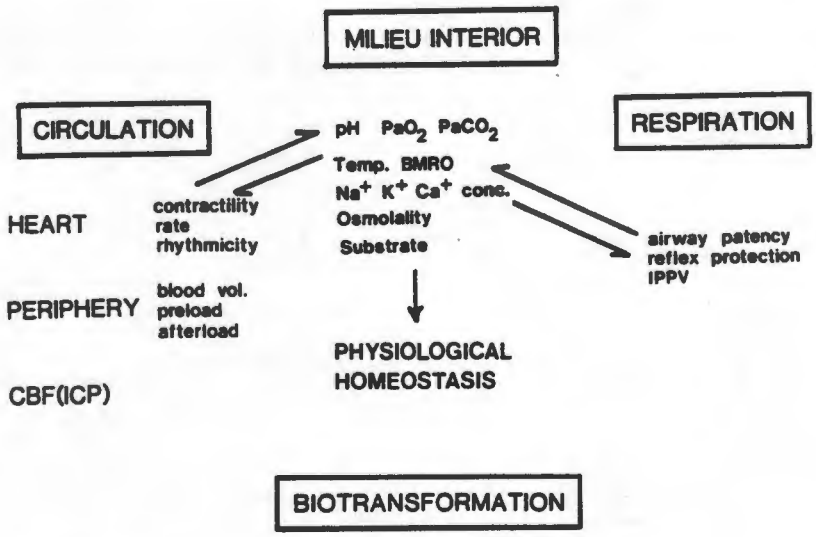


Fig. 3 Interrelated systems in which anaesthetic effects modify control of physiological homeostasis.
CBF (ICP) = cerebral blood flow, intracranial pressure
BMRO = basal metabolic rate for oxygen
IPPV = intermittent positive pressure ventilation.

to view in each case the known ways in which the actions, but more often side-actions, of anaesthetic drugs and the techniques associated with their use may cause complications in the context of the environment of the anaesthetic (Fig. 4).

The interaction between the anaesthetist, his patient and apparatus is complex (Fig. 5) (Harrison, 1983). Each interactive step is a possible locus of failure. It cannot be emphasized too strongly in anaesthetic training programmes that deaths attributable to anaesthesia do not 'just happen'. Invariably there are warning signs. While in the past the early detection of such signs may have required the skill of the very astute clinician, today they are rendered obvious by routinely

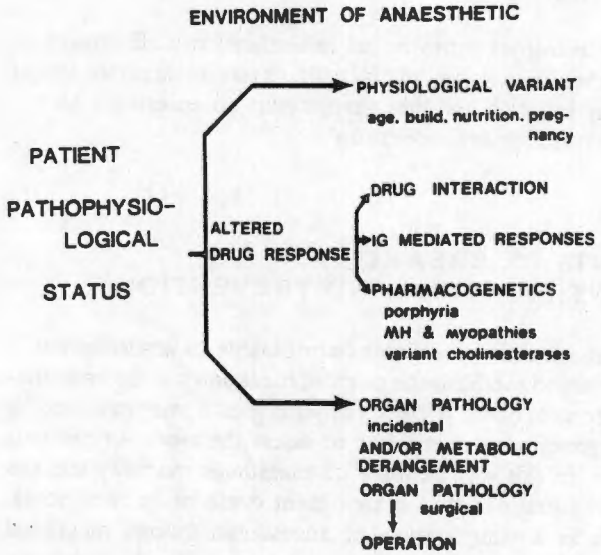
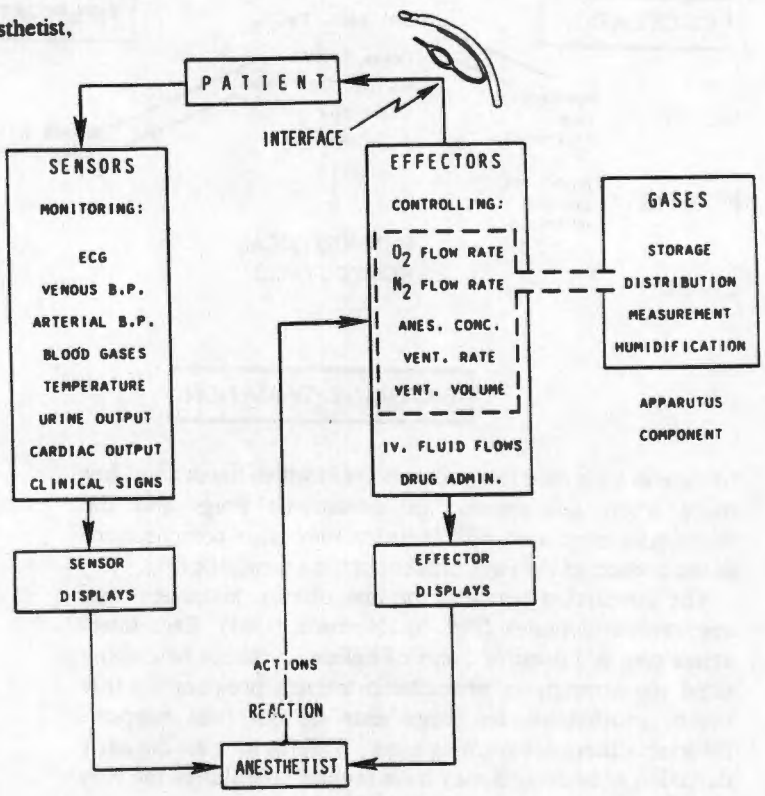


Fig. 4 G = immune globulin
MH = malignant hyptherma.

available vital function monitoring apparatus. No longer is there any excuse for guessing. Timely effective reaction to such signs will prevent tragedy.

Having said all this, we come once again to the sticking points of nomenclature. The lack of comparability between

Fig. 5 Interaction of anaesthetist, apparatus and patient.



published mortality studies which frustrates attempts at establishing a rate of death attributable to anaesthesia applies with equal, if not greater, force to identification and classification of common patterns of anaesthetic related death and the environmental and risk situations that engender them. The following two examples illustrate this problem.

Consider the circumstances when, during anaesthesia, respiratory obstruction develops suddenly because herniation of the endotracheal tube cuff totally obstructs its outlet. Death ensues from hypoxic cardiac arrest before the anaesthetist diagnoses or deals with the situation. In various surveys such an incident has been classified under the following categories:

- technical failure
- equipment failure
- respiratory obstruction
- complication of intubation
- cardiac arrest.

What of the circumstances of the demise of a gravely ill, dehydrated patient shortly after induction of anaesthesia with thiopentone? This will be found classified variously as being due to:

- drug overdose
- wrong or inappropriate drug
- cardiac arrest
- preoperative hypovolaemia or inadequate preparation
- patient's existing disease.

Reporting the findings of the New South Wales Special Committee for the investigation of death under anaesthesia, Holland (1970) noted an average of 4.3 errors per anaesthetic contributory death. Yet when classified for study and publication, most authors still record cases under a single diagnosis of error—continuing a situation so perceptively complained of by Treloar (1956) nearly 30 years ago: 'The causation of a given death is always multiple so that the necessity of selecting a single cause for the purpose of tabulation has become a distressing problem'.

I believe much of the confusion evident in the taxonomy and classification of events related to and causes of death attributable to anaesthesia would be avoided if such were considered in the context of the process of anaesthetic injury delineated by Cooper (1984) (Fig. 6). Occurrences and consequences are then clearly separated. Classification for such should then be designed on a broad basis in such a way that immediately suggests the strategies that would lead to prevention. A suggested classification is:

- Clinical failure—failure in any aspect of patient care directly under the control of the anaesthetist.
  (a) Errors of judgement—in matters of diagnosis, assessment of degree of difficulty and risk, clinical management.

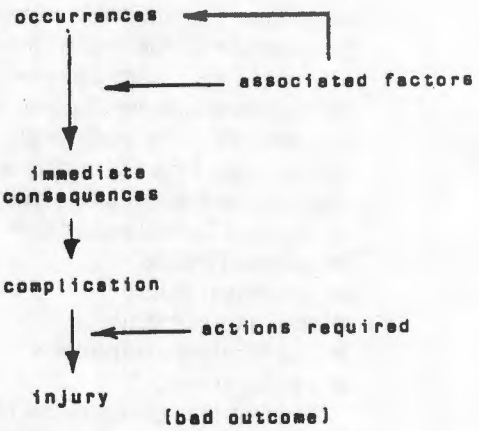


Fig. 6 Occurrence—an event that alone could lead to injury;
Critical incident—a preventable occurrence;
Associated factor—events or circumstances that would not alone cause injury, but could cause injury in conjunction with an occurrence;
Immediate consequence—the physical or physiologic change that results from an occurrence(s);
Complication—the effect of an occurrence(s) that must be treated or must correct spontaneously to avoid injury;
Action required—
Something that must be done to deal with immediate consequences or complications;
Anaesthetic injury—an undesirable result of anaesthetic management.

(b) Technical failure—factors in the performance of clinical techniques.
(c) Observational failure—lack of adequate monitoring.
(d) Unavoidable or fortuitous reaction/incident.

- Organizational failure—allocation of staff inappropriate for task
  —lack of specialist/consultant staff supervision of trainees
  —lack of adequate support facilities such as theatre nursing assistants, recovery room service, etc.
- Equipment failure—failure of any component of the system for storage, distribution and measurement of flow of respirable and anaesthetic gases, dispensing of anaesthetic vapours, the means of providing and measuring adequate pulmonary ventilation, etc.

THE COMMONER CAUSES

In spite of the tremendous variability in the classification and presentation of data in published anaesthetic mortality surveillance studies, certain clinical circumstances which seem to constitute the major hazard or accident situations from which the deaths attributable to anaesthesia arise can be delineated.

The endotracheal tube

Common to both mortality studies and 'critical indent' analyses are problems at the anaesthetic equipment/patient interface—

the endotracheal tube. Complications related to placement or misplacement of an endotracheal tube, or failure to place it, and obstructions due to kinking, cuff herniation, dislodgements and disconnections feature as a very frequent (in many studies the most frequent) cause of death and, indeed, critical incident.

A technique, peculiarly the stock-in-trade of the anaesthetist, has become his unconscious patient's Achilles' heel. The persistent reappearance in mortality studies of the endotracheal tube hazard emphasizes the priority that must be given to ensuring the acquisition of skills and experience-in-depth in endotracheal intubation techniques together with meticulous respiratory monitoring. Perhaps some attention to an old anaesthetic adage may also save life on occasion: 'When in doubt, pull it out, ventilate by mask'.

Neuromuscular blocking agents

Sadly that other stock-in-trade of today's anaesthetist, the muscle relaxant, is still held responsible for many deaths attributable to anaesthesia. These deaths feature as post-relaxant respiratory inadequacy, inadequate reversal, recurarization, respiratory insufficiency or inadequate postoperative care. Considering the now standard provision of postanaesthetic recovery facilities, not to mention intensive care units with expertise in prolonged ventilation, it is hard to understand why this should still be. In many cases the patient suffers from chronic obstructive airways disease, and the anaesthetist, inexperienced and feeling that putting the patient 'on a ventilator' postoperatively is a confession of failure, persists with the hope of permitting the patient to breathe spontaneously to his ultimate detriment. The lesson is obvious—when in doubt 'ventilate'!

Inadequate postoperative supervision

That 'inadequate postoperative supervision' is still held accountable for many anaesthetic related deaths surely needs little comment. While the provision of high intensity nursing care recovery areas within operating room precincts with ready access for the anaesthesiologist has now become standard in hospital design, the safety of the patient is still dependent entirely on the quality of that nursing care and vigilance—the human factor.

Vomiting and regurgitation—the full stomach

The 'full stomach', with its consequent risk of vomiting, regur-

gitation and aspiration, has long been recognized as causing extreme danger, especially in anaesthesia for obstetrical surgical procedures. Though this has not changed, it is gratifying to note that in contrast to so many other causes of death attributable to anaesthesia, some recent mortality surveys show a decreased incidence due to this cause (Dinnick, 1964; Harrison, 1978). While this may be due to increased awareness of the problem, better training and improved techniques, some would attribute it to the increased use of epidural anaesthesia and other regional techniques in obstetrics (Bodlander, 1975).

Circulatory homeostasis

In his control of the patient's circulatory homesostasis the greatest variable for the anaesthetist is the patient's incidental cardiac disease and circulatory status, and the manner in which these affect his reaction to anaesthetic drugs and the conditions imposed by surgery. Most important in this regard is ischaemic heart disease. Lunn & Mushin's study (1982) reveals that this was regarded as significant in 39% of patients submitted to autopsy. It is not pertinent to detail here the many and varied mechanisms and combinations of circumstance by which anaesthetic related factors might precipitate cardiac arrest and possibly death. But it is relevant to draw attention to the most common general circumstance or danger associated with cardiovasuclar deaths attributable to anaesthesia. This is the state of pre-anaesthetic hypovolaemia—often covert. Presenting often as profound 'post-induction hypotension' (a lethal circumstance in the presence of grave ischaemic heart disease) it may be listed as 'drug-induced cardiac arrest' for the simple reason that it is so frequently directly associated with the administration of thiopentone. The situation may also be regarded as 'inadequate preparation for surgery' (Holland, 1970). Under whatever guise, it is prominent in all recent mortality surveys as the situation with the highest anaesthetic accident potential. A less common but not infrequent circumstance is the reverse situation in which liberal fluid replacement *unmonitored* by CVP measurement results in overhydration which manifests as pulmonary oedema on re-establishment of spontaneous respiration at termination of the anaesthetic.

Apparatus technical failure

Considering the number of possible sources of hazard and accident in the system concerned with the supply of oxygen and inhalational anaesthetic to the patient (Feeley, 1976; Wyant, 1978), it is perhaps not surprising that in their 'critical incident' analyses both Cooper *et al* (1978a; 1984) and Craig & Wilson (1981) report equipment failure and technical mishap as the

major threat to the patient's safety. The most common failure was the 'disconnect'. The 'slip-on' so easily becomes the 'slip-off'. If we consider the multiplicity of connections that have evolved in the breathing circuit of today's anaesthetic machine, it is obvious that this is an area that cries out for systematic redesign (Cooper *et al*, 1978b). The redesign and incorporation of safety devices can help in reducing hazard, as evidenced by Cooper *et al*'s (1978a) report that in four of nine incidents of failure of oxygen supply in an anaesthetic machine equipped with a pressure fail safe system this was known to have been activated before the absence of oxygen flow was noticed by the anaesthetist. Surely the time has now come when the incorporation into the patient breathing circuit of such a fail safe pressure alarm device together with an oxygen analyser with low limit alarm should be mandatory in anaesthetic machine design.

Though in the past equipment and technical failure has rarely featured in mortality surveillance studies as a cause of death (Dinnick, 1973) such failure achieves much greater prominence in two more recently published surveys, that of cases referred to by the British Medical Defence Union (Utting *et al*, 1979) and Holland's study of anaesthesia-related mortality in Australia (1984). In the latter equipment failure, other than involving the endotracheal tube, was blamed for 17% of anaesthesia-related deaths.

Factors affecting performance

I have been at pains to stress that, on the whole, death attributable to anaesthesia does not result from accident but from human error and lack of skill. Recently, important contributions towards understanding such errors have been made by Cooper *et al* (1978a, 1984) and Craig & Wilson (1981) through their studies of human factors associated with performance error. Hurry, inattention, carelessness and fatigue are high up on their lists. All of these could doubtless lead to the 'failure to perform normal checks' and 'failure to assess the situation pre-operatively' also listed. Inadequate or substandard performance of the anaesthetist may reflect not so much his own failure but that of the organization, when this is responsible for the allocation of staff inappropriate for the task or failure to provide adequate specialist/consultant staff supervision of trainees.

The unavoidable

Our preoccupation with human error and failure in the causation of anaesthetic related death and morbidity should not blind us to the fact that over the last two decades, three new syndromes which may lead to mortality and morbidity have

been described following exposure to inhalational anaesthetics. These are the syndromes of anaesthetic induced malignant hyperthermia, nephropathy and hepatitis. All could once be regarded as anaesthetic accidents in that they were unpredictable, unavoidable and subject to no specific treatment. However, this is no longer so. Intense research has resulted in a specific therapy for the first together with a knowledge of its genetics; the identification and withdrawal from practice of the causative agent of the second; and while the third is still something of an enigma, volatile agents introduced into practice since halothane bid fair to have minimal, if any, hepatotoxic potential and may well supercede it in practice. In spite of all the attention paid to them, these syndromes find scant mention in mortality surveillance studies. 'Halothane hepatic failure' features only in the Medical Defence Union case study, being held responsible for 3.4% of deaths. Malignant hyperthermia, also reported in this study, is reported in two others—those of Lunn & Mushin (1982) in which three of 58 deaths are attributed to it and Holland's Australian study (1984) which includes seven cases in 20 years from five million anaesthetics.

CONCLUSION

Twenty-three years ago the Lancet opined in an annotation that '. . . the most obvious risks of an operation are those associated with anaesthesia' (Anon., 1962). I have presented evidence to show that the safety of anaesthesia for the patient has indeed increased. In many places the actual incidence of death attributable to anaesthesia seems to have decreased to a level which, having regard for human frailty, is perhaps an irreducible minimum—a circumstance which has provoked the warning from Cooper (1984) that 'the most insidious hazard of anaesthesia today is its relative safety'.

In spite of this, it is sobering to reflect that *actual* numbers of patients who die every year *because* they are anaesthetized are appreciable and that the basic causes in general have not changed with time but simply recur. The causes of these deaths, and of much morbidity, are by and large simple and usually follow the lack of observation of simple precautions and clinical alertness. Extension of surveillance studies to the stages of morbidity and critical incident (Fig. 1) in the process of breakdown to adverse outcome will not only refine the process of hazard identification and the formulation of the strategies of prevention, but will also serve to re-emphasize an aspect of clinical practice and training that seems in danger of being forgotten in our technological age: 'It is the hand that gives the anaesthetic and the eye that watches its effect that matter more than the choice of agent, dosage and method' (Simpson, 1953).

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PART 2.

THE PHARMACOGENETIC STATES OF CONCERN TO THE ANAESTHETIST.

The Malignant Hyperthermia Myopathy.

The Acute Porphyrias.

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7 *September* 1968, 3, 594-595

Hyperpyrexia During Anaesthesia

Summary: Work in pigs has shown that malignant hyperpyrexia during anaesthesia may occur without suxamethonium having been given. A virtually constant feature in reported cases and in our own observations is that all subjects developing hyperpyrexia had received nitrous oxide and halothane.

The baffling and frightening complication of malignant hyperpyrexia occurring during anaesthesia has been described in humans by many authors (Cullen, 1966 ; Davies and Graves, 1966 ; Hogg and Renwick, 1966 ; Lavoie, 1966 ; Relton *et al.*, 1966 ; Thut and Davenport, 1966 ; Purkis *et al.*, 1967) and in anaesthetized pigs by Hall *et al.* (1966). Most of these workers attribute this unpredictable, lethal, and puzzling reaction to suxamethonium, genetically determined. We wish to report six further instances of hyperpyrexia in the anaesthetized pig. No suxamethonium was administered to these pigs as part of the anaesthetic technique. Though of the same breed and from the same farm, these pigs were not siblings. The pigs, healthy 6- to 8-week-old Landrace of 35–40 kg. weight, were being used for experimental isolated liver perfusion and liver transplantation. The anaesthetic technique, the same in each case, was as follows:

Having been starved for 16 hours preoperatively, the pigs were anaesthetized by the inhalation of nitrous oxide, oxygen, and halothane administered by means of a Magill circuit and facepiece, the animals breathing spontaneously. No premedication was given. Nitrous oxide and oxygen were administered at flow rates of 6 litres and 3 litres per minute respectively, while halothane concentration was rapidly increased to 3% during the induction of anaesthesia. As soon as the pig lost consciousness (usually within three to five minutes, depending on the size of the pig) it was removed from the portable pen in which anaesthesia was induced and placed on the operating-table. Anaesthesia was deepened for a further three to five minutes, oral endotracheal intubation then being performed (not an easy task in the pig). A stomach tube was now passed. Shortly after this, monitoring of oesophageal temperature was begun. E.C.G. and arterial and venous pressure monitoring were instituted. Anaesthesia was

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maintained by the inhalation of nitrous oxide and oxygen, 6 litres and 3 litres per minute respectively, with 1–2% halothane vapour administered by an intermittent positive-pressure respiration technique utilizing a non-return system powered by an East-Freeman Autovent. The minute-volume of ventilation was 8 to 9 litres. Ambient temperature of the operating-theatre varied between 15 and 20° C. during the period of these experiments.

The six instances of malignant hyperpyrexia reported here occurred in a total of 34 pigs submitted to anaesthesia. Its occurrence appeared to be quite unpredictable. In each case the rise in temperature began about the time of or shortly after the induction of anaesthesia and was heralded by a blotchy blueness of the skin. (The skin of the normal

TABLE I.—*Temperature Changes (Degrees Centigrade)*

	Minutes after Induction						
	0	15	30	45	60	90	120
Pig 1	*—	—	—	—	44·0	43·4	—
Pig 2	*—	—	—	—	38·0	41·2	42·4
Pig 3	38·0	38·0	40·0	42·0	43·5	44·5	44·5
Pig 4	—	39·0	41·0	42·0	43·0	—	—
Pig 5	—	38·0	42·5	43·0	—	—	—
Pig 6	—	39·0	42·0	>45·5	45·5	45·0	45·0
Time of death				Pig 5 (45 min.)	Pig 4 (60 min.)	Pig 1 (105 min.)	Pigs 3 and 6 (120 min.) Pig 2 (135 min.)

* Early monitoring of temperature was undertaken only after our first experiences of the condition.

TABLE II.—*Acid-base Studies** (*Femoral Arterial Blood-Astrup Technique*)

		Minutes after Induction			
		30	60	90	120
Pig 1	pH	—	6·85	—	—
	P_{CO_2}	—	130	—	—
	B.E.	—	−16·8	—	—
Pig 2	pH	—	7·37	7·12	6·85
	P_{CO_2}	—	40	80	144
	B.E.	—	−1·5	−5·9	−19·0
Pig 3	pH	—	—	7·08	7·1
	P_{CO_2}	—	—	55	67
	B.E.	—	—	−8·4	−7·7
Pig 5. Mixed central venous blood	pH	6·65	—	—	—
	P_{CO_2}	90	—	—	—
	B.E.	> −22·0	—	—	—
Pig 6. Mixed central venous blood	pH	6·44	6·73	6·79	6·99
	P_{CO_2}	>150	>150	71	136
	B.E.	> −22	−4·0	+7·1	−1·5

* (*a*) Studies were begun after femoral arterial cannulation. Pig 4 succumbed before this event. Because of this an early mixed venous specimen was taken from Pig 5. (*b*) The values shown for Pig 3 reflect the acid-base state after the administration of 200 mEq of sodium bicarbonate. Between the 30-minute and 120-minute periods in Pig 6 a total of 375 mEq of sodium bicarbonate was given.

anaesthetized pig has a uniformly pink appearance.) The rise in temperature was rapidly progressive and was associated with a marked and ultimately fatal deterioration in the circulatory condition of the pig. *Pari passu* a gross metabolic and respiratory acidosis was evident. Whereas the limbs of the normal anaesthetized pig are relaxed during anaesthesia—even light anaesthesia—the limbs of the hyperpyrexial pigs were stiffly extended, the muscles in extreme spasm in a way similar to that described by Hall *et al.* (1966) in their pigs and by the Canadian workers (Cullen, 1966 ; Davies and Graves, 1966 ; Hogg and Renwick, 1966 ; Lavoie, 1966 ; Relton *et al.*, 1966 ; Thut and Davenport, 1966 ; Purkis *et al.*, 1967) in the hyperpyrexial humans following the suxamethonium they postulated as being responsible for this condition. We did not observe any marked change in pulmonary compliance. Cultures of blood taken from the first two pigs which displayed this syndrome were negative. In the last two pigs the administration of nitrous oxide and halothane was discontinued as soon as the condition became evident. From then on oxygen alone was administered by intermittent positive-pressure respiration. This made no difference whatsoever to the inexorable progress of the condition.

Details of time, temperature, and acid-base state are presented in Tables I and II.

Discussion

Malignant hyperpyrexia during anaesthesia has been described in both humans and pigs. A new aspect of this problem that emerges from our report is that it can occur in the absence of the use of suxamethonium, the drug on which other workers have focused attention. Examination of the recently published reports of malignant hyperpyrexia during anaesthesia shows that, with one exception, the common factors to all these cases and our own are the anaesthetic agents—a fluorinated hydrocarbon and nitrous oxide. The mechanism by which so immense an amount of heat is so rapidly produced poses a fascinating physiological problem. Does some disturbance of oxidative phosphorylation play a part (Snodgrass and Piras, 1966 ; Wilson *et al.*, 1966) ? Viguera and Conn (1967) failed to produce this picture of malignant hyperpyrexia in chickens, both by the production of muscle spasm and by dinitrophenol-induced uncoupling of oxidative phosphorylation. Even more fundamental than the problem of the mechanism of heat production is the question of the precise identity of the stimulus that initiates this veritable metabolic storm. Most important to the solution of this

problem is the salvaging of one of the pigs that react in this way so that we may see if the event is experimentally reproducible.

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ANAESTHETIC-INDUCED MALIGNANT HYPERPYREXIA AND A METHOD FOR ITS PREDICTION

BY

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SUMMARY

The clinical syndrome of anaesthetic-triggered malignant hyperpyrexia occurring in pigs is described. This condition in the pig is considered to be the same "explosive thermal idiosyncrasy" to general anaesthesia that is manifested by humans. The condition has a strong genetic factor occurring in 25 per cent of Landrace pigs used by us. Sex has no influence on its appearance. The clinical syndrome consists of: (1) tachycardia; (2) muscle rigor; (3) tachypnoea and hyperventilation progressing to apnoea; (4) blotchy cyanosis of the skin; (5) rapid, sustained and extreme rise in temperature; (6) gross acidosis. Prognosis, once the condition is well established, is extremely poor. All attempts at treatment have failed. Histological investigation has shown change in muscle only. The significance of this change is unknown. The muscle of affected pigs shows, in comparison to normal pigs, an abnormal fall in ATP content in response to incubation and to "in vitro" exposure to halothane. This reaction provides a method of predicting the development of the syndrome. Halothane, chloroform and suxamethonium have been identified as triggering agents. The mechanism of heat production is unknown.

Cases of malignant hyperpyrexia or fulminant hyperthermia occurring during the course of clinical general anaesthesia have been described with disturbingly increasing frequency over the last few years (Leading Article, 1968; Hawthorne, Richardson and Whitfield, 1968; Marx et al., 1968). The most frightening aspects of this condition are that:

(1) Mortality is in excess of 70 per cent.
(2) Occurring most frequently in young healthy patients undergoing relatively minor surgical procedures, its occurrence appears to be completely unpredictable.
(3) The stimulus, or trigger, appears to be directly related to the administration of a general anaesthetic.
(4) Because of the complete ignorance of pathogenesis, treatment is empirical, symptomatic and, in general, unsuccessful.

The discovery by Hall and associates (1966) and ourselves (1968) of what we have become convinced is exactly the same abnormal response to general anaesthesia—malignant hyperpyrexia—in the pig is of interest, for quite fortuitously we have been provided with an animal experimental model in which to investigate this frightening condition.

The pig is used by us as the experimental model in a research programme on liver transplantation and isolated liver perfusion. Three breeds of pig have been used: Landrace, Landrace/Large White Cross, and Large White. Pigs of each breed come to us from separate farms. The pigs are used between the ages of 6–10 weeks, weighing between 30 and 45 kg.

ANAESTHETIC TECHNIQUE

Two anaesthetic techniques which differed only in the drugs used were employed. Certain events and manoeuvres were common to both. These were:

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(a) Pre-anaesthetic starvation of 16 hours.
(b) Induction of anaesthesia.
(c) Orotracheal intubation with cuffed endotracheal tube.
(d) Maintenance of anaesthesia with nitrous oxide and oxygen administered by an IPPR non-rebreathing technique utilizing a Bird ventilator (Voss, 1967).
(e) Passage of oesophageal thermometer lead and large-bore stomach tube.
(f) Establishment of arterial and venous pressure and blood-gas monitoring by means of femoral artery and jugular venous catheterization.

The two techniques differed in the inclusion or omission of halothane.

Technique 1.

Halothane with nitrous oxide and oxygen was used both for induction and maintenance of anaesthesia. For induction 3 per cent halothane vapour was utilized, while for maintenance 0.5–2 per cent depending upon the demands of the surgery.

Technique 2.

Anaesthesia was induced with thiopentone sodium (5 per cent solution) administered by an ear vein. During maintenance of anaesthesia with nitrous oxide and oxygen supplemental doses of thiopentone, with in some cases the addition of 3–5 mg doses of tubocurarine, were administered.

In a group of 7 pigs which survived aborted episodes of malignant hyperpyrexia and were the subject of further specific experiment, e.g. identification of triggering agents, monitoring was first established under thiopentone-nitrous oxide-oxygen anaesthesia.

Incidence of malignant hyperpyrexia.

With the two anaesthetic techniques used, the syndrome of malignant hyperpyrexia occurred only in those pigs which were exposed to halothane. The incidence of occurrence of the syndrome in the three breeds of pig when anaesthetized with halothane (technique 1) is set out in table I.

Twenty-three pigs (Landrace 20, Landrace/Large White 3) were anaesthetized with thiopentone, nitrous oxide and oxygen (technique 2). None of these pigs developed the syndrome.

TABLE I
Incidence of malignant hyperpyrexia.

Breed	Total No.	Malignant hyperpyrexia	% incidence
Landrace	85	21	24.7
Landrace/Large White	59	2	3.4
Large White	16	0	0

"An explosive thermal idiosyncrasy" (Wilson et al., 1967) is present in response to exposure to halothane in one quarter of the pure Landrace pigs we have used. It is pertinent to note that the pigs in which Hall and co-workers (1966) found this trait in response to suxamethonium were of the Landrace/Wessex breed (Woolf, N., 1969, personal communication). The appearance of the syndrome in the cross-breed Landrace/Large White pigs we have used has been rare.

As in reported human cases, the sex of the animal had no influence on the appearance of the syndrome.

THE CLINICAL SYNDROME

The clinical syndrome in pigs develops with dramatic speed. The onset, with rare exception, occurs within minutes of exposure to halothane. The changes in physiological parameters are gross and all the manifestations appear almost simultaneously. These are:

(1) Tachycardia.
(2) Stiffness and hardening of the muscles.
(3) Tachypnoea and hyperventilation which rapidly progresses to apnoea.
(4) Blotchy cyanosis of the skin.
(5) A rapid sustained rise in temperature up to as much as 45°C.

Tachycardia.

With the onset of this syndrome a sinus tachycardia of 200–300 beats/min develops and is maintained until very shortly before the death of the animal. Arterial blood pressure is maintained for 30–60 minutes, thereafter declining progressively due to falling cardiac output. Terminally, ventricular arrhythmias, coupling, and finally gross bradycardia precede asystole. Asystole has occurred at varying periods from the commencement of the condition, the shortest being 10 minutes, the longest 165 minutes. Mean survival time was 106 minutes.

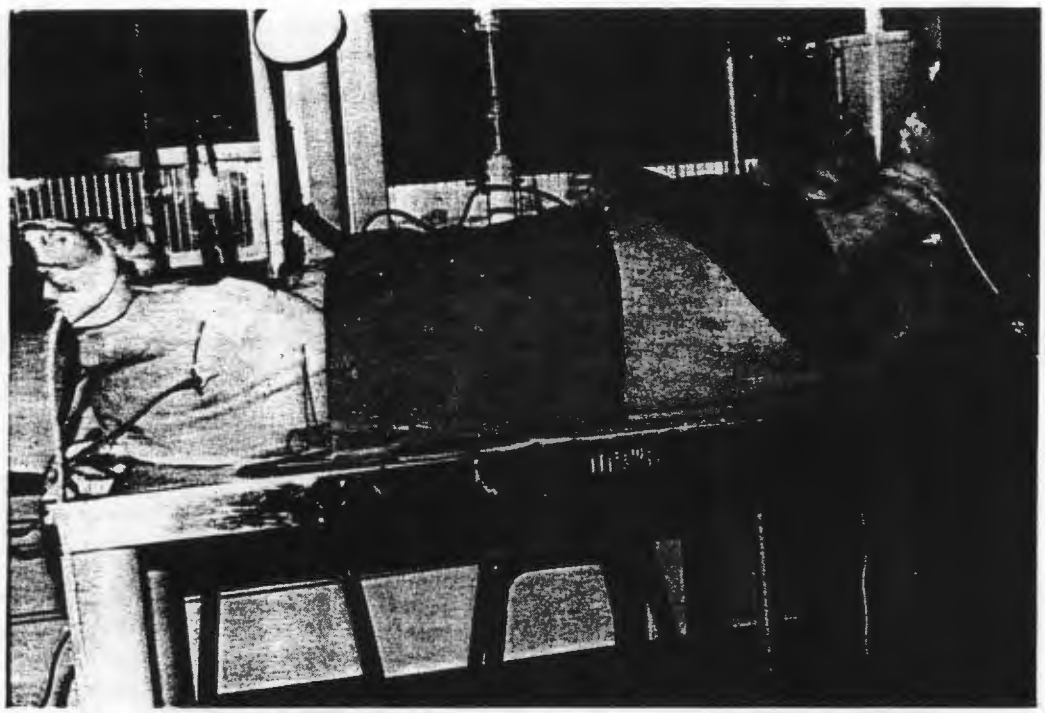


FIG. 1
Susceptible pig anaesthetized with thiopentone. Note relaxed hind limbs.

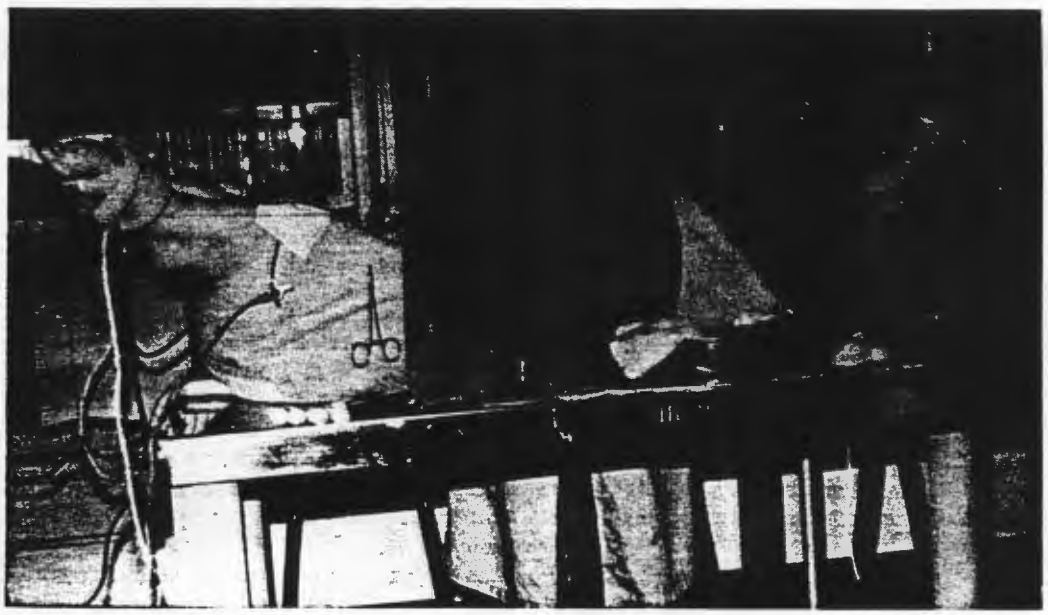


FIG. 2
Same pig as fig. 1, 4 minutes after administration of halothane. Note extension of hind limbs.

Muscle rigor.

Rigor of the muscles occurs rapidly. Though generalized, it is most obvious in the limbs which become rigidly extended, as in rigor mortis (figs. 1 and 2).

The administration of tubocurarine in doses of 6–10 mg has had no effect on this rigor, which is maintained until the death of the animal and beyond. Observation of animals up to 18 hours postmortem has revealed no softening or autolysis of the muscle rigidity.

Tachypnoea and hyperventilation.

Tachypnoea commences with the onset of the syndrome and is rapidly succeeded by apnoea. When attacks of the syndrome have been successfully aborted by the discontinuance of halothane within minutes of its introduction, before the onset of apnoea, the tachypnoea has persisted for up to 30–45 minutes. This tachypnoea is extreme with respiratory rates up to 125 b.p.m.—panting is a better description. In some survivors, if apnoea has ensued following discontinuance of the halothane, tachypnoea has resumed after a period of IPPR with oxygen. In these pigs, though exposure to halothane was extremely brief, deep coma has persisted until the tachypnoea has recommenced.

Blotchy cyanosis.

Cyanosis of the skin, described in some human cases, is a constant early feature in the pig. As measured arterial Po_2 at this stage is not reduced (vide infra) it indicates skin vasoconstriction, with areas of stagnant hypoxia. The skin soon becomes hot to the touch.

Rapid, sustained rise in temperature.

Rise in temperature is rapid and extreme. An example is presented in figure 3. In this experiment a pig which previously survived an aborted attack of the syndrome was re-challenged with halothane after anaesthesia had been induced with thiopentone and monitoring established. The first change in oesophageal temperature was recorded at 6 minutes, with a subsequent rise at a rate of 1°C every 7 minutes. Other examples are to be seen in figures 4 and 5. In these examples, the rate of rise was as fast as 1°C in 5 minutes at one stage. When unmodified by cooling, oesophageal temperatures reached 45° in some pigs before death; 43–44° was the common end point.

Acid base and blood-gas state.

Acidosis was profound and invariable with both gross metabolic and respiratory components; 15–20 minutes from the first signs of the syndrome the arterial acid base state has been typically: pH 6.8; $Pco_2 > 150$ mm Hg; BE < -22 m.equiv/l.

No primary respiratory reason for the gross hypercapnia—pulmonary, lesion or underventilation—was apparent in any animal. As soon as the syndrome commenced IPPR with oxygen was instituted with tidal volumes of 500 ml and minute ventilatory volumes of the order of 10 l./min. Clear air entry to both lungs was noted in all animals. This gross hypercapnia, with its probably accompanying high carbon dioxide output is paralleled in human cases in which carbon dioxide absorbers were observed to become rapidly very hot (Cullen, 1966; Davies and Graves, 1966; Hogg and Renwick, 1966).

Arterial oxygen tension values (Clarke electrode) were above normal in all animals, ranging from 145 mm Hg in pigs breathing 30 per cent nitrous oxide and oxygen to 400 mm Hg when IPPR with oxygen alone had been instituted. These values fell off later when there was evidence of progressive circulatory failure. Mixed venous blood always appeared extremely desaturated. Values observed ranged from 30 to 60 mm Hg.

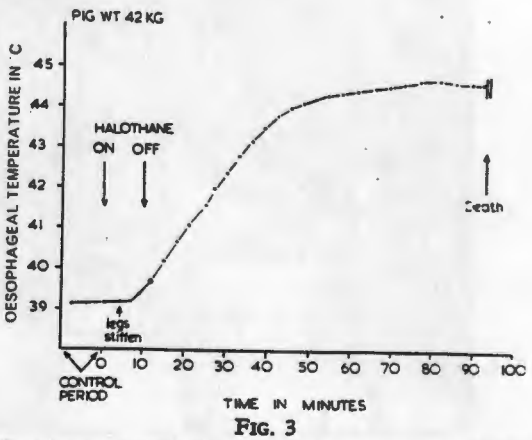


FIG. 3

Continuous record of oesophageal temperature during a control period of thiopentone anaesthesia followed by administration of halothane.

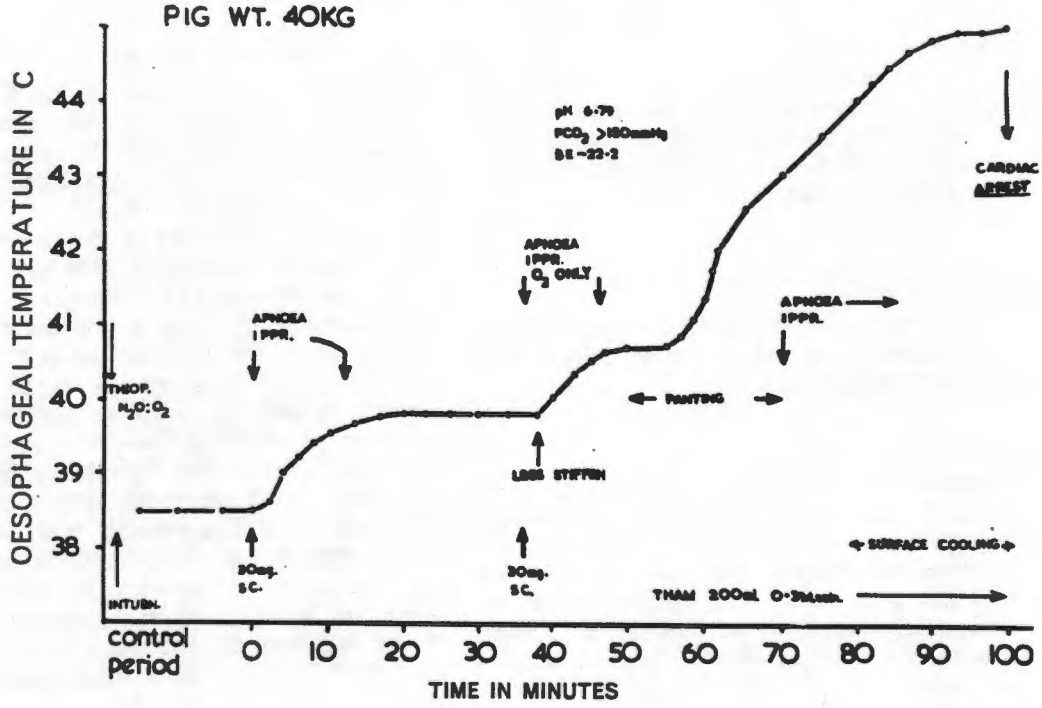


FIG. 4

Record of oesophageal temperature and other events during administration of suxamethonium to a susceptible pig anaesthetized with thiopentone.
Thiop.=thiopentone; S.C.=suxamethonium; Intubn.=intubation.

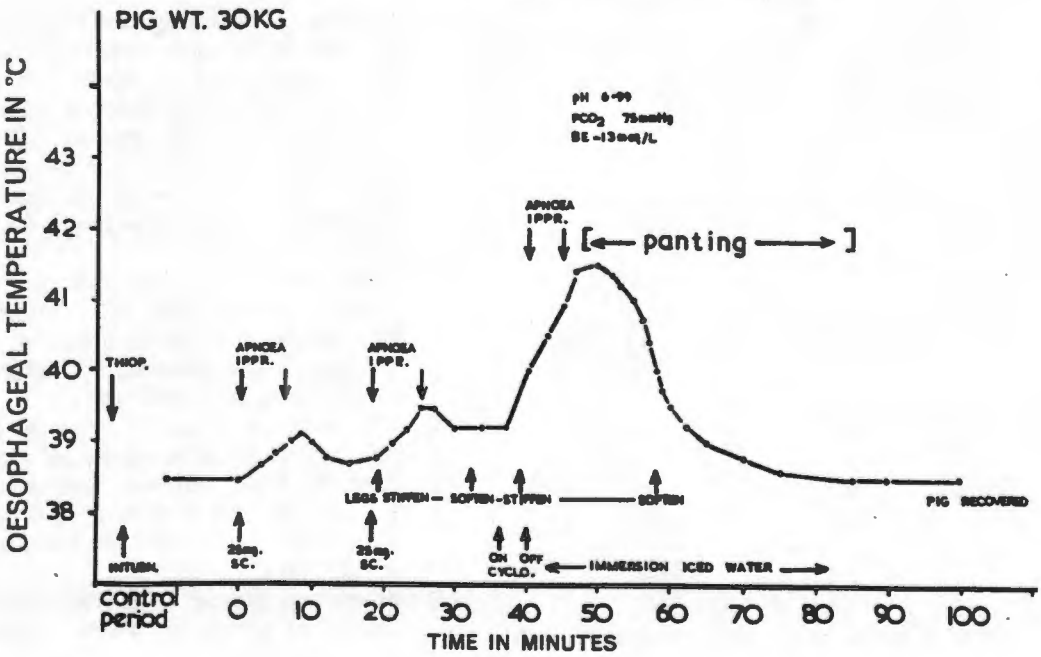


FIG. 5

Record of oesophageal temperature and other events during administration of suxamethonium and cyclopropane to a susceptible pig. Control period anaesthesia-thiopentone.
Thiop.=thiopentone; S.C.=suxamethonium; Cyclo.=cyclopropane; Intubn.=intubation.

Electrolyte changes.

Limited investigation of serum sodium and potassium changes has shown the development of a marked hyperkalaemia. Levels as high as 11–12 m.equiv/l., which could well have accounted for the ultimate cardiac asystole, were observed in some animals.

Blood cultures.

Blood from 2 pigs which died of the syndrome was submitted to bacteriological culture with negative results.

Histology.

Postmortem examination of pigs dying of malignant hyperpyrexia showed no macroscopic abnormalities. Tissue from 4 such pigs was examined histologically (Prof. C. J. Uys); sections of brain, liver, kidney and adrenals showed no changes of note. Only striated muscle showed demonstrable changes (fig. 7). In these sections the majority of fibres appeared normal. However, isolated fibres were shortened and shrunken. At their ends these fibres were separated from adjacent fibres, and in some instances they appeared to have ruptured transversely. These shortened fibres were more intensely eosinophilic than normal fibres. Similar fibres were observed in muscle sections from normal pigs, but in the affected pigs they were more frequent and readily observed.

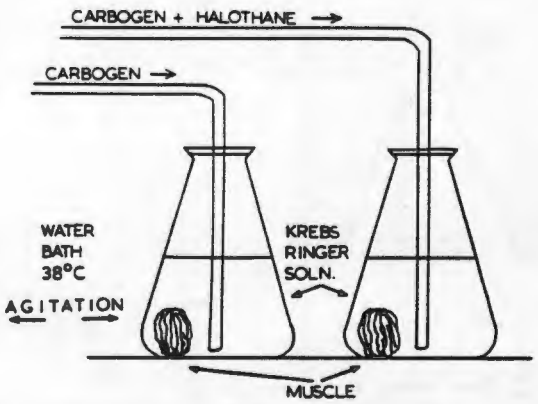


FIG. 6

Method of incubation and exposure of muscle to halothane "in vitro".

PROGNOSIS AND TREATMENT

As with human cases (Wilson et al., 1967), the prognosis in the pig, once the syndrome is established, is extremely poor. Ten minutes after exposure to halothane and commencement of the syndrome, discontinuance of this agent, IPPR with oxygen, active cooling, treatment of the acidosis, and other measures, have had no influence on the inexorably fatal outcome of the condition. Cooling has failed to prevent mortality, even on those few occasions when a drop in temperature was achieved. Doses of sodium bicarbonate, sufficient to correct base deficits, of the order of 40–50 m.equiv/l. of extracellular water administered over 30 minutes have had little effect in changing pH. If this has been shifted up at all, it has rapidly reverted to less than 7. Attempts at hyperventilation have met with no success in lowering the raised P_{CO_2}, boosted as it is, no doubt, by the infusion of sodium bicarbonate. Administration of up to 0.3M of THAM over 20–30 minutes has had as little success in terms of survival.

In the light of some unproven hypotheses of the biochemical lesion, we have administered the following drugs with complete lack of therapeutic effect:

(1) Adrenergic blocking agents: (a) propranolol; (b) propranolol and phentolamine. These drugs were given to block any possible adrenaline-induced or mediated rise in calorigenesis (Havel, 1968; Depocas, 1960; Hsieh, Carlson and Gray, 1957). Pretreatment with these drugs did not prevent the onset of the syndrome, nor did they control it once established.

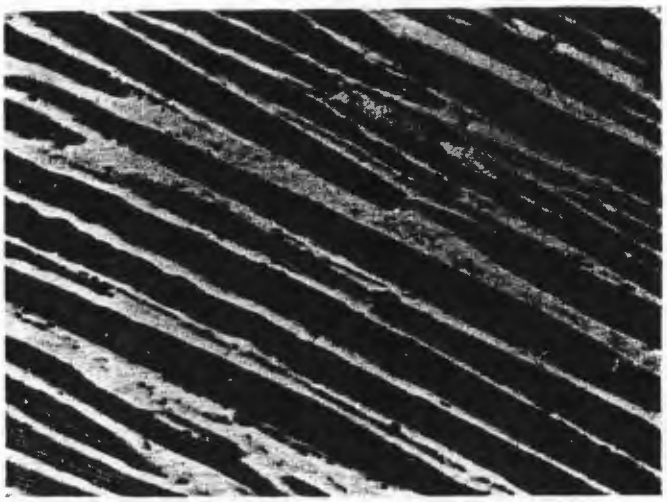
(2) Promethazine. This drug is known to have an "in vitro" antoxidant effect (Slater, 1968). Pretreatment with the drug did not prevent the onset of the syndrome nor did it control the condition once established.

(3) Magnesium sulphate. This drug has an inhibitory effect on mitochondrial respiration (Chance, 1959) and was used because of the suggested role of uncoupling of oxidative phosphorylation in this syndrome (Wilson et al., 1966).

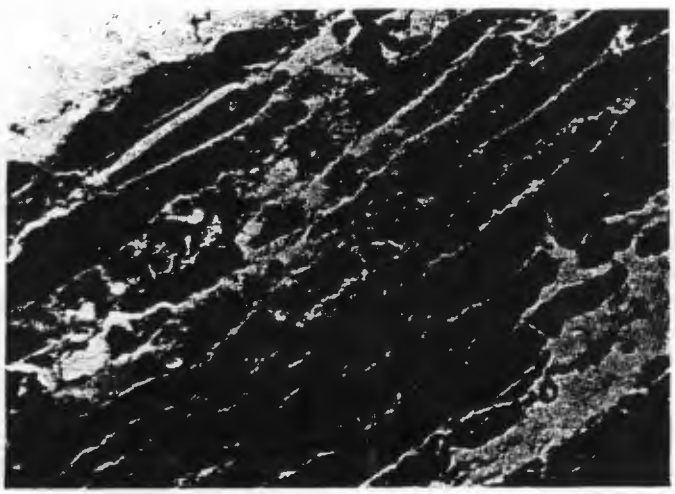
(4) Insulin and glucose. This combination was used in an attempt to combat the observed hyperkalaemia.

FIG. 7
Histological sections of striated muscle. H. & E. stain; magnification ×100.

(A) Normal pig.



(B) Muscle of susceptible pig under control thiopentone anaesthesia.



(C) Muscle biopsied during malignant hyperpyrexia following halothane anaesthesia.

(5) ATP. We observed a fall in muscle ATP during this syndrome (vide infra) and sought to replace it.

Prompt discontinuance of the agent at the earliest moment either of the three major signs appeared (tachypnoea, muscle rigor, tachycardia) left us with 7 initial survivors in 23 examples of this syndrome. These survivors had no treatment. Their survival depended entirely on extremely early diagnosis with discontinuance of halothane within a matter of minutes. In that survival only occurred in cases in which the triggering stimulus —halothane—was discontinued at so early a stage that the syndrome was not properly established, identification of the syndrome could be called in question. However, all survivors developed the full-blown syndrome fatally on a subsequent occasion when re-challenged with halothane or other triggering agent.

THE TRIGGERING AGENT

All 23 instances we have experienced of this syndrome have followed a first exposure to halothane. In the 7 survivors of aborted attacks we have tested other possible triggering agents.

The anaesthetic techniques used in the reported human cases have, in each case, involved multiple agents. These have included thiopentone, halothane, methoxyflurane, cyclopropane, ether, nitrous oxide, and the relaxants suxamethonium and tubocurarine.

We have challenged known susceptible pigs with all these agents individually. Because of the apparent importance of the halogenated hydrocarbon anaesthetic agents, we have tested, in addition, trichloroethylene and chloroform. In the case of a negative response, anaesthesia, temperature and blood-gas monitoring were continued for 1 hour.

A negative response followed administration of thiopentone, nitrous oxide, ether, trichloroethylene, methoxyflurane and tubocurarine. As thiopentone was shown neither to prevent the onset of the syndrome nor to influence its progress once established, monitoring for subsequent specific experiments in survivors was first established under anaesthesia with this agent.

In an experiment complementary to that in which adrenergic blocking agents were administered (vide supra) we challenged a susceptible pig with an intravenous infusion of adrenaline 1:100,000. The syndrome was not triggered.

Positive triggering of malignant hyperpyrexia followed administration of halothane (our standard triggering agent), chloroform, suxamethonium (see fig. 4) and possibly cyclopropane.

The muscle fasciculations that followed administration of suxamethonium were, as in the reported human cases, extremely coarse and tonic, causing the legs to be rigidly extended. The muscle fasciculations that followed the second dose of suxamethonium were the most intense.

A question-mark must be left next to cyclopropane. This drug was tested in a known susceptible pig only after two test doses of suxamethonium had been given (see fig. 5). Malignant hyperpyrexia commenced the moment cyclopropane was administered. But subsequently we demonstrated that fulminant hyperthermia could follow the administration of suxamethonium alone.

AN ASPECT OF MUSCLE FUNCTION

One of the most obvious clinical manifestations of this condition is muscle stiffness, reminiscent of rigor mortis. Szent-Gyorgyi (1944) in his classical studies on muscle concludes that ". . . rigor and insolubility of the actinomyosin are the different consequences of one and the same condition—a lack of adenosine-triphosphate (ATP)". This, and the observation that muscle taken from one of our pigs during a fatal episode of malignant hyperpyrexia showed a low ATP content, led us to study the concentration of ATP in the muscle of susceptible pigs and its behaviour "in vitro" to exposure to halothane.

Twelve Landrace pigs were submitted under thiopentone anaesthesia to muscle biopsy. Two biopsies of approximately 2 g each were taken of the gluteal muscle. Each piece of muscle was immediately divided into three aliquots which were blotted, weighed and then treated as follows.

(1) One aliquot was immediately frozen in liquid nitrogen.

(2) A second aliquot was incubated for 30 minutes at 38°C in 15 ml of Krebs-Ringer solution through which carbogen (oxygen 95 per cent, carbon dioxide 5 per cent) was bubbled (see fig. 6). Thereafter it was frozen in liquid nitrogen.

(3) A third aliquot was treated exactly as the second except for the addition to the carbogen of

4 per cent halothane vapour. This resulted in concentrations of halothane in the solution which ranged between 19 and 29 mg/100 ml. Concentrations of halothane in the solution were estimated by gas chromatography (Gadsden, Risinger and Bagwell, 1965).

Following extraction of the muscle in TCA, the concentration of ATP in mM/g of muscle was now measured in each aliquot using a kit supplied by Boehringer (Kit Ref. CAT. No. 15979TWAC). The aliquot duplicates were then averaged to give the concentration of ATP, (i) in fresh muscle, (ii) after incubation without halothane, (iii) after incubation with halothane.

Following muscle biopsy the pig was allowed to recover consciousness and was returned to its pen. Two days after the muscle biopsy each pig was anaesthetized with halothane, nitrous oxide and oxygen, and its reaction was observed.

RESULTS

Of 12 pigs used, 7 showed no abnormal response to halothane, whilst 5 developed malignant hyperpyrexia. The concentrations of ATP measured in muscle are presented in table II, grouped in relation to the pig's subsequent response to the exhibition of halothane.

The concentration of ATP in fresh muscle was the same in both groups of pigs. Incubation of the muscle with and without halothane caused a fall-off in ATP concentration. In these circumstances, but especially after halothane, fall-off in ATP concentration was greater in muscle from pigs with the hyperpyrexia trait. The constancy of this reaction permitted prediction of the pig's ultimate response to halothane.

DISCUSSION

Anaesthetic-triggered malignant hyperpyrexia poses a nightmare situation for the clinical anaesthetist. We believe that certain susceptible pigs of the Landrace strain suffer precisely the same "explosive thermal idiosyncrasy" to anaesthetic agents as humans and provide an important, if fortuitous, experimental model for the investigation of this condition.

From our experience, it would appear that the one hope for the patient exhibiting this abnormal response lies in its very early recognition, allowing discontinuance of the triggering stimulus. Early signs which presage the onset of the condition are:

(1) Tachycardia.
(2) An abnormal response to suxamethonium such as coarse fasciculations or sustained muscular contraction.
(3) Abnormal muscle tone that does not respond to reasonable doses of tubocurarine.
(4) Tachypnoea and hyperventilation.
(5) Blotchy general skin cyanosis.
(6) A rapid rise in temperature.

Two accompaniments of this syndrome have been identified: (a) gross acidosis; (b) a fall in muscle ATP.

TABLE II

ATP concentration in muscle.

	ATP concentrations in mM/g			% Fall-off in ATP concentrations	
	Fresh muscle	30 min incubation	30 min incub. + halothane	Fresh—30 min incubation	Fresh—30 min incubation + halothane
Normal pigs n=7 Mean ± SD	3.68 ± 0.30	2.27 ± 0.33	2.22 ± 0.43	38 ± 16	39 ± 15
"Hot" pigs n=5 Mean ± SD	3.29 ± 0.36	1.24 ± 0.41	0.82 ± 0.38	61 ± 14	75 ± 12
Significance of difference between similar means (Student's *t* test)	0.05<P<0.10 Not significant	P<0.01	P<0.001	P<0.05	P<0.002

(a) *Acidosis.*

The acidosis which occurs in the presence of an above normal arterial Po_2 is characterized by a rapid fall in base and rise in Pco_2. The former has recently been shown to be due to a severe rapidly developing lactacidosis, and in the absence of any obvious respiratory cause, the latter is assumed to result from buffering of this acid load (Berman et al., 1969). Whether this is the primary result of the heat-producing mechanism, a secondary or coincidental occurrence, remains to be investigated. The high Pco_2 recorded does serve to explain two clinical observations: (1) hyperventilation progressing to apnoea with the reappearance of hyperventilation should the animal survive; (2) persistent unconsciousness of the animal long after the anaesthetic agent has been discontinued and while the animal is being artificially respired with oxygen only. The former is the normal respiratory response to hypercarbia while the latter is probably carbon dioxide narcosis.

Though we have had no success with it in the pig, it would appear reasonable, from the above observations, to use THAM rather than sodium bicarbonate in treating this acidosis.

(b) *The fall in muscle ATP.*

In vitro study of pig muscle showed a greater fall-off in ATP concentration in affected pigs after simple incubation as well as an even greater fall-off after exposure to halothane. This may indicate that not only is the response of these pigs to halothane abnormal, but that the muscle cells have an even more basic abnormality. The fall in ATP may be due to either inhibition of mitochondrial respiration (Fink and Kenny, 1968), or perhaps to uncoupling of oxidative phosphorylation (Snodgrass and Piras, 1966; Wilson et al., 1966) or perhaps to the triggering of energy formation via a non-phosphorylating pathway (Challoner, 1966). Whatever its genesis, the fall in ATP content may be an explanation of the rigor-mortis-like rigidity of the muscle (Szent-Gyorgyi, 1944). Though we are ignorant of its theoretical implications this reaction does have a useful practical application. It provides us with a method of predicting which pig will develop malignant hyperpyrexia when exposed to halothane.

The identity of the triggering mechanism remains an enigma. First, some genetic factor appears necessary to provide the environment in which the triggering substances can act. In our pigs, and those of Hall and associates (1966), this genetic factor appears to be associated with the Landrace breed. A genetic factor has been incriminated in reports of human cases involving near relatives (Barlow, 1968; Britt, Lochner and Kalow, 1969; Denborough et al., 1962; Purkis et al., 1967). Given this genetic environment, there appears to be no single triggering agent. We have identified halothane, chloroform and suxamethonium. There are probably others. Though a comforting molecular similarity may be seen between halothane and chloroform, the intrusion of the chemically dissimilar suxamethonium is bewildering. This seems to rule out any direct chemical triggering mechanism, leaving us the likelihood of some biophysical phenomenon (Allison and Nunn, 1968).

One thing is certain. If by some quirk of fate Raventós (1956) had used Landrace pigs instead rats, mice, dogs, cats and monkeys on which to test his new-found anaesthetic, halothane, it would never have emerged from the laboratory to become the most widely used anaesthetic in the world. For who would dare suggest the clinical use of an agent that killed a quarter of those animals exposed to it?

ACKNOWLEDGEMENTS

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We acknowledge also our indebtedness to Professor A. B. Bull, Head of the Department of Anaesthetics, for figures 1 and 2 and much helpful, constructive criticism; the Department of Chemical Pathology for serum electrolyte estimations, and Dr. Berman of this department for figure 3; the Department of Pathology for histological sections and Professor C. J. Uys of this department for figure 7; S. Wicht, L. Frith and A. Munroe for technical assistance and the J. S. Marais Laboratory for Surgical Research (Director, Professor C. N. Barnard) for the facilities placed at our disposal.

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HYPERPYREXIE MALIGNE INDUITE PAR L'ANESTHESIQUE ET UNE METHODE POUR LA PREVOIR

SOMMAIRE

Le syndrome clinique d'une hyperpyrexie maligne déclenchée par l'anesthésique et se produidant chez des cochons, est décrit. Cet état chez le cochon est considéré comme identique à "l'idiosyncrasie thermique explosive" après anesthésie générale, manifestée par l'homme. Il y a un puissant facteur génétique dans cet état, qui s'est produit chez 25 pourcent des cochons Landrace que nous avons utilisés. Le sexe n'influence pas son apparition. Le syndrome clinique consiste en (1) tachycardie, (2) rigor de la musculature, (3) tachypnée et hyperventilation, progressant vers l'apnée, (4) cyanose en taches de la peau, (5) augmentation rapide, soutenue et extrême de la température et (6) acidose généralisée. Le pronostic est très négatif, dès que l'état s'est bien établi. Tout essai de traitement a échoué. L'étude histologique n'a montré que des modifications dans les muscles. La signification de ces modifications est inconnue. La musculature des cochons atteints présente, comparativement aux cochons sains, une réduction anormale du taux d'ATP par réaction à l'intubation et la soumission "in vitro" à l'halothane. Cette réaction est une méthode permettant de prévoir le développement du syndrome. L'halothane, le chloroforme et le suxaméthonium ont été identifiés comme agents déclencheurs. Le mécanisme de production de chaleur est inconnu.

DURCH NARKOTIKA INDUZIERTE MALIGNE HYPERPYREXIE UND EINE METHODE ZU IHRER VORAUSSAGE

ZUSAMMENFASSUNG

Das klinische bei Schweinen auftretende Syndrom einer durch Narkotika ausgelösten malignen Hyperpyrexie wird beschrieben. Dieser Zustand bei den Schweinen wird als die gleiche "explosive thermale Idiosynkrasie" auf eine allgemeine Betäubung ange-

sehen, wie sie bei Menschen manifest wird. Das Krankheitsbild wird durch einen starken genetischen Faktor bestimmt, der bei 25 Prozent der von uns verwendeten Landrace-Schweine vorhanden ist. Das klinische Syndrom besteht aus: (1) Tachykardie, (2) Muskelrigor, (3) Tachypnoe und Hyperventilation, die in Apnoe übergehen, (4) fleckige Zyanose der Haut, (5) rapider, anhaltender und extremer Temperaturanstieg, (6) stark ausgeprägte Azidose. Wenn das Krankheitsbild sich erst einmal voll entwickelt hat, ist die Prognose außerordentlich schlecht. Alle Behandlungsversuche haben versagt. Eine histologische Untersuchung hat als einzigen Befund eine Veränderung im Muskel ergeben. Die Bedeutung dieser Veränderung ist unbekannt. Im Vergleich mit gesunden Schweinen zeigt sich im Muskelgewebe der betroffenen Schweine als Reaktion auf eine Inkubation und Halothan-Exposition "in vitro" ein abnormes Absinken des ATP-Gehaltes. Diese Reaktion stellt eine Methode dar, mit deren Hilfe man die Entwicklung des Syndroms voraussagen kann. Halothan, Chloroform und Suxamethonium sind als auslösende Agentien identifiziert worden. Der Mechanismus der Wärmeproduktion ist unbekannt.

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Harrison G G, Saunders S J, Biebuyck J F, Hickman R, Dent D M, Weaver V & Terblanche J. Anaesthetic-induced malignant hyperpyrexia and a method for its prediction. *Brit. J. Anaesth.* 41:844-55, 1969.
[Liver Research Group and Departments of Anaesthetics, Medicine, and Surgery, University of Cape Town, South Africa]

The clinical syndrome of anaesthetic-triggered malignant hyperpyrexia (MH) in pigs is described. It is considered to be the same as MH in humans and is likewise genetic in origin. Clinical features are accompanied by profound acidosis. Histological change is found in muscle only. *In vitro*, an abnormal depletion of muscle ATP is observed. Halothane, chloroform, and suxamethonium are identified as triggering agents. [The *SCI*® indicates that this paper has been cited in over 145 publications.]

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January 8, 1987

Of all the complications of general anaesthesia, perhaps the most dramatic, and indeed frightening, is that veritable metabolic storm that characterizes the unexpected, and in the past frequently fatal, syndrome of anaesthetic-induced malignant hyperthermia (MH). First described in 1960,[1] and definitively characterized through case reports over the next six years,[2] this syndrome became the nightmare of the anaesthetist. Rare and sporadic, its occurrence unpredictable, the syndrome boasted a mortality of 70-80 percent. Knowledge of the pathogenesis was lacking, and no specific therapy was available.

As on so many other occasions in the long history of medicine, serendipity now provided the *deus ex machina* that would ultimately help solve this dangerous clinical conundrum—in this case, the "Hot Pig." The authors of this paper, an interdisciplinary group that included surgeons, anaesthetists, physicians, and a laboratory technologist, all members of the Liver Research Group of the University of Cape Town, South Africa, were engaged in a program of experimental liver transplantation utilizing the pig (Landrace and Landrace X Large White) as the experimental model. The anaesthetic protocol was based on halothane.

To the surgeons' annoyance, the physicians' puzzlement, and the anaesthetists' embarrassment, one of the first animals anaesthetized became, for no apparent reason, cyanosed and extremely hot, its legs rigidly extended, and died before the surgical experimental protocol proper could be started. When, frustratingly, a further 5 of the first 34 animals anaesthetized displayed the selfsame reaction, we realized that the anaesthetist had no cause for embarrassment; we had stumbled on a specific syndrome.

Led through Hall's description (a year previous) of the identical reaction in littermate pigs in response to suxamethonium[3] to a consideration of the described syndrome of anaesthetic-induced malignant hyperpyrexia in human patients,[2] we came to the exciting conclusion that we had stumbled on a facsimile in pigs of the human condition. Here, indeed, was the animal experimental model of MH.

This discovery, reported first in a preliminary communication[4] and definitively thereafter in this paper, was seminal. Today, just less than 20 years later, though the minutiae of the pathogenesis of MH have yet to be elucidated, knowledge that has come from worldwide studies in MH swine has led not only to effective pharmacological control of the syndrome,[5] but also to valuable spin-offs in many biomedical areas, in particular those of muscle and membrane physiology, calcium transport, and kinetics, and in the meat industry.

A second reason for this paper's citation is that it carried, *inter alia*, the first report of an identifiable fundamental functional biochemical lesion in the muscle of animals susceptible to MH, i.e., a markedly increased rate of ATP depletion *in vitro*. We were led to investigate specifically the ATP content of muscle by the severity of the muscle rigor that characterizes the syndrome and that one of us (DD) insistently likened to rigor mortis setting in antemortem. Rigor mortis was already known to correlate with ATP depletion.

Further, this paper reported for the first time the laboratory *in vivo* identification of agents that trigger MH.

Though I earned no specific honour or award directly through this publication, it and the research that followed from it doubtless contributed to my later election as a Life Fellow of my university.

It is of some human interest that the authors of this paper (excepting the laboratory technologist) have all subsequently become professors and one has become a university vice chancellor. [For a recent review of the subject, see reference 6.]

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Brit.J.Anaes 41:1062:1969

ANAESTHETIC INDUCED MALIGNANT HYPERPYREXIA

Sir,—In our paper which appeared in your October issue (*Brit. J. Anaesth.*, 41, 844) we mentioned the possibility that cyclopropane might trigger the onset of this syndrome in susceptible pigs. We wish to report that we have now had the opportunity on two occasions to retest this possibility in known susceptible pigs with negative results. Cyclopropane does not trigger malignant hyperpyrexia in susceptible pigs. The triggering of the syndrome we observed following exposure of a susceptible pig to cyclopropane must be accepted as having been due to the previous administration of suxamethonium.

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Changes underlying Halothane-induced Malignant Hyperpyrexia in Landrace Pigs

MALIGNANT hyperpyrexia is a rare but often fatal complication of inhalation anaesthesia in man[1]. The fluorinated hydrocarbon halothane, $CF_3CHBrCl$, is among the many anaesthetic agents which have been implicated[2]. A similar syndrome has been described in a pure Landrace strain of pig[3]. The onset of this phenomenon is rapid (10–15 min after administration of halothane) and there is extreme rigidity of skeletal muscles, a rapid rise in body temperature to 42°–45° C, severe acidosis and a fatal outcome in spite of cooling, muscle relaxants and intravenous alkalis. Once initiated, the process seems independent of the tissue concentration of halothane, which appears to act as a trigger. A few animals have recovered after brief exposure to halothane.

Wilson *et al.* have suggested uncoupling of oxidative phosphorylation as a possible mechanism[3]. The process is augmented by dinitrophenol and halothane is known to uncouple oxidative phosphorylation in normal rat liver mitochondria *in vitro*[4].

All animals studied were of pure Landrace or Landrace–Large White cross strains. No untoward effects of thiopentone were observed in susceptible pigs over periods of up to 150 min. Monitoring was accordingly established under controlled steady state conditions of thiopentone anaesthesia with pigs breathing oxygen. Observations made during thiopentone anaesthesia were compared with those following halothane. Arterial blood samples and pressure readings were taken from a femoral catheter. Venous blood was withdrawn from an indwelling right atrial catheter. Temperatures of the core (oesophagus and rectum), liver, muscle and skin were recorded by thermistor probes. Oxygen uptake was measured with a Benedict–Roth spirometer and respiration was assisted during apnoeic periods by manual chest compression. Carbon dioxide output was determined gravimetrically after absorption in $CaCl_2$/soda-lime. The acid-base status of the blood was measured by the Astrup technique[5]. Total CO_2 content, serum concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, total protein and inorganic phosphate (P_i) were determined by standard clinical laboratory techniques. Blood lactate[6] and pyruvate[7] were determined enzymatically. Blood oxygen tensions were measured with a Clarke electrode. Biopsies of muscle were assayed for glycogen[8] and ATP[9].

Anaesthesia with thiopentone for up to 150 min had no effect on organ temperatures. Within 10 min (usually

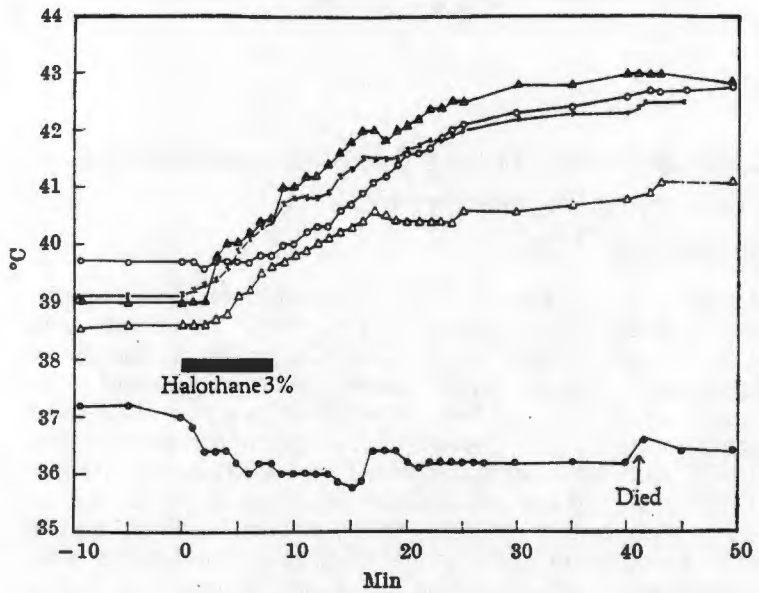

Fig. 1. Temperature changes in organs of pig 82A induced by halothane. While anaesthetized with thiopentone alone, no significant changes took place in those portions of the curves from −150 to −10 min. Halothane, 3 per cent (v/v) in O_2, was commenced at 0 min. and discontinued at 8 min. ▲, Liver; ○, rectum; ×, oesophagus; △, muscle (gluteus); ●, abdominal skin.

3–4 min) of introducing halothane, the temperature of all organs rose, except skin (Fig. 1). Cooling of the latter and cyanotic blotchiness indicated decreased circulation. After discontinuing halothane, the temperature continued to rise and maximum core temperature (oesophagus) was reached at 30–45 min.

O_2 consumption and CO_2 output after giving halothane were compared with control periods of isothermia on thiopentone anaesthesia. The results (Fig. 2) reveal augmented uptake of oxygen, but the disproportionate rise in CO_2 output resulted in high respiratory quotients (1·69 in one experiment). Oxygen consumption rose 20–40 per cent per °C rise in core temperature. Between 38° and 39° C, passive heating raises O_2 consumption 18 per cent in rats[10] and 12–15 per cent in normal human adults[11]. Increased oxygen consumption appeared therefore to be slightly greater than that expected from elevated temperature alone.

Table 1 presents some metabolic changes observed during the development of malignant hyperpyrexia. These changes, initiated by halothane, were: (*a*) Shift of water into the intracellular space; serum Na^+ and total protein concentrations both increased. (*b*) Shift of Ca^{2+} and Mg^{2+} into the extracellular space.

Concentrations of these two cations rose disproportionately with respect to serum protein concentration assuming 50 and 30 per cent, respectively, to be protein bound. (*c*) Release of P_i into plasma. (*d*) Increase in blood glucose. In control animals blood glucose tended to fall

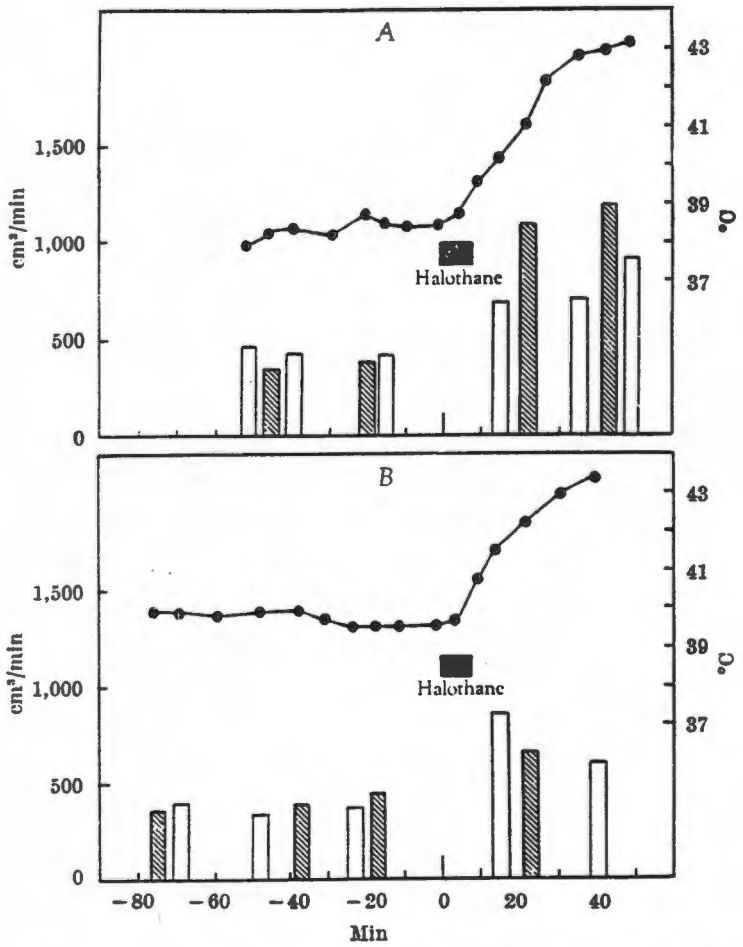

Fig. 2. *A*, Pig 39A, weight 80 kg; *B*, pig 82A, weight 56·1 kg. Effect of halothane on O_2 consumption and CO_2 output. O_2/CO_2 exchange was measured over timed 4 min periods. Halothane, 3 per cent (v/v, in O_2), was given from 0 to 8 min. White columns, O_2; hatched columns, CO_2. ●, Oesophageal temperature.

from values of 60 to 80 to below 50·mg/100 ml. after 2 to 3 h of anaesthesia. (*e*) Uncompensated metabolic acidosis resulting from a rise in plasma lactate. The base deficit was equivalent to the increase in plasma lactate and reflected a pure lactic acid acidosis. A respiratory defect, denoted by lowered arterial pO_2 in spite of administering 100 per cent oxygen, could be partly responsible for elevated pCO_2. In spite of diminished respiratory gaseous exchange, however, raised CO_2 output, measured gravimetrically (Fig. 2), doubtless arose from the reaction: $H^+ + HCO_3^- \rightarrow H_2O + CO_2$. Although both plasma lactate and pyruvate concentrations increased, "excess lactate", calculated according to Huckabee's formula[12], indicated a net shift in the equilibrium $NADH + H^+ + \text{pyruvate} \rightleftharpoons \text{lactate} + NAD^+$, in favour of lactate; presumably the result of accumulation of NADH.

To establish more precisely the interrelation between the observed biochemical and temperature changes, these

Table 1. PIG 39A. EFFECT OF HALOTHANE ON BLOOD COMPOSITION

Time	(min)	−60	−3	+3	+8	+21	+31	+80
Oesophageal temp.	(°C)	38·3	38·4	38·6	39·0	42·0	43·0	44·1
Na^+	(mequiv./l.)	136	142	142	154	151	154	155
K^+	(mequiv/l.)	4·5	4·6	4·5	5·4	8·2	8·4	11·4
Glucose	(mg/100 ml.)	68	68	68	78	112	125	150
Total protein	(g/100 ml.)	6·5	6·5	6·3	6·9	7·7	7·9	6·3
Ca^{++}	(mg/100 ml.)	10·8	11·1	10·3	11·7	13·5	12·9	10·5
Mg^{++}	(mequiv./l.)	1·12	1·44	2·16	2·40	2·00	2·24	2·56
P_I	(mg/100 ml.)	9·6	9·4	9·1	14·5	25·8	29·3	31·7
Lactate	(mequiv./l.)	1·63	1·43	1·70	7·00	19·9	20·9	18·0
Pyruvate	(mequiv./l.)	0·15	0·16	0·17	0·27	0·18	0·20	0·21
"Excess lactate"	(mequiv./l.)	0·62	0·35	0·56	5·18	18·7	19·5	16·6
*p*H		7·36	7·36	7·07	6·92	6·60	6·60	6·82
pO_2 arterial	(mm Hg)	120	350	340	265	200	215	78
pO_2 venous	(mm Hg)	46	55	52	51	28	58	47
pCO_2 arterial	(mm Hg)	49	49	68	119	268*	168*	153*
Base excess	(mequiv./l.)	+3·0	−1·5	−3·2	−7·2	−21·5	< −22·0	< −22·0

Experimental details are as in Fig. 2*A*.
* Calculated from total blood CO_2 and *p*H.

were carefully timed in a separate experiment on a susceptible pig (82A) in the first 10 min after halothane induction. During this period, oesophageal temperature rose from 39·5° to 40·8° C. All the expected changes had occurred within 4 min and Ca^{2+} and Mg^{2+} shifts and P_i and lactate production were apparent at 2 min. It was therefore clear that biochemical changes preceded the onset of pyrexia and were not secondary to heat injury to tissues. The sequence of metabolic changes that we have encountered in pigs sensitive to halothane seems to be completely different from that associated with heat injury[13], in which rise in blood lactate is a late manifestation.

Halothane, in susceptible pigs, initiates massive and uncontrolled glycogenolysis with production of "excess lactate" and a rise in blood glucose. The mechanism responsible for this is not immediately clear, because the usual anaerobic causes of lactate accumulation can be excluded. The animals breathed pure oxygen throughout the experiment and arterial oxygen tensions were maintained above 100 mm Hg in all cases. Blood pressure was over 100 mm systolic, but did fall terminally after lactacidosis had become well established. Cyanotic blotchiness and decline in temperature of the skin, however, provided evidence of a local perfusion defect, but its contribution to the stimulation of glycogenolysis in this and other tissues, for example, muscle, cannot be evaluated. Skin anoxia is considered unlikely to be responsible for the lactacidosis, for it appeared after 8–10 min on halothane (after serum lactate had risen) and was transient. The magnitude of the lactacidosis, moreover, continued to increase after the skin had returned to a healthy pink colour. The possibility remains that muscle rigor resulted in occlusion of blood vessels causing an anaerobic state in muscle. The concentrations of muscle glycogen after halothane fell in two animals from 0·56 and 0·35 to 0·28 and 0·20 g/100 ml., respectively. In one instance muscle ATP fell from 2·7 to 2·2 mg/100 ml., concentrations above those at which fully developed rigor mortis is to be expected[14]. In the pigs we studied, the mechanism of muscle rigor seemed to be intrinsic, for the relaxant, tubocurarine, was ineffective in dispelling the rigidity. Impairment of blood supply to a limb consistently delayed onset of rigidity, suggesting that the effect of halothane on muscle is humoral rather than neural.

For the purpose of discussion of the origin of heat, we may consider pig 82A (weight 56·1 kg). Assuming a water content in the body of 67 per cent by weight and disregarding body solids (whose specific heat is not easily measured), the rise in temperature observed (3·9° C) represents extra heat of 147 kcalories, which—averaged over 40 min—is equivalent to 3·68 kcalories/min. Initially, however (5–10 min), the rate was 10·5 kcalories/min. If this extra heat were strictly related to increased O_2 utilization, 2·18 l./min additional O_2 consumption should have resulted (assuming that 1 l. O_2 consumed generates 4·825 kcalories[15]) whereas the measured increment at

15 min ($T = 41{\cdot}5°$ C) was 0·38 l./min and by 40 min ($T = 43{\cdot}4°$ C) the increment had fallen to 0·22 l./min. Non-oxidative mechanisms seem to be at least partly responsible for the excess heat production. Possibly anaerobic sources of heat include: (*a*) Glycolysis:

$$(\text{Glucose})_n + H_2O \longrightarrow (\text{glucose})_{n-1} + 2 \text{ lactate} + 2H^+$$

for which $\Delta F^{\circ\prime} = -52{\cdot}4$ kcalories/mole[16]. In pig 82A, heat from this source = 21·7 kcalories. (*b*) Neutralization of lactic acid:

$$H^+ + OH^- \longrightarrow H_2O; \quad \Delta H = -13{\cdot}6 \text{ kcalories/g equivalent}$$

In the experimental animal this process provided 11·? kcalories. These calculations are based on the rise in serum lactate concentration and are minimal because the quantity of lactate oxidized was unknown. (*c*) Hydrolysis of high energy phosphate esters.

During anaerobiasis, fall of creatine phosphate in frog sartorius muscle corresponds to rise in its P_i content[17]. Assuming that the released extracellular P_i was derived from compounds possessing high phosphate bond energy, for example creatine phosphate, ATP or phosphoenol pyruvate, capable of yielding 10 kcalories/mole, the rise in serum phosphate from 10 to 30 mg/100 ml. represents 2·4 kcalories in this animal. The sum of accountable anaerobic heat sources amounted to 35·4 kcalories or about 25 per cent of that actually evolved. We are investigating other potential heat sources which could be activated by halothane.

The mechanism by which halothane stimulates excess heat production in susceptible pigs is still obscure. Certain conclusions can, however, be reached which will influence further investigations and possibly management of the similar condition occurring in man.

(1) Excessive heat production is not accountable in terms of oxidative mechanisms and uncoupling of oxidative phosphorylation. Halothane induced an anaerobic state favouring breakdown of glycogen and organic phosphate esters. Anaerobic mechanisms have not previously been considered as possible sources of physiological or pathological heat.

(2) Alterations in the distribution of water and electrolytes and lactic acid production preceded significant rise in temperature and must be regarded as primary and not secondary to non-specific heat damage. They were apparently closely related to the development of muscle rigor. The lactate acidosis was uncompensated and blood pCO_2 rose to levels which could cause narcosis. Immediate treatment with intravenous HCO_3^- is contraindicated because death could ensue from CO_2 poisoning rather than H^+ accumulation.

(3) Known causes of defective tissue oxygenation could not account for the extent of lactate production. Future studies will aim at localizing a possible defect in mitochondrial electron transport.

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animals. We acknowledge financial support from Anglo-American and De Beers Anaesthetic Research Fund.

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ABSTRACTS OF THE SYMPOSIUM ON PAEDIATRIC ANAESTHESIA

AT THE POST CONGRESS MEETING
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Anaesthetic Induced Malignant Hyperpyrexia—Some Observations of the Syndrome in Landrace Pigs

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Malignant hyperpyrexia is a rare and usually fatal complication of general anaesthesia. We have found that certain Landrace pigs, when exposed to halothane or succinylcholine, develop a syndrome exactly analogous to that described in humans (Harrison, *et al.* [1]).

As in man, the salient clinical features of the syndrome in these pigs are:—

(1) gross muscle rigor;
(2) sinus tachycardia;
(3) hyperventilation proceeding to apnoea;
(4) blotchy skin cyanosis;
(5) an explosive and sustained rise in temperature to end levels of 43°-45°C.

These features appear all but simultaneously and the condition proceeds rapidly to fatal termination. Once the syndrome is established, no treatment has been of any avail. However, it has proved possible to abort the condition if the halothane has been discontinued within three to five minutes of the appearance of rigor, tachycardia and hyperventilation. Survivors of aborted attacks have all developed this syndrome again when re-exposed to halothane. This circumstance has allowed us to make serial biochemical observations on the syndrome produced under controlled conditions.

In summary, such observations showed (Berman, *et al.* [2]; [3]) a rise in serum sodium, potassium, calcium and magnesium, glucose, total protein, lactate, pyruvate and excess lactate. Profound acidosis was invariable.

In this example, blood pH fell within 20 minutes from the control level of 7.35 to 6.60. Severe lactacidosis was reflected by a base deficit greater than 22 m.Eq/litre matched by a similar rise in lactate, while $PaCO_2$ rose above 150 mmHg.

We interpreted these findings as indicating:—

(a) a net shift of water into the cells;
(b) leakage of potassium, calcium and magnesium out of the cells; and
(c) massive glycolysis.

The presence of glycolysis was confirmed by the observation of a simultaneous fall in muscle glycogen. Muscle ATP levels also were reduced. Arterial PO_2 levels (the animal being ventilated with pure oxygen) were maintained above 200 mmHg. The high $PaCO_2$ recorded was associated with an increase in CO_2 output and was thought to reflect buffering of the lactacidosis.

Of importance was the fact that these biochemical changes and the muscle rigor preceded the recorded rise in core temperature, and had reached extreme values in advance of the temperature. From this we inferred that the changes observed were not secondary to the effect of heat on the tissues. Further, the

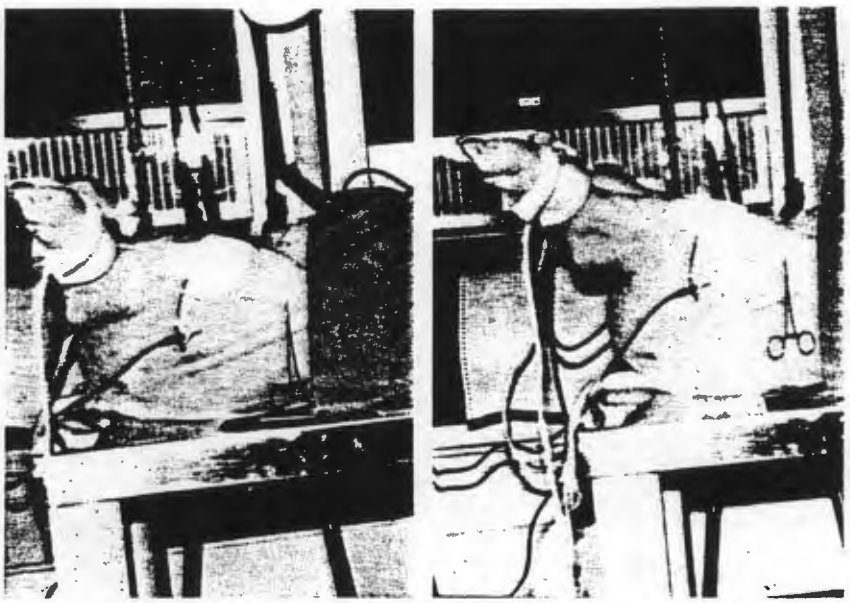

Fig. 1. Onset of muscle rigor in hind limbs of Landrace pig following administration of halothane.

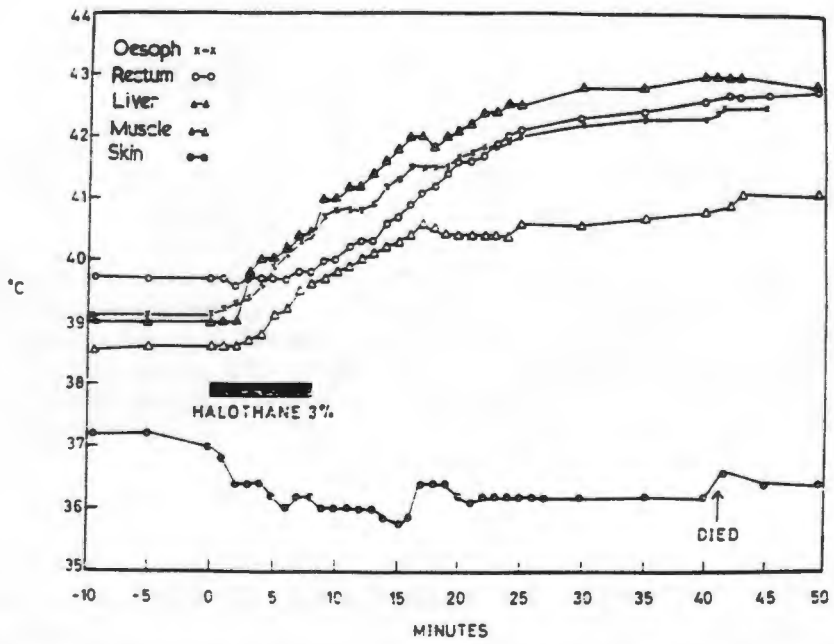

Fig. 2. Temperature changes of malignant hyperpyrexia induced by halothane in Landrace pig.

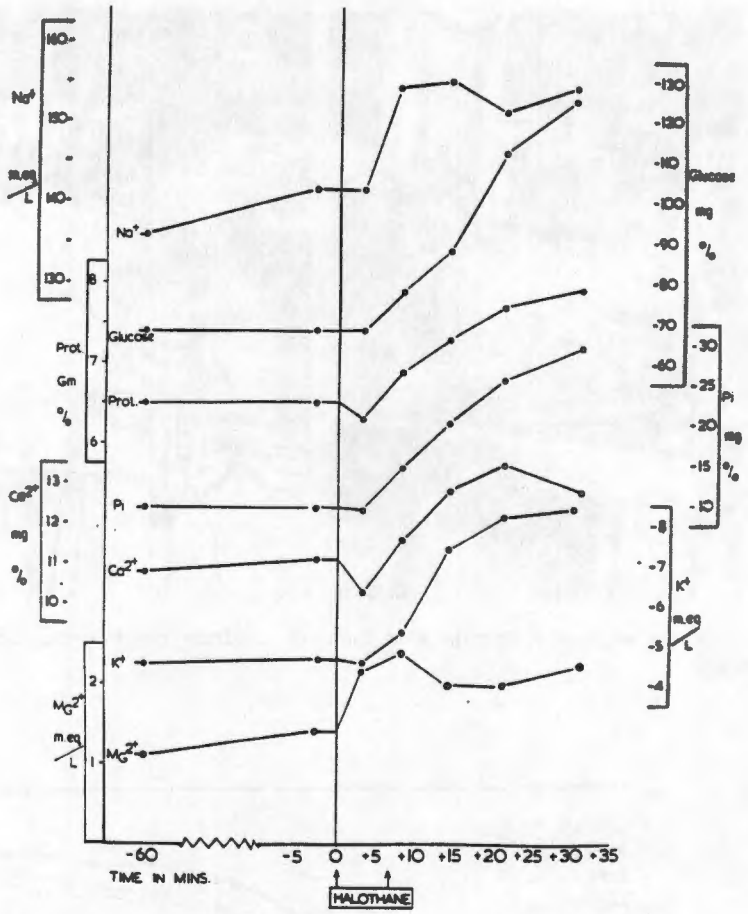

Fig. 3. Biochemical changes associated with halothane induced malignant hyperpyrexia in Landrace pig.

observation that the onset of rigor was delayed in a limb to which the blood supply was impaired, led us to conclude that the mechanism by which halothane initiated the condition was humoral, and considering its refractoriness to non-depolarizing muscle relaxants, the disturbance responsible for the rigor was probably intrinsic. This led us to investigate whether components of the syndrome of malignant hyperpyrexia could be demonstrated in the isolated perfused muscle of a susceptible pig as in the intact animal.

Method

For this purpose we utilized the isolated hindquarters of a known susceptible Landrace pig perfused with oxygenated blood by means of a standard pump oxygenator circuit using a Rygg bag with Sarns roller pumps. For oxygenation, a mixture of 97 per cent oxygen and 3 per cent CO_2 was used in a ventilation/perfusion ratio of two to one. The preparation was attained in the following way:

Under thiopentone-nitrous oxygen anaesthesia, which we have shown does not initiate or influence the syndrome, the aorta was cannulated immediately above the bifurcation separately in a cephalad and caudad direction. At the same level the inferior vena cava was cannulated in a caudad direction. The distal aortic cannula was connected to the arterial outflow from the pump oxygenator, and that from the vena cava to the venous well. The animal was heparinized and bled out via the proximal aortic cannula into the venous well of the pump oxygenator, which had been primed initially with one litre of TisUsol. The inferior vena cava was ligated above its cannula and perfusion of the hind-

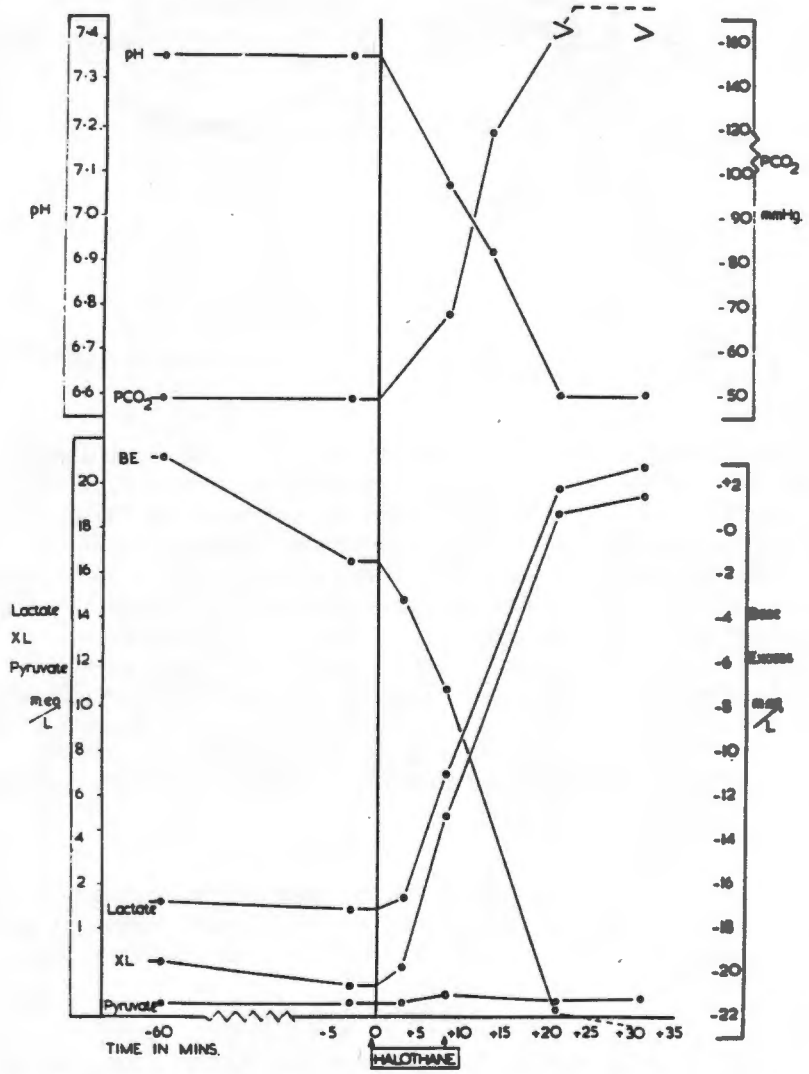

Fig. 4. Acid base changes associated with halothane induced malignant hyperpyrexia in Landrace pig (arterial).

quarters was commenced. Following the exsanguination of the pig, PCV of the perfusing fluid was 20 per cent. As soon as adequate perfusion of the hindquarters was established and the animal had been bled out to the point of cardiac arrest into the oxygenator, the body was completely transected immediately above the pelvis, the gut was removed and haemostasis was secured. The temperature of

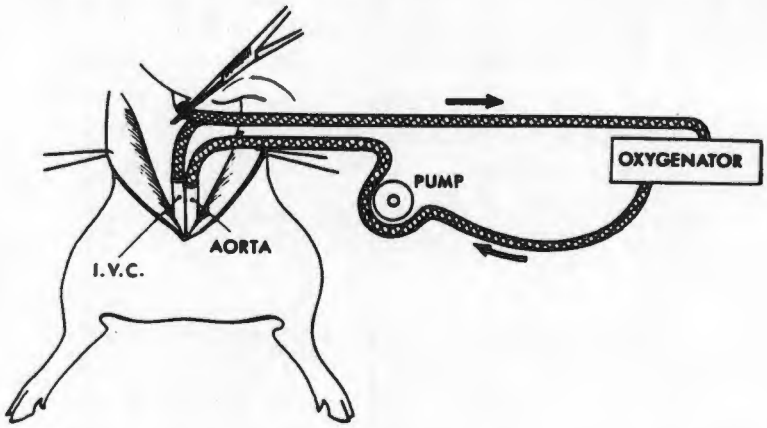

Fig. 5. Isolated hind limbs of Landrace pig perfused with oxygenated blood by means of a standard pump oxygenator circuit.

the arterial blood was kept constant at 38°C by a thermostatically controlled water jacket on the arterial line. Temperature of the venous return was monitored by a thermistor in the venous well. After a short period at 700 ml/min. arterial inflow was maintained constant throughout the experiment at 500 ml/min. This gave an approximate flow of 5-6 ml/100G of muscle per minute. Arterial PO_2 values recorded throughout the perfusion varied between 380-480 mmHg. Following a control period of perfusion of 70 minutes, 3 per cent halothane vapour was introduced into the oxygenator by means of a Fluotec vaporizer and the response of the preparation studied. Biochemical changes were followed in serial blood specimens taken from the venous and arterial lines. In addition, the pH of the venous outflow was continuously recorded from an electrode in the venous well. The experiment was terminated 35 minutes after the introduction of halothane.

Results

Five minutes after the introduction of halothane fasciculation was observed in the muscle, which had been flaccid up to this time. Sustained rigor of the muscle followed and was well developed within a further five minutes.

Serum Electrolytes

Monitored in the venous effluent, biochemical changes exactly similar to those previously observed associated with the syndrome induced in the intact animal developed with rapidity following the introduction of halothane. Following little change during the 70 minute control period of perfusion, changes from control

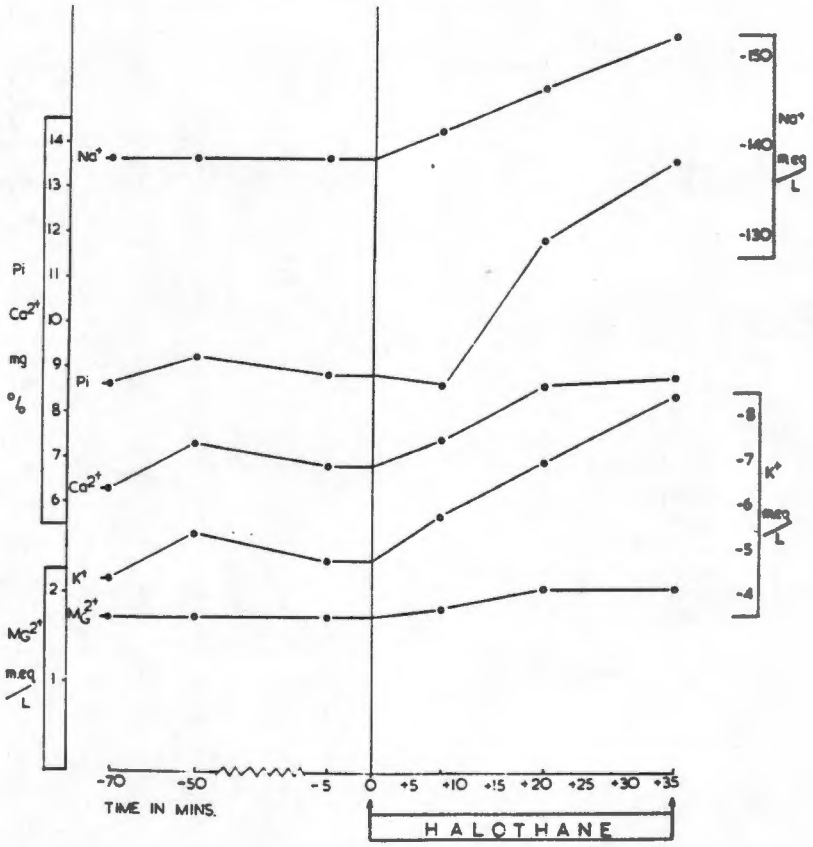

Fig. 6. Biochemical changes in isolated perfused muscle preparation in response to halothane.

levels were noted in the first specimen taken eight minutes after the introduction of halothane. Rises were evident in the serum concentrations of sodium, potassium, calcium, magnesium and Pi.

Acid Base State

Though a degree of acidosis was present during the control period of perfusion, the acid base state remained fairly steady. Introduction of halothane was followed immediately by the characteristic profound and progressive lactacidosis seen in the intact animal. Thirty-five minutes from the introduction of halothane the pH had fallen to 6.575; $PaCO_2$ was in excess of 150 mmHg and the base deficit matched by the level of lactate was greater than 22 m.Eq/L.

Perfusion Pressure

The perfusion pressure remained constant at 30 mmHg throughout the control period of the experiment. The introduction of halothane was followed for a few moments by a slight fall whereafter the pressure rose steadily to reach 40 mmHg after 30 minutes.

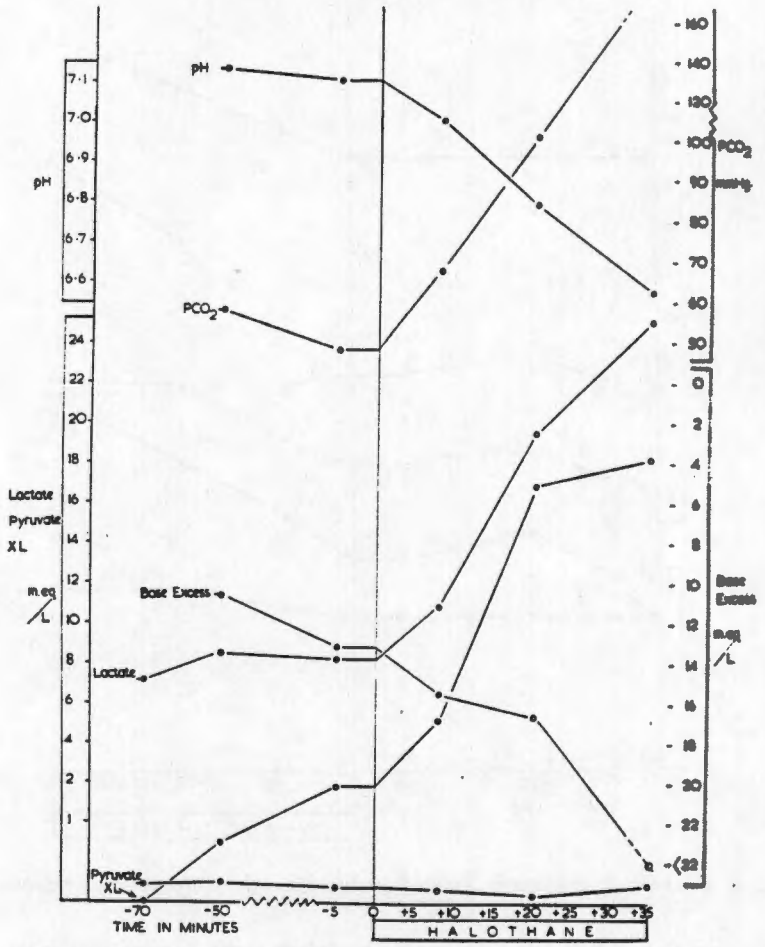

Fig. 7. Acid base changes in isolated perfused muscle in response to halothane (venous return).

Temperature

No change was observed in the temperature of the venous effluent.

Discussion

We did not observe a rise in temperature in the venous effluent. This excepted, the perfused muscle preparation showed the same muscle rigor and biochemical changes that we have previously observed in the syndrome of malignane hyperpyrexia triggered by halothane in the intact animal. From this observation certain conclusions follow:—

(1) The mechanism by which this syndrome is initiated by halothane in susceptible pigs is in its simplest form humoral. Disturbances of the normal integrated mechanisms of heat homeostasis play no primary part in the production of this syndrome.

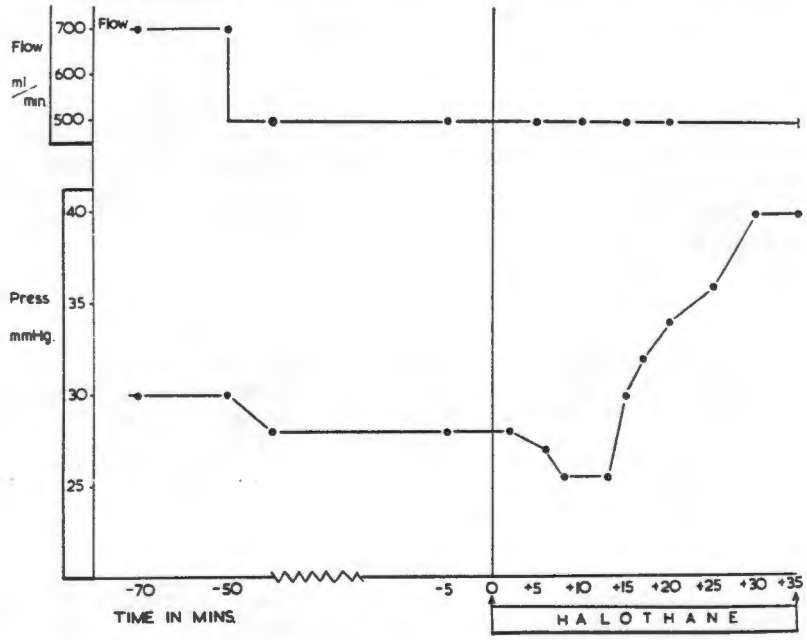

Fig. 8. Changes in arterial pressure during isolated muscle perfusion.

(2) The profound biochemical changes observed are not in any way secondary to hyperthermia, but evidence of an underlying biochemical lesion of which the ultimate rise in body temperature is an accompaniment.
(3) Taking into consideration the prominence of muscle rigor in this syndrome in pigs as well as in man, the proportion of the body mass that is muscle tissue and the evidence that all the changes recorded in the syndrome in the intact animal were shown by this perfused muscle preparation, one may speculate that muscle is the tissue primarily responsible for this syndrome.

Another observation that invites comment is that related to the blood flow in this preparation. In examining the possible reasons for the massive lactacidosis observed, Berman and co-workers [3] questioned the possibility of an anaerobic state in the muscle due to obstruction of blood vessels from some physical effect of the rigor. In this preparation there is evidence of an increased vascular resistance in that progressive rise was observed in the perfusion pressure in the face of a constant blood flow, but the biochemical changes recorded preceded this event.

Lastly, I wish to draw your attention to our observation that in pigs susceptible to malignant hyperpyrexia, both the intact animal and the muscle preparation described here, the muscle rigor that followed exposure to halothane was preceded by fasciculation. In cases of the syndrome recorded in man, both halothane and succinylcholine feature prominently as possible initiators of the condition (Daniels, *et al.* [4]; Wilson, *et al* [5]). We have previously demonstrated that in these pigs both halothane and succinylcholine are triggering agents (Harrison *et al.* [1]). In both pigs and humans, the fasciculation that followed administration of succinylcholine was exaggerated. The elucidation of a common denominator

in the action of these so different agents in the animal—pig or human—susceptible to malignant hyperpyrexia may provide the key to an understanding of this syndrome. Is this observation perhaps a beginning?

Summary

An experiment is described in which the biochemical changes and muscle rigor of the syndrome of anaesthetic induced malignant hyperpyrexia, previously described in the intact animal, are demonstrated in a preparation of isolated perfused muscle taken from a Landrace pig previously shown to be susceptible to this condition.

Acknowledgements

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PALE, SOFT, EXUDATIVE PORK, PORCINE STRESS SYNDROME AND MALIGNANT HYPERPYREXIA—AN IDENTITY ?

G. G. HARRISON*

SUMMARY

Pale, soft exudative pork (PSE pork) is a stress-related syndrome, resulting in undesirable meat quality. Clinical and biochemical features of the porcine stress syndrome (PSS) seemed similar to those described in the syndrome of anaesthetic-induced malignant hyperpyrexia manifested by swine. An investigation is described which seeks to establish if swine susceptible to malignant hyperpyrexia would manifest PSE pork. Changes occurring *post mortem* in the muscle of swine susceptible to malignant hyperpyrexia and killed by exsanguination were studied. These demonstrated that the muscle of swine susceptible to malignant hyperpyrexia shows the same gain in expressible water with a similar pattern of pH fall to that associated with PSE pork.

INTRODUCTION

The condition now called pale soft exudative pork (PSE pork) has long been recognized [1] and is of economic importance in the pork industry. Characterized by rapid *post mortem* glycolysis, accumulation of lactic acid and rapid fall in muscle pH which results in undesirable meat quality, PSE pork has been shown to be a stress related syndrome [2-4].

The porcine stress syndrome (PSS) is also of economic importance: it causes death of swine in transport, is characterized by progressive dyspnoea, increasing body temperature, death within minutes of onset of symptoms and immediate rigor mortis [2]. This acute death syndrome in stress-susceptible swine bears an extremely close clinical similarity to the syndrome of malignant hyperpyrexia which follows exposure of susceptible swine to halothane anaesthesia and succinylcholine [5-7], and even severe exercise [8].

Such association prompts the hypothesis that PSS, PSE pork and malignant hyperpyrexia in swine are indeed manifestations of the same myopathy. It was of interest, therefore, to establish whether malignant hyperpyrexic swine, which, at initiation of the syndrome, manifest extremely rapid glycolysis, lactacidosis and rapid pH fall immediately *ante mortem* [9], would develop PSE pork *post mortem*.

MATERIAL AND METHODS

The *post mortem* changes in muscle in three groups of swine were studied:

Group 1 normal swine (controls) (7 pigs)

Group 2 swine susceptible to malignant hyperpyrexia (MHS) (3 pigs)

Group 3 Swine, susceptible to malignant hyperpyrexia, in which the syndrome was established *ante mortem* (MHAM) (3 pigs)

The inclusion of Group 3 was motivated by a desire to simulate the possible initiation in susceptible swine of the syndrome *ante mortem* by the exercise, struggle and stress of abattoir slaughter. The swine studied were Landrace and Landrace × Large White crossbreds from the Western Cape region.

Susceptibility to malignant hyperpyrexia was tested by screening all animals with a test challenge of halothane anaesthesia [5] one week or more before each experiment.

All pigs were killed by exsanguination from the aorta following induction of general anaesthesia. Those in Groups 1 and 2 were anaesthetized with thiopentone sodium, orotracheal intubation with nitrous oxide and oxygen administered by an intermittent positive pressure ventilation technique. Those in Group 3 were anaesthetized with halothane, thus effectively initiating the syndrome of malignant hyperpyrexia *ante mortem*. Whereas most reported investigations into PSE pork were undertaken on pigs slaughtered in abattoirs by stunning and exsanguination, our in-

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vestigation was undertaken on animals being used in surgical research as blood donors. Hence our use of general anaesthesia. We regarded the subsequent death of the animal by exsanguination as simulating that of abattoir slaughtered pigs.

Immediately following the death of the animal, it was turned prone. A pH electrode (Metrohm Herisau compensator E 388) and thermistor probe (Ellab Type TE 3) were inserted directly into the belly of one longissimus dorsi muscle at the level of L 2—3, while the opposite longissimus dorsi was exposed, and from it serial sections were taken for analysis.

The following observations were made at 30 mins., 1 hour, 1½ hours, 2½ hours, 3½ hours, 5½ hours and 24 hours *post mortem*:—

1. Intramuscular pH.
2. Muscle temperature.
3. Estimation of expressible muscle water[3].
4. Measurement of muscle lactate (Boehringer Mannheim Test Combination—Biochemica, Cat. No. 15972). This measurement was only performed up to 5½ hours *post mortem*. Between 5½ and 24 hours the carcass was refrigerated to 2—4°C.

Though other workers had graded meat on colour, we found the assessment of various grades of "pink" subjective and abandoned the use of this observation.

RESULTS

Expressible water

The changes recorded in expressible water *post mortem* are presented diagrammatically in Figure 1, with the actual amounts and the statistical significance of the relevant differences in Table 1. The groups of animals displayed 3 grades of increasing expressible water content *post mortem* in the order—

Control (1) < MHS (2) < MHAM (3)

Whereas the differences in expressible water content between the MHAM and the control group are significant over the whole time range, that between the MHS group and controls achieve significance after 5 hours. Though the means of the MHS and MHAM groups differ, statistical significance is only

Table 1: CHANGES IN EXPRESSIBLE WATER

Water content expressed as mg/g of muscle

GROUP	HOURS POST MORTEM						
	½	1	1½	2½	3½	5½	24
CONTROL (1)	374	368	359	354	387	368	455
MHS (2)	400	405	389	393	429	453	533
MHAM (3)	482	506	496	540	516	557	575
	Statistical Significance of Differences						
CONTROL—MHS	NS*	NS	NS	NS	NS	P<0,02	P<0,001
CONTROL—MHAM	P<0,01	P<0,002	P<0,01	P<0,01	P<0,05	P<0,001	P<0,001
MHS—MHAM	NS	NS	NS	P<0,05	NS	NS	NS

*NS = not significant

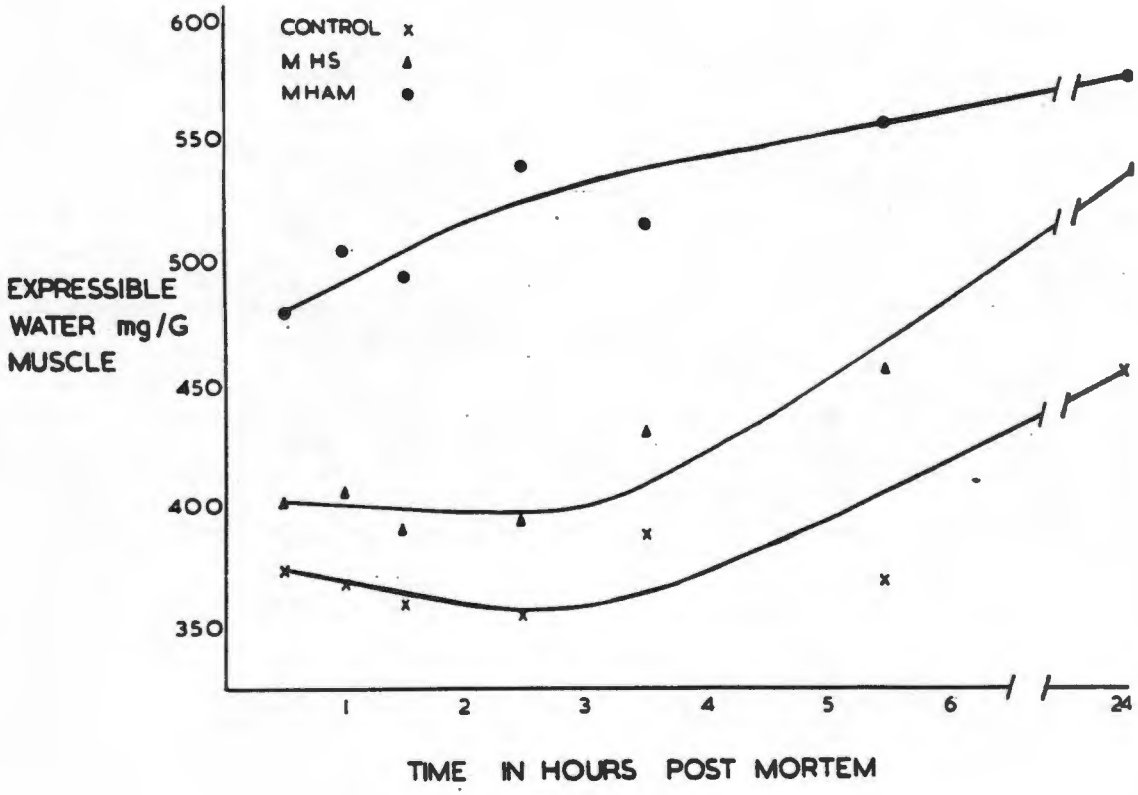

FIGURE 1

achieved at one point. Differences were such, however, that it is felt that statistical significance would have been achieved had more of the MHS animals been available. The expressible water content of both the MHS and MHAM groups at 24 hours is well within the range observed in PSE pork [8, 10].

pH

Changes observed in intramuscular pH are presented diagrammatically in Figure 2 with the actual figures and statistical significance of the relevant differences in Table 2. Though the ultimate pH reached is the same, the rate of fall is different in the three groups of animals in the order—

MHAM (3) > MHS (2) > Control (1)

That of the MHAM group is the most rapid, virtually reaching its lowest limit by the time the first observations were made 30 minutes *post mortem*. That of the MHS group starts at a level near that of the control group but falls rapidly to reach that of the MHAM group by 2½ hours *post mortem*. The control group only reached similar levels at 5½ hours *post mortem*.

The water-holding property of pork has been shown to depend on the rate of pH fall *post mortem*, a reflection of the rate of *post mortem* glycolysis [1-3, 10]. Taking the pH values recorded at 30 minutes, 60 minutes and 90 minutes after exsanguination as reflecting the rate of pH fall, we were able to show a strong inverse correlation between these values and the expressible water content of pork measured at both 5 hours and 24 hours *post mortem*, i.e. the more rapid the pH fall, the higher the expressible water content. The correlation coefficients with their relevant probability values are presented in Table 3.

Lactate

Corresponding with the patterns of pH fall, three grades in the rise of muscle lactate were observed in these groups of animals, in the order, for rate and quantity:

Table 2: CHANGES IN pH

GROUP	HOURS POST MORTEM						
	½	1	1½	2½	3½	5½	24
CONTROL (1)	6,70	6,40	6,23	6,02	5,91	5,82	5,70
MHS (2)	6,42	6,05	5,79	5,61	5,63	5,56	5,58
MHAM (3)	5,78	5,59	5,55	5,53	5,50	5,59	5,77
	Statistical Significance of Differences						
CONTROL—MHS	NS	P<0,02	P<0,02	P<0,05	NS	NS	NS
CONTROL—MHAM	P<0,01	P<0,001	P<0,002	P<0,01	P<0,05	NS	NS
MHS—MHAM	NS	NS	NS	NS	NS	NS	NS

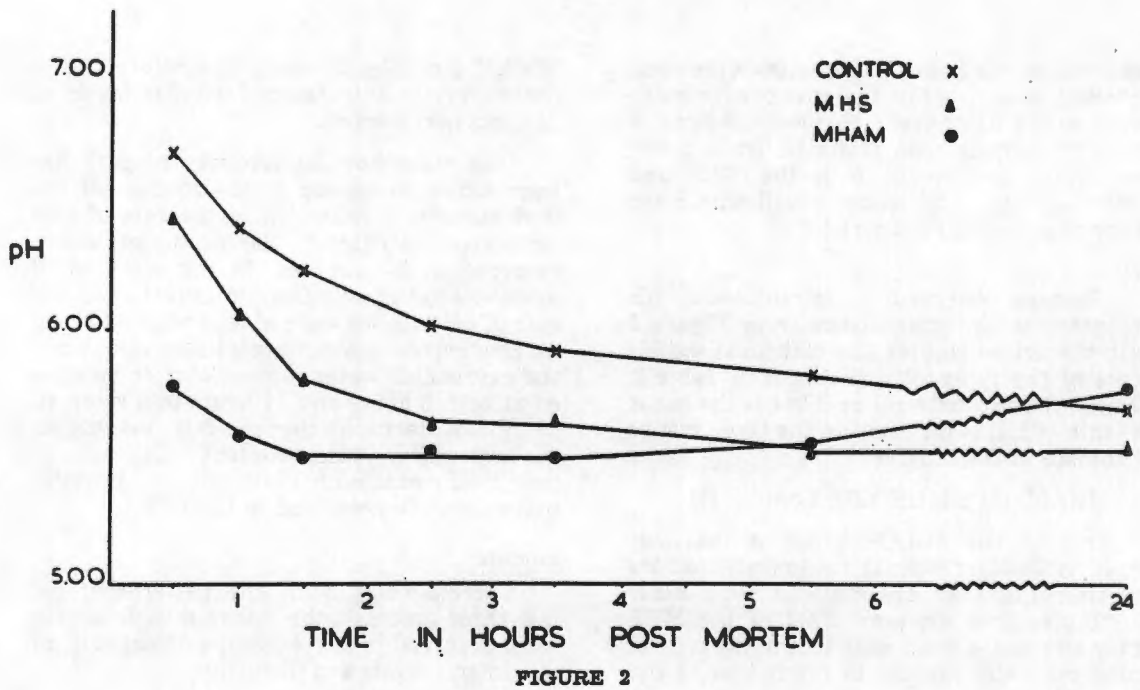

FIGURE 2

Table 3: CORRELATION COEFFICIENTS

EXPRESSIBLE WATER/pH

	pH AT TIME POST MORTEM (P.M.)		
	30 minutes	60 minutes	90 minutes
Expressible water at 5 hours p.m.	r=–0,89 P< 0,001	r=–0,87 P< 0,001	r=–0,83 P< 0,001
Expressible water at 24 hours p.m.	r=–0,67 P< 0,05	r=–0,75 P< 0,01	r=–0,74 P< 0,01

DISCUSSION

The criteria by which PSE pork is judged are primarily colour and content of expressible water[2, 3]. It has been shown that PSE pork is associated with circumstances which result in rapid *post mortem* glycolysis, myolactosis and rapid fall in pH[10, 11]. Such conditions appear to exist in association with the porcine stress syndrone (PSS). Response of stress-susceptible swine to environmental[12], anoxic[13] and exercise stress[8] appears to resemble closely the clinical[5] and biochemical changes[9] observed in the syndrome of malignant hyperpyrexia. Our observation of the *post mortem* changes in muscle of malignant hyperpyrexial swine shows that it manifests the same reduction in water-holding with a similar pattern of rapid pH fall as that associated with PSE pork. In those swine, in which the syndrome was actually initiated before death, the changes were most rapid and severe, the pH reaching its minimum very shortly after death. When the syndrome was not initiated *ante mortem*, the pattern of glycolysis, pH fall and myolactosis was still much faster than that of controls, with a similar loss in water-holding property. Though the patterns of *post-mortem* pH fall displayed by swine susceptible to malignant hyper-

Table 4: CHANGES IN LACTATE

Lactate expressed in μmol/g muscle

GROUP	HOURS POST MORTEM					
	½	1	1½	2½	3½	5
CONTROL	31	40	43	48	58	70
MHS	43	49	50	62	79	100
MHAM	87	99	108	125	120	123
	Statistical Significance of Differences					
CONTROL—MHS	NS	NS	NS	NS	NS	P<0,05
CONTROL—MHAM	P<0,01	P<0,002	P<0,04	P<0,001	P<0,001	P<0,001
MHS—MHAM	NS	NS	NS	NS	NS	NS

MHAM (3)>MHS (2)>Control (1)

These values are presented diagrammatically in Figure 3 with the actual values and statistical significance of the relevant differences in Table 4. The differences between the control group and the MHAM group were highly significant over the whole range. That apparent between the MHS and MHAM group just failed to achieve significance due to the small size of the two groups of animals. Starting at the same level as that of the control group, the MHS pigs show a higher rate of lactate production, the difference from the controls achieving statistical significance at 5½ hours *post mortem*.

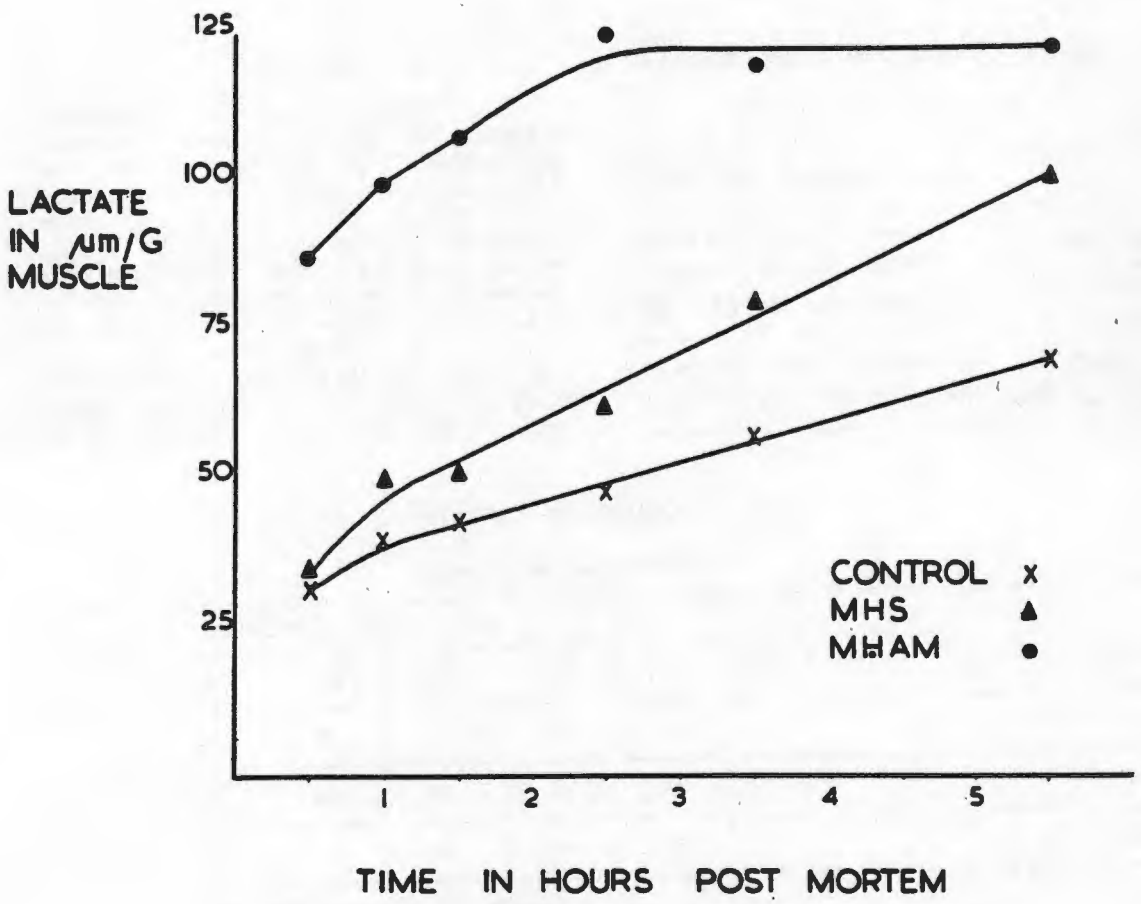

FIGURE 3

pyrexia were similar to those described in PSS by Wismer-Pedersen & Briskey[10] and Toppel[3], we did not observe as low levels, nor the even lower levels described by Lawrie *et al*[11]. This may possibly be the result of our use of anaesthesia before exsanguination, with a resultant tranquil death, whereas stress and struggling usually preceded the stunning and exsanguination at the abattoir as observed in these studies.

Malignant hyperpyrexia swine have been shown to have higher levels of serum CPK[14]. This same enzyme is markedly raised in association wit PSE pork[15]. Our demonstration of the PSE pattern of expressible water in the carcasses of malignant hyperpyrexia-susceptible swine is further evidence that PSS, PSE pork and susceptibility to malignant hyperpyrexia may well be an identity—all expressions of the same myopathy.

Should such an hypothesis be valid, current concepts of the aetiology of malignant hyperpyrexia[16-19] could help towards an understanding of PSE pork and PSS and conversely so.

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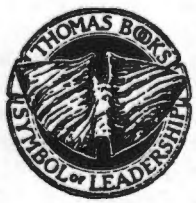

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Chapter 23

THE EFFECT OF PROCAINE AND CURARE ON THE INITIATION OF ANAESTHETIC-INDUCED MALIGNANT HYPERPYREXIA

G. G. HARRISON

THERE IS EVIDENCE that the lesion or abnormality responsible for anaesthetic-induced malignant hyperpyrexia in humans lies within muscle.[1,2,3,4] That this is so in certain Landrace pigs, susceptible to what I believe is the same syndrome, was clearly demonstrated by Harrison *et al.*[5] when they showed that the clinical and biochemical accompaniments of the syndrome previously identified in the intact pig challenged with halothane could be reproduced in an isolated perfused muscle preparation.

The predominant clinical features of the syndrome in pigs are as follows:[6]

1. Gross muscle rigor, particularly evident in the hind legs which become rigidly extended (Fig. 23-1). This rigor is usually preceded briefly by fasciculation of the muscles.
2. An explosive rise in temperature—a rate of 1°C every five to seven minutes with end temperatures of between 43°C and 45°C being usual (Fig. 23-2).
3. Tachycardia.
4. Hyperventilation proceeding to apnoea.

I wish to express my gratitude to Mrs. Carin Verburg and Dr. Colin Beighton for excellent technical assistance and Mr. Brian Sassman for the initial screening and selection of susceptible pigs, as well as post-anaesthetic recovery care. I am indebted to the Liver Research Group and the Department of Surgery of the University of Cape Town for the supply of pigs. Electrolyte estimations were performed by the Department of Chemical Pathology of the University of Cape Town.

This research programme was financially supported by the Anglo American and De Beers Anaesthetic Research Fund and the Joseph Stone Anaesthetic Research Fund.

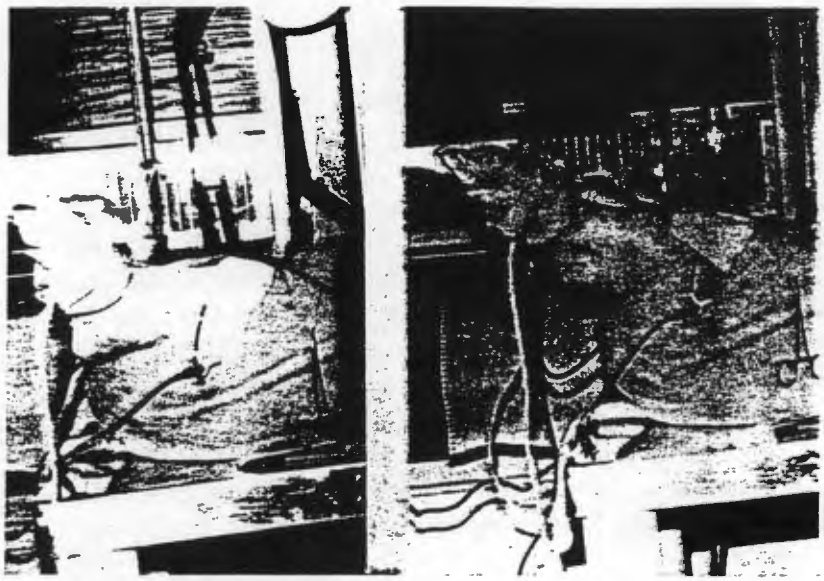

Figure 23-1. Onset of muscle rigor in hind limbs of Landrace pig following administration of halothane. Note the extension.

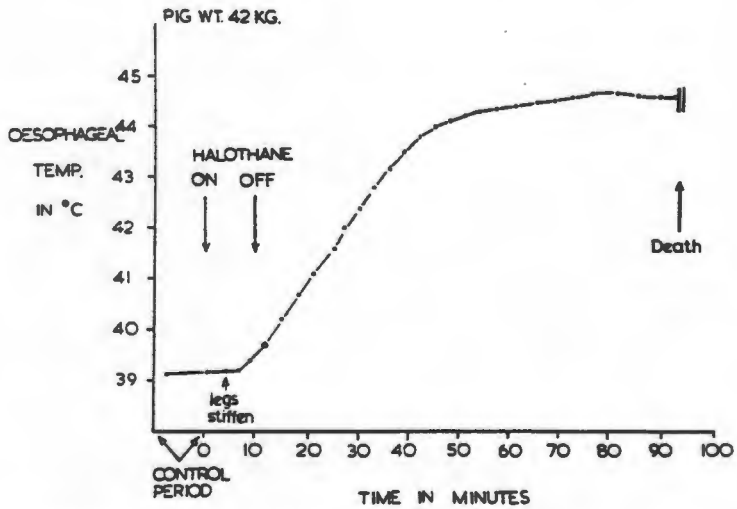

Figure 23-2. Continuous record of oesophageal temperature. Administration of halothane following the control period of Thiopentone anaesthesia.

5. Blotchy cyanosis of the skin, most noticeable on the abdominal wall and snout.

In susceptible animals, the syndrome becomes manifest within 5 to 15 minutes of exposure to halothane. The onset is generally more rapid when anaesthesia is directly induced with halothane, especially if this accompanied by struggling, and is usually slower if anaesthesia is first induced with thiopentone, halothane being exhibited when the animal is quiet. If halothane is discontinued at the very first appearance of rigor, the development of the syndrome may be aborted. If administration of halothane is continued for approximately ten minutes after initiation, the syndrome becomes irreversible resulting inevitably in the death of the animal, usually within 40 to 60 minutes.

Profound acidosis is an invariable accompaniment of the syndrome and develops with bewildering speed, as do also rapid rises in the concentration of serum electrolytes.[7] (Figs. 23-3, 23-4 and 23-5).

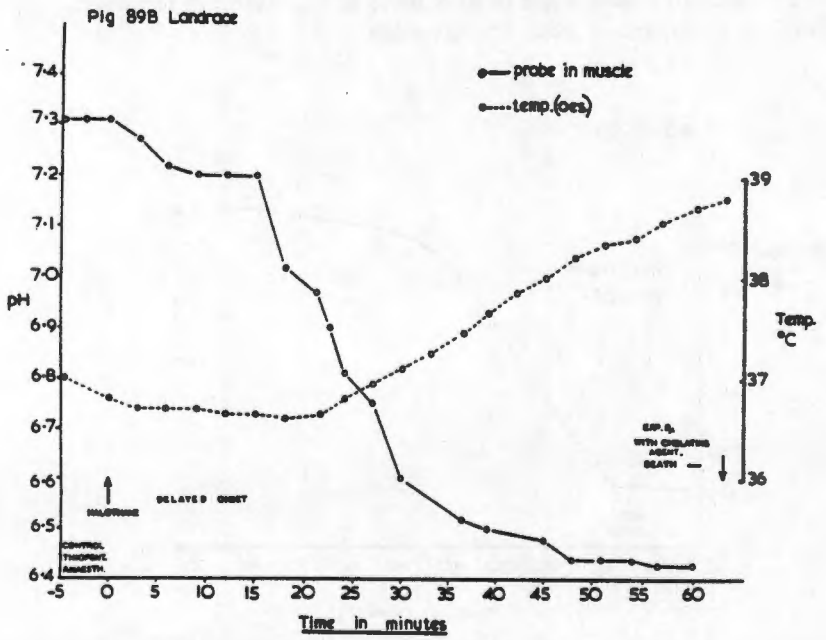

Figure 23-3. Record from free lying intramuscular pH probe.

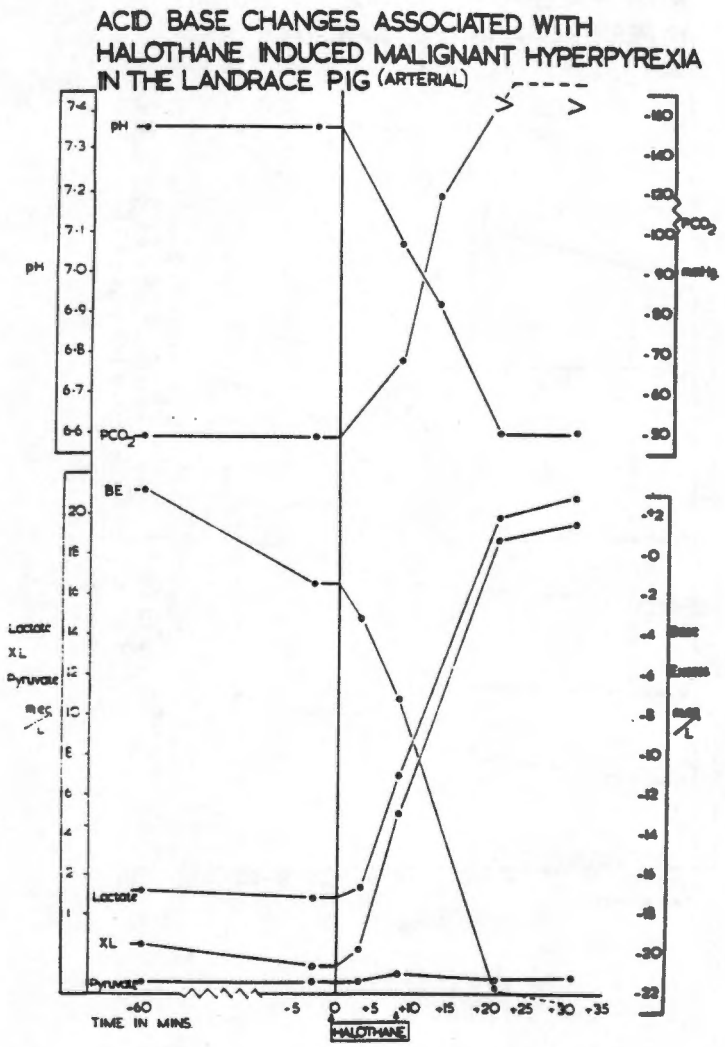
ACID BASE CHANGES ASSOCIATED WITH
HALOTHANE INDUCED MALIGNANT HYPERPYREXIA
IN THE LANDRACE PIG (ARTERIAL)
pH
PCO2
BE
Lactate
XL
Pyruvate
TIME IN MINS.
HALOTHANE

Figure 28-4.

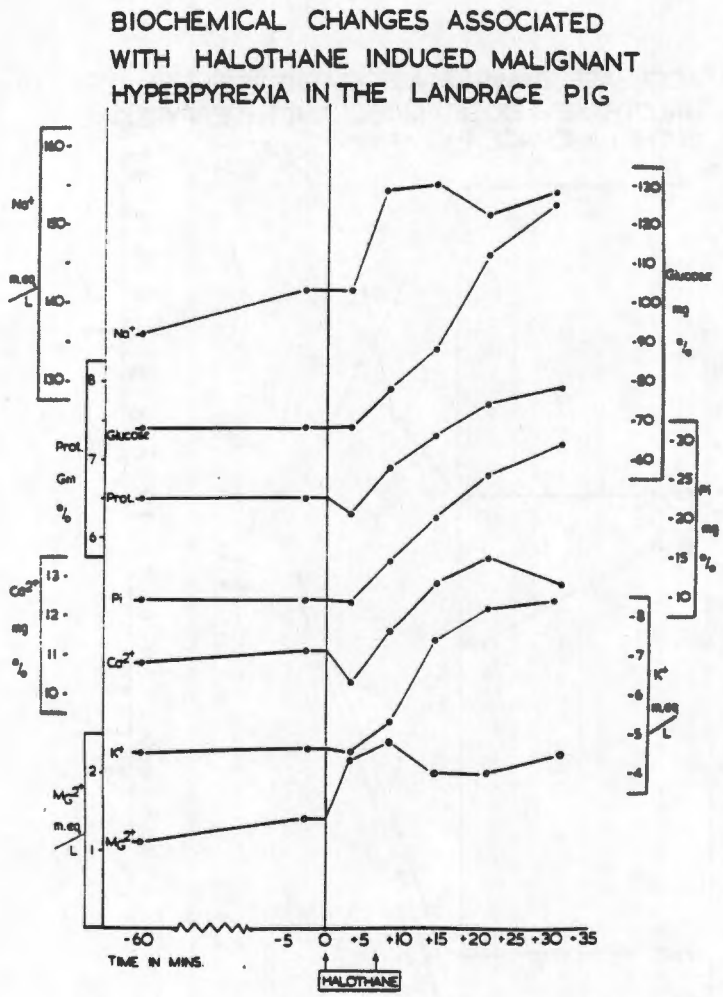

Figure 23-5.

The observation that muscle fasciculation precedes the rigor is important in relation to the agents which initiate or trigger the syndrome in these animals. We have identified halothane and succinylcholine as such agents[6] (Fig. 23-6). Of the many agents that have been associated with the condition in humans, halothane and/or succinylcholine are by far the most prominent, featuring

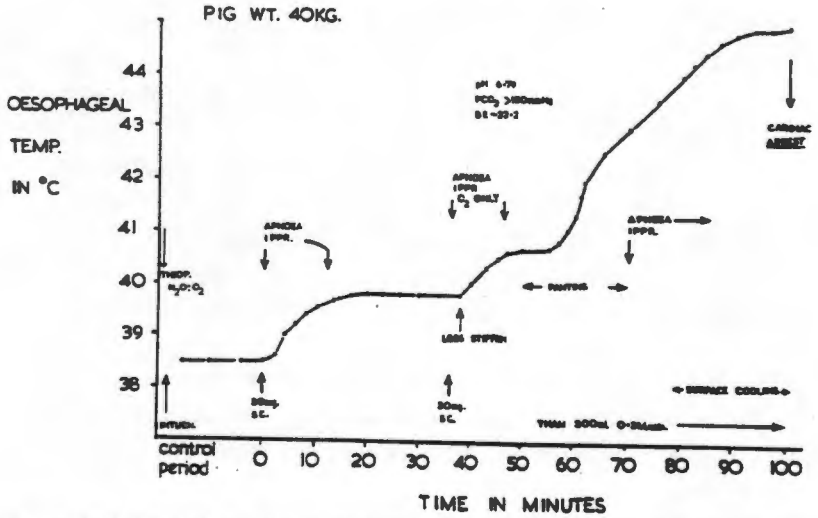

Figure 23-6. Initiation of the syndrome by succinylcholine. S.C. = injection of succinylcholine in dose shown.

in excess of 70 percent of cases.[6,9,10] That these two so dissimilar drugs could have some common denominator or synergism was hinted at many years ago by the work of Sabawalla and Dillon,[11] which showed that halothane caused an increase in the twitch response of muscle to both direct and indirect stimulation, and more recently by Tammisto and Airaksinen[12] who demonstrated an increase in serum CPK level after administration of succinylcholine, which was sevenfold greater when this drug was given in association with halothane than when given with thiopentone alone.

Caffeine has long been known to cause a persistent contraction or rigor of muscle fibre which persists until the caffeine is removed.[13,14] This rigor is due to an enhancement of release and an interference with rebinding of calcium ions by the sarcoplasmic reticulum. This allows a net efflux of calcium from the sarcoplasmic reticulum, a rise in sarcoplasmic calcium concentration, which activates myofibrillar ATPase, and maintains contraction.[15,16]

Procaine is known to block this effect of caffeine on muscle by

preventing calcium efflux. This effect is due to an action on the reticulum and not to a complexing with the caffeine. Once caffeine rigor has become well established, procaine has been said to have no effect.[17] More recently it has been shown that procaine can reverse early caffeine rigor.[15]

In a very significant paper last year, Kalow and co-workers[18] reported the following as the three most fundamental observations made on muscle preparations from human survivors of anaesthetic-induced malignant hyperpyrexia:

1. Such muscle was more sensitive to caffeine rigor than that of normal controls.
2. This effect was enhanced by exposure to halothane.
3. Halothane depressed calcium uptake by the sarcoplasmic reticulum of these patients, whereas it had no effect on that of normal controls.

An obvious question that must follow from these observations is that if halothane and caffeine produce similar changes in sarcoplasmic reticular function and, in fact, enhance each other's action, would not procaine block such action of halothane in the same manner as it is known to block caffeine rigor? Also, what effect would procaine have on succinylcholine initiation of the syndrome?

I thought the answers to these questions would give some clue to the trigger mechanism common to halothane and succinylcholine in this syndrome and so, perhaps, help towards a better understanding of the genesis of the syndrome itself.

The answers were sought in the following experiments on pigs (25-30 kg) known from halothane presceening to be susceptible to malignant hyperpyrexia.

EXPERIMENTAL METHOD

All experiments were carried out under a standard general anaesthetic consisting of induction of anaesthesia with thiopentone, endotracheal intubation and maintenance of anaesthesia with nitrous oxide and oxygen with IPPR.

Under these conditions the influence of pretreatment with tubocurarine and procaine on the initiation of the hyperpyrexic

syndrome by halothane and succinylcholine was studied. The drugs were administered in the following combinations and sequences—each sequence forming a separate experiment. (The number of times each experiment was repeated is given in parentheses.)

Tubocurarine–halothane (1)
Tubocurarine–succinylcholine (1)
Procaine–halothane (6)
Procaine–succinylcholine (2)
Procaine–halothane–succinylcholine (3)

Tubocurarine and succinylcholine were administered as single doses. Procaine was administered as a 0.6% solution by I.V. drip infusion, a loading dose of 30 mg/kg preceding maintenance infusion at rates which varied between 0.2 to 1 mg/kg/min. (Use *in vitro* of 1 to 3.3 mM concentrations is described[19,15]). ECG control was found to be mandatory when using procaine in this order of dosage together with halothane. Bradycardia and hypotension were invariable and it was expedient to support the circulation with an infusion of isoprenaline (concentration 1μg/ml).

The onset of the syndrome was identified as follows:

1. The onset of rigor–monitored crudely by means of a write-out on a revolving drum attached by a string to the pig's hind leg.

2. The rapid rise in muscle temperature–monitored by an intramuscular thermistor probe.

These two observations, rigor and rise of temperature, were considered adequate for identification of the syndrome.

3. Serial biochemical parameters were measured on central venous blood of some pigs.

When the syndrome did not develop following procaine pretreatment, the animal subsequently was reexposed under thiopentone anaesthesia to halothane in the absence of procaine. Such animals all developed the hyperpyrexic syndrome.

In five pigs the effect of procaine infusion on the established syndrome was studied.

RESULTS

The results of these experiments are set out in Table 23-I.

TABLE 23-I
THIOPENTONE, N_2O/O_2 ANAESTHESIA

Pretreatment → / Trigger agent ↓	*NIL*	*Curare*	*Procaine*
Halothane	+	+	—
Succinylcholine	+	—	—
Halothane			
Succinylcholine			+ —
	TREATMENT WITH		
Established syndrome	Cooling alkali etc.	Curare	Procaine
	DEATH IN RIGOR	DEATH IN RIGOR	RIGOR RELAXED SURVIVORS

Note: + = initiation of hyperpyrexic syndrome.
— = no initiation of hyperpyrexic syndrome.

The administration of tubocurarine did not prevent the initiation of the syndrome by halothane, but did prevent its initiation by succinylcholine. Procaine blocked the initiation of the syndrome both by halothane and succinylcholine when these drugs were given separately. When succinylcholine was given in the presence of halothane, fasciculation passed on into rigor and a fulminant rise in temperature occurred in two of three pigs. The rigor was ultimately controlled by continued procaine infusion. The rigor softened with concurrent cessation of temperature rise. The biochemical changes recorded as accompaniments of the syndrome only appeared in the presence of rigor and temperature rise and reverted towards normal when these were overcome.

I will illustrate some of these results (Figs. 23-7, 23-8 and 23-9). In five pigs in which the syndrome was well established with the temperature in excess of 42°C, large doses of procaine led to an ultimate softening of the rigor. No other method of treatment we have used has ever achieved this.[6] Ancillary treatment of administration of alkali was used in all with cooling in one. Two pigs survived (Fig. 23-10).

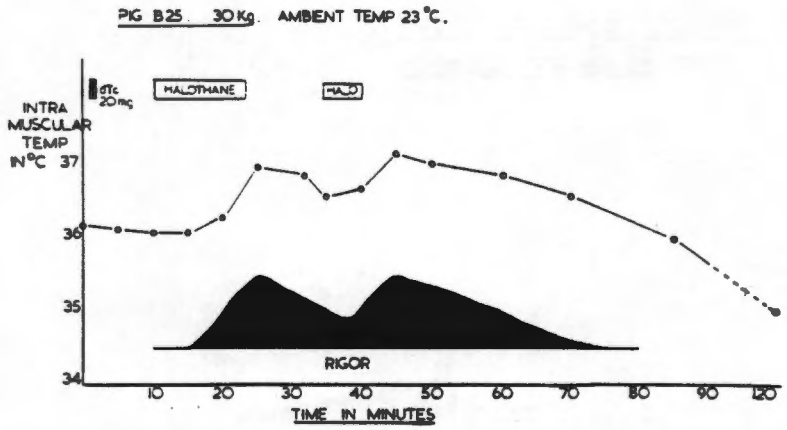

Figure 23-7. Halothane induction of rigor and temperature rise after pretreatment with tubocurarine.

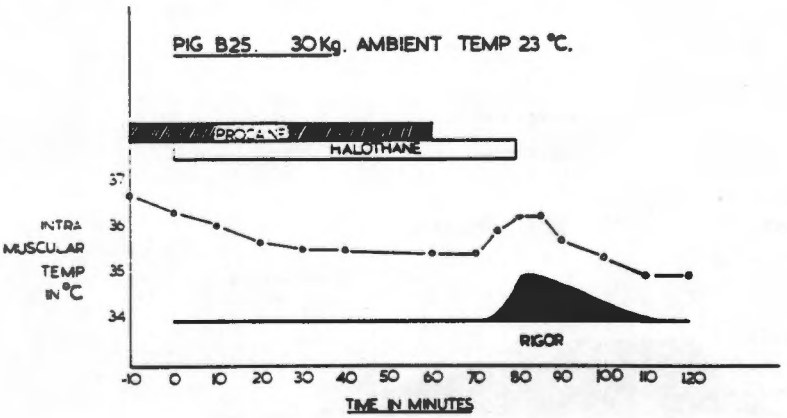

Figure 23-8. Block of halothane induced hyperpyrexia and rigor by pretreatment with procaine.

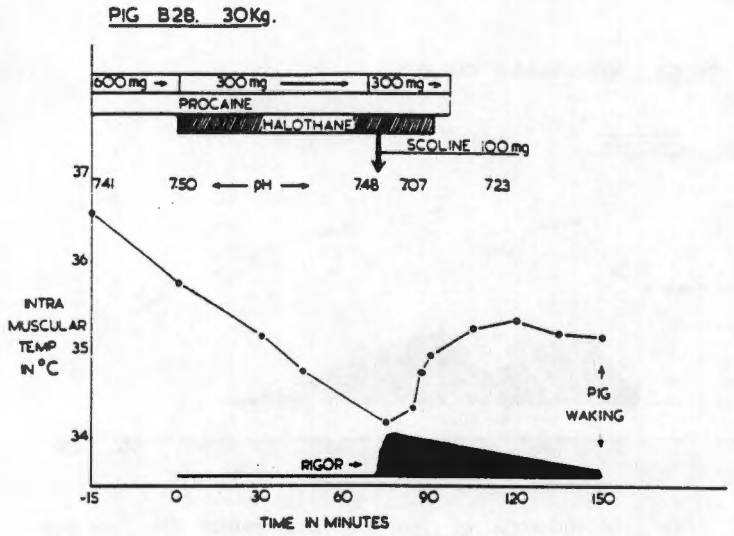

Figure 23-9.

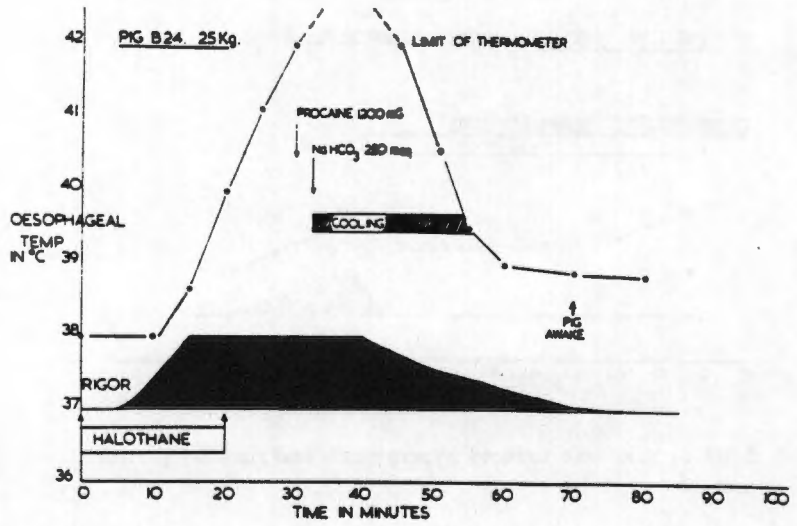

Figure 23-10. Temperature record of established syndrome treated by procaine.

DISCUSSION

These experiments on the initiation of anaesthetic-induced malignant hyperpyrexia by halothane in susceptible pigs show the following:

1. Halothane can trigger the syndrome in the presence of neuromuscular block.

2. Procaine effectively blocks halothane initiation of the syndrome.

We may infer from the first observation that the site of action of halothane must be at some point in the excitation-contraction mechanism distal to the muscle endplate. From the second, considered in association with what we know of the genesis of caffeine rigor, procaine's action thereon, and Kalow's observations on the reticular function of human malignant hyperpyrexic survivors, we may infer that this site is probably sarcoplasmic reticulum. But as halothane has such an action only on certain genetically predisposed pigs,[6] we must postulate that in these animals the sarcoplasmic reticulum itself has some intrinsic functional defect—possibly some defect in the uptake of or binding of calcium from the sarcoplasm. The existence of some such defect is suggested by our earlier observation that the ATP concentration in muscle *in vitro* falls more rapidly in muscle from susceptible pigs than in that from controls. Such a fall could be secondary to excess or more rapid than normal utilisation of ATP, following sarcotubular calcium release. Kalow and co-workers[11] have shown that halothane enhances this defect in susceptible humans. We may postulate that this would allow the sarcoplasmic level of calcium to rise sufficiently in susceptible animals to initiate the contraction process. Once contraction is initiated, the continued presence of calcium ions serves to maintain the rigor and the conditions for the runaway glycolysis, which we regard as a hallmark of the syndrome, will be present. Berman's recent observation[20] that in the very early stage of the syndrome, triggered by halothane, ATP levels are maintained and, in fact, rise slightly, would support this postulate. Once the syndrome is initiated the intrinsic hypoxia and high temperature would further damage

the reticular calcium pump, perpetuating the condition and explaining our observation that once the syndrome is firmly initiated, removal of the cause—halothane—leads to no amelioration of the condition. Halothane serves verily a trigger role. Doubtless the depressant effect of halothane on mitochondrial respiration[21] is but another factor in the early initiation of this vicious cycle.

My findings with regard to the initiation of the syndrome by succinylcholine also fit well the postulate of a reticular calcium binding and/or release defect.

1. The observation that succinylcholine initiation of the syndrome is effectively blocked by the neuromuscular block of tubocurarine indicates that end plate depolarization is necessary to the trigger action of succinylcholine in this condition. The diffuse muscle depolarization and contraction that follow succinylcholine administration doubtless presents the defective sarcotubular pump with more calcium than it can rapidly remove, so contraction persists. It is well documented that the muscle fasciculation that follows the administration of succinylcholine in affected animals and humans is gross and sustained, progressing to rigor. The observation by Sybesma and Eikelenboom[22] that severe exercise alone will initiate the hyperpyrexic syndrome in susceptible animals would also support this postulate that it is the presentation to the reticular pump of an above-threshold task of calcium removal, rather than some action of succinylcholine per se, which is the basic mechanism by which succinylcholine induces the syndrome. Again, once contraction is initiated and maintained by the continued presence of calcium ions in the sarcoplasm, the conditions for the runaway glycolysis with its built-in vicious cycle will be present.

2. Procaine infusion also prevented succinylcholine initiation of the syndrome. It did not prevent the muscle fasciculation induced by succinylcholine, but definitely reduced it in degree. This reduction in the degree of muscle fasciculation may well represent a reduction in the magnitude of calcium ion efflux to a level that could still be effectively removed by the "defective" reticular pump so that relaxation could supervene.

3. My last observation that though procaine blocks the initia-

tion of the syndrome by halothane and succinylcholine individually. it does not when these two drugs are given together may simply represent the result of a summation of effects. Of importance is the observation that the syndrome so initiated appears to be reversible, in contrast to its inexorable course in the absence of procaine. It is relevant that in contrast to the findings of others, Weber and Herz[15] recorded the observation that *in vitro* procaine, besides blocking, could reverse early caffeine rigor.

This work is also relevant to my observations on the effect of procaine infusion on the established syndrome. In five pigs in which the syndrome initiated by halothane was well established with profound rigor and temperature in excess of 42°, procaine infusion resulted in softening of the rigor and cessation of temperature rise. Two of these pigs survived. I report these last observations perhaps prematurely, because to me they provide the first glimmer of hope of some specific treatment in a condition which, though rare, results in a frightening mortality for those afflicted. Investigation into factors which enhance calcium ion uptake by the sarcoplasmic reticulum will, I am sure, lead ultimately to a pharmacologically rational and successful treatment of this dread condition.

SUMMARY

The clinical features of anaesthetic malignant hyperpyrexia in genetically susceptible Landrace pigs are described.

In such pigs it is demonstrated that procaine blocks the initiation of the hyperpyrexic syndrome by both halothane and succinylcholine. Curare pretreatment prevents the trigger action of succinylcholine only.

A preliminary report of the use of procaine in the treatment of the established syndrome is given.

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Anaesthetic-induced Malignant Hyperpyrexia: A Suggested Method of Treatment

GAISFORD G. HARRISON

Summary

Experiments in susceptible Landrace pigs have shown that procaine blocks the initiation of anaesthetic-induced malignant hyperpyrexia by both halothane and succinylcholine. Pretreatment with curare prevents only the trigger action of succinylcholine. In a preliminary study procaine was used to treat the established syndrome in five pigs, two of which survived. On the basis of these findings a treatment regimen can be suggested for patients who develop malignant hyperpyrexia.

Introduction

Though rare, anaesthetic-induced malignant hyperpyrexia continues to attract attention because of its unpredictability, the frightening mortality in those afflicted (in excess of 70%), the puzzle of its pathogenesis, and the complete lack of rational treatment (*British Medical Journal*, 1968). There is evidence that the lesion or abnormality responsible for this syndrome lies within muscle (Satnick, 1969; Denborough *et al.*, 1970; Harrison *et al.*, 1970; Isaacs and Barlow, 1970). This knowledge was greatly extended by Kalow *et al.* (1970) when they reported

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three fundamental observations made on muscle preparations from human survivors of malignant hyperpyrexia. These were that (1) such muscle was more sensitive to caffeine rigor than that of normal controls, (2) this effect was enhanced by exposure to halothane, and (3) halothane depressed calcium uptake by the sarcoplasmic reticulum of these patients whereas it had no effect on that of normal controls.

Caffeine rigor in muscle due to an enhanced release and depressed rebinding of calcium ions by the sarcoplasmic reticulum (Weber and Herz, 1968; Weber, 1968) has long been known to be blocked by procaine (Feinstein, 1963), an effect due to an action of procaine itself on the reticulum and not procaine caffeine complexing.

An obvious question that must follow from these observations is that if halothane and caffeine produce similar changes in sarcoplasmic reticular function, and in fact enhance each other's action, would not procaine block such action of halothane in the same manner as it is known to block caffeine rigor? (Strobel, 1971). Also, what effect would procaine have on succinylcholine initiation of the syndrome, succinylcholine being with halothane the common initiator of the syndrome? (Wilson *et al.*, 1967; Daniels *et al.*, 1969; Harrison *et al.*, 1969; Britt and Kalow, 1970). Further, what was the common pathway by which both halothane and succinylcholine initiated malignant hyperpyrexia?

The answer to these questions was sought in the following experiments on Landrace pigs, known from halothane pre-screening to be susceptible to malignant hyperpyrexia (Harrison *et al.*, 1968, 1969).

Experimental Method

All experiments were carried out under a standard general anaesthetic consisting of induction of anaesthesia with thiopentone, endotracheal intubation, and maintenance of anaesthesia with nitrous oxide and oxygen with intermittent positive-pressure respiration. Under these conditions the influence of pretreatment with tubocurarine and procaine on the initiation of the hyperpyrexic syndrome by halothane and succinylcholine was studied. The drugs were administered in the following combinations and sequences—each sequence forming a separate experiment—the number of times each experiment was repeated is given in parentheses: tubocurarine—halothane (1), tubocurarine—succinylcholine (1), procaine—halothane (6), procaine—succinylcholine (2), and procaine—halothane—succinylcholine (3).

Tubocurarine and succinylcholine were administered as single doses. Procaine was administered as a 0·6% solution by

intravenous drip infusion, a loading dose of 30 mg/kg preceding maintenance infusion at rates which varied between 0·2 to 1 mg/kg/min. (Use in vitro of 1 to 3·3 mM concentrations is described (Weber, 1968; Johnson and Inesi, 1969).) When using procaine in this order of dosage together with halothane bradycardia and hypotension were invariable and it was expedient to support the circulation with an infusion of isoprenaline (concentration 1 μg/ml).

The onset of the syndrome was identified by (1) the onset of rigor, monitored crudely by means of a write-out on a revolving drum attached by a string to the pig's hind leg, and (2) the rapid rise in muscle temperature, monitored by an intramuscular probe. These two observations, rigor and rise of temperature, were considered adequate for identification of the syndrome. Serial biochemical values were measured on central venous blood of some pigs.

When the syndrome did not develop after procaine pretreatment, the animal was re-exposed subsequently under thiopentone anaesthesia to halothane in the absence of procaine. Such animals all developed the hyperpyrexic syndrome.

In five pigs the effect of procaine infusion on the established syndrome was studied and the results were compared with previously tried methods (Harrison *et al.*, 1969).

Results

The results of these experiments are set out in Tables I and II. The administration of tubocurarine did not prevent the initiation of the syndrome by halothane, but did prevent its initiation by succinylcholine. Procaine blocked initiation of the syndrome both by halothane and succinylcholine when these drugs were given separately. When succinylcholine was given in the presence of halothane fasciculation passed on into rigor and a fulminant rise in temperature occurred in two of the three pigs. This rigor was ultimately controlled by continued procaine infusion,

TABLE I—*Thiopentone, N_2O/O_2 Anaesthesia*

Initiating Agent	Pretreatment		
	Nil	Curare	Procaine
Halothane	+	+	−
Succinylcholine	+	−	−
Halothane succinylcholine			+ −

+ = Initiation of hyperpyrexic syndrome.
− = No initiation of hyperpyrexic syndrome.

the rigor softening with concurrent cessation of temperature rise. The biochemical changes recorded as accompaniments of the syndrome appeared only in the presence of rigor and temperature rise and reverted towards normal when these were overcome.

In five pigs in which the syndrome was well established, with the temperature in excess of 42°C, large doses of procaine led to an ultimate relaxation of the rigor. Acidosis was treated with infusion of sodium bicarbonate. One pig was actively cooled. Two pigs survived. No other method of treatment we have used has ever achieved this (Harrison *et al.*, 1969).

TABLE II—*Results of Treatment of Established Syndrome*

Established Syndrome Treated with	Result
Active cooling, correction of acidosis, etc. (Harrison *et al.*, 1969)	Death in rigor
Tubocurarine (Harrison *et al.*, 1969) ..	Death in rigor
Procaine	Rigor relaxed. Temperature rise stopped. Survivors

Discussion

The findings of these experiments that (1) halothane can initiate the syndrome in the presence of neuromuscular block, and (2) procaine effectively blocks halothane initiation of the syndrome add support to the observations of Kalow *et al.* (1970) that a defect in sarcoplasmic reticular calcium release/binding is the probable lesion responsible for the syndrome of anaesthetic-induced malignant hyperpyrexia. The observation that curare as well as procaine blocks the initiation of the syndrome by succinylcholine indicates that endplate depolarization is necessary for this drug's initiation of the syndrome—an observation borne out by Sybesma and Eikelenboom's (1969) observation that severe exercise alone will initiate the hyperpyrexic syndrome in susceptible pigs. The common pathway by which succinylcholine, severe exercise, and halothane initiate the syndrome can be postulated to be a defective reticular calcium-binding mechanism which allows persistence of a high concentration of calcium ions in the sarcoplasm and so persistent rigor.

In contrast to the findings of others, Weber and Herz (1968) recorded the observation that procaine in vitro besides blocking could reverse early caffeine rigor. This is relevant to my observa-

tion that the syndrome when initiated by succinylcholine given together with halothane in the presence of a procaine infusion seems to be reversible, in contrast to its inexorable course in the absence of procaine. It is relevant, also, to the observation that in five pigs in which the syndrome initiated by halothane was well established with profound rigor and temperature in excess of 42°C procaine infusion resulted in softening of the rigor and cessation of temperature rise. Two of these pigs survived.

Bearing in mind the above and what we know of the severe biochemical changes—the runaway glycolysis and profound acidosis—which accompany the syndrome (Berman *et al.*, 1970), I would suggest the following steps in the treatment of this syndrome: (1) discontinuance of anaesthetic agents; (2) rapid correction of acidosis by administration of trometamol or sodium bicarbonate; (3) immediate institution of active and aggressive cooling; (4) administration of procaine by intravenous infusion—a loading dose of 30-40 mg/kg should be followed by infusion at a rate of about 0·2 mg/kg/min until muscle rigor relaxes; support of the circulation by an infusion of isoprenaline will be necessary and E.C.G. monitoring is mandatory; and (5) correction of the hyperkalaemia; if initial correction of the acidosis does not bring the serum K^+ to normal levels administration of insulin and glucose should be resorted to.

This investigation is incomplete and requires further refinement. There may well be a time by which the syndrome is irreversible by procaine. The present early publication of these findings is prompted by the lack of specific treatment for the syndrome based on a knowledge of its pathogenesis. For the future, investigation of factors which enhance calcium ion uptake by the sarcoplasmic reticulum will, I am sure, lead ultimately to a pharmacologically rational and successful treatment of this dread condition.

I wish to express my gratitude to Mrs. Carin Verburg and Dr. Colin Beighton for excellent technical assistance and to Mr. Brian Sassman for the initial screening and selection of susceptible pigs, as well as post-anaesthetic recovery care. I am indebted to the liver research group and the department of surgery of the University of Cape Town for the supply of pigs. Electrolyte estimations were performed by the department of chemical pathology of the University of Cape Town.

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Malignant Hyperpyrexia

The advent of new syndromes in medicine is often treated with scepticism, but though the first case of malignant hyperpyrexia was reported only just over five years ago it has now become fully established as a condition in its own right. Essentially it is characterized by a rapid rise of body temperature in patients undergoing general anaesthesia for surgery. The two drugs most commonly reported as triggering agents are suxamethonium (muscle-relaxant) and halothane (inhalational anaesthetic). Concomitant with the rise in body temperature are the signs of increased muscle metabolism, such as tachycardia, tachypnoea, sweating, and blotchy cyanosis.

There would appear to be one distinctive sign—namely, rigidity—which is present in the majority of patients.[1] In a typical case this rigidity follows immediately on the administration of suxamethonium or alternatively develops insidiously during halothane anaesthesia. If treatment is unsuccessful, it merges into rigor mortis. Indeed the mechanism of the latter condition has been sought to explain malignant hyperpyrexia. In about one-quarter of the patients this rigidity does not appear at any stage until after death, so possibly these cases are different both in signs and in aetiology.

W. Kalow and his co-workers[2] investigated three patients who had suffered malignant hyperpyrexia and found changes of metabolism in muscle biopsy specimens in those patients who had experienced rigidity. The calcium uptake in the sarcoplasmic reticulum was low after exposure of the patient to halothane. They concluded that the lesion in the rigid type of malignant hyperpyrexia was an inability of the sarcoplasmic reticulum to store calcium. This meant that the concentration of calcium in the cytoplasm remained high, the enzyme myosin adenosinetriphosphatase was not activated, and the myofibrils remained forever locked together as in contraction.

P. Furniss[3] has pointed out that the age of the patient is significant when attempting to differentiate between the rigid and non-rigid types of malignant hyperpyrexia. Those reacting with hypertonus are usually young (under 20) and the abnormality is probably hereditary, while most of the non-rigid cases occurred in patients over 20 years old, and the incidence appeared to be sporadic.

Approaching the matter from a different angle, H. Isaacs and M. B. Barlow[4] investigated 99 members of a single family with a history of malignant hyperpyrexia. They found that the resting creatine phosphokinase and aldolase levels were high in many of their patients. They concluded that the high level of muscle enzymes in the serum was evidence of a subclinical myopathy, and that anaesthetic agents like suxamethonium and halothane are capable of damaging muscle metabolism and triggering off a fulminating hyperpyrexic reaction. On this basis they have suggested that the enzyme levels of susceptible patients should be measured preoperatively, and if they are found to be raised the anaesthetic agents that cause the trouble can be avoided.

R. A. Pollock and R. L. Watson[5] have pointed out that the syndrome of malignant hyperpyrexia from anaesthetic agents is very similar to that sometimes observed after giving a combination of tricyclic antidepressants and the monoamine inhibitors. These patients too are usually young (20-30), and within a few hours of starting treatment with the combined therapy they experience sweating and hyperpyrexia, leading to rigidity. The mortality is about 60 to 70%. The authors suggest that these drugs might induce a hypermetabolic state by increasing the intracellular concentration of cyclic adenosine monophosphate (A.M.P.). This would provide an alternative theory to the one based on the uncoupling of oxidative phosphorylation.[6]

In this issue of the *B.M.J.* (page 454) Dr. Gaisford G. Harrison approaches the matter from another angle. He argues that the rigidity produced by caffeine is known to be due to a depressed rebinding of calcium ions by sarcoplasmic reticulum and that this process can be blocked by procaine. Could this also be true for malignant hyperpyrexia? He was able to show that large doses of procaine could abolish the rigidity of experimentally produced malignant hyperpyrexia in the Landrace pig even at a late stage of the condition. This is a most important observation, for as well as adding further confirmation to the calcium theory it offers for the first time a really practical approach to the treatment of the patient. In this respect experience has shown that intensive cooling is required and that general vasodilation is important in reducing the internal temperature of the body. The administration of large doses of procaine

(a well-recognized vasodilator), accompanied by isoprenaline to support the circulation, is an interesting new approach to this problem.

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Pathology of Malignant Hyperpyrexia

Malignant hyperpyrexia, a rare but often fatal complication of general anaesthesia, was first described only some six years ago, but since then reports of the condition have come from many parts of the world. It has been described most often in patients receiving suxamethonium as a muscle relaxant, or halothane, or both.

A rapid rise of body temperature is usually accompanied by signs of increased muscle metabolism such as tachycardia, tachypnoea, sweating, and cyanosis.[1] Typically the rise in temperature is accompanied by increasing muscle rigidity. This may follow immediately after the administration of suxamethonium but comes on more slowly during halothane anaesthesia. However, P. Furniss[2] pointed out that rigidity was not seen in all cases. This symptom was found to be usual in the common, genetically dominant form of the abnormality. It is usually then recognized in childhood and early adult life. A sporadic form is less common, and many of the patients with it are reported to be much older when the condition is first recognized.

W. Kalow and his co-workers[3] studied muscle biopsy samples obtained from patients in whom hyperpyrexia had been accompanied by muscle rigidity and found that the calcium uptake in the sarcoplasmic reticulum derived from such samples was low after exposure to halothane. They suggested, therefore, that the primary lesion is an inability of the sarcoplasmic reticulum to store calcium. Among others, H. Isaacs and M. B. Barlow[4] noted a high resting serum creatine phosphokinase activity in affected members of a family. They suggested that this indicated the presence of a subclinical myopathy. The extensive studies of J. O. King and co-workers,[5] based on the information they obtained from 18 propositi and their families, has given some support to this suggestion. All six survivors tested had high serum creatine phosphokinase levels, and they concluded that all patients who develop malignant hyperpyrexia have one of a number of specific myopathic disorders. In the first group there is evidence of a dominantly inherited myopathy, which they found in 9 of their 18 families, though in 6 of these the myopathy was not clinically detectable in affected individuals, while only 3 showed evidence of overt muscular weakness. The second group of susceptible patients showed clinical and electromyographic evidence of myotonia congenita, and a similar case was reported some years ago by L. J. Saidman and colleagues.[6] In their third group were patients who had associated physical abnormalities including short stature, cryptorchidism, lumbar lordosis, and thoracic kyphosis, as well as evidence of a progressive congenital myopathy.

A disorder similar to the human disease has been described in certain Landrace pigs.[7 8] A finding that confirms the work of Kalow and colleagues[3] in man is that the pigs' muscle is more sensitive to caffeine-induced rigor than is that of normal controls, and this effect is enhanced by exposure to halothane. Recently G. G. Harrison[9] suggested that procaine, given as a 0·6% solution by intravenous drip infusion to susceptible pigs, could sometimes protect the muscle from the effects of halothane and also appeared to have some beneficial effect even after the syndrome had developed. So far these protective and therapeutic effects of procaine have not been confirmed in man.

Controversy continues on whether measurements of serum creatine phosphokinase activity alone are a sufficiently reliable screening test for liability to malignant hyperpyrexia. There is also some dispute about the presence or absence of histological changes in the muscles of affected patients. In a recent study of seven persons from four families F. R. Ellis and colleagues[10] found on muscle biopsy in five of them non-specific myopathic changes. These included variations in fibre size, fibre atrophy with nuclear clumping, mitohondrial aggregates, and fibres with a "moth-eaten" appearance in sections stained for oxidative enzymes, together with variable changes in the motor end-plates. These histological changes correlated well with abnormal muscle contractures induced by halothane or by halothane and suxamethonium in vitro. In one patient, however, in whom there was histological evidence both of myopathy and of muscle contractures induced by halothane, the serum creatine phosphokinase activity was within normal limits. Recently W. G. Bradley and D. Murchison,[11] who studied four persons at risk in two families, found that all but one had a raised serum creatine phosphokinase activity, but none showed histological evidence of myopathy, and only one muscle sample developed contracture when exposed to halothane in vitro. The patient from whom it came had the highest resting serum creatine phosphokinase activity of any of those studied.

The position has been further complicated by the observation of M. A. Denborough and his co-workers, who report in a paper in the *B.M.J.* this week (page 272) that a muscle biopsy obtained from a susceptible patient showed the characteristic histochemical and ultramicroscopic abnormalities of "central core disease"—a myopathy with a characteristic histological appearance of cores in the muscle fibres.[12 13] Abnormal tubular aggregates, probably representing sarcoplasmic reticulum, were also found in the muscle fibres from this patient when they were examined under the electron-microscope.

Evidence is now accumulating to suggest that a number of histological abnormalities in voluntary muscle previously thought to be specific, including the central core anomaly, may have less specificity than was previously thought to be the case. Nevertheless it would be interesting to see whether any further muscle biopsy samples (obtained under local, not general, anaesthesia) from persons liable to suffer from malignant hyperpyrexia show similar histological changes. No cases of malignant hyperpyrexia, so far as is known, have yet been reported in patients suffering from typical Duchenne muscular dystrophy, dystrophia myotonica, or muscular dystrophy of the facio-scapulo-humeral or limb-girdle types. Nevertheless, it is becoming increasingly clear that this curious metabolic anomaly of the musle cell, which predisposes the affected person to this potentially fatal complication during anaesthesia, may be associated with a variety of myopathic disorders which are different clinically and histologically.

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ERYTHROCYTE OSMOTIC FRAGILITY IN HYPERTHERMIA-SUSCEPTIBLE SWINE

G. G. HARRISON AND C. VERBURG

SUMMARY

The observation is reported that hyperthermia-susceptible swine manifest an increased red cell osmotic fragility. The importance of this observation is discussed.

Anaesthetic-induced malignant hyperpyrexia in humans appears to result from some intrinsic abnormality of muscle (Satnick, 1969; Harrison et al., 1970; King, Denborough and Zapf, 1972). Evidence points to this defect being in the sarcoplasmic reticulum (Kalow et al., 1970). A certain strain of Landrace pigs manifests a similar condition and has been used as an animal experimental model for the investigation of various aspects of the disease and trials of therapeutic measures (Berman et al., 1970; Harrison, 1971).

While taking blood specimens for various investigations from such hyperthermia-susceptible pigs, we observed that more often than not these specimens were markedly haemolysed. This observation prompted us to investigate the erythrocyte osmotic fragility of these pigs.

METHODS

Hyperthermia-susceptible swine were initially chosen by observing the reaction of all pigs acquired for the Surgical Research Laboratory to a screening challenge of halothane inhalation (Harrison et al., 1969). Hyperthermia-susceptible swine react in an obvious and highly characteristic fashion by manifesting extreme muscle rigor within about 10 min of exposure to halothane and recover if the halothane is immediately discontinued. Susceptibility to hyperthermia was confirmed in positive reactors by estimation of serum c.p.k. levels, which in hyperthermia-susceptible swine remain persistently raised (Woolf et al., 1970).

The red cell fragility of three such pigs, aged 9–14 weeks, was investigated over a time some weeks after the original test exposure to halothane. Three specimens of blood—a week elapsing beween successive specimens—were taken from each pig under brief thiopentone anaesthesia. This blood was drawn from one of the large veins in the thoracic inlet through a wide-bore needle (15 s.w.g.), care being taken to exert the minimum of suction. The fragility of blood drawn under similar conditions from nine non-reactor pigs, non-reactivity being confirmed by low serum c.p.k. levels, served as a control.

Osmotic fragility was tested by the standard method (Dacie and Lewis, 1968) of estimating colorometrically the degree of haemolysis achieved in serial dilutions of saline solutions. Differences between groups of mean percentage haemolysis at each concentration of saline were tested statistically by a standard Student *t*-test.

RESULTS

The results (table I and figs. 1 and 2) reveal that these hyperthermia-susceptible pigs manifest a greater erythrocyte osmotic fragility than normal. The median corpuscular fragility of the hyperthermia-susceptible swine lay between 0.55 and 0.50% saline, while that of the control pigs was between 0.50 and 0.45% saline. (The human value

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TABLE I. *Mean (and SE) percentage haemolysis at % concentration of saline.*

Type of Pig	1.1	0.95	0.85	0.75	0.65	0.60	0.55	0.50	0.45	0.40	0.30	0.20	0.10
Hyperth.- susceptible pigs (9 samples)	0	0	0.2 ±0.1	0.7 ±0.1	7.8 ±1.1	20.7 ±2.3	38.9 ±2.2	68.2 ±2.6	83.2 ±1.9	84.4 ±1.7	94.6 ±1.1	97.1	100
Control pigs (9 samples)	0	0	0	0.2 ±0.1	5.1 ±1.1	10.0 ±1.0	21.5 ±2.1	37.9 ±2.5	72.0 ±2.9	88.8 ±2.4	94.3 ±2.8	99.3	100
P value			P<0.10	P<0.05	P<0.10	P<0.001	P<0.001	P<0.001	P<0.01				
Significance of difference			n.s.	s.	n.s.	s.	s.	s.	s.	n.s.	n.s.	n.s.	n.s.

s. = significant difference n.s. = not significant difference

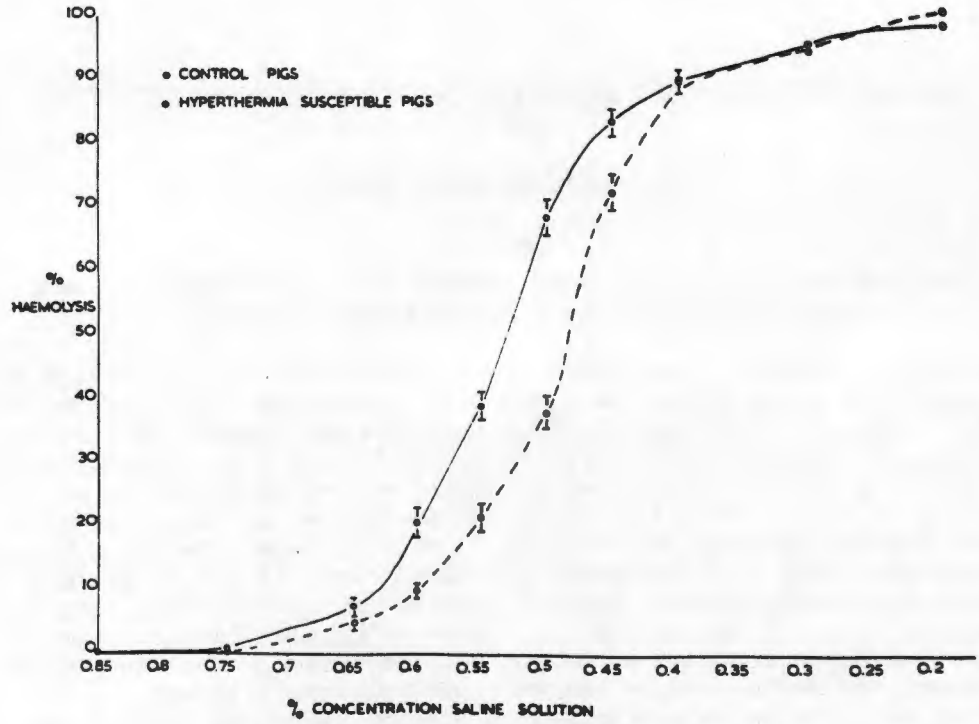

FIG. 1. Red cell fragility curve (mean values with SE).

for comparison lies between 0.445 and 0.40% saline; Dacie and Lewis, 1968.) The increment haemolysis curve (fig. 2) demonstrates a difference of 0.05% saline between peaks of maximal haemolysis.

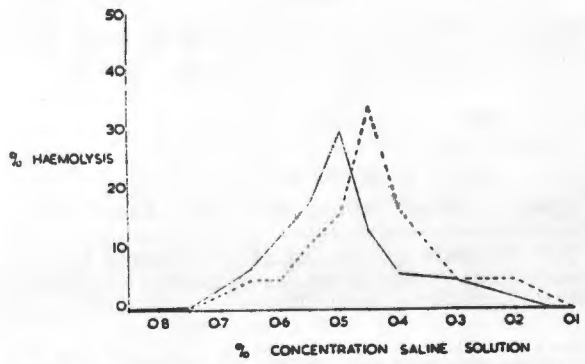

FIG. 2. Increment haemolysis curve. —— Hyperthermia-susceptible pigs. ------ Control pigs.

There is quite marked disparity in the few reported estimations of porcine red cell fragility (Schalm, 1965). As the pig populations on which these reported estimations were performed may well have inadvertently included hyperthermia-susceptible swine (Harrison, 1972), comparisons of fragility in this paper are made only with the screened controls described.

DISCUSSION

Abnormalities in red cell fragility depend to a large extent on the functional state of its surface membrane (Dacie and Lewis, 1968). The marked morphological similarity between this cell membrane and the membranes of cell organelles such as mitochondria, endoplasmic reticulum and Golgi apparatus led to Robertson's concept of these as "unit" membrane (Robertson, 1959). Though there are important functional differences between membranes which doubtless reside in the protein enzymes associated with the phospholipid skeleton, there is indeed a structural organization that is common to most cellular membranes (Fawcett, 1962; Giese, 1968).

It is this that confers relevance to the finding of increased red cell fragility in hyperthermia-susceptible swine reported here. There is evidence that anaesthetic-induced malignant hyperpyrexia results from some intrinsic abnormality in muscle, more specifi-

cally of the sarcoplasmic reticulum. Here is evidence that another "unit" membrane in animals susceptible to anaesthetic induced malignant hyperpyrexia is also defective.

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FRAGILITE OSMOTIQUE ERYTHROCYTAIRE CHEZ LE PORC SUSCEPTIBLE A L'HYPERTHERMIE

SOMMAIRE

Les auteurs rapportent avoir observé que des porcs susceptibles à l'hyperthermie manifestent une fragilité osmotique accrue des globules rouges et discutent l'importance de cette observation.

OSMOTISCHE FRAGILITÄT DER ERYTHROCYTEN BEI HYPERTHERMIE-EMPFINDLICHEN SCHWEINEN

ZUSAMMENFASSUNG

Es wird über die Beobachtung berichtet, daß Hyperthermie-empfindliche Schweine eine gesteigerte osmotische Fragilität ihrer roten Zellen aufweisen. Die Bedeutung dieser Beobachtung wird diskutiert.

FRAGILIDAD OSMOTICA ERITROCITARIA EN CERDOS SUSCEPTIBLES A LA HIPERTERMINA

RESUMEN

Se informa sobre la observación de que los cerdos susceptibles a la hipertermia presentan un incremento en la fragilidad osmótica de sus glóbulos rojos. Se discute la importancia de esta observación.

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Recent Advances in the Understanding of Anaesthetic-Induced Malignant Hyperpyrexia

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Summary. The hypothesis is developed that the lesion responsible for anaesthetic-induced malignant hyperpyrexia is a functional defect in the calcium release/binding mechanism of sarcoplasmic reticulum. Besides the use of pigs susceptible to a similar condition, recent work indicates that caffeinized frog or rat muscle may also serve as an "in vitro" pharmacological model of the myopathy. A regime of treatment is suggested based on current concepts of the pathogenesis.

Jüngste Fortschritte im Verständnis der Anaesthesie-bedingten malignen Hyperpyrexie.

Zusammenfassung. Folgende Hypothese wurde entwickelt: die für eine durch Anaesthesie hervorgerufene bösartige Hyperpyrexie verantwortliche Läsion ist ein funktioneller Defekt im Calcium auf- und abbauenden Mechanismus des sarkoplasmischen Reticulums. Außer dem Gebrauch von Schweinen, die für ähnliche Bedingungen anfällig sind, haben neuere Ergebnisse gezeigt, daß Frosch- oder Rattenmuskeln, die unter dem Einfluß von Coffein stehen, auch als ein "in vitro" pharmakologisches Modell der Myopathie dienen können. Ein Behandlungsprotokoll wird auf der Basis gegenwärtiger Konzepte der Pathogenese vorgeschlagen.

Though rare, anaesthetic-induced malignant hyperpyrexia continues to attract attention because of its unpredictability, the frightening mortality in those afflicted (in excess of 70%), the puzzle of its pathogenesis and the complete lack, until recently, of any approach to a rational treatment [1–3]. Approximately 180 cases are recorded in the world medical literature.

Much of the little we today know of the pathogenesis of the condition has followed the happy chance discovery that certain pigs suffer from the same condition, thus providing us with an experimental model [4]. The condition, which appears to be related to the well known porcine stress syndrome (PSS) which results in pale, soft, exudative pork (PSEP) [5], is found as an autosomal dominant of a varying penetrance in 3 beeds of pig — the Landrace in South Africa Australia and England, the Pietrain in Holland and the Poland China pig in the United States and Canada [4, 6–9].

Early Theories

Initial theories embraced:

1. Disturbance of central heat homeostasis mechanisms.

2. Some form of stress response involving the adrenals.

3. Uncoupling of oxidative phosphorylation.

The first two had little to support them. The third, propounded by Wilson [10] and backed by experimental work in dogs using the uncoupling agent DNP with and without halothane anaesthesia, did seem to fit some of the facts. However, Wang's [11] argument that on purely theoretical grounds uncoupling alone could not produce the heat necessary for the rapid rise in temperature, was followed by the demonstration by Berman [12] in South Africa, Denborough [13] in Australia, Bruker [14] in the United States and Britt, Kalow and Endreyeni [15] in Canada that the mitochondria from affected animals and man were not uncoupled by halothane but, in fact, respiratory control was depressed. There is still a possibility though that uncoupling of oxidative phosphorylation together with mitochondrial abnormalities may be a feature in the non-rigid form of the disease, about one-quarter of human cases [16].

Site of the Lesion

Evidence gradually accumulated that the lesion or abnormality responsible for the syndrome lay within the muscle.

Following up the observation that muscle rigor was such a dramatic event in the syndrome, especially in the pig, we showed that the fall off in ATP content in muscle, when incubated "in vitro" in oxygenated Krebs solution was twice as great in muscle from reactor pigs, and this fall off was increased by the exposure of the muscle to halothane [4]. This indicated some fundamental abnormality in the muscle from reactor pigs. Satnick [17] described a patient who developed malignant hyperpyrexia during the course of an orthopaedic operation, involving the use of a tourniquet on an arm. The whole patient became rigid except the limb in which the circulation was occluded.

Perhaps the clearest demonstration that muscle was the site of the lesion was an experiment performed in our laboratories which showed that the muscle rigor and biochemical accompaniments of the syndrome, previously identified in the intact animal challenged with halothane, (see Figs. 2 and 3) [18] could be reproduced in a preparation of isolated perfused muscle (Fig. 1) [19]. This experiment showed, in addition, that the profound biochemical changes that were associated with the syndrome were not secondary to the hyperthermia, but evidence of an underlying biochemical lesion of which the ultimate rise in body temperature was an accompaniment or result. It also demonstrated conclusively that central heat homeostatic mechanisms and the adrenals had no part in the pathogenesis of this syndrome.

In quick succession thereafter, Isaacs and Barlow [20] found that a high proportion of the families of

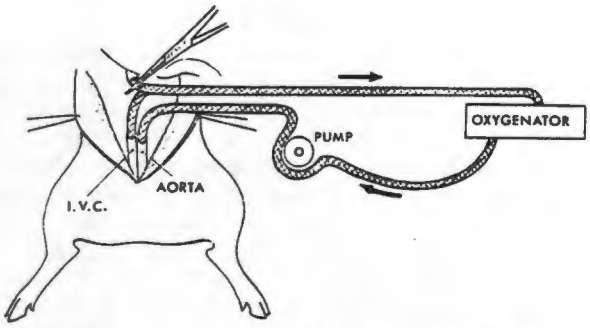

Fig. 1. Pig transected above pelvis. Legs perfused with standard pump oxygenator

victims of the syndrome had above normal serum levels of the muscle enzyme CPK; Denborough [21, 22] confirmed this and demonstrated that the serum levels of the same enzyme (as well as of SGOT and LDH) were grossly raised during and immediately following the actual attack, and Woolf [6] showed that affected pigs had the same raised resting levels of CPK.

In 1970, Kalow and co-workers [23] reported 3 fundamental observations made on muscle preparations from human survivors of anaesthetic-induced malignant hyperpyrexia. These were that:

1. Such muscle was more sensitive to caffeine rigor than that of normal controls.

2. This effect was enhanced by exposure to halothane.

3. Halothane depressed calcium uptake by the sarcoplasmic reticulum of these patients, whereas it had no effect on that of normal controls.

Kalow's finding of the halothane depressed SR calcium uptake was confirmed by similar observations in pig muscle sarcoplasmic reticulum by Bruker [14] and Denborough and co-workers [13].

Caffeine has long been known to cause a persistent contraction or rigor of muscle fibre which persists until the caffeine is removed. This rigor is due to an enhancement of release and an interference with rebinding of calcium ions by the sarcoplasmic reticulum. This allows a net efflux of calcium from the sarcoplasmic reticulum, a rise in sarcoplasmic calcium concentration, which activates myofibrillar ATPase and maintains contraction [24, 25]. Halothane it seemed acted in a somewhat similar fashion to caffeine on a SR abnormally sensitive to this type of action, so leading to rigor in susceptible animals.

Procaine is known to block this effect of caffeine in muscle by preventing calcium efflux. This effect is due to an action on the reticulum, and not due to a complexing with the caffeine. Once caffeine rigor has become well established, procaine has been said to have no effect [26, 27].

These observations led us to investigate the effect that pretreatment with curare (blocking the neuromuscular junction) and procaine (blocking the SR) might have on initiation of the syndrome of malignant hyperpyrexia by halothane and succinylcholine in susceptible Landrace pigs [28]. The results of these experiments (see Tables 1 and 2) showed that the administration of tubocurarine did not prevent the initiation of the syndrome by halothane, but did prevent its initiation by succinylcholine. Procaine blocked initiation of the syndrome both by halothane and

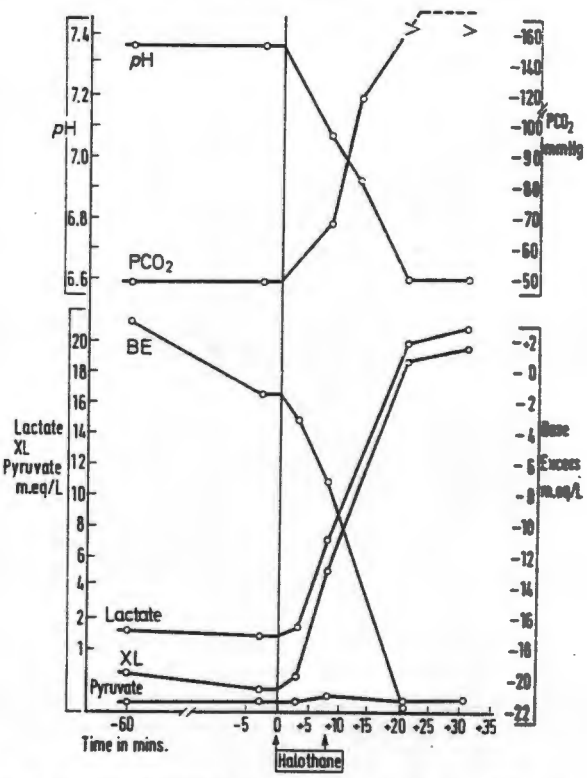

Fig. 2. Changes observed in acid base parameters following initiation of malignant hyperpyrexia by Halothane in a susceptible pig

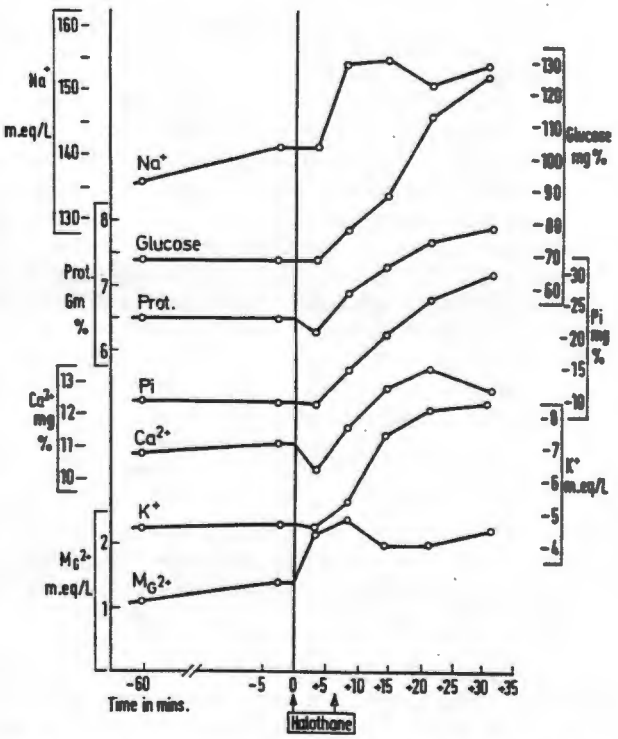

Fig. 3. Changes observed in serum electrolytes following initiation of malignant hyperpyrexia by Halothane and a susceptible pig

Table 1. Thiopentone, N_2O/O_2 Anaesthesia

Initiating agent	Pretreatment		
	Nil	Curare	Procaine
Halothane	+	+	−
Succinylcholine	+	−	−
Halothane succinylcholine			+ −

+ = Initiation of hyperpyrexic syndrome.
− = No initiation of hyperpyrexic syndrome.

Table 2. Results of treatment of established syndrome

Established syndrome treated with	Result
Active cooling, correction of acidosis, etc. (Harrison *et al.*, 1969)	Death in rigor
Tubocurarine (Harrison *et al.*, 1969)	Death in rigor
Procaine	Rigor relaxed. Temperature rise stopped. Survivors

succinylcholine when these drugs were given separately When succinylcholine was given in the presence of halothane fasciculation passed on into rigor and a fulminant rise in temperature occurred in two of the three pigs. This rigor was ultimately controlled by continued procaine infusion, the rigor softening with concurrent cessation of temperature rise. The biochemical changes recorded as accompaniments of the syndrome appeared only in the presence of rigor and temperature rise and reverted towards normal when these were overcome.

In five pigs in which the syndrome was well established, with the temperature in excess of 42°C, large doses of procaine led to an ultimate relaxation of the rigor. Acidosis was treated with infusion of sodium bicarbonate. One pig was actively cooled. Two pigs survived. No other method of treatment we have used has ever achieved this [4].

The findings of these experiments add support to the observations of Kalow *et al.* [23] that a defect in sarcoplasmic reticular calcium release/binding is the probable lesion responsible for the syndrome of anaesthetic-induced malignant hyperpyrexia. The observation that curare as well as procaine blocks the initiation of the syndrome by succinylcholine indicates that endplate depolarization is necessary for this drug's initiation of the syndrome — an observation borne out by Sybesma and Eikelenboom's [9] observation that severe exercise alone will initiate the hyperpyrexic syndrome in susceptible pigs. The common pathway by which succinylcholine, severe exercise, and halothane initiate the syndrome can be postulated to be a defective reticular calcium-binding mechanism which allows persistence of a high concentration of calcium ions in the sarcoplasm and so persistent rigor.

The fact that these reactions to halothane and succinylcholine occur only in certain genetically predisposed pigs (and humans) points in all probability to some genetically determined functional abnormality of the SR membrane. The observation made recently in our laboratory of increased red cell fragility in malignant hyperthermia susceptible pigs shows that other "unit membrane" structures are also defective in these animals [29].

In vitro Models

The susceptible Landrace pig has proved a good animal model for pharmacological and other research into the condition. But susceptible pigs are not always easy to obtain, their supply is not universal and the pig as an experimental animal may prove expensive. For research into this condition to be made possible at more centres, a more readily available and cheaper animal model is essential. Strobel and Bianchi's [30] demonstration that caffeinised frog sartorius muscle behaves in a manner similar to that of susceptible pigs and the use in our laboratory of caffeinised rat rectus muscle repeatedly supramaximally stimulated with similar results [31] promises well for the future in this regard.

Treatment

Though methods of blocking induction of the syndrome contribute to the forming of an hypothesis as to its pathogenesis, such prevention has no practical value in a clinical situation. However, an observation made by Weber and Herz [24] on the effect of procaine on caffeine rigor offers us some hope of finding a rational treatment for this lethal condition. In contrast to the findings of others, they recorded the observation that procaine, besides blocking, could reverse early caffeine rigor.

Following this up, we showed that procaine infusion resulted in the softening of rigor and cessation of temperature rise in pigs in which the syndrome was well established [28]. Recent patients who have survived the syndrome, or improved on treatment, have had procaine infusions included in their treatment [32–34]. In conclusion, therefore, to give some practical expression to our growing understanding of malignant hyperpyrexia biochemically (see Figs. 2 and 3) [18] and pharmacologically, I wish to suggest the following steps in the treatment of the syndrome.

Prevention

Pre-anaesthetic-family history and CPK estimation.

Awareness during anaesthesia-adequate monitoring of temperature.

Note any abnormal reaction to succinylcholine.

In event of any unexplained rise in temperature > 1°C, abnormal reaction to succinylcholine or sudden gross increase in temperature of sodalime canister, *ACT Immediately.*

1. Discontinue anaesthetic.
2. Hyperventilate with oxygen.
3. Correct acidosis with THAM or sodium bicarbonate.
4. Cool patient actively and aggressively.
5. Administer procaine by intravenous infusion — a loading dose of 30–40 mg/kg should be followed by infusion at a rate of about 0.2 mg/kg/min until muscle rigor relaxes or adequate cooling commences; support of the circulation by an infusion of isoprenaline may be necessary and ECG monitoring is mandatory.
6. Correct hyperkalaemia if correction of acidosis does not reduce this to reasonable levels.
7. Look out for and treat on its own merits any disseminated intravascular coagulation and subsequent fibrinolytic syndrome [35, 36].

8. Administer mannitol to protect kidney against the effect of myoglobinuria.

9. Do muscle enzyme studies and investigate patient's family.

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CONTROL OF THE MALIGNANT HYPERPYREXIC SYNDROME IN MHS SWINE BY DANTROLENE SODIUM

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SUMMARY

Experiments are described which demonstrate that dantrolene sodium effectively terminates the syndrome of malignant hyperpyrexia induced in susceptible swine by exposure to halothane. Dantrolene is also shown to block initiation of the syndrome of malignant hyperpyrexia by halothane in MHS swine. Therapeutic use of this drug in patients with anaesthetic-induced malignant hyperpyrexia appears to be indicated.

Anaesthetic-induced malignant hyperpyrexia is a rare, often fatal syndrome affecting man and the pig, which results from a genetic intrinsic functional defect within the muscle fibre (Relton, Britt and Steward, 1973; Harrison, 1973b). Rigor of muscle is its predominant clinical feature.

In 1967, Snyder and associates reported the synthesis of a series of hydantoins which proved to have muscle relaxant properties. One of these, dantrolene sodium* was extensively investigated and its pharmacological effects were demonstrated to follow an action on the intrinsic mechanism of muscle contraction. In addition, it was shown to act only on skeletal muscle and to have no effect on cardiac muscle or smooth muscle (Ellis et al., 1973). Because of this, the effects of dantrolene sodium on the syndrome of malignant hyperpyrexia induced by halothane in malignant hyperpyrexia susceptible (MHS) swine were investigated.

METHOD

In this investigation, use was made of an experimental protocol previously described (Harrison, 1973a). In MHS swine selected by reaction to halothane prescreening and estimation of serum c.p.k. levels, monitoring of vital parameters was established under initial ketamine or ketamine/thiopentone anaesthesia, followed by endotracheal intubation and maintenance of anaesthesia with nitrous oxide and oxygen. IPPV was provided when required by a Blease Pulmoflator.

Monitoring included:

(1) E.c.g.

(2) Observation of rigor (see fig. 1).

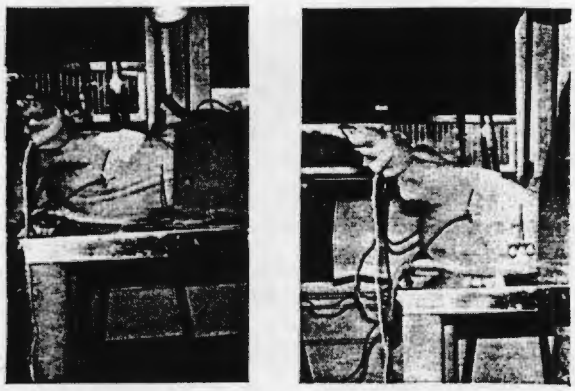

FIG. 1. MHS pig's hind legs before and after onset of rigor following halothane (note extension). The phenomenon may be recorded by attachment of the trotter by string over a pulley to a recording pen on a revolving drum. During rigor the muscle mass is palpably hard.

(3) Temperature measurement by means of a thermistor probe (Ellab, Denmark) inserted deep into the muscle mass of the thigh.

(4) Repeat sampling of mixed venous blood from a right atrial cannula.

Thereafter, the syndrome of malignant hyperpyrexia was initiated by the administration of halothane by IPPV commenced at a concentration of 2.5% and gradually reduced to 0.5% thereafter.

Once the hyperpyrexic syndrome was well established with marked muscle rigor, acidosis and an increase of temperature of 2°C or more, dantrolene sodium (0.5 mg/ml) was administered intravenously; a dosage of 1 mg/kg in early experiments was later increased to as much as 7–10 mg/kg.

The solubility of dantrolene is limited. The

*1-([5-(p-nitrophenyl) furfurylidene] amino) hydantoin sodium hydrate synthesized by Norwich Pharmacal Company Laboratories, New York.

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formulation used in these experiments was that described by Castellion (1973, personal communication):

dantrolene sodium 300 mg
mannitol 26.640 g
sodium hydroxide 48 mg
water to make 600 ml

Blood samples for acid-base, c.p.k. and potassium estimations were taken:

(1) Immediately before exposure to halothane.

(2) When the syndrome was established.

(3) After administration of dantrolene.

Eight such experiments were undertaken using 5 pigs, the experiment being performed three times in one pig, twice in another and once each in the remaining 3 pigs.

It must be appreciated that in this somewhat crude and empiric (though effective) intact animal experiment, judgement of the moment at which to commence treatment was difficult, and precise criteria as to the degree of temperature increase could not be adhered to. The rate at which reactor pigs develop the syndrome differs. While it was desired that the syndrome be well enough established in terms of muscle rigor and increase in temperature, to render any response to dantrolene unequivocal, care had to be taken not to jeopardize the entire experiment by risking sudden death of an animal from cardiac arrest resulting from the concomitant acidosis and hyperkalaemia. In some experiments, not only was the moment of commencing dantrolene dictated by the onset of cardiac arrhythmia, but in two animals "Isoptin" 5 mg (Iproveratril, Knoll, Germany) (shown in previous experiments to have no effect on the syndrome (Harrison, 1972, unpublished data)) was used to control this before the administration of dantrolene. Once well initiated, the syndrome is independent of the concentration of halothane, which appears to act as a trigger (Berman et al., 1970). In five experiments, halothane was discontinued 8–18 min before administration of dantrolene, the syndrome continuing unabated with further increases in temperature of from 0.5 to 1.9°C. In three experiments halothane was continued for 2–4 min after commencement of dantrolene. In neither event did this appear to affect the outcome.

The only ancillary treatment generally applied was the administration of sodium bicarbonate following the onset of rigor. Ambient temperature during these experiments was 21–22°C and with one exception active cooling was not used. In the exception, ice blocks were applied to an animal after the temperature had decreased from 43.8°C to 41°C. Ambient temperature on this day was 25°C.

Complementary to the therapeutic use of dantrolene, its ability to block initiation of the hyperpyrexic syndrome by halothane in MHS pigs was also investigated in two experiments (a week apart) on a single fast reactor pig. In these experiments, following establishment of monitoring under initial ketamine / nitrous oxide / oxygen anaesthesia as described, and treatment of the animal with dantrolene 3 mg/kg, the animal was exposed to halothane inhalation for 90 min. Commencing at 2.5%, the halothane concentration was reduced over 30 min to 1%, at which concentration it was maintained.

RESULTS

The results of these experiments with details of the duration of the malignant hyperpyrexic syndrome before treatment, the actual increase in body temperature, maximum temperature attained, dose of dantrolene and final outcome in terms of survival, are presented in table I. A temperature and events chart of one experiment, typical of all the experiments, is reproduced in figure 2.

In the established syndrome of malignant hyperpyrexia in susceptible pigs, the administration of dantrolene caused:

(1) Rapid loss of muscle rigor commencing within 5 min and usually complete within 20 min.

(2) Immediate cessation of the increase in deep muscle temperature followed by a rapid decrease.

(3) Termination of the progressive, inexorable acidosis characteristic of the syndrome (Harrison et al., 1969) rendering easy the buffering of acidosis developed until the dantrolene administration.

All pigs, except the first, used in the 8 experiments survived. This first pig, after showing a dramatic initial response to what, in the light of subsequent experience, proved to be too small a dose of dantrolene, suffered a recurrence of the syndrome with subsequent death (see table I).

Dantrolene pretreatment of an MHS pig effectively blocked initiation of the hyperpyrexic syndrome by halothane, allowing exposure of the animal to inhalation for 90 min with impunity. The time period of 90 min was chosen arbitrarily as being a period six times in excess of the previously tested "reaction" time of the pig used.

TABLE I

Pig No. and weight	Resting c.p.k. A units/ml at 25°C (normal 0-50)	MH duration before dantrolene (min)	Increase in temp. (°C)	Max. temp. (°C)	Dose of dantrolene (mg/kg)	Temp. decrease in first 20 min (°C)	Final temp. (°C)	Outcome
170 70 kg	364	36	3.2	40.1	1	0.6	42.0	Died
168 120 kg	720	14	2.1	38.5	1	1.9	36.7	Survived
168 125 kg	817	40	2.4	38.8	2.5	2.2	36.4	Survived
182 30 kg	4932	38	3.6	42.2	7	2.0	38.4	Survived
180 39 kg	1480	18	2.0	40.2	6	1.4	38.2	Survived
180 40 kg	1340	21	2.0	40.5	10	1.5	38.4	Survived
180 44 kg	1440	36	3.2	40.0	7	2.6	37.3	Survived
74 30 kg	348	30	3.8	42.8	10	1.8	38.0 Ice packs	Survived

Different weights recorded for the same pig used more than once reflect weight gain with time.

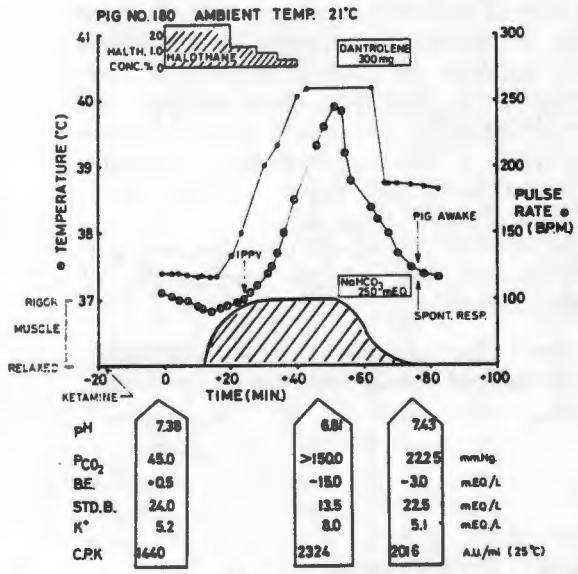

FIG. 2. Temperature (deep muscle) and events chart of typical experiment on MHS pig weighing 45 kg. Dantrolene administered as i.v. drip infusion for duration of square so marked. Biochemical values from mixed venous blood.

DISCUSSION

Untreated, the developed syndrome of malignant hyperpyrexia in pigs has a mortality rate of 100% (Harrison et al., 1969). While earlier work has demonstrated that it was possible to reverse the syndrome with procaine, especially if it was administered early enough (Harrison, 1971), the three out of five (60%) mortality for the established syndrome in pigs so treated described in this paper, has continued in our subsequent animal experiments. To date, procaine is the only drug that has been shown to have any effect on the established syndrome.

These experiments demonstrate that dantrolene has the property of relaxing the muscle rigor which characterizes malignant hyperpyrexia in the pig, and that concomitantly the excess heat and acid production cease. A survival rate of 100% was achieved in the last seven of eight experiments. In contrast to procaine, dantrolene has no effect on the myocardium, a factor which permits its use up to the limits of therapeutic effectiveness.

The pharmacology and toxicology of dantrolene have been extensively investigated in humans (Basmajian and Super, 1973; Chyatte and Birdsong, 1971; Chyatte, Birdsong and Bergman, 1971; Herman, Mayer and Newcombe, 1972) and it has been used extensively in the management of conditions characterized by muscle spasticity. The experiments reported here indicate that dantrolene should prove to be a most effective therapeutic agent in the treatment of malignant hyperpyrexia in humans.

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CONTROLE DU SYNDROME D'HYPERPYREXIE MALIGNE (DANS LES SUIDES MHS) AU MOYEN DU SODIUM DE DANTROLENE

RESUME

On décrit certaines expériences qui démontrent que le sodium de dantrolène met fin effectivement au syndrome d'hyperpyrexie maligne provoqué par l'exposition à l'halothane chez les suidés prédisposés. On montre également que le dantrolène bloque la manifestation initiale du syndrome d'hyperpyrexie maligne par l'halothane dans les suidés MHS. L'usage thérapeutique de ce remède semble indiqué pour les patients atteints d'hyperpyrexie maligne provoquée par un anesthésique.

BEKÄMPFUNG DES MALIGNEN HYPERPYREXIESYNDROMS (BEI MHS-SCHWEINEN) MIT DANTROLEN-NATRIUM

ZUSAMMENFASSUNG

Es werden Untersuchungen beschrieben, welche ergaben, daß Dantrolen-Natrium das bei empfänglichen Schweinen durch Halothan-Einwirkung hervorgerufene maligne Hyperpyrexiesyndrom wirksam kupiert. Außerdem konnte gezeigt werden, daß sich bei MHS-Schweinen die Auslösung eines malignen Hyperpyrexiesyndroms durch Halothan mit Dantrolen blockieren läßt. Das Mittel scheint sich also zur Behandlung von Narkose-bedingten malignen Hyperpyrexiesyndromen zu eignen.

CONTROL DE LOS SINDROMES DE HIPERPIREXIA MALIGNA (EN CERDOS CON SHM) POR SODIO DANTROLENICO

SUMARIO

Se describen los experimentos que demuestran que el sodio dantrolénico acaba eficazmente con el síndrome de hiperpirexia maligna, inducido en un cerdo, suceptible a ella, por exposición a halotano. Se muestra también que el Dantroleno bloquea la iniciación del síndrome de hiperpirexia maligna por halotano en cerdos con SHM. Parece ser apropiado el uso terapéutico de esta droga en pacientes con hiperpirexia maligna, de inducción anestésica.

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*The control and prevention of malignant hyperthermia in MHS pigs: Some experimental observations**

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The stress related myopathy prominent in certain breeds of pig (Nelson, 1973; Harrison, 1972) provides us with what appears to be a close facsimile of the syndrome of anaesthetic induced malignant hyperthermia in man. What differences there are appear to be quantitative rather than qualitative – the expression of the syndrome being more florid and extreme in the pig, a possible consequence of genetic selection for the heavily muscled animal characterized by an extremely rapid rate of growth.

Though muscle from affected pigs can be shown to behave metabolically in an identifiable manner, for example, the consumption of ATP by muscle biopsies taken from malignant hyperthermia susceptible (MHS) pigs and incubated in Krebs-Ringer solution is twice as rapid as that from non-reactor control animals, the simplest and most certain way of identifying the MH susceptible pig is observation of the response of test animals to a challenge exposure to halothane (Harrison et al., 1969). On exposure to halothane MHS pigs show the rapid onset of rigor, the development of a fulminant acidosis and a rapid rise of core temperature. If the halothane is discontinued the moment the rigor becomes evident, the pig will usually survive without treatment. While increased serum CPK levels will be evident at the time, the diagnosis of stress susceptibility will be clinched by the observation of a great increase in CPK activity in the blood measured 24 hr later.

Whatever the differences may be between pig and man in the precise, intrinsic muscle functional lesion, the ultimate common denominator in the genesis of the syndrome is a defect in the control of calcium ion flux in the muscle cell causing a maintained high cytoplasmic level of calcium with persistence of contraction and rigor (Britt, 1975).

Aiming our therapeutic arrow at this point, we were early able to show that pretreatment of MHS swine with procaine (a known inhibitor of SR calcium release) blocked initiation of the syndrome in them by halothane (Harrison, 1973a). Procaine pretreatment was less effective in blocking initiation of the hyperpyrexic syndrome in response to succinylcholine administered together with halothane inhalation. If given early enough in the development of the syndrome, administration of procaine could terminate the rigor. But the general experience of the therapeutic use of procaine in pigs has been disappointing (Hall et al., 1975; Mitchell and Hefferon, 1975). However, in spite of the fact that the effect of procaine in large doses on the myocardium proved a marked drawback, procaine has been therapeutically successfully used in many instances in man (Britt, 1975).

Though its mode of action is unknown, the steroid anaesthetic Althesin, when given by continuous intravenous administration has also been demonstrated by us to block the initiation of the syndrome in MHS swine by halothane, but not by the consecutive administration of succinylcholine. Regrettably, Althesin proved therapeutically useless (Harrison, 1973b).

Following the demonstration of raised catecholamine levels in Pietrain pigs during malignant hyperpyrexia, the Bristol workers demonstrated a similar result with α-adrener-

* This work was financed by the Anglo American and De Beers Anaesthetic Research Fund of the Department of Anaesthetics, University of Cape Town.

gic blockade – initiation of the syndrome could be blocked but once established the syndrome could not be stopped (Hall et al., 1975).

Though Bianchi's (1973) suggestion at the 1971 Toronto Malignant Hyperpyrexia Symposium that diphenylhydantoin be investigated as a therapeutic agent did not bear fruit, it is another hydantoin – dantrolene sodium – that now offers the greatest therapeutic hope in this condition. Following the demonstration by Ellis and Carpenter (1972) that the muscle relaxant properties of dantrolene followed an effect on the excitation/contraction coupling mechanism in skeletal muscle, we showed that administered intravenously to MHS pigs in established hyperthermic rigor, dantrolene controlled and effectively terminated the syndrome (Harrison, 1975). It was also demonstrated at this time that intravenous pretreatment of susceptible animals with dantrolene blocked subsequent initiation of the syndrome by exposure of test animals to halothane.

Survivors of episodes of malignant hyperpyrexia and investigation and identification of their families have given us an increasing population of patients 'at-risk' from malignant hyperpyrexia should they ever require surgery and need an anaesthetic. We deemed it expedient therefore to investigate further the possible prophylactic use of dantrolene in such circumstances. For this purpose we investigated the oral use of the presently available capsules of dantrolene in a litter of 8 specially bred MHS pigs, each of which had been shown to react within minutes to a halothane challenge and to display high levels of CPK activity. The clinical reaction, including observation of core temperature of each pig to exposure to halothane anaesthesia was observed in each of the following circumstances: (1) Following pretreatment on day previous and day of anaesthetic (4 hr preanaesthesia) with oral dantrolene 5 mg/kg. In the absence of reaction anaesthesia was arbitrarily maintained for 1 hr. (2) Without any such pretreatment. Exposure to halothane in these circumstances was discontinued the moment rigor, together with commencing rise in core temperature was observed. This occurred in all un-pretreated animals within 5-10 min.

A period of 2 weeks was allowed to elapse between pretreated and un-pretreated exposures to halothane. In each pig this programme was repeated after a further interval of 7 days.

Fig. 1. *Pigs undergoing test exposure to halothane. That nearest the camera has been pretreated with dantrolene and is in the 30th min of anaesthesia that was continued for 1 hr. That farthest is an unpretreated control. Within 4 min of exposure to halothane, rigor of hind legs is well marked and rise in the core temperature has commenced.*

RESULTS

When pretreated with oral dantrolene, all 8 pigs tolerated anaesthesia with halothane for 1 hr on each of 2 occasions without any abnormal reaction and no rise in core temperature. When the same pigs were exposed to halothane in the absence of dantrolene, all reacted on each of 2 occasions with a rapid onset of rigor with commencing rise of core temperature within 5-10 min (see Fig. 1).

The complete obtundation by dantrolene of the response of MHS reactor pigs to halothane was dramatically illustrated by the measurement of CPK activity during and 24 hr after exposure. Whereas exposure of animals pretreated with dantrolene to 1 hr anaesthesia with halothane resulted in no change in CPK activity, exposure of the same animals without dantrolene treatment to halothane until the onset of rigor (5-10 min only) resulted in a dramatic mean 56-fold rise in CPK activity.

We have demonstrated in addition in 4 MHS pigs, that dantrolene pretreatment even blocked initiation of the hyperpyrexic syndrome in response to the super added stimulus of succinylcholine administered together with halothane – a property that the previously demonstrated halothane blocking drugs, procaine and Althesin, did not possess.

CONCLUSION

If dantrolene can now be shown to act in man as I have demonstrated it to act in the MHS pig, we need no longer fear anaesthetic induced malignant hyperpyrexia. We have the means to cure it and, better still, to prevent it.

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THE PROPHYLAXIS OF MALIGNANT HYPERTHERMIA BY ORAL DANTROLENE SODIUM IN SWINE

G. G. HARRISON

The oral administration of dantrolene for the prophylaxis of malignant hyperthermia (MH) in susceptible swine has been investigated. Complete protection from MH in pigs exposed to the known MH initiating agents, halothane and suxamethonium, was demonstrated.

It is now established that dantrolene sodium can control and prevent the malignant hyperthermia (MH) syndrome induced in malignant hyperthermia susceptible (MHS) swine in response to a halothane challenge (Harrison, 1975; Anderson and Jones, 1976; Gronert, Milde and Theye, 1976). Application of this knowledge in man has been prevented by the lack of an acceptable i.v. formulation of the drug, which is available for clinical use as an oral capsule only. For patients known to be genetically "at risk" from MH (Moulds and Denborough, 1974) there is no completely safe method of general anaesthesia (Ellis et al., 1974; Britt, 1975).

We have investigated the efficacy of dantrolene sodium administered orally as a prophylactic agent against the development of the malignant hyperpyrexic syndrome in susceptible pigs in response to the most common initiating agents, halothane and suxamethonium.

METHODS

The experiments were conducted in a litter of specially bred MHS Landrace pigs. At 6 weeks of age all had been screened and shown to react to a halothane challenge within minutes, with the onset of muscle rigor and a concomitant increase in core (rectal) temperature. All had increased serum concentrations of creatine phosphokinase (c.p.k.) at rest.

The clinical reaction of each pig, including change in temperature, to exposure to halothane anaesthesia was observed in each of the following circumstances:
(1) Following pretreatment on the day before and the day of anaesthesia (4 h before anaesthesia) with dantrolene 5 mg/kg administered orally. In the absence of a reaction, anaesthesia was maintained for 1 h.
(2) With no pretreatment, exposure to halothane was discontinued as soon as muscle rigor, together with an increase in core temperature, was observed.

Rigor and pyrexia were accepted, in these animals, as indicating the onset of MH. The concomitant occurrence of acidosis was observed in specimens of mixed venous blood from venepuncture at the thoracic inlet in all animals which displayed rigor.

Serum c.p.k. was measured (Worthington Statzym c.p.k., Cat. No. 7960) in blood sampled immediately before and after anaesthesia and, in some animals, 24 h later. A period of 14 days was allowed to elapse between pretreated and unpretreated exposure to halothane. Following recovery, the animals were rested for 7 days and thereafter the protocol was repeated.

Dantrolene powder was administered to the test animals in capsules propelled into the pharynx with saline from a syringe by means of a flexible Tygon tube while the animal was restrained. The dose (5 mg/kg) and time of administration in relation to anaesthesia were chosen after consideration of the therapeutic doses used previously (Harrison, 1975) and observations on the relationship of oral dose to blood concentration (Leitman, Haslam and Walcher, 1974).

Halothane, vaporized in nitrous oxide in oxygen, was administered from a Fluotec vaporizer in a Magill circuit by means of a face mask, with the pig breathing spontaneously. Commencing at 3% for induction of anaesthesia, concentrations of halothane were reduced progressively after induction to 1% at approximately 15 min after induction and maintained at this level for the remainder of the anaesthetic.

In a further experiment four of these pigs, premedicated with dantrolene and exposed to halothane anaesthesia for 15 min followed by tracheal intubation, were challenged with two injections of suxamethonium 1 mg/kg i.v. at intervals of 10 min and the reaction observed. IPPV with nitrous oxide in oxygen using a Manley ventilator was instituted until the return of spontaneous respiration.

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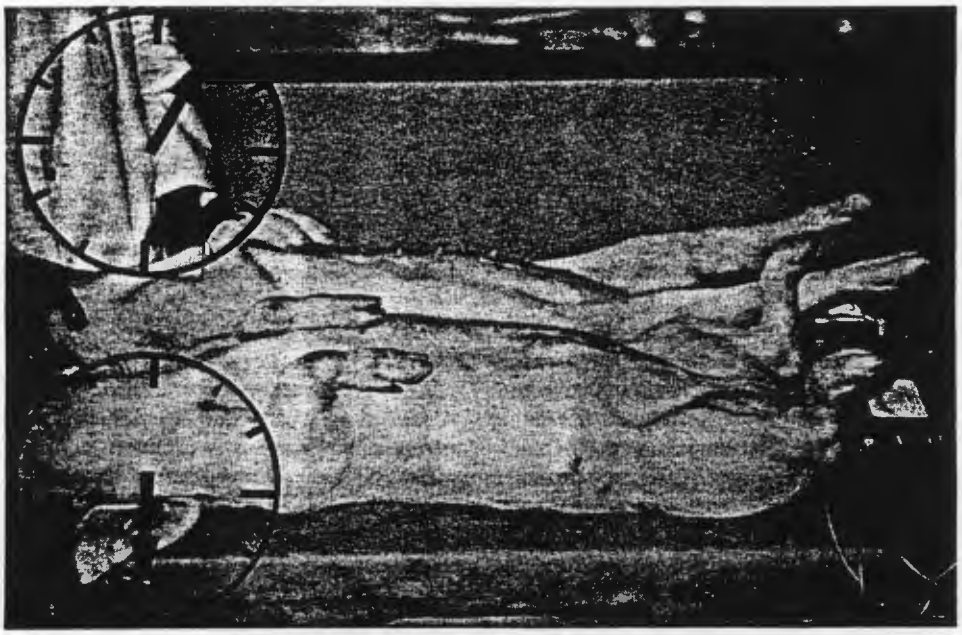

FIG. 1. MHS pigs exposed to halothane anaesthesia. The nearer pig, pretreated with dantrolene, is in the 30th minute of planned 1-h exposure. The other pig was not treated. After 4 min of halothane the characteristic muscle rigor has commenced causing extension of the hind legs. This is accompanied by an increase in rectal temperature.

RESULTS

When pretreated with oral dantrolene all eight pigs tolerated anaesthesia with halothane for 1 h on each of two occasions with no abnormal reaction and no increase in core temperature. When the same pigs were exposed to halothane in the absence of dantrolene, all reacted on each of two occasions with the rapid onset of rigor together with an increase in core temperature within 5–10 min (fig. 1). After discontinuing halothane the temperature was observed until it had reached the peak value. The mean increase of temperature following this short exposure to halothane was 1.2 °C.

C.p.k. activity measured 24 h after exposure to halothane was expressed in relation to the value measured at commencement of the halothane challenge. In four pigs (table I) treatment with dantrolene, followed by exposure to halothane for 1 h resulted in no change in c.p.k. at 24 h; in the absence of dantrolene, exposure to halothane until the onset of muscle rigor only (5–10 min) caused (on average) a 56-fold increase in c.p.k. activity measured at 24 h.

TABLE I. *C.p.k. ratio (24 h after/before anaesthesia)* (n = four pigs)

	Dantrolene pretreatment halothane 60 min	No pretreatment halothane 5–10 min
Mean	0.95	56.0
SEM	0.21	21.7

The challenge of suxamethonium in four animals premedicated with dantrolene and anaesthetized with halothane for 15 min failed to elicit the malignant hyperpyrexia response. All of the pigs recovered. The blocking of initiation of the malignant hyperpyrexic syndrome in MHS pigs by this most severe combined stimulus has not been documented with any other agent to date.

DISCUSSION

Investigation of the family of patients suffering from MH yields a small but increasing number of patients considered to be at risk from MH should they require a general anaesthetic.

It may be argued that the patient's best interests can be served by an awareness of the risks and by avoiding of the most common triggering agents, halothane, methoxyflurane and suxamethonium, coupled with meticulous monitoring of temperature and other vital functions so that corrective measures

may be taken promptly should the syndrome commence. Since there are no general anaesthetic agents that are completely safe in these circumstances, it is frustrating to be denied the use of dantrolene for lack of an acceptable i.v. formulation. The oral use of dantrolene in man is well documented and is safe (Sykes, 1975).

Our experiments demonstrate that dantrolene administered orally in the form presently available protects the MHS pig from MH in response to both halothane and the overwhelming stimulus of suxamethonium administered in addition. We would recommend that premedication of the patient known to be at risk from MH should include oral dantrolene administered at the appropriate time interval.

If dantrolene can be shown to act in man in the same manner as in the pig, we need no longer fear MH. We have at our disposal the means to prevent it and, when an i.v. formulation is available, to cure it.

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PROPHYLAXIE DE L'HYPERTHERMIE MALIGNE PAR ADMINISTRATION ORALE DE DANTROLENE DE SODIUM A DES COCHONS

RESUME

L'administration orale de dantrolène pour la prophylaxie de l'hyperthermie maligne sur des porcs susceptibles d'attraper cette maladie a fait l'objet d'une étude. Il a été démontré que l'on pouvait obtenir une protection complète contre l'hyperthermie maligne des cochons exposés aux agents connus déclenchant l'hyperthermie maligne, tels que l'halothane et le suxaméthonium.

DIE VERHINDERUNG BÖSARTIGER HYPERTHERMIE DURCH MÖNDLICHES DANTROLEN-NATRIUM IN SCHWEINEN

ZUSAMMENFASSUNG

Die Verabreichung per os von Dantrolen für die Verhinderung der bösartigen Hyperthermie in dafür empfänglichen Schweinen war untersucht worden. Es wurde der völlige Schutz gegen die bösartige Hyperthermie in Schweinen, die den bekannten, die bösartige Hyperthermie einleitenden Mitteln Halothan und Suxamethonium ausgesetzt waren, demonstriert.

LA PROFILAXIS DE HIPERTERMIA MALIGNA PORCINA MEDIANTE DANTROLENO SODICO ORAL

SUMARIO

Se ha investigado la administración oral de dantroleno para la profilaxis de la hipertermia maligna (HM) en los cerdos susceptibles. Se demostró la completa protección contra HM en los cerdos expuestos a los agentes conocidos como iniciadores de HM, el halotano y la succinilcolina.

"*I would have everie man write what he knowes and no more.*"—MONTAIGNE

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EDITORIAL

DANTROLENE AND THE TREATMENT OF MALIGNANT HYPERTHERMIA

The availability in the United Kingdom in Autumn 1980 of a stable i.v. preparation of dantrolene for the treatment of malignant hyperthermia (MH) is an important milestone in the management of this rare, but often lethal, complication of anaesthesia. The development of a specific therapeutic agent for the clinical syndrome has followed from experimental studies on the porcine model, although there have been several "false starts," of which the most notable was procaine or procainamide.

Dantrolene, a hydantoin derivative, was synthesized by Snyder and colleagues (1967) and has been used orally to treat muscle spasticity (Dykes, 1975). It acts on skeletal muscle by interfering with the excitation–contraction coupling mechanism, so that the sarcolemmal action potential is no longer coupled or linked to the normal efflux of calcium ions within the muscle cell. Thus, there is a reduction in the availability of calcium ions for the contractile process (for review see Pinder et al., 1977). It is often claimed that the effects of dantrolene are specific for skeletal muscle, but recent studies have shown that dantrolene also depresses smooth muscle (Graves, Dretchen and Kruger, 1978), cardiac muscle (Meyer, Wesseling and Agoston, 1976) and catecholamine release from the adrenal medulla (Cohen and Gutman, 1979). It would be surprising if dantrolene had no effects on other calcium-coupled systems, although these are unlikely to be important at doses used to relieve muscle spasticity.

Harrison (1975) was the first to demonstrate the effectiveness of i.v. dantrolene in the treatment of MH in Landrace pigs. This observation was confirmed in Poland China pigs by Gronert, Milde and Theye (1976) and in Pietrain pigs by Hall, Lucke and Lister (1977) using dantrolene 7.5 mg kg^{-1}. In addition, Gronert, Milde and Theye (1976) showed that the i.v. administration of dantrolene 5 mg kg^{-1} afforded complete protection against porcine MH if given only 10 min before halothane and suxamethonium. Harrison (1977) found that oral dantrolene was also effective in preventing MH if given in two doses of 5 mg kg^{-1}, 24 and 4 h before anaesthesia. The data from the experiments on porcine MH are conclusive; dantrolene is effective both therapeutically and prophylactically.

The application of the results obtained in porcine MH to the clinical syndrome has been delayed by the difficulties in producing a stable i.v. preparation of dantrolene. However, a small pilot study has been undertaken with a suitable i.v. preparation at 45 hospitals in the United States and Canada. The criteria used for confirming the occurrence of MH in this study were very weak (for example, a tachycardia of 20 beat min^{-1} greater than baseline values, arterial $pH < 7.35$, $Pco_2 > 42$ mm Hg) and consequently many of the patients reported were undoubtedly not suffering from MH. Nevertheless there were three cases of MH in which the administration of dantrolene i.v., at doses ranging from 0.9 to 1.6 mg kg^{-1}, was associated with the rapid and complete resolution of the hyperthermic response. It seems likely that not only is dantrolene effective in the treatment of human MH, but also the dose required is considerably less than in the porcine syndrome.

The i.v. preparation of dantrolene will be available in vials containing sodium dantrolene 20 mg, mannitol 3 g and sodium hydroxide to produce a final pH of 9.5 on the addition of 60 ml of water. The use of alkaline mannitol as the solvent for dantrolene is a useful bonus as a diuresis is necessary during the treatment of MH to prevent the deleterious effects of

myoglobin on renal tubular function. It is unfortunate that such a large volume of water is required to dissolve the contents of each vial because valuable time will be lost in preparing the solution. For example, administration of dantrolene 2 mg kg^{-1} to a 70-kg patient will need 420 ml of sterile water.

Oral dantrolene has been used recently as a prophylactic measure in a patient who was MH-susceptible (Pandit, Kothary and Cohen, 1979). Peak blood concentrations of dantrolene occur about 4 h after oral administration with a plasma half-life of 6–8 h (Herman, Meyer and Mecomber, 1972). Various regimes have been advocated for oral prophylaxis with dantrolene but it is my policy to confine pretreatment to the 24 h before surgery when the patient is given about 5 mg kg^{-1}. It is possible that a smaller dose of dantrolene may be equally effective, but it is essential to err on the side of caution until sufficient information is available on the use of oral dantrolene.

At present it is a difficult choice between the administration of prophylactic dantrolene for the MH-susceptible patient or reliance on the use of a "safe" anaesthetic technique. The effective prophylactic dose of dantrolene is similar to that at which adverse side-effects start to occur. A patient who is given oral dantrolene and suffers from any of the following symptoms: dizziness, blurred vision, incoordination, abdominal pain, diarrhoea, nausea and muscle weakness (Pandit, Kothary and Cohen, 1979; author's observations), may question the necessity of this life-saving therapy. Furthermore, the possible interactions of dantrolene with neuromuscular blocking drugs given during anaesthesia have yet to be thoroughly elucidated. A recent study by Flewellen, Nelson and Bee (1980) investigated the effects of dantrolene on neuromuscular blockade produced by tubocurarine. They found that neostigmine did not reverse the dantrolene-induced depression of muscle function and warn that this may contribute to respiratory inadequacy in the period after operation. On balance, the anaesthetist who is meeting the problem of anaesthesia for the MH-susceptible patient for the first time is advised to use oral dantrolene prophylactically. The anaesthetist with experience of such cases can probably rely on the avoidance of known triggering agents.

It is not the purpose of this editorial to describe in detail the treatment of MH (for which, see Ryan, 1977), but to indicate when and how i.v. dantrolene should be used. As soon as MH is diagnosed all potent inhalation anaesthetic agents must be discontinued, and the acidosis treated immediately by hyperventilation and the i.v. administration of sodium bicarbonate. Dantrolene is then injected in aliquots of 1 mg kg^{-1} at intervals of 5–10 min until MH is resolved, as shown by control of the acidosis, tachycardia, temperature and muscle tone. Dantrolene 2–3 mg kg^{-1} i.v. is probably sufficient to treat most cases of human MH, but up to 10 mg kg^{-1} has been used in the porcine syndrome without adverse side-effects (Gronert, Milde and Theye, 1976).

Some anaesthetists who have never seen a case of MH may question the need for purchasing an expensive drug, approximately £1 per mg, with a limited shelf-life of 18 months to 2 years. Dantrolene should be regarded, however, as cheap insurance against a possible anaesthetic death and be readily available in all hospitals in which anaesthetics are undertaken.

Anaesthetists in the United Kingdom who use i.v. dantrolene in MH will be asked to supply all clinical details to Eaton Laboratories, who market the drug, so that the data can be collected and analysed. This service is essential if the correct use of dantrolene is to be established and all anaesthetists are urged to cooperate, even if the diagnosis of MH is not subsequently confirmed.

Progress in the management of MH-susceptible patients has been painfully slow since the syndrome was first described 20 years ago. The use of dantrolene both orally and i.v. offers a unique opportunity to reduce the mortality rate significantly.

I am indebted to Eaton Laboratories, a Division of Morton–Norwhich Laboratories, for details of 19 patients treated with dantrolene in the Multicentre Study in North America.

G. M. Hall

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Dantrolene sodium in the treatment of malignant hyperthermia

A case report

G. G. HARRISON

Summary

The pharmacogenetic syndrome of malignant hyperthermia (MH) in response to general anaesthesia, though rare, is usually fatal when it does occur. A case of anaesthetic-induced MH arising in a 10-year-old girl during intranasal antrostomy and successfully treated with dantrolene sodium (Dantrium; SKF) is described. A treatment protocol for MH is appended. The importance of an awareness on the part of the clinician together with prompt reaction to early tell-tale signs is stressed.

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Of all the complications of general anaesthesia perhaps the most dramatic is the veritable metabolic storm which characterizes the frequently fatal syndrome of anaesthetic-induced malignant hyperthermia (MH), a pharmacogenetic condition associated with a rare myopathy which causes the sufferer little or no disability in the normal course of events.[1] Although extremely rare, the condition has been identified world-wide, the incidence (reported as approximately 1/7 000 to 1/50 000 anaesthetics) appearing to vary geographically with the gene pool.[2] The syndrome has been reported in South Africa,[3,4] and to my certain knowledge more cases have occurred than have been reported in the literature. Because of the extreme rareness of the syndrome, fatalities have resulted from delays in diagnosis, often due directly to a lack of awareness on the part of the clinician.

Occurring most commonly after exposure of genetically susceptible individuals to the volatile anaesthetic agent halothane and the depolarizing relaxant succinylcholine, the syndrome has been associated with all volatile and gaseous anaesthetic agents in clinical use.[1]

The syndrome manifests as a sudden and sustained rise in the patient's core temperature during administration of a general anaesthetic; this is associated in the majority of cases with muscle hypertonus or rigidity and usually preceded by a myotonic response with marked masseter spasm to the injection of succinylcholine. The rise in core temperature is rapid, often fulminant, rates of 1°C every 5 - 10 minutes having been recorded. In the untreated patient, the temperature continues to rise inexorably until the death of the patient, with a core temperature of 42°C. *Pari passu* are observed tachycardia, cyanosis, tachypnoea, and excessive heating of the soda lime cannister (evidencing high CO_2 output), accompanied by gross biochemical changes in the blood, the most prominent being severe acidosis, both metabolic and respiratory, hyperkalaemia and a markedly increased serum level of the muscle enzyme creatine phosphokinase (CPK), which is diagnostic. The reported mortality has been of the order of 70%.

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Until recently, treatment has perforce been symptomatic, but now specific therapy is possible with dantrolene sodium (Dantrium; SKF), an intrinsic relaxant of skeletal muscle with a modifying action on the excitation/contraction/coupling mechanism — the probable site of the functional lesion of MH.[1,5]

The following case report demonstrates the first successful use in South Africa of dantrolene in a patient with MH (Fig. 1).

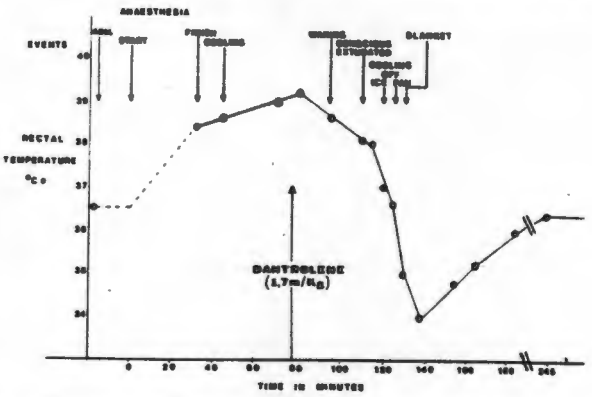

Fig. 1. Temperature and events chart of patient described.

Case report

This case arose in a private hospital in which biochemical investigative back-up was not as readily available or prompt as could have been wished. A Coloured girl aged 10 years, with weight 37 kg, presented for intranasal antrostomy. Apart from the lesion in her maxillary sinuses — the indication for operation — she was clinically normal. Her temperature on admission on the morning of the operation was 36,5°C. She gave a history of having had a general anaesthetic for tonsillectomy 3 years previously, with no untoward effects.

After premedication with oral trimeprazine 84 mg, monitoring of heart rate and blood pressure was commenced and anaesthesia induced with methohexitone sodium 70 mg followed by succinylcholine 40 mg. Thereafter, intermittent positive-pressure ventilation with 100% oxygen and 1% halothane vapour was instituted. Attempted laryngoscopy prior to intubation was frustrated by the onset of gross trismus. Thinking that this might be due to deterioration of the succinylcholine, the anaesthetist thereupon administered a further 30 mg. Trismus persisted, necessitating the use of force with a wooden gag to open the mouth to permit topical analgesia of the larynx and orotracheal intubation, after which the teeth were kept apart with a self-retaining gag. Anaesthesia was maintained with nitrous oxide and oxygen (4 and 2 l/min respectively) and with 1,5% halothane vapour reduced gradually to 0,5%. When spontaneous respiration recommenced after the action of succinylcholine had worn off, tachypnoea (40 breaths per minute) was evident. At the same time a sinus tachycardia of 140/min was noticed and the surgeon observed cyanosis in the blood at the operative site, in spite of adequate ventilation with an inspired oxygen concentration (FiO_2) of 0,33. These conditions persisted. At the conclusion of surgery and anaesthesia, 32 minutes after

induction, the patient's limbs, especially the legs, were noted to be stiff. She felt hot and failed to regain consciousness. Rectal temperature, measured now for the first time, was 38,5°C. Malignant hyperthermia was diagnosed and the patient was transferred to the recovery ward, where an infusion of sodium bicarbonate with furosemide 10 mg was commenced, followed by an infusion of balanced salt solution. Thirteen minutes after the discontinuation of the anaesthetic, active surface cooling was initiated, the patient being covered with ice and with wet sheets on which fans were directed.

In spite of this, 35 minutes later, by which time supplies of dantrolene had arrived from a teaching hospital (the only available source of supply at this time), the patient's rectal temperature had risen to 39,2°C and she was still unconscious and stiff, with a pulse rate of 140/min and a respiration rate of 40.

Dantrolene (1,7 mg/kg to a total dose of 60 mg) was administered intravenously over the next 10 minutes. Almost immediately, muscle tone decreased, cooling commenced, the level of consciousness rose, and respiration slowed. Within 30 minutes of commencing treatment, the patient was wide awake, extubated and the rectal temperature had dropped to 38,2°C. The rate of cooling, initially 1°C in 30 minutes, increased rapidly with a further fall of 4°C over the next 30 minutes in spite of progressive removal of ice, fan, and wet sheets; finally the patient was covered with a bunny blanket (Fig. 1). The patient then warmed spontaneously to 36,5°C within the next 105 minutes, at which level the temperature stabilized. An arterial blood sample taken 35 minutes after administration of dantrolene — when the patient was already awake and cooling and had already had 250 mmol sodium bicarbonate plus 700 ml Plasmalyte and had passed 1 litre of urine — showed that the metabolic crisis had largely passed. The acid-base status at that stage was: pH 7,53; P_{CO_2} 4,8 kPa, P_{O_2} 23 kPa (on 30% O_2), potassium 3,3 mmol/l, sodium 147 mmol/l, but the diagnostic marker of MH was evident in a CPK level of 7 600 IU/l.

On the next day, the patient was well, although complaining of marked stiffness in her leg muscles. Serum enzyme levels at this time were: CPK 575 IU/l (normal < 50 IU/l) and lactic dehydrogenase 384 IU/l (normal 120 - 140 IU/l). The iso-enzyme-5 level was raised, compatible with a muscle lesion.

Discussion

Although not as completely documented in many respects as one would have liked, this case warrants reporting, for it highlights many important clinical aspects of the rare and so often fatal MH syndrome.

The individual susceptible to MH need not respond on every occasion to an appropriate stressor with an attack of MH, i.e. a history of previous general anaesthesia with no untoward effects (as in this case) does not exclude the possibility of an attack, as was once commonly thought.[6] The sensitivity of the 'trigger' varies in degree, not only between patients but in the same patient at different times. The anaesthetic administered to this patient 3 years previously for tonsillectomy was halothane, inhaled for a relatively short time — ± 30 minutes. The 'trigger', not too sensitive at this time, was only 'fingered' and not pulled. On the present occasion, two doses of succinylcholine and a 2 MAC initial concentration of halothane meant that the 'trigger' was verily pulled.

This case also illustrates the danger inherent in the common reaction of the anaesthetist when the first dose of succinylcholine is 'ineffective' (as manifested by masseter spasm), which is to give another dose from a different ampoule in the belief that the first had deteriorated pharmacologically. Caffeine stimulation tests on muscle biopsy specimens have shown that 50-100% of patients displaying this abnormal masseter spasm reaction in response to succinylcholine suffer from MH susceptibility.[7]

The importance of the sudden onset of unexplained sinus tachycardia during anaesthesia as a warning and early diasgnostic sign of MH is also illustrated. It follows the rapid rise in circulatory catecholamine levels shown to accompany initiation of the syndrome.

Cyanosis in the presence of adequate F_{IO_2} and pulmonary ventilation should also alert the anaesthetist to the possibility of MH.

The specific therapeutic efficacy of dantrolene sodium in the established MH syndrome is also well illustrated. It is as if it simply turns off the switch of muscle hypermetabolism that halothane/succinylcholine turns on in the genetically susceptible individual.

Since MH when it occurs is a life-threatening emergency, I conclude this case report with a summarized treatment protocol. Prevention and early effective treatment depend on an awareness of the condition by the clinician, and rapid, appropriate reaction to the earliest warning and diagnostic signs, before core temperature has risen more than 0,5°C, is often sufficient to abort an attack. Such early signs are: (*i*) masseter spasm following administration of succinylcholine, (*ii*) unexplained sudden sinus tachycardia, (*iii*) tachypnoea if the patient is breathing spontaneously, (*iv*) cyanosis in the presence of adequate ventilation and F_{IO_2}, (*v*) heating of the soda lime cannister, and (*vi*) any unexplained rise in the core temperature of 0,5°C or more.

The following treatment protocol should be instituted: (*i*) discontinue anaesthesia, (*ii*) hyperventilate the patient with 100% oxygen, (*iii*) administer sodium bicarbonate (having first taken a blood specimen for diagnostic and baseline biochemical parameters) to correct the fulminant acidosis (a dose sufficient to correct a base deficit of 10 mmol/l can safely be given in the first instance); (*iv*) cool the patient aggressively — use ice and wet sheets with fans, infuse iced intravenous solutions, irrigate any open body cavity with iced saline; (*v*) infuse dantrolene sodium intravenously, 1 - 2 mg/kg every 10 - 15 minutes, until there is (*a*) decrease in muscle tone, (*b*) fall in core temperature, and (*c*) slowing of the heart rate; (*vi*) maintain circulatory homeostasis with intravenous balanced salt solutions (iced), (*vii*) look for and treat associated complications such as myoglobinuria (treat with furosemide or mannitol) and disseminated intravascular coagulation (treat with fresh-frozen plasma).

Once the syndrome is controlled, the patient should be kept in an intensive care unit for 24 hours in case of possible recrudescence of the condition, which has happened. Later, first-degree relatives should be screened for susceptibility to the MH syndrome.

A drawback to increasing the general availability of dantrolene, is its expense (± R420,00 per treatment pack of 12 x 20 mg ampoules) and its relatively short shelf-life (2 years from manufacture in the USA). While no national policy has yet been agreed to in this regard, a fairly effective and relatively inexpensive scheme can be instituted in cities. For example, in Cape Town one treatment pack is held at each of the teaching hospitals, Groote Schuur and Tygerberg Hospitals, whence it can be transported to any other hospital in the town timeously in case of need, as happened in this case. Immediately a dose of the drug is used, it is replaced within 24 hours by the pharmaceutical firm concerned. However, in view of the wide geographical spread of the country hospitals where anaesthetics are administered, and at any of which the syndrome might occur, the cost-effectiveness of dantrolene can be called into question. I do not have a ready solution to this dilemma.

I wish to thank Dr Y. Bosman for permission to publish and Smith, Kline & French for supplies of dantrolene sodium.

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Dantrolene sodium in the treatment of malignant hyperthermia

To the Editor: A recent issue of the *SAMJ* carried a report of the first successful use in this country of dantrolene sodium (Dantrium; SKF) in the treatment of anaesthetic-induced malignant hyperthermia (MH).[1]

This covert genetic myopathy is rare, especially in South Africa. To reinforce in our colleagues an awareness that there are individuals in our community susceptible to MH (such awareness being essential to early, life-saving diagnosis of the developing syndrome), we report briefly on a second patient successfully treated with dantrolene within 14 months of the case previously reported. The patients were not related.

An 8-year-old Coloured girl weighing 23 kg required general anaesthesia for Harrington rod correction of congenital lumbar scoliosis. She had been anaesthetized without complications 7 years before for incision and drainage of an abscess. Apart from the lumbar scoliosis, she was healthy.

The events during the anaesthetic are displayed in chart form in Fig. 1. On arrival in the operating theatre after oral premedication with trimeprazine tartrate 72 mg and droperidol 3 mg, an oral

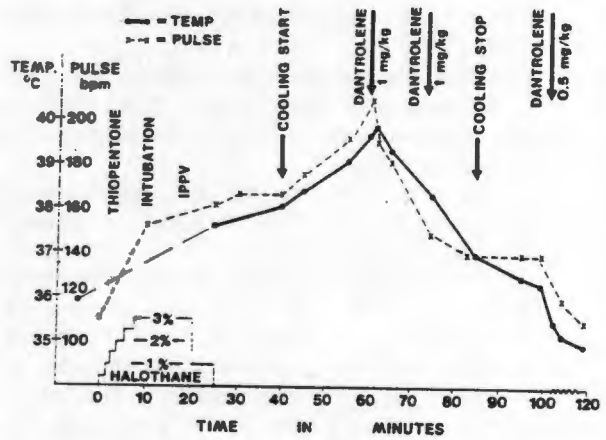

Fig. 1. Anaesthetic events chart.

temperature of 35,8°C was recorded. Monitoring of the blood pressure and ECG was commenced, and general anaesthesia was induced by the intravenous injection of thiopentone 100 mg, followed by inhalation with spontaneous respiration of nitrous oxide/oxygen (4:2) together with halothane vapour of increasing concentration. Commencement of the inhalational anaesthetic was accompanied by drawing up of the arms and other muscle movements. The anaesthetist, interpreting these as signs of waking, rapidly increased the concentration of halothane vapour to 3% in an endeavour to achieve smoother induction of anaesthesia. Ten minutes from the start endotracheal intubation was performed following lignocaine topical analgesia of the larynx. The halothane concentration was thereafter reduced to 1% and intermittent positive-pressure ventilation (IPPV) commenced. The pulse rate was noted to have risen to 150/min (sinus tachycardia). Twenty minutes after induction difficulty in catheterization of the bladder drew attention to generalized muscular rigidity. This and a further rise in the pulse rate to 160/min prompted thoughts of MH. A rectal probe now inserted showed the temperature to be 37,5°C and rising. Halothane was discontinued and the patient ventilated with oxygen alone. At 40 minutes, with the rectal temperature 38°C, the pulse rate 165/min and a marked generalized increase in muscle tone, active surface cooling with wet sheets, ice and fans was instituted. An arterial blood sample taken at this time showed the gross metabolic acidosis characteristic of an MH crisis — pH 7,07, partial arterial carbon dioxide pressure 61,9 mmHg, base excess -14 mEq, partial arterial oxygen pressure 214 mmHg (on 100% oxygen). Infusion of sodium bicarbonate was commenced, and in all over the next 40 minutes 200 mEq was administered. In spite of the active surface cooling the patient's temperature continued to rise inexorably and by 58 minutes after induction had reached 39,6°C (pulse rate 210/min). At this stage dantrolene 2 mg/kg in 1 mg/kg aliquots was administered. The muscle spasm immediately relaxed, the pulse rate slowed and after a further 0,2°C rise to a peak of 39,8°C (the overall temperature rise was 4°C in 60 minutes, 1,8°C since the start of cooling) the temperature began to fall. Twenty-five minutes after the administration of dantrolene the temperature had fallen to 37°C, the pulse rate was 140/min, and active cooling was discontinued. The patient was now waking and shortly after was allowed to breathe spontaneously and extubated. An afterdrop of 2°C followed cessation of active cooling, rectal temperature bottoming out at 35°C 30 minutes later, after which it returned to 37°C over the next 3 hours. Further dantrolene (0,5 mg/kg) had been given when cooling was discontinued. In addition to sodium bicarbonate, 5 g mannitol was infused and a good urinary output maintained. Throughout the crisis the blood pressure remained stable between 120 and 130 mmHg systolic.

As so often happens in such crises the Astrup apparatus broke down immediately after the first blood specimen had been analysed so that final precise real-time acid-base control was not possible and progress was judged on clinical grounds. Other biochemical assays of interest and diagnostic import were for serum potassium, which rose to 5 mEq/l during the crisis, and creatine phosphokinase (CPK), which rose to 92 IU/l in mid-crisis and to 5510 IU/l in 24 hours (normal value < 50 IU/l); these were accompanied by more modest rises in lactate dehydrogenase (LH) (191-1 540 IU/l) (normal 100-350 IU/l) and aspartate aminotransferase (AST) (19-357 IU/l) (normal < 40 IU/l) values. One week later the CPK value had fallen to 187 IU/l and LH and AST to virtually normal levels.

Clinical points worthy of comment are: (*i*) the history of previous uncomplicated general anaesthesia, although it was of short duration and the patient was only 1 year old at the time; (*ii*) misinterpretation of the initial muscle contraction as indicating lightening anaesthesia rather than the onset of MH; and (*iii*) unexplained sudden sinus tachycardia as an early diagnostic pointer.

The operation was safely carried out 10 weeks later under general anaesthesia. Intravenous dantrolene was used for pre-induction and the use of volatile anaesthetic agents was avoided; instead a pancuronium/fentanyl/nitrous oxide/oxygen IPPV technique was utilized following induction of anaesthesia with thiopentone. Investigation of the family — parents and 2 siblings — has shown 1 sibling to be susceptible to MH.

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1. Harrison GG. Dantrolene sodium in the treatment of malignant hyperthermia. *S Afr Med J* 1981; 60: 909.

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DANTROLENE—DYNAMICS AND KINETICS

G. G. HARRISON

Although Bianchi's perceptive suggestion [2] at the First International Symposium on Malignant Hyperthermia in 1971, that diphenyl hydantoin should be investigated as a possible therapeutic agent for Malignant Hyperthermia (MH), did not bear fruit, it was the introduction of another hydantoin—dantrolene sodium—that later revolutionized the treatment and prognosis of MH. Dantrolene was synthesized originally by Snyder and associates [49] during the investigation of a series of substituted furans that had muscle relaxing properties, and its skeletal muscle relaxant properties were shown by Ellis and coworkers [11–13] to stem from a selective depressive action on the intrinsic mechanisms of excitation–contraction coupling (ECC). This demonstration motivated the successful therapeutic trial of dantrolene in porcine MH [24, 26], followed later by its equally successful use in the MH syndrome in man [32].

At the time this syndrome first came to world notice, during the decade of the 1960s, the mortality from MH was of the order of 80%. During the 70s, increasing awareness of the syndrome with resultant earlier diganosis and treatment, albeit non-specific, led to an improvement in prognosis, recorded mortality decreasing to 28%. This decade, following the introduction to practice of dantrolene sodium, the mortality recorded following MH is now but 7% and these subjects, on review, all manifested serious avoidable errors in therapeutic management. Today, with correct management utilizing dantrolene sodium, survival from MH is the expected norm [4, 23].

Chemistry

Dantrolene sodium, 1-{[5-(p-paraphenyl) furfurylidene] amino} hydantoin, is pharmacologically the most active member of a long series of 1-[(5 aryl furfurylidene) amino] hydantoins synthesized for their muscle relaxant properties (fig. 1). Dantrolene is the only analogue in the series to achieve clinical usage, although others have been tested [51].

The molecular structure of dantrolene is planar, with the exception of the phenyl ring, which is rotated approximately 30° out of the plane of the furan ring [13] (fig. 1).

Highly lipid soluble, but extremely poorly soluble in water, the drug was originally dispensed in capsular form for administration by mouth—a circumstance compatible with its originally envisaged use for the pharmacological control of chronic states of muscle spasm. Once its efficacy in the control of MH had been demonstrated, its wide-spread use for this purpose was sadly delayed some years by the need to develop an i.v. formulation appropriate to the clinical circumstances of MH.

Today, for this purpose, the drug is supplied in ampoules containing 20 mg of lyophylized dantrolene sodium, an orange powder, together with mannitol 3 g (which improves the solubility) and sufficient sodium hydroxide to yield a pH of 9.5 when the contents are dissolved in 60 ml of water. The final concentration of dantrolene in this solution is 0.33 mg ml^{-1}.

Pharmacodynamics

The primary pharmacological action of dantrolene is the relaxation of skeletal muscle, with reduction in the force of contraction. This, although dose-dependent, reaches a plateau at a level well short of total paralysis of muscle, regardless of the dose [21]—a circumstance attributed to the limited water solubility of the drug [13].

The loci at which drug action could have such an effect in the complex interactions responsible for skeletal muscle contraction are shown diagrammatically in figure 2 and provide the background against which will be presented findings, from the extensive investigation to which dantrolene has

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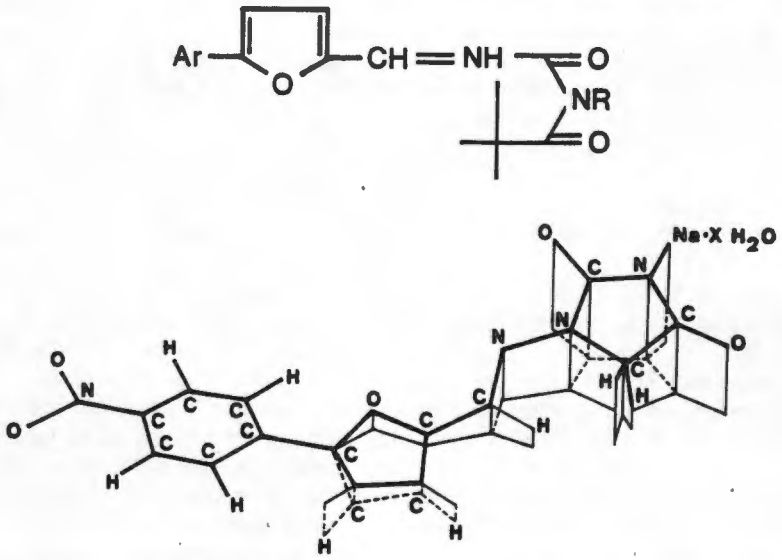

FIG. 1. Top: Structural formula of derivative aryl amino hydantoins. Below: Ground state conformation of 1-{5-(p-nitrophenyl) furfurylidene} amino hydantoin sodium hydrate: dantrolene sodium.

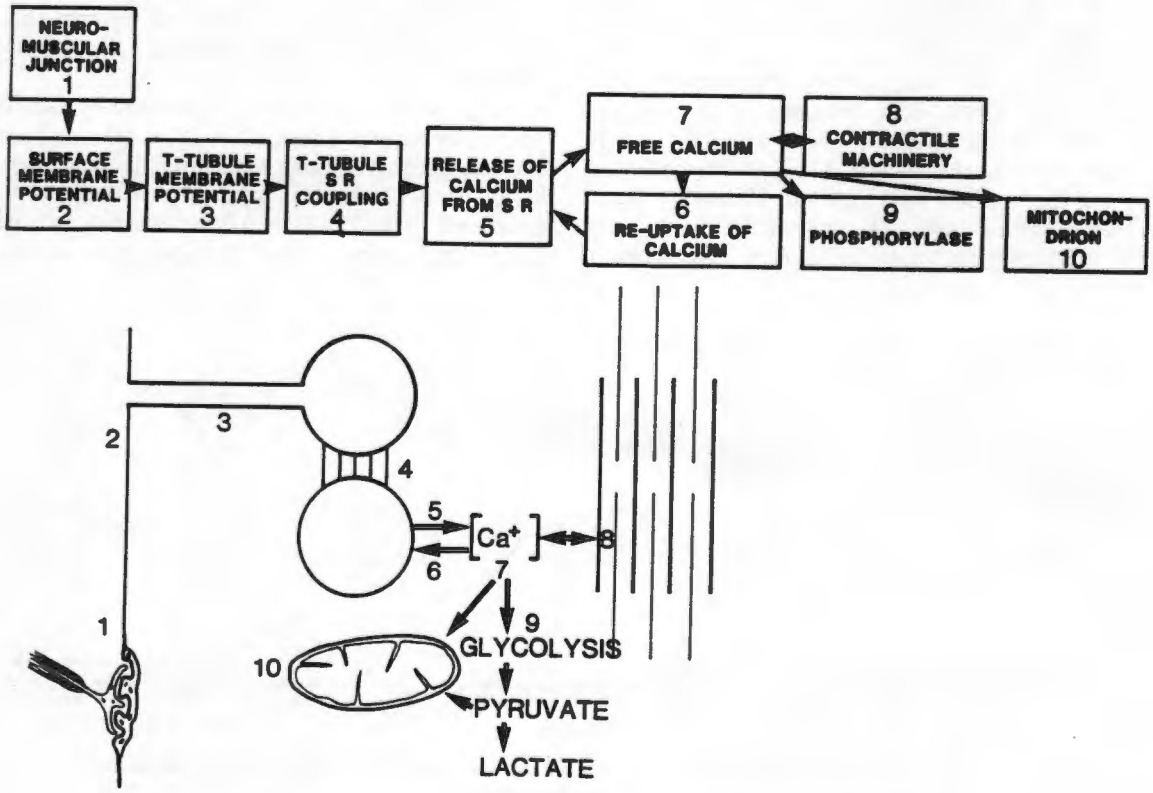

FIG. 2. Sequential steps in skeletal muscle excitation–contraction coupling.

been submitted, that are most relevant to our present understanding of its mode of action.

Early work, predominantly that of Ellis and associates [11–13] showed the relaxant action of dantrolene to be unusual and to result from a depressive action within the muscle cell at one or more loci in the ECC train, specifically affecting the sarcoplasmic reticulum (SR). Briefly, these conclusions were based on observations that dantrolene:

(1) did not act on the CNS [13,56];

(2) had no effects on spinal polysynaptic reflex responses [13];

(3) acted directly and specifically on skeletal muscle and had no effect on cardiac or smooth muscle, nor did it affect respiration ventilation [13,15,21];

(4) had no effect on the neuromuscular junction [5,13,56] and produced no change in "train-of-four" ratios [21];

(5) had no effect on the electrical properties of sarcolemma or T-tubular mechanisms, nor was the EMG affected [13,35];

(6) caused dissociation of muscle ECC by interfering directly with SR calcium release or the preceding "trigger calcium", or both. These deductions, based originally on observation of the interactive effects of dantrolene with those of caffeine, procaine and EDTA on *in vitro* induced muscle twitch and tetanus [13], were confirmed by the direct observation by van Winkel [55] and Desmedt and Hainout [7] that dantrolene reduced SR calcium release, both in rate and amount, without completely abolishing it. Ellis and Carpenter [13] postulated that the effect on trigger calcium might be the basis for the specificity of action of dantrolene on skeletal muscle, as opposed to its lack of action on cardiac and smooth muscle, in which sarcolemmal calcium transients constitute the more important mechanism in ECC;

(7) had no effect on SR calcium uptake [13,53,55].

Morgan and Bryant's conclusions [39] based on the foregoing and their own observations of the effects of dantrolene on rheobasic potential, chronaxie and on voltage dependent "charge movements", provided the best concept, at that time, of the mode of action of dantrolene. They proposed that dantrolene acted to cause skeletal muscle relaxation by interference with ECC at no fewer than two sites, the SR membrane and the voltage-dependent "charge movements" of the T-tubular–SR coupling. At the first site the effect is to decrease the release of calcium out of the SR, possibly by decreasing the mobility of a natural calcium ionophore. At the second site the effect is produced by an increased rate of movement of the "charge movement particles" to the OFF position.

Whatever its mechanism, depression by dantrolene of the rate and amount of SR calcium release finds its most dramatic expression in the abrupt termination of the MH syndrome it engineers—illustrated in figures 3 and 4 by animal biochemical and human clinical data [24,29]. This action,

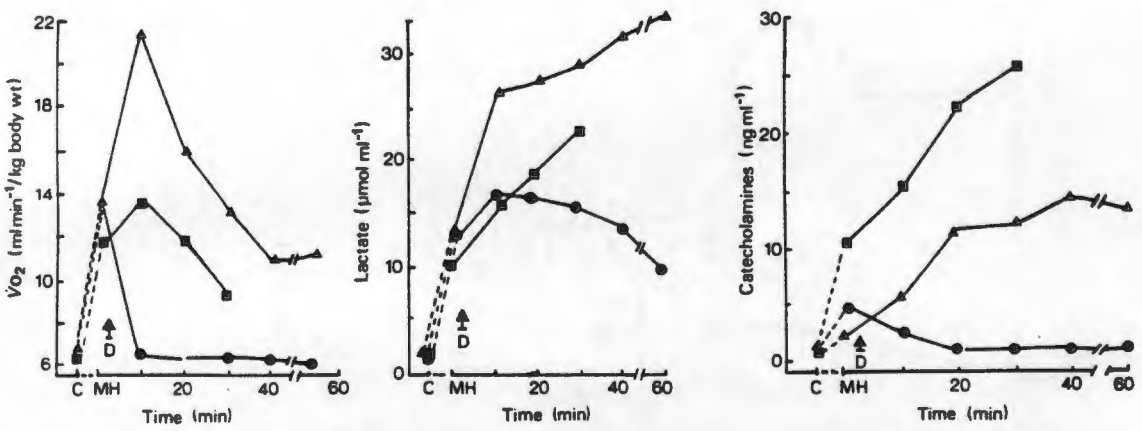

FIG. 3. Effects of dantrolene administration (D) on the $\dot{V}O_2$, lactate production and catecholamine secretion of MHS swine provoked into malignant hyperthermia by anaesthesia with halothane. ■ = Untreated; ▲ = supportive treatment only; ● = supportive treatment and dantrolene 7.5 mg kg^{-1}. (From Gronert, Milde and Theye [24] with permission.)

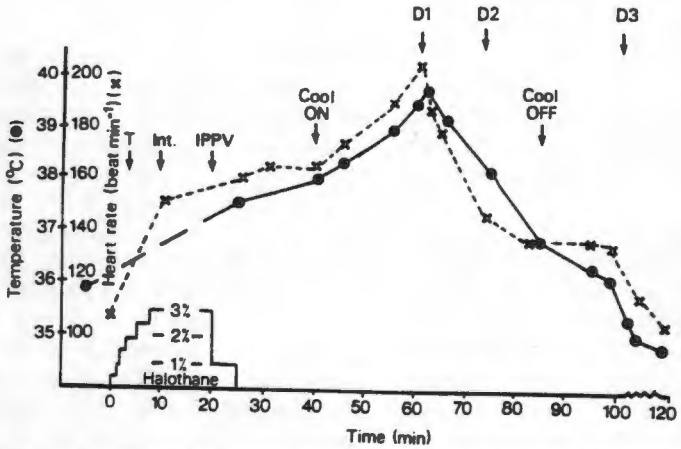

FIG. 4. Malignant hyperthermia: illustrative events chart from a 9-yr-old female patient admitted with congenital kyphoscoliosis (see associated conditions), for insertion of Harrington Rods. Note: Onset tachycardia; progression of syndrome despite discontinuation of halothane and institution of cooling; immediate response to dantrolene (delay in administration occasioned by its being obtained from neighbouring hospital). The operation was successfully performed 6 weeks later, when prophylactic i.v. dantrolene was given with induction. T = Thiopentone; Int. = intubation. Cool ON = Start of cooling; Cool OFF = discontinuation of cooling; D1, D2 = dantrolene 1 mg kg^{-1}; D3 = dantrolene 0.5 mg kg^{-1}. (From Harrison and Chapman [29], with permission).

which has been shown by direct measurement by Lopez [37] to correlate with a dantrolene-induced decrease in myoplasmic free calcium, has the attributes of the turning off of a switch and itself carries implications for both the pathogenesis of MH and the mode of action of dantrolene. Considered together with observed lack of effect of dantrolene on SR calcium uptake, it implies that continued SR calcium release—as against decreased SR uptake—is fundamental to the genesis of MH. Once this is terminated, the function of other calcium modulating mechanisms appears adequate to return myoplasmic free calcium to resting concentrations, permitting relaxation of muscle and termination of the syndrome.

An attractive current hypothesis identifies an abnormality in the mechanism of calcium-induced calcium release (CaIR) from the SR as the causative functional lesion of susceptibility to MH [16, 17]. This putative third SR calcium channel, which has no physiological role in the mechanism of skeletal muscle contraction, has been shown to have a lower than normal threshold and to respond with a faster than normal rate of calcium release, in subjects susceptible to MH [31]. As some similarity had been demonstrated between caffeine (and halothane) and CaIR [16], it seemed not unreasonable to expect that dantrolene would decrease the latter, as it has been shown to inhibit the former [13, 43].

This, however, has not proved to be the case [40]. Examining the effects of dantrolene on SR calcium release induced by caffeine, calcium jump and depolarization, Danko and colleagues [6] showed these to be complex and clearly distinguishable from those of the conventional SR calcium channel blocking drugs such as amethocaine and procaine, which inhibit all these modalities equally [1]. While inhibiting caffeine- and halothane-induced SR calcium release, dantrolene produced no effect on CaIR and exhibited a time-dependent dual effect on depolarization-induced calcium release—an initial inhibition being followed by enhancement. Associated with these findings was a correlative pattern in the binding of dantrolene to both SR and T-tubular membranes. Two kinetically distinguishable binding sites were identifiable on the T-tubular membranes, the slow binding site being related to the effects of the drug on depolarization-induced calcium release. Of that portion which bound to the SR, little competed with caffeine binding sites, suggesting that much of the binding of dantrolene was to non-specific sites unrelated to calcium release.

Considering the above in the light of their therapeutic activity in the MH syndrome, one may speculate that the difference between the

uncertain action of the conventional SR calcium-blocking drugs such as procaine and the completely reliable action of dantrolene [28] must lie in the latter's more complex action involving, as it does, a major effect on the T-tubular–SR coupling mechanism. Such a postulate carries its own implications for the conceived pathogenesis of MH.

In this regard it is important to note that, while dantrolene has no effect on normal sarcolemma, its action on the sarcolemma of MHS muscle is of relevance. Gallant, Godt and Gronert [22] have demonstrated that, whereas exposure to halothane has no effect on the sarcolemma of normal muscle, that of MHS muscle responds to such exposure by a depolarization of between 5 and 15 mV—a reaction that is antagonized by dantrolene. While this degree of depolarization, with its accompanying increase in calcium permeability, would not be sufficient of itself to initiate contraction in normal muscle, it could so do in the presence of SR that displayed a low threshold CaIR mechanism such as is postulated to characterize MHS muscle [44].

All in all, therefore, it seems that, although dantrolene provides a specific and life-saving treatment for the syndrome of MH, we are still a long way from understanding the finer details of its mode of action and, correspondingly, those of the pathogenesis of MH.

Effective antagonism of the muscle relaxant properties of dantrolene sodium is provided by germine monoacetate (GMA) [34].This reversal follows no direct pharmacological antagonism, but is the result of the property of GMA in causing repetitive firing of muscle in response to single stimulation and, so, repetitive myoplasmic free calcium release. In essence, it produces the effects of a brief tetanus, which is well documented as being depressed by dantrolene less than single twitch [13, 42].

Pharmacokinetics

Following its ingestion by mouth, approximately 70% of dantrolene is absorbed, peak plasma concentration being reached in 6 h [10]. However, especially in children, there is great variation between patients in the plasma concentrations achieved relative to the oral dose [36]. This fact doubtless accounts for the reported failure in prophylactic efficacy for MH of dantrolene administered by mouth [19, 47].

After i.v. administration of dantrolene to the conscious patient, the plateau maximal depression of muscle twitch response (75% depression) and the maximal depression of grip strength (42% depression) coincides with the administration of an accumulative dose of 2.4 mg/kg body weight. This achieves a blood dantrolene concentration of 4.2 $\mu g\ ml^{-1}$. Thereafter the elimination half-life ($T_{\frac{1}{2}}^{\beta}$) is 12 h, although the blood concentration is maintained at a steady value within the therapeutic range for about 5 h. Residual dantrolene concentration in the blood at 24 h after such a dose is 1.7 $\mu g\ ml^{-1}$ and this is reflected subjectively by patients, in a feeling of weakness. This may persist for up to 48 h, by which time the residual blood concentration of dantrolene has decreased to 0.3 $\mu g\ ml^{-1}$ [21].

Dantrolene is microsomally metabolized in the liver, through both oxidative and reductive pathways. The former results in hydroxylation of the hydantoin ring to form 5-hydroxydantrolene (5HD), while reduction of the nitro- moiety of the benzene ring results in the formation of aminodantrolene, which is then acetylated to form the reduced acetylated derivative of dantrolene (RAD) [36]—metabolites which themselves manifest some muscle relaxant properties [14].

Excreted in both the urine and the bile, dantrolene is 79% excreted as 5HD, 17% as RAD, while 4% of the dose appears unchanged in the urine [10, 36].

Adverse effects and drug interactions

Although dantrolene is documented as having no effect on the CNS, the commonest adverse reactions it causes are symptoms of dizziness, lightheadedness and drowsiness. These feelings can accompany both acute i.v. and chronic oral administration of the drug [21, 36]. There is, as yet, no suitable explanation for these changes in the sensorium. It is suggested that they may exemplify similar changes in the sensorium reported to accompany inhibition of neuromuscular transmission *per se* by other drugs [21]. While these unpleasant side effects may be associated with nausea and vomiting, the latter more commonly follows oral ingestion of the drug, in which case they may be accompanied by diarrhoea [10].

The major adverse reactions that have been documented following administration of dantrolene, have all accompanied its chronic administration by mouth. Perhaps most important have been reports of hepatic dysfunction [18, 45, 54].

However, several reports to the contrary lead one to question whether the drug alone was responsible for the hepatic dysfunction observed. To cap the reports of Dykes [10] of no change in serum biochemistry, urine function or haematological indices following prolonged administration by mouth, and the failure of Flewellen and colleagues [21] to find any change in SGOT and SGPT concentrations to accompany acute i.v. administration of dantrolene, Durham, Gandolfi and Bentley [9] have reported their failure to produce any evidence of dantrolene hepatotoxicity in mice, even after enhancement of any hepatotoxic potential by the inhibition of acetylation, depletion of glutathione, induction of biotransformation and the promotion of reductive metabolism. Further, Sorensen and Acosta [50] have failed to produce in hepatocyte cultures any evidence of hepatotoxicity from dantrolene.

Other adverse reactions include reports of the relatively common and non-specific drug reaction of an acneiform rash associated with prolonged oral ingestion of dantrolene [10].

The most important adverse interaction of dantrolene with another drug is that with verapamil—a drug which may be considered indicated in the management of MH associated-cardiac tachyarrhythmias. While dantrolene alone has no myocardial effects, Saltzman and others [48] and Lynch and colleagues [38] reported marked myocardial depression in swine and dogs after administration of verapamil in the presence of dantrolene, and *vice versa*. Although this reaction has yet to be described in man, simultaneous administration of verapamil and dantrolene should be regarded as contraindicated in any event, as the cardiac arrhythymias that accompany MH invariably respond to the termination of the syndrome with dantrolene and correction of the associated acidosis and hyperkalaemia.

As dantrolene causes a decrease in the force of muscle contraction, some mechanical synergism with drugs which block neuromuscular transmission can be expected. Driessen, Wuis and Gielen [8] reported an unexpected interaction of dantrolene with the non-depolarizing neuromuscular blocking agent, vecuronium, in which the presence of dantrolene caused marked prolongation of the neuromuscular junction recovery time as monitored by the evoked EMG—a modality not normally affected by dantrolene. They speculated that this effect may result from decreased transmitter mobilization at the neuromuscular junction because of impairment of calcium release from storage sites within the cholinergic terminals. Little more can be said of this reaction until there has been considerably more investigation.

Therapeutic use of dantrolene

Dantrolene was originally introduced to therapeutics for the long term functional control of chronic states of skeletal muscle spasm such as follow stroke or characterize cerebral palsy. That it may still have some use in this regard is born out by the very favourable report from Ketel and Kolb [30] of its successful long term use in the treatment of stroke patients with spasticity limiting return of function.

However, the most important therapeutic use of dantrolene today is in the treatment of MH, in which circumstance it is indeed life-saving [23]. It has now proved equally life-saving in the management of the functionally related neuroleptic malignant syndrome [25]. More recently, its use has been suggested as an adjunct in the treatment of tetanus, especially at the stage at which the patient is weaned from conventional neuromuscular blocking and antispasmodic drugs [52].

For i.v. use, dantrolene is supplied as an orange powder (together with sodium hydroxide and mannitol), in a vial containing 20 mg, which is dissolved by the addition of 60 ml of water. The resulting solution, pH 9–10, is irritant to veins and should be injected to a fast-running i.v. infusion or large vein. Effective therapeutic concentrations of dantrolene for the treatment of MH are achieved after the administration of 2.4 mg kg^{-1}. If necessary, this dose may be repeated at 15-min intervals, until relaxation of muscle rigor, control of tachycardia and arrhythmia and cessation of temperature increase are achieved, or a total dose of 10 mg/kg body weight has been given. It is seldom that a dose exceeding 4 mg kg^{-1} is required.

Although patients may complain of muscle weakness even up to 48 h after administration, this will not be of an order which imperils ventilatory capacity, or coughing.

Once controlled, the MH syndrome may recur in the post-anaesthetic period. Dantrolene administration should be repeated immediately at the first sign of such occurrence. In view of this possibility, some advocate a repeat prophylactic dose of dantrolene 2.4 mg kg^{-1} after 10–12 h—the elimination half-life of the drug.

In the treatment of MH, it must be emphasized that after a timely administration of dantrolene, after early diagnosis, little or no supportive therapy is necessary. The need for therapy is directly, perhaps exponentially, proportional to the delay in diagnosis and the institution of treatment. Such supportive measures and the treatment of MH generally are dealt with elsewhere in this issue and will not be discussed further here.

Following control of the syndrome the patient must be closely monitored and sedated for 24 h after initial recovery, for fear of recurrence of the syndrome.

Pre-anaesthetic prophylactic dantrolene for the MHS patient

The application of simple guidelines will ensure safe anaesthesia for the individual known to be susceptible to MH. These guidelines are:
(1) Obtundation of the patient's "trigger" sensitivity.
(2) Utilization of meticulous vital function monitoring.
(3) Avoidance of agents known to trigger MH.
(4) Immediate availability of i.v. dantrolene.

While it once seemed obvious that the prophylactic administration of dantrolene was the necessary and most efficacious way of achieving the first of these desiderata [27], this is now subject to some controversy. Certainly, the use of oral pre-anaesthetic prophylactic dantrolene regimens [3]—which may involve admission to hospital for a 2–4 day programme of administration of the drug, from which the patient may suffer many unpleasant symptoms and which does not guarantee prophylactic systemic blood concentrations of dantrolene [19, 20, 47]—are no longer to be recommended. Indeed, given the application of correct anaesthetic technique with meticulous vital function monitoring, together with the immediate availability of i.v. dantrolene, many would question the necessity for the prophylactic administration of dantrolene at all, especially if the planned surgical procedure is relatively short. However, when the surgery contemplated is prolonged, there are those who still think the patient's best interests are served if dantrolene is administered prophylactically. In these circumstances, it should be administered i.v. in a dose of 2.4 mg kg^{-1} during the induction of anaesthesia and—to avoid unpleasantness for the patient—after consciousness has been lost [29].

When this regimen is adopted, it must be noted that the effect that pre-anaesthetic prophylactic dantrolene might have on the outcome of *in vitro* contracture testing of muscle biopsied in these circumstances, is controversial. While, *a priori*, an invalidating inhibitory effect on both caffeine- and halothane-induced contractures is to be expected, and indeed has been reported [3, 33], the contrary has also been documented [41, 46]. These reported discrepancies are in all probability dose (and tissue concentration) dependent.

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A Pharmacological *in Vitro* Model of Malignant Hyperpyrexia

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SUMMARY

Caffeine causes contracture in muscle. Drugs which initiate malignant hyperpyrexia in susceptible pigs, lower the threshold concentration of caffeine at which this contraction occurs. The implications of and uses for this finding are described.

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Anaesthetic-induced malignant hyperpyrexia has been shown to be a disorder of muscle.[1-3] Certain pigs of the Landrace strain in South Africa[4] and Australia,[5] the Poland China strain in America,[6] and Landrace Wessex in England,[7] have been shown to suffer from a similar disorder, and have served as useful models of this puzzling lethal condition. But pigs as experimental animals are expensive, and supplies of animals of the specific susceptible strain sporadic. Attempts at direct breeding of this specific strain have not proved very successful in our hands, and so we searched for a more readily obtainable and cheaper animal experimental model.

Whatever the precise nature and site of the defect of malignant hyperpyrexia myopathy, Kalow *et al.*[2] showed that it produces responses in muscle rather similar to those which follow application of caffeine, a conclusion which gained pharmacological support from my demonstration that initiation of the syndrome in susceptible pigs could be effectively blocked by procaine.[3] Strobel and Bianchi[8] demonstrated that if frog sartorius muscle was exposed to subcontracture-producing doses of caffeine, subsequent administration of halothane to the preparation led to rigor in a manner analogous to the response of the muscle of hyperpyrexia-susceptible pigs. As the only known subjects of malignant hyperpyrexia are mammals, I chose to investigate rat rectus as an *in vitro* model. The rat is a readily available and inexpensive laboratory animal, and the rectus is easily cut into strips of parallel fibres.

METHOD

The apparatus is shown diagrammatically in Fig. 1. One-gram strips of rat rectus were mounted in a vertical bath of approximately 120 ml volume, containing pharmacological solution (Table I), to which 5 mg/100 ml of calcium chloride was added. This solution was oxygenated with carbogen (3% CO_2 and 97% O_2) bubbled through a dispersion grid. The pH, monitored by a pH probe (Metrohm) was buffered to 7,4 by the addition of trihydroxymethylaminomethane as needed. Temperature was maintained at 22° - 32°C. Muscle strips were mounted vertically between a fixed lower clamp and a platinum wire attached to the cross beam of a Devices displacement transducer, counterbalanced by a 1,5 g weight, this arrangement permitting isotonic contraction of the muscle. The muscle attachments served as electrodes of a stimulator, programmed to stimulate the muscle supramaximally at 0,25 Hz. Muscle contractions were recorded on a Beckman 10″ recorder. Caffeine stock solutions were made by the acid solution method of Frank *et al.*[9] Anaesthetic agents were added by passage of the carbogen stream through the appropriate vaporizer, provision being made by a venting side arm to keep gas flows within the performance tolerance of the vaporizer. Relaxant drugs used were from commercially available solutions.

TABLE I. COMPOSITION OF SOLUTION

Salt	Concentration (mEq/L)	Salt	Concentration (mEq/L)
Sodium	137,6	Potassium	5,8
Magnesium	1,6	Chloride	142,3
Sulphate	1,6	Phosphate	1,1

RESULTS

Caffeine in increasing concentration causes enhanced muscle contraction, followed by shortening and rigor of the muscle (Fig. 2). If a muscle is exposed to a subcontracture-producing concentration of caffeine, subsequent exposure to halothane causes shortening (Fig. 3). If, on the other hand, the muscle strip is first exposed to halothane and caffeine is then added, a reciprocally synergistic effect is evident and shortening of the muscle occurs at a lower concentration of caffeine than when caffeine alone is added (Fig. 4). By noting the concentration of caffeine at which shortening occurred after exposure to halothane—or any other anaesthetic—this effect could be quantitated. I have called this concentration of caffeine at which shortening started to develop the 'caffeine threshold'. As this level differed slightly between rats, I have in each case used muscle from the same rat as its own control. The normal caffeine threshold was found to be 1 - 1,5 mM caffeine.

The effect of various anaesthetic agents and relaxants on the caffeine threshold was investigated. Results are given in Table II. From these it will be seen that agents which have been shown to initiate the syndrome of malignant hyperpyrexia in susceptible pigs[4] lower the caffeine

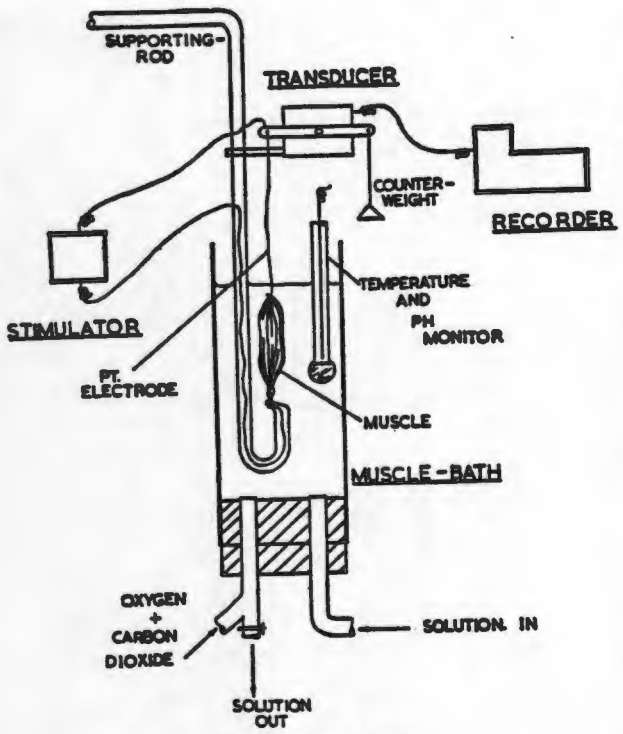

Fig. 1. Muscle bath.

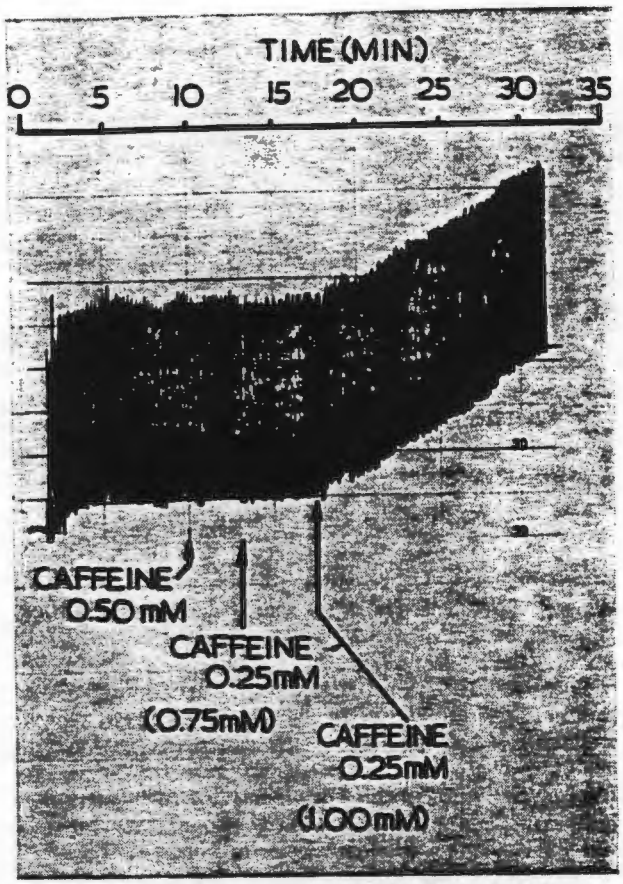

Fig. 2. The effect on muscle of increasing caffeine concentration.

threshold of this muscle preparation. There is discrepancy between the agents that have been incriminated as trigger agents in humans[20] and those that have been shown to so act in susceptible pigs.[4] Though many more agents have been incriminated in the human disease, the commonest agents in humans—halothane and succinylcholine—are those which strongly initiate the condition in pigs and lower the caffeine threshold in this preparation. (Chloroform, which does so in pigs and which lowered the caffeine threshold preparation in our *in vitro* experiment is no longer used in humans). A notable exception is methoxyflurane. Though this agent markedly lowered the caffeine threshold in our preparation and has been recorded as being a strong inducing agent in susceptible human subjects, I have failed to initiate malignant hyperpyrexia with it in susceptible pigs.

It is also of interest to note that though Chalstry and Edwards[21] have described pancuronium as a trigger agent in pigs, I have failed to confirm this.

CONCLUSIONS

These observations appear to support Kalow's hypothesis that the functional lesion of malignant hyperpyrexia in susceptible subjects is a caffeine-like defect in the function of the sarcoplasmic reticulum. It seems that certain anaesthetics cause a similar alteration in membrane function of the sarcoplasmic reticulum. If susceptible human subjects, pigs, or muscle preconditioned by caffeine, are exposed to such agents, the defect is accentuated by a synergistic action, so causing rigor and precipitating malignant hyperpyrexia.

TABLE II. EFFECT OF ANAESTHETICS AND RELAXANTS ON CAFFEINE THRESHOLD OF RAT MUSCLE

Agent	Effect on rat muscle caffeine threshold	Initiation of malignant in susceptible	
		Pigs	Humans
Halothane	↓	++	++
Chloroform	↓	++	++
Methoxyflurane	⇊	−	++
Cyclopropane	↓	±	+
Trichlorethylene	−	−	−
Ether (diethyl)	−	−	±
Fluroxene	↑	−	−
Succinylcholine	↓	++	++
Curare	−	−	−
Gallamine	−	−	−
Pancuronium	−	−	−

↓ = lowers.
— = no effect.
↑ = raises.
\+ = initiates malignant hyperpyrexia.
Pigs — tested in our laboratory; humans — described in literature.

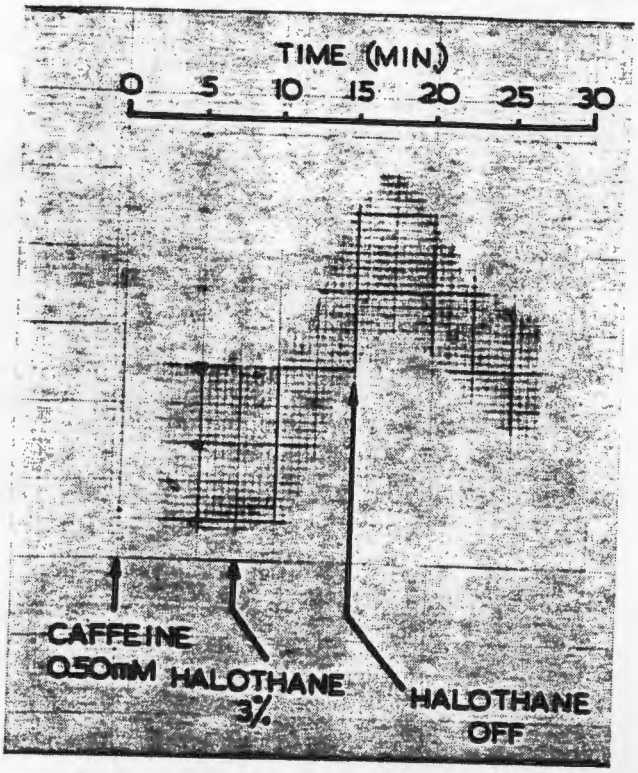

Fig. 3. Muscle exposed to halothane after subcontracture concentration of caffeine.

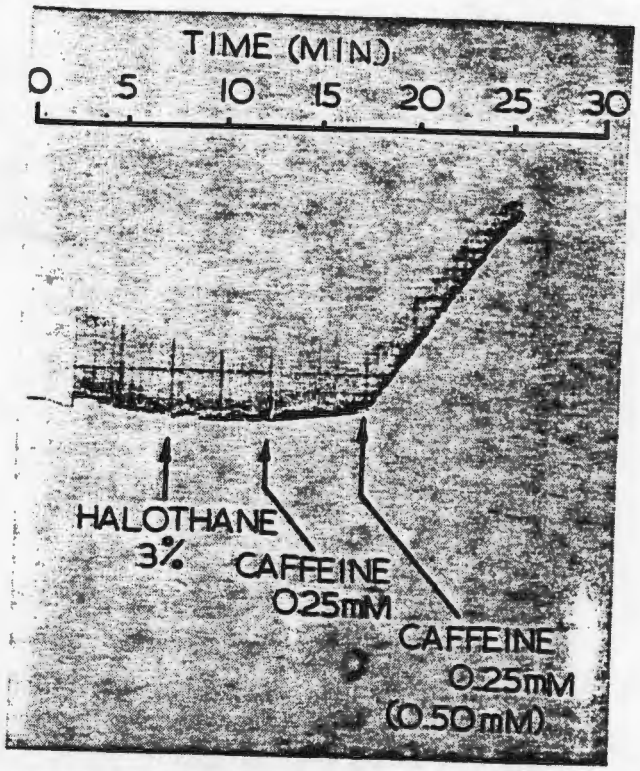

Fig. 4. Caffeine threshold of muscle first exposed to halothane.

A practical use for this *in vitro* rat muscle preparation would be as a screening test for new anaesthetic agents for malignant hyperpyrexia-inducing properties. Further uses of this model are the investigation of the efficiency of agents such as procaine to block initiation of malignant hyperpyrexia, or perhaps of even more importance in the therapeutic field, the development from this of a preparation of sustained rigor on which agents which might actively relax rigor could be sought and tested.

I should like to thank Mr Jan Korrubel of the Department of Bio-engineering, Groote Schuur Hospital, for construction of the muscle stimulator as well as modifications to the transducer. The project was supported financially by grants from the Anglo American and De Beers Anaesthetic Research Funds.

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ALTHESIN AND MALIGNANT HYPERPYREXIA

G. G. HARRISON

SUMMARY

Althesin is shown to block initiation of malignant hyperpyrexia by halothane in susceptible swine. It has no effect on the established syndrome, nor does it prevent the initiation of hyperpyrexia by suxamethonium.

The growing number of cases of anaesthetic-induced malignant hyperpyrexia with its genetic associations has brought with it a growing number of individuals known to be at risk from this condition, should they present for anaesthesia and surgery. In this context, the observation of Hall, Trim and Woolf (1972) that Althesin appears to block initiation of malignant hyperpyrexia by halothane/suxamethonium in susceptible pigs, is of great clinical importance. As there are differences in the response of different strains of malignant hyperthermia susceptible (MHS) pigs (Hall, Trim and Woolf, 1972; Harrison et al., 1969; Jones et al., 1972) and because the observations of Hall's group were made in few pigs exposed to halothane for 15 minutes only, it seemed important to confirm these observations in another strain of MHS pig during a longer exposure to halothane and, perhaps of more importance, to investigate the effect of Althesin on the established syndrome.

METHOD

MHS pigs were selected from the general pig intake of our Surgical Research Laboratory by exposure of all to a challenge dose of halothane (Harrison et al., 1969). Those displaying the characteristic rigor were chosen, susceptibility to malignant hyperpyrexia being confirmed by estimation of the serum CPK (Woolf et al., 1970). Of the six pigs chosen (8–16 weeks old) all had resting CPK values in excess of 450 i.u./l. rising to 1500–2000 i.u./l. following exposure to halothane. The weights of pigs selected varied from 18 to 70 kg.

Anaesthesia was induced in the experimental animals by administration of Althesin 400–500 μl/kg intravenously via an ear vein. Following endotracheal intubation, the animals were ventilated with a nitrous oxide and oxygen mixture by means of a Blease Pulmoflator. Thereafter, Althesin was administered continuously as an intravenous infusion (50 μl/ml) via a cannula in the right atrium, which was also used for blood sampling when required.

Initiation of the syndrome of malignant hyperpyrexia in response to halothane and suxamethonium challenges was judged by the onset of tell-tale rigor in the hind legs and a rise in intramuscular temperature recorded by a thermistor probe (Elab) inserted deep into the thigh muscles.

Six MHS pigs so anaesthetized were challenged with halothane inhalation, 2% decreasing to 0.5%, for 1 hour. Althesin was administered throughout at rates varying between 5–15 μl/kg/min, but increasing to as much as 50 μl/kg/min in one pig to abort onset of the syndrome which appeared imminent. After 1 hour, halothane and Althesin were discontinued until the animals showed signs of waking. Thereupon halothane inhalation was recommenced to confirm the malignant hyperpyrexic response to halothane in the absence of Althesin. In a repeat experiment in one survivor, after 30 minutes halothane anaesthesia the animal was challenged with suxamethonium 50 mg.

RESULTS

Althesin administration successfully blocked initiation of malignant hyperpyrexia by halothane in five of the six MHS pigs challenged. The susceptibility to malignant hyperpyrexia of all pigs used was confirmed by the onset of rigor and rise in temperature in response to re-exhibition of halothane after discontinuance of Althesin. This confirmatory initiation of the syndrome proved reversible by timely discontinuance of the halothane in all but one of these five animals (fig. 1). In one animal, Althesin administration (5–10 μl/kg/min—possibly too low a dose) failed to block the onset of fatal malignant hyperpyrexia.

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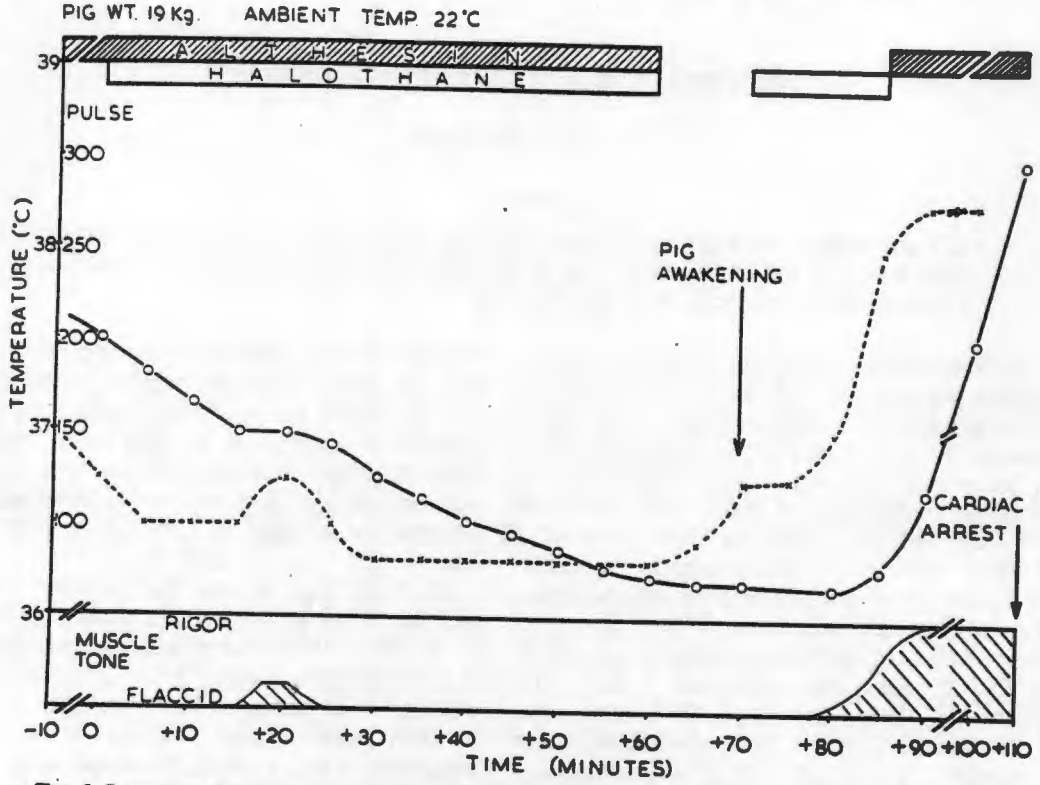

FIG. 1. Intramuscular temperature, pulse and muscle tone record of MHS pig exposed to halothane after administration of Althesin. Terminal exposures to halothane caused irreversible malignant hyperpyrexia.

Althesin also failed to prevent the onset of fatal hyperpyrexia in the animal to which suxamethonium was administered after 30 minutes of halothane inhalation. The use of massive doses of Althesin (30–50 μl/kg/min) in this animal, as also in another in which the syndrome was established, had no effect—except perhaps that the rate of temperature rise was slower than usual (Berman et al., 1970). This may have resulted from the vasodilatation caused by Althesin (du Cailar, 1972) permitting a greater heat loss.

DISCUSSION

Althesin blocks initiation of malignant hyperpyrexia by halothane in MHS pigs. The results of our experiments differ a little from those of Hall, Trim and Woolf (1972) in that in ours Althesin failed to block the trigger action of suxamethonium given in the presence of halothane. Further, continuous Althesin administration as against a single dose, was necessary to protect pigs successfully against the trigger action of halothane. But it must be pointed out that the reactor response of the pigs used by Hall's group and of ours differ. Whereas our MHS pigs responded to a challenge of halothane alone (Harrison et al., 1968) those used by Hall's group require suxamethonium in addition (Hall et al., 1966).

In view of the above, Althesin would appear to be the preferred anaesthetic induction agent in patients who have survived episodes of malignant hyperpyrexia, or are known from family history and raised CPK levels to be at risk (King, Denborough and Zapf, 1972). Such use of Althesin would, however, not justify the subsequent use of halothane in a patient at risk.

It is disappointing in the extreme that Althesin, with its lack of effect on cardiac output (Savege et al., 1972), appears to be useless in the treatment of the established syndrome of malignant hyperpyrexia, for the treatment currently in vogue—the use of procaine in large dose (Harrison, 1971)—requires, because of its myocardial depressant effects, great circumspection.

ACKNOWLEDGEMENTS

I am indebted to: Anglo American and de Beers Anaesthetic Research Fund for financial assistance. Glaxo Allenbury (S.A.) Ltd for supplies of Althesin. The Department of Surgery, University of Cape Town and its J. S. Marais Surgical Research Laboratory, for supply of MHS pigs. Mr B. Sassman for initial screening of the pigs. To all of these my grateful thanks.

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MINAXOLONE AND MALIGNANT HYPERTHERMIA

Sir,—Althesin has been shown to block initiation of the malignant hyperthermia syndrome by halothane in MHS swine (Hall, Trim and Woolf, 1972; Harrison, 1973), and recommended for use in human subjects susceptible to the condition (Honda et al., 1977).

It was of interest, therefore, to ascertain whether minaxolone, the water-soluble steroid anaesthetic (Aveling et al., 1979; McNeill, Clarke and Dundee, 1979) possessed similar properties.

We report here the results of our investigation in four MHS swine of the effects of continuous infusion of minaxolone on the initiation of the MH syndrome by halothane. The experimental programme used was similar to that described previously (Harrison, 1973) with modification which included:

(1) Direct arterial pressure monitoring via femoral artery cannulation.
(2) Use of a cerebral function monitor.
(3) GLC assay of blood concentrations of minaxolone during the experiment.

Dosage of minaxolone was as follows: induction—1.4–2 mg kg^{-1}; maintenance infusion—40–100 $\mu g\ kg^{-1}\ min^{-1}$; total dose (45–60 min)—4–6 mg kg^{-1}.

Intravenous concentrations of minaxolone varied from 0.66 to 1.55 $\mu g\ ml^{-1}$ in most, and 2.84 $\mu g\ ml^{-1}$ in one pig where the infusion rate was greatly increased in an attempt to abort the onset of MH.

Although minaxolone itself did not provoke the onset of the syndrome, in all four experiments it failed to block initiation of the MH syndrome in response to the administration of halothane, the syndrome being well established within 20 min of exposure in each case.

We cannot as yet define the role, if any, of cremophor (the solvent for Althesin) in these contrasting actions of Althesin and minaxolone.

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RESPONSE OF MHS SWINE TO I.V. INFUSION OF LIGNOCAINE AND BUPIVACAINE

G. G. HARRISON AND D. F. MORRELL

SUMMARY

We report the negative response of MHS swine to i.v. infusions of lignocaine and bupivacaine yielding plasma concentrations which equal or exceed those reported in humans during extradural analgesia. It is concluded that local anaesthetic techniques using the amide-linked local anaesthetics administered in conventional dosage are safe to use in patients known to be genetically susceptible to malignant hyperthermia.

While it is accepted that the anaesthetic technique of lowest risk for the patient known to be genetically susceptible to malignant hyperthermia is some form of regional or local analgesia, strictures against the use of the amide-linked group of anaesthetic drugs (lignocaine and bupivacaine) are still voiced (Britt, 1973; Britt, Kwong and Endrenyi, 1977a; Relton, Britt and Steward, 1973; Strobel, 1977).

Such strictures are based on the work of Bianchi and others which demonstrated that lignocaine, in contrast to procaine and other ester-linked tertiary amine local anaesthetics, potentiated caffeine and caffeine/halothane induced contractures of muscle *in vitro*, enhancing also the oxygen uptake of such preparations (Bianchi and Boulton, 1967; Novotny and Bianchi, 1967; Bianchi, 1968; Strobel and Bianchi, 1971).

Clinical evidence cited in support of such strictures is scant and consists of "unpublished data" and "personal communication" reporting rare incidents of putative initiation of malignant hyperthermia by the administration of amide-linked anaesthetics to MHS individuals (Kalow et al., 1970; Britt, Kwong and Endrenyi, et al., 1977b).

In many hospitals today, the most readily available local anaesthetic drugs are usually members of the amide-linked group—lignocaine, mepivacaine and bupivacaine. This circumstance, and the fact that the concentration of lignocaine which produced potentiation of caffeine induced muscle rigor *in vitro*, was about 100-fold greater than the convulsive threshold *in vivo* in man (Foldes et al., 1960; Sasyniuk and Ogilvie, 1975), and was so greatly in excess of concentrations likely to be found systemically after conventional clinical use, stimulated us to examine the validity of these strictures against the use of such local anaesthetics in the MHS patient. We used the MHS Landrace pig as the experimental model.

We report here the response of MHS Landrace swine to the i.v. infusion of lignocaine and bupivacaine in doses which produced serum concentrations equal to and greater than those reported in humans following extradural and other major regional blocks or topical analgesia of the larynx.

METHODS

Four MHS Landrace swine, screened and identified as described previously (Harrison, 1977), were used in this investigation. The reactions of these animals were observed in response to the infusion of ligocaine on 10 occasions (two pigs three times, two pigs twice) and bupivacaine on eight occasions (four pigs twice). Subsequently, each pig was submitted to a confirmatory halothane challenge.

The plan for each infusion was as follows:

Anaesthesia was induced with thiopentone administered via an ear vein, the trachea was intubated and anaesthesia was maintained with nitrous oxide in oxygen administered by intermittent positive pressure ventilation using a Manley ventilator. When necessary, supplementary doses of thiopentone were administered. Body temperature was recorded from the probe of an Electric Universal Thermometer (TE3, ELLAB, Denmark) placed deep in the medial muscle mass of the thigh. A catheter was introduced into a jugular vein via a cut-down in the neck and advanced to the level of the right atrium. This catheter served for blood sampling and for the i.v.

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administration of drugs. Blood samples (mixed venous blood) from this site were analysed for:

(1) Acid–base studies at 2, 5, 10, 15 and 30 min after local anaesthetic infusion (interpolation technique).

(2) Plasma concentrations of lignocaine/bupivacaine at 2, 5, 10, 15 and 30 min after infusion. Lignocaine and bupivacaine were assayed by the gas–liquid chromatographic method of Tucker (1970). A calibration curve with standard solutions was prepared from a blank specimen for each pig on each occasion. Using cyclizine hydrochloride as an internal standard and with quantitation by peak height ratios correlation coefficients were consistently greater than 0.998. (Pye Unicam GCV Chromatograph with dual FID and DP88 computing integrator using 2.1-m columns packed with 5% OV101.)

The test drugs, lignocaine and bupivacaine, were administered as follows: *Lignocaine*—initial dose 1.5 mg kg^{-1} followed by infusion of 30 $\mu g\ kg^{-1}\ min^{-1}$ in initial experiments, increased in later experiments to 2–3 mg kg^{-1} and 120 $\mu g\ kg^{-1}\ min^{-1}$. *Bupivacaine*—1 mg kg^{-1} administered over 5–10 min.

In view of the importance of the free base form of the anaesthetic to its contracture-producing ability (Bianchi, 1968), we deliberately induced severe metabolic alkalosis in two test animals by infusions of sodium bicarbonate 4.2% in addition to the routine use of hyperventilation.

Criteria of the malignant hyperthermic syndrome sought included onset of muscle rigor resulting in characteristic extension of hind legs (Harrison, 1975); supraventricular tachycardia; rapid progressive increase in muscle temperature; and metabolic acidosis.

RESULTS

No sign of the onset of malignant hyperthermia syndrome was observed in any animal in response to the i.v. infusion of lignocaine or bupivacaine.

The plasma concentrations of lignocaine and bupivacaine assayed during infusion are recorded in figure 1. Mixed venous pH values during the majority of infusions (13) were in the range 7.4–7.5. The ranges 7.5–7.6 and 7.6–7.7 were noted in two infusions each, and 7.2–7.3 in one.

At the conclusion of the experiment all animals, when exposed to halothane, developed MHS syndrome to an extent which required treatment with dantrolene i.v. to ensure their survival.

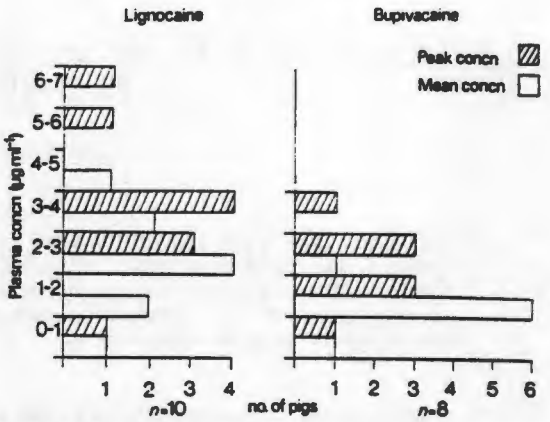

FIG. 1. Peak and mean plasma lignocaine and bupivacaine concentrations observed following i.v. infusion.

DISCUSSION

When local anaesthetic techniques are used, with the notable exception of bilateral intercostal nerve blocks, the greatest plasma concentrations of local anaesthetic drugs are recorded in association with extradural anaesthesia.

In the case of lignocaine, concentrations from 2.4 $\mu g\ ml^{-1}$ (Tucker and Mather, 1975) to 4.5 $\mu g\ ml^{-1}$ (Covino and Vassallo, 1976) are reported. With bupivacaine, plasma concentrations reported range from the former authors' report of 0.7 $\mu g\ ml^{-1}$ to 1.49 $\mu g\ ml^{-1}$ reported by Moore and others (1976). Exposure of MHS swine to such circulating concentrations and greater failed to evoke any untoward response. Our findings, considered with the lack of well-documented human cases of malignant hyperthermia provoked by lignocaine or bupivacaine, cast doubts on the validity of the strictures advocated on the use of the amide-linked group of local anaesthetic drugs in patients known to be susceptible to malignant hyperthermia, and suggest that these drugs may be safe when given in conventional clinical dosage.

ACKNOWLEDGEMENTS

MHS pigs were supplied by the J. S. Marais Laboratory of the Department of Surgery of the University of Cape Town and financial support came from Sterling Winthrop S.A. (Pty) Ltd. Assays were performed by Jeanne Meyer.

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REACTION DE PORCS SUSCEPTIBLES A L'HYPERPYREXIE MALIGNE A UNE INFUSION INTRAVEINEUSE DE LIGNOCAINE ET DE BUPIVACAINE

RESUME

Nous signalons dans cet article la réaction négative de porcs susceptibles à l'hyperpyrexie maligne (MHS), aux infusions de lignocaïne et de bupivacaïne entraînant des concentrations dans le plasma équivalentes ou supérieures à celles signalées chez les humains pendant une analgésie extradurale. On en a conclu que les techniques d'anesthésie locale basées sur les agents anesthésiants locaux de la classe des amides peuvent être administrés sans danger et aux doses habituelles aux patients que l'on sait être génétiquement susceptibles à l'hyperthermie maligne.

REAKTION VON MHS-SCHWEINEN AUF INTRAVENÖSE INFUSION VON LIGNOCAIN UND BUPIVACAIN

ZUSAMMENFASSUNG

Wir berichten über die negative Reaktion von MHS (anfällig auf maligne Hyperprexie)-Schweinen auf intravenöse Infusionen von Lignocain und Bupivacain in Plasmakonzentrationen, die diejenigen erreichen oder übertreffen, die bei Menschen während extraduraler Analgesie gemeldet werden. Es wird geschlossen, dass Lokalanästhesiemethoden, bei denen amidhältige lokale Betäubungsmittel in konventionellen Dosen verwendet werden, gefahrlos bei Patienten angewendet werden können, die genetisch auf maligne Hyperthermie anfällig sind.

RESPUESTA DE CHANCHOS CON HMS A INFUSIONES I.V. DE LIGNOCAINA Y DE BUPIVACAINA

SUMARIO

Informamos sobre la respuesta negativa del chancho con HMS a infusiones i.v. de lignocaina y de bupivacaina que tuvieron por resultado concentraciones en el plasma iguales o mayores de las registradas en seres humanos durante la analgesia extradural. Se llega a la conclusión de que las técnicas de anestesia local mediante agentes anestéticos locales del grupo de las ámidas administrados según dosis convencionales pueden usarse en toda seguridad en pacientes conocidos por ser susceptibles genéticamente a la hipertermia maligna.

Br. J. Anaesth. (1986), 58, 444–446

THE SCREENING OF ATRACURIUM IN MHS SWINE

D. F. MORRELL AND G. G. HARRISON

SUMMARY

The potential role of atracurium besylate as a trigger or attenuator of the malignant hyperthermia syndrome was tested in six MHS Landrace swine. Animals were tested for susceptibility and then exposed to atracurium given as an i.v. bolus both alone and concomitantly with 2% halothane. In no instance could the syndrome be triggered by atracurium nor did it convincingly attenuate the syndrome when triggered by halothane.

The non-depolarizing neuromuscular blocking drugs tubocurarine (Britt, Webb and Le Duc, 1974), gallamine (Ryan, 1979) and pancuronium (Chalstrey and Edwards, 1972; Waterman, Albin and Smith, 1980) have all been incriminated at some time as triggers of the malignant hyperthermia syndrome (MH) in susceptible individuals or swine. Although these original claims are now a matter of contention (Short et al., 1976; Denborough, 1979; Harrison, 1980) they do provide the *raison d'être* for the screening of all newly-developed non-depolarizing neuromuscular blockers for MH triggering potential.

We report the outcome of such an assessment of the recently developed non-depolarizing agent, atracurium besylate, in MHS Landrace swine. The design of the study differs from that of a previous study (Lucke, 1983) in that our animals were challenged (a) when atracurium-induced neuromuscular blockade was maximal and neither clinically waning nor reversed, and (b) with halothane alone, avoiding suxamethonium with its associated interaction with non-depolarizing agents at the neuromuscular junction (Harrison, 1973).

MATERIALS AND METHODS

Six MHS Landrace swine were selected on a positive MH response to a brief "barnyard" exposure to mask-administered halothane, the syndrome being reversed with dantrolene (Harrison, 1975). Weights ranged from 22 to 35 kg.

For all investigations, anaesthesia was induced in the pen with thiopentone administered via an ear vein on the first and via an internal jugular catheter on subsequent occasions. Following endotracheal intubation, anaesthesia was maintained with 70% nitrous oxide in oxygen administered via a volume preset ventilator set to maintain normocarbia (as assessed by arterial gas analysis) supplemented by intermittent thiopentone.

Catheters were inserted to an internal jugular vein and carotid artery via a cut-down in the neck to provide: a source for samples of blood for the measurement of blood-gas tensions and acid–base balance, a continuous waveform display and digital read-out of arterial and venous pressures (Statham P23 strain gauges), and a route for the infusion of drugs and fluids. ECG monitoring (Hellige Servomed) was maintained using chest wall electrodes.

Temperature was recorded from an i.m. thermistor needle probe deep in the thigh muscle mass (ELLAB).

The atracurium challenge consisted of a 1.0-mg kg^{-1} i.v. bolus in all instances except one, when 0.8 mg kg^{-1} was administered inadvertently. The halothane challenge was by way of introduction of 2% halothane (Fluotec Mark 2 vaporizer) to the fresh gas supply.

Established malignant hyperpyrexia was identified by 20% increases in resting heart rate and mean arterial pressure, a stiffening of the hind quarters and the development of a combined metabolic and respiratory acidosis. Those animals which were destined for subsequent challenges were salvaged with titrated amounts of i.v. dantrolene, i.v. sodium bicarbonate and hyperventilation with 100% oxygen.

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Before the challenge all animals were homeostatically stable, particular attention being paid to indices of cardiovascular function, and acid–base balance.

The design of the study was such that it could test the MH triggering potential of atracurium, and the modification of the triggering potential of halothane by atracurium.

Two animals (Nos 1 and 2) were challenged *de novo* with atracurium and then 1 h later with halothane and a second bolus dose of atracurium.

The remaining four animals were challenged initially with halothane in order to provide control times for the onset of the syndrome, and 2 days later were rechallenged with atracurium. The last two (Nos 5 and 6) were given a third challenge of simultaneously-administered halothane and atracurium 1 h after the second.

RESULTS

The results are set out in table I.

Animals Nos 1 and 2 were unaffected by the initial dose of atracurium, but the syndrome was triggered in its full fulminant form following exposure to halothane, the second dose of atracurium affording no protection. The syndrome, once established, was allowed to run its course without being fuelled by further halothane or reversed with dantrolene and was characterized by progressive limb stiffness, cyanosis, hypertension, tachycardia, muscle temperature increasing to above 40 °C, a combined metabolic and respiratory acidosis, and eventual death following the development of ventricular arrhythmias.

MH was triggered in 8–45 min by halothane in animals Nos 3–6. The subsequent exposure to atracurium failed to trigger the syndrome when given alone but, despite a slight increase in the time required (from 15 to 20 min), the syndrome appeared on cue when animals Nos 5 and 6 were given their third challenge with halothane (plus atracurium) 1 h later.

Creatinine phosphokinase concentrations in those animals which survived were markedly increased on the day following the manifestation of MH. No attempt was made to compare the increase following halothane alone with that following halothane administered concomitantly with atracurium.

TABLE I. *Time (min) to trigger MH syndrome. Hal. = 2% halothane; Atra. = atracurium; CPK = Creatine phosphokinase measured before and 24 h after initial halothane challenge (normal: 10–75 u litre^{-1}); Neg. = MH not triggered; — = Investigation not performed; d = Death from untreated MH*

Pig No.	Hal.	Atra.	Atra. +Hal.	CPK (u litre^{-1}) Before	CPK (u litre^{-1}) After
1	—	Neg	25	374	d
2	—	Neg	10	78	d
3	8	Neg	—	816	1470
4	45	Neg	—	—	2180
5	15	Neg	20	8090	24900
6	15	Neg	20	313	10490

DISCUSSION

Our study demonstrated that atracurium neither initiated nor attenuated the MH syndrome in MHS swine in response to the inhalation of halothane, an action similar to that of other non-depolarizing neuromuscular blockers when studied in the same model (Harrison, 1979). Although there is inherent danger in extrapolating from animal data, it would seem that the drug is safe for use in the human MHS subject.

An aspect of the action of atracurium in the circumstances of MH that still needs be addressed is whether its action would be prolonged by the very severe acidosis or shortened by the hyperthermia of the established MH syndrome. However, such action *per se* is unlikely to affect prognosis.

A side issue perhaps worthy of comment concerns the uselessness of resting serum creatinine phosphokinase (CPK) concentrations in swine as a diagnostic criterion of MH (Mitchell and Heffron, 1975), or as a correlate of rapidity of response to halothane exposure. This is well exemplified by examination of the resting (before) concentrations of CPK in our animals (table I), in particular that of animal No. 2, in which a virtually normal CPK concentration accompanied one of the most rapid reactions to halothane and actual death from halothane-induced MH.

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The Screening of Propofol in MHS Swine

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This study investigated the use of propofol in swine genetically susceptible to malignant hyperthermia (MH). Thirteen animals were exposed to 2% halothane in inspired air, a propofol infusion of 12 mg·kg^{-1}·hr^{-1} for up to 45 min, or a combination of both. When MH was triggered the animals were treated with dantrolene. Mean onset time of malignant hyperthermia on exposure to halothane alone was 7.2 min ± 3.0 min and onset with propofol and halothane was 5.0 ± 2.5 min. In no instance did propofol alone trigger the syndrome nor was there a statistical difference ($P < 0.05$) in onset time when the drug was used in conjunction with halothane. On the basis of these results, we conclude that propofol is almost certainly safe for use in humans who are susceptible to MH.

Key Words: ANESTHETICS, INTRAVENOUS—propofol. HYPERTHERMIA—malignant.

The precipitation of malignant hyperthermia (MH) in genetically susceptible (MHS) individuals by exposure to certain anesthetic drugs and adjuvants renders essential the screening for such property of all newly developed anesthetics (1). We report here on the screening in MHS swine of the newly introduced intravenous anesthetic propofol (2) to determine its potential for eliciting or preventing or attenuating halothane induction of MH.

Methods

Specially bred MHS Landrace swine (20–65 kg weight), initially identified by their positive MH response to a brief "barnyard" mask exposure to halothane (1), were selected for this study. Under thiopental/N_2O/O_2 anesthesia with appropriate monitoring, the animals were exposed to propofol and halothane in the following protocol in random order and evidence of the onset of MH was sought: 1) To halothane alone; 2) To propofol alone for 45 min; 3) To propofol for 45 min followed by halothane alone; and 4) To both propofol and halothane simultaneously, with propofol given ten min before exposure to halothane.

In each of the above circumstances except the second, halothane was to be continued until triggering of MH occurred or, failing this, for 60 minutes. Exposures were separated by 72 hours to ensure adequate recovery of the animals. Individual animals were submitted to procedures 1, 2 and 3 or 1, 2 and 4.

All animals were anesthetized with 4 mg/Kg i.v. thiopental (ear vein on first occasion, jugular cannula subsequently), intubated and mechanically ventilated with N_2O/O_2 (FiO_2 = 0.4) using a non-rebreathing circuit. Minute volume was regulated to maintain $Paco_2$ in the range 40–45 mm Hg. Pao_2 ranged from 180–220 mm Hg while the mean pH during control periods was 7.412 (+/−0.05).

The internal carotid artery and jugular vein were cannulated by cut-down in the neck providing access to arterial blood samples for serial measurements of blood gas tensions and acid/base status (Radiometer), arterial and venous pressure monitoring (Hellige), and administration of fluids and drugs. The ECG was monitored using skin electrodes, the temperature by means of a thermistor probe (ELAB) inserted deep into the thigh muscle mass, and F_ECO_2 by sampling of gases at the proximal tracheal tube mount by capnography (Morgan).

Following anesthesia and the establishment of a hemodynamic steady state, propofol was administered as a continuous intravenous infusion of 12

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Table 1. MH Onset Times

Drug Exposure Protocol	MH Onset Times (in minutes) Mean	SD	Number of Animals
Halothane	7.2	± 3.0	13
Propofol	No MH	—	13
Propofol→Halothane	8.7	± 4.4	7
Propofol + Halothane	5.0	± 2.5	6

$mg \cdot kg^{-1} \cdot hr^{-1}$ (the upper limit of the recommended human dose) (2). Halothane 2% in inhaled air was administered into the fresh gas flow by means of a Fluotec Mark 2 vaporizer.

The onset of MH (1) was manifested by all of the following: 1. Muscle fasiculation proceeding to rigor, seen in the supine pig most clearly by extension of the hind legs; 2. A gross increase (>2%) in F_ECO_2 (a manifestation of the development of metabolic and respiratory acidosis); 3. Tachycardia and an increase in blood pressure (manifestations of the systemic release of cathecolamines); 4. A progressive fulminant increase in body temperature of 1°C/5–10 min, terminating halothane exposure after a rise above 40°C.

In these experiments the commencement of a progressive extension of the hind legs was recorded as the time of onset of MH. Increases in F_ECO_2 and core temperature were measured thereafter as confirmatory evidence of the onset of the syndrome. Further evidence of the initiation of the syndrome was sought in the gross increase in serum creatine phosphokinase (CPK) levels 24 hours later.

When triggered, MH was allowed to continue until an increase in core temperature at 1–2°C had occurred. The animals were then treated, following discontinuance of halothane, by the administration of dantrolene, 4.2% sodium bicarbonate, and hyperventilation with 100% oxygen.

The statistical significance of differences in mean times to onset of MH was tested by Student's *t* test ($P < 0.05$).

Results

Results are summarized in Table 1. Exposure to halothane triggered MH in all animals. No animal developed MH when exposed to propofol alone. Administration of propofol failed to prevent or attenuate halothane triggering of the syndrome (protocols 3 and 4), there being no statistically significant difference in times of onset. On each occasion that MH was clinically evident its presence was subsequently confirmed by many-fold increases in serum CPK levels sampled at 24 hours.

Discussion

Despite their heterogeneity, no intravenous anesthetic agent has been shown to trigger MH in susceptible patients (1). However, an effect on halothane provocation of MH has been attributed to althesin, thiopental and etomidate. Althesia has been reported to block it (3), thiopental to attenuate (4) and etomidate to enhance the time of onset of MH (5).

Our observations show that propofol, does not trigger MH in MHS swine nor has it any modifying effect on halothane provocation of the syndrome. These data indicate that propofol may be safely used as an anesthetic for individuals known to be susceptible to MH.

Study of propofol in MHS swine for its effects alone and on halothane induction of MH as described indicates that propofol neither elicits nor modifies halothane induction of malignant hyperthermia in MHS swine. We conclude that propofol may be safely used as an anesthetic in patients known to be susceptible to MH.

We acknowledge with thanks our indebtedness to Brian Sasman for the screening, anesthesia of and establishment of monitoring in our experimental animals.

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Propofol in malignant hyperthermia

SIR,—In-vitro evidence suggests that propofol may be safe for individuals genetically susceptible to malignant hyperthermia (MH).[1] In-vitro data support this belief and I now report clinical data from 19 patients susceptible to MH.[2,3]

Patients undergoing a biopsy of muscle for diagnostic in-vitro contracture testing (IVCT) for susceptibility to MH were anaesthetised according to a standard protocol, which included propofol for induction and maintenance of anaesthesia. Patients also received diazepam premedication, alfentanyl analgesia, atracurium neuromuscular block, endotracheal intubation and ventilation with N_2O/O_2 (FIO_2 0·3), and reversal of neuromuscular block with neostigmine and glycopyrrolate. Of 19 patients, 9 have satisfied IVCT criteria for MH susceptibility.[4] Duration of anaesthesia ranged from 60 to 90 min. No patient showed signs of MH.[5] These findings suggest that propofol is safe in those susceptible to MH.

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Acute calcium homeostasis in MHS swine

To elucidate a pathogenesis for the reduction in bone calcium content observed in MHS individuals, we studied the acute calcium homeostasis of MHS swine. This was achieved by the serial measurement, with a calcium selective electrode, of calcium transients in Landrace MHS (five) and control Landrace/large white cross MH negative (five) swine following IV bolus injection of calcium gluconate 0.1 mmol·kg^{-1} – a dose which induced an acute 45 per cent increase in plasma ionised calcium. Experimental animals were anaesthetised with ketamine 10 mg·kg^{-1} IM, thiopentone (intermittent divided doses) 15–25 mg·kg^{-1} (total) IV and N_2O/O_2 (FIO_2 0.3) by IPPV to maintain a normal blood gas, acid/base state. The plasma ionised calcium decay curve observed in MHS swine did not differ from that of control normal swine. Further it was noted that the induced acute rise in plasma ionised calcium failed to trigger the MH syndrome in any MHS swine. It is concluded that the mechanisms of acute calcium homeostasis in MHS swine are normal. An explanation for the reduction in bone calcium content observed in MHS individuals must be sought, therefore, through study of the slow long-term component of the calcium regulatory process. In addition, the conventional strictures placed on the use, in MHS patients, of calcium gluconate are called in question.

Key words
HYPERTHERMIA: malignant hyperthermia, MHS swine;
MALIGNANT HYPERTHERMIA: calcium homeostasis, bone physiology.

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Although the primary site of the functional lesion responsible for malignant hyperthermia (MH) resides in the skeletal muscle organelles, there is evidence which suggests a more widespread and general membrane defect.[1] Inter alia, reduction in bone calcium content and, by inference, abnormalities in osteocyte function have been reported in patients susceptible to MH.[2] These observations, considered in the light of the central role of bone in the homeostasis of plasma calcium concentration,[3] led us to investigate the acute calcium homeostasis of MHS swine.

Methods

The experimental animals, five especially bred Landrace MHS swine, weight 30–50 kg, selected initially based on a positive MH response to brief "barnyard" exposure to mask administration of halothane[4] were formally retested under anaesthesia in the laboratory before use in the definitive experiments and the onset of MH (for criteria see later) in response to halothane was timed.

Five large white/Landrace crossbred swine which displayed a negative reaction to similar testing served as controls.

Anaesthesia

For all experiments, animals were anaesthetised with ketamine 10 mg·kg^{-1} IM followed by IV thiopentone via an ear vein and then tracheal intubation. Anaesthesia thereafter was maintained with N_2O/O_2 (FIO_2 0.3) delivered by mechanical ventilation via a non-return circuit, the volume of ventilation being adjusted to maintain $PaCO_2$ in the range 40–45 mmHg. Intermittent supplemental doses of thiopentone were administered at any sign of lightening anaesthesia. Total doses of thiopentone ranged from 15–25 mg·kg^{-1}. Mean pH during the control period of the experiments was 7.41 ± 0.05 (n = 30). PaO_2 ranged from 180–210 mmHg. Catheters inserted into the carotid artery and internal jugular vein via cut-down in the neck provided access to blood samples for calcium, blood gas and acid/base assay (Radiometer), arterial and venous pressure monitoring (Hellige) and the route for fluid and drug administration.

The ECG was monitored via skin electrodes and temperature was recorded from a thermistor needle probe (Ellab) placed deep in the thigh muscle mass.

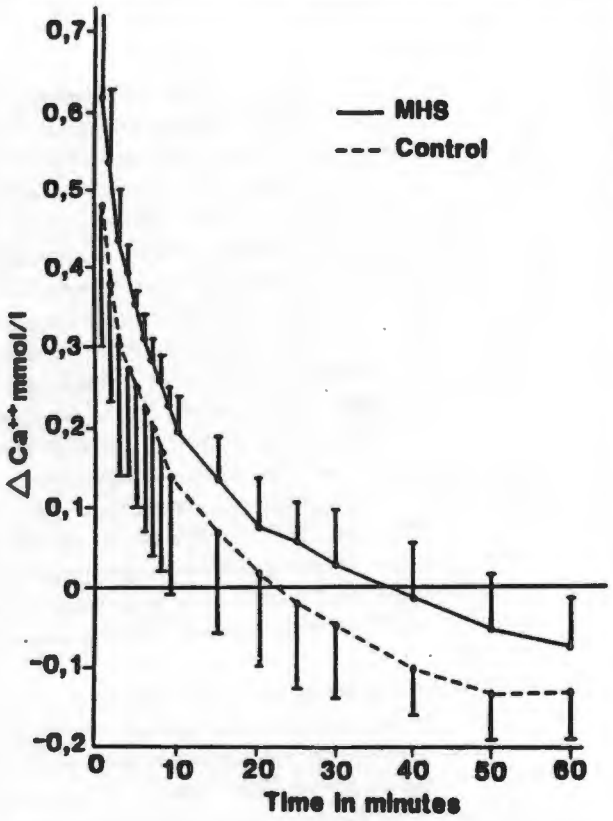

FIGURE Transient response of MHS and normal swine to the bolus injection of calcium gluconate 0.1 mmol·kg^{-1}. Plot is of difference from control values (Mean ± SD).

Protocol

Following establishment of steady state conditions under anaesthesia and three control blood samples, a bolus of calcium gluconate (0.1 mmol·kg^{-1}) was administered via the jugular vein infusion. Thereafter blood was sampled serially for 60 minutes at the following time intervals: one minute for the first ten; five minutes for the next 20, and ten minutes for the last 30.

The dose of calcium gluconate chosen was such as would induce an appreciable increase in plasma ionised calcium, without invoking cardiac arrhythmia.

Calcium concentration was measured by calcium selective carrier disc electrodes,[5] all samples being read in duplicate and the mean result recorded. Values expressed are of ionized calcium.

Additionally, the possible onset of MH was monitored for, the following criteria of the syndrome being sought: >20 per cent increase in heart rate and/or mean arterial pressure, development of combined respiratory and metabolic acidosis (>20 per cent increase in $PaCO_2$), increase in muscle core temperature of >0.5° C and extension of the hind legs.

Results

Data points on the constructed plasma ionised calcium concentration decay curve (change from control values) (Figure) reflect mean and SD of values at each time interval in experimental and control groups. The significance of differences observed between MHS and control animals at individual data points was tested with Student's t test after equivalence of variance had been tested.

Following bolus injection of 0.1 mmol calcium gluconate·kg^{-1}, peak concentration in plasma ionised calcium was reached within the first minute, the mean rise above control values being 34 per cent (1.40 mmol·L^{-1}–1.88 mmol·L^{-1}) and 45 per cent (1.33 mmol·L^{-1}–1.90 mmol·L^{-1}) respectively in normal and MHS swine. This was followed by a fast transient response which restored the ionised calcium concentration to control levels in 25 and 35 minutes respectively, followed by an overshoot below control levels. This decay curve was in every way analagous to our previously published observations on normal swine.[5]

No difference of statistical significance was demonstrated between the normal and MHS pig plasma ionised calcium decay curves.

The acutely induced rise in plasma ionised calcium concentration failed to trigger the MH syndrome in any animals.

Discussion

The mechanisms involved in the control of plasma ionised calcium are arranged in a hierarchical fashion from the minute-to-minute to long term.[3] Our investigation examines the initial calcitonin-dependent fast transient response ($t_{\frac{1}{2}}$ of 15 minutes) of MHS swine, a response mediated by the cells lining the fluid compartment of bone.

Our observations show this response in MHS swine to be no different from normal controls and to differ in no way from our previously published data on the normal response.[5]

These findings provide no explanation for the reduced bone calcium content of MHS patients reported by Britt, Harrison and MacNeill[2] which must be related therefore to a possible dysfunction of the slow long-term component of the calcium regulatory process.

Our incidental observation of the failure of an acutely induced rise in plasma ionised calcium concentration to trigger the onset of MH in susceptible swine, in line with similar observations recently reported,[6] calls in question the strictures placed in the past on the use of calcium gluconate in MHS patients.[7]

Acknowledgements

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Résumé

Afin d'élucider pathogénèse pour la réduction dans le contenu osseux en calcium observé chez les personnes SHM, nous avons étudié l'homéostase aiguë du calcium chez le porc SHM. On a mesuré de façon sériée, à l'aide d'une électrode sélective de calcium, les changements de calcémie chez cinq porcs SHM Landrace et chez cinq porcs témoins Landrace/à-crois-blanche-large HM négatif, l'effet d'une injection de 0.1 mmol·kg^{-1} de gluconate de calcium IV – dose qui a provoqué une augmentation aiguë de 45 pour cent dans le calcium ionisée plasmatique. On a anesthésié les animaux expérimentaux avec de la kétamine IMI 10 mg·kg^{-1} (total), du thiopental IV (dose intermittente 15–25 mg·kg^{-1}) et du N_2O/O_2 (FIO_2 0.3) par VPPI afin de maintenir un état de gaz sanguin acido-basique normal. La courbe décroissante du calcium plasmatique ionisé que l'on a remarquée chez le porc SHM ne différait pas de celle du porc-témoin normal. De plus, on a remarqué que la provocation d'une augmentation aiguë dans le calcium ionisé plasmatique n'a pas réussi à déclencher le syndrome HM chez aucun des porcs SHM.

On en conclut donc que les mécanismes d'homéostases aigus de calcium chez les porcs SHM sont normaux. On doit donc chercher à expliquer la réduction du contenu osseux en calcium remarqué chez les personnes SHM en étudiant la composante lente et à long terme du processus régulatoire du calcium. De plus, on remet en question les mises en gardes conventionnelles concernant l'utilisation du gluconate de calcium chez les patients SHM.

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The Effects of Calcium Channel Blocking Drugs on Halothane Initiation of Malignant Hyperthermia in MHS Swine and on the Established Syndrome

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SUMMARY

We have investigated in malignant hyperthermia susceptible swine in vivo the effects on halothane initiation of the MH syndrome and on the established syndrome of five calcium channel blocking drugs — nifedipine, nisoldipine, diltiazem, verapamil and flunarazine. Nifedipine alone caused attenuation of halothane-initiated malignant hyperthermia to the extent of blocking onset of the syndrome in 29% of animals for the 60 minute period of exposure. In the face of the established malignant hyperthermia syndrome, all the drugs tested were therapeutically completely ineffective.

Key Words: malignant hyperthermia, MHS swine; halothane, calcium channel blockers, nifedipine, nisoldipine, diltiazem, verapamil, flunarazine

The pathogenesis of malignant hyperthermia (MH) rests on a cycle that results in a sustained rise in myoplasmic free calcium.[1,2] Of the many factors that may contribute to this, studies have identified in MH susceptible (MHS) swine and humans a halothane induced or enhanced dysfunction of sarcolemma involving increased calcium permeability[3-7] associated with sarcolemmal depolarisation.[8] Considered in the light of the hypothesis that the underlying functional lesion responsible for the susceptibility to MH is an abnormally low threshold calcium induced calcium release from the sarcoplasmic reticulum,[9-11] such abnormalities of sarcolemmal calcium permeability could constitute the mechanism by which the syndrome is triggered.[4]

In these circumstances, should the action of calcium channel blocking drugs (CCB) on skeletal muscle match their action on cardiac and smooth muscle,[12,13] it could be anticipated that pretreatment of MHS animals with CCBs would block initiation of the MH syndrome in response to their exposure to halothane. Support for such a postulate comes from in vitro studies which demonstrate both inhibition and reversal of halothane induced contractures in MHS muscle by diltiazem.[14,15]

To test this hypothesis we have investigated in MHS swine in vivo the effects on halothane triggering of the MH syndrome of pretreatment with CCBs representative of each of the four classes of these agents.[12,16] In

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view of the therapeutic implications of the in vitro studies, we investigated also the effects of these CCBs on the established syndrome.

The drugs investigated included:

1. nifedipine and nisoldipine,
2. diltiazem,
3. verapamil,
4. flunarazine.

METHODS

Animals and experimental protocol

Specially bred MHS Landrace swine of 30-50 kg weight, initially identified at the post-weaned stage by brief 'barnyard' mask exposure to halothane,[17] served as the experimental animals. Under general anaesthesia and appropriate monitoring (see later) these animals were exposed to halothane inhalation and the consequent MH reaction time was observed in a protocol wherein each animal served as its own control as follows:

1. under general anaesthesia alone,
2. under general anaesthesia, with CCB treatment preceding halothane exposure. In these circumstances administration of halothane was continued until the onset of MH or, failing this, for 60 minutes,
3. under general anaesthesia alone.

Steps in this protocol were separated by an interval of three to five days, so as to ensure complete elimination of previously administered drugs, particularly dantrolene (elimination half-life 10-12 hours). The average of times 1 and 3 was recorded as the halothane/MH reaction control time for comparison with 2 — that observed following CCB pretreatment. Experiments were conducted on groups of 2-3 pigs at a time, depending on the availability of the MHS strain of swine. Choice of pretreatment drug was random.

To test the effect of CCBs on the established MH syndrome, following establishment of anaesthesia and monitoring, MH was induced by the exposure of test animals to halothane for sufficient length of time to ensure its persistance after discontinuance of the halothane,[18] whereafter the test CCB was administered IV and the effect observed.

Anaesthesia

For all experiments animals were anaesthetised initially with ketamine 10 mg/kg by intramuscular injection (dorsal neck muscles), followed shortly by IV thiopentone injection (ear vein), endotracheal intubation and N_2O/O_2 ($F_IO_2 = 0.4$) administered by IPPV with a non-return circuit ventilator, the volume of ventilation being regulated to maintain P_{CO_2} in the range 40-45 mmHg. P_{O_2} ranged from 180-220 mmHg, while mean pH during the control periods was 7.41 (SD 0.05) ($n = 60$). Intermittent supplemental doses of thiopentone were administered at any sign of lightening anaesthesia. Total dose of thiopentone utilised ranged from 15-25 mg/kg.

Monitoring

Carotid artery and jugular venous cannulation via cut-down in the neck provided access to blood samples for serial blood gas and acid/base assay (Radiometer), arterial and venous pressure monitoring (Hellige) and the route for fluid and drug administration. Expired CO_2 concentration, sampled at the endotracheal tube mount, was monitored by capnography (Morgan), ECG via skin electrodes and temperature by means of a thermistor (Ellab) inserted deep in the thigh muscle mass.

CCB pretreatment

After establishment of control conditions under anaesthesia, the test CCB was administered IV. Thereafter ten minutes was allowed for haemodynamic changes to stabilise. In view of the vasodilating effects of most CCBs, fluid loading with balanced salt solution was needed to achieve this. Thereafter test exposure to halothane 2% was commenced. After ten minutes halothane concentration was reduced to 1%.

Dosage of drug

For both preventative pretreatment and treatment of the established syndrome, the dosage of CCBs utilised was in excess to an extent that could be tolerated — of the relevant human therapeutic dose. Doses utilised were as follows (human dose in parenthesis):

nifedipine	100 μg/kg (20 μg/kg)
nisoldipine	6 μg/kg (2-4 μg/kg)
diltiazem	500 μg/kg (150 μg/kg)
verapamil	400 μg/kg (50-100 μg/kg)

In the case of flunarazine, an oral formulation only was available. A dose of 1.2

mg/kg/day (human 0.15-0.3 mg/kg/day) was administered for four days before exposure to halothane. Because of its oral formulation, the testing of flunarazine in the established syndrome was not possible.

Onset of halothane triggered MH — timing

The onset of the MH syndrome is manifested by:[17]

1. muscle fasciculation proceeding to rigor, manifest in the supine pig most clearly by extension of the hind legs,
2. a gross increase in F_ECO_2 (a manifestation of the development of metabolic and respiratory acidosis),
3. sudden tachycardia and rise in blood pressure as a manifestation of the accompanying systemic catecholamine surge,
4. progressive fulminant rise in body temperature.

In these experiments the commencement of a progressive extension of the hind limbs was recorded as the 'time of onset' of the syndrome. While the rise in F_ECO_2 and onset of tachycardia are normally equally early (and confirmatory) signs of initiation of MH, their use in marking onset of the syndrome in this experiment was invalidated by the use of CCBs. In the former case hypotension, consequent on the combined use of CCB with halothane, resulted in an initial fall in F_ECO_2 before the rise consequent on the syndrome became evident, while in the latter, use of CCB greatly attenuated any response to the catecholamine surge.

Time of onset of temperature rise was used as some indication of the severity and speed of the induced intramuscular reaction, as was also the rate of temperature rise.

Once triggered, the syndrome was allowed to continue until a rise in core temperature of 1-2 degrees C had occurred. Thereafter, following discontinuance of halothane, salvage of the experimental animals was ensured by administration of dantrolene sodium.

The statistical significance of differences from the control reaction were tested as follows:

1. proportion of experimental groups in which CCB pretreatment blocked MH triggering, by the Chi-square technique,
2. comparison of times of onset and the rate of clinical reaction by Students t test for equal and unequal variances as appropriate.

The number of pigs with the established syndrome in which the different drugs were tested were: nifedipine 6, nisoldipine 3, diltiazem 4, verapamil 4.

RESULTS

These are summarised in Tables 1 and 2.

TABLE 1

Effect of calcium channel blocking drugs in modifying the response to susceptible pigs to exposure to halothane

	Control	Nifedipine	Nisoldipine	Diltiazem	Verapamil	Flunarazine
Number of pigs tested	31	14	4	6	4	3
Number in whom exposure to halothane failed to produce MH	0	4*	0	1	0	0

*$P < 0.05$

TABLE 2

Interval in which pigs developed MH between the commencement of exposure to halothane and onset of leg extension and temperature rise (minutes). Mean values with standard deviations in brackets

	Pretreatment drug					
	None	Nifedipine	Nisoldipine	Diltiazem	Verapamil	Flunarazine
Leg extension	4(4)	13(15)	5(3)	7(4)	4(2)	12(9)
Temperature rise	7(10)	15(15)	6(3)	13(2)	4(1)	15(16)
Numbers of pigs in each group	31	10	4	5	4	3

Of the drugs tested, pretreatment with nifedipine alone blocked halothane initiation of MH for the 60 minutes of exposure in a statistically significant proportion of test MHS swine (4/14).

After nifedipine pretreatment administration of halothane commonly caused hypotension. Severe hypotension (MAP < 40 Torr) was manifested by three of the four pigs whose MH response to halothane was blocked by nifedipine — a statistically significant proportion compared with the incidence of a similar degree of hypotension in animals whose MH response to halothane was not blocked by nifedipine ($P < 0.05$).

In reactor pigs no significant attenuation was apparent following pretreatment with any CCB, in the time of onset of MH as judged by commencement of leg extension or commencement of temperature rise, nor in the rate of temperature rise — the latter averaging 1°C per seven minutes.

None of the drugs tested displayed any therapeutic efficacy in the presence of established MH.

DISCUSSION

The therapeutic role suggested some years ago for the conventional CCBs by Bikhazi, Thomas and Foldes,[19] a suggestion which found support from the in vitro observations of Iwatsuki, Koga and Amaha,[14] and Ilias et al.,[15] is clearly refuted by this in vivo demonstration of the therapeutic ineffectiveness of CCBs of all classes in established malignant hyperthermia. While such has been reported previously with regard to verapamil,[20,21] examples of each class of CCB[12,16] were included in our investigation.

Considering current concepts of the pathogenesis of the established MH syndrome,[1,2] such findings are not surprising. Recent work on sarcolemmal function,[5,6,7,8] however, does provide some rationale for the expectation that CCBs would cause some attenuation of halothane initiation of malignant hyperthermia.

Again, with the exception of nifedipine, our observations belie the expectation. Nifedipine alone showed a statistically significant effect in blocking halothane triggering of MH for 60 minutes but acted thus on a minority of test animals. The single instance of diltiazem blocking initiation of the syndrome, though not statistically significant, should be noted. With so low a rate of response, however, it was thought that proceeding to a greater number of animals would be unlikely to provide further information of phenomenological or clinical import.

Why the action of nifedipine should differ from that of other CCBs tested is open to speculation. In that nifedipine is thought to act on the postulated voltage dependant outer calcium channel gate — as against the action of the others on the inner phosphorlyation dependent gate — these findings may be construed as supporting the concept of an MH triggering mechanism based on a halothane induced, depolarisation based, increase in sarcolemmal calcium permeability. On the other hand, the apparent correlation of blocking of MH onset with extreme hypotension (systolic MAP < 40 Torr) may imply a non-specific reduction of exposure of the effector organ, the muscle, to the trigger, halothane, consequent on reduced cardiac output. We are planning to explore this postulate further in the future.

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HISTORICAL NOTES

Malignant hyperthermia

An historical vignette

G. G. HARRISON AND H. ISAACS

Summary

This paper reports previously unpublished accounts of the deaths in 1915 and 1919 of two members of the same family during general anaesthesia. The deaths were subsequently ascribed to a 'hereditary susceptibility' to chloroform. Contemporary evidence is presented which suggests that these deaths were among the very earliest examples of malignant hyperthermia to be described.

Key words

History; chloroform.
Complications; death.
Hyperthermia; malignant.

The report by Denborough and Lovell, in 1960 [1], of a family who manifested a strange predisposition to death during general anaesthesia, led ultimately to the identification of malignant hyperthermia (MH) as a clinical pharmacogenetic entity [2]. Subsequent experience has indicated that all volatile anaesthetics, especially the newer halogenated agents as well as chloroform, are capable of triggering the MH crisis in genetically susceptible individuals [3], and this has begged the question why MH had not been noted before 1960 as a genetic entity. Letters that have recently come to light do seem to provide evidence that, some 40 years before Denborough and Lovell's classic description, a Dr G.A. Jones of Buckinghamshire did observe and record an example of such a familial fatal idiosyncrasy to chloroform anaesthesia but, sadly, never published his observations.

In 1987 a 2.5-year-old child died tragically from MH while subjected to general anaesthesia for conservative dentistry of a minor nature. Subsequent muscle biopsy *in vitro* contracture testing (IVCT) of the proband's close family showed the child's father, uncle and cousin (the latter's son) all to be susceptible to MH (MHS). A cousin of the child's grandfather, (Fig. 1 (6)), had believed that members of her family had a strange and dangerous sensitivity to general anaesthetics and a family tree was revealed (Fig. 1) which showed circumstantial evidence of the MHS trait in all four generations. This by itself can no longer be regarded as extraordinary (MHS transmission is autosomal dominant and many such pedigrees have been reported since Denborough and Lovell's original description [1]). However, letters written in 1919 and earlier about the circumstances of the first two deaths during anaesthesia of members of this family, those of Figure 1 (1) in 1915 and her son, (Fig. 1 (3)), in 1919, are of considerable historical interest. The first of these deaths occurred in 1915, but the letters were written in 1919 following the death of this patient's son, when the similar circumstances of the latter's death motivated re-examination of those of the former.

The death of the latter, (Fig. 1 (3)), occurred in February 1919, at Marlborough during an operation for the exploration of a ruptured kidney. The following is an extract from the report submitted by the anaesthetist, Dr E. Penny:

> 'The anaesthetic was chloroform and ether given on an open Schimmelbusch mask and the amount used during the operation was almost exactly one ounce of the former and two ounces of the latter, the duration of the administration being half an hour. The patient, who did not seem at all nervous, passed normally and quickly under the anaesthetic but when the surgeon was about to begin the operation, it was noticed that a curious muscular rigidity existed in the arms, legs and abdominal muscles, the patient being fully under the anaesthetic and breathing quietly and freely. This

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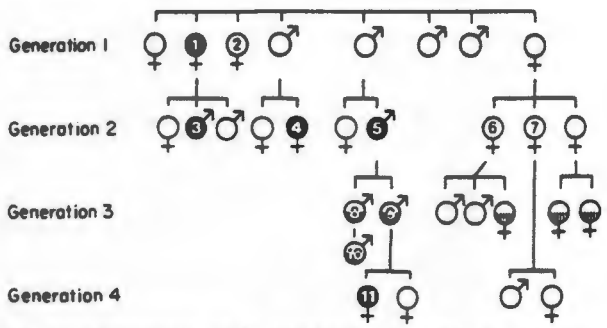

Fig. 1. Genealogical table (abridged). ●, died of malignant hyperthermia (MH) or? malignant hyperthermia; ◒ *in vitro* contracture testing (IVCT) MH susceptible; ◒, IVCT MH negative.

muscular condition persisted during the operation which consisted in the first stage of exploring the peritoneal cavity in front of the injured kidney and in the second stage in cutting down to the kidney from behind. Just as the second stage was being completed a violent and persistent spasm of all the respiratory muscles of the thorax occurred and the movements of the chest ceased suddenly and absolutely. Artificial respiration was at once resorted to but no re-establishment of natural breathing could be obtained, though it was evident no impediment existed to the passage of air through the larynx. The heart continued to beat for a time after the fatal spasm occurred ...'.

On receipt of this report, Dr G.A. Jones of Little Seeleys, Beaconsfield, Buckinghamshire, the anaesthetist concerned 4 years earlier with the death under anaesthesia of this patient's mother, wrote to her husband this letter, from which extracts follow:

'... Mrs ...'s operation was a rather long one in two stages, the first being abdominal for the fixing of the womb to the abdominal wall and the second vaginal for still further preventing prolapse ... I began with gas and ether but changed to a mixture of chloroform and ether as is usually done in operations of any length. The first stage was done in half-an-hour to three-quarters-of-an-hour, and there was nothing then in the condition to cause the slightest anxiety ... The second stage took about half-an-hour and it was not until near the end that any alarming symptoms showed themselves. There was a marked tendency to spasm of the muscles of the arms and jaw and apparently those of respiration, and the breathing stopped fairly quickly. The condition of the pupils had indicated a medium rather than deep anaesthesia i.e. they were contracted, not dilated as in chloroform overdose. When breathing stopped, the pulse was present though rather rapid. Stimulants and artificial respiration were tried without avail. Tracheotomy was tried as a last resort.

There is a strong similarity in the cases of mother and son as regards the curious rigidity and spasm ... but it is very rare indeed for any spasm of the muscles of respiration to occur. It looks as if in both cases there was a tendency of abnormal rigidity coupled with a susceptibility to chloroform.

The sister of the house told me afterwards that Mrs ... had had attacks of muscular spasm apparently suggesting the condition known as Tetany. ...

The fact of a fatality occurring to both mother and son is very suggestive, but I cannot find any mention of hereditary susceptibility definitely ascertained in the standard text book on Anaesthetics ... One is inclined to advise that the two other children should be thoroughly examined by a first-class physician who might be able to say whether they had anything of the kind, but its a condition very difficult to recognise. I think chloroform should be absolutely barred if an operation were necessary and as little ether given as possible on an open mask after an injection of morphine. Yours sincerely, etc.'

Later, Dr Jones wrote to the 'sister of the house' (Fig. 1 (2)) when she had become the guardian of the remaining children: 'Dear Miss ..., With reference to your nephews, I consider that in view of what occurred in the case of their mother and brother, they should never have chloroform for an operation in any circumstances, but that with either gas and oxygen or ether by the open method preceeded by an injection of Morphia gr 1/6 and atropine gr 1/20 the risks should be practically negligible. Yours &c.'

In 1925 a third member of the family, (Fig. 1 (4)), died while anaesthetised undergoing an appendicectomy. Clinical details of this fatality have been lost. However, this succession of three deaths under anaesthesia in this family led them to take the precaution of showing the above letters to the matron of the Johannesburg Hospital in 1933 when another member of generation 2 (Fig. 1 (7)), entered the nursing profession. Some years later, yet another member of generation 2 (Fig. 1 (5)) (the grandfather of the child whose death motivated this report), died while anaesthetised. In this case, details of which also are now sadly lacking, the patient died immediately postoperatively after a gastric operation.

This family's history begs the question 'Was Jones' fatal familial susceptibility to chloroform indeed MH?' In describing the clinical circumstances associated with the deaths of 1915 and 1919, neither Jones nor Penny make any mention of their patients' body temperature, an omission that could be regarded as invalidating any retrospective diagnosis of MH. Yet it must be remembered that at that time monitoring of the patient's temperature during anaesthesia and surgery was not routine practice, if indeed it was ever recorded at all under these circumstances. Further, both accounts lay particular stress on the appearance of that other cardinal manifestation of MH, muscle rigor, implying that respiration ceased because of the rigidity of the muscles of respiration. Jones also mentions the history given by a 'sister of the house' that her sister had complained of bouts of muscle spasm, a feature well-documented in some subjects of the MHS trait [4].

When the Jones and Penny accounts are considered in the light of objective contemporary evidence of the death from MH of Figure 1 (11), the IVCT-diagnosed MH trait in her father, (Fig. 1 (9)), her uncle, (Fig. 1(8)), and her cousin, (Fig. 1 (10)), together with the histories of the anaesthetic-related deaths of her grandfather, (Fig. 1 (5)), and his cousin, (Fig. 1 (4)), the balance of probability must lead us to conclude that these cases of 'hereditary susceptibility' to chloroform, identified by Jones, were none other than historically very early examples of MH, indeed among the very earliest to be described [2] albeit unpublished till now.

Table 1. Results of diagnostic tests.

Family member	% halothane	Contracture, g.	Caffeine concentration mmol.l^{-1}	Contracture, g.
8	0.5	0	0.5	0.5
	1.0	0.2	1.0	1.3
	2.0	1.3	2.0	> 5.0
	3.0	6.4	3.0	
9	0.5	0	0.5	0.9
	1.0	0.7	1.0	1.8
	2.0	2.8	2.0	> 5.0
	3.0	8.0	3.0	
10	0.5	0	0.5	0.1
	1.0	0	1.0	0.3
	2.0	2.2	2.0	0.8
	3.0	0.5	3.0	1.6

All displayed normal histology and histochemistry.

Appendix, data relevant to Figure 1

MH proband, family member Figure 1 (11), died during anaesthesia with halothane and nitrous oxide/oxygen approximately 45 min after the onset of a fulminant episode of MH, which exhibited the classic features of tachycardia, muscle rigidity, cyanosis and hyperthermia; the core temperature reached 42°C antemortem. To date, seven members of the family have subsequently been screened for susceptibility to MH by IVCT of biopsied skeletal muscle utilising the methods and diagnostic criteria of the European MH Group [5, 6].

In our laboratories, once the muscle is in the tension study apparatus and stimulated to contract utilising a pulse of 0.1 amp at 0.25 Hz, the muscle is left for 5 min to establish a regular baseline before individual muscle strips are subjected to challenge by halothane and caffeine. At the end of the procedure the challenging substances are removed and the muscle, still being stimulated, is allowed to return to baseline confirming the contractility and viability of the muscle strip.

Muscle of three members of the family (see Fig. 1) has satisfied the diagnostic criteria for susceptibility to MH. These results are in Table 1.

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MALIGNANT HYPERTHERMIA

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*Porcine Malignant Hyperthermia**

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Much of what we understand today about the pathogenesis and management of the anesthetic-induced malignant hyperthermia (MH) syndrome in man has followed the happy chance discovery that the well-known stress-related myopathy manifest in certain breeds of pig provides a close facsimile of this syndrome in man. The possibility that strains of certain breeds of pig might serve as a valid animal experimental model of this rare, puzzling, and lethal complication of general anesthesia in man was first suggested by the observation by Hall and co-workers [44] and Harrison and associates [53] of muscle rigor associated with fulminant hyperthermia occurring in the anesthetized pig. In Hall's report this reaction was a response to the administration of succinylcholine (preceded by halothane), while in Harrison's it followed administration of halothane alone. The chance nature of these discoveries, reported from centers as far apart as Cambridge (U.K.) and Cape Town (S.A.), can be judged from the fact that it was an investigation into the pathogenesis of atherosclerosis in the first instance and liver transplantation in the second that motivated the use of pigs as experimental animals and thus their being anesthetized. Similar syndromes have since been reported in other species—the dog [83], the cat [21], and the horse [63]—but so rarely as to constitute scientific curiosities. Only in the pig does the condition occur with any regularity, a possible consequence of the genetic selection practiced by breeders to produce a heavily muscled animal characterized by a rapid rate of growth with high feed efficiency [76].

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Though the identity of the syndromes of anesthetic-induced MH in the susceptible pig and in man has been questioned [5, 11, 57], the pig has proved to be a practical experimental model of the condition [69]. What differences there are appear to be quantitative rather than qualitative—the expression of the syndrome being more florid and extreme in the pig than in man.

CLINICAL SYNDROME IN THE PIG

The following predominant clinical features of the hyperthermic syndrome in the pig all develop pari passu:

1. Gross muscle rigor, usually preceded by fasciculation. This rigor is particularly evident in the hind legs, which become rigidly extended in a few minutes (Fig. 1).
2. An explosive and sustained rise in core temperature (Fig. 2). Though rate of increase varies among pigs, a rate of 1°C. every 5 to 7 minutes is commonplace, with temperatures reaching 43 to 45°C. antemortem.

FIGURE 1. *Progressive rigid extension of MHS Landrace pig hind legs produced by the onset of muscle rigor provoked by exposure of this animal to halothane after a control period of anesthesia with thiopentone and nitrous oxide. This phenomenon may be recorded by attaching trotter by string over pulley to a recording pen on a revolving drum.*

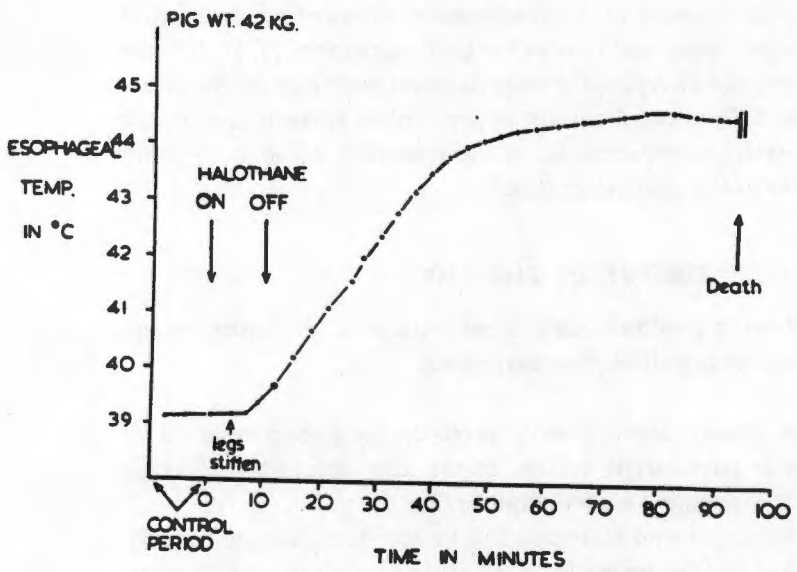

FIGURE 2. *Record of esophageal temperature in MHS Landrace pig during the course of an MH syndrome provoked by exposure to halothane after a control period of thiopentone and nitrous oxide anesthesia. Note the "trigger" effect of halothane. (From Harrison et al. [54].)*

3. Sinus tachycardia. A rapidly developing sinus tachycardia at a rate usually in excess of 200 beats per minute is often the earliest detectable harbinger of the syndrome. Tachycardia continues until shortly before death in the untreated animal.
4. Open-mouth breathing and hyperventilation that proceed within minutes to apnea necessitating the institution of intermittent positive-pressure ventilation if the animal is to survive for more than 10 to 15 minutes.
5. Blotchy cyanosis of the skin, most noticeable on the abdominal wall and snout.

In susceptible animals the syndrome usually becomes manifest within 5 to 15 minutes of exposure to the stressor or triggering agent (e.g., halothane). This interval depends to a large extent on the time it takes for the tissue tension of the stressor to reach the threshold level; hence the onset is faster in small than in large animals.

Onset is more rapid when the induction of anesthesia is accompanied by struggling and excitement [2, 89], circumstances that alone may initiate the syndrome [61, 93]. On the other hand, onset is slower when induction of anesthesia is quiet, such as it may be with intravenous agents preceded by suitable sedative premedication (e.g., azeparone or ketamine).

If exposure to the stressor (e.g., halothane) is stopped within minutes of the first appearance of any of the major clinical manifestations, the syndrome is spontaneously reversible. If, however, exposure to the stressor is continued for more than several minutes thereafter, the syndrome rapidly becomes irreversible and will continue independently of the tissue concentration of the stressor, which can now be said to have acted as a trigger.

Profound acidosis develops with bewildering speed and is accompanied by equally rapid and gross changes in serum biochemistry —changes that precede and reach extreme values in advance of the recorded rise in core temperature [4]. The acidosis is primarily an uncompensated metabolic acidosis due to a gross rise in plasma lactate ("excess lactate" in terms of Huckabee's formula [59]) reflected by the appropriate base deficit (Fig. 3). The phenomenal rise in Pco_2, a reflection of the $H^+ + HCO_3^- \rightarrow H_2O + CO_2$ reaction, is doubtless responsible for the open-mouth breathing–hyperventilation—apnea sequence, in effect CO_2 narcosis. The great speed at which these changes occur is graphically illustrated in Figure 4, in which a pH probe in pig hind limb muscle records a drop in extracellular fluid pH from 7.2 to 6.6 in a period of 12 minutes, while the experiment recording arterial blood acid-base changes illustrated in Figure 3 shows a fall in arterial blood pH from 7.35 to 6.6 in 20 minutes. The changes recorded in serum biochemistry values (Fig. 5) include rises in NA^+, K^+, Ca^{++}, Mg^{++}, total protein, glucose, intracellular P (P_I), and lactate and indicate: (1) shift of water into the cells (with concomitant rise in Na^+ and total protein); (2) leakage of K^+, Ca^{++}, Mg^{++}, and P_I out of the cells; and (3) glycolysis. The rise in serum K^+ is so large that it may well contribute to the ultimate death of the animal in hyperthermic rigor.

Untreated, the syndrome is inevitably fatal; death occurs within

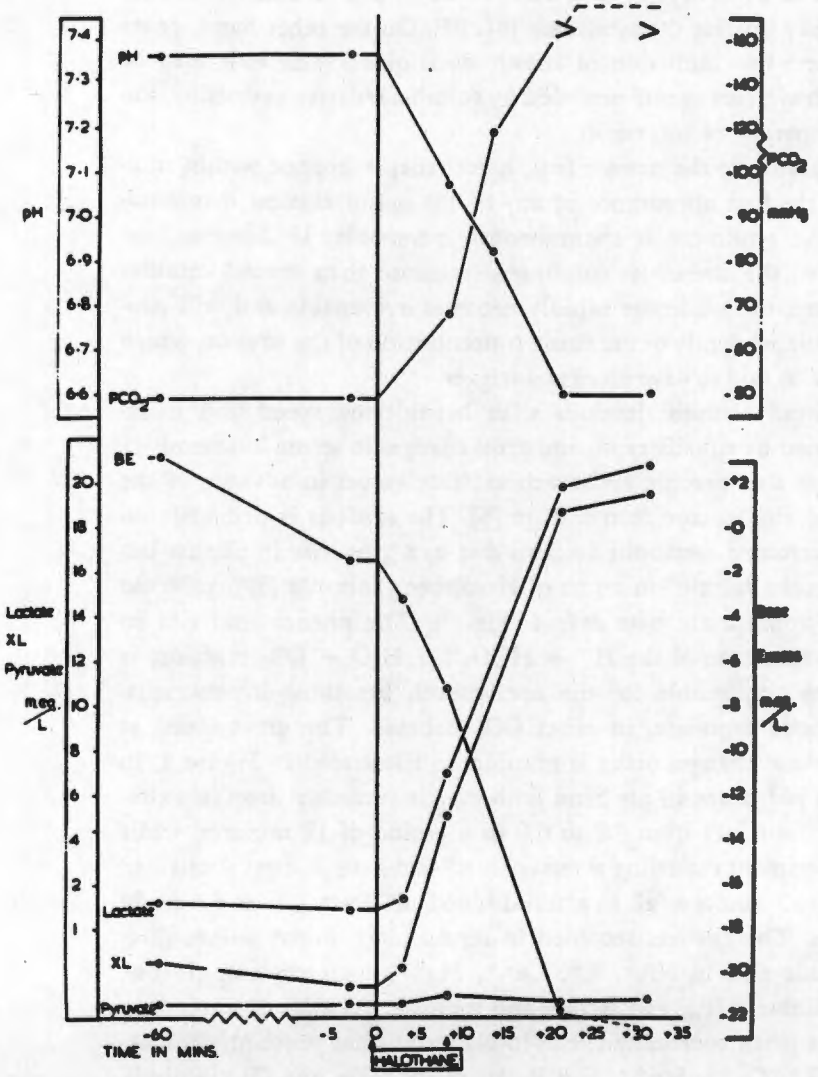

FIGURE 3. *Arterial blood acid-base changes recorded in an MHS Landrace pig during the course of an MH syndrome provoked by halothane after a control period of thiopentone and nitrous oxide anesthesia.* XL = *Huckabee's "excess lactate" [59]. (From Harrison [48].)*

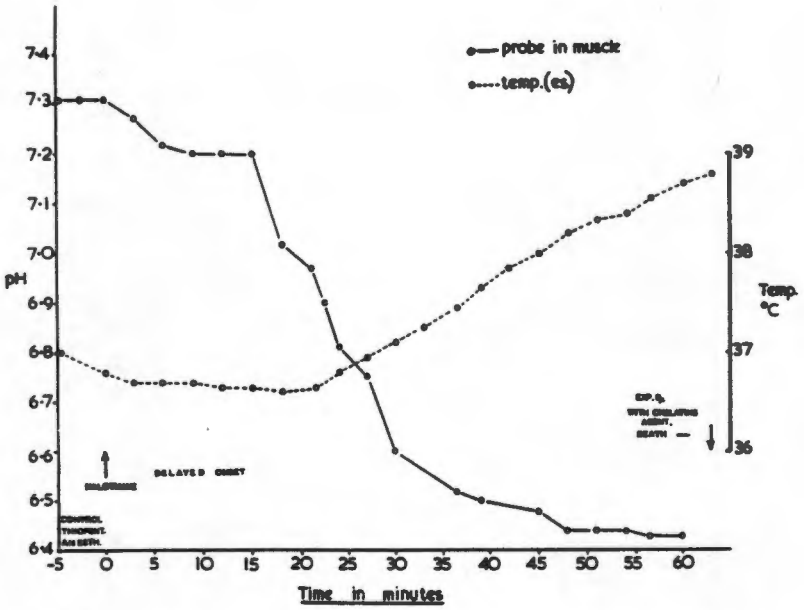

FIGURE 4. *Changes in pH, recorded from an intramuscular pH probe, together with simultaneous record of esophageal temperature during the course of an MH syndrome provoked by exposure to halothane in a MHS Landrace pig. Note that experimental treatment with a chelating agent caused the death of the animal. (From Harrison [48].)*

15 minutes if the animal has been breathing spontaneously and within 40 to 60 minutes if it has been mechanically ventilated.

THE MALIGNANT-HYPERTHERMIA-SUSCEPTIBLE STRAIN

It was soon apparent that this "explosive thermal idiosyncrasy" [97] was a genetic trait displayed only by certain pigs of a few breeds, mostly the leaner, heavily muscled, high-growth-rate breeds. Breeds in which this trait of malignant hyperthermia susceptibility (MHS) has been described include the Danish Landrace, Dutch Pietrain, Poland China, and crossbreeds of these strains [76]. Its occurrence in

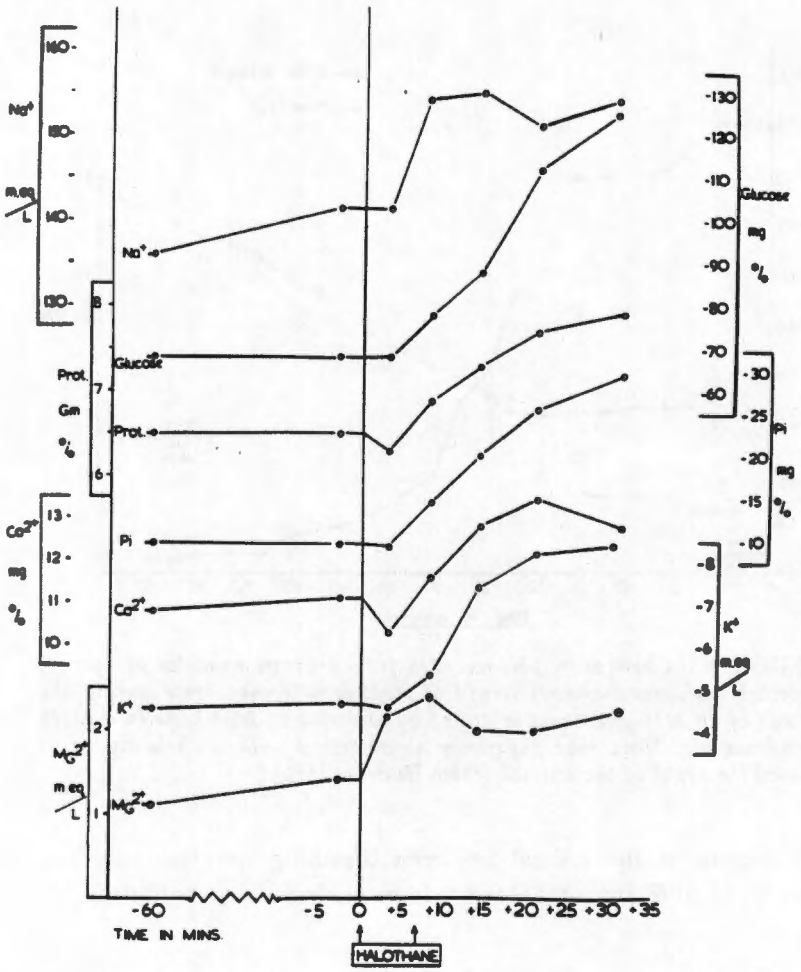

FIGURE 5. *Changes in serum biochemistry determinations during the course of an MH syndrome in the same animal as in Figure 3. (From Harrison [48].)*

Yorkshires has also been recorded [19]. MHS was originally regarded as a single autosomal dominant trait [44, 61, 94], but the graded phenotype variations produced in recent breeding experiments now indicate the involvement of more than one gene, or at least a complex or strongly modified single dominant gene, in the inheritance of porcine MHS [14a, 94a]. Similarly, it is now proposed that the condition in humans manifests a pattern of inheritance that is polygenetic and multifactorial [25a].

One of the difficulties facing the researcher intent on breeding MHS swine is the fact that stressors that provoke the syndrome include excitement and severe exertion—a circumstance that often results in what may be termed "purple porcine passion," with the subsequent demise of a valued MHS boar.

STRESSORS OR TRIGGERING AGENTS

Knowing what we do today of the pathogenesis of MH, it is not unreasonable to suspect that any of the conventional volatile anesthetics could trigger the syndrome in susceptible animals. Indeed, in humans the syndrome has been reported in association with all such anesthetics [11, 26] though some appear to be more potent as triggers than others.

Investigation and identification of possible stressors, or triggering agents, has been one of the first aspects of the condition to be investigated in MHS swine. The number of agents identified as stressors is relatively limited, though perhaps the search has not been sufficiently persistent or exhaustive.

Of the conventional volatile agents, we have investigated halothane, methoxyflurane, trichlorethylene, chloroform, cyclopropane, diethyl ether, and nitrous oxide [54, 55]. More recently we have conducted as yet unreported investigations of fluroxene and ethrane. Prompt initiation of the syndrome follows exposure of MHS pigs to halothane, chloroform, and ethrane. Fluroxene has proved to be a weak and inconsistent stressor.

In direct contrast to the volatile agents, not only do none of the intravenous agents tested (thiopentone, althesin, and ketamine) act

as stressors, but one, althesin, when administered by continuous intravenous infusion, consistently blocks initiation of the syndrome [49].

Among the muscle relaxant drugs tested, the only stressor is succinylcholine. Though in Hall and co-workers' original report the stressor action of succinylcholine was noted only in an environment of halothane anesthesia [44], in pure Landrace strain [54] and Pietrain pigs [66], succinylcholine alone has proved to be a potent single stressor. In these animals the MH stressor action of succinylcholine is characteristically preceded by a grossly abnormal myotonic reaction to the drug. This myotonic reaction appears to be necessary for the MH-triggering action of succinylcholine, because if it is blocked by the preliminary administration of a nondepolarizing relaxant, the syndrome fails to develop [40, 48]. As can be anticipated from this observation, nondepolarizing relaxants do not act as MH triggering agents in MHS swine. Not only have we failed to confirm the existence of porcine MH in response to the administration of pancuronium reported by Chalstrey and Edwards [17], but Hall and associates [40] have even demonstrated a degree of protection exerted by pancuronium against the halothane-induced syndrome. Short and colleagues [84] go so far as to recommend pancuronium for elective use in anesthesia for MH swine.

In contrast to MHS Landrace and Pietrain swine, MHS Poland China pigs fail to develop MH in response to succinylcholine alone [78]. Succinylcholine, however, may act as an additive stressor in animals that fail to react initially to a halothane challenge. This differing response to stressors displayed by the various genotypes probably reflects varying degrees of severity of the same basic dysfunction in the mechanisms controlling the normal muscle intracellular Ca^{++} flux (see later discussion).

While the possibility of catecholamine-mediated effects on thermogenesis soon engaged our attention (with negative results) [54], the Bristol group has investigated the problem also. Following their demonstration that circulating levels of catecholamines increased during the MH syndrome in Pietrain swine [66], these workers successfully provoked the syndrome by α-adrenergic agonist infusion

[41] with the corollory demonstration that initiation of the syndrome was blocked in response to other stressors by α-adrenergic blockade [67].

Though Williams and co-workers [92, 93] proposed circulating norepinephrine as the key triggering factor for the hyperthermic syndrome provoked by psychological or exercise stress, Hall, Lucke, and Lister could provoke it in the anesthetized MH Pietrain pig with infused norepinephrine only when this was accompanied by the β-adrenergic blockade of propranolol [41].

PALE, SOFT EXUDATE OF PORK, PORCINE STRESS SYNDROME, AND PORCINE MH—AN IDENTITY?

The occurrence of pork with unfavorable meat qualities (pale, soft exudative pork [PSEP]) has long been the subject of concern and research in the meat industry [64]. This condition (judged primarily by its color and the amount of expressible water) occurs when conditions are present that lead to rapid postmortem glycolysis with concomitant myolactosis and a fall in carcass muscle pH that is more rapid than normal [65, 100]. The identification of these conditions in association with both the porcine stress syndrome (PSS) [88] and the anesthetic-induced syndrome of MH in swine [48] immediately suggested that these separately described syndromes were by manifestations of the same underlying myopathy. Sybesma and Eikelenboom's provocation of MH with halothane in stress-susceptible Pietrain swine [87], and our demonstration of the converse (changes in expressible water of the PSEP type in carcasses of MHS Landrace swine [47]—a finding confirmed in Poland China swine by Nelson and co-workers [79]), provide perhaps the simplest evidence in support of this hypothesis. More elegant perhaps is the evidence that the response of stress-susceptible swine to environmental [32], psychological [93], exercise [87], and anoxic stress [68] is identical with the clinical and biochemical changes observed in the MH syndrome.

The association in swine of susceptibility to stress and susceptibility to MH has been proposed as evidence that the condition in the pig and in man are different, for man does not seem to display the

former [57]. However, until more is known about the causes of sudden death unassociated with general anesthesia in members of MHS families [98, 99], and about those unfortunate persons who die of "heat stroke" with associated muscle necrosis (crushed-muscle syndrome) following severe, unaccustomed exercise (e.g., route marching of new military recruits), this argument cannot be supported, for perhaps these episodes are human manifestations of a stress syndrome similar to that observed in swine.

In this regard, an interesting analogy that invites speculation is the condition of capture myopathy of wild game and its relation to the hypothermia and stress syndromes in swine and man [58]. Here is a condition with many of the features of PSS but one that usually occurs in response to a more severe stimulus. The relevance of the comparison lies in the possible application to wild game management (especially for endangered species) of therapeutic measures useful in the pig and man. Would the addition of dantrolene to the sedative and tranquilizing drugs used in darting animals requiring culling and transport reduce losses due to this condition?

SCREENING AND IDENTIFICATION OF MHS SWINE

Clinical and Biochemical

The demonstration that muscle from MHS pigs could be shown in vitro to behave metabolically in a manner different from that of normal nonreactor pigs, i.e., that the rate of adenosine triphosphate (ATP) depletion in incubated muscle biopsies was approximately double (Fig. 6), provided the basis for the first diagnostic screening test for the MHS trait [54]. However, equally reliable identification of susceptible animals follows the simple clinical observation of a test animal's response to a challenge exposure of halothane. Susceptible animals usually commence developing muscle rigor with its consequent characteristic extension of the hind legs (see Fig. 1) within 5 to 15 minutes, whereupon discontinuance of halothane ensures spontaneous reversal of the syndrome and recovery of the animal. When no abnormal response is evident, halothane is continued for 45 minutes before the test is regarded as negative. We prefer test-

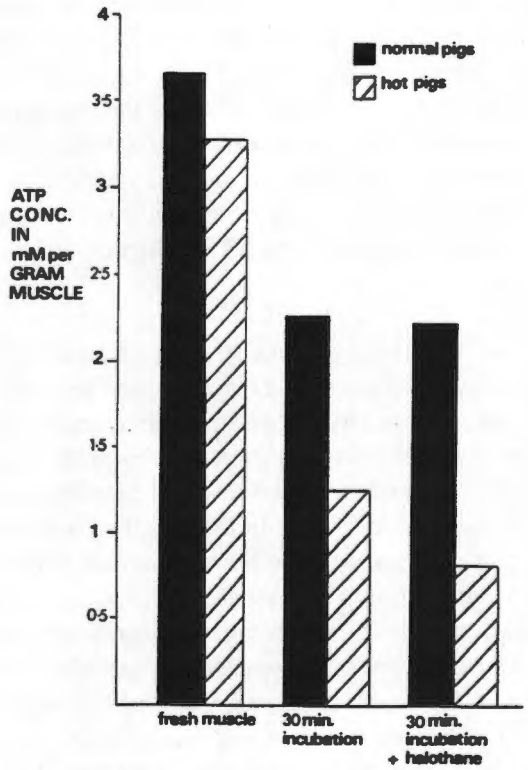

FIGURE 6. *The effect on muscle biopsy ATP content of incubation in oxygenated Krebs-Ringer's solution and exposure to halothane. Hot pig = MHS pig.*

ing animals during the immediate postweaning stage at 4 to 6 weeks of age.

Though mean levels of serum creatine phosphokinase (CPK) are higher in MHS than in nonreactor pigs [101], the variability in values observed renders the level recorded in any individual pig of little diagnostic significance [72]. What is of diagnostic value is the observed change in the level of serum CPK measured on induction of anesthesia when compared with that measured 24 hours later. In MHS swine the increase is truly immense—being of the order of 20- to 100-fold—even if exposure to the anesthetic is brief [51, 101].

Difficulty arises in pigs such as those originally described by Hall and associates [44] in which initiation of the MH syndrome requires administration of succinylcholine in addition to halothane, for this reaction is uncontrollable and usually results in the death of the animal. Such an event may be circumvented by the intraarterial (femoral) injection of a small dose of succinylcholine, with resultant gross myotonic response in the susceptible animal confined to one leg [43, 80]. The diagnosis of MHS may then be confirmed on muscle biopsy by resorting to the original in vitro ATP depletion test.

Histological and Histochemical Screening

Harking back to the hypothesis that the stress syndrome and MH in the pig are manifestations of the same myopathy, and the fact that conventional light and electron microscopy have demonstrated some characteristic features in muscle biopsies from human MHS patients [15, 45, 60], we hoped that such investigation would provide a means of confirming the diagnosis of MHS trait in the pig. The outcome of such investigation, though negative, was interesting. Comparison of the histology on conventional light and electron microscopy of muscle from previously screened MHS and nonreactor Landrace pigs revealed no differences (the histologist viewed the material "blind"). Muscles from both strains, however, showed features that would be considered abnormal in human muscle, e.g., fiber grouping, heterogeneity in fiber size, and occasional cell necrosis. Perhaps, like high CPK values, these are features of the rapid muscle growth characteristic of this breed.

Hematological Screening

Finally, some help in screening for the MHS trait may come from Rasmusen and Christian's recent finding that stress susceptibility appears to be associated primarily with one porcine blood type—the H system [81]. This is a complex system of blood groups in the pig controlled by at least seven alleles with five different blood factors. The MHS characteristic is associated with only two genotypes of this system, H a/a (negative for A or O) and H −/− (positive for A or O). On the other hand, resistance to PSS is associated with three other

genotypes of this system, all positive for A or O, H a/a, H a/c, and H c/—.

PATHOGENESIS

Site of Lesion

Some idea of the magnitude of heat production responsible for the meteoric rise in core temperature which characterizes the hyperthermic syndrome in swine could be derived from the observations of Berman and colleagues [4] and Williams and associates [93]. Berman and co-workers recorded the average heat output by a 56-kg. pig in hyperthermic rigor as 0.275 J. kg.$^{-1}$ min.$^{-1}$, this figure being three times greater, 0.78 J. kg.$^{-1}$ min.$^{-1}$, during the first 10 minutes of the syndrome. Williams and colleagues more recently recorded a 17-fold increase in heat production from normal resting levels in susceptible swine provoked into the syndrome merely by being caged, measuring an average heat output in excess of 0.47 J. kg.$^{-1}$ min.$^{-1}$.

With rigor of skeletal muscle—the other prominent feature of the syndrome—it was not long before the results of various investigations pointed to muscle, not only as the site of the lesion responsible for MH but also as the primary source of heat production. In our laboratory we showed several years ago that muscle from MHS swine behaves metabolically in a manner different from that of normal swine. Thus the in vitro ATP consumption of incubated biopsies was twice as great as that of normal muscle [54] (see Fig. 6). In similar in vitro muscle experiments, Nelson and associates [80] showed that this rapid in vitro ATP consumption in muscle biopsies is accompanied by an appropriately rapid production of lactate.

Further evidence of the primary involvement of muscle came from our demonstration that the rigor and biochemical changes of the syndrome could be reproduced in response to halothane in a preparation of isolated perfused MHS pig hind limb—in effect, a preparation of isolated perfused muscle [52] (Fig. 7). These observations, later confirmed in vitro by Moulds and Denborough [74], drew attention away from the stress-related endocrine aspects of the syndrome as a primary event. The disturbances in pituitary and

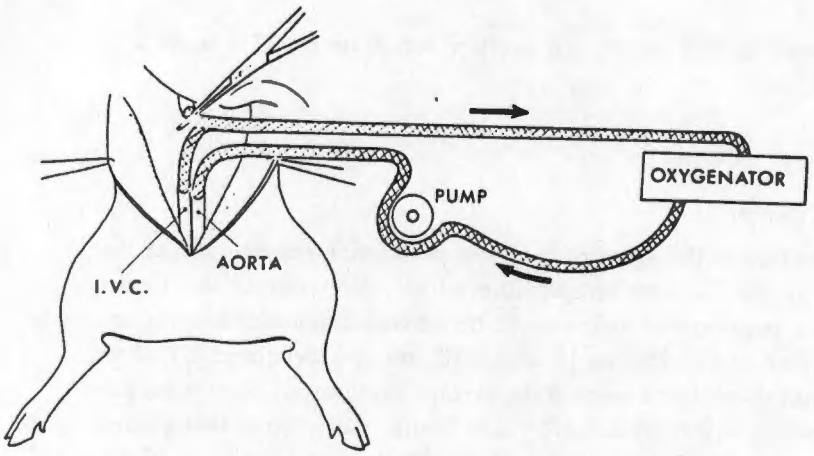

FIGURE 7. *Preparation of isolated perfused hind limbs. Following exsanguination of the animal from the proximal aorta into a pump oxygenator, the hindquarters were perfused through the distal aorta with venous return via the inferior vena cava. The animal was then transected at the lumbar sacral region. Exposure to halothane was achieved by introduction of halothane vaporizer into the oxygen line of the pump oxygenator. (From Harrison et al. [52].)*

adrenal function, which have been the subject of so much research into the PSS and PSEP aspects of this syndrome [70], together with the reported concomitant increases in circulating catecholamines [66, 92], would seem to be either associated defects or defects that are secondary to the primary lesion in muscle.

Any doubts that may have lingered on this score have since been effectively laid to rest by Gronert and co-workers [38], who demonstrated in the MHS pig that total sympathetic denervation fails to block initiation of the syndrome in response to a stressor, but does block the usual accompanying rise in circulating catecholamines. In addition, they demonstrated the corollary that administration of dantrolene, which effectively blocks initiation of the syndrome, has no effect on stress-provoked increases in circulating catecholamines.

The fact that muscle is the source of the increased heat output was validated by Clarke and colleagues [20] when they showed a differ-

ence of 2°C. in the temperature of venous blood outflow compared to that of arterial inflow in the hind limbs of hyperthermic pigs. The next step after these observations, a demonstration that muscle was responsible for the increase in whole-body oxygen uptake measured during the syndrome, has now been taken by Gronert and associates [35].

The astounding explosive heat production which characterizes the syndrome directed early attention to the possibility that uncoupling of oxidative phosphorylation, with the further implication of a defect in mitochondrial function, was the mechanism responsible [96]. Attractive as this hypothesis was, Wang and colleagues' argument [91] that on purely theoretical grounds uncoupling of oxidative phosphorylation could not account for all the heat output was followed by the demonstration by many workers [5, 13, 14, 16, 22] that mitochondria isolated from muscle of both MHS humans and pigs displays normal respiratory control, and the changes in mitochondrial function produced by exposure to halothane—a depression of nicotinamide-adenine dinucleotide–linked substrate metabolism—were not different from those produced in normal mitochondria.

Accelerated Glycolysis

Evidence of massive muscle glycolysis led Berman and co-workers [4, 5] to an in vivo study of the glycolytic intermediates in the hind limb muscle of MHS swine together with simultaneous observations of oxygen uptake and CO_2 production during the course of the hyperthermic syndrome provoked by exposure to halothane (Fig. 8). They concluded that anaerobic mechanisms are responsible for as much as 50 percent of the extra heat production that accompanies the syndrome, while less than 10 percent of the heat production can be accounted for by aerobic mechanisms. These conclusions fitted well with observations by workers investigating the PSS and PSEP manifestations of the syndrome. They observed that stress-susceptible swine have a larger proportion of fast white fibers per unit mass of muscle than non-stress-susceptible animals, such muscle having a lower capacity for oxidative metabolism and an increased potential for anaerobic glycolysis [70].

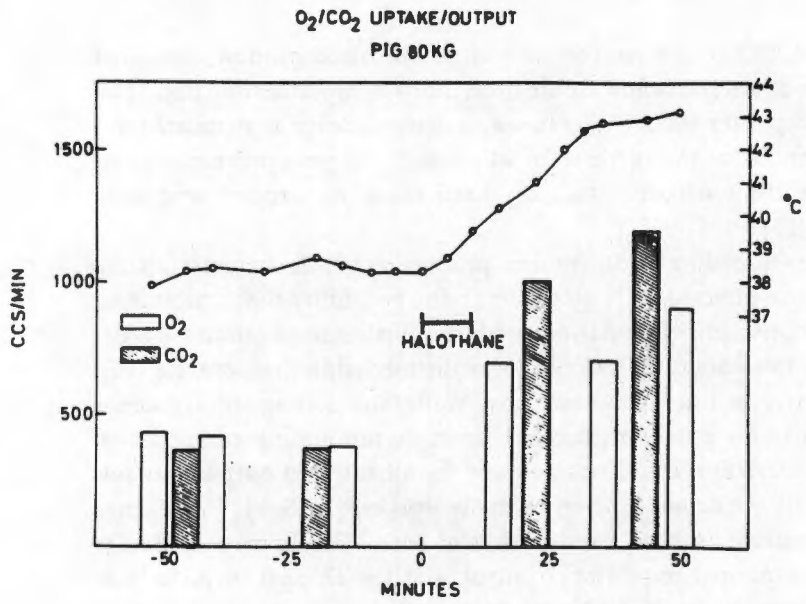

FIGURE 8. *Oxygen uptake and carbon dioxide output with simultaneous esophageal temperature recorded during the course of an MH syndrome provoked by exposure to halothane in a Landrace pig. Control period of anesthesia was with thiopentone and nitrous oxide. Note that the increased oxygen uptake is accompanied by a far greater output of CO_2. (From Berman et al. [4].)*

Further studies of the phase of accelerated glycolytic flux [3] showed that there was a rise in all the glycolytic intermediates measured. No single enzymatic step appeared to be specifically activated. It was postulated that this accelerated glycolysis was due to a "flash activation" of glycogen phosphorylase by intracellular Ca^{++} release (see later discussion).

Other Mechanisms of Heat Production

Besides glycolysis, other anaerobic mechanisms suggested as contributory to the heat production were neutralization and buffering

of excess lactic acid, entropy, change of muscle filament shortening [5], and hepatic lactate gluconeogenesis [22].

The resynthesis of glucose in the Cori cycle is a major route of lactate disposal in the body. During exercise (and the conditions of rigor with accelerated glycolysis are very similar to conditions imposed by severe exercise), enhanced gluconeogenesis can limit the resultant lactic acidosis. It has been suggested by Exton [29] that the "oxygen debt" phenomenon is but a reflection of ATP consumption associated with lactate gluconeogenesis. A factor that doubtless contributes to the extreme and rapid lactic acidosis that characterizes the MH, PSS, and PSEP syndromes is the finding by Dimarco and co-workers [24] that the livers of stress-susceptible swine have a greatly reduced rate of lactate gluconeogenesis and oxidation to CO_2—a rate less than half that of normal animals. A factor in the genesis of PSEP, this deficiency in lactate gluconeogenesis also surely contributes to the rapidity with which the MH syndrome becomes irreversible (see later discussion).

Clarke and co-workers [20] have set forth the challenging hypothesis that accelerated substrate cycling of fructose-6-phosphate is the source of the increased heat production and ATP depletion in muscle that characterize the hyperthermic syndrome. Following their demonstration that the recycling of fructose-6-phosphate in bumble bee flight muscle through reactions catalyzed by phosphofructokinase and fructodiphosphatase generates heat to maintain the thoracic muscle temperature necessary for flight (Fig. 9), they have produced evidence that a similar accelerated rate of fructose-6-phosphate cycling occurs in the hind limb muscle of pigs during the hyperthermic syndrome. They accordingly suggest that this is the mechanism of heat production and depletion of ATP observed during the hyperthermic syndrome. However, the amount of heat released by the rates of substrate cycling they observed in hyperthermic pig muscle is not sufficient to account for the total heat output observed by them during the syndrome. To account for the difference, they propose substrate cycling at other sites in the glycolytic pathway as a further potential source of heat generation—an interesting hypothesis that still awaits proof [92].

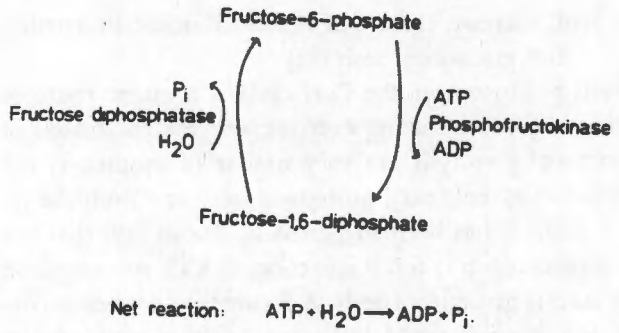

FIGURE 9. *Substrate cycling in bumblebees and MHS swine. (After Clarke et al. [20].)*

Of import in understanding the genesis of rigor is Berman and Kench's observation [5] that ATP levels in muscle are in fact well maintained early in the course of the syndrome when muscle rigor is already established, and only fall after an appropriate depletion of creatine phosphate. This has dispelled the idea developed from our earlier in vitro muscle studies [54] and those of Nelson and co-workers [80] that a decrease in ATP level was the primary cause of muscle rigor.

INTRACELLULAR Ca^{++} FLUX

The key to this excess metabolic activity was found initially by Kalow and co-workers [62] when they reported the following three fundamental observations made on muscle preparations from human survivors of anesthetic-induced MH:

1. Such muscle was more sensitive to caffeine-induced rigor than that from normal controls.
2. This effect was enhanced by exposure to halothane.
3. The uptake of Ca^{++} by the sarcoplasmic reticulum (SR) from such patients was depressed by exposure to halothane, an event that produced no change in SR from normal patients.

Their hypothesis that "malignant hyperthermia is caused by an inborn error in skeletal muscle which renders the muscle susceptible to disturbances of intracellular Ca^{++} distribution" still holds today.

The attractiveness of this hypothesis is that this one event, an increased myoplasmic Ca^{++} flux, can explain both of the principal manifestations of the syndrome: (1) muscle rigor from Ca^{++} inhibition of troponin and activation of myofibrillar adenosine triphosphatase; and (2) hypermetabolism from flash activation of glycogen phosphorylase. These are the two events from which all other accompaniments of the syndrome flow.

Evidence of the release of Ca^{++} from intracellular binding sites was deduced by Berman and Kench [5] from their observation of a rise in plasma Ca^{++} and a concomitant decrease in total muscle Ca^{++} during progression of the syndrome in MHS swine. That drugs known to lower myoplasmic Ca^{++} or reduce its release—procaine [48] and more recently dantrolene sodium [50]—block initiation of the syndrome in response to known activators in vivo and halothane-induced rigor in vitro [74] added not only pharmacological support for Kalow's etiological hypothesis but pointed the way toward specific techniques of therapy and prophylaxis [50, 51].

Brief consideration of the role of Ca^{++} in the muscle excitation-contraction coupling train indicates that membrane dysfunction, genetic or drug induced or both, at any or all of three sites, the SR, sarcolemma, or mitochondria, could lead to the postulated sustained high myoplasmic Ca^{++} concentration.

Sarcoplasmic Reticulum

That no overt rigor has been observed in smooth muscle in association with the hyperthermic rigor of skeletal muscle in MH immediately suggests the pathogenic role of SR dysfunction in this condition. In contrast to skeletal muscle, the main flux of excitation-contraction coupling Ca^{++} in smooth muscle is from the extracellular fluid (ECF) across the sarcolemma. SR is much less well formed in this type of muscle than in skeletal muscle and of much less consequence in the excitation-contraction coupling. Such dysfunction could lead to sustained levels of Ca^{++} in the myoplasm from either increased

release or defective resorption, or both. Kalow's observation that halothane depresses Ca^{++} uptake by SR in MHS humans, supported by similar observations of Brucker and associates [16] and Denborough and co-workers [22] in MHS pigs, seemed to confirm such a hypothesis. Paradoxically, however, SR both from human MH patients [10] and from MHS swine [5, 12, 80, 86] has been shown in vitro to have an increased capacity for Ca^{++} resorption, which is improved by exposure to halothane. These contradictory findings may be due to differences in experimental technique but may also be seen as a chronic adaptation of this organelle to a high myoplasmic Ca^{++} level, the result of a defect in some other intracellular Ca^{++}-binding organelle, e.g., increased sarcolemmal Ca^{++} release or leak, or decreased mitochondrial Ca^{++} uptake.

Direct evidence that the SR membranes of MHS swine are indeed different from normal has now been reported by Berman and colleagues [6], who have shown that they display differences in both density and lipid composition. More recently these same workers, in describing the phenomenon of proton inactivation of Ca^{++} transport by SR membranes [7], have provided one ready explanation for the rapid establishment of the vicious circle which characterizes the MH syndrome once the initial rise in myoplasmic Ca^{++} has occurred. This phenomenon is doubtless the explanation for the observation reported by Britt and associates [12] that, whereas SR preparations from muscle biopsies taken from MHS pigs before exposure to halothane displayed enhanced Ca^{++} uptake, the Ca^{++} uptake of similar SR preparations from muscle biopsies from MHS swine taken after their exposure to halothane (and initiation of MH) was markedly reduced.

Sarcolemma

The well-documented raised serum levels of CPK in MHS subjects, both pig and human, have been proposed as evidence of increased porosity of their sarcolemma [11].

Experimental evidence in support of this hypothesis comes from in vitro investigation of responses of muscle of susceptible subjects (human [75] and porcine [77]) to halothane in conditions of varying

Ca^{++} concentration. The failure of halothane to provoke contracture in a Ca^{++}-free solution, with restoration of this property by the addition of Ca^{++} to the muscle-bath solution, implies that sarcolemmal Ca^{++} transit plays some part in this process.

Mitochondria

The considerable investigation that has been made of the primary energy-producing function of mitochondria in relation to MH has been mentioned already. Much less study has been made of the other function of mitochondria relevant to this syndrome—Ca^{++} accumulation.

Britt and colleagues [13] observed that MHS porcine mitochondria possess a greatly reduced ability to accumulate Ca^{++}—on the order of 8 to 10 times less—than mitochondria from the normal pig. This Ca^{++} accumulation is not significantly affected by exposure to halothane. Such a reduction of Ca^{++}-accumulating capacity can be assumed to enhance the effects of any excess intracellular Ca^{++} release on appropriate stimulus and should be regarded as contributing to the overall pathogenesis.

More recently, Cheah and Cheah [18] reported their observation that the efflux rate of Ca^{++} from mitochondria of the MHS pig in circumstances of anaerobiosis, in this instance immediately postmortem, is twice that of stress-resistant animals and that this Ca^{++} efflux rate from the mitochondria is enhanced by their exposure to halothane. As the circumstances of the MH syndrome rapidly engender anaerobic conditions in the cell, these observations are no doubt pertinent to the pathogenic role in MH syndrome of the high level of myoplasmic Ca^{++} maintained and the establishment of irreversibility.

Generalized Membrane Defect

Though there are important functional differences between the membranes of intracellular organelles, there is indeed a structural organization that is common to most [30, 34, 82]. That the membrane "defect" responsible for MH is not confined to muscle was

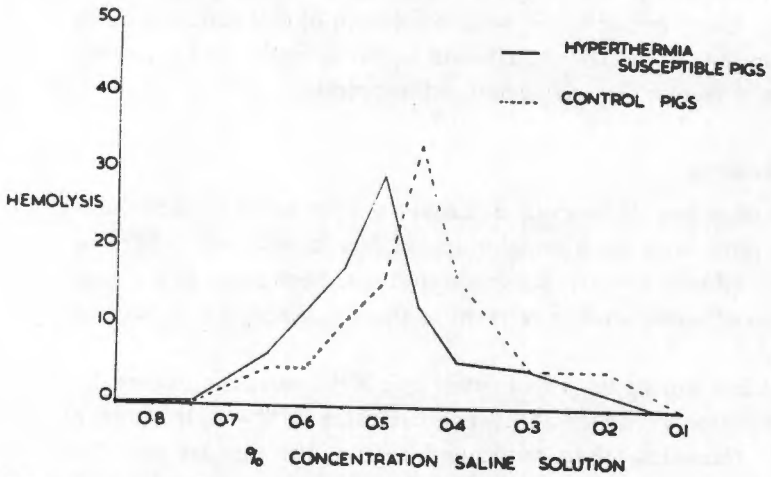

FIGURE 10. *Increment hemolysis curve demonstrating increased fragility of MHS pig erythrocytes. (From Harrison and Verburg [56].)*

suggested by Harrison and Verburg's observation [56] of increased osmotic fragility of MHS porcine erythrocytes (Fig. 10).

Observation of other calcium-mediated reactions becomes relevant to the question of a more generalized membrane "defect." Prompted by Malaisse's proposal that the regulation of insulin secretion depends on the level of Ca^{++} in the beta cells of the pancreas, Denborough and co-workers [23] studied insulin secretion in MHS humans and found that they had an increased insulin response to a glucose load, an observation yet to be investigated in the MHS pig. The observed increase in circulating catecholamines in porcine MH [66] may well have the same implications, judging from the work of Douglas and Rubin [25] on the role of Ca^{++} in the secretory response of the adrenal medulla.

IRREVERSIBILITY–THE VICIOUS CIRCLE

The hyperthermic syndrome is described as "malignant" because of its rapid progression to irreversibility. Applied for a sufficient time, any activating stimulus acts as a trigger; the syndrome there-

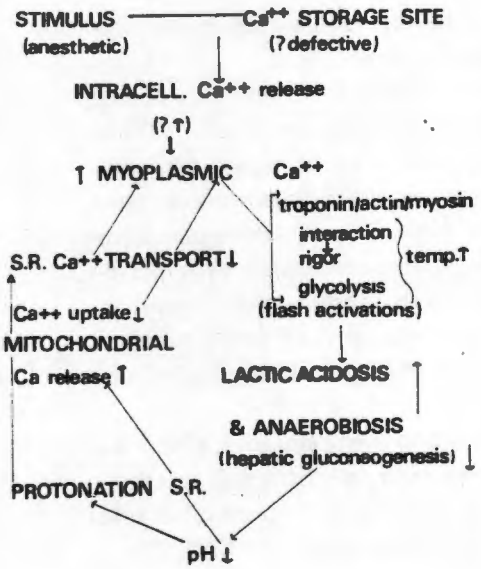

FIGURE 11. *The vicious circle.*

after continues independent of the stimulus. Consideration of some of the observations of muscle cell organelle function and hepatic gluconeogenesis already referred to allows us to form a reasonable working hypothesis of the evolution of this state (Fig. 11).

The initial stressor acts by causing the release of Ca^{++} (possibly an excess in relation to the magnitude of the stimulus) into the myoplasm from a genetic functionally defective SR and possibly sarcolemma. This causes muscle contraction and flash activation of glycolysis, events which produce heat and lactate. Defective hepatic gluconeogenesis [24] results in a reduced rate of lactate clearance by the liver, and consequently severe intracellular acidosis, soon causing the intracellular pH to fall below the critical level of pH 6 at which Berman and co-workers [7] showed that irreversible inactivation of Ca^{++} transport by the SR occurs. In conjunction with the reduced Ca^{++}-accumulating capacity of the mitochondria [13], these events lead to the maintenance of a high level of Ca^{++} in the myoplasm. Activation of muscle and glycolysis continues, contraction becoming rigor. At the same time, the anaerobic conditions engendered

by these events in the muscle cell cause an increased rate of Ca^{++} efflux from the mitochondria [18], adding yet another factor contributing to inexorable irreversibility of the syndrome.

The high level of myoplasmic Ca^{++} maintained may not be the only factor leading to irreversibility of the syndrome. Fuchs [33] has now demonstrated that the Ca^{++} control system of human actomyosin is inactivated by temperature increases of the magnitude encountered in intact muscle during the hyperthermic syndrome. This thermal inactivation of the Ca^{++}-regulating mechanism of actomyosin is potentiated by decline in ATP levels. Thus, two key events in the MH syndrome, pyrexia and fall in muscle ATP level, can perpetuate muscle rigor independently of the intracellular Ca^{++} concentration.

In addition, attention has been drawn to the stimulating effects on muscle and metabolism of the vast increase in circulating catecholamines observed in Pietrain pigs with MH and their possible role in events leading to irreversibility of the syndrome [41, 73, 92, 95].

THERAPY

Pharmacological support for Kalow's hypothesis of the central role of Ca^{++} flux in the genesis of human MH came from the observation that procaine, a known inhibitor of SR Ca^{++} release, blocks initiation of the syndrome in MHS swine. This pointed the direction from which specific therapy for the syndrome might emerge [46] (Fig. 12). In spite of initial reservations based on the lack of effect of procaine on established rigor in vitro [8, 31] and the negative inotropic effect of procaine in large doses on the myocardium, procaine has acquired a history of successful therapeutic use in MH in man [11, 15]. However, in porcine hyperthermia the therapeutic use of procaine has proved ultimately disappointing [36, 39, 43, 71].

Though its mode of action is unknown, the steroid anesthetic Althesin, when given by continuous intravenous infusion, has a similar action to that of procaine, blocking initiation of the porcine MH syndrome in response to halothane but not to halothane combined with succinylcholine. Disappointingly, as with procaine in the por-

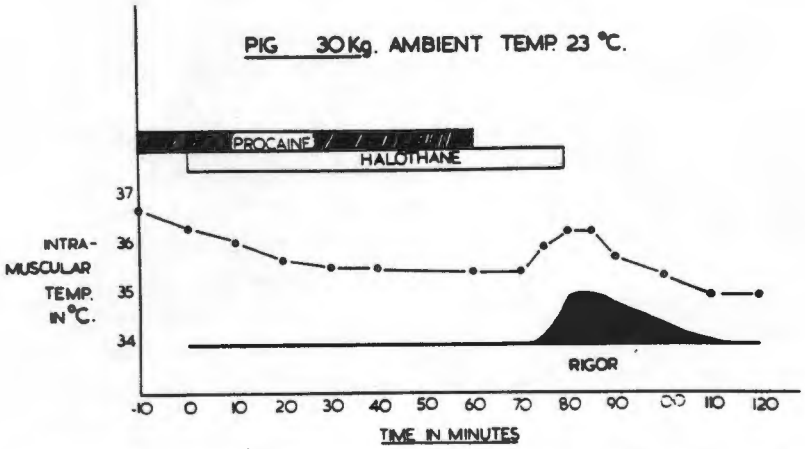

FIGURE 12. *The original in vivo demonstration in MHS Landrace pig of block of halothane-induced rigor by procaine (continuous intravenous infusion). Events recorded (from above downward): procaine infusion, halothane administration, intramuscular temperature, and rigor (recorded as extension of hind limb). Note the onset of rigor and rise in temperature when procaine was discontinued. (From Harrison [48].)*

cine syndrome, it has proved therapeutically useless once the syndrome is established [49].

Attention has already been drawn to the interrelated observations reported by the Bristol group of high circulating levels of catecholamines, α-adrenergic agonist trigger action [41], and prevention of that action by α-adrenergic blockade [67] in porcine MHS. As was the case with Althesin, α-adrenergic blockade could not be extended to offer therapy for the established syndrome.

Though Bianchi's suggestion [9] in 1971 that diphenylhydantoin should be investigated as a therapeutic agent did not bear fruit, it is another hydantoin—dantrolene sodium—that now offers the greatest therapeutic hope. Interested in drugs that might be of use in relaxing spastic muscle states, Snyder and associates [85] reported in 1967 the synthesis of a series of hydantoins which proved to have muscle relaxant properties. One of these, dantrolene sodium, was extensively investigated, and its muscle-relaxant properties were shown to

be due to an effect on excitation-contraction coupling. Further, probably as a result of its limited water solubility, dantrolene, no matter what the dose, did not produce complete muscle paralysis in vitro or in vivo [27]. Interestingly, its effects were on skeletal muscle only, and no effect on cardiac or smooth muscle was apparent [27, 28].

These properties immediately excited my curiosity about the possible use of dantrolene sodium in treating MH. My investigation of this drug produced results excitingly different from those of other drugs previously investigated [50, 51]. Administration of dantrolene sodium blocked initiation of the syndrome in response not only to halothane and succinylcholine separately, but also to succinylcholine administered in addition to halothane (Fig. 13). Furthermore, when administered to animals in established hyperthermic rigor, dantro-

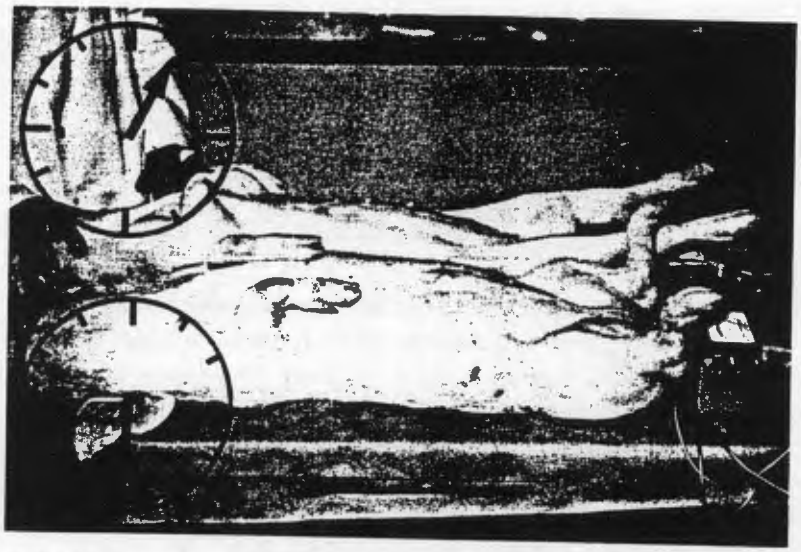

FIGURE 13. *Blocking effect of oral dantrolene on halothane-induced rigor in MHS swine. The nearer pig, pretreated with dantrolene, is in the thirtieth minute of a planned one-hour exposure to halothane. The legs are relaxed and temperature is falling. The other pig was not so treated. After 4 minutes of halothane inhalation, muscle rigor has commenced, causing a characteristic extension of the hind legs. This is accompanied by an increase in rectal temperature. (From Harrison [51].)*

lene sodium effectively terminated the syndrome (Fig. 14). These findings, now confirmed in Poland China swine [1, 37] and Pietrain pigs [42], have yet to be applied to humans, such application having been prevented by the lack of a suitable intravenous formula for clinical use in humans. Development of a lyophilized form of the drug promises to overcome the difficulties imposed by its limited

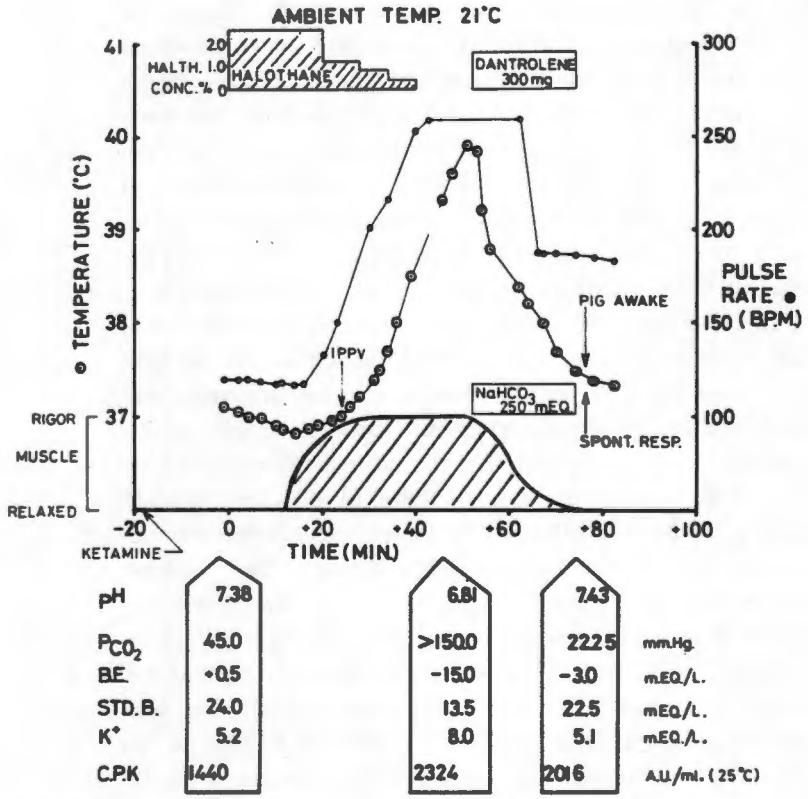

FIGURE 14. *Temperature (deep muscle) and events chart showing in vivo termination by intravenous dantrolene of the established MH syndrome provoked by exposure to halothane in Landrace swine. Dantrolene administration commenced at the 51st minute coincident with the left margin of the block so marked. The presence of rigor was recorded as extension of hind limb. Biochemical values recorded were from mixed venous blood. (From Harrison [50].)*

water solubility, and the results of the first clinical trials may be known by the time this issue is published. If dantrolene can be shown to act in man in the same manner as in the pig, we need no longer fear MH. We will have at our disposal the means to prevent it and cure it.

I want to conclude this chapter with a brief consideration of how present knowledge on the action of dantrolene sodium may affect our ideas about the pathogenesis of the syndrome of MH. Consideration of the effects of dantrolene in vivo on muscle twitch responses and caffeine contracture led Ellis and Carpenter [27] to suggest that dantrolene acts on the trigger Ca^{++} mechanism of excitation-contraction coupling. This suggestion would conveniently explain its apparent specificity of action in skeletal muscle and its lack of action on smooth or cardiac muscle. In skeletal muscle a small sarcolemmal Ca^{++} flux triggers a large intracellular Ca^{++} efflux from the SR, whereas in cardiac and smooth muscle the sarcolemmal Ca^{++} flux is the large component. Thus a small depression of Ca^{++} flux at this point in the excitation-contraction coupling train would have a large functional effect on skeletal muscle, whereas in cardiac and smooth muscle a similar small depression would have only an insignificant effect. If this is so, it implies that an element of sarcolemmal defect is at least part of the essential lesion of MH. On the other hand, recent work by van Winkel [90] on isolated SR has demonstrated that dantrolene depresses not only the rate of Ca^{++} release on stimulation but also the total amount of Ca^{++} released—without completely inhibiting such release. In addition, dantrolene had no effect on SR Ca^{++} uptake. These observations not only support the central role of abnormal Ca^{++} flux in the genesis of MH but also suggest that the principal defect is situated in the SR. In light of van Winkel's findings, the fact that the hyperthermic syndrome is readily reversed by dantrolene sodium seems to imply that continued release of Ca^{++} into the myoplasm may be an important factor in the genesis of the syndrome. Once this release is effectively stopped, there is still sufficient functional ability in the various Ca^{++} modulating mechanisms to restore the myoplasmic level once more to that which permits relaxation and thus termination of the syndrome.

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PORCINE MALIGNANT HYPERTHERMIA—THE SAGA OF THE "HOT" PIG

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ABSTRACT

Much of what we understand today of the pathogenesis and therapeutic management of the anaesthetic induced malignant hyperthermic syndrome in man followed from the happy chance discovery, approximately twenty years ago, that the well-known stress syndrome of swine was a very close facsimile of this puzzling and fatal syndrome in man. In both, the syndrome was genetically based, a fact that allowed for the breeding of a valid animal experimental model of human malignant hyperthermia. So extensive has been the use of the MHS pig model that today an account of porcine hyperthermia per se would indeed be the whole story of the evolution of our present understanding of the human MH syndrome, the subject of this book.

This chapter confines itself to the more purely porcine aspects (if that is possible) of the syndrome. It includes descriptions and discussions of:

1. The syndrome in the pig including clinical signs, biochemical accompaniments and variations in the syndrome.
2. The identity of the separately described stress myopathies of swine—porcine malignant hyperthermia, porcine stress syndrome and pale, soft, exudative pork.
3. Modes of inheritance.
4. The MHS pig and other experimental models of human MH.
5. Pharmacological triggering, attenuation, blocking and reversal of porcine malignant hyperthermia.
6. Pathogenesis of the vicious cycle of malignant hyperthermia in relation to the stress syndromes of the pig and man.

INTRODUCTION

"All animals are equal; only some animals are more equal than others". The pig Napoleon, Animal Farm (George Orwell).

Of all the complications of general anaesthesia perhaps the most dramatic and indeed frightening is that veritable metabolic storm which characterizes the unexpected, in the past frequently fatal, syndrome of anaesthetic-induced malignant hyperthermia. First described in 1960 in Denborough's et al report (1) (later elaborated in 1962 (2)) of a family which displayed an apparent hereditary predisposition to death attributable to general anaesthesia, this syndrome became definitively characterized over the next six years through the reports of Saidman et al (3) and those in the Canadian Anaesthetists' Society Journal of September 1966 (4) which presented the collected experience of six groups of authors of eleven cases of this fatal condition. Variously referred to up to this date as "hyperpyrexia", "hyperthermia" or even "extreme hyperthermia during anaesthesia", in this publication we find this syndrome referred to for the first time as "Malignant Hyperpyrexia" (5,6), later changed by common usage to "Malignant Hyperthermia" (MH). Since this time little has been added to the clinical manifestations we recognize of the syndrome.

As on so many other occasions in the long history of medicine, serendipity now provided the deus ex machina that would ultimately help solve this dangerous clinical conundrum—the "hot" pig. The possibility that strains of certain breeds of pig might serve as a valid animal model of MH was suggested, also in 1966, by the observation reported by Hall and co-workers (7) of muscle rigor and hyperthermia in littermate pigs, anaesthetized with halothane in response to the injection of succinylcholine, followed shortly by Harrison and co-workers' (8) report of the same reaction but now in response to halothane alone. As with the human cases this response appeared to be pharmacogenetic.

These discoveries were seminal. Today, just less than twenty years later, though the minutiae of the pathogenesis of malignant hyperthermia have yet to be elucidated, knowledge that has come from studies worldwide in malignant hyperthermia susceptible (MHS) swine has led not only to effective

pharmacological control of the syndrome, but also to valuable spin-offs in many biomedical areas, in particular those of muscle and membrane physiology, calcium transport and kinetics and the meat industry.

THE CLINICAL SYNDROME IN PIGS

The malignant hyperthermia syndrome in swine has two prerequisites:

1. Possession of the operative gene or genes.
2. Exposure to a specific stressor for an adequate time.

The specific stressors originally described--halothane anaesthesia alone or succinylcholine administration during halothane anaesthesia--identified genetically based differing grades of severity of lesion and response.

Once established the syndrome rapidly becomes self-sustaining and irreversible, continuing independently of withdrawal of the stressor which can be then seen to have acted as a trigger (8,9).

Clinical Signs

The syndrome is manifested by a group of inexorably progressive clinical signs together with a myriad of biochemical changes which immediately precede them (10).

The clinical features include:

1. Cyanosis of the snout and blotchy cyanosis of the skin, most marked on the anterior abdominal wall. This blotching disappears as the syndrome becomes established, often "pinking up" before it does so.
2. Twitching and sporadic fasciculation of muscles, most obvious in the thigh, proceeding usually within minutes to progressive extreme rigor of muscle. This rigor causes the limbs, most markedly the hind limbs, to become rigidly extended. Rarely, rigor may hold a limb in flexion. Triggering of the syndrome by succinylcholine is characterized by extremes of coarse muscle fasciculation and myotonia.

3. Sinus tachycardia proceeding often to extreme rates of the order of 240 beats per minute. This is accompanied in the early stages of the syndrome by a rise in blood pressure. Cardiac arrhythmias, usually of the nature of ventricular tachyarrhythmias, appear variably, sometimes very early, at others terminally.

4. An explosive sustained rise in core temperature. Change in temperature may be earliest observed by means of a thermistor probe sited deep in the thigh muscle mass. The rate of temperature rise varies between animals. In highly susceptible strains rates of rise of $1^{o}C$ every five to seven minutes are common, reaching $43–45^{o}C$ ante mortem.

5. Open-mouthed breathing and hyperventilation. This manifests only in pigs allowed to breathe spontaneously. Apnoea supervenes within ten to fifteen minutes.

Biochemical Accompaniments (11)

The underlying hypercatabolism of the syndrome and its effects are reflected in the conventional indices of acid/base, blood gas, electrolyte and substrate status. Of these, changes observed in the first two can be considered diagnostic. These changes in biochemical parameters are characterized by a rapid progression, preceding and reaching extreme values ahead of the rise in core temperature. Profound, rapidly worsening metabolic acidosis—lactacidosis—with concomitant rise in PCO_2 from its buffering, is the hallmark of the syndrome. Blood and tissue pH may fall from 7.4 to 6.8 and lower in as little as ten minutes (10). The hypercapnia, readily monitored by capnography, is the earliest and most sensitive marker of initiation of the syndrome (12). Capnography, therefore, should be considered an essential monitoring device in the clinical situation in humans. Reduction in PaO_2 accompanies the acidosis. While this need not be great, the reduction in PO_2 of the mixed venous blood is, leading to a greatly increased $P(a-V)O_2$ gradient. The A-a gradient may also widen (13), all this in the face of a marked increase in O_2 uptake (9,14).

Rises recorded in all serum electrolytes, total protein, glucose and lactate indicate a shift of water into cells, a leak of Na^{+}, K^{+}, Ca^{++}, Mg^{++} and Pi out, and glycogenolysis (11,14). Of

particular import to the clinical management and prognosis is the rise in serum K^+. As the syndrome progresses, increasing permeability of the sarcolemma is manifest by rapidly rising serum levels of the muscle enzymes creatine kinase (CK) and lactic dehydrogenase (LDH) (12,15). Within skeletal muscle the hypercatabolic state is marked by a rapid fall off in the high energy intermediates creatine phosphate (CP) and adenosine triphosphate (ATP) (11,12,15). Untreated the syndrome is inevitably fatal.

Animals permitted to breathe spontaneously may die in fifteen minutes. Artificially ventilated animals can survive up to forty, even sixty minutes.

Variations in the Syndrome

Variations in (1) sensitivity of the triggering mechanism; and (2) rate of progression of the established syndrome, occur in response to environmental, pharmacological and genetic factors.

Excitement from the stress of manhandling and struggling has a profound effect on both the sensitivity of the triggering and the speed of progression of the hyperthermia crisis and may of itself spontaneously initiate the syndrome (16). Such conditions are the inevitable accompaniment of the conventional barnyard screening procedure for MH susceptibility viz brief mask anaesthesia with halothane. In these circumstances the syndrome may be fired by an exposure to halothane of as short a duration as 30–40 seconds. Skin blotching becomes very marked and muscle rigor occurs almost before the onset of anaesthesia. It is often difficult to distinguish between the stiff leg of conscious induction struggling and the extended leg of muscle rigor—one merges so imperceptibly with the other. The syndrome rapidly becomes irreversible and dantrolene (17) may be needed for salvage. Highly reactive animals should be carefully observed during recovery from a halothane challenge for fatal recrudescence of the syndrome can follow return of consciousness and muscle activity. The same may indeed occur after initially successful treatment with dantrolene.

Sedation with hypnotics, tranquillizing or anxiolytic drugs (18-20) on the other hand, reduces triggering sensitivity and delays initiation of the syndrome, though the speed of progression once the syndrome is established, may be unaffected. Variations in these same aspects of the syndrome may also be induced non-specifically by other groups of drugs such as non-depolarizing relaxants (21) or those which lower blood pressure and perhaps reduce cardiac output and limb blood flow (G.G. Harrison, unpublished). In these circumstances triggering of the syndrome by halothane may be delayed by as much as 45 minutes or longer or even prevented altogether, requiring ultimately the injection of succinylcholine to fire the syndrome (vide infra: Triggering agents).

Genetic factors are the major determinants of variations in the manifested syndrome (22). Though malignant hyperthermia susceptibility was originally thought of as something of an all-or-none phenomenon (23), controlled breeding experiments have since produced phenotypes with graded responses in both the sensitivity of the triggering mechanism and thereafter in the rate and extent of progression of the established syndrome from what we may call the hair-triggered "rocket" to the "slow burner" (22,24,25). In the case of the former, the syndrome is fired by exposure to halothane alone while in the latter the addition of succinylcholine (as was the case with Hall's historic pigs (7)) is necessary. These genetically based variations in response occur in all the MH affected breeds—Landrace, Poland China and Pietrain.

PORCINE STRESS MYOPATHY—PMH, PSS AND PSEP

Following its identification it was soon established that this Porcine Malignant Hyperthermia (PMH) response to halothane and/or succinylcholine, was heritable and could be evoked only in selected strains of relatively few breeds of pigs (26). Further, clinical and biochemical features of the syndrome were identical to those of the Porcine (sudden death) Stress Syndrome (PSS) (27) long recognized in the same breeds of pig in response to stress, excitement, severe exercise and/or environmental extremes alone (28-30). The breeds, worldwide, in which this genetic trait is manifest include the Landrace, Pietrain, Poland China and crossbreeds of these strains,

all breeds genetically selected for rapid growth, high feed efficiency, heavy muscling and high muscle/fat ratios. Ironically, the improved profitability of pig husbandry sought through such high grade genetic selection has often been reduced by losses incurred from the concomitant increased incidence of PSS. For example, annual losses of the order of 300 million dollars have been reported in the USA from this condition and PSEP (vide infra) (31).

Also of long time concern to the meat industry and subject of much research is the occurrence of pork with unfavourable meat quality in that it is pale, soft and watery. Studies of pork meat quality in different countries have shown its presence in as much as 20-35% of carcasses (31-33). Variously called "wasseriges fleisch", "muscle degeneration" or "pale, soft, exudative pork" (PSEP) (34) it occurs in carcasses which manifest accelerated glycolysis with concomitant myolactosis and rapid fall in pH in the immediate one to two hours post slaughter (35,36). The pale appearance, altered texture and increased waterholding quality of the muscle fibre is thought to be due to denaturation of the sarcoplasmic protein consequent on the low muscle pH while the carcass is still warm (37).

The identification of these same conditions in association with both PSS (27) and the MH syndrome (38) immediately suggests that these three separately described syndromes were simply manifestations of the same covert myopathy. This hypothesis has been amply supported by the demonstration of halothane-provoked MH syndrome in stress susceptible swine (28), the PSEP meat condition and its biochemical accompaniments post mortem in pigs dying of malignant hyperthermia (39) and finally the correlation of enzyme markers of malignant hyperthermia susceptibility with the incidence of PSEP (33,40).

The inference from this concept that susceptibility to PSS and PSEP could now be simply identified, considerably facilitated research in the field of pork quality control. By identification of susceptible animals at weanling stage, progressive pig farmers have been enabled to breed out from their herds this undesirable trait.

Screening for Malignant Hyperthermia Susceptibility

The simplest, most practical and reliable screening test for MHS remains the crude "barnyard" halothane challenge, clinical observation of the test animal's response to brief mask halothane inhalation—bearing in mind the caveat expressed earlier. Animals having a degree of susceptibility worth noting for meat quality "breeding out" or laboratory "breeding in" purposes will react with skin blotching and muscle rigor within five minutes.

Of simple blood sampling tests none have proved to be specifically diagnostic. Sadly, the variability observed in the muscle enzyme markers of this myopathy, even the most specific, CK, is such that the level recorded in an individual pig is of little diagnostic significance (33,41). For laboratory purposes collaborative diagnostic data may be sought "in vitro" from muscle biopsy. These tests (42) are based on identifying and quantifying two abnormalities manifest by MHS muscle: (1) sensitivity to caffeine induced contracture; and (2) an increased rate of ischaemically induced ATP depletion. They are time consuming and not practical for large scale screening of animals. However, Verburg's et al (12) observation of consistently low levels of creatine phosphate in MHS muscle may provide the basis for a quicker and more widely applicable screen for animals, especially if the method could be miniaturized to assay creatine phosphate in needle biopsy specimens.

Inheritance Patterns

Inheritance of this readily "in vivo" identifiable myopathic trait was thought initially to involve a simple autosomal dominant allelic gene, inconsistencies in transmission being explained as varying expressivity and incomplete penetrance (7,15,43). Subsequently, planned breeding experiments have produced evidence for the existence of two sharply differing modes of inheritance—a circumstance indeed analogous to that in humans (44). While autosomal dominant inheritance; with some modifications, is supported by most of the published evidence (including this author's Landrace breeding experience) strong evidence for a recessive mode of transmission has been reported by others.

Modifications to the concept of a single gene autosomal transmission have followed from the nicely graded clinical manifestations of the MH syndrome correlated with "in vitro" muscle caffeine and caffeine/halothane responses demonstrated by the five MHS phenotypes bred by Britt and co-workers (24) from Poland China (MHS) and Yorkshire (MH resistant) crosses. These gradations in phenotype response followed from the offspring of crosses manifesting response characteristics that were the mean of those of the individual parents and not segregating out into groups which followed the response characteristics of one or other individual parent. The identification of five phenotypes in two generations indicated that two gene alleles (at least) must be involved and the gradations observed meant that such genes must be co-dominant.

Support for this hypothesis of co-dominance in porcine MH alleles comes from Nelson's et al (25) report of similar graded phenotype reactions in first generation MHS (Poland China) and MH negative mini swine (Pittman-Moore/Hartford) crosses, similarly identifiable clinically, with corroborative graded "in vitro" halothane and caffeine/halothane contractures.

Observing in addition to graded clinical severity of the syndrome, graded elevations in basal metabolic rate in MHS phenotypes, Williams and co-workers (22) also proposed the existence of more than one co-dominant gene, but accepted the possibility of a strongly modified single dominant gene. Like Nelson et al they warn against the validity of accepting the barnyard halothane challenge alone in identifying MHS phenotypes in breeding studies and themselves only consider an animal completely MH negative when its response to succinylcholine administration as well as to halothane is negative and basal metabolic rate is not raised.

The likelihood of a multifactorial inheritance pattern is further suggested by evidence of cross-linkage of this myopathic trait with other characteristics. Rasmussen and Christian (45) and Jorgensen et al (46) report the association of MH susceptibility with only one porcine blood group, the H system. Susceptibility to MH occurs in only two of the phenotypes of this complex seven allele

system while resistance or negative response is associated with three others. Andersen (47) goes further and infers from his data a linkage in Landrace swine of the MHS gene with that for phosphohexose isomerase.

While all the above is compatible with the general thesis of autosomal dominant or codominant inheritance, good evidence of the antithesis—a recessive pattern of transmission—has also been published.

Eikelenboom and co-workers (48) especially, as well as Christian (49) and Ollivier et al (50) have reported from their breeding programs with Dutch Landrace and Pietrain swine, strong evidence of recessive transmission of MH susceptibility, proposing the involvement of a single major recessive gene with complete penetrance.

An explanation for the co-existence of two distinct patterns of inheritance is not as yet forthcoming. That these differences may be associated with breed is obviously not the case—certainly in broad terms—for although studies involving the Poland China breed have all evidenced the dominant pattern of inheritance, both dominant and recessive modes have been demonstrated in Landrace, Landrace crossbreeds and Pietrains.

Though intellectually dissatisfying, it seems that for the moment we must accept that in swine, as in the human, there are two subclinical or covert stress-associated myopathic states which may manifest the malignant hyperthermic syndrome as the functional final common pathway. Considering the subsets of musculoskeletal disorder as well as the various overt myopathies and muscular dystrophies associated with the susceptibility of humans to malignant hyperthermia (51), it is perhaps not surprising that the inheritance pattern of MH susceptibility in swine has turned out to be considerably more complex than appeared at first sight.

Anaesthetic-induced syndromes clinically similar to the malignant hyperthermia syndrome of swine and humans have been described rarely in other species such as the horse (52,53), dog (54) and cat (55). In contradistinction to the porcine and human syndrome, genetic factors do not appear to play an obvious role in

these biological curiosities and the repeatability of the reaction in the individual animal has not been established (invariably the affected animal has died).

Though the topic of MH in other species is discussed by Klein and Rosenberg elsewhere (see Chapter 6), I wish to allude briefly here to a common regularly appearing stress-related myopathy in other species which has some superficial resemblance to that of swine though the initiating factors are entirely environmental and genetic factors appear to play no part. This is the so-called "capture myopathy" of wild animals, in particular ungulates (described in nineteen species) and birds (56). Indeed, a similar condition has been observed also in sharks (Thurman, South African Association of Marine Biologists, personal communication). Precipitated by the alarm, stress and extreme muscular exertion of flight that precedes the darting, sedation, immobilization and capture of wild game, the syndrome presents as shock, prostration, muscle stiffness ("spastic paresis" is another name) with moderate hyperthermia proceeding to coma and death. Sharks, taken live but exhausted from entanglement in beach protection nets and released in aquaria, swim with ever increasing muscle stiffness until completely incapacitated.

The principal biochemical derangement in all these species is a profound lactacidosis which together with raised serum levels of muscle enzymes, CK, LDH, alanine amino transferase (ALT) and aspartate amino transferase (AST), point to the initiation of anaerobic metabolism in muscle and membrane damage (57). Skeletal muscle lesions very similar to those of PSEP appear post mortem. Once induced, the mortality from this syndrome, as is that from PMH, is exceptionally high—rates of up to 100% of affected animals having been reported. For this reason an understanding of the syndrome and its prevention is now of enormous importance for game conservation as attempts at capture of remaining nuclei of rare species for relocation or restocking can well be, and indeed have been, rendered abortive by this unacceptably high mortality rate. Fortunately, the steps in the reaction chain lack the extreme run-away characteristics of the porcine syndrome. Adequate sedation by a variety of tranquillizers or anxiolytics, if achieved

with a minimum of alarm, stress and pursuit, prevents the condition. Harthoorn's demonstration (58) that control of the acidosis alone with sodium bicarbonate will abort the established condition, as well as being of great practical importance indicates that in all probability the pathogenesis of this condition differs fundamentally from the PSS it superficially resembles (vide infra Pathogenesis). Further understanding of this condition and its relationship, if any, to PSS must surely follow from observation of the effects of halothane and succinylcholine on stressed and unstressed animals, as well as from the effects of dantrolene on the established condition (doubtless a very expensive exercise). But all this is still for the future.

MODELS OF MALIGNANT HYPERTHERMIA

Proposed in 1968 as an animal model for the study of the MH syndrome in man (8), use of the MHS pig has stood the test of time. With few exceptions, all the features of the syndrome observed in man—clinical, biochemical and ultracellular—have been documented in susceptible swine and vice versa. Some differences there may be but, being largely quantitative rather than qualitative, they do not appear to be fundamental.

The clear association of the porcine syndrome with stress, both psychological and environmental, was once held to distinguish it from human MH. This distinction has since been rendered considerably less clear by Wingard's (59,60) identification of instances of unexpected sudden deaths in families of MH probands as exemplifying a "Human Stress Syndrome".

Another difference that has been postulated twixt MH in pig and man stems from differences recorded in levels of serum Ca^{++} observed at various stages of the syndrome (11,61). However, as levels recorded by various workers have been very variable, a real systematic difference is difficult to sustain (51).

While in the most reactive animals the trigger mechanism—being responsive to environmental as well as pharmacologic activation—may be regarded as more sensitive than in man, not all the drugs associated in man with initiation of the syndrome have been demonstrated to fire the syndrome in the MHS pig. This may

well be because the association of particular drugs with the triggering of MH in man is largely anecdotal usually involving greater or lesser degrees of the polypharmacy of clinical anaesthesia, whereas in the pig model drugs can be deliberately tested singly. However, as MHS humans have been shown to develop the syndrome in response to all the drugs demonstrated to trigger MH in susceptible swine, this model does provide a useful positive screen for drugs that would be potentially harmful to the MHS human.

In using the MHS pig model, especially for drug screening, it is important that the caveats previously expressed (vide supra Variations in the syndrome) be observed if misleading, even spurious, results are to be avoided. The conclusion that certain phenothiazine and neuroleptic drugs displayed MH blocking properties (19,20) is an example of such an error. These deductions were based on animal halothane exposure times of minutes only. Experimental protocols should be so designed that drug induced attenuation or delay in onset of MH can be clearly distinguished from MH blocking or prevention. Uneventful exposure to halothane of adequate duration, sixty to ninety minutes, terminated by an MH free succinylcholine challenge is necessary before true MH blocking properties be attributed to any drug. (vide infra Triggering, Attenuation, Blocking and Reversal).

Other animal models of MH both "in vivo" and "in vitro", based primarily on two concepts of its pathogenesis, have been proposed. The first of these sought to give experimental expression to Wilson and co-workers' (62) proposal that "...this explosive thermal idiosyncracy" was due to "some disturbance of biological oxidation, possibly uncoupling of oxidative phosphorylation". Dinitrophenol pretreated dogs (63) and rats (64) were utilized to study halothane induced or exacerbated uncoupling of oxidative phosphorylation. This mechanism, and thus model, was soon discounted by the demonstration of several workers (10) that mitochondria isolated from MHS muscle, both porcine and human, display normal respiratory control and that halothane induced depression of NAD linked substrate metabolism is no different in MHS or normal muscle.

The second more relevant group of animal models is based on Kalow and co-workers' (65) early fundamental observations that muscle from survivors from MH was more sensitive "in vitro" to caffeine induced contracture than normal muscle, a sensitivity enhanced by exposure to halothane; observations that carried with them the inference that the functional lesion of MH was a caffeine-like disturbance of SR Ca^{++} flux. This was given further expression by the demonstration 'in vitro' that muscle strips from laboratory animals including frogs (66) and rats (67) pretreated with subcontracture producing concentrations of caffeine responded to halothane and indeed other MH triggering drugs in a manner similar to MHS muscle. These conditions were produced also "in vivo" in the rabbit (68). These models were proposed inter alia as suitable screens for the MH trigering or blocking potential of drugs. The caffeine-like effects of ryanodine were also used to generate models of MH both "in vivo and vitro" (69) but these were less successful.

Lastly, the myotonic/hyperpyrexic response of domestic poultry to succinylcholine which has been shown to be blocked by the same drugs that attenuate MH (70) has also been proposed as constituting a suitable MH drug screen.

However, none of these models can be said to emulate the reactions of human MH as accurately as does the MHS pig. At best they may each be said to mirror some fragment, and some fragment only, of the MH responses. As such one must question their relevance.

Anaesthesia for the MHS Pig

The conventional method of inducing anaesthesia in the pig for laboratory procedures, i.e. physical restraint followed by the injection of thiopentone into an ear vein, carries with it the risk that, in the most sensitive MHS strains, the physical manhandling and struggle necessary to achieve, this may initiate MH. This may be avoided by the preliminary intramuscular injection—into the dorsal muscles of the neck—of ketamine 10 mg/kg, a procedure that requires a minimum of manhandling or skill. Within five minutes the animal will be recumbent and unresponsive, allowing easy

access to ear veins. It must be allowed that we have not found ketamine to be a particularly suitable anaesthetic in the pig for general surgical procedures but for this purpose it serves well. General anaesthesia is then induced with thiopentone (up to 10 mg/kg), endotracheal intubation is performed and IPPV with nitrous oxide/oxygen (FIO_2 0.3) instituted. Thereafter anaesthesia is maintained with nitrous oxide and oxygen supplemented by intermittent thiopentone as required. ECG and intramuscular temperature monitoring are established. Arterial and venous access for arterial and venous pressure and biochemical monitoring as well as drug and fluid administration are achieved by means of jugular vein and carotid artery canulation. If the time taken for establishment of the planned experimental protocol or for achieving steady state conditions is prolonged, in order to avoid the MH attenuating effects of large doses of thiopentone (vide infra) its use is discontinued to be replaced by continuous infusion of etomidate (0.5–1.0 mg/kg/minute). At the conclusion of the experimental procedure the endotracheal tube is left in situ with the pig breathing spontaneously until it is conscious once more.

THE PHARMACOLOGICAL TRIGGERING, ATTENUATION, BLOCKING AND REVERSAL OF PMH

Anaesthetics

Triggering of anaesthetic induced MH in susceptible swine is a property displayed by potent volatile anaesthetics only. Intravenous anaesthetics not only fail to initiate the syndrome but in many instances modify, even block, its onset in response to the active volatile agents.

Although the volatile agents most active in the MHS pig include the halogenated alkanes and ethers, no correlation of their activity as triggers of MH is evident with any physical characteristic—solubilities, various partition coefficients or MAC values. Prompt initiation of the syndrome in susceptible swine follows their exposure to halothane, chloroform, enflurane (10), isoflurane (G.G. Harrison, unpublished), and sevoflurane (71). Fluoroxene and cyclopropane have been documented as weak and inconsistent triggers (10). However, not all of the conventional

inhalational anaesthetics have been shown to act as triggers of MH in susceptible swine. Strangely, methoxyflurane (MOF) as well as trichloroethylene, diethyl ether and nitrous oxide have failed to initiate the syndrome in exposed animals. Each of these, particularly MOF, has been documented as triggering MH in man (72).

Triggering of MH in swine has not been ascribed to any of the conventional intravenous anaesthetic agents, including ketamine. On the contrary, one, the steroid, althesin (73), administered as a continuous infusion, blocks initiation of the syndrome in response to halothane alone, while another, thiopentone, slows and attenuates its onset (18,21). In neither circumstance is the trigger action of succinylcholine affected. Surprisingly, the water soluble analogue of althesin, minaxolone (74) has no similar attenuating effect.

Somewhat out of line with other intravenous agents is etomidate (75) which, a recent report alleges, enhances speed of onset and progression of the halothane induced syndrome though itself is not a trigger.

Muscle Relaxants

Of the relaxant drugs tested in MHS swine, it is the depolarizing drugs only--succinylcholine and decamethonium--that have been shown to trigger MH. In the most sensitive MHS strains, those that respond with MH to halothane alone, succinylcholine alone is also a potent trigger though two pulse doses may be necessary (23). In the less sensitive strains that fail to respond with MH to halothane alone, succinylcholine acts as the specific additive stressor to trigger MH though alone it, too, fails to fire the syndrome (10). In these circumstances triggering of the syndrome appears to depend on the widespread endplate depolarization with resultant induced muscle contraction, because if this is blocked by preliminary administration of a non-depolarizing relaxant the syndrome fails to develop (76).

A corollary of this action of succinylcholine would appear to be the triggering of the syndrome in sensitive strains by excitement and exercise alone, as is also the exercise-mediated reduction in response time and increase in the severity of the syndrome in response to halothane (16,28).

Of the non-depolarizing muscle relaxants, none have been documented as triggers of MH in swine with the possible exception of pancuronium. There is one report of the apparent precipitation of MH by this drug which is invalidated however by the concomitant use of halothane (77). Further it has not been confirmed by other workers (10).

Atracurium, the non-depolarizing relaxant that differs markedly from others in its mode of biodegradation, has also now been screened in MHS swine (78,79) and shown to lack any MH triggering potential.

In contrast to their blocking effects on succinylcholine-induced MH, non-depolarizing relaxants do not prevent initiation of the syndrome by the potent volatile anaesthetics. Some do attenuate or delay onset of the syndrome. In particular, attenuation of halothane-induced MH by pancuronium (21,80) has led to its being recommended as the muscle relaxant of choice in both MHS animals and man (81).

Attenuation, to the extent of prevention, has been claimed for the pre-administration of metocurine (82) though examination of the documented results validates attenuation only.

Catecholamines

Induction of MH by stress, fright and severe exercise immediately calls in question the part played by catecholamine response. The answers are still far from clear cut. Early on the Bristol group demonstrated that increased levels of circulating catecholamines accompanied induction of MH in swine (83). Thereafter they reported initiation of the syndrome in response to the infusion of alpha adrenergic agonists (84) and the converse, the prevention of MH in susceptible animals by alpha adrenergic blockade (85). Williams and co-workers (29,31) have consistently and vigorously proposed circulating norepinephrine as the key

triggering agent, yet the Bristol group could induce MH in susceptible Pietrains with infusion of epinephrine only in the presence of beta adrenergic blockade (84).

At the same time there is incontrovertable evidence that the biochemical markers and muscle rigor of MH can be induced in isolated perfused muscle (86) and "in vitro" in muscle strips (87) in the absence of any catecholamine intervention. This circumstance was nicely illustrated by Gronert's et al (88) demonstration "in vivo" that total sympathetic denervation failed to block initiation of the syndrome with no accompanying rise in catecholamines and the converse that dantrolene blocked initiation of MH in response to a stressor but not the accompanying rise in the circulating catecholamines.

While it is most likely that the surge of catecholamines that accompanies initiation of MH is a secondary phenomenon which plays an amplifying role, a primary triggering role for the catecholamines is possible if their effects on skeletal muscle (89)--facilitation of prejunctional acetylcholine release, amplification of glycogenolysis energy transduction and 3/5 cyclic AMP mediated change in the rate of SR Ca^{++} uptake--are considered in the light of present concepts of pathogenesis (vide infra).

Calcium Channel Blocking Drugs

Of the many factors involved in the pathogenesis of MH, evidence of sarcolemmal dysfunction involving halothane-induced depolarization (90) and increased calcium permeability (91-93) has led to the postulate that calcium channel blocking drugs might be effective therapeutically or at least prevent or attenuate initiation of the syndrome by halothane.

Indeed, in the case of verapamil (92) and diltiazem (94) some in vitro evidence does appear to support this hypothesis. However, later studies in vivo in MHS swine failed to show therapeutic effect for any calcium channel blocking drugs. Gallant et al (95) clearly demonstrated that verapamil has no therapeutic or prophylactic effect. These observations were supported by a study in this author's laboratory (96) which showed that in

addition to verapamil, other calcium channel blocking drugs—nifedipine, diltiazem, nisoldipine and flunarazine—also lacked any therapeutic effect on the established MH syndrome.

However, in the latter study, nifedipine and diltiazem were shown to manifest the property of blocking and attenuating, respectively, halothane initiation of the syndrome. While diltiazem merely delayed the time of onset of MH in response to halothane, in the case of nifedipine attenuation was of an order that prevented onset of the syndrome in response to halothane for the planned sixty minute period of exposure in 36% of animals and markedly delayed the onset in the remainder.

These effects were achieved only with doses far in excess of the human therapeutic dose.

Negative Responses of Note

Besides drugs that trigger MH in the MHS pig model, it is relevant to comment on some that unexpectedly do not. Such observations bring in question past strictures placed on the use in the MH syndrome or MHS patients of certain drugs—strictures based originally on anecdotal clinical association ("personal communication" and "unpublished" data) backed by theoretical considerations of the effects of such drugs on membrane Ca^{++} fluxes.

An important example of such negative response is that now reported to amide-linked local anaesthetic drugs. The use of this group of drugs, perhaps the most widely used local anaesthetic agents in clinical practice and in many hospitals the only local anaesthetic agents available, has long been regarded as contra-indicated in the MHS patient. Such strictures are now called in question by the demonstration by two groups of workers (97,98) that the intravenous infusion into the MHS pig of lidocaine and bupivacaine, up to monitored toxic levels failed to trigger the MH syndrome. This negative response indicates that this important group of drugs, in conventional doses, is safe to use in the MHS patient.

Digitalis, equally long claimed as deleterious in the MH syndrome and contra-indicated in the MHS patient, is another such drug. Gronert et al (99) have screened digitalis in the MHS pig model supported by cardiopulmonary bypass to obviate its cardiotoxic effects. In this model digitalis infused to the supratoxic levels of 61 ng/ml failed to stimulate any increase in whole body metabolism or by inference that of skeletal muscle. In the same preparation calcium chloride too, infused to serum levels in excess of 15 mEq/L, similarly failed to trigger the syndrome.

Blocking and Reversal

In considering specific pharmacological reversal of the established syndrome in the MHS pig—the bottom line in terms of human therapy—two drugs only, procaine and dantrolene warrant consideration. The action of each in these circumstances supports Kalow's original broad hypothesis (65) of the central role of increased myoplasmic Ca^{++} ion flux in the genesis of MH.

Procaine, the classical inhibitor "in vitro" of caffeine induced muscle contracture, was early shown "in vivo" to block initiation of MH in the MHS pig by halothane (76). For this effect large doses were necessary. Along with all the other drugs to which attention has been drawn for demonstrably attenuating onset of the syndrome in response to halothane, procaine also failed to prevent triggering of MH by the administration of succinylcholine additional to halothane though it did modify this reaction. If administered in large doses early in the progress of the established syndrome, procaine has been documented as terminating it (100), an action supported by similar "in vitro" action on halothane induced muscle contracture (87). In spite of the disappointing inconsistency of this reaction documented in the MHS pig (101,102) and the fact that large doses with grave cardiac negative inotropic effects were required, procaine did acquire a history of successful therapeutic use in MH in humans (61).

Dantrolene, a drug which reduces the rate and total amount of SR Ca^{++} release on appropriate stimulus (103) is the only drug to have demonstrated the property of blocking both halothane and halothane/succinylcholine induced MH "in vivo" in the MHS pig (17).

Moreover it is the only drug to date to reverse the established MH syndrome consistently, even if administered fairly late in its progress. Like the "turn-on" quality of the triggering mechanism of MH, dantrolene's action on the established syndrome and all its biochemical accompaniments "in vivo" has all the "turn-off" attributes of a switch (104-107).

PATHOGENESIS—THE VICIOUS CYCLE

The physical and biochemical events that characterize MH are witness to the induction of a profound hypercatabolic -state in skeletal muscle which continues inexorably until total substrate depletion (10).

The fundamental lesion responsible is functional and in its simplest terms cannot be better described than it was in 1970 by Kalow et al (65), "...an inborn error in skeletal muscle which renders the muscle susceptible to disturbance in intracellular calcium distribution". In short, control of the myoplasmic flux of excitation-contraction coupling Ca^{++} is abruptly lost, resulting in the cytosolic $[Ca^{++}]$ being sustained at contracture producing levels. Drugs such as caffeine which serve to raise myoplasmic $[Ca^{++}]$ enhance this process, while those such as dantrolene and procaine which serve to lower cytosolic $[Ca^{++}]$ counteract the rigor and lead to survival.

Brief consideration of the role of calcium in muscle excitation/contraction coupling and energy transduction (108) indicates that membrane dysfunction, genetic or drug induced, at any or all three sites—the sarcolemma, the SR or the mitochondria—could cause the postulated rise in myoplasmic Ca^{++}. The extensive investigations that have been undertaken into these functional domains, together with the insight they give into the pathophysiology of malignant hyperthermia are discussed elsewhere in this volume in chapters by Britt (Chapter 2), Cheah (Chapter 3) and Nelson (Chapter 4).

However, to complete the picture here of PMH, PSS and its reultant PSEP and perhaps to distinguish it from capture myopathy, it remains necessary to summarize briefly the MH cascade to

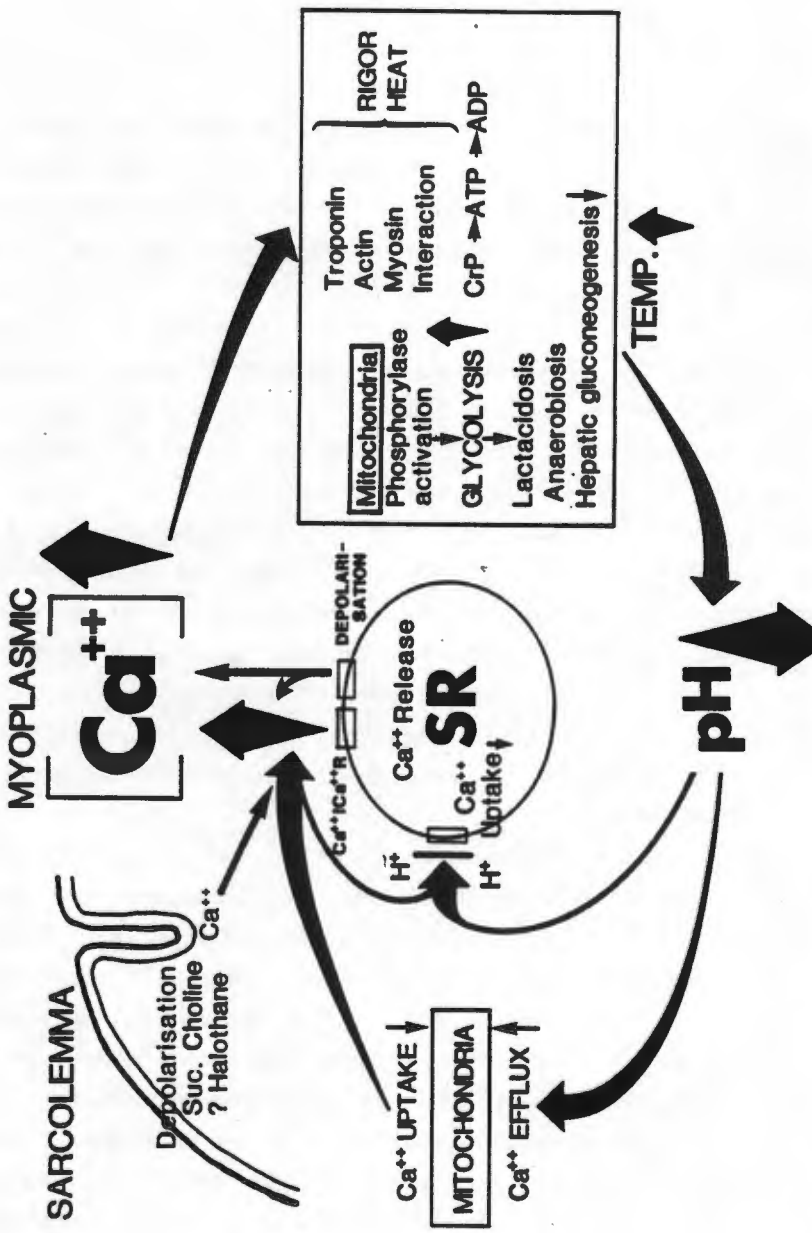

FIGURE 1. The intracellular events of the vicious cycle of MH. SR = sarcoplasmic reticulum; $Ca^{++}ICa^{++}R$ = calcium induced calcium release; H^+ = acid inhibition of SR calcium uptake

irreversibility and, in particular, the recent work which brings us a little closer to an understanding of the trigger mechanism (Figure 1).

Though it has long been established that physiological contraction of skeletal muscle is elicited by Ca^{++} released from the SR and the molecular mechanisms of how the released Ca^{++} triggers off the contractile reaction--the troponin/myosin/actin interaction--have been fairly well worked out, the mechanism by which calcium is released from the SR is the least understood part of the entire series of events through which spread of an action potential in a muscle cell leads to contraction (109).

Following Bianchi's proposal that the coupling of T tube depolarization to SR Ca^{++} release was by means of "trigger calcium" possibly released from sarcolemmal sites, Endo (109) and Ford and Podalsky (110) independently identified a mechanism of Ca^{++} induced SR Ca^{++} release. This mechanism was demonstrated in both skeletal and cardiac muscle. As the threshold cytosolic $[Ca^{++}]$ for contraction in skeletal muscle was 6-9 x 10^{-7}M peaking to 10^{-5} M at peak twitch while the threshold of Ca^{++} induced Ca^{++} release was 10^{-4}M, they concluded that the mechanism did not play a primary role in normal physiological contraction of skeletal muscle and possibly constituted an independent calcium channel. However, the mechanism did appear to be relevant in the excitation/contraction coupling of cardiac muscle.

Consideration of the factors which affect Ca^{++} induced Ca^{++} release suggested a strong relevance for this mechanism in the genesis of MH. Of the physiological variables, Mg^{++} was paramount--Ca^{++} induced Ca^{++} release being inversely and its threshold positively correlated (111). The action of caffeine-enhancement of twitch response progressing with increasing concentration to contracture--as well as halothane-enhancement of muscle twitch--were both shown to result from an enhancement of the Ca^{++} induced Ca^{++} release phenomenon. On the other hand inhibition of Ca^{++} induced Ca^{++} release with a rise in its threshold was shown to be the basis of the classical caffeine inhibitory reactions of procaine and tetracaine.

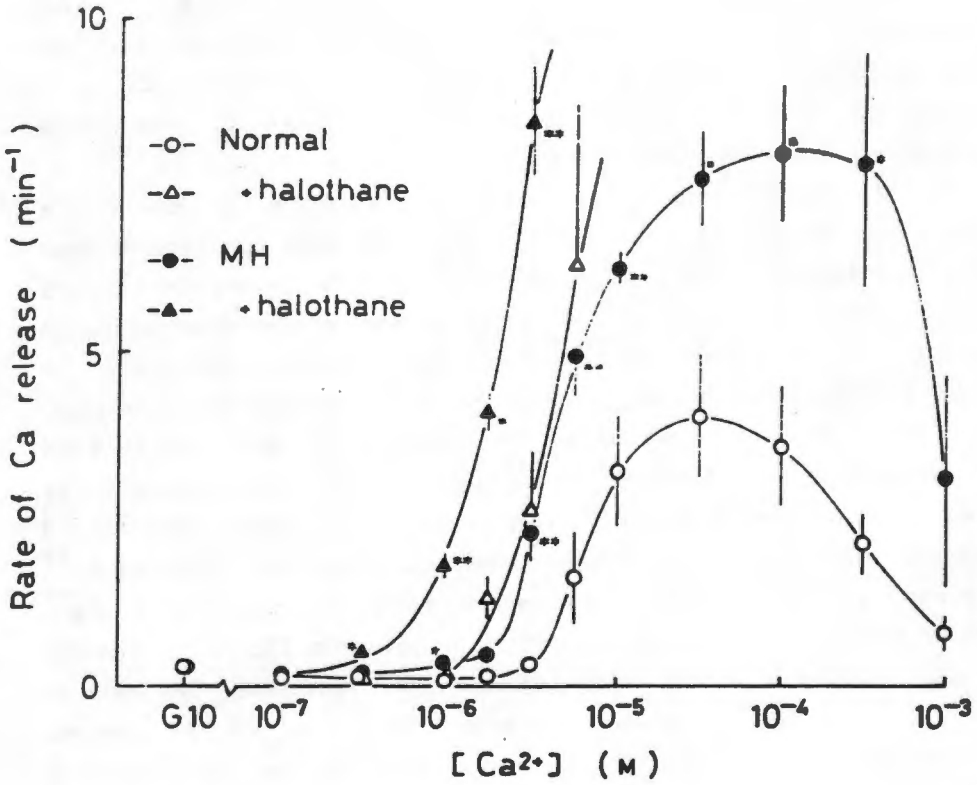

FIGURE 2. Dependence of Ca^{++} release from SR on free [Ca^{++}] in normal (open symbols) and MH (closed symbols) fibres at 37°C.
Without halothane: circles (n=4–11)
With 0.01% halothane (v/v) applied during Ca^{++} induced Ca^{++} release (n=22–5)
Mean and SE shown.
***P 0.05**
(From Endo et al., reference 112, with permission).

Endo's group have now reported from studies of human MH muscle (112) corroborative evidence of the involvement of Ca^{++} induced Ca^{++} release in the genesis of MH. They have demonstrated that muscle of a human MH patient displayed a lower threshold for Ca^{++} induced Ca^{++} release than normal and in addition, a greater

maximum rate of Ca^{++} release at optimum $[Ca^{++}]$. Confirmatory findings from studies in MHS pig muscle are reported by Do Han Kim and co-workers (113).

The higher sensitivity of the Ca^{++} induced Ca^{++} release mechanism of MH fibres compared with normal, together with the greater maximum rate of Ca^{++} release at optimum $[Ca^{++}]$ is illustrated in the Figure from Endo's report (Figure 2). Halothane was shown to enhance this effect.

Of great interest in explaining many of the vagaries of the triggering of MH by various stressors as well as factors that provide attenuation of the response, are the results of Endo's calculation of simultaneous SR Ca^{++} uptake and release rates in relation to cytosolic $[Ca^{++}]$. These showed that even in circumstances of moderately enhanced Ca^{++} induced Ca^{++} release, such as in normal fibres under the influence of halothane or even MH fibres without halothane, Ca^{++} uptake rates were substantially greater than release rates. This ensured that even when $[Ca^{++}]$ was increased by release through channels other than those of Ca^{++} induced Ca^{++} release, eg. by depolarization, the rate of SR Ca^{++} uptake was still adequate to return myoplasmic $[Ca^{++}]$ to resting levels. Whereas in MH fibres influenced by halothane, the rate of Ca^{++} induced Ca^{++} release exceeded the rate of SR uptake throughout most of the $[Ca^{++}]$ range so that net Ca^{++} release proceeded spontaneously to levels of $[Ca^{++}]$ that produced contracture. Further, in circumstances of enhanced Ca^{++} induced Ca^{++} release it is not difficult to imagine circumstances in which the balance between rates of uptake and release are finely balanced, even unstable, so that any further increase in $[Ca^{++}]$ produced by other factors such as succinylcholine induced depolarization or even severe exercise, could provide the positive feedback loop to tip the balance to contracture (Figure 3).

The concept that the primary genetic abnormality which characterizes susceptibility to MH lies in the domain of SR Ca^{++} release mechanisms, fits well with the observed action of dantrolene viz that it depresses both the rate and total amount of SR Ca^{++} released in response to a stimulus yet has no effect on SR Ca^{++} uptake (114). The prompt termination of the established syndrome

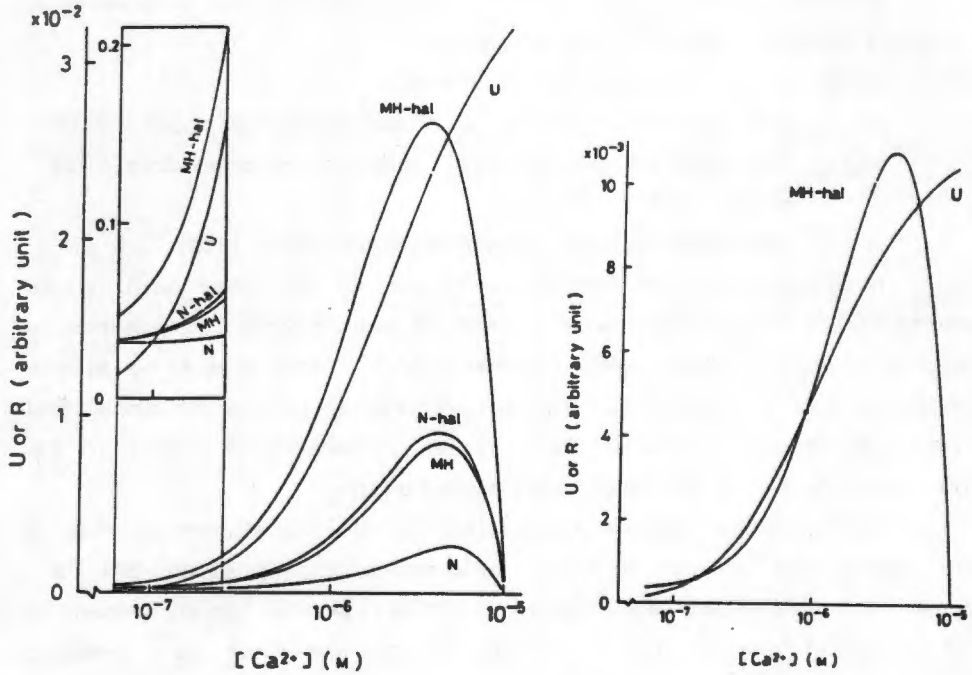

FIGURE 3. Left – dependence of rate of Ca^{++} uptake, U, and that of Ca^{++} release, R, on free [Ca^{++}] in a model system. (See Ref. 109 for calculations.)
Right – demonstration of possible existence in MH muscle under certain circumstances of three crossover points between Ca^{++} uptake rate and Ca^{++} release rate which may explain succinylcholine induced aggravation of MH.
Lowest and highest points are stable. Middle point represents unstable state, from which departure of [Ca^{++}] in either direction will proceed further until stable point is reached. An increased [Ca^{++}] concentration will result in sustained contracture.
(From Endo et al., reference 112, with permission).

that follows administration of this drug implies continuing SR Ca^{++} release as the key factor in its genesis. Once terminated, the function of other Ca^{++} modulating mechanisms is adequate to restore myoplasmic [Ca^{++}] to resting levels, permitting muscle relaxation and termination of the syndrome.

In addition to this primary mechanism other factors of note that serve to swell the cascade to irreversibility are (Figure 1):

1. Acid inhibition of SR Ca^{++} transport (115), an event enhanced by halothane (116);
2. Thermal inactivation of Ca^{++}/actomyosin control mechanisms, an event enhanced by ATP depletion (117);
3. Abnormalities manifest by MHS swine:
   a) reduced mitochondrial Ca^{++} accumulating ability (118);
   b) increased anaerobically induced mitochondrial Ca^{++} efflux (119);
   c) decreased hepatic gluconeogenesis rate (120).

In these terms the variations of the MH syndrome both in the sensitivity of triggering and the rate of progression in response to genetic, environmental and pharmacological factors can be simply conceptualized as reflecting inherent variations in the threshold and magnitude of Ca^{++} induced Ca^{++} release phenomenon based in its turn possibly on an SR structural abnormality.

In slaughter animals susceptible to MH, post mortem rise in myoplasmic [Ca^{++}] due both to anaerobically depressed SR Ca^{++} uptake and increased mitochondrial efflux (greater than normal in MHS animals) exceeds the threshold of the abnormal Ca^{++} induced Ca^{++} release mechanism which then provides the milieu for the excessive and rapid glycolysis and lactacidosis essential for the production of PSEP.

Attention has been drawn to the superficial resemblance capture myopathy bears to this porcine stress myopathy. It was noted, however, that while an end stage vicious cycle was apparent, it could be interrupted by simple control of the acidosis alone. Such observation would not support the presence in this case of an abnormal Ca^{++} iduced Ca^{++} release mechanism. Acid inactivation of SR Ca^{++} transport in response to the severe lactacidosis of excessive muscular activity, together with the effects of raised circulating catecholamine levels, appears to provide an adequate explanation for the manifestations of this syndrome.

CONCLUSION

Since its original identification, elucidation of the pathogenesis of this frightening, yet fascinating, originally irreversible and fatal, hypercatabolic state that is Malignant Hyperthermia, has provided an ongoing stimulus for research by investigators from the whole field of biomedical sciences from the clinical anaesthetist to the membrane chemist, from the veterinary scientist to the geneticist.

The story recounted in this volume of the development and progression of knowledge and ideas in these various areas, from identification of muscle as the site of the lesion and abnormal SR Ca^{++} release mechanisms as a basis of the vicious cycle, to calcium flux depressant drugs as therapy, is an exciting history. Much indeed has been a gift of "pearls from swine".

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General Anaesthesia

Fifth Edition

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Malignant hyperthermia

Gaisford G. Harrison

Malignant hyperthermia (MH) is a covert pharmacogenetic myopathy which incommodes the sufferer little, if at all, in the normal course of events but, in response to exposure to general anaesthesia, manifests in the susceptible individual as a life-threatening syndrome of muscle hypercatabolism and rigor, accompanied by a rapid and inexorably progressive rise in body temperature to extremes of the order of 42°C. Untreated, this syndrome is invariably fatal. Its rapid progression to irreversibility, in spite of withdrawal of the original stimulus, characterizes this 'hyperthermic' syndrome as 'malignant'.

History

This condition was first recognized in 1960 in Australia by Denborough and Lovell (1960) (Denborough *et al.*, 1962), who described it as a fatal reaction to anaesthetics which showed the hereditary characteristic of autosomal dominant transmission. MH was recognized independently and contemporaneously in the USA by Locher, but the evidence was published only much later (Henschel and Locher, 1977). As a clinical entity the syndrome gained definite shape from descriptions of cases by Ruttle (1962), Saidman, Harvard and Eger (1964) and finally by several authors in the September 1966 issue of the *Canadian Anaesthetists' Society Journal* (Multiple authors, 1966).

Few, if any, cases of this syndrome, as here defined, were reported before 1960. Perhaps the explanation lies in the introduction into clinical practice in the late 1950s of suxamethonium (succinylcholine) and halothane, the two agents subsequently shown to be the most potent—almost specific—agents for triggering MH. Much of our knowledge is based on the chance discovery of the 'hot pig' (Harrison, 1979).

The possibility that strains of certain breeds of pig might serve as a valid animal model of MH was suggested by the observation, in litter mate pigs anaesthetized with halothane, of muscle rigor and hyperthermia in response to the injection of suxamethonium reported by Hall and co-workers (1966); thereafter, Harrison and co-workers (1968) reported the same reaction but in response to the administration of halothane alone.

This MH response in pigs, later identified with the porcine stress syndrome, was shown to be heritable, confined to selected strains of a few breeds (Landrace, Pietrain and Poland China) and to mirror the human reaction in every way. Syndromes clinically similar to the MH syndrome of swine and humans have been described rarely in other species such as the horse (Klein, 1975; Waldron-Mease and Rosenberg, 1979), dog (Short and Paddleford, 1973) and cat (De Jong, Heavner and Amory, 1974). In contradistinction to the porcine and human syndrome, genetic factors do not appear to play an obvious role in these biological curiosities.

Epidemiology

The MH sundrome, though rare, occurs world wide in most broad racial groups, its incidence displaying a 40-fold range from 1:5000 to 1:200000 depending demographically on the gene pool (*Table 1*). However, the natural history of the condition is such as to defeat all attempts at establishing the true incidence of MH susceptibility (MHS). Thus MH is not an 'every time' phenomenon—one-third to one-half of MHS patients give a history of previous reaction-free general anaesthesia (Halsall, Cain and Ellis, 1979; Kalow, Britt and Chan, 1979)—a circumstance that must lead to an underestimate of the true incidence, while gene isolation in small communities may produce the reverse.

Whilst no age group can be said to be exempt (Britt, 1985), the syndrome manifests most commonly in the second decade, seemingly coincidental with the period of major growth in skeletal muscle bulk. MH is exceptionally rare below the age of 3 and over the age of

TABLE 1 Incidence of malignant hyperthermia

Region	*Country and reference*	*Incidence/No. of anaesthetics*
North America	Canada and USA (Britt, 1985)	1/15 000–150 000
Pacific Basin	Japan (Kikuchi *et al.*, 1978)	1/7000–110 000
	New Zealand (Newson, 1972)	1/5000
Europe	UK (Ellis, 1981)	1/200 000
	Austria (Walter, 1986)	1/23 000
	Switzerland (Kalow *et al.*, 1979)	1/95 800
	Denmark (Ørding, 1985)	1/220 000

50 years. Although few cases of the MH syndrome proper have been reported in the newborn, a growing body of evidence points to some association between 'cot deaths' or the sudden infant death syndrome (SIDS) and susceptibility to MH. Recent retrospective studies have revealed susceptibility to MH in 30 per cent of the parents of victims of SIDS (Denborough, Galloway and Hopkinson, 1982; Ording, Ranklev and Fletcher, 1984; Peterson and Davis, 1986).

The clinical syndrome

During clinical anaesthesia onset of MH is presaged usually by a surge of sympathetic activity (*Figure 1*). This manifests clinically as the sudden appearance of unexplained tachycardia and possibly tachyarrhythmias. An accompanying cyanosis (in the presence of adequate pulmonary ventilation and inspired oxygen concentration greater than 0.3) reflects the immediately increased oxygen demand triggered by muscle hypercatabolism. This in turn results in a greatly increased carbon dioxide output which, monitored most effectively by capnography, provides the earliest evidence of this syndrome's onset (Baudendistel *et al.*, 1984; Dunn, Maltry and Eggers, 1985). Failing this, clinical evidence of the event would be hyperventilation in the spontaneously breathing patient going on to apnoea and an excessively hot soda-lime cannister.

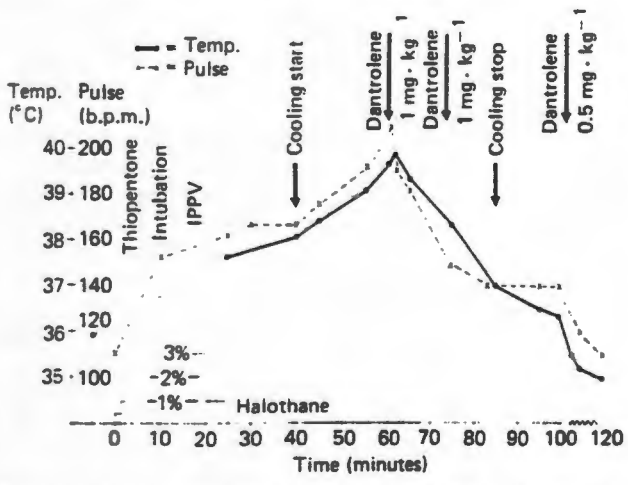


Figure 1 Events chart of an illustrative case of MH in a 9-year-old girl with congenital kyphoscoliosis, for insertion of Harrington rods. Note: onset of tachycardia; progression of syndrome despite discontinuance of halothane and institution of cooling, immediate response to dantrolene (delay in administration occasioned by its being obtained from neighbouring hospital). Operation successfully performed 6 weeks later. Prophylactic intravenous dantrolene was given with induction. (From Harrison and Chapman (1982), with permission.)

Muscle hypertonus or rigor appears in a majority of cases (over 80 per cent). This is preceded—if the drug has been used—by an exaggerated myotonic response to suxamethonium. In these circumstances the myotonic response presents characteristically as masseter muscle spasm (MMS) which may cause difficulty with tracheal intubation. Of itself, MMS is now recognized as reliable evidence of the presence of MHS. Onset of the syndrome can be prevented or aborted at this point by discontinuation of the anaesthetic or change to an MH safe technique (*see* later). Of all patients who manifest MMS, 60–70 per cent have been shown to be MHS on subsequent diagnostic screening (Ellis and Halsall, 1984; Flewellyn and Nelson, 1984a; Rosenberg and Fletcher, 1986).

Muscle rigor, though a cardinal component of the syndrome, may rarely be absent—the so-called 'non-rigid' variety of MH. As muscle rigor and fever both result from the same underlying mechanism, it is probable that this 'non-rigid' variety of MH represents a variation in the chronological presentation of the syndrome, rather than in the basic disease process as proposed by Kalow, Britt and Chan (1979).

Pari passu the patient develops a progressive, often fulminant, pyrexia. Rates of core temperature rise of 1°C every 5–10 minutes are not unusual (Steward, 1979; Ellis, 1981). Once established, this reaction soon cascades into a vicious cycle and continues independently of the action of the triggering agent (Berman *et al.*, 1970). Untreated, the patient's temperature rises inexorably until death supervenes at the extreme of 41°C and more. Paradoxically, death from MH may occur at normothermia, even mild hypothermia. When triggering of the syndrome has been delayed (Beldavs *et al.*, 1971; Murphy *et al.*, 1984) the patient may well have cooled during anaesthesia to 34°C or lower before the syndrome has fired. Thereafter death may follow cardiac arrhythmia due to the catecholamine surge and/or hyperkalaemia which accompany the syndrome, before the temperature has risen above normal level. Likewise, the onset of the syndrome may be masked by induced hypothermia; for example, during cardiopulmonary bypass (MacGillivray *et al.*, 1986).

Biochemical accompaniments

The underlying muscle hypercatabolism and its effects are reflected in the conventional indices of acid–base,

blood gas, electrolyte and substrate status. The changes recorded are characterized by their rapid progression, preceding and reaching extreme values ahead of the rise in core temperature (Berman *et al.*, 1970).

Profound metabolic acidosis—lactic acidosis—with concomitant rise in P_{CO_2} from its buffering, is the hallmark of the syndrome. A fall in arterial P_{O_2} usually accompanies this acidosis. This fall need not be great, but, because of excessive muscle oxygen utilization, reduction in venous P_{O_2} is disproportionately great, leading to the prominence of cyanosis as a clinical sign.

Elevations are recorded in the serum levels of all electrolytes, total protein, glucose and lactate. These indicate glycogenolysis, glycolysis, a shift of water into the cells and a leakage of sodium, potassium, calcium, magnesium and inorganic phosphorus (Berman and Kench, 1973; Verburg *et al.*, 1984). Of all the above, gross acidosis and hyperkalaemia are of the greatest clinical import.

As the syndrome progresses, increasing permeability of the sarcolemma becomes manifest by rapid rise in serum levels of the muscle enzymes creatine phosphokinase (CPK) and lactic dehydrogenase (LDH) as well as alanine transaminase (ALT) and aspartate transaminase (AST). The rise in serum levels of CPK particularly—often tenfold at 24 hours—is considered diagnostic (Denborough, 1979).

An ominous consequence of the progressive intrinsic muscle damage and sarcolemma permeability is the release into the circulation of myoglobin and tissue thromboplastins. The myoglobinuria, renal failure and disseminated intravascular coagulopathy (DIC) which may follow, worsen the prognosis immeasurably (Gronert, 1980).

Differential diagnosis

With the onset of MH, the rate of progression of alarming signs and pyrexia are usually of such a dramatic nature that there is little else with which the syndrome can be confused. However, some thought should be given to possible alternative diagnoses.

In small children, in particular, the possibility of a response to an adverse heat environment should be considered. This could be related to operating room temperature and humidity, a faulty humidifier in the anaesthetic circuit or a warming blanket. Other possible pyrexial reactions to consider are those associated with surgically induced bacteraemia or septicaemia, the infusion of pyrogens or reaction to blood transfusion (Crider, Nightingale and Hill, 1986). Thyroid storm (crisis) is another possible differential diagnosis.

Clinically the late stage of the neuroleptic malignant syndrome (NMS)—a hyperpyrexic syndrome which follows overdosage with antipsychotic neuroleptic drugs—virtually identifies with MH and responds to the same treatment. However, the patient's history and circumstances surrounding its onset will distinguish it (Guze and Baxter, 1985).

MH triggering agents

There are three prerequisites for the precipitation of an MH crisis:

1. Possession of the MH gene.
2. Exposure of the susceptible individual to a specific trigger agent for an adequate time.
3. A 'qualifying' factor which influences the sensitivity of the patient trigger.

The circumstances which enhance sensitivity of the patient trigger increase systemic catecholamine levels. Such circumstances are preanaesthetic anxiety and stress (Gronert, Thompson and Onofrio, 1980; Fletcher *et al.*, 1981), heavy muscular exercise as in the injured sportsman (Kalow, Britt and Chan, 1979) and environmental heat stress (Ording, Hald and Sjontoft, 1985). By contrast, drugs which control or reduce such stress-related responses, depress the sensitivity of the MHS patient trigger and attenuate the onset of the syndrome.

MH can be triggered in susceptible patients by all volatile anaesthetic agents (Britt and Kalow, 1970). The most potent are the newer halogenated agents—halothane, methoxyflurane, enflurane, isoflurane and sevoflurane. Fluroxene, cyclopropane and ether have been documented as only weak and inconsistent triggering agents (Harrison, 1987).

Although nitrous oxide has been implicated rarely in episodes of MH in humans (Ellis, Clarke and Appleyard, 1974; Waite, Ballard and Yonfa, 1985), its identification as an MH trigger must be questioned. Not only has it been used with impunity in countless MHS patients, but also it has failed to evoke positive response in MHS swine even when administered at hyperbaric levels (Gronert and Milde, 1981a).

None of the commonly used intravenous anaesthetic agents has been identified as a trigger of MH, although some reservations have been expressed in regard to the use of ketamine (Gronert, 1980) and etomidate (Suresh and Nelson, 1985).

Of the neuromuscular blocking agents, the depolarizing relaxant suxamethonium is virtually a specific trigger of MH in the susceptible patient, especially when administered in the presence of a potent volatile agent. However, a single dose of suxamethonium, of itself, will not trigger the syndrome. Repeat doses, usually administered misguidedly in response to MMS, and/or the presence of halothane are necessary for initiation of the syndrome (Harrison, 1981; Ellis and Halsall, 1984). In the event of an MMS response, suxamethonium must not be repeated (*see* 'The clinical syndrome', above).

Non-depolarizing relaxants do not trigger MH (Hall, Lucke and Lister, 1976; Morrell and Harrison, 1986). The triggering of MH attributed to tubocurarine in two often-cited cases (Britt, Webb and Le Duc, 1974) is questionable on many grounds (Harrison, 1973; Gronert, 1980), particularly as no further cases have been reported. Non-depolarizing relaxants do not prevent initiation of the syndrome by the potent volatile anaesthetics, but attenuation or delay in onset of the syndrome has been attributed to pancuronium, which

some regard as the muscle relaxant of choice for the known MHS patient (Hall, Lucke and Lister, 1976; Gronert and Milde, 1981b). In spite of the association of both atropine (Pollock, 1973) and cholinergic agents (Gronert, Milde and Taylor, 1980) with the provocation of MH, untoward reactions have not been generally reported following standard reversal of non-depolarizing neuromuscular blockade in the MHS patient.

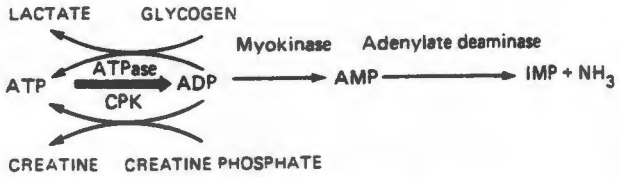
The identification of drugs with MH-triggering potential has depended —initially at least—on anecdote and often isolated 'personal communication' (Britt and Kalow, 1970). Considered in relation to the 'anxiety' factor and against the background of the polypharmacy inherent in clinical anaesthesia, the identification as triggering agents of drugs but rarely associated with MH must be questioned. The screening of drugs for MH-triggering potential in the MHS pig model has shown that human MHS patients react positively to all drugs identified positively in swine, although MHS swine do not react positively to all drugs implicated in the triggering of MH in humans (Harrison, 1987). MHS swine can show exquisite sensitivity of MH triggering that is not found in humans. When such animals fail to respond to a putative triggering agent, consistently and unequivocally, the circumstances of its original identification should be examined. On these grounds, strictures long applied to the use of amide-linked local anaesthetic drugs, digitalis and calcium gluconate in the MHS patient (Britt, 1983) now call for review (Wingard and Bobco, 1979; Harrison and Morrell, 1980; Gronert *et al.*, 1986).

Pathogenesis

The MH reaction is based on the induction of an abrupt and sustained rise in myoplasmic free calcium (Lopez, 1986; Lopez *et al.*, 1986), an event which initiates muscle contracture, activates glycolysis and mitochondrial activity, and sets in train a series of events which interact to form a vicious cycle. The lesion responsible is a genetic functional derangement in the excitation/contraction/coupling (ECC) mechanism distal to the neuromuscular junction (Gronert, 1980; Cheah and Cheah, 1985).

Heat production

Rise in myoplasmic free calcium above a threshold value activates the troponin/actin/myosin interaction leading to muscle contraction—soon to persist as contracture when free calcium elevation is sustained. Later, with rise in temperature, this reaction becomes progressively independent of free calcium, an event that may ultimately contribute to its irreversibility (Fuchs, 1975). Contracture is fuelled by powerful ATPase systems and hydrolysis of adenine nucleotides as follows:



No abnormalities have been identified in the contractile proteins themselves (Heffron, 1984; Walsh *et al.*, 1986).

Pari passu, rising cytosolic free calcium flash activates glycogen phosphorylase and massive glycolysis ensues, muscle glycogen being depleted stoichiometrically with the production of lactate which may rise 15- to 20-fold (Berman *et al.*, 1970; Berman and Kench, 1973). At an early stage, heat output may increase 15- to 17-fold (Williams, Houchins and Shanklin, 1975) to equal conditions of very severe exercise in a trained athlete. But, in contrast to the tenfold increase in O_2 uptake recorded by the athlete, the patient in MH records at this time only a threefold increase in O_2 uptake and consequently, due to predominantly anaerobic glycolysis, manifests a severe acid–base disturbance (Gronert and Theye, 1976).

Mitochondria have a high affinity for calcium but its sequestration is an energy-consuming, respiration-dependent process which replaces and takes primacy over that of phosphorylation (Stadhouers *et al.*, 1984). Exposed to this high calcium load, mitochondria of MHS muscle display only 60 per cent of the normal capacity to synthesize ATP (Ruitenbeek *et al.*, 1984). *Ab initio*, increased consumption of ATP exceeds mitochondrial capacity for its regeneration. Supported initially at the expense of creatine phosphate—resting levels of which are reduced in MH (Verburg *et al.*, 1984)—ATP levels soon decline rapidly (Berman and Kench, 1973). Once these are depleted, various membrane ion pumps (e.g. in mitochondria and sarcoplasmic reticulum) are deprived of substrate, membrane integrity fails and the biochemical conditions of rigor mortis ensue.

Excitation/contraction/coupling

ECC and its related energy transduction are an integral function of the sarcolemma, sarcoplasmic reticulum and the mitochondria (Sandow, 1965; Endo, 1977). In MHS muscle, defects have been identified at all points in this cycle (*Figure 2*). Most relevant to the MHS crisis are those of the sarcoplasmic reticulum, the organelle which plays the primary role in the regulation of myoplasmic free calcium.

Sarcoplasmic reticulum (SR)

Extensive investigations of SR calcium reuptake function in MH (Gronert, 1980) have not revealed convincing evidence for a mechanism primarily responsible for initiating the surge in myoplasmic free calcium—the

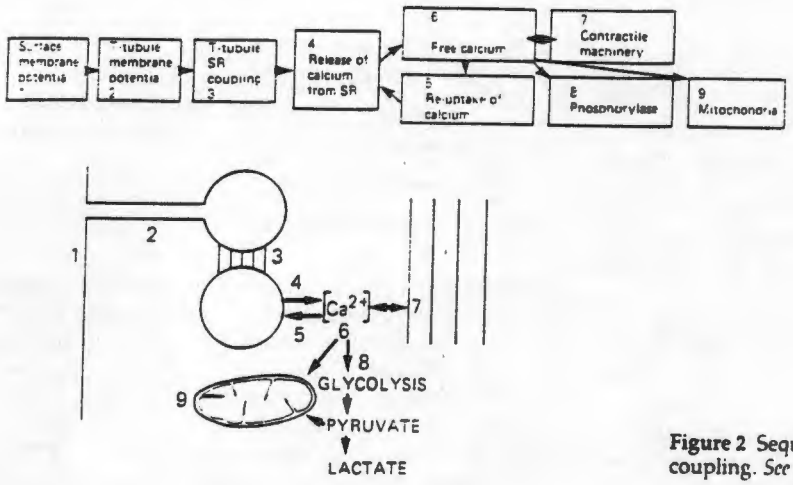


Figure 2 Sequential steps in skeletal muscle/excitation/contraction/coupling. *See* text for discussion.

MH triggering process. Now, advances in knowledge of the mechanisms of SR calcium release rather than uptake, and the modifications of these in MHS humans and swine, have provided a basis for a rational hypothesis of the pathogenesis of MH. This concerns the mechanism of calcium-induced calcium release (CaIR) from the SR, identified independently by Ford and Podolsky (1970) and Endo (1977). In his review of SR function, Endo (1977) proposed the hypothesis that an abnormality of this CaIR mechanism—low threshold and increased rate of calcium release—was the functional lesion responsible for MH. This hypothesis has now been effectively validated by the reported identification of low threshold, enhanced rate CaIR from SR of seven MHS patients (Endo *et al.*, 1983; Horiuti *et al.*, 1986), backed by similar findings in MHS swine and humans by Kim and co-workers (Kim *et al.*, 1984; Kim, Sreter and Ikemoto, 1986). In each case these effects were enhanced by exposure of the SR to halothane. The results of the calculations of Endo *et al.* of simultaneous SR calcium uptake and release rates in relation to cytosolic free calcium (Endo *et al.*, 1983), which reveal circumstances in which they may be finely balanced, even unstable, are of great interest in explaining many of the vagaries of the triggering of MH.

Paradoxically, a phenomenon of normal (rather than MHS) SR calcium uptake may play a contributory role in the establishment of the MH vicious cycle. This is the phenomenon of proton or acid inactivation of SR calcium transport reported by Berman, McIntosh and Kench (1977), which the early fall in myoplasmic pH would soon invoke. Additionally, the pH threshold of this reaction is raised in the presence of halothane (Diamond and Berman, 1980).

Sarcolemma

Abnormalities that have been identified in the SL function of MHS muscle manifest as halothane-induced, increased permeability to calcium (Moulds and Denborough, 1974; Gruener and Blanck, 1980; Stadhouers *et al.*, 1984). Exposure to halothane induces a 5–15 mV depolarization of sarcolemma which is both preventable and reversible by dantrolene (Gallant, Godt and Gronert, 1979). Whilst this degree of sarcolemmal depolarization and increase in calcium permeability would not be sufficient of itself to initiate contraction in normal muscle, it could well do so in the presence of the low threshold CaIR of MHS muscle (Okumura, Crocker and Denborough, 1980).

Other membranes

The functional abnormalities in mitochondrial, SR and sarcolemmal membranes suggest the possibility of a generalized membrane defect in MHS subjects. The increased erythrocyte osmotic fragility of MHS swine (Harrison and Verburg, 1973; Heffron and Mitchell, 1981; O'Brien *et al.*, 1985), backed by findings of electron spin resonance spectroscopy which indicate abnormalities in the bilayer organization of MHS erythrocyte ghosts (Louis, 1986), as well as abnormalities in bone calcium storage (Britt, Harrison and MacNeil, 1979) and increased insulin response to glucose loads—a calcium-mediated response (Denborough *et al.*, 1974)—are all claimed as evidence of such an underlying generalized membrane defect, but final proof is still lacking.

Other enzymes

The activities of several muscle enzymes, other than those already considered, have been examined in MH reactors (Gronert, 1986). In general, initial positive findings have been shown by subsequent investigation to lack specificity.

The role of the sympathetic nervous system

The induction of MH in susceptible swine by stress or severe exercise alone, a circumstance very occasionally

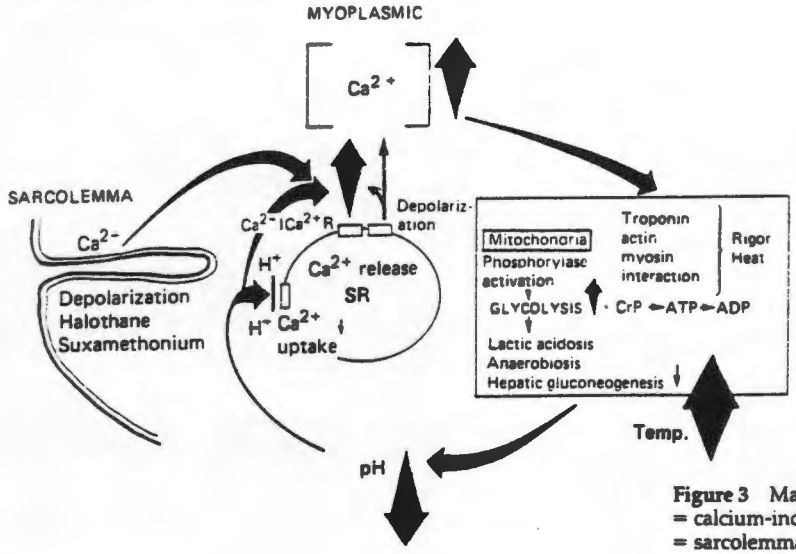


Figure 3 Malignant hyperthermia—the vicious cycle. $Ca^{2+}ICa^{2+}R$ = calcium-induced calcium release; CrP = creatine phosphate; SR = sarcolemma.

mirrored in the human (Gronert, Thompson and Onofrio, 1980; Wingard, 1981), and the prominence of tachycardia and other sympathetic phenomena as the first clinical signs of the syndrome's onset, all point to a major role for the sympathetic nervous system and endogenous catecholamine secretion in MH. Indeed, surging increases in circulating catecholamine levels have been shown to accompany the syndrome's onset (Lister, Hall and Lucke, 1974; Gronert and Theye, 1976). Although a primary role has been proposed for this catecholamine surge (Lucke *et al.*, 1978; Williams *et al.*, 1985), the bulk of evidence favours its being a secondary phenomenon (Gronert, Milde and Theye, 1977; Harrison, 1979; Gronert, Milde and Taylor, 1980) which plays an important amplifying role in the course of this syndrome (Bowman and Nott, 1969).

In conclusion, the pathogenesis of MH can be summarized best (*Figure 3*) as a vicious cycle based on derangements in ECC mechanisms, of which the sudden induction of a sustained rise in myoplasmic free calcium is the principle.

Treatment

If onset of the syndrome is diagnosed early in its course, before its vicious cycle is irreversibly established (i.e. up to 10 or so minutes from onset), mere discontinuance of the triggering anaesthetic may be sufficient to abort the syndrome. Failing this, the therapeutic success of specific measures is ensured by their early application.

Treatment consists of (1) specific therapy to arrest the primary dysfunction (continued SR free calcium release), and (2) supportive therapy to treat the secondarily induced phenomena. The complexities and urgency of the treatment protocol are such as to require the services of more than one person. The anaesthetist will need assistance.

Specific therapy

The advent of dantrolene sodium, a hydantoin, has revolutionized the treatment and prognosis of MH, and in practice its use has completely superseded that of agents previously used such as procaine, procainamide and steroids. Synthesized originally by Snyder and associates (1967) for use in spastic muscle states, Ellis's conclusion that dantrolene's muscle relaxant properties stemmed from a depressive action on ECC (Ellis and Bryant, 1972; Ellis and Carpenter, 1972), motivated its successful therapeutic trial in porcine (Harrison, 1975, 1977; Gronert, Milde and Theye, 1976) and later human MH (Kolb, Horne and Martz, 1982).

Dantrolene binds to and acts on the SR and the T-tubular membrane (Figure 2, sites 4 and 3), reducing the rate and amount of calcium release from the former and altering the T-tubular/SR coupling 'charge movements' of the latter (Morgan and Bryant, 1977; Danko *et al.*, 1985; Harrison, 1988). As triggering agents switch MH on, so cantrolene, quite literally, switches it off (*Figures 4* and *1*).

For clinical use dantrolene is presented as an orange powder (together with sodium hydroxide and mannitol) in vials of 20 mg which require to be dissolved in 60 ml of diluent, preferably sterile water. The resulting solution, pH 9–10, is irritant to veins and should be injected into a fast-running intravenous infusion or large vein. Effective therapeutic levels of dantrolene follow the administration of $2.4\,mg \cdot kg^{-1}$ (Flewellyn *et al.*, 1983). If necessary, this dose may be repeated at 15-minute intervals, until relaxation of muscle rigor, control of tachycardia and arrhythmia, and cessation of temperature rise are achieved or a total dose of $10\,mg \cdot kg^{-1}$ has been given. It is seldom that a dose exceeding $4\,mg \cdot kg^{-1}$ is required.

Although patients may complain of muscle weakness (even up to 48 hours after administration), this will not

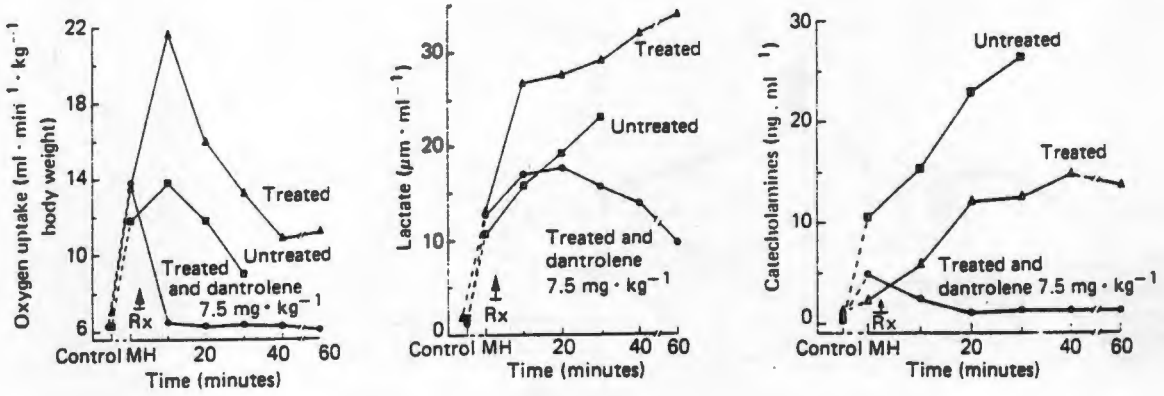


Figure 4 Effects of dantrolene administration (Rx) on oxygen uptake, lactate production and catecholamine secretion of MHS swine provoked into malignant hyperthermia by anaesthesia with halothane. ■ untreated; ▲ supportive treatment only; ● supportive treatment and dantrolene. (From Gronert, Milde and Theye (1976), with permission.)

be of an order which imperils respiratory ventilatory capacity or coughing, as dantrolene does not block SR calcium release completely and its effects plateau short of muscle paralysis.

Conveniently, dantrolene has no adverse cardiovascular effects (Ellis *et al.*, 1975; Gallant *et al.*, 1985). Metabolized by the liver, its subsequent renal excretion results in orange urine, an event which might cause some alarm if not anticipated.

Once controlled, the syndrome may recur in the postoperative period. Dantrolene administration should be repeated immediately at the first sign of such occurrence. In view of this possibility, some advocate a repeat prophylactic dose of dantrolene ($2.5\,mg \cdot kg^{-1}$) after 10–12 hours ($T_{½\beta}$ 12 hours).

Neither hepatic dysfunction nor any other of the rare complications of prolonged high dosage oral administration of dantrolene follows its acute short-term use (Flewellyn *et al.*, 1983; Britt, 1984).

A therapeutic role has also been suggested for the conventional calcium channel blocking agents (Bikhazi, Thomas and Foldes, 1979). Despite some *'in vitro'* support for this suggestion (Iwatsuki, Koga and Amaha, 1983; Ilias *et al.*, 1985), *'in vivo'* these agents have been devoid of therapeutic effect (McGrath, Lee and Remple, 1984; Gallant *et al.*, 1985; Harrison, Wright and Morrell, 1986), although nifedipine pretreatment did attenuate halothane induction of MH in susceptible swine.

Finally, in terms of specific therapy, should dantrolene be unavailable—and only in this event—the use of procaine ($30–40\,mg \cdot kg^{-1}$ i.v.) or procainamide should be considered, bearing in mind that this may result in a consequential need for cardiac inotropic support (Harrison, 1971).

Supportive therapy

It must be emphasized that after timely administration of dantrolene, following early diagnosis, little or no supportive therapy is necessary. The need for such therapy is directly, perhaps exponentially, proportional to the delay in diagnosis and institution of treatment.

If not already established, ECG and temperature monitoring must be instituted immediately, peripheral and central venous and arterial lines inserted for intravenous fluid replacement, as well as circulatory and biochemical monitoring. The bladder needs to be catheterized. These sources will provide the information appropriate to the control of the supportive therapy, of which the most important steps are to:

1. Discontinue the administration of all anaesthetic agents, hyperventilate with oxygen.
2. Correct metabolic acidosis with sodium bicarbonate to pH 7.2–7.3. It is appropriate to assume and to correct a base deficit of $6\,mmol \cdot l^{-1}$ immediately while awaiting the first biochemical results. Correction of hyperkalaemia usually follows control of acidosis. Cardiac arrhythmias usually do not require individual treatment but respond to dantrolene control of the syndrome. Should this not be the case, procainamide is the drug of choice. Although use of verapamil may seem logical, it cannot be recommended because, in the presence of dantrolene, it may cause profound myocardial depression (Gallant *et al.*, 1985).
3. Institute aggressive cooling. Whilst application of ice is traditional, consideration should be given, rather, to the more efficient cooling by fan evaporation of a fine spray of tepid water from the body surface (paradoxically, warm air is employed because this maintains vasodilatation, assisting heat dissipation, while the low thermal capacity of air ensures negligible heat input) (Lloyd and Scott, 1985). If ice has been used, active cooling should be discontinued at 38°C.
4. Induce diuresis by the administration of mannitol in the interests of protecting the kidney against the effects of myoglobinuria. Should treatment of the acidosis have involved heavy sodium loading, the use of frusemide (furosemide) is more appropriate.
5. Infuse fresh frozen plasma if the syndrome has been

long lasting and there is any hint of the development of DIC.

Once the patient's temperature has reached normal levels, the arterial P_{CO_2} is normal and metabolic acidosis has been corrected, spontaneous respiration can be allowed to recommence and the patient is extubated once conscious.

The patient must be closely monitored and sedated for 24 hours after initial recovery, for fear of recurrence of the syndrome.

Mortality and prognosis

The mortality from MH at the time it first came to world notice was of the order of 80 per cent. During the 1970s, increasing awareness of the syndrome, with resultant earlier diagnosis and treatment, led to an improvement in prognosis, mortality dropping to 28 per cent. This decade, following the introduction of dantrolene sodium, the mortality recorded from MH is now only 7 per cent—all such cases on review manifesting serious errors of therapeutic management. Today, with correct management, survival from MH should be 100 per cent (Britt, 1985; Gronert, 1986).

Inheritance

Although it was its genetic aspects which first led to identification of MH, the details of its mode of hereditary transmission are still unclear. Whilst early evidence supported the concept of autosomal dominant transmission involving a single gene allelle—what inconsistencies there were being attributed to incomplete penetrance and varying expressivity (Kalow and Britt, 1973; Denborough, 1979)—all the evidence now points to the mode of transmission being multigenic (Ellis, Cain and Harriman, 1978; Kalow, Britt and Chan, 1979; McPherson and Taylor, 1982). Although the condition is more common in males, any apparent sex linkage has been shown to be spurious.

Associated conditions

Approximately 30 per cent of MH sufferers manifest one of a group of non-specific musculoskeletal disorders such as strabismus, congenital ptosis, kyphoscoliosis, subluxation of patellae and recurrent joint dislocation. Whilst this association allows us to recognize a group of patients at risk from MH if anaesthetized, it has contributed nothing to characterization of the mode of MH transmission.

Although sufferers from the classic overt organic myopathies—myotonia congenita or dystrophica, Duchenne muscular dystrophy, central core disease and others—are prone to complications when subjected to general anaesthesia, it is surprising how rarely such complication is MH (Gronert, 1980; Brownell *et al.*, 1983; Wang and Stanley, 1986). The Denborough myopathy, in which transmission is recessive, and central core disease, are the only overt myopathies with which the MH syndrome is consistently associated. The Denborough myopathy is found in boys, small for age who display cryptorchidism, kyphosis, lordosis, pectus carinatum, neck webbing, low-set ears and antimongoloid obliquity of the palpebral fissure (Denborough, 1979). Arthrogryposis multiplex congenita has been anecdotally associated with MH. This association is tenuous, however, and it remains to be proven that the incidence of MHS in this group of patients is any different from that in the background population (Baines, Douglas and Overton, 1986).

Evaluation of susceptibility—preanaesthetic diagnosis

Preanaesthetic identification of the individual susceptible to MH is prerequisite for the prevention of the syndrome. This involves diagnostic screening of the family of the MH proband. Because transmission of MH is dominant, those relatives who have a 50 per cent probability of possessing the gene—siblings, parents and children—should be screened. Thereafter, screening is extended in a similar manner from any individual identified as MHS (Ellis, 1981).

Invasive diagnostic tests (involving open muscle biopsy)

The original demonstrations that *in vitro* biopsy muscle strips from MHS pigs and humans reacted pharmacologically differently from normal muscle, in a manner which could be quantified, provided the basis not only for early insights into the pathogenesis of MH but also for diagnostic screening tests. These reactions were:

1. Excessive rate of ATP hydrolysis (Harrison *et al.*, 1969).
2. A lower threshold and increased reactivity to caffeine-induced contracture (Kalow *et al.*, 1970).
3. Contracture induced by exposure to halothane (Ellis *et al.*, 1971; Moulds and Denborough, 1972).
4. Lower threshold to potassium chloride depolarization-induced contracture (Moulds and Denborough, 1974).

From these, differing test protocols have been formulated, based on evaluation of the isometric contracture tension developed *in vitro* by dissected fascicles of muscle, in response to serial increase in concentration of caffeine and/or halothane (Moulds and Denborough, 1974; Ellis *et al.*, 1978; Britt, 1979; Gronert, 1979). Although the *in vitro* contracture test (IVCT) has proved the only reliable diagnostic screening procedure for MH, the gradations observed between normal and MHS muscle in contracture response to caffeine, halothane, and halothane and caffeine in combination have led to difficulties in the definition of precise diagnostic criteria (Nelson, Flewellyn and Gloyna, 1983).

Based on the findings of Rosenberg and Reed (1983), that IVCT to halothane alone and to caffeine alone provided the most discriminant diagnostic procedure, the European MH Group (1984, 1985) have advocated a standard IVCT protocol consisting of (1) static caffeine test, (2) static halothane test, and (3) dynamic halothane test. Results are reported in terms of the threshold caffeine or halothane concentration at which contracture first appears (tension increase >0.2 g). Critical values are 2 mmol·l^{-1} caffeine and 2% (0.44 mmol·l^{-1}) halothane. Specimens scoring less than both indices are classified as MHS, those greater than both as MHN (negative). All others are classed as MHE (equivocal), to be kept under review.

Histology

Muscle is the only tissue in MH subjects in which histological abnormalities have been found. About two-thirds of patients have abnormal skeletal muscle histology indicative of a myopathy (Harriman, 1979). These abnormalities, though protean and non-specific (Isaacs, Frere and Mitchell, 1973; Isaacs and Heffron, 1975), may contribute to the definite diagnosis of equivocal cases (MHE).

Non-invasive tests

For the purpose of this discussion, venous blood sampling is not considered invasive.

Many non-invasive tests have been developed but all lack specificity. Assay of serum CPK activity has been the most widely used of these.

CPK activity

It is ironic that the observation of elevated levels of serum CPK—the marker muscle enzyme by which Isaacs and Barlow (1970) and Denborough *et al.* (1970) originally identified susceptibility to MH as a subclinical hereditary myopathy—should ultimately come to be regarded as having little, if any, usefulness as an MHS diagnostic screen.

Raised serum CPK levels may be found in 80 per cent of either known MH patients or affected relatives (Britt, 1979) but also in 10 per cent of normal individuals (Britt, 1985). Repeat estimations have shown that levels of CPK activity vary within the same patient and that 50–60 per cent of known MH reactors display normal values at some time (Ellis *et al.*, 1975; Britt, 1985). Hopes that study of CPK isoenzymes would reveal a diagnostic pattern (Zsigmond *et al.*, 1972) were also vain (Addis *et al.*, 1978). Now Paasuke and Brownell (1986) recommend that CPK assay be abandoned in MH diagnostic protocols, for fear that misleading results may lead to medical mismanagement.

In one circumstance, however, the observation of elevated serum CPK activity does serve a useful diagnostic purpose. If a close relative of a known MH reactor has raised serum CPK activity for which there is no other cause, that individual should be regarded as susceptible to MH—IVCT may be dispensed with.

Blood

Attempts at basing MH diagnostic screening tests on various abnormalities that have been observed in MHS porcine and human blood cells have proved unsuccessful. Not only has the increased osmotic fragility of MHS swine erythrocytes (Harrison and Verburg, 1973; Heffron and Mitchell, 1981) not been found in MHS humans (Zsigmond, Penner and Kothary, 1977) but also hopes that platelets with their ATP/calcium/actin/myosin contractural process would mirror the functional lesion of MH muscle and so constitute a diagnostic screen (Solomons and Mason, 1984) have been shown to be illusory (Giger and Kaplan, 1983; Lee, Adragna and Edwards, 1985; Britt and Scott, 1986).

Anaesthesia for the MHS patient

The application of three simple guidelines will ensure safe anaesthesia for the individual identified as susceptible to MH:

1. Obtundation of the patient's 'trigger' sensitivity.
2. Utilization of meticulous vital function monitoring.
3. Avoidance of known MH-triggering agents.

An essential corollary to the above is the immediate availability of the drugs and equipment necessary for treatment of MH should it be initiated.

Obtunding the patient trigger

From previous discussion, it is obvious that anaesthetic safety for the MHS patient can be enhanced by preanaesthetic control of any apprehension and anxiety the patient may evince. For this, personal reassurance of the patient is as important as the use of appropriate sedative and anxiolytic drugs. For this latter purpose the benzodiazepines, droperidol and opiates are all suitable, although the phenothiazines are best avoided (Moyes, 1973).

The preanaesthetic prophylactic use of dantrolene is the subject of some controversy. Certainly, the use of oral dantrolene—a 3-day preanaesthetic protocol from which the patient may suffer many unpleasant symptoms and which does not guarantee prophylactic systemic drug levels—is no longer to be recommended (Flewellyn *et al.*, 1983; Ruhland and Henkle, 1984; Flewellyn and Nelson, 1984b). If prophylactic dantrolene is desired, it should be administered intravenously in a dose of 2.4 mg·kg^{-1} with the induction of anaesthesia (Harrison and Chapman, 1982). Given the application of a correct anaesthetic technique with meticulous monitoring and immediate availability of intravenous dantrolene, many question the necessity of

prophylactic administration at all. However, it is generally agreed that when prolonged or major surgery is contemplated, the MHS patient's interests are best served if dantrolene is given prophylactically.

Utilization of meticulous vital function monitoring

This should include capnography and monitoring of ECG, blood pressure and core temperature. If the surgery is major or prolonged, provision should also be made for serial acid–base and the blood gas monitoring.

Avoidance of known MH triggering agents

Local or regional anaesthesia

Although it has long been taught that anaesthesia with the lowest risk for the MH patient is provided by regional techniques, the decision to use such should be based on the merits of the technique itself. Unfamiliar techniques should not be embarked upon simply because the patient is susceptible to MH.

General anaesthesia

A blanket ban on all potent volatile inhalational agents and suxamethonium is the only constraint on general anaesthetic techniques for the MHS patient.

Lastly, the individual who presents for anaesthesia with a history of MH in his close family, but whose susceptibility status has not yet been decided by IVCT, should be treated as susceptible to MH until proved otherwise.

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A Genetic Membrane Disease

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Chapter 5

THE DISCOVERY OF MALIGNANT HYPERTHERMIA IN PIGS — SOME PERSONAL RECOLLECTIONS

Gaisford G. Harrison

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I. INTRODUCTION

It is ironic that although shock-like, stress-related syndromes had long been recognized in domestic swine and, because of their economic implications for the meat industry, they had been the subject of much veterinary research,[1,2] the identification of their manifestation of anesthetic-induced malignant hyperthermia (MH) was so entirely fortuitous. This discovery had to await the exposure of pigs to general anesthesia with halothane and succinylcholine occasioned by their use in medical research. Based, as it was, on observations by two independent groups of workers in places as far apart as Cambridge (Great Britain) and Cape Town (South Africa), the chance nature of this discovery that genetic strains of certain breeds of swine could serve as a valid experimental animal model of human MH can be judged from the fact that in the former instance the pigs were anesthetized for an investigation into atherogenesis, in the latter for experimental liver transplantation. Further at this time, about 1966, except for Denborough's original identification of this syndrome in a family in Australia,[3,4] published reports of MH in humans had been confined to the North American continent.[5,6] No cases had yet been documented as such in Great Britain or South Africa. Because so much of what we understand today of the pathogenesis of this pharmacogenetic, membrane-based myopathy MH, has come from experimental studies of malignant hyperthermia-susceptible (MHS) swine, a brief account of the circumstances surrounding the discovery of the "hot pig" is of interest and relevance to this volume.

II. THE DISCOVERY

The establishment of a program of liver transplantation in man was a primary objective of the Liver Research Group, an interdisciplinary group formed at the medical school of the University of Cape Town, South Africa in 1968.* To this end initial experience was sought in an animal experimental model. For this purpose we chose the domestic pig instead of the more usual surgical experimental animal, the dog. There were several reasons for this besides the very practical one that our research group's surgical leader already had considerable experience in experimental liver transplantation in swine, which he had acquired while working for a period at the medical school of the University of Bristol (Great Britain). First, the pig, in marked contrast to other animals, shows little evidence of rejection following liver homotransplantation, even of liver of nonrelated donors and in the absence of

* The original members of this group concerned with the "hot pig" discovery were J. Terblanche (surgeon), S. J. Saunders (physician), J. F. Biebuyck (anesthesiology resident), D. M. Dent (surgical resident), R. Hickman (surgical resident), and the author (anesthesiologist). In later years each of these attained professorial appointment in his/her particular discipline.

immunosuppression. This strange exception applies only to liver; kidneys, skin, and other organs being rejected, as usual, in these circumstances. Second, on surgical handling, pig liver does not develop venoconstriction with resultant venous outflow block as does that of dogs. Last, pigs were readily obtainable from local farm breeding lines in almost unlimited quantities and were cheap to maintain, and blood for transfusion during experimental surgery was readily obtainable from the local slaughterhouse.

The first animal models to be used in this project were locally bred Landrace piglets, 6 to 8 weeks old weighing 30 to 40 kg. These we anesthetized in the following manner. Having been starved for 16 h preoperatively (for surgical reasons), the unpremedicated pig was anesthetized in its transport pen by the inhalation of N_2O with O_2 (flow rates 6 and 3 l per minute, respectively) with halothane (concentration rapidly increased to 3%) delivered via a snout mask attached to a semi-open circuit, with the pig breathing spontaneously. When the pig lost consciousness, usually within 3 min, it was removed from its pen and placed supine on the operating table. Here, after anesthesia had been deepened for a further 3 to 5 min, orotracheal intubation was performed — a task considerably more difficult in the pig than in man — followed by the passage of a stomach tube and an esophageal thermistor for temperature monitoring. ECG monitoring was then instituted followed by femoral arterial and jugular venous catheterization for the measurement of arterial and venous pressure, arterial blood gas, acid/base and electrolyte monitoring, and to provide the conduit for blood and fluid replacement during surgery. For the surgery, anesthesia was maintained with N_2O/O_2 with halothane (1 to 2%) delivered via a nonreturn circuit by IPPV, powered by a standard automatic ventilator. Ventilatory volumes were adjusted to maintain normo- to mild hypocapnia and apnea. This method provided satisfactory operating conditions in the pig without the use of muscle relaxants. These we chose to avoid so as not to compound the difficulties in relaxant reversal and postoperative respiratory problems consequent to the inevitable early abdominal distension. Ambient temperature in the operating theater varied between 15 to 20°C. Thus the scene was set.

We had anesthetized but a few pigs in this manner without complication, when one reacted to anesthesia in the most bizzare and puzzling manner.[7] As the animal lost consciousness, instead of the usual quieting of respiration, it commenced panting, developing at the same time a blotchy skin cyanosis, despite good auscultated air entry and an FIO_2 of 0.3. Instead of the expected flaccidity, its legs became rigidly extended, so much so that once placed on the operating table, it had all the appearance of a pig in rigor mortis (Figure 1). Orotracheal intubation presented more than its usual difficulty because of masseter spasm and was no sooner successfully accomplished than the animal ceased breathing. IPPV was commenced and, as a good but fast pulse was palpable, we continued with our experimental protocol.

Commencement of ECG monitoring showed a sinus tachycardia of 200 beats per minute while the first blood sample from the femoral artery catheterization

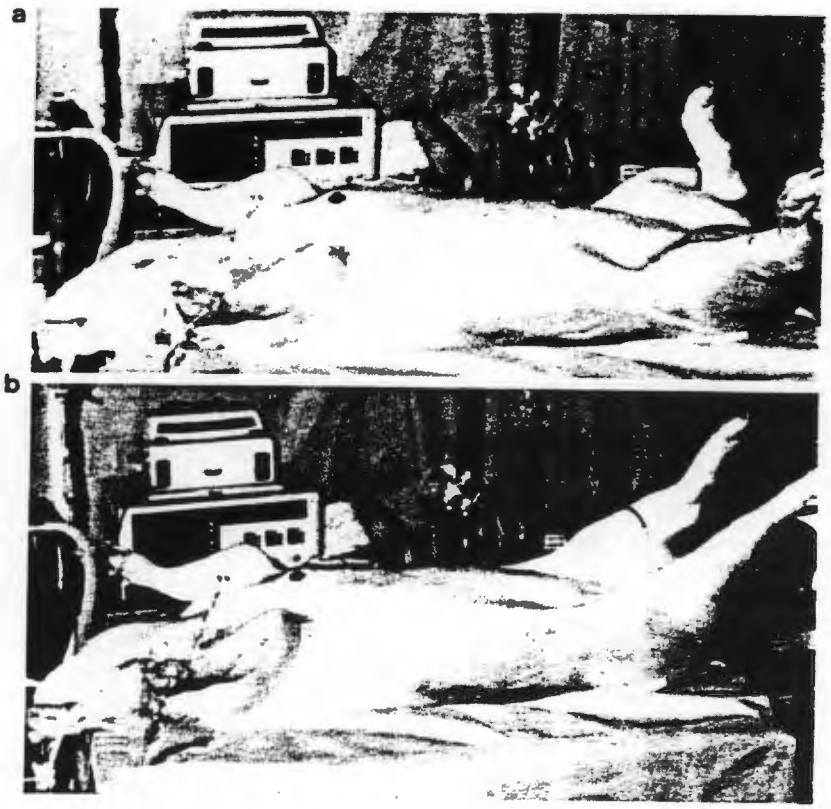


FIGURE 1. The characteristic ante mortem "rigor mortis" that first drew attention to PMH as an entity. (a) MHS pig anesthetized with thiopentone and (b) same pig after 10 min of inhalation of halothane.

revealed a metabolic acidosis so gross (pH 6.85) that we thought it had to be an artifact and immediately repeated it — with the same result. Afterwards, copious amounts of sodium bicarbonate made no impression at all on the acidosis. About 55 min had elapsed from the induction of anesthesia before the surgeon performed the laparotomy and immediately remarked on the extreme heat of the abdominal organs. (He insisted that steam arose from the peritoneal cavity.) The esophageal thermistor, just activated, registered the core temperature as an unbelievable 44°C! Blood pressure and circulation, which had been well maintained until this point, now commenced to fail. After several bursts of multifocal ventricular premature beats, the pig finally demised in ventricular fibrillation after 105 min of anesthesia.

Sporadically and quite unpredictably this same sequence of events was to be repeated exactly in 6 of the first 34 animals anesthetized in this manner. Therapeutic interventions proved fruitless and all died with the longest survival being 135 min, the shortest a mere 45 min from commencement of anesthesia.

At death, core temperatures, which during anesthesia had risen 1°C/5 to 10 min, ranged from 42.5 to 45°C (Figure 2) and all suffered severe metabolic acidosis — pH ≤ 6.85, PCO_2 ≥ 150 mmHg, base excess < −22 mEq/l — an acidosis seemingly uncorrectable by large amounts of sodium bicarbonate (Figure 3). We could discern no cause for all of this. Careful review of the clinical events of each death together with laboratory checks for such possible reasons as septicemia or pyrogens in infused fluids all proved negative.

Besides the unpredictability of its occurrence, the most ominous and frustrating aspect of what we were now coming to recognize as a specific syndrome was the fact that, although it was seemingly initiated in some way by the induction of anesthesia, once established discontinuance of all anesthetic agents and ventilation of the animals with O_2 alone made no difference whatsoever to its inexorably fatal progress. The anesthetic had, in some way, triggered a self-sustaining pathological process.[8]

The early random introduction of an alternate anesthetic technique omitting halothane and utilizing intermittent thiopentone with small doses of curare together with N_2O/O_2 by IPPV, soon demonstrated that when it did occur, the syndrome was only associated with the administration of halothane.

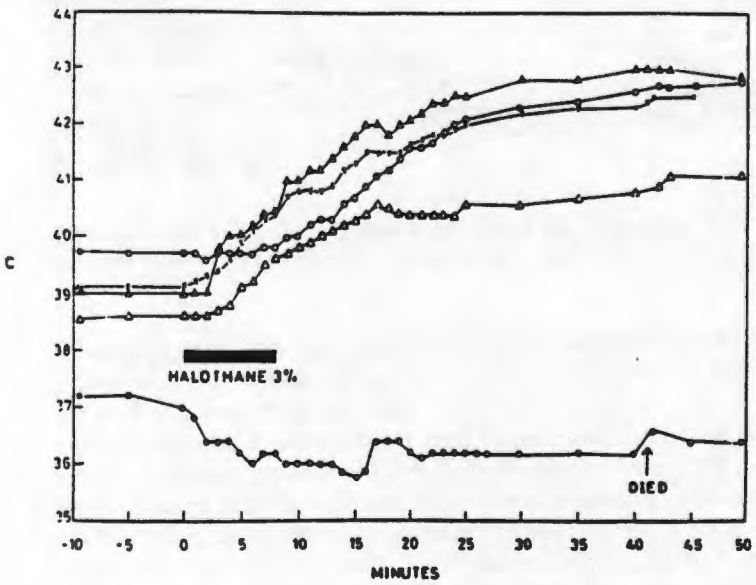


FIGURE 2. Multisite temperature changes recorded following halothane initiation of MH in an MHS pig following a control period of thiopentone anesthesia. Note extreme rapidity of MH onset and speed of temperature rise in internal organs. Skin vasoconstriction (blotchy cyanosis) has prevented a similar rise in skin surface temperature. X = esophagus; ○ = rectum; ▲ = liver; △ = muscle (gluteus); and ● = abdominal skin (surface). (From Berman et al. *Nature*, 225, 653, 1970. With permission.)

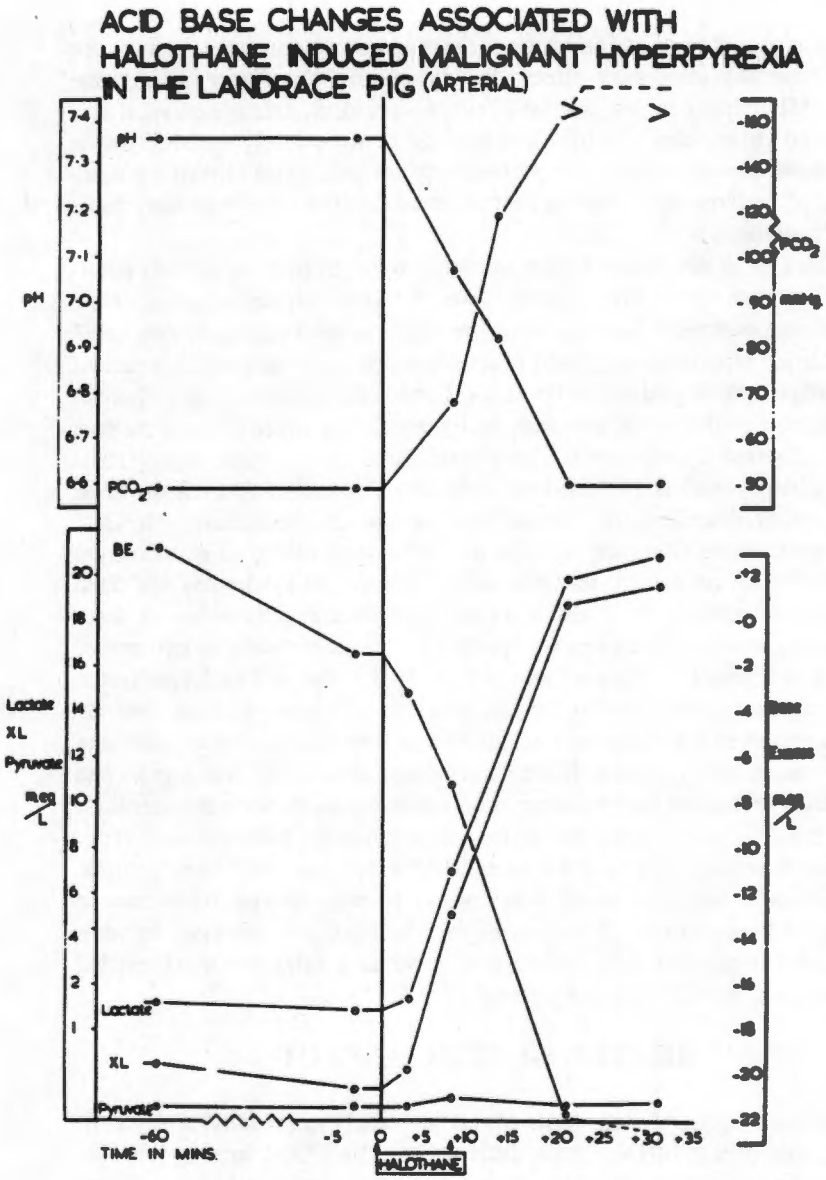


FIGURE 3. Arterial blood acid/base changes recorded in an MHS Landrace following initiation of MH after control period of thiopentone anesthesia. XL = excess lactate. Note speed and extremes of pH and related changes. (From Harrison, G. G., *Int. Symp. Malignant Hyperthermia*, R. A. Gordon, B. A. Britt, and W. Kalow, Eds., Charles C Thomas, Springfield, IL, 1971, 271. With permission.)

Addition of curare to the halothane technique did not prevent the onset, nor did it have any therapeutic effect.[8] Besides pointing the finger at halothane as the MH trigger in our original anesthetic protocol, this observation also identified an anesthetic technique that could be used safely on MHS swine — a technique we were to use subsequently for setting up *in vivo* the monitoring procedures and achieving control period steady states for investigations in such swine.

Clues as to the nature of this syndrome were given to us by two publications current at the time. The first was the 1966 September issue of the *Canadian Anaesthetic Society Journal* (now *Canadian Anaesthetic Journal*).[9] This carried reports by six groups of anesthesiologists — originally presented at a symposium organized by Dr. R. A. Gordon in Toronto — of 11 human fatalities following a syndrome induced by anesthesia, called then for the first time malignant hyperpyrexia. The second came from a preliminary communication by Hall and co-workers to the *British Medical Journal* the same year, which described an "unusual reaction to suxamethonium chloride" observed in three littermate pigs that had been anesthetized as experimental animals.[10] The component features and outcome of the syndromes described by these authors, in man and in swine, described exactly what we were observing; however, there was an important difference between the two groups of pigs. Whereas the "unusual reaction" in Hall's pigs at Cambridge (gross myotonia) was precipitated by the administration of suxamethonium chloride after a period of uncomplicated halothane anaesthesia, in our pigs halothane alone evoked the syndrome. In the human cases which, like Hall's pigs, had a genetic component both halothane and suxamethonium were incriminated. These findings led us to the conclusion that this bizzare hypercatabolic state, initiated in some swine by exposure to halothane, could well be a facsimile of the newly described lethal syndrome in humans of anesthetic-induced malignant hyperpyrexia. If they could be identified and salvaged in some way, we thought that such swine would serve as a valid and much needed experimental model of the syndrome.

III. THE GENETIC ASPECT

Establishing the genetic basis of this seemingly unpredictable susceptibility of our pigs to MH was made difficult for us by a local farming practice. The local pig breeders all corralled their post-weaned piglets in large groups according to weekly age. This made it impossible for us, at this stage, to identify accurately the pedigrees of the pigs supplied to us. Also, varying sources of supply resulted in our using Large White and Large White/Landrace Cross swine in addition to the original pure Landrace strain. As the number of pigs anesthetized increased, however, the genetic aspects of MH susceptibility become more obvious on a statistical basis. When this number had

reached 160, we found MH had occurred in 25% of the pure Landrace, 3% of the Landrace/Large White Cross and not at all in the pure Large White breed.[8] This vulnerability of the Landrace was further borne out when an inquiry of Dr. Hall's group in Cambridge brought the reply that their "stiff" pigs that suffered this unusual reaction to suxamethonium were Landrace/Wessex Cross breeds. (N. Woolf, personal communication).

Very soon reports of Porcine MH (PMH) from Europe,[11] America,[12,13] and Australia[14] showed that, far from being rare, this condition was widespread. In a genetic context, though, susceptibility to MH was limited, in that it was manifested by selected strains of but two major swine breeds other than Landrace. These were the Pietrain and Poland China breeds. All three are breeds genetically selected for the qualities of rapid growth, heavy muscling with high muscle/fat ratio, and high feed efficiency. These are the very breeds that manifest, in high incidence, the recognized stress-related syndromes, porcine stress syndrome (PSS), and pale, soft, exudative pork (PSEP). Indeed it was not long before subsequent research produced good evidence to support the hypothesis that PSS, PSEP, and PMH were all manifestations of the same covert genetic myopathy.[15]

MH-like syndromes have been documented but rarely in species other than swine, most notably the dog and the horse.[16] In these species, in contrast to MHS swine, evocation of the syndrome has needed much longer exposure of the animal to MH-triggering agents, progression of the syndrome has been slower, repeatability of the reaction in individual animals has not been established, and the role of genetic factors has not been as obvious as in swine or humans.

IV. SELECTION AND BREEDING

The association of PMH with stress syndromes and, once established, its rapid progress to a "malignant" irreversibility caused us much frustration in our early attempts to establish the MHS pig as the experimental model of the human condition. This required prospective diagnosis and thereafter inbreeding of the susceptible strain. Then, as now, observing the pig's response to the inhalation of halothane was the only reliable method of identifying MH-susceptible animals.[15] However, excitement from the stress of struggling while being manhandled profoundly enhances the sensitivity of the triggering and the speed of progression of the syndrome and may even be sufficient, of itself, to evoke it.

In these circumstances, MH may be fired by an inhalation of halothane for as short a time as 30 to 40 s. Muscle rigor with accompanying blotchy skin cyanosis occurs almost before the onset of anesthesia, so much so that it is often difficult to distinguish between the stiff leg of conscious induction struggling and the extended leg of MH rigor, so imperceptibly does one merge with the other. To compound these difficulties, the syndrome, having been aborted by very early discontinuation of halothane inhalation, may often

recrudesce in highly reactive animals in the early period of recovery from anesthesia. Today, piglets that react so severely are salvaged easily and consistently by the i.v. administration of dantrolene,[17] but in those pre-dantrolene days we suffered the frustration of losing animals often in the diagnostic screening process.

Our first attempts at breeding the MHS strain were embarrassingly and laughably unsuccessful. In all innocence we had fed and pampered two MHS Landrace boars for 6 months before we discovered they had been castrated! Unbeknown to us, male pigs destined for the meat market are routinely castrated shortly after being weaned, in the interests of improving meat palatability and quality and, on superficial examination, signs of castration in young pigs are not that obvious to the uninitiated. Thereafter, having acquired a noncastrated young MHS boar and nurturing him to reproductive age, our next breeding attempt was frustrated by the syndrome of "purple porcine passion". Our boar was so highly stress-susceptible, that his excited attempts at procreation evoked fulminant MH that led to his becoming stiff, hot, and blue and his immediate and premature demise. Our breeding frustrations were not confined to boars. Of the first three MHS gilts that we carefully reared to reproductive age, one had a vaginal abnormality, one developed sway back, and the third refused to mate.

V. SOME EARLY INVESTIGATIONS

Fortunately, we were not dependent for our supply of MHS swine on our own initially futile attempts at breeding this strain. The random incidence of the MHS gene in the Landrace and Landrace/Large White Cross swine we acquired for the surgical research program — 25 and 3%, respectively — ensured a supply of MHS animals sufficient for our early MH investigational needs. Later, by efficient screening and elimination of stress-susceptible animals, the local pig breeders were to reduce this incidence greatly but, by then, we had established our own MHS breeding line.

The early investigations of MH using the MHS pig model were especially fruitful and cost effective. In this exciting and virgin field, fundamental conclusions could be and were made from relatively simple, unsophisticated, and inexpensive experiments. In a short time these led to the identification of skeletal muscle both as the site of the lesion and the primary source of the heat production.[18] In briefly recalling some of these early fundamental investigations using the MHS pig model to conclude this account, as these are personal recollections, I am confining my remarks to our own investigations of the "hot pig." Of course, as MHS swine were identified widely geographically, similar research was being undertaken contemporaneously in Europe, England, America, and Australia with results that were confirmatory and/or complementary to our own, but I do not have the space here to review these.

In pigs dying from MH, the only tissue to show histological abnormalities (conventional light microscopy) was skeletal muscle. These changes were

consistent with fiber damage and destruction from severe rigor.[8] The character of this rigor, clinically so like that of rigor mortis (see Figure 1), which was then known to correlate with depletion of ATP from muscle, led us initially to investigate specifically ATP content of MHS muscle and the effects on this of exposure to halothane. This relatively simple investigation revealed the presence of a fundamental, identifiable functional lesion in the skeletal muscle of the MHS swine. The rate of depletion of ATP from biopsies incubated *in vitro* was double that of muscle from non-MH pigs, and this depletion rate was enhanced by exposure to halothane (Figure 4). Ironically, as we soon found, the depletion of ATP *in vivo* was **not** by itself the cause

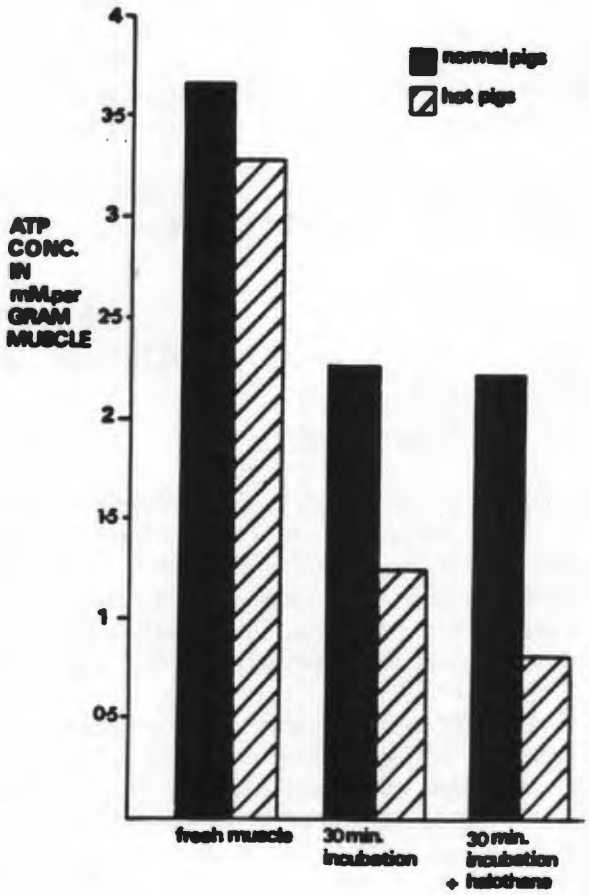
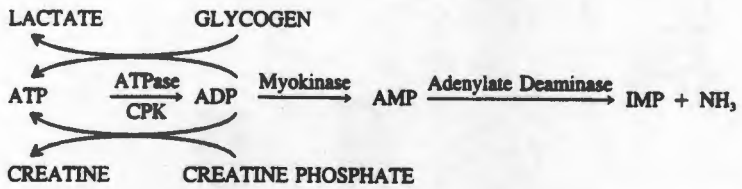


FIGURE 4. The effect *in vitro* on ATP content of muscle from normal and MHS swine of incubation in Krebs-Ringer solution and exposure to halothane. (From Harrison, G. G., *Porcine Malignant Hyperthermia in Malignant Hyperthermia*. B. A. Britt, Ed., International Anesthesiology Clinics, Vol. 17(4), Little, Brown, Boston, 1979, 25. With permission.)

of the muscle rigor. ATP levels in MHS muscle *in vivo* were well maintained early in the course of the MH syndrome initiated by halothane, at a time when muscle rigor was already well established. The levels of ATP fell only after there had been an appropriate depletion of creatine phosphate (Figure 5), which by itself correlated with a concomitant depletion of glycogen.[19]

This realization came from the subsequent, more sophisticated studies of the correlated dynamic *in vivo* biochemical changes in blood and serial muscle biopsies (liquid N_2 frozen) that accompanied progression of this syndrome to its fatal end.[18,19] The depletion of glycogen, which accompanied the concomitant development of gross acidosis (see Figure 3) with a stoichiometric increase in lactate (Figure 6), was the outcome of mass glycolysis that rapidly became anaerobic (Figure 7). Anaerobiasis was calculated as being responsible for at least 50% of the increased heat production. Anaerobic mechanisms had not previously been considered as a source of physiological or pathological heat. At the same time, no evidence was found of the uncoupling of oxidative phosphorylation that had been suggested by some as the source of the increased heat.[20]

The muscle rigor was fueled by the activation of the powerful myofibrillar ATPase and consequent hydrolysis of adenine nucleotides.



The originally observed depletion of ATP was the outcome of its rate of consumption in this system far exceeding its regeneration potential by anaerobiasis. Deprived of substrate by this reaction, various membrane ion pumps — in the sarcoplasmic reticulum (SR), mitochondria, and sarcolemma (SL) — progressively failed, leading to the a loss of membrane integrity, with the ultimate production of the biochemical conditions that characterize rigor mortis.

Both of these interrelated events: (1) activation of myofibrillar ATPase and rigor and (2) activation of glycolysis, were explainable, it was suggested, by the rapid release of calcium from intracellular sites. We provided pharmacological support for this contention that the release of calcium from intracellular binding sites was the primary event in the onset of the MH syndrome, by a demonstration *in vivo* that the triggering of the MH syndrome in MHS swine by halothane and succinylcholine was effectively blocked by the administration of procaine.[21] This drug was then well known to block caffeine-induced SR calcium release and so caffeine-induced muscle rigor *in vitro*, a reaction for which Kalow et al.[22] had shown the muscle of MH patients to have an abnormal sensitivity.[22] These observations provided the

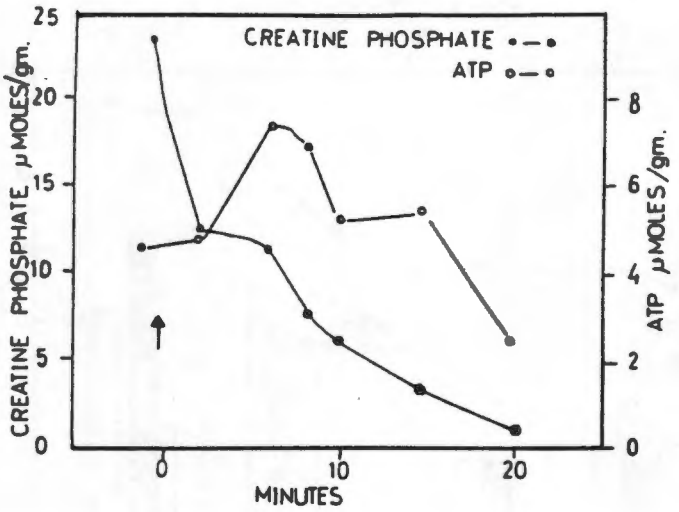


FIGURE 5. *In vivo* ATP and creatine phosphate content of serial muscle biopsies during progress of PMH provoked by inhalation of halothane. (From Berman, M. C. and Kench, J., *Int. Symp. Malignant Hyperthermia*, R. A. Gordon, B. A. Britt, and W. Kalow, Eds., Charles C Thomas, Springfield, IL, 1973, 287. With permission.)

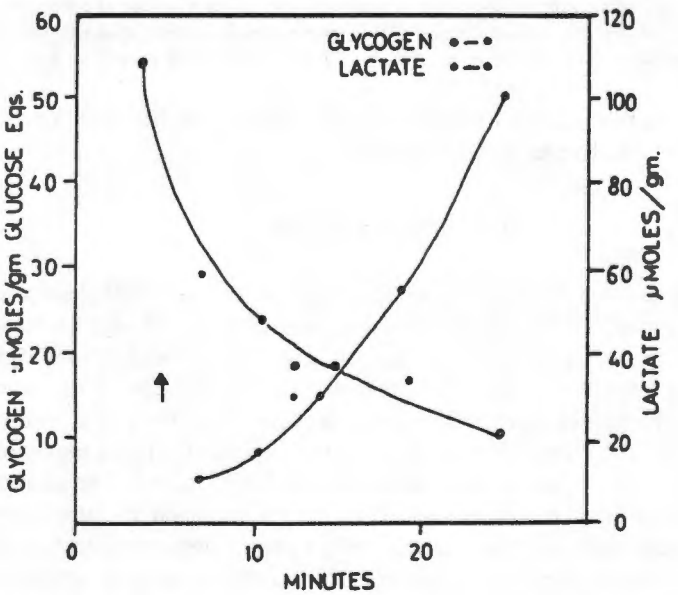


FIGURE 6. *In vivo* glycogen and lactate content of serial muscle biopsies during progress of PMH provoked by inhalation of halothane. (From Berman, M. C. and Kench, J., *Int. Symp. Malignant Hyperthermia*, R. A. Gordon, B. A. Britt, and W. Kalow, Eds., Charles C Thomas, Springfield, IL, 1973, 287. With permission.)

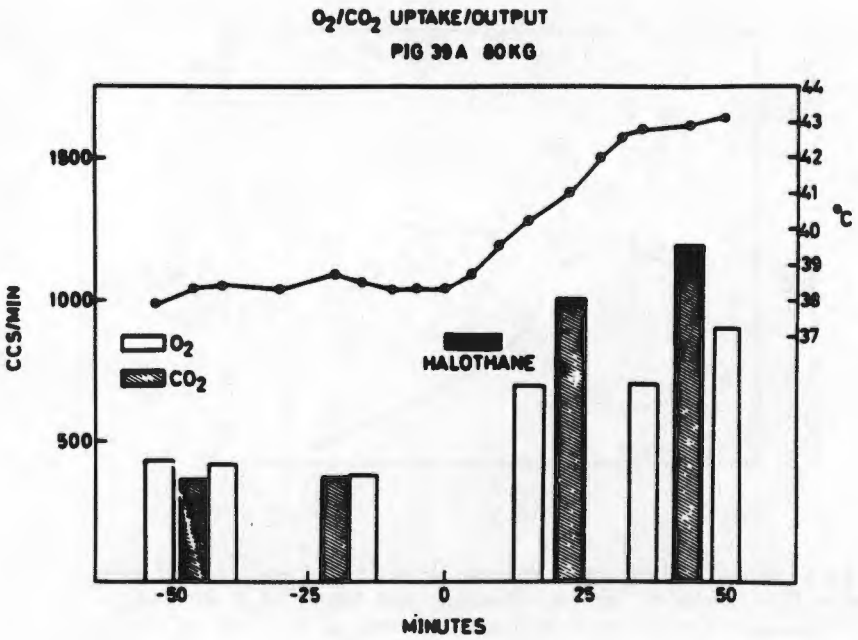


FIGURE 7. Oxygen uptake and CO_2 output with simultaneous esophageal temperature recorded during a course of PMH evoked by inhalation of halothane after a control period of thiopentone anesthesia. Note the disproportionate increase in CO_2 output compared to O_2 uptake indicating onset of anaerobiasis. (From Berman et al., *Nature*, 225, 653, 1970. With permission.)

rationale for the first and only specific therapy suggested for MH[23] before the introduction of dantrolene several years later.[17]

VI. CONCLUSION

Proposed in 1968 as an animal model for the study of the MH syndrome in man, the use of MHS swine has stood the test of time. Though the final minutiae of the pathogenesis of MH have still to be elucidated, knowledge that has come from worldwide extensive studies of PMH has shown it — and by inference the human syndrome — to be the functional result of a genetic membrane defect/dysfunction in the triadic SR/T-tubular junctional region of the myocyte.[24,25] In certain circumstances, this leads to a failure of the normal control of the calcium-release component of the excitation/contraction/coupling mechanism leading to the induction of an abrupt and sustained rise in myoplasmic free-calcium. This event initiates muscle contracture, activates glycolysis and mitochondrial activity, and sets in action a series of events that interact to form a vicious cycle.[26] Using the MHS pig as the pharmacological test bed, these insights into the pathogenesis of MH have been accompanied by the development of an effective therapy for the syndrome,

a drug that reduces the rate and amount of SR calcium release, dantrolene.[17] This addition to the anesthetists' armamenterium has potentially eliminated MH as the major cause of unavoidable mortality attributable to anesthesia that it once was. Today, with correct management, survival from MH should be the norm.

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Chapter 8

THE PORCINE HYPERTHERMIA AND STRESS SYNDROMES

Gaisford G. Harrison

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I. INTRODUCTION

Malignant hyperthermia in swine (PMH) is the specific drug-induced syndrome of hypercatabolism and muscle rigor exhibited by swine, the subjects of a covert pharmacogenetic myopathy known as malignant hyperthermia susceptibility (MHS) or more generally, stress susceptibility (SS). The discovery of this lethal syndrome in swine was serendipitous and, as with man, it had to await the chance exposure of genetically susceptible animals to the classic MH-triggering agents, halothane and/or succinylcholine, occasioned by their use in medical research (see Chapter 5).

Evoked in these swine, the MH syndrome displays all the features of its counterpart in man, validating the use of MHS swine as the animal experimental model of this life-threatening syndrome in man.[1,2] There may be some differences but, being quantitative rather than qualitative, they do not appear to be fundamental.[3] MH-like syndromes have been observed in species other than swine and man, but with a rarity that renders them but biological curiosities. Only in MHS swine does the syndrome occur predictably and reproducibly. Most of our knowledge of the acute biochemical changes that characterize the human syndrome and hypotheses of its pathogenesis in this volume will have been derived by extrapolation from controlled experiments conducted on MHS swine.

II. STRESS SYNDROMES IN SWINE

Whereas in man MH has been documented in most broad racial groups, in swine affliction with the MHS genes is confined, strangely, to selected strains only of five major pig breeds, the Landrace, Pietrain, Poland China, Yorkshire, and Duroc.[4] These are the selfsame breeds that are subject to the long recognized porcine (sudden death) stress syndrome (PSS) and its resultant postslaughter carcass condition of pale, soft, exudative pork (PSEP) — pork that exhibits unfavorable meat quality, being pale, soft, and watery.[1]

Documented separately chronologically, PSS, PMH, and PSEP are now accepted as manifestations of the same covert myopathy. PSS and PMH both present as the florid MH syndrome, in the former instance evoked naturally by severe stress such as engendered by social order fighting, exercise, manhandling, hot environment, etc. and in the latter, artificially by exposure to certain drugs. PSEP follows from the accelerated postmortem glycolysis, concomitant myolactosis, and abnormally rapid postmortem fall in muscle pH peculiar to this myopathy. This, it is proposed, alters the muscle water-holding capacity and texture by denaturation of the sarcoplasmic protein.[5,6]

III. INHERITANCE AND IDENTIFICATION

Considering the economic losses sustained by pig breeders around the world from the effects of PSS,[7] losses preslaughter in transport and post-

slaughter from PSEP, it is strange how inconclusive our knowledge is of the inheritance patterns of this myopathy. These patterns have turned out to be considerably more complex than appeared at first sight. There are good data to support hypotheses of both autosomal dominant and recessive modes of inheritance.[1,8]

A major difficulty that arises in the conduct of breeding experiments is the manner in which SS is identified. In many studies, especially in the past, animals were identified as SS retrospectively postslaughter by the presence of PSEP.[9] Since the discovery of PMH, this method has been superseded by the more objective *in vivo* barnyard halothane test; the observance of the animal's response to brief mask inhalation of halothane.[1] However, the correlation between genotype and phenotype is not absolute.[4] For this reason some investigators only consider an animal completely MHS/SS negative when response to succinylcholine in addition to that to halothane is negative.[10] To these criteria Williams adds the requirement of an unraised basal metabolic rate.[11]

Of simple blood sample tests, none have proved to be specifically diagnostic. The variability observed in the serum levels of the muscle enzyme markers of this myopathy is such that the levels recorded in an individual pig are of little diagnostic significance.[12-14] As in humans, the only diagnostic screening test with any pretensions to sensitivity and specificity of a high order involves *in vitro* contracture testing (IVCT) of muscle biopsy material.[15] While these have been used to evaluate some breeding experiments,[10,16] logistic difficulties obviate its widespread use in this sphere.

In terms of autosomal dominant inheritance, current data of graded genotype response support the view that this is multigenic (at least two gene allelles), the genes involved being codominant. The likelihood of a multifactorial pattern of inheritance is further suggested by evidence of the cross-linkage of this myopathic trait with other characteristics, e.g., blood group.[17] On the other hand, workers, especially in Europe, report data from breeding programs in the Dutch Landrace and Pietrain breeds that strongly support the existence of a recessive inheritance pattern in these swine involving a single major recessive gene exhibiting complete penetrance. In these studies, identification of MHS/SS was not corroborated with IVCT.[8,18]

Although all the reported studies in Poland China swine have evidenced a dominant inheritance pattern, both dominant and recessive patterns have been documented in the Landrace, Pietrain, and crossbreeds of these. The present data seem to support the hypothesis that swine, as do humans, suffer covert subclinical myopathic states both dominantly and recessively transmitted that manifest the MH syndrome as the functional final common pathway.

The recent localization of the functional defect responsible for the MH reaction to the ryanodine receptor calcium release channel of the sarcoplasmic reticulum (see Section VI.B.) has afforded us a rational and more accurate insight into the transmission of the PMH/SS trait. In the human, the gene for

the ryanodine receptor (ryr 1) has been localized to chromosome 19q 13.1 and susceptibility to MH has been linked to mutations of this gene.[4a] In the pig, the ryr 1 gene and the PMH (hal) locus have been localized to chromosome 6p11-q21. Fujii et al[4] have demonstrated a single point mutation in this gene to be correlated with the PMH/SS phenotype in the five major breeds which manifest the condition — knowledge which has allowed the development of a simple, accurate, and noninvasive diagnostic test that can provide the basis for elimination of this mutation if desired or, in view of its association with leanness and muscularity, its controlled retention as the N/n genotype.

IV. THE CLINICAL SYNDROME

Evoked in highly reactive MHS/SS swine by stress alone, or *ab initio* by the inhalation of halothane alone, the PMH syndrome is manifested by the following clinical signs that progress simultaneously and worsen rapidly:

1. Generalized blotchy cyanosis of the skin with well-marked cyanosis of the snout
2. The onset of sinus tachycardia often to extreme rates of 200 beats/min; untreated, this progresses through multifocal ventricular premature systoles to ventricular fibrilation
3. Distressed open mouth breathing with increasing hyperventilation that progresses to apnea in 15 to 20 min
4. Tail twitching and sporadic fasciculation of muscle, most obvious in the thighs; this progresses inexorably to generalized muscle rigor that causes a characteristic rigid extension of the hind legs (see Figure 1 in Chapter 5). So rigid do the legs become that they are impossible to flex passively. In the absence of endotracheal intubation, this generalized rigor may be accompanied by laryngospasm.
5. An explosive sustained rise in muscle and core temperature at rates as extreme as 1°C per 5 to 10 min, reaching 43 to 45°C antemortem (see Figure 1).

Left untreated. other than for the provision of IPPV when apnea supervenes, death usually follows 45 to 100 min from initiation of the syndrome.

If exposure to halothane is discontinued within minutes of the first appearance of any of the major clinical signs, the syndrome is spontaneously reversible. This maneuver is the basis of the simplest diagnostic screening test for MHS already referred to, the so-called "barnyard test" (see Section III). If, however, exposure to the triggering agent is continued for more than several minutes thereafter, the syndrome rapidly becomes irreversible and will continue independent of the stressor drug. This can now be seen to have acted as a trigger.

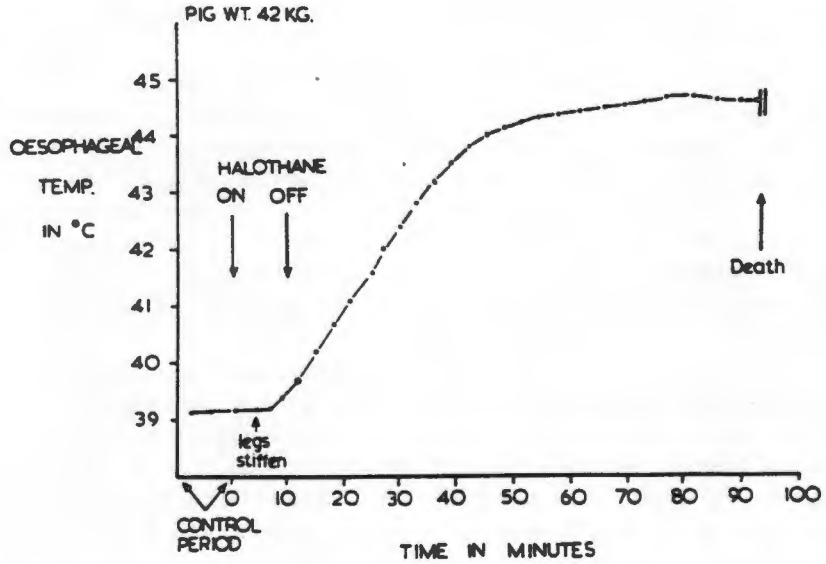

FIGURE 1. Esophageal temperature recorded in a Landrace pig during halothane-induced MH following a 30-min control period of thiopentone anesthesia. N_2O (60%)/O_2 (40%) was administered throughout by IPPV. Maximum rate of temperature rise 0.15°C/min or 1°C/6 min, 42 s. (From Harrison, G. G., Saunders, S. J., Biebuyck, J. F., Hickman, R., Dent, D. M., Weaver, V., and Terblanche, J., *Br. J. Anesth.*, 41, 844, 1969. With permission.)

A. MODIFICATIONS OF THE SYNDROME

When the unpremedicated MHS pig is anesthetized with halothane in this manner, the MH reaction commences within minutes, as soon as halothane concentration in the tissues rises. Subjecting the animal to manhandling stress or severe exercise sensitizes the triggering reaction and shortens the onset time markedly.[19] On the other hand, for reasons that are not yet clearly understood (see Section IX), pretreatment of the MHS pig with hypnotic[20] or anxiolytic drugs causes a variable delay in the onset of MH in response to the exhibition of halothane and may prevent that by stress.[21,22] However, once triggered, the speed of progression of the syndrome is unaltered. Usually of the order of 15 to 20 min, this delay or latent period may extend for 45 min or more, and in some circumstances the triggering of MH by halothane alone may even be prevented. Such pretreatment does not affect the triggering of the syndrome by the additional administration of succinylcholine.

The sensitivity of the MH-triggering mechanism is also affected by the genetic factors already discussed. Gradations in genotype-determined triggering sensitivity documented range from those that exhibit delay to those that fail to be triggered by halothane alone.[16] The latter, like Hall's historic pigs,[23] require the superimposition of succinylcholine depolarization to trigger the syndrome.

V. THE BIOCHEMICAL ACCOMPANIMENTS

Observed in the laboratory *in vivo* under controlled conditions, "switch on" of the MH syndrome is signaled by an array of interrelated and rapidly progressive changes in biochemical parameters that are indicative of the abrupt ignition of a hypermetabolic state accompanied by rigorous contracture in skeletal muscle. These changes are reflected by the conventional indices of acid/base, blood gas, electrolyte, and substrate status in blood as well as by changes observed in serial liquid nitrogen frozen muscle biopsies. They proceed to extreme values ahead of the physical rise in muscle and core temperature.[24,29]

Profound, rapidly worsening lactacidosis together with concomitant hypercapnea, the result of its physiological buffering, is the hallmark of this syndrome (see Chapter 5). The latter, readily monitored by capnography, is perhaps the earliest indication of the syndrome's switch on. A rise in plasma lactate and in oxygen uptake are equally early. Blood and tissue pH may fall from control levels to 6.8 units and lower in a time as short as 10 min (Figure 2).

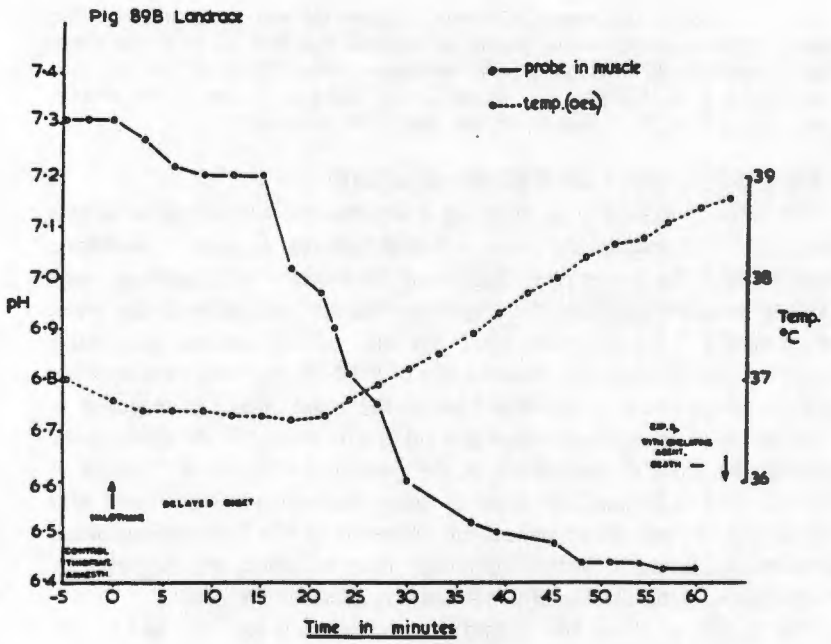

FIGURE 2. Simultaneous record of muscle pH (free-lying pH probe deep in thigh muscle) and esophageal temperature from Landrace pig during halothane-induced MH following 30-min control period of thiopentone anesthesia. N_2O (60%)/O_2 (40%) was administered throughout by IPPV. Note virtual "free fall" of pH that precedes commencement of temperature rise by 5 min. Death of this pig followed promptly on administration of Ca^{2+} chelating agent. (From Harrison, G. G., *Int. Symp. Malignant Hyperthermia*, R. A. Gordon, B. A. Britt, and W. Kalow, Eds., Charles C Thomas, Springfield, IL, 1973, 271. With permission.)

That the rise in plasma lactate precedes the rise in muscle and core temperature distinguishes MH from heatstroke and heat injury. In these conditions the rise in lactate is a late phenomenon occurring only after core temperature exceeds 41°C and correlates with the breakdown of compensatory mechanisms.

Though PaO_2 may not be reduced much, marked increase in tissue oxygen utilization results in an early and marked decrease in mixed venous PO_2 long before the terminal drop in cardiac output and the prominence of central cyanosis as a clinical sign.

Early elevation of plasma protein and Na^+ concentration tally with a shift of water into the cells. Elevation of plasma K^+ and Mg^{2+} also occurs early, the rise in the former being clinically important in the generation of the cardiac arrhythmias that characterize the terminal phases of the syndrome. Plasma Ca^{2+} and inorganic phosphate also rise, though the former has been reported variable.

Commencement of accelerated glycogenolysis and glycolysis is signaled by a rise in plasma glucose that correlates with depletion in muscle glycogen, itself stoichiometric with the increase in plasma lactate. Examination of serial muscle sections shows the whole glycolytic pathway to be accelerated with no single enzymatic step being specifically activated.[30] *Pari passu* with excessive glycolysis within skeletal muscle, the level of creatine phosphate falls within minutes, to be followed by that of ATP as both glycogen and creatine phosphate become exhausted. While ADP levels remain unaltered, AMP and inosine increase as the syndrome progresses, the end result being continuous unrequited nucleotide hydrolysis. This is a process that ends in producing the ante mortem biochemical conditions that characterize rigor mortis.[25]

Increasing permeability of the sarcolemma (SL) becomes evident in the rising plasma levels of the muscle enzymes, specifically CPK but also LDH, AST, and ALT.[26,28] In treatment-salvaged pigs, a severalfold rise in serum CPK 24 h after the experimentally induced syndrome serves as confirmation of the previous induction of MH.[31]

A surging increase in circulatory catecholamines accompanies onset of the syndrome and is doubtlessly responsible for the early tachycardia and later cardiac arrhythmias. It must be noted that chronologically this rise in circulating catecholamines follows that of lactate and PCO_2.[27]

VI. PATHOGENESIS

This progression of biochemical changes is the outcome of a single event — the induction of an abrupt and sustained rise in myoplasmic free Ca^{2+}. The validity of this hypothesis, founded until comparatively recently on experimental inference, has now been confirmed by direct observation. Utilizing ion-specific microelectrode impalement of MHS myocytes, Lopez and coworkers have demonstrated a rise in myoplasmic free Ca^{2+} to accompany the onset of MH rigor and a fall, induced by dantrolene, to accompany control of the syndrome and relaxation of muscle (Figure 3).[33,34]

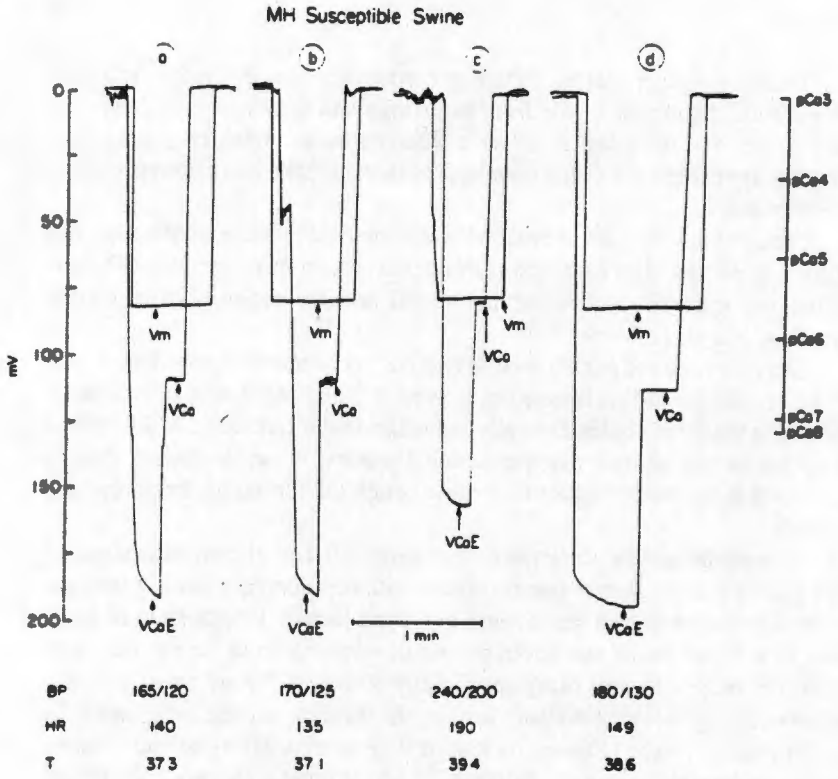

FIGURE 3. Changes in membrane potential, resting myoplasmic free calcium concentration, blood pressure (BP), heart rate (HR), and temperature (T) recorded during MH in MHS swine and its reversal with dantrolene. Responses were recorded (a) before halothane exposure during control anesthesia with thiopentone/fentanyl/N_2O, O_2/pancuronium; (b) after halothane exposure; (c) after reversal of pancuronium effect with muscle contracture well established; and (d) after dantrolene (2 mg/kg). Vm = resting membrane potential (KCl microelectrode); VCa = myoplasmic resting free calcium concentration (VCaE − Vm); and VCaE = potential measured by Ca^{2+}-specific microelectrode (Vm + VCa). (From Lopez et al., *Muscle Nerve*, 11, 82, 1988. With permission.)

The attainment of a threshold concentration in myoplasmic free Ca^{2+}, approximately 6 to 9 × $10^{-7}M$, activates the ATPase-dependent troponin/actin/myosin interaction leading to muscle contraction, soon to persist as contracture when the free Ca^{2+} concentration is not only sustained but rises even higher to $10^{-5}M$.[35] This reaction becomes progressively independent of Ca^{2+} when muscle temperature exceeds 40°C, an event among many that results in the syndrome's ultimate irreversibility.[36]

The early rise in plasma lactate (15- to 20-fold) and modest increase in oxygen uptake,[24,27] which accompany the vast increase in heat output (up to 17-fold),[37] signal the early onset of anaerobiasis. That this occurs in the absence of hypoxia long constituted an enigma.[38] Now, this too is seen as

but one further sequela of the sustained rise in the level of myoplasmic free Ca^{2+}.

The sequestration of Ca^{2+}, for which mitochondria have a high affinity, is an energy consuming, respiration-dependent process that takes primacy over and replaces that of phosphorylation. Exposed to the high Ca^{2+} load, the mitochondria of MHS muscle display only 60% of the ATP-generative capacity of normal muscle.[39,40] *Ab initio*, the increased consumption of ATP by skeletal muscle adenine nucleotide hydrolysis exceeds the mitochondrial capacity for its regeneration. This situation may be worsened late in the syndrome when reduction in muscle blood supply for various reasons may actually reduce available oxygen.[41] The rapid depletion of muscle ATP content is a major factor in the establishment of the vicious cycle that finally becomes irreversible when ATP concentration falls to half the original resting level (Figure 4).[38]

Precise identification of the thermogenic reactions involved and their respective contributions has not been possible. The reason is that we are unable to observe steady-state conditions in metabolism and circulation during the acutely progressing MH syndrome as well as because of uncontrolled heat losses. Factors that have been identified as contributory to the heat generation (equivalent to maximal muscle exertion) are[25,38,42]

1. Hydrolysis of adenine nucleotides
2. Aerobic and anaerobic glycolysis
3. Buffering of the resultant H^+ ion production
4. Entropy of muscle contraction

It is relevant here to mention two attractive early hypotheses of the mechanisms of heat production in MH that are now discounted. Historically, the first serious hypothesis of a pathogenesis for MH[43] proposed the induction of uncoupling of oxidative phosphorylation, a proposal subsequently extensively investigated. The subsequent demonstration by many investigators of normal respiratory control in MHS muscle mitochondria has long since confirmed Wang and coworkers' original objections to this hypothesis on purely theoretical grounds.[44] The bulk of evidence now does not support a role for abnormalities in mitochondrial respiration in the pathogenesis of MH.[45]

The second hypothesis proposed a role for, and produced some evidence of, the futile recycling of fructose $6PO_4$ and fructose 1,6-diphosphate, the mechanism responsible for the preflight warming to operating temperature of bumble bee flight muscle.[46] Sadly, the amount of heat released by the rate of cycling observed in MHS pig muscle was insufficient to account for the heat output observed in MH. Further, the accelerated substrate cycling of glycolysis in MH is not futile but is in response to the additional ATP demands of the Ca^{2+} pumps.[42]

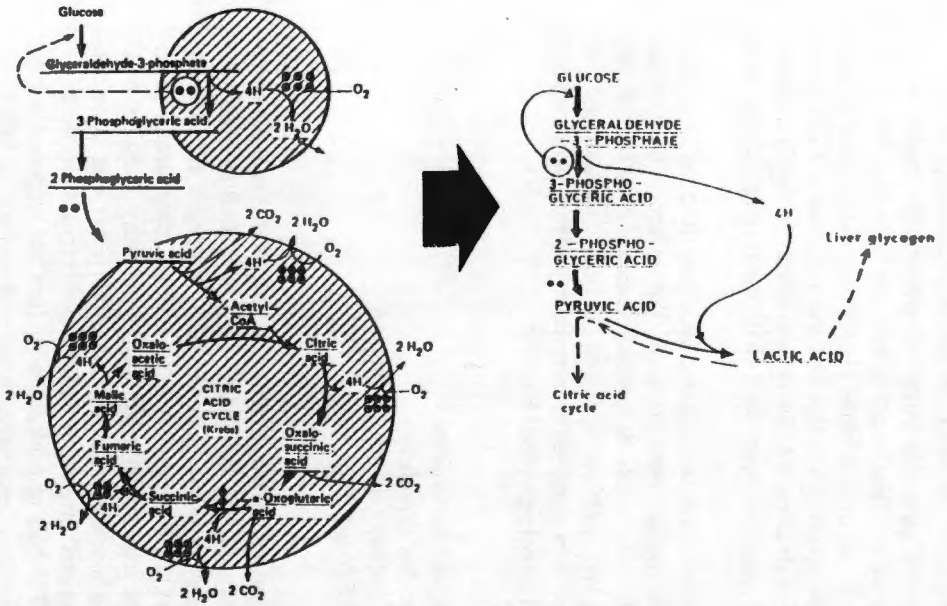

FIGURE 4(A). The key biochemical characteristics of MH, early and severe lactacidosis (despite adequate oxygenation) with concomitant energy substrate — glycogen and ATP — depletion are based on Ca^{2+} load depression of mitochondrial ATP generation, reducing the ATP yield of 1 mol of glucose from 38 with mitochondrial activity to 2 mol without. In this abbreviated diagram of cytosolic (Emden-Meyerhoff) and mitochondrial (Krebs) energy transduction pathways, the latter reactions are enclosed in the shaded circles. Underlining indicates that two molecules are formed from one of glucose. ATP generation is indicated by one black dot/mol. (From Nunn, J. F., *Applied Respiratory Physiology*, 2nd ed., Butterworths, London, Boston, 1977, pp. 378, 379. With permission). **(B)** The effect *in vitro* on ATP concentration of muscle from normal and MHS swine of incubation in Krebs-Ringer solution and exposure to halothane. (From Harrison, G. G., *Porcine Malignant Hyperthermia in Malignant Hyperthermia*, Little, Brown, Boston, 1979, 25. With permission.)

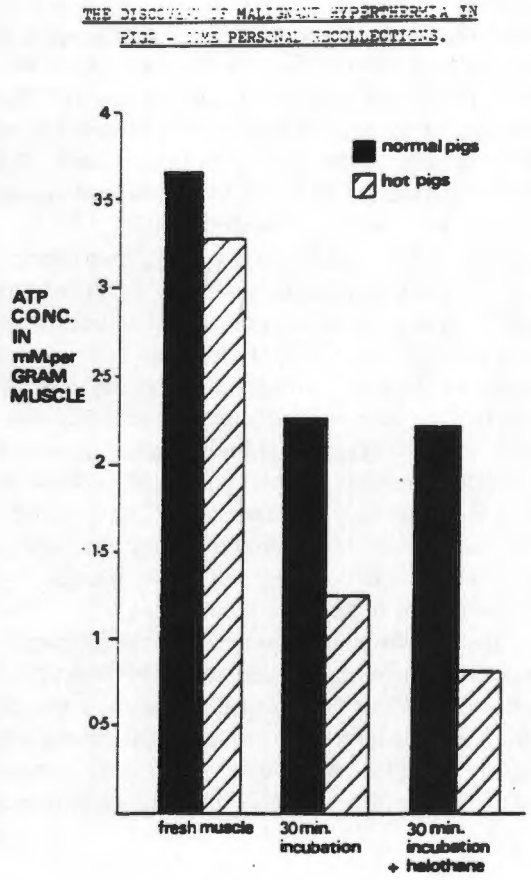

FIGURE 4(B).

A. MYOPLASMIC FREE Ca^{2+}

The abrupt and runaway rise in myoplasmic free Ca^{2+} that drives the events of the MH crisis appears to be the response to triggering of the excitation/contraction/coupling (ECC) mechanisms of skeletal muscle afflicted by genetically induced dysfunctions of one or more of its complex sequential reactions. Indeed, dysfunction has been identified in MHS muscle at many ECC steps and in the organelles involved. some of primary import, others secondarily induced.

B. SARCOPLASMIC RETICULUM

Most relevant to the MH crisis is dysfunction of the sarcoplasmic reticulum (SR), the organelle which plays the principal role in the regulation of ECC myoplasmic Ca^{2+} fluxes. Though studies of the Ca^{2+} transport, uptake, and binding functions of MHS-SR have failed to yield a convincing expla-

nation for the initiation of the MH process, recent characterization of SR Ca^{2+} release mechanisms and their modifications in MHS muscle now seems to provide the basis for an acceptable hypothesis of this event.[45,47]

The phenomenon of calcium-induced calcium release (CICR) exhibited by the triadic terminal cisternal SR of MHS skeletal muscle has been shown to exhibit substantial differences from that of normal muscle. These differences are described and discussed at length in subsequent chapters in this volume and accordingly are briefly summarized here.

This CICR function of MHS-SR displays a lower than normal threshold Ca^{2+} concentration for triggering, together with an enhanced rate of Ca^{2+} release.[35,48-52] Parallel findings have been documented from studies of the ryanodine receptor with which the CICR function has been identified.[53]

It has been suggested by some workers that the observed faster Ca^{2+} release by MHS-SR may be based on a desuppression rather than an actual activation mechanism.[54] This suggestion is based on the observation that in MHS pig SR, Ca^{2+}/CaM-dependent phosphorylation of a 60-kDa protein that normally inhibits CICR release is lower than normal. Responsible for this is a reduction in a 55-kDa subunit of the protein kinase that catalyzes phosphorylation of the 60-kDa CICR inhibitor. The Ca^{2+} permeability of MHS SR has also been shown to be higher than normal.

Associated with these findings, measurements of the SR membrane order parameter, utilizing electron paramagnetic resonance spectroscopy, have demonstrated that halothane, in a less than clinical anesthetic concentration, induces disorder in the MHS SR lipid bilayer to an extent greater than normal. These abnormalities are all enhanced by exposure of the SR to halothane and are uniquely inhibited by dantrolene, the only drug known to control the MH phenomenon.[49-52]

C. THE VICIOUS CYCLE

The rapid establishment of the vicious cycle which characterizes the PMH and SS syndromes can be more readily explained today in the light of recent elucidation of the structure, properties, and function of the ryanodine receptor. This receptor, a tetrameric protein which spans the sarcotubular gap, serves as the principal Ca^{2+} release channel of the SR.[50a] It is encoded by a 15.3 kb gene located in swine on chromosome 6. In all five breeds of swine which manifest it, the MHS phenotype is associated with a single point mutation (bp 1843) of a T/A for C/G.[4] The resultant codon encodes cysteine instead of arginine — a change which renders the ryanodine receptor channel leaky, in degree varying with the homo- or heterozygous expression of the gene. The channel also displays a greater sensitivity (of an order of magnitude) to the action of channel opening agents such as Ca^{2+}, ATP, and caffeine. It is postulated that the anesthetic triggers of MH and/or the endocrine responses to stress could act either directly on this abnormal channel or indirectly by raising the myoplasmsic concentration of physiological channel gating agents. Once opened, this MHS channel is unresponsive to Ca^{2+} and Mg^{2+} induced

closure, so engendering the sustained rise in myoplasmic [Ca^{2+}] which drives the vicious cycle (Figure 5).

The abrupt termination of the syndrome by dantrolene, which itself lacks any effect on SR Ca^{2+} reuptake, implies that continued Ca^{2+} release, as against impairment of SR Ca^{2+} uptake, is fundamental to the genesis of MH. Once this is terminated by dantrolene, the functions of other Ca^{2+}-modulating mechanisms of the SR and mitochondria, are sufficient to return free Ca^{2+} concentration to resting levels — at least early in the syndrome — permitting relaxation of muscle and termination of the syndrome.

D. THE MILIEU OF IRREVERSIBILITY

Though dysfunction of SR itself provides a sufficient basis for the establishment of the MH vicious cycle; other functions (normal) and dysfunctions (peculiar to MHS genotype) of ECC mechanisms and the organelles involved also contribute to the milieu of irreversibility.

A primary role in the initiation of MH is no longer assigned to reduction in SR Ca^{2+} reuptake function, but, secondarily induced, it may well contribute significantly to perpetuation of the syndrome once established.

Inhibition, with ultimate inactivation, of SR Ca^{2+} reuptake occurs in response to the biochemical sequelae of the MH syndrome through three mechanisms.

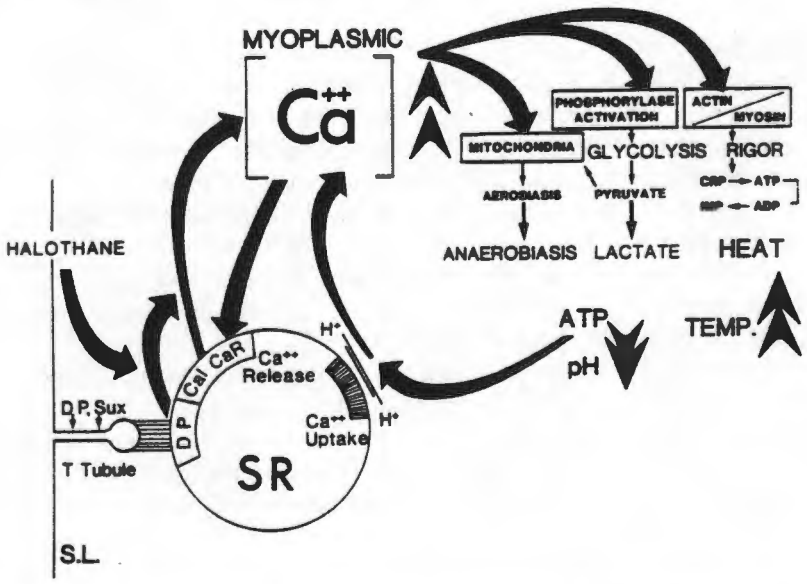

FIGURE 5. The vicious cycle of MH (see text for description): SL = sarcolemma; SR = sarcoplasmic reticulum; DP = depolarization; Sux = Succinylcholine; H^+ = protonation of SR; CICR = calcium-induced calcium release; CRP = creatine phosphate; ATP = adenosine triphosphate; ADP = adenosine diphosphate; IMP = inosine monophosphate; and Temp. = Temperature.

1. Proton or acid inactivation of SR Ca^{2+} transport induced by the early, rapid fall in intracellular pH, the threshold for which is itself raised in the presence of halothane;[55,56] while this phenomenon is a function of normal muscle, in MHS swine it may well be enhanced by reduced lactate clearance consequent on the defective hepatic gluconeogenesis they have been shown to manifest[57]
2. Substrate depletion consequent on the exhaustion of ATP[38]
3. Increase in membrane permeability secondary to the free fatty acid (FFA) release engendered by excessive Ca^{2+}-activated membrane associated phospholipase A_2 activity[58]

E. MITOCHONDRIA

Though a primary role in the pathogenesis of MH for enhanced endogenous levels of calmodulin-dependent phospholipase A_2, as postulated by Cheah[58] is not considered likely,[42] an increase in membrane Ca^{2+} permeability secondary to the FFA engendered by its Ca^{2+} activation (as in 3 above) may well promote the irreversibility of the established syndrome. The consequent failure of the mitochondria as a major intracellular Ca^{2+} sump is then manifested by (1) the reduction (8- to 10-fold) in mitochondrial Ca^{2+} accumulating ability observed in the MHS genotype[59] and (2) the increased rate (normal $\times$ 2) of mitochondrial Ca^{2+} efflux induced by anaerobiasis in MHS swine, an important event in the production of PSEP in MHS slaughter animals.[60]

F. SARCOLEMMA

Though perhaps of more relevance to the triggering of MH, a dysfunction identified in MHS sarcolemma (SL) may also make its contribution toward the establishment of irreversibility. The earlier findings that nondepolarizing neuromuscular blockade prevented succinylcholine triggering of MH and was capable of attenuating that by halothane suggested the importance of continuing SL depolarization as a factor in the triggering of the syndrome by exercise.[1] Associated with this, MHS-SL has been shown to manifest: (1) a lower than normal mechanical threshold, further enhanced by exposure to halothane[61,62] and (2) deficient Ca^{2+} pump activity.[63]

In addition, halothane has been shown to exert a mild depolarizing action on MHS-SL (5 to 15 mV) not apparent on normal SL, an effect countered by dantrolene.[64] Though postulated in the past to play a role in "awake" or anaesthetic-induced triggering,[42] this action has recently been shown to play no part, per se, in halothane induction of contracture in MHS muscle either *in vitro* or *in vivo*.[65]

G. CONTRACTILE PROTEINS

While the above mechanisms may all contribute *ab initio* to the establishment of the vicious cycle, another phenomenon demonstrable in normal muscle plays a part later, once muscle temperature has risen to 40°C and

above. This is thermal inactivation of the Ca^{2+} dependence of the actin/myosin contractile interaction; a reaction which, under the circumstances of MH, is potentiated by the sharp decline in ATP content.[30]

H. OTHER MEMBRANES

Demonstrable dysfunction in the membranous elements of all the organelles involved in muscle ECC suggests the possibility that the MHS/PSS state may be associated with a generalized genetic membrane defect. To date, blood is the only tissue of which studies by many investigators has produced consistent evidence for such a hypothesis.[42]

The finding that MHS swine erythrocytes display an increased osmotic fragility[66-68] has been proposed as support for such a hypothesis. However, the importance of this finding, in a primary sense, to any general concept of the pathogenesis of MH is immediately called in question by the fact that it is not mirrored in the human patient susceptible to MH.[69]

Recently more subtle abnormalities have been identified in the plasmalemma of MHS swine lymphocytes[70,71] and erythrocytes[72,73] that are present also in those of human MHS subjects. These concern a halothane-induced increase in membrane Ca^{2+} permeability associated with evidence derived from electron paramagnetic resonance spectroscopy that halothane induces a greater degree of fluidity in MHS membranes than in those of normal animals. These changes induced by halothane in MHS plasmalemma are of a similar nature to those induced in MHS-SR[49,51,52] with the difference that, whereas in the latter they occur in response to less than clinical anesthetic concentrations of halothane, the former require a concentration of halothane severalfold in excess. While this evidence may strengthen the case for the existence in the MHS state of a more generalized genetic membrane defect involving tissues other than muscle, it is most unlikely — from this evidence — that this plays any part in the pathogenesis of the MH crisis.

VII. THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM

The readiness with which MH can be evoked in MHS swine by excitement, stress, and exercise, considered together with the prominence of tachycardia and other sympathetic phenomena as the first clinical signs of its onset and the correlated increase observed in circulating catecholamines,[27,74] all suggest that activity of the sympathetic nervous system and endogenous catecholamine secretion must play a major role in the genesis of PMH. However, the question of whether sympathetic activity plays a primary role or is a secondary event has long been the subject of controversy.[42]

In spite of the extensive theory developed by Williams and colleagues and Lucke and coworkers incriminating norepinephrine as the primary factor in MH both as a basic abnormality and as a trigger,[75,76] the bulk of evidence favors a secondary role.

This evidence includes the complementary demonstrations that rigor and the biochemical markers of MH were induced by halothane in an isolated perfused MH muscle preparation,[77] that the syndrome was provoked by halothane in totally sympathectomized MHS swine with no rise in circulating catecholamine,[78] and the corollary that the syndrome was not initiated by the infusion into MHS swine of adrenergic agonists.[32,79] Further, as already mentioned (see Section V), the rise observed in circulating catecholamines is documented to chronologically follow the first metabolic changes observed.[27]

Though assigned a secondary role, when viewed in the context of the physiological effects catecholamines have on skeletal muscle — amplification of glycogenolysis energy transduction and 3,5 cyclic AMP-mediated change in the rate of SR Ca^{2+} uptake[80] — activity of the sympathetic nervous system must be accepted as playing an important amplifying role in the development of MH.

VIII. CAPTURE MYOPATHY

As it bears a superficial resemblance to PSS/PMH, a brief consideration of the life-threatening, stress-related syndrome manifested by wild game, particularly ungulates and birds, known as "capture myopathy"[81] is relevant here. Lacking the genetic constraints of PSS, this syndrome is precipitated in normal animals by the alarm, stress, and extreme muscular exertion of the flight from pursuit that precedes capture, or the darting and sedation-induced immobilization necessary for capture, of wild game. It presents as shock, prostration, muscle stiffness, or "spastic paresis", with moderate hyperthermia preceding coma and death. The principal biochemical derangement is a profound lactacidosis which, together with raised serum levels of muscle enzymes, points to the initiation of anaerobic glycolysis in muscle and membrane damage. Postmortem skeletal muscle may have an appearance very similar to PSEP.[82]

However, it has been noted that while capture myopathy does manifest an MH-like. end-stage vicious cycle, this can be aborted by the simple expedient of combating the acidosis alone by the administration of $NaHCO_3$.[83] Further, it can be reliably prevented by adequate precapture sedation with a variety of anxiolytic drugs, if such is achieved with minimal alarm and pursuit stress. It is suggested that, in the circumstances of capture myopathy, entry into the MH-like vicious cycle is by way of proton inactivation of SR Ca^{2+} reuptake (see Section IV.D). The ultimately uncompensated lactacidosis being the result of excessive muscular activity, is also amplified by the effects of the persistently stress-raised levels of circulating catecholamines.

IX. THE TRIGGERING OF PMH

In the description earlier in this chapter of the clinical characteristics of PMH/PSS. attention was drawn to the variations in triggering sensitivity that

are produced by certain circumstances. These were, on the one hand, the enhancement of triggering sensitivity produced by stress, excitement, and exercise and, on the other, its attenuation in response to sedation, anxiolysis, and anesthesia. It is likely that these responses are but a reflection of the induction or inhibition of the capture myopathy scenario in the milieu of MHS muscle with its dysfunctional CICR and other ECC mechanisms. Circumstances most relevant to the enhancement of the sensitivity of triggering would follow from prolonged increases in myoplasmic free Ca^{2+} secondary to continuing multiple depolarizations, accentuated by catecholamine facilitation of prejunctional release and amplification of glycogenolysis.[80]

X. DRUG SCREENING IN MHS SWINE

The identification of drugs with MH-triggering potential depended — at least initially — on anecdote, "personal communication", and unpublished data, often uncorroborated. As ethical considerations prohibited any thought of prospective controlled clinical trials in humans, it is probable in the context of the polypharmacy inherent in modern anesthesia, that many such identifications were not valid. Once incorrectly identified as exhibiting MH-triggering potential, a drug could remain permanently so labeled, its identification as such being copied uncritically from text to text. Objective, retestable data from the pharmacological screening of drugs in MHS swine have ameliorated this situation.

MHS swine can be inbred to display a uniformity of response and sensitivity of the MH-triggering mechanism that is not found in humans.[1,10] Further, in such a model, drugs can be tested singly in contrast to the environment of polypharmacy that pertains in human clinical anesthesia. For the clinical anesthetist, it is of importance to note that human MHS patients react positively to all drugs that test positive as triggers of MH in the MHS pig model, though the converse does not hold.[84] This circumstance is most likely explainable by the above circumstances rather than by inherent species differences in drug response.

In the MHS pig model, the most potent triggers of MH are the newer volatile halogenated alkanes and ethers: halothane, ethrane, isoflurane, and sevoflurane. Of historical interest, so too is chloroform. Fluroxene (now withdrawn) and cyclopropane feature as weak and inconsistent triggering agents.[1] Strangely, methoxyflurane, trichlorethylene, diethyl ether, and nitrous oxide — all documented as MH triggers in the human — do not act this way in swine. Indeed, in swine nitrous oxide fails to display any MH-triggering activity even when administered under hyperbaric conditions.[85, (Harrison and Watson, unpublished data)]

In the pig model, MH triggering has not been ascribed to any of the conventional i.v. anesthetic agents, including ketamine which has been questionably associated with initiation of human MH.[86] Indeed two such agents, althesin (now withdrawn) to a greater extent[20] and thiopentone to a lesser

extent,[87] block or attenuate MH initiation by volatile anesthetics. Propofol, the most recent introduction into clinical anesthetic practice, has tested neutral to MH, neither stimulating nor attenuating its initiation.[88]

Of muscle relaxant drugs, depolarizing succinylcholine alone manifests the potential to trigger the syndrome. In the most highly reactive strains (those that react to halothane alone) succinylcholine alone also initiates the syndrome, though this may require two pulse doses. In those less reactive strains in which the syndrome is not precipitated by exposure to volatile anesthetics alone, succinylcholine acts as the specific added stressor to trigger the syndrome. None of the nondepolarizing relaxants trigger the syndrome, and, as already mentioned, pretreatment with them effectively blocks succinylcholine triggering.[1,84]

The attribution of MH-triggering potential to several drugs by anecdotal clinical association backed, perhaps, by some theoretical consideration of their effects on membrane Ca^{2+} fluxes, has been mentioned. Recent MHS pig screening of some of these drugs has revealed such inference to be invalid in the case of several clinically very useful drugs.

Perhaps first among these is lignocaine and the amide-linked group of local anesthetics (LA). Arguably, the most widely used LA agents in clinical practice and in many hospitals the only local anesthetic agents available, amide-linked LAs have long been documented as putative MH-triggering agents. That this important group of drugs is, in conventional doses, safe to use in the MHS patient has now been validated by a failure to trigger MH in the pig model with amide-linked LAs infused i.v. to monitored toxic levels.[89,90]

Digitalis is another such drug. In a sophisticated MH pig model utilizing cardiopulmonary bypass to obviate the drug's cardiotoxic effects, Gronert and coworkers[91] have demonstrated that digitalis infused i.v. to supratoxic, cardiac arresting levels (61 ng/ml) lacks any effect on whole body metabolism, and by inference that of skeletal muscle.

Similarly, in the same preparation $CaCl_2$ infused to cardiac arresting levels (15 mEq/l) was also shown to lack the MH-triggering potential it was long assumed to have.

Last, and perhaps most importantly, the MHS pig model has served as the pharmacological test bed in which the strange specificity of dantrolene in the control and prevention of MH was established.[92] The demonstration in rats by Ellis and coworkers[93] that the skeletal muscle relaxing properties of dantrolene stemmed from a selective depressive action on the ill-understood intrinsic mechanisms of ECC motivated his suggesting its therapeutic trial on the MH syndrome established in susceptible swine. In these animals, dantrolene proved to be the only drug capable of reliably terminating the MH syndrome and of completely blocking its inception by volatile anesthetics and/or succinylcholine. As triggering agents switched MH on in the susceptible animal, so dantrolene switched it off. These findings were subsequently

successfully applied to the treatment of human MH.[92] Dantrolene, we now know, binds to and acts on the SR and T-tubular membranes, reducing the rate and amount of calcium release from the former, altering the T-tubular/SR coupling "charge movements" of the latter and reducing at least the halothane component of CICR.[53,54]

These actions of dantrolene, superficially those of a Ca^{2+} channel blocker, are exceedingly complex and are still poorly understood. They are clearly distinguishable from those of the conventional Ca^{2+} channel blocking drugs, e.g., verapamil, nifedipine, diltiazem, etc., which act at an SL site and have no effect on MH,[94] and also the classic SR Ca^{2+} channel blockers, e.g., procaine and amethocaine, which do have a blocking but poor therapeutic effect on MH.[92] This virtual specificity of the actions of dantrolene in preventing and controlling the MH syndrome carries with it the implication that on their elucidation depends our final concept of the pathogenesis of the syndrome.

XI. FUTURE DIRECTIONS

Since its original identification in 1960, MH has provided a continuing stimulus for research by investigators in many disciplines, from the clinical scientist to the membrane chemist. Now that these endeavors have brought a worldwide awareness of the condition, a basic broad understanding of its pathogenesis, and a proven specific pharmacological cure, one may well question the direction of future research relevant to MH. For the clinician, priority must surely be given to the development of a diagnostic test for susceptibility to MH that is noninvasive and widely available, yet specific and sensitive. For the biological scientist, there remains the more intricate task of further unraveling the molecular mechanisms of SR calcium release. The solution to these problems in the future will doubtlessly come from the continued study of MH in swine.

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SCREENING OF CERTAIN ANAESTHETIC AGENTS FOR THEIR ABILITY TO ELICIT ACUTE PORPHYRIC PHASES IN SUSCEPTIBLE PATIENTS

G. H. Blekkenhorst, G. G. Harrison, E. S. Cook and L. Eales

SUMMARY

The activity of δ-aminolaevulinic acid synthetase (E.C. 2.3.1.37) (ALA–S) was measured in rat liver after the simultaneous administration of various anaesthetic agents and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) *in vivo*. Flunitrazepam, Althesin and phenobarbitone caused a significant increase in the activity of the enzyme which was not observed with propanidid, etomidate and minaxolone. It is suggested that DDC-treated rat, which resembles latent human variegate porphyria, may be a more valid method of testing drugs for their ability to elicit acute porphyric phases in susceptible individuals. The anaesthetic agents which induced the activity of hepatic ALA–S in this model are not recommended in patients with genetic hepatic porphyria.

The porphyrias are a group of disorders of haem metabolism characterized by specific patterns of haem precursor overproduction, accumulation and excretion, each pattern defining a particular form of porphyria. Variegate, or South African genetic porphyria, is one of the forms particularly common in southern Africa. It has been estimated that there are more than 8000 susceptible individuals (Dean, 1971) with a frequency of 1 in 250 in one region. The acute phase of this condition and of two other forms of the hereditary "hepatic" porphyrias, acute intermittent porphyria and hereditary coproporphyria, may be life-threatening. It is usually caused by exposure of the susceptible individual to drugs, particularly barbiturates (Eales, 1971). The frequency of acute porphyric attacks in South Africa has been reduced dramatically by effective family surveys, when asymptomatic but biochemically positive members of families have been warned to avoid precipitating agents (Eales, 1971). Identification of drugs which are porphyrogenic is also necessary to prevent life-threatening acute porphyric crises. Previously this has been largely a process of trial and error or anecdote, but it is now possible to identify the porphyrogenicity of drugs in the laboratory.

Rats given a relatively low dose of 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) exhibit a condition which resembles latent human variegate porphyria. The animals become sensitive to drugs which can precipitate the metabolic disorder such that, biochemically, the reaction is typical of the human attack (De Matteis, 1973). We have used the DDC-primed rat to test the porphyrogenicity of phenobarbitone and the i.v. anaesthetic agents Althesin, etomidate, flunitrazepam, propanidid and minaxolone by determination of the activity of hepatic δ-aminolaevulinic acid synthetase (E.C. 2.3.1.37) (ALA–S). This is the initial and rate-limiting enzyme of haem biosynthesis (Granick and Urata, 1963) and is increased in the three hereditary "hepatic" porphyrias (Elder, Gray and Nicholson, 1972; Meyer and Schmid, 1973).

MATERIALS AND METHODS

Male Wistar rats (weight 180–220 g) were starved for 24 h but water was freely allowed. In each experiment the five animals in the control group each received arachis oil 1 ml; the second group of five received the drug under study; a third group of five received DDC 100 mg kg^{-1} suspended in arachis oil and the fourth group received DDC 100 mg kg^{-1} simultaneously with the drug under study. The drugs were administered i.p. in the doses: phenobarbitone (Gardenal, May and Baker, Port Elizabeth, South Africa) 50 mg kg^{-1}; propanidid (Fabantol, Bayer, Leverkusen, Germany) 100 mg kg^{-1}; etomidate (Ethnor, Halfway House, South Africa) 5 mg kg^{-1}; flunitrazepam (Rohypnol, Roche, Isando, South Africa) 0.5 mg kg^{-1}; Althesin (Glaxo–Allenbury, Wadeville, South Africa) 12 mg kg^{-1} and minaxolone (Glaxo Group Research, Greenford, Middlesex, England)

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15 mg kg^{-1}. The volume of the drug injected was usually 1 ml and drugs were diluted if necessary in sodium chloride solution (0.15 mmol litre; saline).

After 17 h, the animals were sacrificed by decapitation, exsanguinated and the livers removed, blotted free of blood and washed with ice-cold saline. For measurement of ALA-S, the liver was weighed, chopped into small pieces, washed twice with ice-cold saline and homogenized in Tris : HCl buffer 0.01 mol litre^{-1}, pH 7.4 containing sodium chloride 0.15 mmol litre^{-1} and EDTA 0.5 mmol litre^{-1} using a motor-driven Teflon pestle in a glass mortar to give a 10% (w/v) homogenate. The assays for ALA-S were performed as described previously (Pimstone, Blekkenhorst and Eales, 1973) using a modification of the method of Strand and others (1972b).

The significance of difference between mean values was assessed by Student's t test.

RESULTS

A preliminary experiment established that a dose of DDC 100 mg kg^{-1} gave a maximal increase in ALA-S activity when administered with phenobarbitone, a known porphyrogen (Eales, 1971). When administered alone, this dose of DDC gave only a modest increase in ALA-S activity.

The activity of hepatic ALA-S in rats treated with the anaesthetic agents together with DDC is shown in figure 1. Phenobarbitone and DDC caused an increase in the activity of the enzyme but no change was observed in the activity when propanidid and DDC were administered compared with the activity of ALA-S when DDC alone was given. No significant difference in hepatic ALA-S activity was observed when etomidate and minaxolone were given together with DDC, but flunitrazepam and Althesin given with DDC caused an increase in the activity of hepatic ALA-S ($P < 0.05$).

The activities of the enzyme in rats treated with only the drug under test were in all cases found to be not significantly different from those in rats receiving oil only.

DISCUSSION

The results of this study demonstrate that phenobarbitone and the anaesthetic agents flunitrazepam and Althesin, when given simultaneously with DDC to rats cause a significant increase in the activity of hepatic ALA-S compared with the activity of the enzyme when DDC is given alone. On this basis, these drugs must be regarded as porphyrogenic and contraindicated in patients with genetic porphyria.

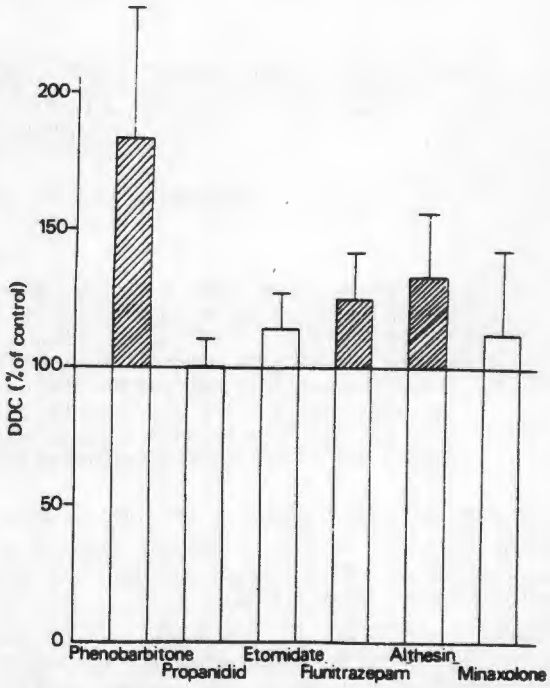

FIG. 1. Hepatic δ-aminolaevulinic acid synthetase (ALA-S) activity in male rats treated simultaneously with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) and various drugs. Each result represents the mean for five animals; bars indicate SD. One hundred per cent of DDC control = ALA 479 ± SD 58 (n = 10) nmol formed per hour per gram of liver. Hatched bars indicate a significant difference ($P < 0.05$) when compared with DDC controls.

A similar investigation (Parikh and Moore, 1978) showed that repeated doses of various anaesthetic agents, including Althesin and etomidate, increased hepatic ALA-S activity. The experimental approach adopted by these workers may not be correct to screen drugs for their porphyrogenicity, since it appears that increased activity of hepatic ALA-S results from the coexistence of two requirements, each of which alone may have little or no effect on the enzyme. These are: first, an effect on haem synthesis and, second, exposure to lipid-soluble inducers of haemoprotein synthesis (Maxwell and Meyer, 1978). Thus when rats are primed with small doses of DDC which cause a partial block at the ferrochelatase level (De Matteis, 1973), and a lipid-soluble inducer of haemoprotein synthesis such as phenobarbitone is administered, a potentiation of ALA-S activity is observed. However, single doses of

the inducing drugs administered to animals with an intact haem biosynthetic pathway have little effect on hepatic ALA-S activity (De Matteis, 1978; Maxwell and Meyer, 1978).

Similarly, inducing drugs can superimpose their effects on ALA-S in the hereditary hepatic porphyrias. The basis genetic defects in the haem biosynthetic pathway have been shown to be decreased uroporphyrinogen synthetase in acute intermittent porphyria (Strand et al., 1972a), decreased coproporphyrinogen oxidase in hereditary coproporphyria (Elder et al., 1977) and a decreased ferrochelatase activity (Becker et al., 1977) and decreased protoporphyrinogen oxidase, or both (Brenner and Bloomer, 1979), in variegate porphyria. When these drugs are administered to patients with the metabolic defect, the acute porphyric phase becomes manifest, with an inappropriate increase in hepatic ALA-S activity (Meyer and Schmid, 1973).

The determination of hepatic ALA-S in the DDC-primed rat, which biochemically resembles latent variegate porphyria, appears to be a reliable technique in screening drugs for their potential porphyrogenicity. Phenobarbitone is the classic porphyrogenic agent, while propanidid is known from clinical use to be free of this disadvantage. The inclusion of these drugs among those tested serves to an extent as a control of the method. This method appears more sensitive than determination of hepatic haem precursors after administration of DDC and drugs (Eales and Blekkenhorst, 1978), when large variations in the individual responses of rats to the drugs were observed. It should be pointed out, however, that induction of ALA-S by drugs is a dose-related phenomenon, and a large species variation in the therapeutic or toxic effects of chemical substances exists. Furthermore, there is an interspecies variation in drug metabolism and pharmacological response especially to liposoluble drugs (Brodie and Reid, 1971), all of which must be considered when extrapolating these findings to man. In the present experiments, large doses were used, but less than the known lethal doses of the anaesthetic agents. The *in vivo* screening technique presented here is more valid than the exquisitely sensitive chick embryo liver cell culture (Granick, 1966) and chick embryo *in vivo* (Anderson, 1978) screening systems, in which metabolites which may augment synthesis of ALA-S cannot be readily excreted. It is conceivable that some drugs classed as porphyrogenic using these methods might be safe to use in the hereditary hepatic porphyrias.

In this investigation of the porphyrogenicity of newer i.v. anaesthetic agents, we conclude that etomidate and minaxolone are probably non-porphyrogenic in susceptible patients, whereas Althesin and flunitrazepam are likely to be dangerous. Nevertheless, the ultimate test of porphyrogenicity of these anaesthetics can only be the response of individuals who suffer genetic porphyria to administration of these agents.

ACKNOWLEDGEMENT

We thank the South African Medical Research Council for financial support.

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DEPISTAGE DE CERTAINS AGENTS ANESTHESIANTS SUSCEPTIBLES DE PROVOQUER DES PHASES PORPHYRIQUES AIGUES SUR DES MALADES SUJETS A CE GENRE DE REACTION

RESUME

On a mesuré l'activité de l'acide δ-aminolévulinique synthétase (E.C. 2.3.1.37) (ALA-S) dans le foie d'un rat après l'administration simultanée de divers agents anesthésiants et de 3,5-diéthoxycarbonyle-1,4-dihydrocollidine (DDC) *in vivo*. Le flunitrazépam, l'Althésine et le phénobarbitone ont provoqué une augmentation significative de l'activité de l'enzyme, ce que l'on n'a pas observé avec le propanidide, l'étomidate et le minaxolone. Ceci laisse penser que le rat traité au DDC, lequel ressemble à la porphyrie humaine diversicolore latente, peut constituer une méthode plus valable d'essayer les médicaments susceptibles de provoquer des phases porphyriques aiguës sur les individus sujets à ce type de réaction. Les agents anesthésiants qui ont provoqué l'activité de l'ALA-S hépatique dans ce modèle ne sont pas recommandés pour les malades souffrant de porphyrie hépatique génétique.

PRÜFUNG GEWISSER NARKOSEMITTEL AUF IHRE EIGENSCHAFT, AKUTE PORPHYRIEPHASEN IN ANFÄLLIGEN PATIENTEN HERVORZURUFEN

ZUSAMMENFASSUNG

Die Aktivität von δ-aminolävulinische Säure-Synthetase (E.C. 2.3.1.37) (ALA-S) wurde in der Rattenleber gemessen nach gleichzeitiger Verabreichung verschiedener Narkosemittel und von 3,5-Diethoxycarbonyl-1,4-dihydrocollidin (DDC) *in vivo*. Flunitrazepam, Althesin und Phenobarbiton bewirkten einen wesentlichen Aktivitätsanstieg des Enzyms, der bei Propanidid, Etomidat und Minaxolon nicht beobachtet wurde. Man nimmt an, dass eine mit DDC behandelte Ratte, die eine menschenähnliche Porphyrie aufweist, eine gültigere Methode zur Überprüfung von Drogen auf ihre Eigenschaft hin darstellt, akute Porphyriephasen bei anfälligen Individuen hervorzurufen. Die Narkosemittel, die in diesem Modell die Aktivität von hepatischer ALA-S hervorriefen, werden für Patienten mit genetischer hepatischer Porphyrie nicht empfohlen.

SELECCION DE CIERTOS AGENTES ANESTESICOS CON ARREGLO A SU HABILIDAD PARA ELUCIDAR LAS FASES PORFIRICAS EN PACIENTES SUSCEPTIBLES

SUMARIO

Se midió la actividad de la sintetasa de acido δ-aminolevulínico (E.C. 2.3.1.37) (ALA-S) en el hígado de rata, después de la administración simultánea de varios agentes anestésicos y de 3,5-dietoxicarbonil-1,4-dihidrocollidina (DDC) *in vivo*. El flunitrazepam, la Altesina y la fenobarbitona ocasionaron un incremento significativo en la actividad de la enzima, que no se observó con la propanidida, etomidate ni con la minaxolona. Parece ser que el tratamiento de la rata con DDC, que se asemeja a la variedad de porfiria latente en el ser humano, es un método de mayor validez para la verificación de drogas respecto a su habilidad para elucidar las fases porfíricas agudas en individuos susceptibles. Los agentes anestésicos que indujeron la actividad del ALA-S hepático en este modelo, no se recomiendan para pacientes con porfiria hepática de tipo genético.

Br. J. Anaesth. (1985), 57, 420–423

PORPHYRINOGENICITY OF ETOMIDATE AND KETAMINE AS CONTINUOUS INFUSIONS

Screening in the DDC-primed Rat Model

G. G. HARRISON, M. R. MOORE AND P. N. MEISSNER

SUMMARY

The porphyrinogenicity of etomidate and ketamine administered as continuous i.v. infusions was screened in the DDC-primed rat model of latent variegate porphyria. Ketamine produced no change from control in 5-aminolaevulinate synthase (ALAs) activity and haem intermediate production in either untreated or DDC-primed rats, and would appear to be safe for use in the patient with genetic porphyria. Etomidate, while producing no significant changes in these parameters in untreated rats, caused a statistically significant 47% increase in hepatic ALAs activity with a corroborative 85% increase in coproporphyrin and a 40% increase in protoporphyrin content, in DDC-primed rats. On these grounds, etomidate must be regarded as potentially porphyrinogenic when administered as a continuous infusion for total i.v. anaesthesia.

The acute porphyrias are a group of inherited metabolic diseases, acute attacks of which are often triggered by the use of certain drugs (Moore and Disler, 1983). Prevention of life-threatening crises of acute porphyria in genetically susceptible individuals depends on identification of those individuals, and identification of porphyrinogenic drugs. In the past, information on the latter was largely anecdotal, but additional objective evidence can now be obtained by observing, in liver homogenates of experimental animal models, the effects of the drug in question on 5-aminolaevulinate synthase (ALAs) activity and the production of the haem biosynthetic porphyrin intermediates (Parikh and Moore, 1978; Blekkenhorst et al., 1980).

Prominent among known porphyrinogenic drugs are the barbiturates most commonly used for induction of general anaesthesia. Of the recently introduced i.v. anaesthetic induction agents, two (Althesin and flunitrazepam (Rohypnol)) have now also been incriminated, while animal screening of a third (etomidate) has produced results that are suggestive of porphyrinogenicity (Disler et al., 1982). Parikh and Moore (1978) also had concluded that this drug was porphyrinogenic, following their demonstration in the rat that repeated i.p. doses of etomidate caused an increase in hepatic ALAs activity. However, in the study of Blekkenhorst and colleagues (1980), in the 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC)-primed rat model which simulates latent variegate porphyria, the mean increase in hepatic ALAs activity that followed single dose i.p. administration of etomidate just failed to achieve statistical significance. In humans, no corroborative examples of acute porphyria precipitated by etomidate have yet been reported.

The short disposal half-life of etomidate makes it particularly suitable for use not only for i.v. induction of general anaesthesia when administered as a single dose, but also for total i.v. anaesthesia when administered as a continuous i.v. infusion. In view of this and the above findings, it seemed to us important to extend the screening of etomidate for porphyrinogenicity from single dose administration to continuous i.v. infusion in the DDC-primed rat model.

Control conditions of total i.v. anaesthesia were produced by continuous infusion of ketamine, a drug generally regarded as non-porphyrinogenic

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(Disler et al., 1982).

METHODS

Male Wistar rats (weight 200–300 g) were submitted to i.v. anaesthesia for 6 h by continuous infusion of the test drug to a cannulated femoral vein by means of a mechanically driven syringe pump.

Twelve rats were anaesthetized with etomidate and 12 with ketamine, the dose of anaesthetic having been established empirically as that which maintained adequate depth of anaesthesia without causing anaesthetic death. With etomidate, 4 mg i.p. was adequate for anaesthesia for femoral vein cannulation, whereafter anaesthesia was maintained by continuous i.v. infusion of 10–15 mg kg^{-1} h^{-1}. With ketamine the doses were 100 mg kg^{-1} i.p. and 10–15 mg kg^{-1} h^{-1}, respectively. Both drugs were diluted so that the volume of fluid administered approximated 2 ml h^{-1}.

Spontaneous respiration of air was maintained throughout anaesthesia. Experiments were conducted using pairs of rats one from each group anaesthetized simultaneously. Six animals in each group were treated for 3 days before anaesthesia with DDC administered orally at a dose of 50 mg kg^{-1} dissolved in 1 ml of corn oil. All animals were starved for 24 h before anaesthesia, but were permitted water *ad libitum*.

After 6 h of anaesthesia, animals were sacrificed and their livers removed, blotted free of blood and washed with ice-cold saline. Hepatic ALAs activity was assayed and full porphyrin analysis of hepatic homogenates carried out.

Assays

ALAs activity was measured by the method of Moore and others (1980), in which the production of ^{14}C-labelled ALA from ^{14}C-labelled glycine is measured after separation of ALA by high voltage electrophoresis. Incubations were carried out using liver homogenate at 37 °C for 1 h.

Porphyrin analysis. Porphyrins were measured, using HPLC, by the method of Seubert and Seubert (1982). The methyl esters of porphyrins used as standards were obtained from Porphyrin Products (Logan, Utah) and dissolved in chloroform. The separations were performed on a Waters liquid chromatograph with a Model 6000-A solvent delivery system, a Model 710-B WISP automatic injector, a Model RCM-100 module and Radial-PAK silica cartridges, 10-μm particle size and a Model 420-AC fluorescence detector (Waters Associates, Milford, Mass.). The chromatograms were evaluated with a Waters Model 730 Data Module.

Liver samples for porphyrin analysis were prepared as follows: known weights of liver were esterified overnight, in the dark at room temperature in 10 volumes of 5% sulphuric acid in methanol solution. After centrifugation the supernatant was neutralized with 5% ammonia solution in a separating funnel where the porphyrin esters were extracted into chloroform, washed with distilled water and dried by draining over anhydrous sodium sulphate. Before injection to the chromotograph, each sample was evaporated to dryness in a Bucchi rotary evaporator and redissolved in exactly 1 ml of chloroform.

TABLE I. *Untreated rats anaesthetized for 6 h with ketamine or etomidate. No value was significantly different from control (unanaesthetized)*

	ALAs (mmol h^{-1}/g protein)	Uroporphyrin (mmol/g liver)(wet)	Coproporphyrin (mmol/g liver)(wet)	Protoporphyrin (mmol/g liver)(wet)
Unanaesthetized				
n	12	3	3	3
Mean	57.5	0.0075	0.2314	0.8046
SD	21.0	0.0044	0.1116	0.0814
Ketamine				
n	6	5	5	5
Mean	53.0	0.02672	0.2193	0.7613
SD	22.0	0.0450	0.1293	0.1936
Etomidate				
n	6	5	5	5
Mean	69.0	0.0487	0.2975	0.9115
SD	12.0	0.0584	0.1000	0.2936

TABLE II. *DDC-primed rats anaesthetized for 6 h with ketamine or etomidate. *Significant difference from control (unanaesthetized)*

	ALAs (mmol h^{-1}/g protein)	Uroporphyrin (mmol/g liver)(wet)	Coproporphyrin (mmol/g liver)(wet)	Protoporphyrin (mmol/g liver)(wet)
Unanaesthetized				
n	6	3	3	3
Mean	426.1	0.0271	0.679	9.2536
SD	122.3	0.0238	0.1709	1.0582
Ketamine				
n	6	5	5	5
Mean	473.0	0.0544	0.6950	9.1044
SD	77.0	0.7123	0.2799	1.0369
Etomidate				
n	6	5	5	5
Mean	625.0	0.2869	1.2598	12.9715
SD	105.0	0.2666	0.4452	1.3720
*P**	<0.02	ns	<0.05	<0.01

Control data were obtained from similar assays undertaken on six unanaesthetized rats, half of which were pretreated with DDC. The significance of differences between sample means was established by means of Student's *t* test.

RESULTS

Anaesthesia with ketamine caused no significant change when compared with unanaesthetized controls in either untreated (table I) or DDC-primed (table II) rats. Hepatic ALAs activity and porphyrin content were the same in both groups.

By contrast, 6 h of etomidate anaesthesia, which produced no change from control in untreated animals (table I), caused a marked increase in all these parameters in DDC-primed rats (table II, fig. 1). The 47% increase in hepatic ALAs activity and corresponding 85% increase in coproporphyrin and 40% increase in protoporphyrin content were statistically significant. The almost 10-fold increase in uroporphyrin failed to achieve statistical significance because of the large variance in the values.

DISCUSSION

The essential criterion for judging the porphyrinogenicity of a drug in the DDC-primed rat model of latent variegate porphyria is a demonstrable increase in hepatic ALAs activity in response to administration of the drug (Blekkenhorst et al., 1980). On these grounds we must infer from our experiments that etomidate is potentially porphyrinogenic. The 47% increase in ALAs activity observed in response to etomidate was accompanied by a corroborative increase in resultant haem precursors. The failure of the observed increase in uroporphyrin to achieve statistical significance reflects the large variation in haem precursor

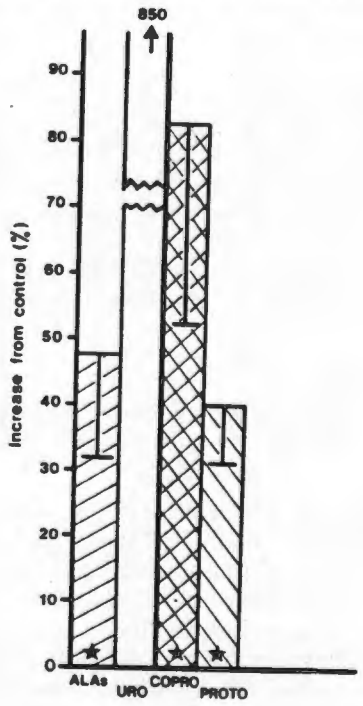

FIG 1. Effect of a 6-h infusion of etomidate on 5-aminolaevulinate synthase (ALAs) activity and haem intermediates in DDC-primed rats. 0 = control. URO = uroporphyrin; COPRO = coproporphyrin; PROTO = protoporphyrin. *Significantly different from control. Vertical bar = standard error of difference.

response to drug administration, previously observed by Eales and Blekkenhorst (1978), and the small numbers of animals used in the present trial.

Identification of a porphyrinogenic drug in an animal model is far removed from proof that it is potentially dangerous to the human sufferer from genetic hepatic porphyria. We identify only that, in this particular animal model, the drug in question displays a property known to characterize those drugs which do precipitate crises in susceptible humans. Drug metabolism differs profoundly between species, and even animals so apparently similar as rats and mice display marked variation in the response of their haem biosynthetic pathway to the effects of various drugs.

Final proof of the porphyrinogenicity of a drug should come from a clinical report of the initiation of an acute crisis in a susceptible individual following exposure to the drug. Regrettably, even this premise may be false—there are well documented inconsistencies.

Disler and colleagues (1982) devised the following simple classification of drugs to guide clinicians in the assessment of porphyrinogenicity:

Category A Drugs reported in terms of clinical experience as dangerous or safe by three or more authorities. Category B As above but only two or fewer authorities.	Associated with corroborative experimental animal data
Category C Drugs evaluated only in the experimental rat model. Category D Drugs evaluated in chick embryo liver cell culture or "*in ovo*".	No corroborative reports of human cases

On the basis of our present observations and those of Parikh and Moore (1978), etomidate qualifies for a "C" rating. From this we infer that, although its use in susceptible individuals cannot be definitely contraindicated, it should be so used only when no convenient alternative is available. Thereafter, the patient should be observed and appropriately screened for a period, using biochemical tests, in order to detect any nascent crisis in good time.

Although ketamine has been implicated as porphyrinogenic in a single case report (Wetterberg, 1976), it is considered safe by many authorities (Rizk, Jacobson and Silvay, 1977; Parikh and Moore 1978; Silvay, Miller and Tausk, 1979). Our present study supports this belief in its safety for use in the patient with porphyria.

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FOCUS ON INFUSION

INTRAVENOUS ANAESTHESIA*

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SPECIAL PATIENT GROUPS II

Chairman — *Dr F Servin (France)*

Propofol anaesthesia in pharmacogenetic states

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Two of the pharmacogenetic conditions of direct interest to the anaesthetist are of particular concern because their abnormal drug responses may be immediately life-threatening. These conditions are malignant hyperthermia susceptibility and the acute porphyrias. For each, the policy for the prevention of crises dictates: (1) identification of the susceptible individual, and (2) identification of the drugs which provoke an abnormal reaction and, the corollary, identification of the drugs that are safe to use. With this view, I will review the response to propofol of individuals subject to these two conditions.

Malignant hyperthermia susceptibility (MHS)

Introduction

Susceptibility to this covert myopathy, which is autosomally dominant, is identifiable in relatives of those who have suffered malignant hyperthermia (MH) syndrome by *in vitro* contracture testing (IVCT) of biopsied skeletal muscle. The anaesthetic agents and adjuvants which trigger the syndrome include all volatile agents — in particular, the halogenated compounds — and the non-depolarizing muscle relaxant, succinyl choline or suxamethonium. Triggering of MH has not been attributed to any of the intravenous anaesthetic drugs, but three have been shown to influence it [1]: (1) etomidate may shorten onset time, (2) thiopentone may lengthen it, and (3) 'Althesin' blocks it — a property which made this drug the anaesthetic of choice in the MHS patient. The introduction of propofol into clinical practice contemporaneously with the withdrawal of 'Althesin' due to its vehicle, 'Cremophor', suggested it as an obvious replacement.

The first objective evidence that propofol was likely to be safe in MHS individuals was provided by Denborough and Hopkinson's brief report in 1988 [2] of propofol's lack of effect on IVCT of muscle biopsied from two MHS swine and two MHS humans. Further evidence to support this conjecture was added in 1989 by the simultaneous publications of Krisovic-Horber *et al.* [3] and ourselves [4], which reported the negative *in vivo* response to propofol of batteries of MHS swine. We concluded from this evidence that it would be safe to proceed with a clinical trial of propofol in human MHS patients.

Methods

Over the past three years, all patients presenting to our unit for muscle biopsy for diagnostic IVCT for susceptibility to MH, have been anaesthetized according to a standard protocol which included the use of propofol for the induction and maintenance of anaesthesia by continuous infusion. Included also were Valium premedication, alfentanyl analgesia, atracurium neuromuscular block (NMB), endotracheal intubation and IPPV with N_2O/O_2 (FiO_2=0.3) and NMB reversal with neostigmine/glycopyrrolate. Vital function monitoring included ECG and automated non-invasive blood pressure recording, capnography, inspired gas and pulse oximetry, and nasal thermometry. Dantrolene was immediately available in the operating room.

Vital function signs of MH were specifically looked for, including (1) rise in $FECO_2$; (2) fall in SaO_2; (3) signs of sudden sympathetic excitation (tachycardia and hypertension); (4) muscle rigidity; and (5) rise in core temperature.

Results

Of 21 patients anaesthetized for muscle biopsy for IVCT, 10 displayed the IVCT criteria of MHS [5]. The time course of operation and anaesthesia ranged from 60–90 minutes. No patient displayed any evidence of MH triggering, nor were any other complications noted.

Conclusions

These observations, considered together with the cited *in vitro* and *in vivo* MHS swine data and other recent clinical reports [6,7,8], allow us to conclude that propofol may be used safely to anaesthetize individuals genetically susceptible to MH.

The acute porphyrias

Introduction

This group of conditions results from autosomally dominant hereditary dysfunction of various enzymes concerned with haem biosynthesis [9]. Three clinical entities are of concern to the anaesthetist, in that drug exposure may precipitate an acute attack. These conditions are (1) acute intermittent (Swedish) porphyria (AIP); (2) variegate (South African) porphyria (VP); and (3) hereditary coproporphyria (HCP) — the rarest.

In these conditions, acute crises occur when stimulation of haem synthesis in the presence of the dysfunctional enzyme causes concentrations of precursors, especially alpha-amino-laevulinic acid (ALA) and porphobilinogen (PBG), to rise steeply. Drugs which precipitate crises, do so by stimulating the activity of ALA synthetase (ALAs) — the rate-limiting enzyme. This stimulation is usually the indirect result of a reduction in the level of haem — the negative feed-back repressor of ALAs — but some drugs do have a direct stimulant action on ALAs.

As was the case with MH, propofol was introduced into practice at about the time when propanidid (the only intravenous induction agent safe for porphyrics) was withdrawn from practice due to reactions to its vehicle, Cremophor.

The demonstration in an animal model that propofol was non-porphyrinogenic [10], motivated our assessment of its potential for safe clinical use in human subjects susceptible to the acute porphyrias. A multicentre, prospective clinical trial was conducted using propofol as the intravenous anaesthetic induction agent in 13 sufferers from VP and 21 non-porphyric control patients who presented for surgery. The results of this trial have now been published [11]. All patients were monitored clinically and biochemically immediately pre- and post-anaesthetic for up to five days. The biochemical parameters monitored included the concentrations of urinary porphyrins and their precursors, ALA and PBG, as well as the porphyrin content of plasma and stools. A statistical comparison was made of the concentrations of these compounds measured pre-anaesthetic (day 0) and on days 1, 3 and 5 post-anaesthetic (Fig. 1).

While ALA, PBG and porphyrin concentrations in the group of porphyric patients were at all times significantly higher than those in the control group, at no stage did they exceed the limits established (in our laboratory) for VP patients in remission. Nor was any significant increase over baseline levels of porphyrin precursor or porphyrin output found post-anaesthetic in either the porphyric or control groups of patients. No adverse clinical reactions suggestive of acute attack were noted post-anaesthetic in any porphyric patient.

We concluded from these observations that propofol is safe to use as an anaesthetic induction agent in patients suffering VP and probably, therefore, in patients subject to the other acute porphyrias.

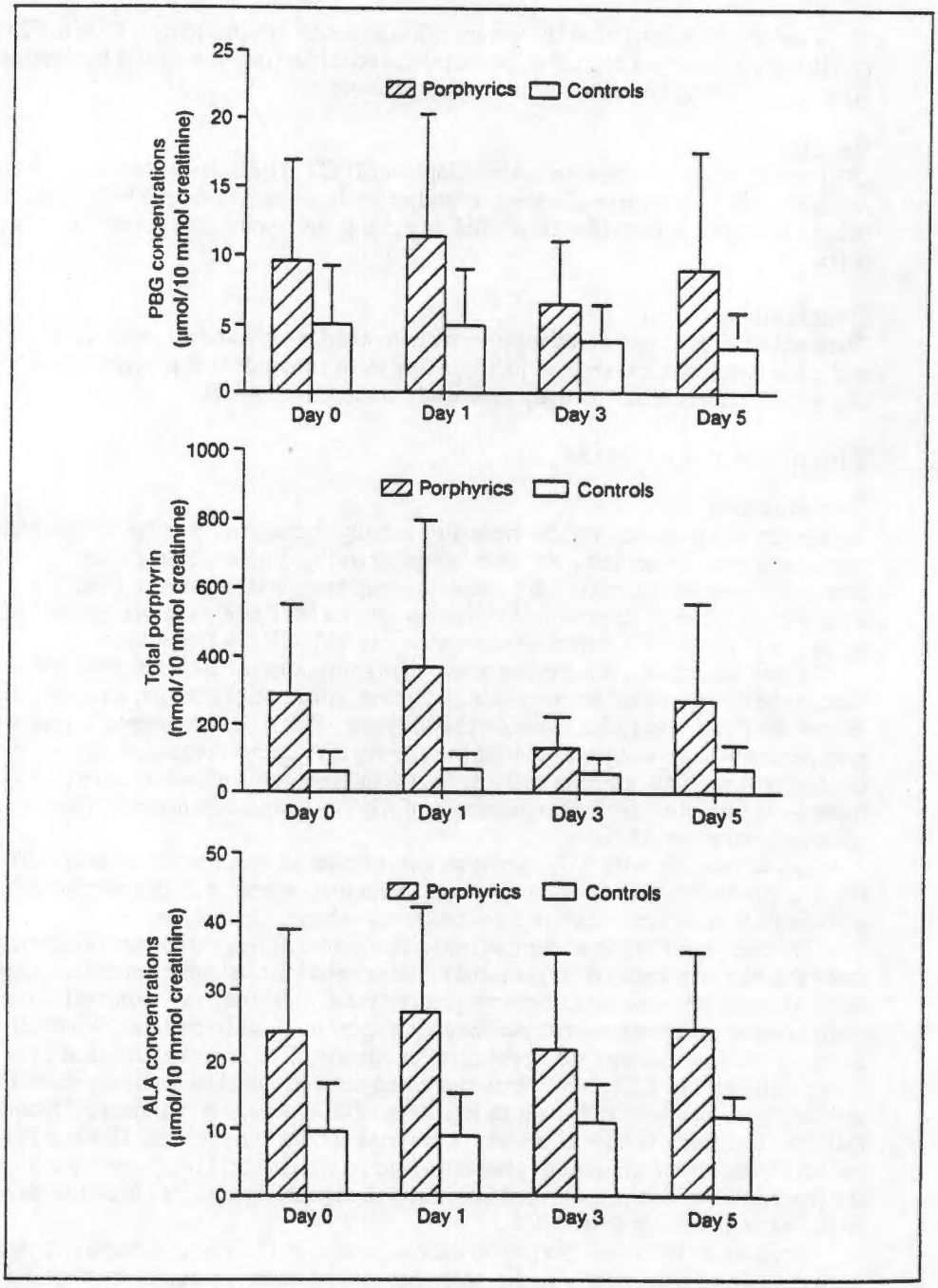

Fig. 1. Fluctuations in the mean (± SD) urinary concentrations of ALA, PBG and total porphyrins in VP subjects and non-porphyric controls, following induction of anaesthesia with propofol. (Derived from [8]).

During the course of this trial, eight reports were published of the safe use of propofol in single patients subject to the other acute porphyrias — seven with AIP and one with HCP [12–19]. To six of these patients, administration was by incremental doseage or continuous infusion. In addition, one discordant report appeared [20] which documented an

increase in urinary porphyrins and their precursors following an anaesthetic with propofol administered by continuous infusion to a VP patient, the third anaesthetic received by the patient in as many days. However, the increases observed, which we considered moderate [21], were not associated with any of the clinical signs of acute porphyria.

While propofol's exceedingly short metabolic half-life makes it ideally suited for continuous infusion techniques and for total intravenous anaesthesia (TIVA), this aspect of its use had not been directly addressed in Parikh and Moore's animal screening [10] or our human trial of the drug [11]. As this property could have a bearing on the expression of a drug's potential porphyrinogenicity, we have studied this aspect in the diethoxycarbonyl dihydrocollidine (DDC) pretreated rat model of VP. This investigation, as yet unpublished, was undertaken in collaboration with my colleagues, Drs. Meissner and Davis.

Methods

In this animal model, blockage of ferrochelatase activity by pretreatment with DDC produces conditions in the rat which simulate those in patients subject to VP. We studied the effects on haem biosynthesis of TIVA with propofol of four hours' duration, and compared these with the effects of a similar administration of the classic porphyrinogen, phenobarbitone.

Drugs were administered by automated syringe-pump via a femoral vein catheter. Dosages given to groups of 12 rats each, were (1) propofol, i.p. loading 133 mg/kg, followed by i.v. infusion of 13–10 mg/kg/h, or (2) phenobarbitone, i.p. loading 133 mg/kg, followed by i.v. infusion of 200 μg/kg/h. One hour before this procedure, 15 mg/kg of DDC in 01.arachis (5 mg/ml) was administered i.p. to half of each group and a similar volume of 01.arachis alone to the remainder. After the four-hour drug infusion, the animals were killed and hepatectomized and the concentrations of uro-, copro- and protoporphyrin in liver homogenates were assayed. Normal (control) concentrations of these intermediates were derived from a matched group of animals not subject to drug exposure.

Results

Results are summarized in Fig. 2. The classical stimulation of haem biosynthesis by phenobarbitone is shown by the consistent and marked increases in concentrations of all three porphyrins compared with non-drug-exposed controls. In particular, concentrations

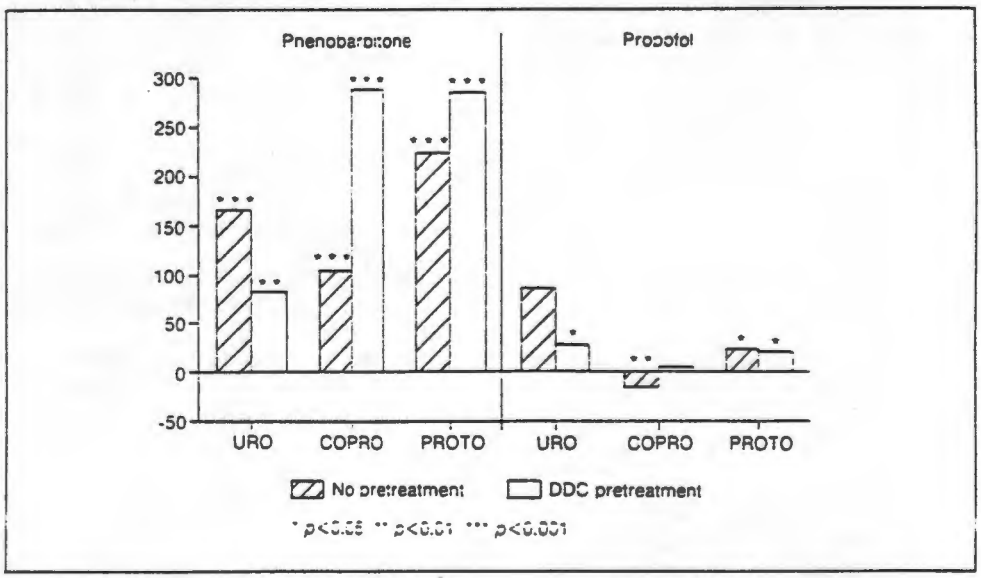

Fig. 2. Percentage change from control (non-drug-exposed) levels in the concentrations of uro-, copro- and protoporphyrins in liver homogenates of rats subjected to four-hour TIVA with phenobarbitone and propofol.

of the distal porphyrin intermediates, copro- and protoporphyrin, were significantly enhanced by DDC pretreatment, rising from 105% and 225%, respectively, in excess of those in non-drug-exposed controls in the absence of DDC, to 288% and 285% after DDC pretreatment.

By contrast, the pattern of response to propofol infusion showed no evidence of consistent stimulation of haem biosynthesis, and the small changes that were recorded were unaffected by pretreatment with DDC.

Conclusions

We conclude that these findings, considered in the light of the human evidence cited above, confirm propofol's lack of porphyrinogenicity. We consider it safe to use for the induction and maintenance of anaesthesia in patients subject to the acute prophyrias.

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PROPOFOL AS AN I.V. ANAESTHETIC INDUCTION AGENT IN VARIEGATE PORPHYRIA

P. N. MEISSNER, G. G. HARRISON AND R. J. HIFT

SUMMARY

The choice of an i.v. anaesthetic induction poses problems for the anaesthetist confronted with a patient with one of the acute porphyrias. We undertook a prospective clinical trial in 13 variegate porphyric subjects using propofol as an anaesthetic induction agent. Urinary porphyrin precursors and porphyrins were measured before operation and 1–5 days after operation. Stool and plasma porphyrin concentrations were measured over the same period. Comparison of these data in the porphyric patients and in 21 control subjects over the trial period revealed no significant change in porphyrin or porphyrin precursor output after operation. Urinary porphyrin precursor concentrations did not exceed the limits established for variegate porphyric patients in remission, and there were no changes in the stool and plasma porphyrin profiles or any symptoms of an acute porphyric attack. We conclude that propofol did not appear to be porphyrinogenic when used for the induction of anaesthesia in 13 patients with variegate porphyria.

KEY WORDS

Anaesthetics, intravenous: propofol. Complications: porphyria.

The porphyrias are a group of disorders in which there is disturbance of haem biosynthesis, resulting in excessive production of haem pathway intermediates, the porphyrins and their precursors δ-aminolaevulinic acid (ALA) and porphobilinogen (PBG). The underlying abnormality may be hereditary or acquired and reflects a defect of any one of the steps of haem formation (fig. 1) [1]. The clinical features of the porphyrias are two-fold: *photocutaneous sensitivity*, caused probably by tissue accumulation of the porphyrins, and the *acute attack* [2, 3]. The latter presents with mild to severe abdominal and neuropsychiatric symptoms and may be fatal. Such an attack is often precipitated by drugs. However, patients may develop spontaneous attacks in response to stress, infections, fasting and endogenous hormonal fluctuations [4, 5]. Three types of porphyria may exhibit these acute symptoms: *acute intermittent porphyria* (AIP), *hereditary coproporphyria* (HCP) and *variegate porphyria* (VP). The acute attack is always associated with increased production and excretion of ALA and PBG [6]. These compounds are strongly implicated, therefore, in the genesis of acute symptoms, although a causal relationship has not been established unequivocally; increased urinary concentrations of ALA and PBG do serve, however, as markers of such an attack.

Although not encountered commonly, the porphyrias are of particular importance to the anaesthetist, as anaesthetic agents are among those incriminated most strongly in the precipitation of the acute attack. The i.v. induction agents in particular pose problems. The barbiturates, thiopentone and methohexitone, are extremely dangerous in porphyria [7]. Propanidid was used safely as the standard i.v. induction agent for porphyric patients for more than 25 years. One author has reported an adverse effect on porphyria [8], but propanadid has been withdrawn from use because of a high incidence of anaphylactoid reactions. Althesin, withdrawn from use for the same reason, had been shown in both animal experiments and human subjects to be porphyrinogenic [8, 9]. Testing of etomidate in

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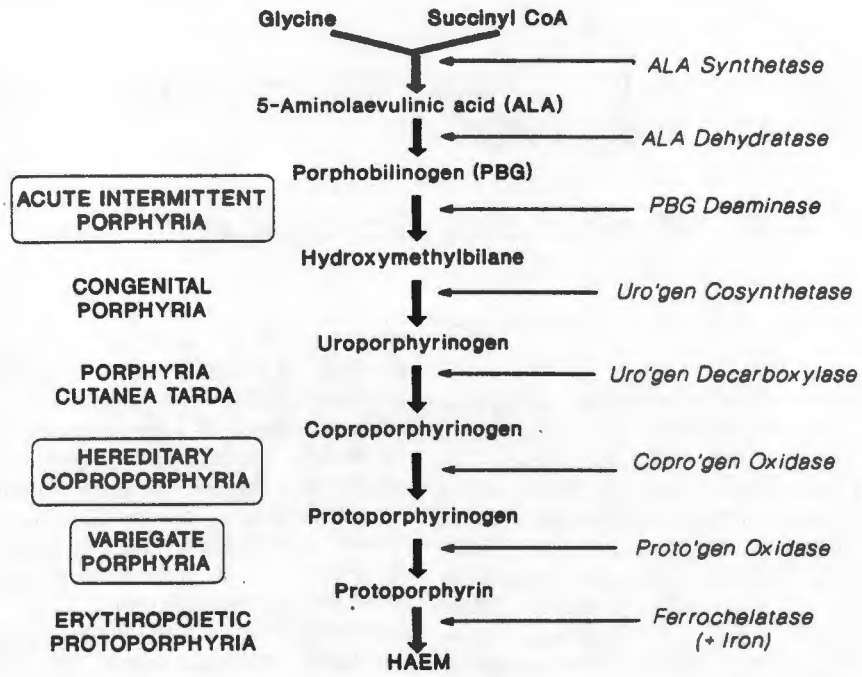

FIG. 1. The haem biosynthetic pathway, showing the enzymatic abnormalities resulting in the various types of porphyria. The three porphyrias which may exhibit acute symptoms are shown within boxes.

animal experiments also suggests porphyrinogenicity [10, 11]. Ketamine has shown no evidence of porphyrinogenicity in animal models, even after 6 h of infusion in the diethoxycarbonyl-dihydrocollidine (DDC)-primed rat model [11], but, even though it possesses excellent analgesic properties, it has other drawbacks which preclude its use as a routine induction agent.

Propofol, the most recently introduced i.v. agent, possesses many of the properties which characterize the ideal anaesthetic agent [12] and in addition, porphyrinogenicity could not be demonstrated in a rat model [13]. We report here a prospective controlled trial of propofol as an i.v. anaesthetic induction agent in porphyric subjects.

PATIENTS AND METHODS

We studied porphyric subjects (ASA grades I and II; aged 16–65 yr; normal renal function) requiring general anaesthesia for elective surgery who presented to the participating anaesthetists during a period of 1 year. As far as could be ascertained, no patient had been exposed to any porphyrinogenic drugs in their recent clinical history (past 6 months). Patients excluded from the study were those with severe underlying disease, an allergy to propofol, previous adverse experience with general anaesthesia, those undergoing operations on bladder or bowel, and those who were pregnant. These exclusion criteria were designed to ensure that there were no circumstances that could have an influence on the measurement of excreted porphyrins or precursors, or on porphyrin metabolism generally.

The diagnosis was confirmed in each patient by the demonstration of diagnostic changes in stool, urine and plasma concentrations of porphyrin. A control group was recruited consisting of people without a history of porphyria and in whom porphyrin analysis was normal. All subjects gave written, informed consent.

No restrictions were placed on the anaesthetic procedure except that induction should be with propofol and that porphyrinogenic drugs should be avoided thereafter. The mean induction dose of propofol administered to the 13 patients was 2.45 mg kg^{-1} (range 1.6–3.33 mg kg^{-1}). Thereafter, 10 received halothane and three enflurane. Atracurium or suxamethonium was used for muscle relaxation and fentanyl, alfentanil and morphine for analgesia. Patients were observed

for any clinical symptoms suggestive of the acute attack for up to 5 days after anaesthesia and were asked to report any untoward developments thereafter.

Random specimens of urine were obtained before operation (day 0) and on days 1, 3 and 5 after operation. Stool and plasma specimens were obtained also, wherever possible, on the same days. All specimens were protected from light to avoid spontaneous interconversion or photodegradation of the precursors or porphyrins.

ALA and PBG were measured by an established ion-exchange technique [14] using test kits (Biorad, Munich, West Germany). Urinary, stool and plasma porphyrins were extracted, esterified, separated by thin-layer chromatography and measured by fluoroscanning, according to established methods [15–17]. Urinary concentrations of precursor and porphyrin were expressed as μmol/10 mmol creatinine and nmol/10 mmol creatinine, respectively, to take account of variability in urinary concentration. Changes in concentration of precursor or porphyrin excretion between days 0 and 1, 0 and 3, and 0 and 5 were calculated. Data from each individual patient were examined to assess whether values remained within the range of values for non-acute VP. The significance of any variation in porphyrin, ALA or PBG concentration from day 0 to day 1, 3 or 5 was assessed with Student's *t* test (two-tailed). Similarly, variations in the VP group were compared with those in the control group, as were changes in the porphyric subjects' urinary concentrations of precursor and porphyrin from day 0 to days 1, 3 and 5 compared with those in the control group.

Subjects

Only patients with VP, the most prevalent form of porphyria in South Africa, presented for surgery during the study period. Thirty-one subjects believed by their physicians to have VP were enrolled. Twelve were excluded because biochemical evidence of porphyria was lacking or because data were incomplete. A further six were excluded from analysis because, despite an abnormal porphyrin profile, the diagnosis was not established beyond doubt. In most instances this was where the diagnosis rested on a mild increase in faecal excretion of protoporphyrin alone. Thus data were obtained for 13 subjects with unequivocal VP. These were compared with 21 control subjects.

TABLE I. *Urinary ALA, PBG and total porphyrin (TP) concentrations (mean (SD))*

Group	ALA concn (μmol/10 mmol creatinine)	PBG concn (μmol/10 mmol creatinine)	TP concn (nmol/10 mmol creatinine)
Propofol controls ($n = 21$)			
Day 0	9.8 (5.8)	5.0 (3.9)	55.3 (50)
Day 1	8.9 (5.2)	5.0 (3.8)	63.8 (40)
Day 3	10.7 (4.6)	3.9 (2.4)	56.8 (39)
Day 5	11.7 (2.0)	3.5 (2.3)	70.5 (58)
Propofol VP ($n = 13$)			
Day 0	24.1 (12.9)	9.6 (7.4)	285 (243)
Day 1	26.8 (15.6)	11.4 (10.1)	371 (424)
Day 3	21.3 (12.4)	6.6 (3.9)	128 (70)
Day 5	24.4 (9.6)	9.1 (7.8)	277 (257)
Normal population ($n = 97$)	14.0 (12.7)	5.2 (4.4)	112 (110)
Non-acute VP population ($n = 228$)	29.3 (27.6)	8.1 (23.6)	473 (924)

RESULTS

It has been held traditionally that subjects with VP have normal urinary concentrations of ALA and PBG during remission. We have become aware that this is not so. We therefore redefined our normal ranges (mean, 2 SD) separately for a group of 221 subjects with VP known to be free of acute symptoms, and 93 non-porphyric subjects. These data were drawn from results from our laboratory over the past 5 years. The redefined normal ALA and PBG upper limits for VP subjects free of acute symptoms were 78 and 55 μmol/10 mmol creatinine, respectively. The upper limits for non-porphyric subjects were 40 and 14 μmol/10 mmol creatinine, respectively.

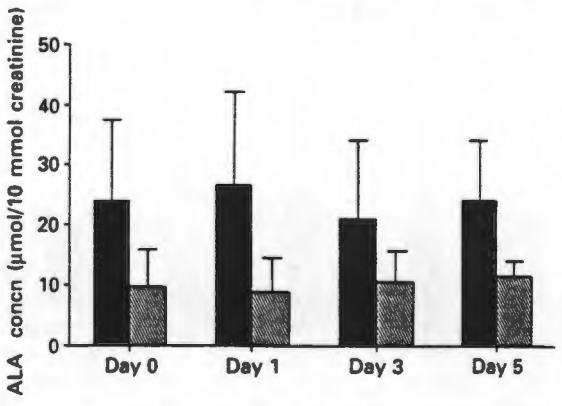

FIG. 2. Fluctuation of the mean (SD) urinary concentration of ALA for the control (▨) and porphyric (■) groups. Individual values fell within the upper limits of "normal" for each respective group at all times.

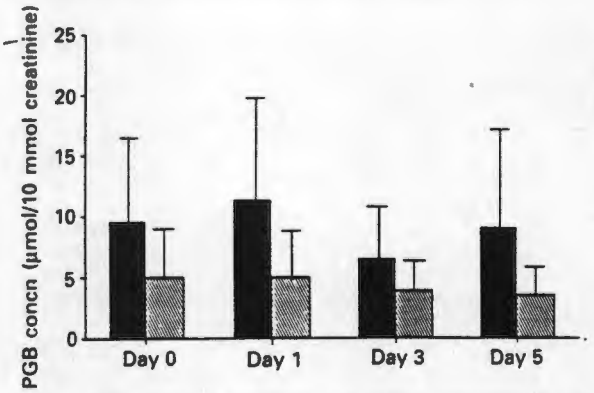

FIG. 3. Fluctuation of the mean (SD) urinary concentration of PBG for the control (▧) and porphyric (■) groups. Individual values fell within the upper limits of "normal" for each respective group at all times.

The mean concentrations of ALA, PBG and porphyrin in the porphyric patients were greater than those in the control group at all times (table I), including day 0 ($P < 0.001$), but ALA and PBG concentrations did not exceed the limits established for VP patients in remission (figs 2, 3). No significant increase in output of porphyrin or porphyrin precursor over the baseline was found after operation ($P > 0.1$); the porphyric group behaved similarly to the control group in this respect. The mean urinary concentrations of total porphyrin of the porphyric group varied greatly and were greater than those of the control group (fig. 4). Again, the change from day 0 to the postoperative days was not significant. No change in pattern of excretion of urinary porphyrin to the earlier haem pathway intermediates was demonstrated by thin-layer chromatography (data not shown). Such a shift would be expected if the pathway were stressed. There were no changes in stool and plasma porphyrin profiles (data not shown).

No symptoms suggestive of an acute attack of porphyria were noted in any subject at any stage.

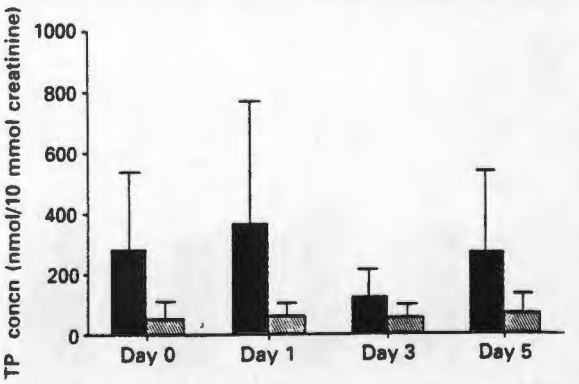

FIG. 4. Fluctuation of the mean (SD) urinary concentration of total porphyrin (TP) for the control (▧) and porphyric (■) groups. The concentrations in the porphyric group are high, indicative of the underlying porphyric status of the VP patients.

DISCUSSION

To be considered safe for use in the acute porphyrias, ideally a drug should meet the following criteria: first, it should not cause increase in production of porphyrin or porphyrin precursor, and should not induce ALA synthetase or diminish free haem concentrations in laboratory test systems, including various types of cell culture [6, 18–20] and laboratory animals rendered porphyric by the administration of agents such as DDC [6, 9, 21, 22]; second, the drug should be devoid of porphyrinogenic potential in human subjects—its administration should not be followed by symptoms of the acute attack or by a significant increase in excretion of porphyrin and precursor. Such data are usually derived from personal experience, anecdotal evidence or from individual case reports. Few, if any, drugs have had porphyrinogenicity tested prospectively in controlled trials in porphyric patients. Thus doctors must often base their decision to use a particular drug in a porphyric subject on incomplete or possibly inaccurate data. Also, experience in human subjects and laboratory testing sometimes give conflicting results [6, 8, 23].

Propofol has been reported not to induce hepatic ALA synthetase in the rat [13] and therefore, by implication, to be non-porphyrinogenic, but it induces hepatic ALA synthetase in DDC-primed chick embryos [20 and personal communication, Deybach JC]. However, this system is considered by some to be extremely sensitive and may produce seemingly false positive results [6]. There have also been conflicting reports on its safety in humans. In one study, propofol was used to induce anaesthesia in a patient with AIP with apparent safety [24], whereas another case report indicated that urinary concentrations of porphyrin and PBG increased in a VP patient anaesthetized with propofol [25]. However, this patient had been exposed to several anaesthetics over a few days, including an infusion of propofol, and the changes in concentrations of

porphyrin and PBG were unconvincing [26, 27]. The patient did not experience acute symptoms.

This study represents a clinical trial undertaken to assess the safety of propofol for the induction of anaesthesia in patients with an acute porphyria. The lack of significant increase in concentrations of ALA and PBG following administration of propofol suggests that the drug has low porphyrinogenic potential in porphyric subjects, at least when used in the dose and manner reported here. This is supported further by failure to alter the urinary, stool and plasma profiles of porphyrin either quantitatively or qualitatively. No symptoms suggestive of the acute attack were noted. We conclude, therefore, that propofol, as used for the induction of anaesthesia in 13 patients with VP, did not appear to be porphyrinogenic. However, the number of patients studied was small and the disease is unpredictable, so care should always be taken when using *any* drug in porphyria. We cannot extrapolate our findings to the use of propofol for maintenance of general anaesthesia by continuous infusion until further clinical experience has been obtained. In the light of the case report referred to above [25], we suggest that caution be exercised with propofol for this purpose.

Comment is necessary on the choice of halothane for maintenance of anaesthesia in this study. Two reports and some experimental data warn of its risk in porphyric patients [7, 20, 28], but this conflicts with other experimental data [10] and much clinical experience accumulated by our and other units, where it has been used safely in porphyric patients on numerous occasions. As 10 of our patients received halothane, this study presents substantial evidence that halothane, in addition to propofol, may be used safely in the acute porphyrias.

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REVIEW ARTICLE

Anaesthesia for the porphyric patient

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The porphyrias feature prominently among the pharmacogenetic conditions of concern to the anaesthetist, in particular the three so-called 'acute' prophyrias: acute intermittent porphyria, variegate porphyria and hereditary coproporphyria. The drug-induced acute attack may be life-threatening, and some drugs in common use in anaesthetic practice are highly dangerous in porphyria. Many of the drugs in the anaesthetist's formulary are listed as potentially porphyrinogenic, yet in many instances the evidence for such listing is tenuous. An awareness of porphyria is essential to any anaesthetist, and is particularly relevant in northern Europe, with its high incidence of acute intermittent porphyria, and in South Africa which enjoys the highest incidence of variegate porphyria in the world. Here variegate porphyria manifests among ethnic groups of pure or mixed western European origin with an estimated 10 000–20 000 individuals being affected [1–5].

The acute attack of porphyria may be prevented by (i) the identification of individuals at risk and (ii) the identification of drugs that are porphyrinogenic and their subsequent avoidance in porphyric patients.

The many changes in the anaesthetic drug formulary over the last decade, both deletions and additions, provide a *raison d'etre* for a specific review of the drugs currently used in anaesthetic practice. While this review will not cover the detailed description, diagnosis and treatment of porphyria, the pitfalls in diagnosis require emphasis. Laboratory screening may not necessarily exclude porphyria unequivocally. Latent carriers may express the classic porphyrin excretion patterns extremely subtly or demonstrate no sign of altered porphyrin metabolism at all. Such carriers are at risk of an acute attack. Included in the latent group would be all prepubertal children of porphyric families who, though normal on biochemical screening, may be precipitated into an acute attack by exposure to porphyrinogenic drugs [6]. We would suggest that the only clinical defence is for the anaesthetist to treat all patients who have a family history of porphyria, or who have signs and symptoms suggestive of porphyria, as potentially at risk, irrespective of age or biochemical status.

Porphyrinogenicity of drugs commonly used in anaesthesia

By combining and comparing data from several published drug lists [5,7–14] with the personal experience of medical practitioners managing porphyric patients and information derived from the screening of drugs in animal and tissue culture models, we have attempted to evaluate for the anaesthetist the relative safety of drugs in everyday use for the porphyric patient. It is worth mentioning that such an exercise is often frustrating because new drug lists very often emerge by modification of existing lists. Thus the original source of information sometimes appears to have been 'lost in the mists of time' and inaccuracies in reporting may have been unknowingly perpetuated.

It must be noted that with the data presently available to us, any list classifying drugs as potentially safe or potentially unsafe will be incomplete. There are those drugs whose use in porphyric individuals had not been recorded, or investigations of their porphyrinogenicity in experimental models not undertaken. The list of 'safe' drugs, in particular, may be understated as generally it is unfavourable reactions to drugs rather than a lack of reaction that are reported in the literature. On the same basis, some drugs reported as 'unsafe/dangerous' may be so listed because many instances of their safe use in porphyric patients have not been reported, whereas a single unfavourable reaction may have been.

A list of drugs in every day use in clinical anaethesia is shown in Table 1, classified in descending order of safety as safe, possibly safe, contentious, probably unsafe and unsafe. Some of these are discussed in greater detail below.

Intravenous induction agents

It is the induction of anaesthesia that has provided the major problems when anaesthetising the porphyric patient. Those stock-in-trade intravenous anaesthetic induction agents, the barbiturates, have long been clearly contraindicated [7,15–19].

Propanidid, though recently listed as 'contentious' because it was reported as unsafe in one review [8], was

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Table 1. Safety of drugs commonly used in clinical anaesthesia for patients with acute porphyrias.

Drug group	Safe (S) Possibly safe (PS)		Contentious (C) No data (ND)		Unsafe (U) Probably unsafe (PU)	
Intravenous induction agents	Propofol	PS	Ketamine	C	Barbiturates	U
	Midazolam	PS			Etomidate	PU
Inhalation agents	Nitrous oxide	S	Halothane	C	Enflurane	PU
	Cyclopropane	S	Isoflurane	ND		
	Diethyl ether	S				
Muscle relaxants	Curare	S	Atracurium	ND	Alcuronium	PU
	Suxamethonium	S	Pancuronium	C		
	Vecuronium	PS				
Neuromuscular blockade reversal	Atropine		Glycopyrronium	ND		
	Neostigmine	S				
Local anaesthetics	Procaine	S	Lignocaine	C	Mepivacaine	PU
	Amethocaine	PS	Prilocaine	C		
			Bupivacaine	C		
Analgesics	Morphine	S	Alfentanil	ND	Pentazocine	U
	Pethidine	S	Sufentanil	ND	Tilidine	U
	Fentanyl	S				
	Buprenorphine	S				
	Naloxone	PS				
	Paracetamol	S				
Anxiolytics	Temazepam	S	Diazepam	C	All other benzodiazepines	U
	Lorazepam	PS	Triazolam	C		
	Droperidol	S	Oxazepam	C		
	Phenothiazines	S				
Anti-arrhythmics	Procainamide	S	Lignocaine	C	Verapamil	U
	β-blockers	S	Mexilitine	ND	Nifedipine	U
			Bretylium	ND	Diltiazem	U
			Disopyramide	C		
Other cardiovascular drugs	Adrenaline	S	β-agonists	ND	Hydralazine	U
	Phentolamine	S	α-agonists	ND	Phenoxybenzamine	U
			Sodium nitroprusside	ND		
Bronchodilators	Corticosteroids	PS	Hexaprenaline	ND	Aminophylline	U
	Salbutamol	S				
Gastric—for Caesarean section	Metoclopramide	PS	Ranitidine	C	Cimetidine	PU
	Domperidone	S				

generally accepted as the induction agent of choice [9,18,20] and had been safely used in South Africa as the standard intravenous induction agent for porphyric patients for 25 years. Unfortunately, it was withdrawn from practice because of a high incidence of anaphylactoid reactions consequent on its Cremophor formulation. The withdrawal from practice of Althesin for the same reasons was of little consequence, for this drug had been shown to be potentially porphyrinogenic in experimental models [20].

Etomidate provided the next hope for a safe intravenous induction agent. Laboratory studies initially suggested it to be nonporphyrinogenic [20] and it has been used in several porphyric patients without any apparent clinical harm (personal experience, G.G. Harrison). Further evidence from experimental models, however, is against it [10,21] and one corroborative report of acute human crisis following its use has been reported [22]. It is, nevertheless, worthwhile to note that the drug has an extremely short half-life and that its use as an induction agent only may not provide a stimulus of sufficient strength and duration to stimulate haem synthesis and evoke an acute attack of porphyria. Only if no alternative were readily available would we suggest the cautious use of etomidate with careful monitoring of the patient both clinically and biochemically after anaesthesia.

Ketamine has shown no evidence of porphyrinogenicity in rat models [23,24], even after infusions of 6 h in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) primed rat model [21]. It has been used with apparent safety as an induction agent in porphyric patients by some anaesthetists [8,25–27] and owes its listing here as a 'contentious' drug to one report from Poland of a porphyric crisis following its use [11], and evidence that it is porphyrinogenic in chick embryo experimental systems [9,28]. A further report of an adverse reaction in a human patient is of doubtful significance since the patient was already in an established acute attack of porphyria [29]. However, though possessing excellent analgesic properties, some of ketamine's other effects are drawbacks to its routine use as an induction agent, especially in adults, and its 'contentious' listing for use in porphyria is thus perhaps appropriate.

Propofol, the most recently introduced intravenous anaesthetic, possesses many of the properties that characterise the ideal anaesthetic agent, in particular, a short metabolic half-life [30]. The lack of porphyrinogenic potential it demonstrated in a rat model [19] has now been confirmed in a prospective controlled clinical trial of its use as an induction agent in variegate porphyria [31]. Eight other reports have documented the safe use of propofol in single patients with porphyria: seven in acute intermittent porphyria [32–38] and one in hereditary coproporphyria [39]. In six of these patients administration was by incremental dosage or continuous intravenous infusion. However, propofol appears to be an inducer of hepatic aminolaevulinate (ALA) synthase in DDC-primed chick embryos [12], and one adverse case report in a variegate porphyria patient documented an increase in urinary porphyrin concentration and porphyrin precursor concentration following its administration as a continuous intravenous infusion [40]. This was, however, the third anaesthetic received by this patient in as many days, and the increases documented were modest and unaccompanied by any clinical signs of an acute attack. Subsequently, the question of the porphyrinogenic potential of propofol administered as a continuous intravenous infusion, rather than as a single induction bolus, has been studied by our group [41]. In a DDC-primed rat model a 4 h infusion of propofol failed to evoke porphyrinogenesis. From the animal and human data cited above, it would seem that propofol can now fill the gap created by the withdrawal of propanadid, as a safe intravenous anaesthetic for use in patients with porphyria. We would recommend, however, that patients should, in any case, be carefully monitored for any signs of increased porphyrinogenesis after anaesthesia.

The benzodiazepines, which include drugs that may be used for the intravenous induction of anaesthesia as well as for sedation and anxiolysis, display a strange lack of homogeneity in their porphyrinogenic potential. Acute attacks have been reported to follow the use of diazepam, chlordiazepoxide, flunitrazepam and nitrazepam [8,11,13,17,42–44]. While some corroborative data from animal screening tests exist in the case of the first three, other experimental systems have suggested that they are nonporphyrinogenic [9]. Indeed, the safe use of diazepam for sedation during porphyric crises has been documented [7–9, 17] leading to its listing as 'contentious' rather than 'unsafe'. Lorazepam, which is available in an intravenous formulation has not demonstrated porphyrinogenicity on experimental animal screening [45]. Midazolam, a drug more suitable as an intravenous induction agent because of its kinetic profile, appears to be nonporphyrinogenic in the DDC-primed chick embryo liver system [9] and has been used in porphyric patients without ill-effect [46].

Volatile agents

Some volatile agents once listed, may now be omitted. For reasons unrelated to porphyrinogenicity, chloroform, fluroxene and methoxyflurane have all been withdrawn from use.

Enflurane has been classed as porphyrinogenic on animal data alone [23]. As yet, no acute attacks in humans have been ascribed to it, though we do know of its use in porphyrics (personal experience, G.G. Harrison). However, without good clinical data to the contrary it is likely to remain as 'possibly unsafe'.

Halothane, perhaps still the most widely used inhalational anaesthetic, owes its contentious listing to two adverse clinical reports and to some experimental data [11]. This conflicts with other experimental data [23] as well as a large body of clinical experience accumulated by our unit as well as others [24, 25, 47, 48], and also with the convincing clinical and biochemical evidence for its safety in porphyric patients shown by a recent controlled clinical trial [31]. In the absence of any safer inhalational agent, we would recommend halothane as the agent of choice in porphyria.

Muscle relaxants

Suxamethonium and tubocurarine are known to be safe [9,43,49] but there is a suggestion that pancuronium may be harmful [8,50]. Alcuronium, one of the most widely used relaxants, has been classed as unsafe by some authorities [9,10] but we think its continued uncomplicated use by many anaesthetists in the porphyric patient warrants its classification rather as 'contentious'. No data are available on any of the other muscle relaxants in current use. Most have been administered to porphyric patients without report of acute attacks, negative evidence that allows one to assume their safety, until data to the contrary are published.

Narcotic analgesic agents

With the exception of pentazocine and tilidine, which are unsafe, the narcotic analgesics of which the morphine analogues are the most relevant to the anaesthetist, are of proven safety. Fentanyl is nonporphyrinogenic in chick embryo systems [9] and has been used safely in porphyrics [11]. Pethidine, though implicated in an acute attack in one case [13], has a long record of safe clinical use and is the analgesic of choice for the symptomatic relief of the acute porphyric crisis.

Local anaesthetics

Although lignocaine has been found to be porphyrinogenic in chick embryo and rat liver experimental systems [18,23] it has nevertheless been used frequently as a local anaesthetic by ourselves in the porphyric patient without ill effect. Procaine has been recommended as a local anaesthetic for porphyrics since there have been no adverse reports. However, as we are not aware of any local anaesthetics, either amide or esters, having induced an acute attack of porphyria in humans we do not think the use of lignocaine, amethocaine and bupivacaine is contraindicated in porphyrics.

Cardiac anti-arrhythmic agents

There are adequate substitutes for lignocaine when used for local anaesthesia but its ability to provide short-term control of ventricular arrhythmias is not so easily replaced. As stated, the evidence for its porphyrinogenic potential is derived from experimental systems, without any corroborative evidence of human porphyric crises. As ventricular arrhythmias during anaesthesia pose an immediate threat

to life that far outweighs the likelihood or risk of an episode of acute porphyria, we feel that its use is justified in these circumstances.

Drug structures and porphyrinogenicity

New drugs are constantly being introduced into clinical practice, yet screening of their porphyrinogenic potential is not compulsory for their registration by any statutory authority. As there is little or no homogeneity in the molecular structure of drugs which stimulate porphyrinogenesis, there is no way of predicting which new drugs should be regarded with caution when administered to the porphyric. This places ethical constraints on the use of new and as yet untested drugs in the porphyric patient. Although the giving of new drugs may be justified when no satisfactory alternative safe drug is available, it would be more difficult to justify in the context of modern clinical anaesthesia, where safe alternatives are widely available.

Inadvertent use of a porphyrinogenic drug

Occasionally a porphyric patient will be inadvertently exposed to a potentially dangerous agent. This may arise, for example, when the personal or family history is unavailable and when thiopentone has been used for anaesthesia, or where the anaesthetist has only noticed a Medic-Alert disc after induction of anaesthesia.

Under such circumstances there is no specific prophylactic therapy. However, since carbohydrate loading is known to suppress the synthesis of porphyrins and is effective treatment for the established acute attack, we would recommend that this be employed in the hope of ameliorating a subsequent attack. Large amounts of carbohydrates should be given, aiming at 2000 kcal/24 hours, preferably enterally, or, if necessary, intravenously. At the very least, a 10% dextrose infusion should be used. Haematin, the most effective therapy for the acute attack, has not been assessed as a prophylactic agent under these circumstances.

It is essential that the patient's condition is carefully monitored for a period of 5 days following the administration of the inducing drug. A sample of urine should be tested with Ehrlich's aldehyde reagent daily as this will detect elevated porphobilinogen, thus signalling the onset of the acute attack. Other complaints such as severe abdominal pain, or the passage of red urine are suggestive of the onset of an attack and should be managed accordingly. A publication [14] and notes from our Centre giving further information on porphyria, the acute attack and its treatment are available from the authors on request.

Conclusion

The very real problem anaesthesia once posed for the porphyric patient is reflected in the number of acute porphyric attacks admitted to Groote Schuur Hospital, Cape Town, between 1950 and 1971 when 31 of 145 admissions for the acute attack were precipitated by the induction of anaesthesia with thiopentone. Two proved fatal. Since that time the identification of patients at risk by the biochemical testing of all members of proband families has lead to a dramatic reduction in the number of acute porphyric admissions [4,5]. Significantly, for the past 20 years only one of these has been related to anaesthesia.

We believe the 'bogey' image that the patient suffering from variegate porphyria, acute intermittent porphyria or hereditary coproporphyria has long held for the anaesthetist and surgeon is no longer justified. Provided patients at risk are clearly identified before anaesthesia by a combination of family history, clinical and biochemical examination, anaesthetists today have at their disposal a sufficient number of drugs that are safe to use, allowing them to approach the task of anaesthetising the porphyric patient with equanimity.

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PART 3.

THE EFFECTS OF ANAESTHETICS ON THE LIVER.

Studies of Hepatic Drug Metabolism of relevance to post-Halothane Hepatitis and The Hepatotoxicity of Anaesthetic Agents.

The Treatment of Fulminant Liver Failure.

SOUTH AFRICAN MEDICAL JOURNAL.
40:15 - 17:1966.

POSTOPERATIVE JAUNDICE WITH SPECIAL REFERENCE TO HALOTHANE*

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Halothane was introduced into anaesthetic practice in 1956.[1] Structural similarity to chloroform, a halogenated hydrocarbon, with known hepatotoxic propensities,[2] immediately posed the question of potential hepatotoxicity.

The early experimental work of Raventos[3] was soon followed by extensive experimental and clinical investigations by a host of workers.[4-19]

Their findings suggested that halothane was a safe non-hepatotoxic agent, with hepatotoxic propensities no greater than that of other general anaesthetic agents, e.g. ether.

From 1958, however, the above happy reputation fell under a cloud, when an increasing number of reports of both fatal and non-fatal cases of hepatic necrosis, following halothane anaesthesia, appeared in the literature by various authors,[20-26] though in many instances the connection between administration of halothane and the appearance of postoperative liver damage appeared tenuous.

Because of these reports we decided to investigate all cases of postoperative jaundice in Groote Schuur Hospital.

METHOD

All clinical jaundice occurring postoperatively at Groote Schuur Hospital over a period of 10 months was investigated.

A record was kept of the anaesthetic agents administered to these patients and other factors known to be capable of producing postoperative jaundice.[27,28]

The number of anaesthetics administered during this period was recorded. As an estimate of the incidence of the use of halothane during the period of this survey, we used as a sample a census of anaesthetic agents used in this hospital for one month.

Because of the multiplicity of factors that may result in jaundice after cardiac surgery with cardio-pulmonary bypass, all such patients were omitted from this investigation, in spite of the fact that halothane is used as the routine anaesthetic for the bypass phase of such surgery in this hospital.[29]

Anaesthetics administered for outpatient surgery in the Casualty Department were also excluded, because of the possibility of inadequate follow-up, although halothane was used in 50% of anaesthetics administered in the Casualty Department, and no cases of postoperative jaundice were registered from this source during the time of this survey.

Patients presenting for ophthalmological surgery, where halothane was used as a routine, provided a separate group in this investigation.

RESULTS

A follow-up of 16,500 consecutive inpatients, anaesthetized over a period of 10 months, produced 68 cases of clinically obvious postoperative jaundice. Biochemical investigation was carried out in 29 cases.

Halothane had been included in the anaesthetic technique in only 7 cases. while the remainder had received a variety of anaesthetic techniques of which the majority were a thiopentone-N_2O-O_2-relaxant-IPPR with CO_2 absorption sequence. with or without the use of minimal amounts of ether (Table I).

The possible factors involved in the causation of jaundice in our cases are summarized in Table II.

Of the 7 patients who had received halothane, adequate cause for the jaundice other than the association of halothane was apparent in each case. A brief case report of the salient features of each follows:

Case Reports

Case 1. Male aged 60 years. Emergency nephrectomy. Transfused with 4 pints of blood, 2 of which were transfused as an emergency—they were compatible on Bromelin testing, but

*Paper read at the 45th South African Medical Congress (M.A.S.A.), held in Port Elizabeth, July 1965.

because of a rare blood group factor were later found to be incompatible on Coombs test.

TABLE I. ANAESTHETIC TECHNIQUES USED

	No. of cases of postoperative jaundice
1. Thiopentone-succinylcholine-N_2O-O_2-ether-d.tubocurarine	38
2. Thiopentone-succinylcholine-N_2O-O_2-ether-gallamine	12
3. Thiopentone-succinylcholine-N_2O-O_2-ether	6
4. Thiopentone-succinylcholine-N_2O-O_2-d.tubocurarine	5
5. Thiopentone-succinylcholine-N_2O-O_2-halothane-gallamine	3
6. Thiopentone-succinylcholine-N_2O-O_2-halothane-d.tubocurarine	4
Total	68

TABLE II. FACTORS WHICH WERE CONSIDERED AS IMPORTANT AETIOLOGICAL FACTORS

1. *Type of surgery:*	
Upper abdominal—	
(*a*) Gallbladder and common bile duct	14
(*b*) Liver	4
(*c*) Stomach and oesophagus	14
(*d*) Major vascular	14
(*e*) Other abdominal surgery	12
All other surgery	10
2. *Massive blood transfusion*	42
3. *Hypotension occurring during anaesthesia*	7
4. *Recurrent surgery*	11

Case 2. Male aged 48 years. Oesophagectomy performed. The patient was transfused with 6 pints of blood during surgery. Halothane was used for only ± a ½-hour and in concentrations of ½ to 1% V/V in the inhaled mixture VOC.

Case 3. Male aged 32 years. Thoracoplasty done for a bronchopleural fistula following pneumonectomy for extensive pulmonary tuberculosis. Seven pints of blood were transfused. The patient had halothane for ± 15 min. to facilitate closure.

Case 4. Male aged 50 years. The patient had 3 thoracotomies in 5 weeks for bleeding, following a decortication of the lung. A total of 52 pints of blood was transfused in a period of 5 weeks. There was jaundice after the second thoracotomy which persisted intermittently—*pari passu* with blood transfusion—till 4 days after the third operation. Halothane had been used with the first two operations.

Case 5. Female aged 38 years. Exploration of the common bile duct. Jaundice appeared on the first postoperative day and lasted for 3 days. Chemistry suggested an obstructive jaundice.

Case 6. Female aged 26 years. Oesophagectomy performed for a stricture—8 pints of blood transfused. One week later the oesophageal suture line leaked. A gastrostomy was performed and the chest drained and oesophageal leak closed. Two pints of blood transfused. A thiopentone-succinylcholine-N_2O-O_2-halothane-d.tubocurarine sequence was used in both these cases. Clinical jaundice was noticed 4 days after the second operation and persisted for 3 weeks. Sixteen days after the second operation a laparotomy was performed for jejunal obstruction under thiopentone-succinylcholine-N_2O-O_2-d.tubocurarine anaesthesia. Three pints of blood were transfused.

In this patient, halothane may well appear to have been a factor, but the surgical trauma and blood transfusion would appear to be greater factors.

Case 7. Female aged 28 years. Ruptured liver repaired under thiopentone-succinylcholine-N_2O-O_2-halothane-gallamine anaesthesia. Jaundice appeared on the first postoperative day. At surgery there was excessive manipulation of the liver. In addition, 12 pints of blood were transfused in the peri-surgical period.

All these patients recovered.

To test the possible influence the administration of halothane might have on the incidence of postoperative jaundice, we matched the incidence of the use of halothane in the background surgical population with the frequency of its administration in those cases of postoperative jaundice registered (Table III).

TABLE III. PATIENTS WITH POSTOPERATIVE JAUNDICE

	Patients with postoperative jaundice	*One month's sample of anaesthetics*
Anaesthetics with halothane	7 (10·3%)	333 (24·8%)
Anaesthetics without halothane	61	1,008
Total	68	1,341

It will be seen that whereas halothane was administered in 24·8% of all anaesthetics, it had been used in 10·3% of the cases which showed postoperative jaundice. (This difference is highly significant – $x^2 = 6{\cdot}94$ $P<0{\cdot}01$). Halothane was associated with postoperative jaundice in a significantly lower proportion of cases than is reflected in the general incidence of its use.

Halothane in Ophthalmological Surgery

Because of the low incidence of postoperative vomiting after its use, halothane has been used as the anaesthetic of choice for all ophthalmological surgery in this hospital for some years. Following 1,670 consecutive halothane anaesthetics for ophthalmological surgery, no cases of postoperative jaundice were recorded.

DISCUSSION AND CONCLUSIONS

From the above, we think that it is fair to infer that the influence of the use of halothane *per se*, on the occurrence of postoperative jaundice is insignificant. The occurrence of postoperative jaundice appears to be determined by other factors.

Factors which appeared to be more significant in the production of jaundice were the type of surgery and the quantity of blood transfused.

It may be argued that halothane and other anaesthetics were not used for comparable cases, e.g. in most major vascular and abdominal surgery, in persons prone to develop postoperative jaundice, a thiopentone-N_2O-O_2-relaxant-IPPR-CO_2 absorption technique was used. This is to some extent true, but many of the published cases of postoperative jaundice attributed to halothane involved relatively minor surgery.[22,30,31] Our experience, that jaundice occurred most frequently associated with major abdominal surgery, and more particularly major upper abdominal surgery, is in keeping with the experience of many authors, e.g. Zamcheck,[32] Geller and Tagnon.[27] Sutton,[28,29] and Torrance,[26] who described marked disturbance of liver function, raised serum bilirubin levels and even histological changes in the liver following upper abdominal surgery and particularly following gastric and biliary surgery. They ascribed these changes to varying degrees of interference with the blood supply to the liver and to direct trauma to the liver incumbent with surgery in this region.

Massive blood transfusion was the next most common factor, 42 of the 68 jaundiced patients having received more than 2.500 ml. of blood, with a range of up to 30

litres. This is in keeping with the findings of Geller and Tagnon.[27] In addition to obviously increased bilirubin production associated with the transfusion of stored blood, there was definite evidence of a hepatic element in those patients who became jaundiced.

Hypotension of more than 10 minutes' duration during anaesthesia occurred in 7 patients, and some of these patients were exposed to vasopressors, both of which have been described as factors in the production of liver damage, or predisposing to liver damage by anaesthetic agents including halothane, according to such authors as Anlyan *et al.*,[35] Sims *et al.*,[36] Stephen *et al.*[37] and others.

Eleven of the patients were exposed to recurrent surgery, which was associated in several of our cases with major abdominal surgery and massive blood transfusion.

Lastly, it is pertinent to mention the 1,670 anaesthetics for ophthalmological surgery. These patients all received halothane and no cases of postoperative jaundice were registered.

It is interesting to note, in relation to this present controversy, how in 1932 Guthrie and Robertson[38] questioned the use of ether as an anaesthetic agent for gallbladder surgery because of the high incidence of postoperative liver necrosis.

SUMMARY

1. An investigation into the occurrence of postoperative jaundice in a follow-up series of 16,500 anaesthetics is discussed.

2. Note was made of the anaesthetics administered, the incidence of the use of halothane, both in the cases of jaundice and the background surgical population, and the occurrence of other factors known to cause jaundice.

3. It is concluded that the influence of the use of halothane *per se*, on the occurrence of postoperative jaundice, is not significant.

We wish to thank Prof. J. H. Louw and the Heads of departments and firms in the Division of Surgery, and the Departments of Obstetrics and Gynaecology for their help and cooperation in our obtaining the data on which this paper is based. Also, Prof. A. B. Bull, Head of the Department of Anaesthetics, for much encouragement and advice, and Dr. J. G. Burger, Superintendent, Groote Schuur Hospital, for permission to publish this paper.

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IN DIE VERBYGAAN : PASSING EVENTS

Dr. B. A. Bradlow, of Johannesburg, has been elected a Fellow of the American College of Cardiology, a designation which is rarely given to foreign graduates outside North America.

* * *

Dr. A. C. G. B. Anderson, formerly full-time consultant in the Department of Anaesthesia at Groote Schuur Hospital, Cape Town, has now joined Drs. J. K. Beneke and J. le R. Haumann, specialist anaesthetists, in practice at 300 Medfontein, St. Andrew's Street, Bloemfontein.

* * *

Cape Western Branch (M.A.S.A.), Alan Sichel Cup Golf Tournament. The Alan Sichel Cup Golf Tournament was held at the Rondebosch Golf Course on 9 December 1965. A total of 35 players participated in the competition, and the prizes were presented by Dr. A. W. Sichel.

The winner of the Cup was Dr. I. Levine, and the runner-up was Dr. R. Gasson. The Prize for the Longest Drive was won by Dr. I. Levine.

The winners of the Stableford Competition (run concurrently) were Drs. I. Levine and I. Kirk, and the runners-up were Drs. C. Rauch and P. S. Willers. The prize for nearest to the pin was won by Dr. J. Mervis.

The proceeds of R52 were donated to the Benevolent Fund, and the organizers wish to thank the Captain of the Rondebosch Golf Club for the courtesy of the use of the course.

WORLD LIST OF FUTURE INTERNATIONAL MEETINGS

ALTERATIONS AND ADDITIONS NOTIFIED DURING NOVEMBER 1965

12th Congress for Laboratory Medicine, Bad Kissengen, Germany (Fed. Rep.), 4 - 8 May 1966. Dr. med. W. Albath, Katharinengasse 3, 87 Würzburg, Germany (Fed. Rep.).

2nd International Symposium on Plastic and Reconstructive Surgery of the Eye and Adnexa, New York, 16 - 20 May 1966. Manhattan Eye, Ear and Throat Hospital, 210 East 64th Street, New York, NY 10021, USA.

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14 *March* 1970, 1, 668–671

Multiple Halothane Exposure and Hepatic Bromsulphthalein Clearance

Summary: Multiple exposures to halothane have been shown to have delayed effects on bromsulphthalein (B.S.P.) clearance. Rats were exposed to repeated halothane anaesthetics, and their livers were subsequently isolated and perfused. B.S.P. retention in the perfusate of these isolated livers was greatly increased one to three weeks after the last halothane exposure. In similarly pretreated animals at the same time period the B.S.P.–glutathione conjugating enzyme activity in homogenates of the livers was found to be depressed. These findings did not occur after multiple diethyl ether exposures or following a single exposure to halothane.

INTRODUCTION

The question of multiple exposures has become an extremely important one relative to the mechanism by which liver damage may be caused following halothane anaesthesia (Little, 1968). Attention has again been drawn to this problem by several recent reports (Trey *et al.*, 1968; Klatskin and Kimberg, 1969; Rodriguez *et al.*, 1969), in addition to many previous individual surveys and case reports (Tygstrup, 1963; Sherlock, 1964; Morgenstern *et al.*, 1965; Griner, 1966; Subcommittee on National Halothane Study, 1966).

The controversy surrounding the possible role of halothane in the causation of postoperative liver dysfunction has centred on, among other factors, the inability of clinical and statistical surveys to eliminate the numerous potential causes of liver dysfunction during this period (viral hepatitis, blood transfusion, intraoperative hypotension and hypoxia, septicaemia, and the concurrent administration of potentially hepatotoxic drugs).

The present investigation was designed in an attempt to eliminate these other potential causes of liver dysfunction by the study of isolated perfused livers and liver homogenates from animals previously exposed to halothane on several occasions. Abnormalities of bromsulphthalein (B.S.P.) handling by the livers of such animals were observed, and the study was modified to confirm the findings and to attempt to isolate the abnormality present in the liver.

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Materials and Methods

Animals

Male albino rats (weight 375–400 g.) of Wistar and Sprague-Dawley strains, housed in conditions of controlled temperature and humidity and fed on a standard laboratory diet, were used as liver donors for both the isolated perfusion and liver homogenate experiments. The animals were allowed free access to water and food up to the beginning of the experiments. The animals were divided into three groups:

Group 1.—Control animals.

Group 2.—Animals exposed to multiple (3 or 5: see Results) 60-minute periods of 1% halothane vapour v/v (Vapor vaporizer, Dräger) in oxygen, on alternate days.

Group 3.—Animals exposed to multiple (3 or 5: see Results) 60-minute periods of 4% v/v diethyl ether vapour (E.M.O. vaporizer) in oxygen, on alternate days.

To achieve these periods of light surgical anaesthesia (Duncan and Raventós, 1959) in groups 2 and 3, batches of six animals were anaesthetized in an exposure chamber, anaesthetic mixtures being produced at a rate of 8 l./min., which is several times greater than the total average minute volumes of the rats. During the periods of anaesthesia there was no evidence of hypoxia or hypercarbia, as estimated by blood gas determinations on samples obtained by cardiac puncture from random animals. The animals regained full consciousness within minutes of the termination of the anaesthetic.

These animals showed no obvious ill effects after the anaesthetic exposures, continued to gain weight, and behaved normally until their use as liver donors at varying periods after the exposures.

Isolated Liver Perfusions

Isolated liver perfusions were performed according to the method of Miller *et al.* (1951) as modified by Fisher and Kerly (1964). The method, discussed in detail by the latter authors, involves the cannulation of the bile duct and portal vein followed by the removal of the liver and its subsequent perfusion in a sealed cabinet. The interior of the cabinet is humidified, and thermostatically controlled at a temperature of 38°C. The liver, mounted on a glass platform with the diaphragmatic surface down, is perfused via the portal vein. The outflow via the hepatic vein runs into a reservoir below this platform, from where it is recirculated by a finger pump to the glass "thin film" oxygenator above the liver. A 95% oxygen and 5% carbon dioxide mixture is passed into the oxygenator at a flow rate of 750 ml./min.

In the present perfusions the surgical procedure on the donor rats (in all groups) was performed under light diethyl ether and oxygen anaesthesia. The livers were not exposed to halothane or diethyl ether, however, during the perfusions in this series. Perfusate volume used was 100 ml., consisting of pooled Wistar and Sprague-Dawley albino rat blood obtained by cardiac puncture from multiple donors, diluted to a packed cell volume of 25% with a balanced salt-bicarbonate solution.* Perfusate *p*H (7·35-7·42). P_{O_2} (150-175 mm. Hg.), and P_{CO_2} (35-40 mm. Hg) were monitored at 15-minute intervals by the Astrup technique and kept within the given ranges. The portal vein pressure was 18 cm. perfusate, and the measured hepatic vein flow was constant (1·25-1·5 ml./g. liver wet weight/min.) during the period of B.S.P. estimation.

Apart from limited sampling of the perfusate for blood gas and acid-base estimations, the perfusate volume in these perfusions was kept constant. A different series of perfusions was undertaken to establish criteria of adequate perfusion and assess other metabolic effects of the anaesthetic agents (various criteria reflecting changes in carbohydrate and protein metabolism).

B.S.P. (supplied by Hynson, Westcott and Dunning, Inc., Baltimore) was added, at a dosage of 1·25 mg./100 g. donor rat weight, to the perfusate reservoir 30 minutes after the beginning of the perfusion. At this time steady blood (1·25-1·5 ml./g. liver wet weight/min.) and bile flow (80-90 microlitres/g. liver/hour) were established. Perfusate samples were then withdrawn from the reservoir at 1, 3, 5, and 30 minutes after the addition of B.S.P.

B.S.P. concentration in the perfusate was measured spectrophotometrically, the samples having been subjected to a technique (Henry *et al.*, 1959) of acetone precipitation of proteins. This technique allows accurate B.S.P. estimations in the presence of haemolysis. Free plasma haemoglobin levels ranging from 50 to 75 mg./100 ml. were present in the perfusate during the period of B.S.P. estimations.

Liver Homogenates

In an attempt to establish which of the three mechanisms involved in the handling of B.S.P.—that is, hepatic uptake, conjugation, or excretion—was responsible for the findings from the isolated perfusion experiments, liver homogenates from livers of animals previously exposed to halothane and diethyl ether were used to estimate levels of hepatic B.S.P.–

*Plasmalyte-B (Baxter) containing (mEq/l) Na 130, Cl 109, K 4, Mg 3, bicarbonate 28.

glutathione conjugating enzyme activity. The enzyme activity in the soluble supernatant fraction of liver was assayed by the method of Combes and Stakelum (1962), which involves incubating (for five minutes) homogenized aliquots of liver with B.S.P. and glutathione, and subsequently identifying the proportions of free B.S.P. and B.S.P.—glutathione by paper chromatography of the supernatant fluids.

Quantification of the B.S.P. on the chromatograms is performed by elution of the relevant bands of paper, the concentration of B.S.P. in the eluate being determined spectrophotometrically. In the present study aliquots of liver of 400-600 mg. were used (from freshly decapitated animals), and 0·5 mg. of B.S.P. and 4 mg. of glutathione were the quantities of substrates added for incubation. The data are expressed as milligrams of B.S.P. conjugated per gramme of liver wet weight per five minutes.

Duplicate slices of liver were assayed for glutathione content by the method of Grunert and Phillips (1951).

Results

Isolated Liver Perfusions

The figures for B.S.P. percentage retention in the perfusate given in Tables I-IV represent the retention 30 minutes after the introduction of the dye to the perfusate reservoir.

The control figures in each table refer to B.S.P. estimations in isolated liver perfusions where the donor animal had not been previously exposed to an anaesthetic agent. These control animals were housed in the same conditions as the other groups, and control perfusions were interspersed at varying times among the perfusions of livers previously exposed to anaesthetic agents.

The results of perfusions performed at varying times after three halothane exposures (as described in Group 2 above) are set out in Table I. The statistically highly significant increased B.S.P. retention at one and three weeks after the halothane exposures did not occur at the same periods after diethyl ether exposure (see Table II).

The three-week interval following anaesthetic exposure was chosen to assess the effect of a single halothane exposure, and Table III shows that B.S.P. retention in this group did not differ significantly from the controls.

The fact that B.S.P. percentage retention values returned to the control perfusion range six weeks after the halothane exposures (see Table I) prompted the investigation from which data are presented in Table IV. Animals were exposed to halo-

thane on three occasions, and after an interval of six weeks were re-exposed to halothane on three further occasions (using the technique described under Group 2). This procedure resulted again in an increased B.S.P. percentage retention at the one-week and three-week post-exposure periods, but the figures do not differ significantly from those in Table I.

TABLE I.—*Mean Perfusate B.S.P. Percentage Retention* (± *S.D.*) *after Three Halothane Exposures*

	Control (No Previous Halothane)	Interval After Exposure (Weeks)			
		1	3	4	6
Percentage retention ..	5 ± 3	18 ± 3	29 ± 6	14 ± 3	3 ± 2
n*	5	3	3	3	3
P†	—	<0·001	<0·001	<0·005	N.S.

*Number of perfusions performed. †Refers to relevant values *v.* control (Student's *t* test).

TABLE II.—*Mean Perfusate B.S.P. Percentage Retention* (± *S.D.*) *after Three Diethyl Ether Exposures*

	Control	Interval After Exposure	
		1 Week	3 Weeks
Percentage retention	5 ± 3	6 ± 2	3 ± 2
n	5	3	3

TABLE III.—*Mean Perfusate B.S.P. Retention* (± *S.D.*) *after a Single Halothane Exposure*

	Control	3 Weeks After Exposure
Percentage retention	5 ± 3	6 ± 3
n	5	3

TABLE IV.—*Mean Perfusate B.S.P. Percentage Retention* (± *S.D.*) *after Three Halothane Exposures, Followed by a six-week Interval, and Three Further Halothane Exposures*

	Control	Interval After 2nd Three Exposures	
		1 Week	3 Weeks
Percentage retention	5 ± 3	18 ± 2	21 ± 5
n	5	3	3

B.S.P.-GLUTATHIONE CONJUGATING ENZYME ASSAY

Results of estimations B.S.P.–gluthathione conjugating enzyme activity in homogenates of livers from groups of animals, previously exposed to halothane and to diethyl ether,

are presented in Table V. The control data represent similar estimations on animals which had never been exposed to an anaesthetic agent.

TABLE V.—*B.S.P.-Glutathione Conjugating Enzyme Activity (Mean ± S.D.) after Multiple (5 ×) Anaesthetic Exposures (mg. B.S.P. Conjugated/g. Liver Wet Weight/5 Minutes)*

		Controls	Interval After Exposure	
			2 Weeks	3 Weeks
After halothane	Enzyme activity	2·10 ± 0·62	1·49 ± 0·28	1·68 ± 0·40
	n	26	5	10
After diethyl ether	Enzyme activity	2·10 ± 0·62	1·93 ± 0·36	1·70 ± 0·35
	n	26	7	6

n = Number of animals investigated.

Statistical analysis of these results shows significant differences between the means of controls and those at two weeks after multiple halothane exposures ($P < 0.05$) and between controls and the three-week sample ($P < 0.01$). The control levels of enzyme activity and those following multiple diethyl ether exposures show no statistical difference. The glutathione content of duplicate slices of liver, at the two-week and three-week post-halothane exposure periods, does not differ significantly from the control levels (Table VI).

TABLE VI.—*Liver Glutathione Content (Mean ± S.D.) Following Multiple (5 ×) Halothane Exposures (mg. Glutathione/g. Liver Wet Weight)*

	Controls	Interval After Exposure	
		2 Weeks	3 Weeks
Glutathione levels	3·46 ± 0·58	3·84 ± 0·45	3·34 ± 0·20
n	6	6	3
P (*v.* control)	—	N.S.	N.S.

DISCUSSION

The present investigation shows what appears to be a delayed hepatic effect of multiple halothane exposure. Several studies showing increased B.S.P. retention in the early postoperative period following single and multiple halothane anaesthetics have previously been reported in man (Brindle *et al.*, 1957; Virtue *et al.*, 1958; Burnap *et al.*, 1958; Carson *et al.*, 1959; Griffiths and Ozguc, 1964; Joseph, 1964; Keéri-Szántó, 1965), in dogs (Brindle *et al.*, 1957), in sheep (Hull and Reilly, 1968), and in calves (Middleton *et al.*, 1966). During the immediate postoperative period raised B.S.P. retention has also been shown to occur following other anaesthetics: chloroform (Griffiths and Ozguc, 1964), cyclopropane (Prince *et al.*, 1965),

and methoxyflurane (Keéri-Szántó, 1965). In these reports, however, the observations have been confined to the immediate postoperative period (up to seven days), and have not extended over the number of weeks reported in this communication.

The precise mechanism of the increased B.S.P. retention during the liver perfusions following previous halothane exposure in this study is not clear. There is considerable evidence (Combes and Stakelum, 1962) to support the view that the removal of B.S.P. from plasma is dependent on the simultaneous operation of two processes: (*a*) uptake into a hepatic storage compartment, and (*b*) excretion into bile.

It is now generally agreed (Combes and Stakelum, 1962) that the major pathway of B.S.P. metabolism in man, rat, dog, and other species involves conjugation with the tripeptide glutathione, composed of glutamic acid, cysteine, and glycine. Nevertheless, despite the observation that most of the B.S.P. excreted into bile is conjugated, the finding of free B.S.P. in bile leaves it uncertain whether conjugation is important for, or merely incidental to, hepatic uptake and biliary excretion of B.S.P. Combes and Stakelum (1961) identified in liver an enzyme that catalyses conjugation of glutathione with B.S.P. A depression of this enzyme activity has been shown in the present investigation to occur after multiple halothane exposures. This depression parallels the increase in B.S.P. retention seen in the perfused livers in similar circumstances. Impairment of B.S.P. conjugation may also be a reflection of low hepatic glutathione levels. Such levels did not occur in livers of animals reported here, where glutathione levels after halothane anaesthesia were within the normal range.

The enzyme shown to be depressed following multiple halothane exposure is not specific for the conjugation of B.S.P. This enzyme also catalyzes the conjugation of glutathione with other compounds (Combes, 1964), such as bromobutane, benzyl chloride, and *p*-fluoronitrobenzene. It is thus possible that the enzyme may be concerned with the conjugation of trifluoracetic acid, which has been reported as a metabolite occurring during the biotransformation of halothane (Stier and Alter, 1966; Rehder *et al.*, 1967). The inference is that as a result of previous exposure to halothane certain enzymes are depressed, permitting accumulation of hepatotoxic metabolites, perhaps chlorinated derivatives which have yet to be identified. The possibility of enzyme changes of this nature resulting in hepatic injury following halothane has been suggested by Zimmerman (1968). The present findings, showing biochemical derangement following previous halothane exposure, are opposed to the criteria (Sherlock, 1964) which, in classifying halothane hepa-

totoxicity as a "sensitivity" or, allergic effect, exclude its reproducibility in experimental animals.

Two further mechanisms of interference with B.S.P. clearance merit attention. Alterations in the characteristics of serum albumin (Crawford and Hooi, 1968) and the relative deficiency of the hepatic cytoplasmic organic anion acceptor protein Y (Levi *et al.*, 1969) have been shown to interfere with B.S.P. uptake by the liver. The effect of halothane on these criteria would be of interest.

Finally, species variations are naturally a pertinent criticism of the type of study presented here. A mixture of Wistar and Sprague-Dawley rats (as supplied in these laboratories) have been used in this study. Wistar rats have been reported as having genetic variations in enzyme patterns (Javitt, 1965), but it should be emphasized that in this study the same animals were used as controls as were exposed to anaesthetic agents.

Despite such factors, these findings are regarded as significant in that they serve as a pointer to further directions in animal studies and possible application to comparable human situations.

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Massive Lethal Hepatic Necrosis in Rats Anesthetized with Fluroxene, after Microsomal Enzyme Induction

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Rats in which the level of cytochrome P450 is raised by phenobarbital pretreatment die of massive hepatic necrosis when exposed to fluroxene anesthesia. This observation has great relevance to man when consideration is given to the great number of commonly used therapeutic agents which induce increased levels of cytochrome P450. Fluroxene anesthesia should not be used for any patient taking such drugs. (Key words: Enzyme induction; Fluroxene; Liver, necrosis; Phenobarbital.)

UNTIL 1964, inhalational anesthetics in common clinical use, with the exception of trichloroethylene,[1] were considered biologically inert though pharmacologically active. In that year the publication by Van Dyke and associates[2] of their fundamental discovery that all inhalational anesthetics in common use were biotransformed, immediately allowed the possibility that mechanisms of anesthetic toxicity in the liver might have a biochemical basis not previously considered. Subsequent studies by the same workers[3-4] identified the endoplasmic reticulum of the liver cell as the site of, and the nonspecific hydroxylating enzyme cytochrome P450 as the agent for, these reactions.

A pilot study in the rat into the effects on liver function and histology of anesthetics administered in the presence of enhanced activity of this system[5] surprised us by revealing fulminant lethal hepatic necrosis in rats anesthetized with fluroxene, an anesthetic regarded until recently as innocuous to the liver.[6] We confirmed these findings in the following experiment.

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Method

Two groups of ten Wistar rats (female, 200–300 g) each were exposed to fluroxene anesthesia after the levels of cytochrome P450 in the livers had been increased by pretreatment with phenobarbital, ip.[7] The livers were subsequently examined histologically.

Group I rats were pretreated with phenobarbital, 50 mg/kg/day, ip for three days and thereafter (on day 4) anesthetized with fluroxene for one hour, this program being repeated three times. Survivors were sacrificed on the third day after the third anesthesia (fig. 1). Group II animals were pretreated with phenobarbital, 80 mg/kg/day, ip, for three days and thereafter (on day 4) anesthetized with fluroxene for three hours (fig. 2). Animals in both groups were starved for 24 hours before anesthesia (day 3), having been allowed food (Epol Balanced Rations) and water *ad lib.* at all other times.

With each of these primary experimental groups, three groups of control animals were associated (figs. 1 and 2). In the first control group (ten animals each) the two patterns of fluroxene anesthesia—1 hour × 3 or 3 hours × 1—with the 24-hour periods of preanesthetic starvation, were studied without phenobarbital pretreatment. In the second control group (ten animals each) the two schedules of phenobarbital administration (50 mg/kg/day and 80 mg/kg/day) were studied without exposure to fluroxene anesthesia. After sacrifice at the relevant time, the livers were examined histologically. A third group of animals (15 and 5) was used for estimation of the cytochrome P450 levels achieved by the two schedules of phenobarbital administration. In these animals cytochrome P450 content of liver homogenates was measured after the two schedules of phenobarbital ad-

GROUP I

PROGRAM		NO. OF RATS	DAY: 1 – 3		4	---	15
ENZYME INDUCTION + FLUROXENE 1 HOUR		10	Ph B 50 mg/Kg/day	STARVE	FLUROXENE 1 HOUR	X3	SACRIFICE
CONTROL	FLUROXENE 1 HOUR	10	–		–		
CONTROL	ENZYME INDUCTN.	10	Ph.B 50 mg/Kg/day		sacrifice 5	sacrifice 5	sacrifice 5
CONTROL	CYT. P450 LEVEL	15	Ph.B 50 mg/Kg/day				

FIG. 1. Group I program.

GROUP 2

PROGRAM		NO. OF RATS	DAY: 1 – 3		4	---	8
ENZYME INDUCTION + FLUROXENE 3 HOURS		10	Ph.B 80 mg/Kg/day	STARVE	FLUROXENE 3 HOURS		SACRIFICE
CONTROL	FLUROXENE 3 HOURS	10	–		–		
CONTROL	ENZYME INDUCTN	10	Ph.B 80 mg/Kg/day		sacrifice		
CONTROL	CYT. P450 LEVEL	5	Ph.B 80 mg/Kg/day				

FIG. 2. Group II program.

ministration by the method of Schoene *et al.*[8] as modified by Blekkenhorst.[9] For comparison, normal hepatic cytochrome P450 content was estimated in 11 untreated rats (not reflected in tables 1 and 2) on the same diet and after 24 hours of starvation.

The rats were anesthetized in groups of five in a dessicator 28 cm in diameter. The total volume of the dessicator was 27.3 liters, 4 liters of which comprised the volume below the grid, which was filled with standard anesthetic soda lime. Fluroxene, 3 per cent, in oxygen, was passed through the dessicator at a flow rate of 5l/min. This concentration of fluroxene was just sufficient to maintain a light plane of anesthesia, control animals regaining consciousness 2–3 minutes after discontinuance of the anesthesia.

TABLE 1. Liver Weights in Group II, Expressed as Percentage of Body Weight

	Control, No Treatment	Phenobarbital, 80 mg/Day × 3 + Fluroxene, 3 Hours	Fluroxene, 3 Hours	Phenobarbital, 80 mg/Day × 3
Number of rats	5	10	10	5
Liver weight				
MEAN	2.5	5.45	3.94	3.2
SD	±0.12	±0.44	±0.36	±0.18

Results

All ten rats of Group II died during or immediately after their three-hour exposure to fluroxene, as did six of Group I, 12–24 hours after their first one-hour exposure. Their livers were enlarged (table 1) and showed massive central and midzonal necrosis (figs. 3, 4, and 5). Four Group I animals, though clinically sick after their first exposure to fluroxene, recovered and survived two further one-hour exposures.

Liver cytochrome P450 content was increased from control "no-treatment" levels by both schedules of phenobarbital pretreatment (table 2). The Group I schedule (50 mg/kg/day) increased cytochrome P450 levels by a factor (on average) of 1.5. The Group II schedule (80 mg/kg/day) increased cytochrome P450 content by a factor of 2.6 (difference statistically significant).

No rat in the control groups anesthetized with fluroxene without enzyme induction died, but the livers all showed demonstrable histologic changes (fig. 6), the most characteristic being feathery degeneration of the cytoplasm with widespread vacuolation.

The livers of rats pretreated with phenobarbital without exposure to fluroxene showed only the changes in liver weight (table 1) and histology described previously by Burger and Herdson.[10]

Discussion

This study shows that fluroxene anesthesia in the rat in the presence of increased levels of hepatic cytochrome P450 causes a rapidly lethal fulminant hepatic necrosis, and that fluroxene anesthesia alone causes histologically detectable changes in the liver. This is indeed a remarkable finding, considering that this drug was originally introduced into clinical practice as long ago as 1953,[11] that in subsequent clinical use in man it has accumulated an impressive record of clinical safety, and that, let alone there being any suggestion of hepatotoxicity, it has been recommended as the anesthetic of choice for hepatic[12] and renal[13] transplantation. Because of this one might seriously question the relevance to man of these findings in the rat, were it not for the recently published report[14] of the death from massive hepatic

TABLE 2. Cytochrome P450 after Phenobarbital Pretreatment

	Control, No Treatment	Group 1			Phenobarbital, 80 mg/Day for 3 Days ×1
		Phenobarbital, 50 mg/Day for 3 Days ×1	Phenobarbital, 50 mg/Day for 3 Days ×2	Phenobarbital, 50 mg/Day for 3 Days ×3	
Number of rats	11	5	5	5	5
Cytochrome, P450, nM/g liver					
MEAN	44	70	62	70	116
SD	±7.9	±35.0	±5.9	±19.4	±28.7

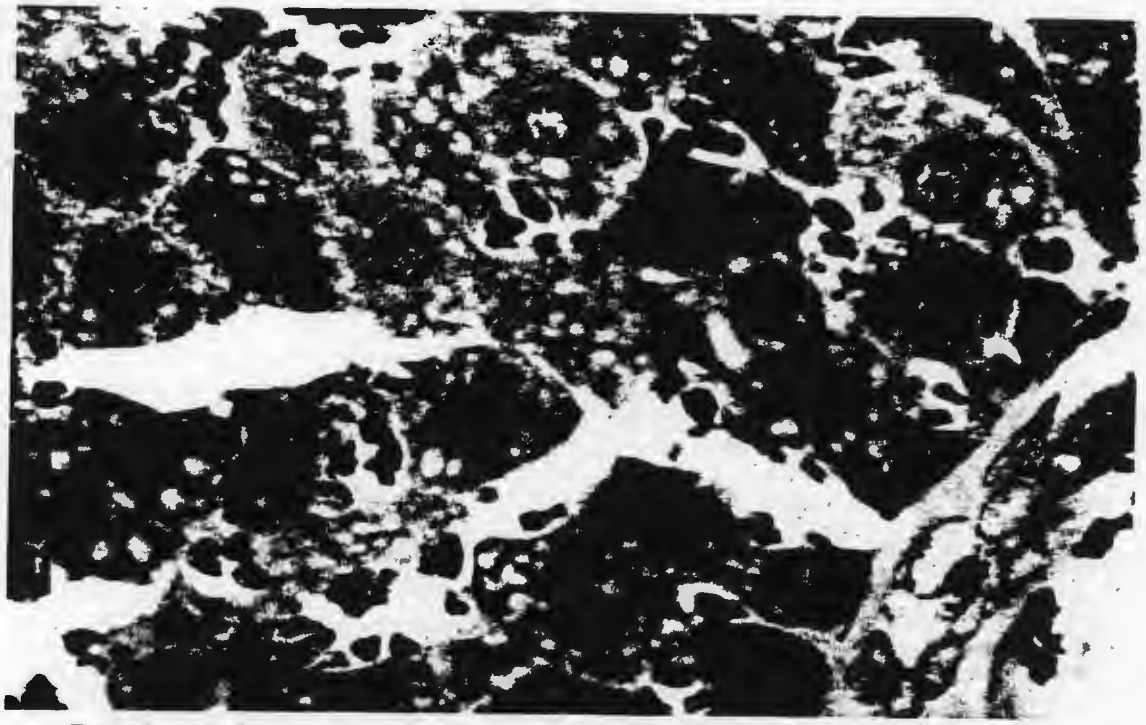

FIG. 3. Section of the liver of a Group II rat dying after 3 hours of exposure to fluroxene after enzyme induction, central vein at lower right. Note the cloudy swelling and acute early fatty infiltration. Two already-dead cells with darkly staining nuclei and dark cytoplasm are visible to right of center. Hematoxylin and eosin, ×400.

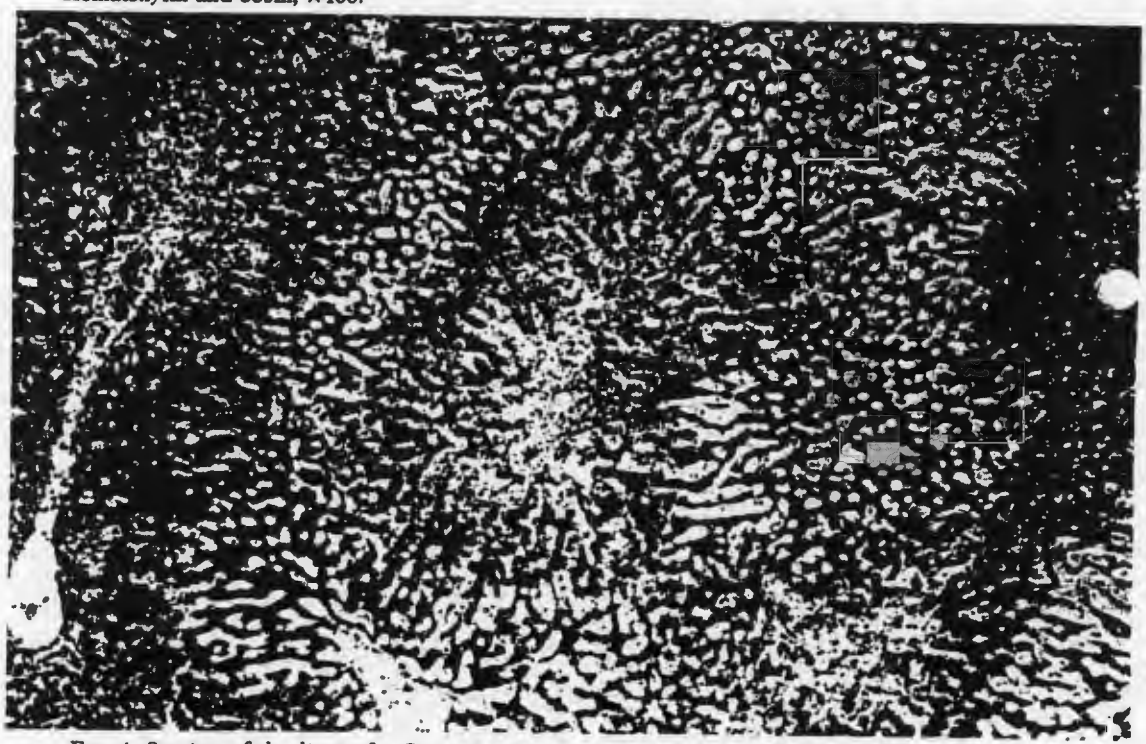

FIG. 4. Section of the liver of a Group I rat dying 24 hours after one hour of exposure to fluroxene after enzyme induction. Note the central and midzonal necrosis with dilated peripheral sinusoids. Hematoxylin and eosin, ×40.

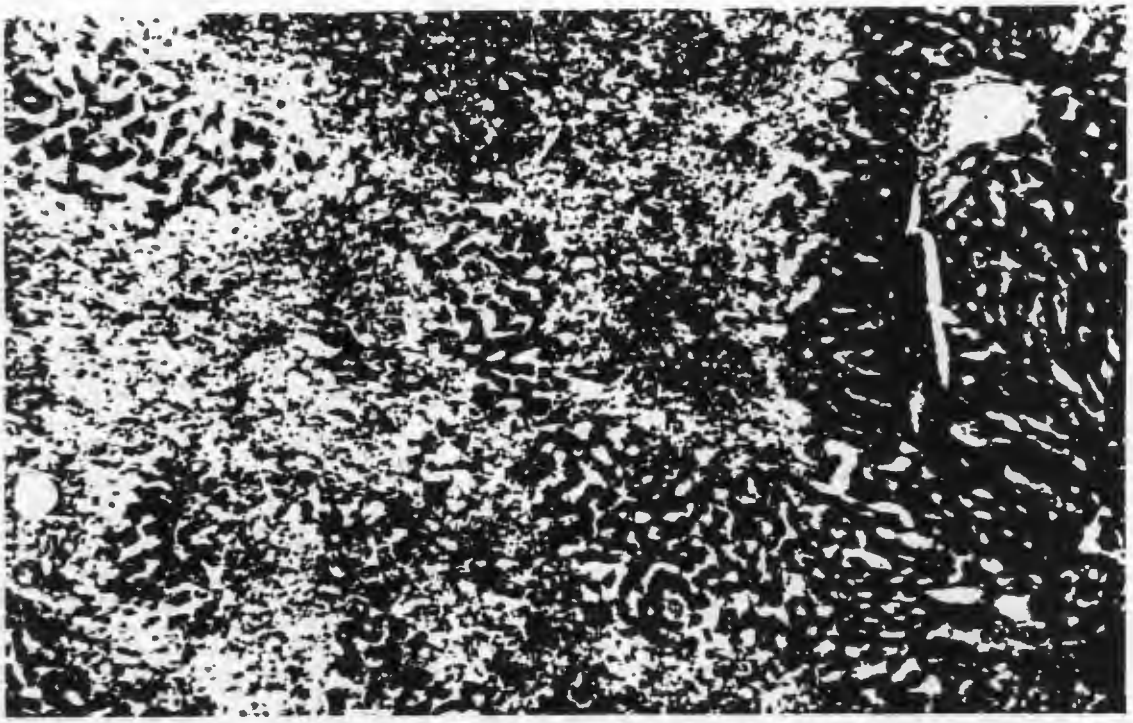

FIG. 5. Section of the liver of a Group I rat surviving a program of 3 × 1 hour exposures to fluroxene after enzyme induction. The rat was sacrificed on the third day after the last exposure. Note the complete central and midzonal necrosis with survival of peripheral zone cells. Hematoxylin and eosin, ×40.

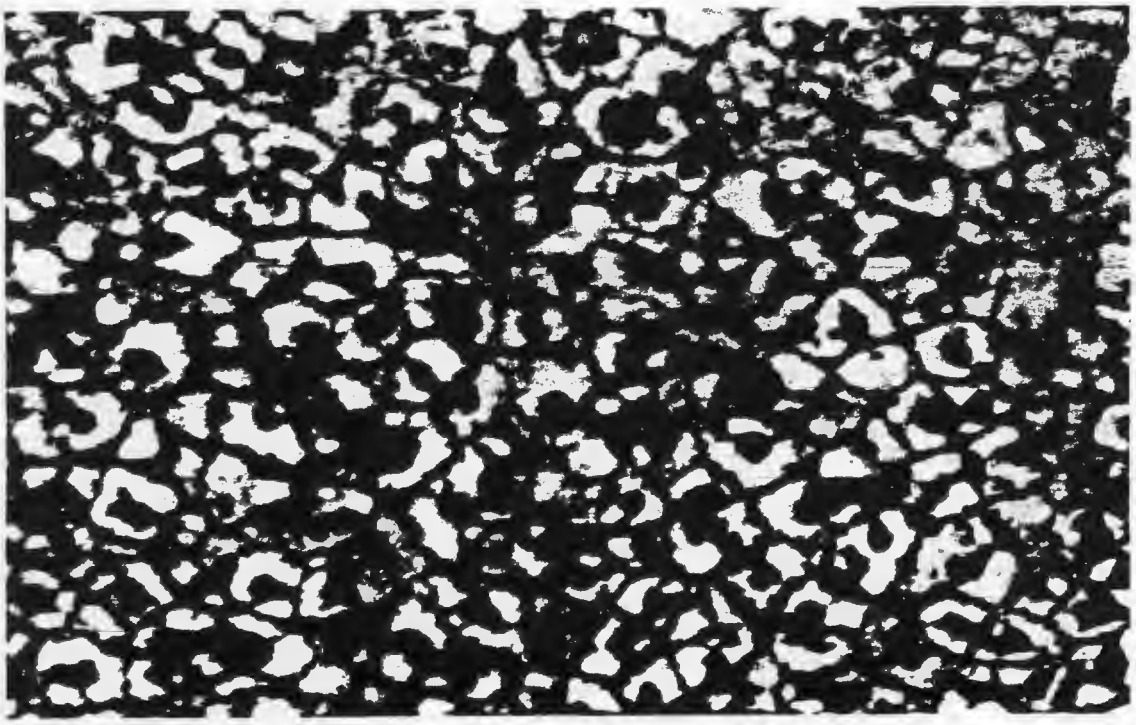

FIG. 6. Section of the liver of a rat from the control group sacrificed after 3 × 1 hour exposures to fluroxene without enzyme induction. Note the generalized vacuolation and feathery degeneration, giving a "chicken-wire" appearance. Hematoxylin and eosin, ×200.

TABLE 3. Some Drugs with Enzyme-inducing Properties

Hypnotics and sedatives	Barbiturates
Central nervous system stimulants	Amphetamines
Anticonvulsants	Meprobamate
	Chlordiazepoxide
Antipsychotics	Chlorpromazine
Hypoglycemic agents	Tolbutamide
Anti-inflammatory agents	Phenylbutazone
Antihistamines	Diphenylhydramine
Steroid hormones	

necrosis following fluroxene anesthesia for a gastrectomy of an epileptic patient treated with phenobarbital and diphenylhydantoin, drugs with potent enzyme-inducing properties.

The more fundamental question posed by our observations relates to the mechanism of hepatic damage and its relationship, if any, to that which follows other drugs such as carbon tetrachloride and chloroform, and to the whimsical hepatotoxicity of halothane.

Fluroxene is subject to biotransformation in the liver.[15,16] Evidence from our study that massive necrosis of the liver occurred only in animals in which fluroxene biotransformation would have been increased fits well with the observations of Cascorbi and Singh-Amaranath[17] that toxicity of fluroxene in mice was enhanced by enzyme induction and decreased by enzyme depression. All this evidence would seem to point to the probability that it is some reaction in or metabolite resulting from the biotransformation of fluroxene which is the toxic agent.

What this reaction or metabolite may be is a matter of conjecture. Though Blake and co-workers[18] have shown that trifluorethanol and trifluoroacetic acid, both products of fluroxene transformation, are toxic in dogs and mice, their aminals did not show any hepatic necrosis, although mild cloudy swelling and fatty accumulation are described as occurring in the dogs. To explain the gross hepatic necrosis we observed, we speculate that free radical formation[19] or epoxidation of the vinyl radical[20] of fluroxene as a step in its transformation to CO_2 may take place and result in lipid peroxidation of the cell organelle membranes, this leading ultimately to the massive cell destruction we saw.

Speculation aside, the clinical implication of our observations is clear. Fluroxene anesthesia should not be used for any patient who is on a regime of treatment with a drug that has enzyme-inducing properties[7] (table 3).

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Anesthesia AND Analgesia

.....CURRENT RESEARCHES

Fluroxene (2, 2, 2-Trifluoroethyl Vinyl Ether)
Toxicity: A Chemical Aspect

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Fluroxene (2, 2, 2-Trifluoroethyl Vinyl Ether) Toxicity: A Chemical Aspect

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Fluroxene is highly toxic to several animal species. This toxicity is enhanced by induction of raised levels of hepatic microsomal enzymes. Experiments in rats are described which seek to assess the relative contribution to this toxicity of the individual component groups of the fluroxene molecule. Though results point to the trifluoroethyl moiety of fluroxene as that aspect of the molecule most responsible for the observed mortality, reduction of the vinyl group modifies the pattern of liver injury. That the liver necrosis, manifest following fluroxene anesthesia in the presence of microsomal induction, is alone the direct cause of the acute death of experimental animals is questioned.

THE anesthetic, fluroxene, has recently been demonstrated to be toxic to several animal species under certain circumstances.[1] Rats, guinea pigs, and mice have been shown to display toxic effects when chronically exposed to concentrations of fluroxene as low as 30 parts per million (1/2000 MAC).

That this toxicity is the result of some aspect of fluroxene biotransformation, rather than an effect of fluroxene itself, is suggested by the demonstration of increased manifestations of toxicity following hepatic microsomal enzyme induction, with a decrease in these when microsomal enzyme activity is suppressed.[2,3]

There is some evidence to suggest that formation of the highly toxic trifluoroethanol from the fluorinated ethyl moiety of fluroxene is responsible for these toxic effects.[4,5] But, whereas fluroxene anesthesia in the presence of enhanced hepatic microsomal mixed function oxidase levels resulted in florid liver necrosis,[6] direct exposure of animals to trifluoroethanol resulted in the death of the animal without similar histologic changes in the liver.[5] We questioned, therefore, the contribution to the manifest toxicity that might result from the catabolism of the vinyl moiety of fluroxene.

To this end, we exposed groups of rats in

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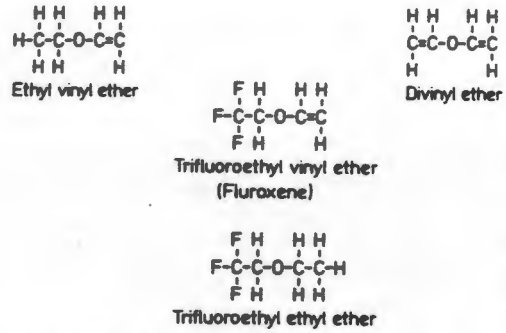

FIG 1. Structural formulas of fluroxene and the 3 related compounds studied.

which levels of microsomal enzymes had been increased (together with appropriate control groups) to fluroxene anesthesia and to 3 related compounds, each of which comprised some aspect of the fluroxene structure. We noted thereafter manifestations of toxicity as determined by (1) death or survival of the animal and (2) examination of liver histology.

The structural formulas of fluroxene and the 3 other anesthetic compounds exemplifying modifications of aspects of this structure (fig 1) are as follows:

1. Fluroxene: 2,2,2-trifluoroethyl vinyl ether.*

2. Vinamar, or ethyl vinyl ether, the nonfluorinated analog of fluroxene.†[7]

3. Vinesthene, or divinyl ether.‡[8]

4. Trifluoroethyl ethyl ether (TFEE), a chemically reduced form of fluroxene in which the vinyl moiety is hydrogenated to an ethyl group.

METHODS

The experimental animals were 200 to 300-gm male Sprague Dawley rats. Liver microsomal enzyme levels were enhanced by the daily intraperitoneal administration of phenobarbital (80 mg/kg/day) for 3 days preanesthetic. The concentration of phenobarbital sodium in saline solution used was 10 mg/ml, giving injection volumes of approximately 2 ml. Control rats were injected with similar volumes of physiologic saline solutions.

Having been allowed food and water ad lib until then, rats were starved for 24 hours after the 3rd phenobarbital injection, at which time (day 4) they were anesthetized. In the event of survival, this program was repeated. Survivors were then sacrificed by cervical dislocation on the 3rd day thereafter.

Animals were anesthetized in groups of 5 for periods of 3 hours in a standard dessicator 28 cm in diameter with a volume of 27.3 L, of which 4 L, the volume below the grid, was filled with standard anesthetic soda lime. Additional chemical CO_2 absorption was provided for by the placement of extra vertically standing perforated containers of soda lime between the animals.

The anesthetic agents were carried by a flow of O_2 (5 L/min) passed through a vaporizer and the anesthetic chamber in series. In such circumstances, the level of ambient CO_2 in the anesthetic chamber was less than 1%. In the case of ethyl vinyl ether and divinyl ether, because of their limited availability, a lower O_2 flow (2 L/min) was used.

Animals were exposed to anesthetic and subanesthetic concentrations of fluroxene (3 and 0.5%) delivered from a Fluoromartec* vaporizer, or to concentrations of the other agents sufficient to maintain a very light level of anesthesia, in equianesthetic concentrations. This level of anesthesia was defined as that at which response to paw-pinching became obtunded. TFEE, which produced anesthesia in rats, was synthesized in our laboratory by reduction of commercially available fluroxene with hydrogen in the presence of a palladium catalyst. Reduction of the vinyl function was checked by IR spectrophotometry, the material being treated to remove peroxides and redistilled before use.[9] All agents were screened to exclude the presence of peroxides.

After the death or sacrifice of experimental animals, the livers were immediately excised and fixed in formalin. Thereafter, sections of liver were stained with hematoxylin-eosin (H & E) and submitted to light microscopy.

RESULTS

The tabulated results, in terms of anesthetic agent, number of 3-hour exposures, mortality, and histology (table) reveal that all enzyme-induced rats exposed to fluroxene, both in anesthetic and subanesthetic concentrations, died after a single 3-hour exposure. Similar mortality followed expo-

*Ohio Medical Products.
†Fluka, AG.
‡British drug houses.

TABLE

Anesthetic		Microsomal state: + = with induction 0 = no induction	Duration of anesthetic, hours	Number of exposures	Mortality	Histology
Fluroxene	3%	+	3	1	20/20	Periportal congestion, gross centrizonal necrosis or marked cell damage in all.
	0.5%	+	3	1	10/10	Periportal congestion, gross centrizonal necrosis or marked cell damage in all.
	3%	0	3	2	0/20	Periportal hydropic changes, 8/20.
	0.5%	0	3	2	0/10	Normal histology.
TFEE		+	3	1	12/12	Frank centrizonal necrosis, 2/12. Marked fatty infiltration ± areas of focal necrosis and cell damage, 10/12
		0	3	1	0/12	No centrizonal necrosis. Miliary fatty infiltration and periportal hydropic degeneration, 6/12.
Vinamar		+	3	2	0/9	No necrosis. Periportal hydropic degeneration, 2/9.
		0	3	2	0/10	No necrosis. Periportal hydropic degeneration, 5/10.
Vinesthene		+	3	2	0/5	No necrosis. Periportal hydropic degeneration, 4/5.
		0	3	2	0/5	No necrosis. Periportal hydropic degeneration, 4/5.

sure of enzyme-induced rats to TFEE. This mortality, which occurred during or shortly after anesthesia, was associated with histologic evidence of severe centrizonal liver damage: frank necrosis, chromatolysis, fatty infiltration, pyknosis, and cloudy swelling (figs 2 and 3). Congestion, especially in the periportal areas, was a marked feature, particularly of those enzyme-induced animals anesthetized by fluroxene. The incidence of frank necrosis was much lower in enzyme-induced animals after anesthesia with TFEE, in which circumstance marked fatty infiltration with centrizonal pallor was the most consistent finding (fig 4). The magnitude of histologic liver damage in some animals after TFEE did not appear sufficient to account for death.

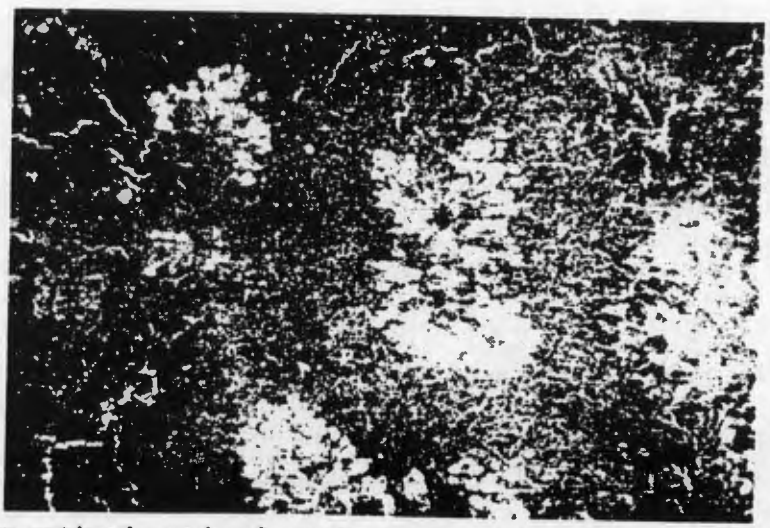

FIG 2. Diffuse centrizonal necrosis and congestion in enzyme-induced rat dying 8 hours after 3-hour exposure to 0.5% fluroxene. H & E x 160.

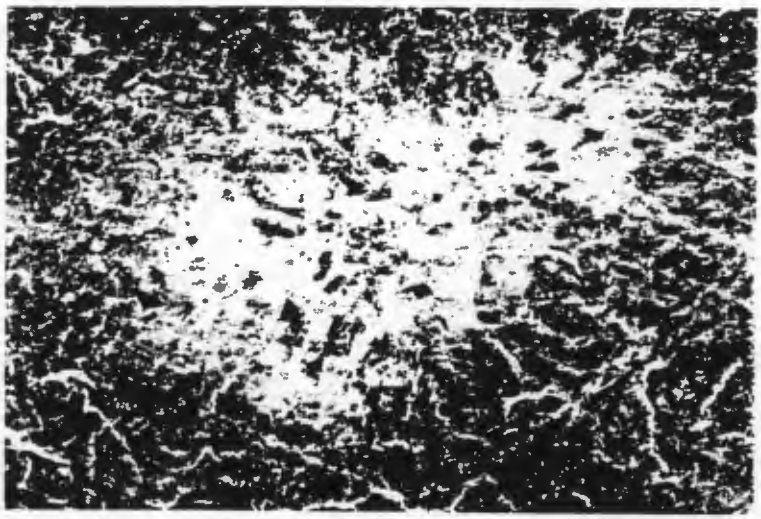

FIG 3. Early centrizonal changes in enzyme-induced rat dying at termination of 3-hour exposure to 3% fluroxene. Note chromatolysis, pallor, congestion, and occasional pyknotic cells. H & E x 400.

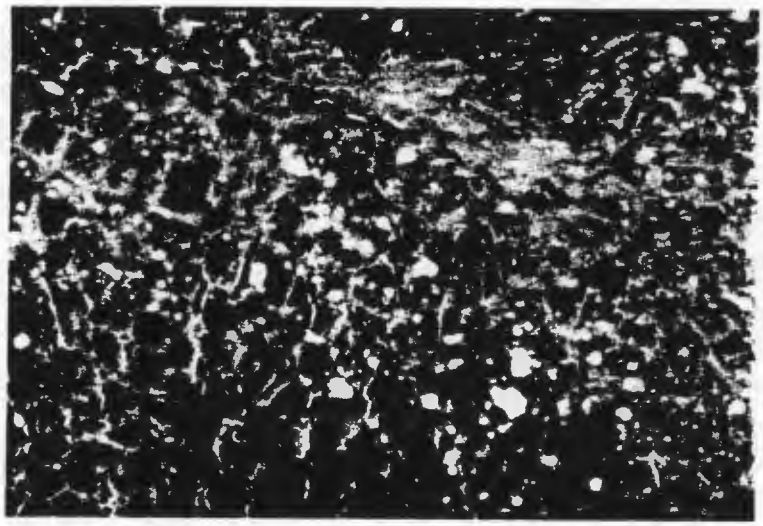

FIG 4. Centrizonal pallor, chromatolysis, and fatty infiltration in enzyme-induced rat dying 12 hours after 3-hour exposure to TFEE. H & E x 800.

No mortality occurred in the relevant fluroxene- or TFEE-anesthetized, uninduced controls, nor in any induced or uninduced animal exposed to vinamar or vinesthene. Nor was there evidence of centrizonal necrosis in any of these animals. However, the livers of some showed a nonspecific periportal hydropic change, apparent in both enzyme-induced and uninduced control animals.

DISCUSSION

Following phenobarbital induction of microsomal enzymes—

1. Fluroxene, even in subanesthetic concentrations, causes florid liver necrosis and death.

2. Modification of the vinyl group of fluroxene to an ethyl moiety does not lessen this mortality, though it does produce a different and less extreme pattern of liver damage.

3. Neither mortality nor histologic liver necrosis followed repeated exposures of animals to ethyl vinyl ether or divinyl ether.

Considering as the index of toxicity the mortality which occurred in the presence of enzyme induction following exposure of rats

to the test agents, it is immediately apparent that the trifluoroethyl moiety is the common denominator, no mortality resulting in its absence. However, the modification of the histologic pattern of liver injury following the use of TFEE rather than fluroxene suggests that the vinyl group must in some way be involved in the induction of the liver damage which, in itself therefore, may not be the direct cause of death in the experimental animals. This is in agreement with our earlier report that catabolism of the vinyl group of fluroxene, *in vivo* or *in vitro*, results in the destruction of hepatic cytochrome P450.[8,9] It is surprising, in view of the apparent damage caused to the liver by the biotransformation of the vinyl group in fluroxene, that neither ethyl vinyl ether nor divinyl ether produces similar liver damage. This aspect is presently under investigation.

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THE ROLE OF CYTOCHROME P-450 IN THE TOXICITY OF FLUROXENE (2,2,2-TRIFLUOROETHYL VINYL ETHER) ANAESTHESIA *IN VIVO**

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Abstract—Induction of type P-450 cytochromes in rats by i.p. injections of phenobarbital potentiated the toxicity (100% mortality) of the normally non-toxic anaesthetic fluroxene (2,2,2-trifluoroethyl vinyl ether). The toxic effects were eliminated by administration of 2-allyl-2-*iso*propylacetamide prior to anaesthesia. Hepatic microsomal cytochrome P-450 levels of the dead rats were markedly diminished relative to unanaesthetised induced controls. Induction by 3-methylcholanthrene and 3,4-benzpyrene did not potentiate toxic effects of fluroxene but anaesthesia after mixed induction with 3-methylcholanthrene and phenobarbital manifested toxicity more rapidly than induction with phenobarbital alone. When 2,2,2-trifluoroethyl ethyl ether was used as the anaesthetic similar toxic effects were observed except that levels of type P-450 cytochromes were not depressed at the time of death and induction with 3-methylcholanthrene did potentiate toxic effects with this anaesthetic. We interpret these results to indicate that cytochrome P-450 catalyses an essential step in the production of toxic metabolites from fluroxene and that elevated concentrations of the enzyme are required to potentiate the toxicity. Apparently, cytochrome P-448 does not metabolize fluroxene and elevated levels of this enzyme therefore do not potentiate the toxicity of fluroxene anaesthesia. The ability of fluroxene to destroy cytochrome P-450 resides in its vinyl group while the toxic metabolite arises from the trifluoroethyl moiety.

Fluroxene (2,2,2-trifluoroethyl vinyl ether) is a volatile anaesthetic agent first introduced into clinical practice in 1953 [1]. Although it has accumulated a considerable record of safe clinical usage in man with no evidence of organ toxicity [2, 3], recent work has shown it to be toxic to many animal species [4, 5, 6] and even to man under certain circumstances [7, 8, 9, 10]. This toxicity, which includes hepatotoxicity [11], is markedly enhanced by pretreatment of the experimental animal with phenobarbital. The toxicity is considered to result from biotransformation of fluroxene into toxic metabolites such as trifluoroethanol glucuronide [12, 13] or trifluoroacetaldehyde [14, 15] rather than to be an effect of fluroxene itself [4, 16]. Since phenobarbital is known to induce the proliferation of the hepatic endoplasmic reticulum and the biosynthesis of the components of the cytochrome P-450 drug metabolizing pathway [17], it appeared possible that hepatic microsomal cytochrome P-450 could be involved in potentiating the toxicity of fluroxene anaesthesia [11], especially in view of the localization of fluroxene metabolism in the hepatic endoplasmic reticulum [12]. We will use the terms 'cytochromes P-450' or 'type P-450 cytochromes' to represent both cytochrome P-450 and cytochrome P-448 (see later). We have consequently investigated the effect of induction of cytochromes P-450 on the toxicity of fluroxene and 2,2,2-trifluoroethyl ethyl ether (TFEE) anaesthesia in rats with a view to investigating the role of the cytochromes P-450 in potentiating these toxic effects, and determining the mechanisms of production of toxic effects.

MATERIALS AND METHODS

Materials. Sodium phenobarbital (PB) and 3-methylcholanthrene (MC) were obtained from Maybaker, S.A. and Eastman-Kodak, respectively. 3,4-benzpyrene (BP) was from Sigma Chemicals. 2-Allyl-2-isopropylacetamide (AIA) was a generous gift from Hoffman-La Roche, Nutley, New Jersey. Ampoules of sterile saline (0·9%, w/v) were obtained from Petersen, Ltd., S.A. Fluroxene was supplied by Ohio-Medical Products, Madison, Wisconsin. 2,2,2-Trifluoroethyl ethyl ether (TFEE) was prepared by hydrogenation of fluroxene using our previously published method [18]. The TFEE was tested for the presence of peroxides with 5% aqueous KI (w/v) immediately before use. Cylinders of CO and O_2 were supplied by Afrox Ltd. All other chemicals were analytical grade reagents. Water was distilled and deionized.

Animals. Male Wistar rats weighing between 175 g and 300 g were used in all experiments; animals were permitted free access to Epol Laboratory Chow (protein min. 20%, fat 2·5%, fibre max. 6%, calcium 1·4%,

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phosphorus 0·7%) and water. Induction of cytochrome P-450 was by i.p. injection of sodium phenobarbital (80 mg/kg/day in 0·9% sterile saline), 3,4-benzpyrene (40 mg/kg/day in corn oil) or 3-methylcholanthrene (40 mg/kg/day in corn oil) for three consecutive days. One group of rats received sodium phenobarbital (80 mg/kg) for 1 day only. In the case of a simultaneous induction by sodium phenobarbital and 3-methylcholanthrene, the animals received the above doses in separate i.p. injections for 3 consecutive days. Control animals were injected i.p. with vehicle for 3 days. All animals were fasted overnight after the final injection, and where indicated, anaesthetised the following morning. Animals receiving AIA were injected subcutaneously with a dosage of 200 mg/kg (20 mg/ml in 0·9% sterile saline) in the loose skin of the neck 1 hr prior to anaesthesia. Rats were anaesthetised in groups of ten or less in a special perspex anaesthetic chamber (30 × 30 × 60 cm). Animals were placed on a fenestrated floor raised 8 cm from the base of the chamber, the space below the fenestrated floor being occupied by standard anaesthetic sodalime. The anaesthetic (fluroxene or TFEE), vaporised by a 5 l/min stream of oxygen through a Cyprane Fluoromatek vaporiser, was passed as a continuous stream into the anaesthetic chamber through an inlet port sited at one corner beneath the fenestrated floor and exhausted through an exhaust port sited at the diagonally opposite upper corner. A flow rate of this order was shown to result in CO_2 concentration of <0·1%. Animals were exposed to a 2 hr period of anaesthesia of a level approximately equivalent to that which would be attained by the minimum anaesthetic concentration of the respective agent, this being achieved by exposure of animals to a 3% concentration of fluroxene or TFEE. Unanaesthetised control animals in these experiments were exposed to air. In subsequent experiments in which unanaesthetised control rats were exposed to O_2 only, no differences in the parameters under consideration were noted. Toxicity was judged by death after anaesthesia. Animals not dying were sacrificed by cervical fracture. Liver weight was determined immediately after excision of the liver.

Preparation of microsomes. Livers were excised from animals within minutes of death. A portion of the liver was fixed in buffered aqueous formaldehyde (10% v/v) for histological investigation and the remainder was utilized for the preparation of microsomes by differential ultracentrifugation [19]. Protein concentration was determined by the method of Lowry *et al.* [20], as modified by Chaykin [21] using bovine serum albumin as a standard.

Spectrophotometry. Microsomal suspensions (protein concentration 2 mg/ml) were divided between two cuvettes of 1-cm optical path length. Difference absorbance spectra of microsomal suspensions were recorded at room temperature in a Unicam SP1800 recording spectrophotometer using the cell holder adjacent to the photomultiplier. Microsomal cytochrome P-450 content was determined by the CO difference spectral method of Omura and Sato [22]; it should be noted that total type P-450 cytochromes were measured, and that these values include the contributions of both cytochromes P-450 and P-448 to the total.

RESULTS

Toxicity of fluroxene anaesthesia. The effects of various inducing agents of cytochromes P-450 on the toxicity to rats of fluroxene anaesthesia are reported in Table 1. Injection of saline alone or in conjunction with exposure to 3% fluroxene anaesthesia for 2 hr did not produce toxic effects and did not affect cytochrome P-450 content per mg of microsomal protein or the ratio of liver to body weight relative to untreated control animals.

Phenobarbital treatment of animals for three days, as described in Methods, resulted in a 2·6-fold increase in cytochrome P-450 levels and a slight increase in liver weight but produced no toxic effects. All rats induced with phenobarbital and subsequently anaesthetised with fluroxene died between 1 and 3 hr after the termination of anaesthesia (100% mortality). Immediately after death, these animals were shown to have a 2·8-fold decrease in cytochromes P-450 content and markedly enlarged livers, relative to unanaesthetised phenobarbital induced controls.

The toxic effects of fluroxene anaesthesia observed in phenobarbital pretreated animals were prevented by treatment of the animals with AIA 1 hr prior to anaesthesia. No deaths were observed in this latter group. The animals appeared normal at the time of sacrifice and up to that time demonstrated none of the symptoms of the phenobarbital induced fluroxene anaesthetised animals (e.g. crouching, staring coat [23], glassy eyes, sluggishness, bleeding eyelids). Furthermore, fluroxene anaesthesia apparently does not elicit any marked effect on cytochromes P-450 content or liver weight in phenobarbital induced AIA treated rats, relative to unanaesthetised phenobarbital induced AIA treated animals.

Pretreatment of animals with phenobarbital for 1 day only, elevated cytochromes P-450 levels approximately 2-fold relative to control animals and was in itself non-toxic. Fluroxene anaesthesia of animals induced in this manner was less toxic (40% mortality), and the animals that did not die subsequent to anaesthesia appeared normal prior to sacrifice at 190 hr.

Injection with corn oil, the vehicle for induction with polycyclic hydrocarbons, does not affect the hepatic microsomal cytochromes P-450 levels but does increase liver weight relative to saline-injected or untreated controls. The above effects were not altered by subsequent fluroxene anaesthesia.

Injection of 3,4-benzpyrene or 3-methylcholanthrene suspended in corn oil increased levels of type P-450 cytochromes approximately 2-fold and increased liver weight relative to corn oil controls. Subsequent fluroxene anaesthesia was non-toxic and did not affect cytochrome levels and liver weight of 3,4-benzpyrene induced animals but did slightly reduce the cytochrome levels of those rats pretreated with 3-methylcholanthrene. After five days the cytochrome content and liver weight of the fluroxene anaesthetised rats decreased to within the range found for control animals. The appearance and behaviour of the anaesthetised animals was completely normal at the time of sacrifice.

Mixed induction by injection of 3-methylcholanthrene plus phenobarbital elevated levels of both cytochromes P-448 and P-450 and resulted in increased

Table 1. Toxicity of fluroxene anaesthesia

Pretreatment* (no. rats)	Anaesthetised	Time of death† (hr)	Toxicity	Cyt P-450 (nmol/mg microsomal protein)	wt. liver/ wt. body (%)
Untreated (2)	—	—	—	1·27 ± 0·12	2·76 ± 0·22
Saline (2)	—	K24	—	1·31 ± 0·10	3·07 ± 0·29
Saline (2)	+	K24	—	1·32 ± 0·02	3·47 ± 0·63
PB (4)	—	K4	—	3·42 ± 0·34	3·39 ± 0·05
PB (9)	+	1–3	+	1·23 ± 0·13	4·63 ± 0·44
PB + AIA (2)	—	K–2§	—	0·81 ± 0·02	3·83 ± 0·08
PB + AIA (2)	—	K24	—	0·91 ± 0·02	4·82 ± 0·01
PB + AIA (2)	—	K166	—	0·99 ± 0·04	3·20 ± 0·18
PB + AIA (2)	+	K24	—	0·75 ± 0·15	4·70 ± 0·10
PB + AIA (2)	+	K141	—	0·98 ± 0·07	2·69 ± 0·02
PB (3)‡	—	K0	—	2·33 ± 0·17	4·07 ± 0·16
PB (2)‡	+	18	+	1·41	4·26
PB (3)‡	+	K190	—	0·98 ± 0·14	4·14 ± 0·29
Corn Oil/saline (2)	—	K2	—	1·28 ± 0·12	3·84 ± 0·31
Corn oil/saline (2)	+	K2	—	1·16 ± 0·15	3·72 ± 0·08
PB + MC (2)	—	K2	—	4·87 ± 0·32	5·10 ± 0·38
PB + MC (5)	+	2	+	1·92 ± 0·17	6·96 ± 0·44
Corn Oil (2)	—	K24	—	1·27 ± 0·12	3·75 ± 0·16
Corn Oil (2)	+	K24	—	1·10 ± 0·20	3·97 ± 0·05
BP (2)	—	K24	—	1·86 ± 0·09	4·49 ± 0·02
BP (6)	+	K24	—	1·57 ± 0·21	4·31 ± 0·31
BP (2)	+	K140	—	1·31 ± 0·03	3·41 ± 0·21
MC (3)	—	K24	—	2·47 ± 0·12	4·81 ± 0·08
MC (2)	—	K190	—	1·34 ± 0·09	4·88 ± 0·15
MC (3)	+	K24	—	1·52 ± 0·29	4·89 ± 0·79
MC (2)	+	K190	—	1·51 ± 0·04	4·62 ± 0·32

Anaesthesia for 2 hr at 3% fluroxene. All values are means ± S.D. For experimental details see text.

* Abbreviations used are: BP, 3,4-benzpyrene; MC, 3-methylcholanthrene; PB, phenobarbital; AIA, allyl-*iso*-propylacetamide. The first two compounds are injected in corn oil, the remainder in saline.

† From the end of anaesthesia or, if not anaesthetized, from the equivalent time. K indicates that the animals were killed.

‡ Injected with only 1 dose of PB (80 mg/kg) 1 day prior to anaesthesia.

§ Killed at a time equivalent to the beginning of anaesthesia.

hepatic weight and cytochromes P-450 content in excess of that seen for either type of induction individually [24], but was in itself non-toxic (0% mortality). Fluroxene anaesthesia of these induced animals resulted in 100% mortality within 2 hr of the end of anaesthesia. A marked decrease in cytochrome levels and an increase in liver weight relative to the unanaesthetised but similarly induced animals were also noted.

Toxicity of TFEE anaesthesia. The effects of TFEE anaesthesia on rats with elevated levels of cytochrome P-450 and/or P-448 are reported in Table 2. Anaesthesia with TFEE did not affect animals injected with vehicle except for a slight increase in liver weight in the case of saline.

In phenobarbital-induced rats, anaesthesia with TFEE was toxic: 100% mortality was observed within 35 hr after termination of anaesthesia at which time a slight decrease in cytochromes P-450 content and an increase in liver weight were evident. Treatment of phenobarbital-induced rats with AIA prior to anaesthesia with TFEE completely protected the animals from the toxicity of anaesthesia (0% mortality). Cytochrome levels and liver weights in unanaesthetised and anaesthetised rats receiving phenobarbital and AIA were comparable.

TFEE anaesthesia of 3.4-benzpyrene induced animals was non-toxic (0% mortality) and did not alter the levels of type P-450 cytochromes but did diminish liver weight relative to that found for unanaesthetised benzpyrene treated controls. 3-Methylcholanthrene induction did potentiate the toxicity of TFEE anaesthesia (80% mortality) without markedly affecting cytochrome P-450 levels but decreased the liver weights in the animals that died. Increasing the duration of TFEE anaesthesia to 160 min for 3-methylcholanthrene induced rats increased mortality to 100%. Administration of AIA after 3-methycholanthrene induction did not noticeably diminish levels of type P-450 cytochromes but did overcome the toxic effects of TFEE anaesthesia.

Induction with phenobarbital and 3-methylcholanthrene together potentiated the toxicity of TFEE anaesthesia more than phenobarbital alone. TFEE lowered the levels of type P-450 cytochromes of these induced rats only very slightly.

DISCUSSION

Our experiments on the effects of various microsomal inducing agents on the toxicity of the anaesthetic fluroxene were initiated to explore the role of the cytochromes P-450 in potentiating this toxicity and

Table 2. Toxicity of 2,2,2-trifluoroethyl ethyl ether (TFEE) anaesthesia

Pretreatment* (no. rats)	Anaesthetised	Time of death† (hr)	Toxicity	Cyt P-450 (nmol/mg microsomal protein)	wt. liver/ wt. body (%)
Untreated (2)	—	—	—	1·27 ± 0·12	2·76 ± 0·22
Saline (2)	—	K24	—	1·31 ± 0·10	3·07 ± 0·29
Saline (3)	+	K24	—	1·25 ± 0·05	3·79 ± 0·25
PB (4)	—	K24	—	3·41 ± 1·08	4·82 ± 0·42
PB (8)	+	0–35	+	2·49 ± 0·63	5·33 ± 0·58
PB + AIA (2)	—	K–2§	—	0·81 ± 0·02	3·82 ± 0·08
PB + AIA (2)	—	K46	—	0·97 ± 0·08	4·13 ± 0·22
PB (2)	—	K46	—	2·13 ± 0·08	3·84 ± 0·14
PB + AIA (3)	+	K46	—	1·22 ± 0·14	3·83 ± 0·01
Corn Oil/Saline (2)	+	K24	—	1·32 ± 0·08	3·82 ± 0·28
Corn Oil/Saline (2)	—	K2	—	1·28 ± 0·12	3·84 ± 0·31
PB + MC (2)	—	K26	—	4·80 ± 0·69	5·69 ± 0·39
PB + MC (5)	+	17–26	+	4·09 ± 0·29	5·19 ± 0·22
Corn Oil (2)	—	K24	—	1·27 ± 0·12	3·75 ± 0·16
Corn Oil (2)	+	K24	—	1·31 ± 0·14	3·81 ± 0·22
BP (2)	—	K24	—	2·25 ± 0·05	4·75 ± 0·05
BP (3)	+	K24	—	2·13 ± 0·43	3·27 ± 0·23
BP (2)	+	K145	—	1·38 ± 0·11	4·15 ± 0·25
MC (5)	—	K48	—	1·72 ± 0·09	5·55 ± 0·35
MC (4)	+	27–76	+	1·96 ± 0·44	3·56 ± 0·40
MC (1)	+	K144	—	1·03	5·05
MC + AIA (3)	—	K–2§	—	1·74 ± 0·32	4·85 ± 0·33
MC + AIA (3)	—	K164	—	1·92 ± 0·16	5·20 ± 0·48
MC + AIA (3)	+	K164	—	1·94 ± 0·09	5·53 ± 0·37
MC (5)‡	+	17–24	+	3·24 ± 0·34	4·19 ± 0·20

Anaesthesia for 2 hr at 3% TFEE. All values are means ± S.D. For experimental details see text.

* Abbreviations used are: BP, 3,4-benzpyrene; MC, 3-methylcholanthrene; PB, phenobarbital; AIA, allyl-*iso*-propylacetamide. (The first two compounds are injected in corn oil, the remainder in saline.) TFEE, 2,2,2-trifluoroethyl ethyl ether.

† From the end of anaesthesia or if not anaesthetized from the equivalent time. K indicates animals were killed.

‡ Anaesthesia for 2 hr 40 min at 3% TFEE.

§ Killed at a time equivalent to the beginning of anaesthesia.

to determine the mechanism whereby the toxicity is generated.

Type P-450 cytochromes are a heterogeneous group of enzymes that metabolize an extremely wide variety of substrates. In general, this class of enzymes catalyses the detoxification of hydrophobic xenobiotics by converting them to relatively more hydrophilic water soluble compounds which are more readily excreted from the body, although in some instances the metabolites are more toxic than the parent compound [25]. A variety of compounds have been demonstrated to induce the biosynthesis of cytochromes P-450. These inducing agents are classified into two major groups: (1) those which induce cytochrome P-450 and (2) those which induce a similar enzyme, cytochrome P-448 (also called cytochrome P_1-450) [26]. Phenobarbital is an example of the first type of agent. This compound also enhances the proliferation of the endoplasmic reticulum and elevates the levels of NADPH-cytochrome *c* reductase and cytochrome b_5 per mg of microsomal protein [17]. The second class of inducers includes polycyclic aromatic compounds such as 3,4-benzpyrene and 3-methylcholanthrene which do not increase the proliferation of the endoplasmic reticulum or the synthesis of microsomal NADPH-cytochrome *c* reductase or cytochrome b_5. These polycyclic hydrocarbons apparently only induce the synthesis of cytochrome P-448 which differs from cytochrome P-450 with regard to substrate specificity, spectral properties and sensitivity to inhibitors [26]. Mixtures of these two (or more) type P-450 cytochromes are apparently always present in hepatic microsomes, regardless of the pretreatment of the source animal. In phenobarbital induced and uninduced animals, cytochrome P-450 predominates, while in polycyclic hydrocarbon treated animals, cytochrome P-448 predominates [26]. We will use the terminology 'cytochromes P-450' or 'type P-450 cytochromes' to refer to both cytochrome P-450 and P-448, whereas 'cytochrome P-450' or 'cytochrome P-448' refers to the specific enzyme mentioned.

The observed effects of the various inducing agents in potentiating the toxicity of fluroxene anaesthesia can now be considered in relation to the mechanism of toxicity. The fact that uninduced rats, with levels of hepatic microsomal cytochromes P-450 of approximately 1 nmol/mg microsomal protein, are not susceptible to the toxic effects of fluroxene anaesthesia, while phenobarbital pretreated rats, with approximately a three fold elevation of cytochromes P-450 levels are highly susceptible (100% mortality), (Table 1) implies that the metabolism of fluroxene is enhanced as a consequence of the inductive properties

of phenobarbital. Since phenobarbital induces a number of microsomal proteins, the enzyme(s) catylyzing the rate-limiting step of this metabolism is (are) not determined by this experiment. The effect of AIA, however, in overcoming the toxic potentiating effects of phenobarbital (Table 1) on fluroxene anaesthesia unequivocally demonstrated an essential role for cytochrome P-450, since AIA specifically degrades cytochrome P-450 while not affecting other microsomal proteins (e.g. cytochrome b_5 or NADPH–cytochrome *c* reductase) or reversing other non-microsomal effects of phenobarbital [27, 28]. Thus, while other microsomal enzymes definitely play a role in the metabolism of fluroxene, elevated levels of cytochrome P-450 are essential in potentiating the toxicity of fluroxene. Apparently, cytochrome P-450 is involved in an essential step of the metabolism producing the toxic metabolite and mechanisms such as those involving the effect of phenobarbital on other subcellular components can be excluded.

The failure of induction by 3-methylcholanthrene or 3,4-benzpyrene, which produces elevated levels of cytochrome P-448, to potentiate the toxicity of fluroxene (Table 1), is apparently not a consequence of insufficient concentrations of type P-450 cytochromes. In these animals the total type P-450 cytochrome levels are approximately 2 nmole/mg microsomal protein which is similar to the level obtained following phenobarbital induction for one day, a treatment which does produce toxic effects subsequent to anaesthesia (Table 1). There are several possible explanations for the toxicity differences observed between induction by phenobarbital or by 3-methylcholanthrene and 3,4-benzpyrene, which are still consistent with an essential role for cytochrome P-450:

(a) Cytochrome P-448, in contrast to cytochrome P-450, may be protecting the organism by converting fluroxene to non-toxic metabolites.

(b) In view of the different specificities of cytochromes P-448 and P-450, cytochrome P-448 may metabolize fluroxene slowly compared to cytochrome P-450, or not at all.

(c) 3-Methylcholanthrene may produce physiological or biochemical changes which are not directly involved with the microsomal system but which prevent fluroxene anaesthesia from mediating toxic effects.

The first possibility involving the production of different and non-toxic metabolites by cytochrome P-448 appears to be highly unlikely in view of the results of studies with mixed induction by phenobarbital and 3-methylcholanthrene (Table 1). The double induction potentiates the toxic effects of fluroxene anaesthesia even more rapidly than induction by phenobarbital alone (time of death subsequent to anaesthesia is used as an index of toxicity). In contrast, in view of the competition between cytochromes P-450 and P-448 for the substrate fluroxene in the doubly induced animals, it would be expected that a reduction of toxicity would occur if scheme (a) were operative. We can suggest no situation, where the suggestion outlined in scheme (c) could arise, to explain the results observed. Confirmation of scheme (b) requires establishing whether cytochrome P-448 binds to and metabolizes fluroxene. Our studies *in vitro* of the interaction of fluroxene with microsomes induced for cytochrome P-448 or P-450 indicate that although cytochrome P-450 forms a spectrally detectable complex with and metabolizes fluroxene, cytochrome P-448 does neither [29]. Apparently, then, the failure of the induction of rats with 3-methylcholanthrene or benzpyrene to potentiate the toxicity of fluroxene follows from the failure of cytochrome P-448 to metabolize the fluroxene.

The results of preanaesthetic induction of hepatic microsomal cytochromes on the toxicity to rats of TFEE anaesthesia were compared with the results of the investigations of fluroxene anaesthesia, to identify the portion of the fluroxene molecule in which the potential toxicity resides (Tables 1 and 2). Since TFEE and fluroxene anaesthesia exhibited similar effects under conditions of no induction, benzpyrene induction, phenobarbital induction or mixed induction with phenobarbital and 3-methylcholanthrene (Tables 1 and 2), it is clear that the potential toxicity resides in the trifluoroethyl moiety of fluroxene rather than in the vinyl group which is not present in TFEE. There is ample support for the metabolism of the fluroxene molecule to trifluoroethanol glucuronide or trifluoroacetaldehyde *in vivo*, and the toxicity of trifluoroethanol and physiological derivatives thereof has been demonstrated [13]. However, although our results indicate that the toxicity arises from a metabolite of the trifluoroethyl portion of the fluroxene molecule, we have no evidence that the toxic effects observed in this study arise directly from a particular metabolite such as trifluoroethanol or its glucuronide or trifluoroacetaldehyde as proposed elsewhere [4, 14, 15].

The unexpected observation that induction with 3-methylcholanthrene potentiates the toxicity of TFEE is difficult to explain especially in view of the failure of 3,4-benzpyrene to produce a similar effect We have concluded from *in vitro* studies that hepatic microsomal cytochrome P-448 does not bind or metabolize TFEE [29]. It could be postulated that the cytochrome P-450 component of the type P-450 cytochromes present after induction with 3-methylcholanthrene in these experiments is of a high enough concentration to potentiate the toxicity of TFEE. This simple explanation is, however, unlikely in view of the failure of 3-methylcholanthrene induction to potentiate fluroxene toxicity. This aspect is undergoing further investigation.

Comparison of the toxicity of fluroxene and TFEE indicates that since equivalently-pretreated animals expire earlier after fluroxene than after TFEE anaesthesia, the former agent is probably metabolised more rapidly by cytochrome P-450. In confirmation, we have demonstrated that fluroxene is a better substrate for hepatic microsomal cytochrome P-450 *in vitro* [29]. Even though fluroxene is metabolized by microsomal enzymes it does not induce elevated levels of cytochromes P-450 [12].

Although the vinyl group of the fluroxene molecule has no apparent role in the observed toxicity of fluroxene anaesthesia, this moiety appears to be necessary for the fluroxene induced destruction of cytochrome P-450 observed *in vivo* (c.f. Table 1 and Table 2), since TFEE, without the vinyl group, does not produce any similar destruction. Fluroxene-mediated destruction of cytochrome P-450 is of the greatest magnitude in phenobarbital or phenobarbital-plus-3-

methylcholanthrene-induced animals. It is, however, not clear from the *in vivo* studies whether this destruction arises from elevated concentrations of cytochrome P-450 or other microsomal enzymes (e.g. NADPH–cytochrome *c* reductase) in these animals prior to anaesthesia. We have, however, duplicated this phenomenon in isolated hepatic microsomes and have concluded that the destruction results from the interaction of fluroxene with ferrocytochrome P-450 [18]. The levels of other microsomal enzymes (e.g. NADPH–cytochrome *c* reductase and cytochrome b_5) are unaffected by fluroxene *in vitro*.

Previous reports concerning compounds capable of destroying cytochrome P-450 *in vivo* and *in vitro* confirm the ability of a monounsaturated carbon-carbon bond to function in this manner [30, 31]. Similarly, the vinyl portion of fluroxene is also essential for the destruction of cytochrome P-450 *in vitro* and *in vivo* since TFEE elicits no effect on cytochrome P-450 levels [18]. Cytochrome P-450 is apparently more susceptible to the destructive effects of fluroxene than is cytochrome P-448.

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An investigation into the hepatic cytochrome P-450 catalysed metabolism of the anaesthetic fluroxene (2,2,2-trifluoroethyl vinyl ether)*

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ABSTRACT

The role of the different cytochromes P-450 in the metabolism of the anaesthetic agent fluroxene, and the mechanism of production of toxic effects seen after pre-treatment of the animals with phenobarbital prior to anaesthesia, have been investigated.

Male rats were anaesthetized with fluroxene, or with 2,2,2-trifluoroethyl ethyl ether, or with ethyl vinyl ether in an attempt to ascertain the *in vivo* toxic effects of the three anaesthetic agents. The resultant hepatic histology is reported. A study of the binding and metabolism of fluroxene by isolated rat hepatic microsomes was also made. We conclude that it is elevated levels of cytochrome P-450 which potentiate the toxicity of fluroxene anaesthesia in phenobarbital treated animals and that cytochrome P-448 does not bind or metabolize fluroxene. The potential toxicity of the fluroxene molecule is considered to reside in the trifluoroethyl moiety, while the vinyl group of fluroxene appears to play a role in the observed liver damage.

INTRODUCTION

Enzymes situated in the hepatic endoplasmic reticulum protect the organism against an accumulation of lipophilic xenobiotics by converting these compounds to relatively more water-soluble metabolites which can readily be conjugated with glucuronic acid or sulphuric acid or directly excreted by the kidney. The most common metabolic change catalysed by these enzymes is hydroxylation which is the major function of the non-specific hepatic microsomal drug metabolizing system in which the heterogeneous class of proteins known collectively as cytochrome P-450 acts as the terminal oxidase. Molecular oxygen is utilized and NADPH supplies the reducing equivalents for the hydroxylation of substrates by cytochrome P-450 (Gillette *et al.* 1972).

Induction or inhibition of the activity of these enzymes has a considerable influence on the metabolism and possible toxic effects of exogeneous compounds. Inducing agents

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for cytochromes P-450 fall into two major groups: those which induce cytochrome P-450, e.g., phenobarbital, and those which induce a similar but spectrally distinct enzyme known as cytochrome P-448, or cytochrome P_1-450, e.g., the aromatic polycyclic hydrocarbon, 3-methylcholanthrene (Conney 1967; Gillette *et al.* 1972).

While metabolism of drugs and other xenobiotics by cytochrome P-450 often results in the detoxification of these substances, on occasion relatively more active and toxic metabolites arise which produce liver injury, e.g., in the case of carbon tetrachloride (Judah *et al.* 1970). Fluroxene (2,2,2-trifluoroethyl vinyl ether) is an inhalation anaesthetic which is frequently used in clinical practice but which has recently been shown to be toxic under certain conditions to laboratory animals (Cascorbi & Singh-Amaranath 1972; Stevens & Gibbons 1973; Johnston *et al.* 1973) and to man (Harris & Cromwell 1972; Reynolds *et al.* 1972; Tucker *et al.* 1973; Wollman & Surks 1973). Fluroxene anaesthesia, while not normally toxic to rats, causes their death and produces hepatotoxic symptoms after pre-treatment of the animals with phenobarbital (Harrison & Smith 1973). Since phenobarbital enhances hepatic cytochrome P-450 levels, this toxicity is considered to result from enhanced biotransformation of the anaesthetic by the cytochrome P-450 enzyme system to one or more toxic metabolites.

We have attempted to elucidate the role of the different cytochromes P-450 in the metabolism of fluroxene by investigating the effects of induction of cytochromes P-450 and P-448 on the toxicity of fluroxene *in vivo* and by investigating the interactions of fluroxene with cytochromes P-450 and P-448 in isolated rat hepatic microsomes. In an effort to determine the mechanism of production of the toxic effects, investigations with 2,2,2-trifluoroethyl ethyl ether (TFEE) and ethyl vinyl ether (EVE) were also carried out.

MATERIALS AND METHODS

2-Allyl-2-isopropylacetamide (AIA) was a gift from Hoffman — La Roche, Nutley, New Jersey. 2,2,2-trifluoroethyl ethyl ether (TFEE) was prepared by hydrogenation of fluroxene at 206,8 kPa hydrogen using activated palladium on carbon as catalyst.

Fluroxene was purchased from Ohio-Medical Products, Madison, Wisconsin, and ethyl vinyl ether from Fluka, A. G.

INDUCTION AND ANAESTHESIA

Male rats weighing between 175-300 g were used in all experiments; animals were allowed free access to Epol Laboratory Chow and water. Induction of cytochrome P-450 was by intraperitoneal injection of sodium phenobarbital (80 mg/kg/day in 0,9 per cent sterile saline) and cytochrome P-448 by injection of 3-methylcholanthrene (40 mg/kg/day in corn oil) for three consecutive days. Control animals were injected similarly with saline or corn oil only.

Animals were fasted overnight and anaesthetized or sacrificed 24 hours after the final injection. Animals receiving AIA (200 mg/kg in 0,9 per cent sterile saline) were injected subcutaneously in the loose skin of the neck one hour prior to anaesthesia. Rats were anaesthetized for 2 hours in a perspex anaesthetic chamber. The anaesthetic agent, vapourized by a stream of oxygen at 5 l/minute, was in sufficient concentration to maintain light surgical anaesthesia. Ambient levels of CO_2 were below 1 per cent.

PREPARATION OF MICROSOMES

Livers were excised from animals within minutes of death or sacrifice and a portion of the liver was reserved for histological investigation (fixative, 10 per cent buffered formol saline; stain, haematoxylin and eosin). Microsomes from the livers of rats used for *in vivo* studies were prepared by differential ultracentrifugation (Holtzman & Carr 1972) and microsomes for biochemical studies were prepared by gel filtration on Sepharose 2B equilibrated with 0,15 M KCl-0,02 M Tris-HCl buffer pH 7,4 (Tangen *et al.* 1974). Protein concentration was determined using bovine serum albumin as a standard (Lowry *et al.* 1951). Microsomal cytochromes P-450 content was determined by the method of Omura and Sato (1964).

BIOCHEMICAL STUDIES

All spectrophotometry was carried out using a Unicam SP1800 recording spectrophotometer. Difference absorbance spectra of microsomal suspensions were recorded at room

Table 1
Toxicity, cytochromes P-450 concentrations and histological changes after induction and anaesthesia

Pre-treatment* (No. rats)	Anaesthetic* agent	Time of death† (hr)	Cyt P-450§ (nmol/mg microsomal protein)	Histology
Saline (17)	None, FL, TFEE or EVE	K24	1,27 ± 0,06	Hydropic change in all. Mild fatty infiltration in livers of TFEE anaesthetized rats.
PB (4)	—	K4	3,42 ± 0,34	Centrizonal hydropic change.
PB (9)	FL	1-3	1,23 ± 0,13	Periportal congestion, gross centrizonal necrosis. Mild fatty infiltration in all areas.
PB + AIA (2)	—	K-2‡	0,81 ± 0,02	Marked hydropic change, 'punched out' vacuoles in cytoplasm.
PB + AIA (2)	FL	K24	0,75 ± 0,15	Marked hydropic change, 'punched out' vacuoles in cytoplasm. No additional change.
PB (8)	TFEE	0-35	2,49 ± 0,63	Widespread fatty infiltration. Foci of frank necrosis 1/9 rats.
PB (9)	EVE	K24	Not determined	No necrosis. Periportal hydropic degeneration 2/9 rats.
Corn oil (2)	—	K24	1,27 ± 0,12	Minimal cytoplasmic vacuolation.
Corn oil (2)	FL	K24	1,10 ± 0,20	Minimal cytoplasmic vacuolation.
MC (3)	—	K24	2,47 ± 0,12	Minimal cytoplasmic vacuolation.
MC (2)	FL	K24	1,52 ± 0,29	Centrizonal chromatolysis. Midzonal hydropic change.

*Abbreviations used are: PB, phenobarbital; AIA, 2-allyl-2-isopropylacetamide; MC, 3-methylcholanthrene; FL, fluroxene; TFEE, 2,2,2-trifluoroethyl ethyl ether; EVE, ethyl vinyl ether.
†From termination of anaesthesia, or equivalent time if no anaesthesia. K indicates animals were killed; in other cases toxicity caused death.
‡Killed at a time equivalent to commencement of anaesthesia.
§Total cytochromes P-450 including cytochromes P-450 and P-448.

temperature by equally dividing portions of the suspension between two 1 cm path length cuvettes. Varying quantities of fluroxene or TFEE were added to the reference cuvette, and the magnitude of the resultant difference spectrum was measured as the difference in absorbance between the peak at approximately 390 nm and the trough at approximately 420 nm, and designated $\triangle A$.

The rates of metabolism of fluroxene or TFEE by hepatic microsomes were determined by monitoring NADPH consumption. Equal quantities of microsomal suspension were divided between two 1 cm path length cuvettes and equilibrated at 28 °C. Varying quantities of fluroxene or TFEE were added to the sample cuvette and the reaction initiated by the addition of 50-100 µl of NADPH solution (0,12-0,24 mM final concentration). NADPH oxidation was monitored spectrally at 340 nm and was corrected for background rates of endogenous NADPH oxidation in the presence of fluroxene or TFEE in an atmosphere of $CO\text{-}O_2$ (80: 20; v/v) (Stripp *et al.* 1972).

RESULTS

IN VIVO STUDIES

The effects of the inducing agents of cytochromes P-450 in potentiating the toxicity of the three anaesthetic agents to rats are shown in Table 1. The hepatic microsomal cytochromes P-450 content per mg of microsomal protein and the histological changes following induction with subsequent anaesthesia are tabulated.

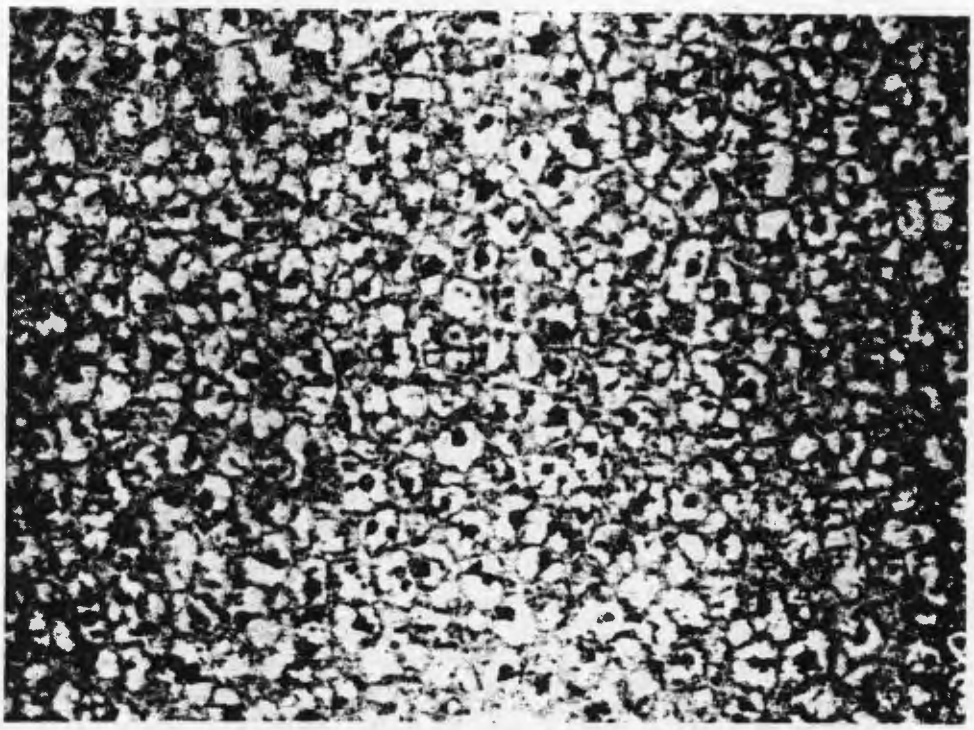

Figure 1. Section from liver of a rat given an intraperitoneal injection of saline followed by TFEE anaesthesia. Note the hydropic change without marked hepatic swelling. (H & E × 250).

Treatment with saline alone or in conjunction with anaesthesia by fluroxene, TFEE or EVE did not produce toxic effects and did not affect hepatic cytochrome P-450 content per mg of microsomal protein. In all cases, histological investigation revealed minor hydropic changes, (e.g., Figure 1).

Phenobarbital pre-treatment of animals resulted in an approximately threefold increase in cytochrome P-450 levels. All rats induced with phenobarbital and subsequently anaesthetized with fluroxene died within 1-3 hours after the termination of anaesthesia. After death, these animals exhibited a large decrease in hepatic cytochrome P-450 content, and there was marked histological evidence of liver damage. Severe congestion was an outstanding feature especially in the periportal zones where hepatocytes appeared atrophied and compressed by grossly dilated sinusoids. Centrizonal hepatocytes were swollen with pale pink featureless cytoplasm. Foci of necrosis were present and mild fatty infiltration was noted in all areas (Figure 2).

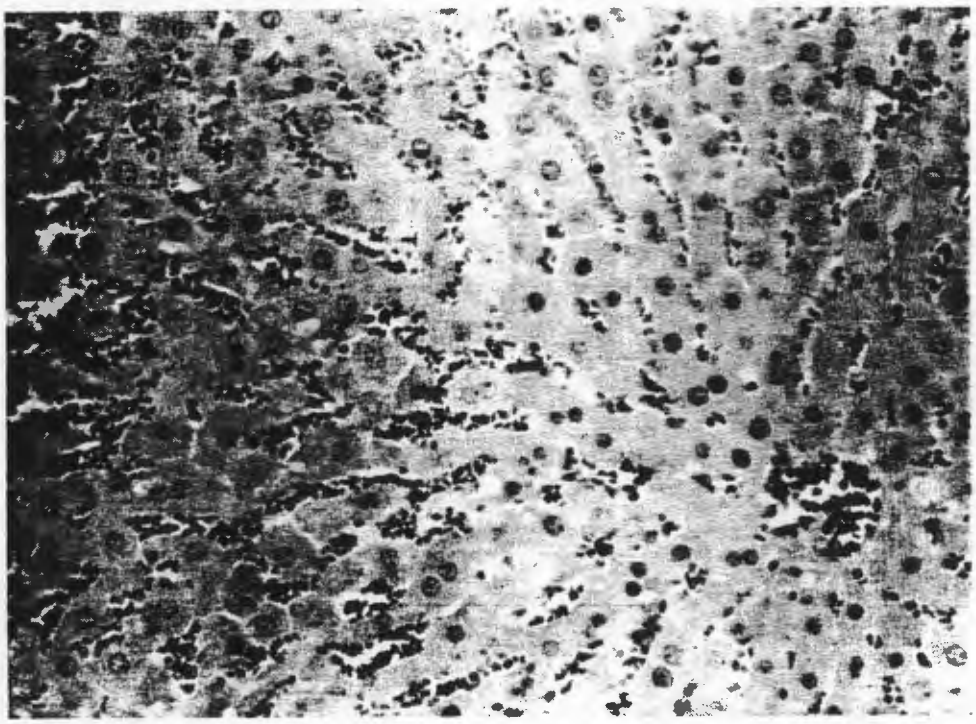

Figure 2. Section of liver from a rat induced with phenobarbital followed by fluroxene anaesthesia showing periportal congestion with atrophy of hepatocytes and centrizonal swelling of hepatocytes with homogeneous pale-staining cytoplasm and very mild fatty change. (H & E × 250).

The toxic effects of fluroxene anaesthesia observed in phenobarbital pre-treated animals were prevented by treatment of the animals with AIA one hour prior to anaesthesia. No deaths were observed in this latter group and animals appeared normal at the time of sacrifice. The marked histological changes observed with fluroxene anaesthesia of phenobarbital pre-treated animals were also prevented, the only features observed being those shown by phenobarbital and AIA treated anaesthetized controls.

Anaesthesia of phenobarbital induced rats with TFEE also resulted in the death of the rats: 100 per cent mortality was observed within 35 hours after termination of anaesthesia at which time a slight decrease in hepatic cytochrome P-450 content was evident. Histologically, frank hepatic necrosis was rare, the most consistently observed change being widespread fatty infiltration, a change not considered adequate to account for the death of the animals.

In the group of animals pre-treated with phenobarbital and anaesthetized with EVE no mortality occurred and there was no evidence of centrizonal necrosis in any of the livers. The non-specific periportal hydropic changes which were observed were also apparent in uninduced anaesthetized control animals.

Injection of 3-methylcholanthrene suspended in corn oil increased levels of cytochrome P-448 such that overall cytochromes P-450 levels were enhanced approximately twofold, and minimal cytoplasmic vacuolation was observed in the hepatic histology. Subsequent fluroxene anaesthesia was non-toxic and diminished the hepatic cytochrome content only slightly. Livers exhibited a mild version of the changes following phenobarbital induction and fluroxene anaesthesia. Necrosis was not a prominent feature and no congestion was seen. Swollen pale pink homogeneous staining centrizonal hepatocytes and hydropic change in swollen midzonal cells were manifested.

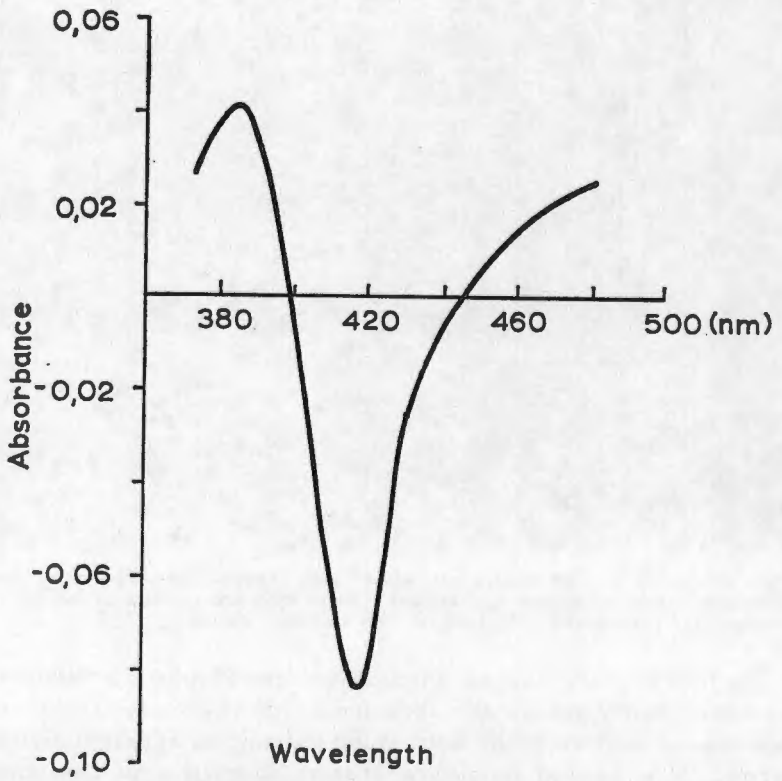

Figure 3. Difference spectrum of phenobarbital induced rat hepatic microsomes (2,0 mg protein/ml) with fluroxene ($6{,}0 \times 10^{-3}$ M), in 0,02 M tris/HCl, pH7,4. Cytochrome P-450 concentration 2,7 nmoles/mg protein.

IN VITRO STUDIES

Fluroxene binds to cytochrome P-450 of hepatic microsomes from untreated, phenobarbital treated and 3-methylcholanthrene treated rats, resulting in the appearance of a 'Type 1' difference spectrum (e.g., Figure 3), the extent of which increases with increasing fluroxene concentration until saturating concentrations of the anaesthetic are reached (Figure 4). Induction of cytochrome P-448 with 3-methylcholanthrene does not affect the extent of binding of fluroxene to hepatic microsomal cytochromes P-450 relative to control microsomes whereas induction of cytochrome P-450 with phenobarbital increases the maximal extent of binding of fluroxene approximately twofold (Figure 4). Lineweaver-Burk type plots of the data from the difference spectral studies are linear

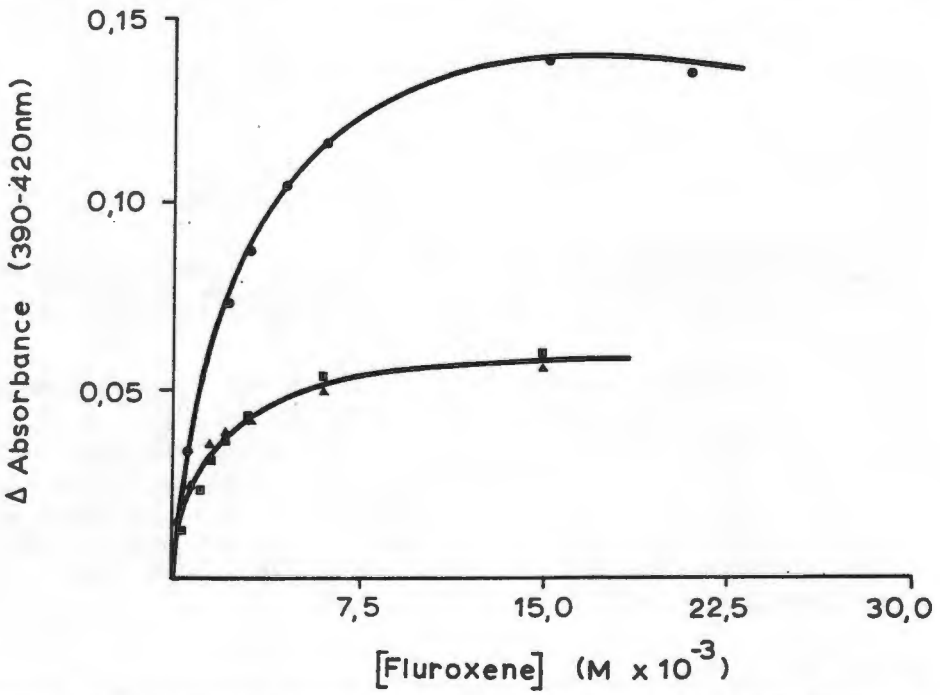

Figure 4. Effect of increasing fluroxene concentration on the extent of its difference spectrum with cytochromes P-450 of uninduced (■), phenobarbital induced (●) and 3-methylcholanthrene induced (▲) rat hepatic microsomes, the respective cytochromes P-450 concentrations being 1,08 2,70 and 2,14 nmoles/mg protein. Microsomal concentration 2,0 mg protein/ml, buffer 0,02 M tris/HCl, pH 7,4.

and permit calculation of the spectral dissociation constants (K_s) (Schenkman 1970) for the binding of fluroxene to hepatic microsomal cytochrome P-450 (Figure 5), the K_s values being the same from uninduced, phenobarbital induced and 3-methylcholanthrene induced hepatic microsomes (Table 2).

Fluroxene stimulates NADPH consumption by hepatic microsomes from uninduced, phenobarbital induced and 3-methylcholanthrene induced rats, and this enhancement of NADPH consumption is inhibited by an atmosphere of CO-O_2 (80:20; v/v) or 10 mM KCN and is not affected by 1 mM KCN. Rates of metabolism of fluroxene

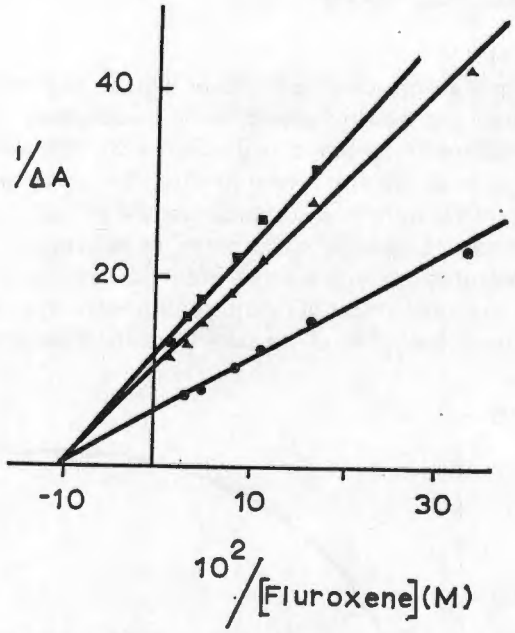

Figure 5. Lineweaver-Burk plots of data from fluroxene difference spectral studies with uninduced (■), phenobarbital induced (●) and 3-methylcholanthrene induced (▲) rat hepatic microsomes, the respective cytochromes P-450 concentrations being 1,20 3,34 and 2,12 nmoles/mg protein. Microsomal concentration 2,0 mg protein/ml, buffer 0,02 M tris/HCl, pH 7,4.

monitored by the disappearance of NADPH are used to calculate the Michaelis constants, K_m, under different conditions of induction (Figure 6) by means of Lineweaver-Burk plots. The K_m values obtained with microsomes induced for elevated cytochromes P-450 or P-448 levels do not differ significantly from that obtained with control microsomes. Phenobarbital induction elevated V_{max} relative to uninduced controls roughly in proportion to the increase in cytochrome P-450 content. The V_{max} value from microsomes induced with 3-methylcholanthrene did not differ from that of the uninduced control value (Figure 6 and Table 2).

Table 2
Effects of induction of hepatic microsomal cytochromes P-450 on the binding and metabolism of fluroxene (2,2,2-trifluoroethyl vinyl ether)

Induction*	Cyt P-450† (nmol/mg microsomal protein)	K_s (M)	K_m (M)	ΔA_{max}‡ (O.D.)	V_{max}§ (nmol NADPH/min/mg microsomal protein)
NONE	1,1	$9,3 \times 10^{-4}$	$7,6 \times 10^{-4}$	0,06	4
PB	2,7	$9,5 \times 10^{-4}$	$6,3 \times 10^{-4}$	0,13	13
MC	2,1	$9,4 \times 10^{-4}$	$9,5 \times 10^{-4}$	0,07	3

*Abbreviations used are: PB, phenobarbital; MC, methylcholanthrene.
†Total cytochrome P-450 including cytochromes P-450 and P-448.
‡$\Delta A = A_{peak} - A_{trough}$ of difference spectrum, S.D. ± 0,02.
§S.D. ± 1 nmol/min/mg microsomal protein.

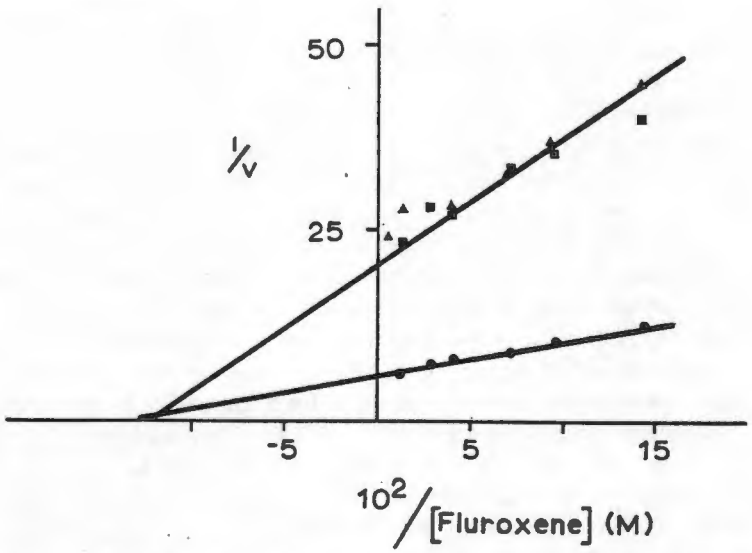

Figure 6. Lineweaver-Burk plots illustrating the metabolism of fluroxene by uninduced (■), phenobarbital induced (●) and 3-methylcholanthrene induced (▲) rat hepatic microsomes, the respective cytochromes P-450 concentrations being 1,02 3,34 and 2,14 nmoles/mg protein. v represents the rate of metabolism as monitored by the corrected rates of NADPH oxidation presented as ΔA_{340nm}/min. Microsomal concentration 2,0 mg/ml, buffer 0,02 M tris/HCl, pH 7,4 and temperature 28°C.

DISCUSSION

Phenobarbital induces the synthesis of hepatic cytochrome P-450, but not cytochrome P-448, and also causes the proliferation of the endoplasmic reticulum and elevates the levels of NADPH-cytochrome P-450 reductase and cytochrome b_5 per mg of microsomal protein (Conney 1967). In contrast, the polycyclic hydrocarbon inducers, of which 3-methylcholanthrene is an example, enhance the levels of hepatic cytochrome P-448 but do not cause proliferation of the endoplasmic reticulum or the synthesis of microsomal NADPH-cytochrome P-450 reductase or cytochrome b_5. Cytochrome P-448 differs from cytochrome P-450 with regard to substrate specificity, spectral properties and sensitivity to inhibitors (Fujita & Mannering 1971). Mixtures of these two (or more) type P-450 cytochromes are always present in hepatic microsomes, but cytochrome P-450 predominates in phenobarbital induced and uninduced animals, whilst cytochrome P-448 predominates in 3-methylcholanthrene treated animals (Fujita & Mannering 1971). We will use the term 'cytochromes P-450' to refer to both cytochrome P-450 and cytochrome P-448, and the terms 'cytochrome P-450' or 'cytochrome P-448' to refer to the specific type.

The fact that fluroxene anaesthesia does not lead to mortality in uninduced rats with hepatic microsomal cytochromes P-450 levels of approximately 1 n mole/mg microsomal protein but always does so in the case of phenobarbital pre-treated rats with approximately a threefold elevation of cytochromes P-450 implies that it is the cytochrome P-450 catalysed metabolism of fluroxene to one or more toxic products which is responsible for the toxic effects observed. Since phenobarbital induces a number of microsomal proteins, the enzyme(s) involved in the rate limiting step of this metabolism is (are) not determined by this experiment. The effect of AIA, however, in overcoming the toxic potentiating effects of phenobarbital on fluroxene anaesthesia unequivocally demonstrates an

essential role for cytochrome P-450 since AIA specifically degrades cytochrome P-450 while not affecting other microsomal proteins (e.g. cytochrome b_5 and NADPH-cytochrome P-450 reductase) or reversing other non-microsomal effects of phenobarbital (De Matteis 1970; Levin *et al.* 1972). Thus elevated levels of cytochromes P-450 are essential in potentiating the toxicity of fluroxene, probably because an increased rate of production of a toxic metabolite(s) results when cytochrome P-450 levels are elevated. Other microsomal enzymes undoubtedly play a role in the metabolism of fluroxene but are apparently ineffective in potentiating the toxicity of fluroxene in the absence of high cytochrome P-450 concentrations. The *in vitro* studies in which uninduced and phenobarbital induced isolated hepatic microsomes were demonstrated to bind and metabolize fluroxene proportionately to their cytochrome P-450 concentration provide confirmatory evidence for the increased *in vivo* rate of metabolism of fluroxene when cytochromes P-450 levels are elevated by phenobarbital (Table 2).

In uninduced rats anaesthetized with fluroxene, toxic levels of the active metabolite(s) are apparently not reached, perhaps because further metabolism or excretion is able to balance the relatively slow rate of production of the metabolite(s). This would explain the histological evidence that fluroxene causes minimal damage to livers of uninduced rats whereas the damage to livers of phenobarbital treated animals is marked.

In animals pre-treated with 3-methylcholanthrene to induce cytochrome P-448, the cytochromes P-450 levels of 2,4 nmol/mg microsomal protein obtained are similar to the levels following phenobarbital induction for one day, a treatment which does produce toxic effects subsequent to fluroxene anaesthesia (observation in our laboratory). Failure of pre-treatment by 3-methylcholanthrene to potentiate fluroxene toxicity is thus not then a consequence of insufficient cytochromes P-450 but must follow from the inability of cytochrome P-448 to metabolize fluroxene.

The studies of the binding of fluroxene to hepatic microsomal cytochromes P-450 from uninduced, and 3-methylcholanthrene induced animals clearly demonstrate that there is no binding of fluroxene to hepatic microsomal cytochrome P-448 *in vitro*, since 3-methylcholanthrene induced microsomes with markedly enhanced cytochrome P-448 levels do not exhibit enhanced difference spectra relative to uninduced controls (Figure 4 and Table 2). The constancy of the spectrally determined binding constant, K_s, (Table 2) for fluroxene binding to untreated and induced microsomes provides evidence that the same cytochrome P-450 type is binding the fluroxene in all the microsomal preparations but in the case of 3-methylcholanthrene induced microsomes this is only the minor component of the overall microsomal cytochromes P-450.

In utilizing the rate of oxidation of NADPH as a monitor of the metabolism of fluroxene, correction was made for CO insensitive endogenous NADPH oxidation in accordance with the method of Stripp *et al.* (1972) who have investigated the relationship between the rates of cytochrome P-450 mediated metabolism of drugs and the rates of oxidation of NADPH. The results of inhibition studies with KCN indicate that the hepatic desaturase system is not involved in the metabolism of fluroxene (Shimakata *et al.* 1971).

Since phenobarbital induced microsomes show markedly enhanced maximum rates of metabolism of fluroxene relative to uninduced controls while 3-methylcholanthrene induced microsomes show no change in maximum rates relative to control microsomes (Table 2), it can be concluded that cytochrome P-448 does not catalyse the metabolism

of fluroxene. The failure of 3-methylcholanthrene induction to potentiate the toxicity of fluroxene anaesthesia *in vivo* can be explained, then, by the inability, observed *in vitro*, of cytochrome P-448 to bind and metabolize fluroxene..

The close agreement between the average values of K_m (0,8 mM) and K_s (0,9 mM) for the interaction of fluroxene with all microsomes investigated indicates that the 'Type I' difference spectrum observed is probably indicative of substrate-cytochrome P-450 complex formation.

The histological investigation reveals that hepatic damage following fluroxene anaesthesia of 3-methylcholanthrene treated animals was in the form of a mild version of the damage seen following similar anaesthesia of phenobarbital treated animals. This demonstrates that the damaging metabolite(s) was (were) not present in toxic concentrations and this confirms that the elevated levels of cytochrome P-448 could not potentiate the toxicity of fluroxene.

In attempting to determine the mechanism of production of the toxic effects of fluroxene mediated by elevated cytochrome P-450 levels, use was made of two other anaesthetic agents structurally related to fluroxene. 2,2,2-trifluoroethyl ethyl ether is the reduced form of fluroxene in which the vinyl moiety of fluroxene has been hydrogenated to an ethyl group. Ethyl vinyl ether is the non-fluorinated analogue of fluroxene.

Exposure of rats to TFEE anaesthesia following phenobarbital induction resulted in the death of the animals but after a longer period than in the case of fluroxene anaesthesia. There was evidence of liver damage in both groups (Table 1). The patterns of this liver damage differed, however, in that those exposed to fluroxene, exhibited centrizonal necrosis and congestion, as described previously (Harrison & Smith 1973) whereas with those exposed to TFEE, fatty change was the consistent pattern. From a comparison of the investigations of fluroxene and TFEE it is evident that the potential for mortality resides in the trifluoroethyl moiety rather than in the vinyl ether moiety which is absent in TFEE. This is confirmed by the inability of ethyl vinyl ether (which has no trifluoroethyl group) to cause mortality or liver damage in rats which have been induced to elevated cytochrome P-450 levels. There is ample evidence for the metabolism of the fluroxene molecule to trifluoroethanol glucuronide *in vivo*, and the toxicity of trifluoroethanol and its physiological derivatives has been established (Blake *et al.* 1969). There is however no unambiguous proof at present that the toxic effects observed in this study arise directly from the toxicity of trifluoroethanol.

The difference in the histological pattern of liver injury following the use of TFEE rather than fluroxene suggests that the vinyl group of fluroxene must be involved in some way in the production of this liver damage which, in itself, is probably not the direct cause of death in the experimental animals. In Table 1, it can be seen that anaesthesia with fluroxene causes a large decrease in hepatic cytochromes P-450 levels within 3 hours post anaesthesia, whereas the same is not observed within 35 hours after TFEE anaesthesia. The apparent destruction of cytochromes P-450 mediated by the vinyl group of fluroxene is therefore probably related to the observed liver damage. That EVE, possessing a vinyl group, does not produce similar liver damage is surprising but this phenomenon is under investigation at present.

The fatty changes observed in the livers of phenobarbital pre-treated animals anaesthetized with TFEE are not considered adequate to account for the deaths of the animals. Whether the actual mode of death following fluroxene or TFEE anaesthesia is hepatic

failure, or a more widespread acute toxic effect on other organs cannot be ascertained from these studies, although the liver is the site of production of the toxic metabolite(s) and therefore of crucial importance.

In conclusion, these studies demonstrate that the toxicity of fluroxene anaesthesia potentiated by phenobarbital induction results from the enhanced rates of metabolism of the anaesthetic due to elevated cytochrome P-450 levels. The type P-450 cytochrome involved in the metabolism of fluroxene is cytochrome P-450 in contrast to cytochrome P-448 which neither binds nor metabolizes the anaesthetic. The potential cause of mortality in the fluroxene molecule resides in the trifluoroethyl portion and the causative agent in the observed liver damage is probably the vinyl moiety.

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Some Aspects of the Hepatic Metabolism of Ethrane

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SUMMARY

From our studies of the rat we report observations on three aspects of the interaction of ethrane and liver.

1. Interaction of ethrane with cytochrome P450 *in vitro*.

2. The effect of ethrane administration on glutathione levels *in vivo*.

3. The effects of repeated ethrane administration in the absence and presence of microsomal enzymic induction on liver morphology.

We conclude that ethrane has little or no propensity for damaging the liver.

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Date received: 20 July 1976.

In the evaluation of any new anaesthetic agent, investigation of its interaction with the liver is of prime clinical relevance. Three aspects of ethrane and liver interaction were of special interest to us:

The interaction of ethrane with hepatic cytochrome P450 *in vitro*. As with other lipophilic xenobiotics, the biotransformation of volatile anaesthetics is a function of the hepatic microsomal cytochrome P450. In addition to establishing the equilibrium constants K_s and K_m for the binding and metabolism of ethrane by cytochrome P450, we were interested to establish whether or not this reaction was accompanied by any destruction of cytochrome P 150. Our recent observations of fluroxene-mediated destruction of cytochrome P450[1] and the similar observation of Brown *et al.*[2] in the case of chloroform, suggest that destruction of cytochrome P450 in this reaction may in some way be related to the drug's hepatotoxic potential.

The effect of ethrane administration on liver glutathione levels *in vivo*. Reduced glutathione is the free radical scavenger that protects the structural lipid of the hepatocyte from interaction with reactive intermediates that might result from microsomal drug metabolism. Such reaction, which has been shown to follow exposure of animals to bromobenzine, paracetamol, chloroform, carbon

tetrachloride,[2] and fluroxene, all of which produce liver necrosis, results in a fall in the level of hepatic glutathione. We regard the response of liver glutathione levels to exposure to a drug, and the further effect on this of enhancement of the drug's metabolism by microsomal enzymic induction, as an index of the potential of the drug to produce reactive and possibly hepatocyte-damaging metabolites.

The effects on liver histology of ethrane anaesthesia in both the absence and the presence of phenobarbitone-induced enhanced levels of microsomal activity. Though somewhat crude, we regard the histological examination of the liver as an essential correlate of the investigation of any drug and liver interaction, with the further postulate that, should any harmful reaction be possible, it would, as in the case of chloroform, paracetamol, and fluroxene, be worsened and made more obvious by artificial enhancement of the metabolism of the drug.

METHODS

Interaction of Ethrane with Cytochrome P450

The interaction of ethrane with cytochrome P450 was monitored by methods previously described.[3,4]

Calculations: Binding constants and Michaelis constant were calculated from plots of [S] versus [S]/V or [S]/absorbance change.

Effect of Ethrane on Liver Glutathione and Histology

The effects of ethrane anaesthesia on liver glutathione levels *in vivo* and liver histology were studied in male LE rats weighing 200 - 250 g, both in the presence and absence of microsomal enzymic induction. Liver microsomal enzymes were enhanced by the daily intraperitoneal injections of phenobarbitone (80 mg/kg/d) for 3 days before anaesthesia. The concentration of phenobarbitone in saline was 10 mg/ml, which resulted in volumes for injection of approximately 2 ml. Control rats (which had received no pretreatment) were injected with similar volumes of physiological saline. Having been allowed food and water *ad lib.* until then, the rats were starved for 24 hours after the third injection of phenobarbitone, at which time (day 4) they were anaesthetised with ethrane 1,5%.

The anaesthetic chamber was a rectangular perspex box, length 60 cm × breadth 30 cm, depth 30 cm, within which a fenestrated floor was raised 8 cm from the base. The space below the floor was filled with standard anaesthetic soda lime. A further fenestrated metal box of dimensions 12 cm^3 containing soda lime stood on the floor between the experimental animals. Ethrane was vaporised from a Cyprane vaporiser by a stream of oxygen at the rate of 5 litres per minute, which was introduced into the chamber through a port below the floor at one corner and exhausted from the chamber via the top corner diagonally opposite. Ambient carbon dioxide was monitored and was always less than 1%.

In the glutathione studies, groups of 3 rats each which had been pretreated, and controls which had received no pretreatment, were removed from the anaesthetic chamber at 30, 90, and 180 minutes, and were immediately killed by cervical dislocation, after which they were hepatectomised. Glutathione levels were then measured in the liver by the method of Cohn and Lyle.[5] The level of glutathione for each period of time was taken as the mean value for 6 animals.

In the histological study, animals were subjected to 3-hour periods of anaesthesia with 1,5% ethrane at weekly intervals for 3 weeks, i.e. three 3-hour periods of anaesthesia in 3 weeks. All rats were killed by cervical dislocation on the third day after the third anaesthetic exposure. Thereafter the livers were removed, fixed in 10% formol saline, sectioned, stained with haematoxylin and eosin and examined histologically. The 60 rats (30 control, 30 enzyme-induced) used in the histological study were anaesthetised in groups of 10.

RESULTS

Interaction of Ethrane with Cytochrome P450 (Table I)

Ethrane binds to and is metabolised by hepatic microsomal cytochrome P450. The binding is a typical 'type I' with a λ_{max} equal 385 nm, λ_{min} equal 418 nm (Fig. 1).

TABLE I. INTERACTION OF ETHRANE WITH HEPATIC MICROSOMAL CYTOCHROMES P450

		Microsomal proteins		
Binding	Type I		0 min	30 min
K_s(mM)	1,3 ± 0,2			
$\triangle A_{max}$ (OD)	0,17 ± 0,2	Cytochrome †	3,0	3,0
K_m(mM)	0,6	Cyt. *b*5	0,76	0,73
V_{max}*	10 ± 1	NADPH Cyt. *c* reductase ‡	0,108	0,101

* nmol NADPH/min/mg/mic. protein
† nmol/mg mic. protein
‡ nmol cyt. *c* reduced/min/mg mic. protein

The K_s for this interaction is 1,3 mM. Ethrane is metabolised by the cytochrome P450-dependent enzymic system as judged by ethrane enhancement of CO-inhibitable NADPH consumption. The K_m for this process is 0,6 mM, V_{max} is 10 nmol NADPH/min/mg microsomal protein. *In vitro*, this interaction with ethrane was unaccompanied by any change in the levels of any of the microsomal proteins measured.

Effect of Ethrane on Hepatic Glutathione Levels

In contradistinction to chloroform and fluroxene, ethrane administration, even in the presence of microsomal enzyme induction, caused no fall in the hepatic glutathione level (Fig. 2).

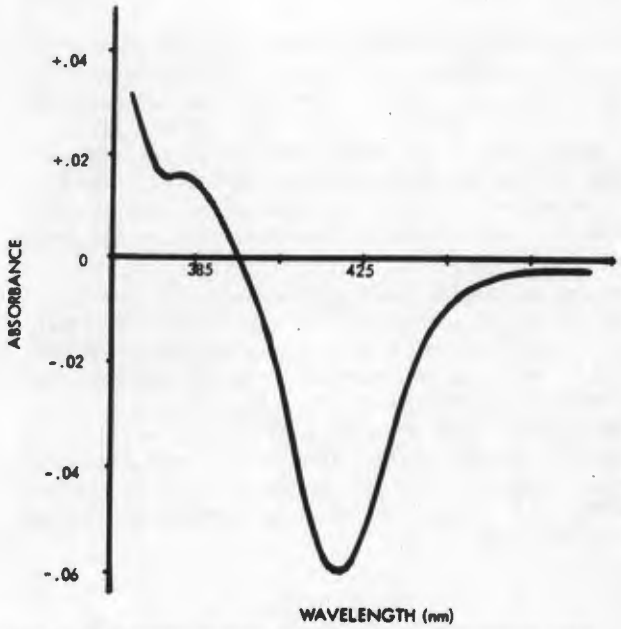

Fig. 1. Difference spectrum of induced microsomes with ethrane.

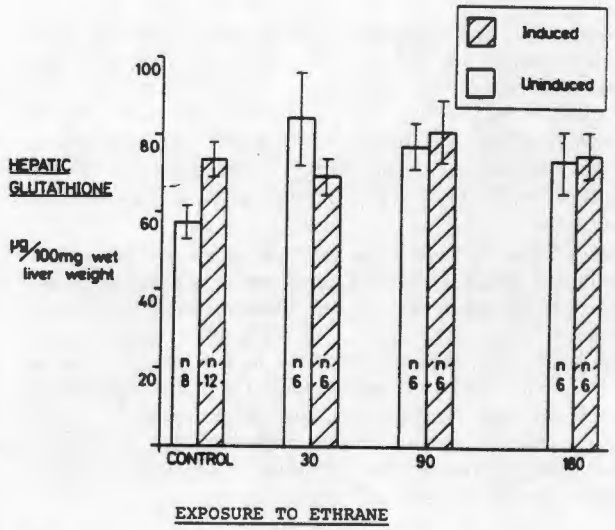

Fig. 2. Liver glutathione levels measured in phenobarbitone-induced and uninduced rats after exposure to ethrane/O_2 anaesthesia.

Effect of Ethrane Administration on Liver Morphology

Of 60 rats (30 enzyme-induced, 30 control, no pretreatment) exposed to three 3-hour periods of anaesthesia with ethrane 1,5%, none died. At sacrifice on the third day after the last anaesthetic exposure, there was no histological evidence of liver damage in any section of liver from either the enzyme-induced or the control group of rats.

DISCUSSION

Ethrane has been shown to interact with and be metabolised by the hepatic microsomal cytochrome P450-dependent pathway *in vitro*. The type and extent of binding, K_s, $\triangle A_{max}$, and the metabolic measurements K_m, V_{max} are similar to those found for the interaction of other volatile halogenated hydrocarbon anaesthetics with cytochrome P450 *in vitro*. This interaction of ethrane with cytochrome P450 is unaccompanied by any destruction of the microsomal electron transfer components measured. This metabolism, even when it is enhanced by microsomal enzyme induction, is not accompanied by any fall in liver glutathione levels. These observations are consistent with the conclusion that the hepatic metabolism of ethrane does not result in the production of any highly reactive, possibly organelle-damaging intermediates, such as result from the metabolism of the anaesthetics chloroform and fluroxene and other agents such as bromobenzine, paracetamol, and carbon tetrachloride.

Such a conclusion correlates with the normal hepatic histology observed after repeated exposure to ethrane, even in circumstances of enhanced biotransformation of ethrane. In conjunction with the low rate of biotransformation observed for ethrane[6] — lower than that of other volatile agents except for its isomer, isoflurane — our observations are consistent with ethrane's possessing little or no direct hepatotoxic potential.

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This work was undertaken with the technical assistance of Mr Brian Sasman, whose help we acknowledge with thanks.

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The Effect of Exposure to Halogenated Anaesthetics on Liver Glutathione Levels in Rats

An Index of Hepatotoxicity

G. G. HARRISON, V. MANCA

SUMMARY

Having studied anaesthetic drug interactions in rats, we report the effects of halogenated anaesthetics on the liver glutathione levels and histology, as well as the results of the enhancement of these effects by microsomal enzyme induction. The anaesthetic agents studied included methoxyflurane, halothane, ethrane, chloroform and fluroxene. While exposure of rats to methoxyflurane, halothane and ethrane produced no significant changes in hepatic glutathione levels, or in liver histology, exposure to chloroform and fluroxene produced marked depression of liver glutathione, especially after microsomal enzyme induction. Furthermore, rats exposed to these agents after enzyme induction developed gross centrilobular necrosis and died. It is suggested that the study of the effects of any new anaesthetic agent on liver glutathione levels could be a valuable screening test of its hepatotoxic potential, before its clinical trial.

S. Afr. med. J., 55, 555 (1979).

Reduced glutathione is a free radical scavenger that protects the structural lipid of the hepatocyte from interaction with highly reactive intermediates that might result from microsomal drug metabolism. This reaction, which is accompanied by a fall in the level of liver glutathione, has been shown to follow exposure of animals to many drugs which are known to produce liver damage and necrosis, e.g. bromobenzene, paracetamol, carbon tetrachloride and chloroform.[1] The effect of exposure to a drug on the level of liver glutathione and the further effect on this of enhancement of the drug's metabolism by microsomal enzyme induction, may be regarded as an index of the potential of biotransformation of that drug to produce reactive and possibly hepatocyte-damaging metabolites, i.e. a measure of its hepatotoxic potential.

We studied *in vivo* the effects of exposure to halogenated anaesthetics on the levels of liver glutathione and on liver histology in rats, as well as the results of enhancement of such effects by microsomal enzyme induction. The correlation of these findings with the reported effects of the same drugs on hepatic cytochrome P-450 content is discussed in relation to preliminary screening tests for new anaesthetic drugs.

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MATERIALS AND METHODS

Male Long-Evans rats weighing 200 - 250 g were exposed to the anaesthetics methoxyflurane, halothane, ethrane, chloroform and fluroxene.

Animals were anaesthetized in a rectangular Perspex anaesthetic chamber measuring 60 x 30 cm, with a raised fenestrated floor 8 cm from the base. The space below this floor was filled with standard soda lime. The anaesthetics tested were vaporized in standard clinical vaporizers to 1 MAC[2] by a stream of oxygen (5 l/min), which was passed into the chamber through a port below the floor at one corner and exhausted from the chamber via the top diagonal corner. Ambient carbon dioxide was monitored and was always less than 1%.

Microsomal enzyme induction was achieved by the intraperitoneal injection of phenobarbitone 80 mg/kg in a 1% solution administered daily for 3 days before anaesthesia. Control animals were injected with similar volumes of saline. Pretreated and control animals were treated simultaneously in duplicate chambers.

To study the effects of the anaesthetics on liver glutathione, groups of 5 rats each, pretreated with phenobarbitone and not pretreated, were subjected to periods of anaesthesia of 30, 90 and 180 minutes. At the conclusion of these periods of anaesthesia they were immediately killed by decapitation. The livers were removed and glutathione levels were assayed in liver homogenates by the method of Cohn and Lyle.[3] A group of 5 rats sacrificed at commencement of the period of anaesthesia served as controls for each experiment. In the case of ethrane, hepatic glutathione was assayed in two groups of 5 rats each for each time period. A further control adopted was the estimation of liver glutathione in groups of 2 rats each confined in the anaesthetic chamber ventilated with oxygen alone at a flow rate of 5 l/min for 0, 30, 90 and 180 minutes. The values from 5 such experiments (10 values at each time period) were pooled.

In the histological study, 2 groups of 10 animals each, with and without phenobarbitone pretreatment, were subjected to 3-hour periods of anaesthesia with 1 MAC of the test anaesthetic at weekly intervals for 3 weeks, i.e. 3 x 3-hour 1 MAC anaesthetics. Animals were sacrificed 24 hours after the last exposure, the livers were then removed and fixed in 10% formal saline, sectioned and stained with haematoxylin and eosin and subjected to standard light microscopy. In the case of pretreated animals subjected to fluroxene and chloroform anaesthesia, this protocol could not be completed, because all these animals died after one exposure.

RESULTS

The effects on the levels of hepatic glutathione of the anaesthetics tested are presented in Table I, and are expressed graphically as the percentage change from control levels in Figs 1 and 2.

In rats confined to the anaesthetic chamber and breathing oxygen alone a statistically significant fall in hepatic glutathione levels occurred — a mean of 25% from control values (95% confidence limits 7 - 43%). Statistically significant falls of the same order in hepatic glutathione levels were observed after a 90-minute exposure of untreated rats to halothane, and in both untreated and treated rats exposed to ethrane. In each instance the mean fall in glutathione levels was less after 180 minutes' exposure, and was no longer statistically significantly different from the control value (Fig. 1).

Hepatic glutathione levels are subject to diurnal variation. so it is important to note that we performed our experiments at approximately the same time each day — the 90-minute period occurring at about midday.

Three of the anaesthetics tested — methoxyflurane, halothane and ethrane — therefore produced changes that were statistically no different from those which followed exposure of rats to oxygen alone, owing perhaps to diurnal variation. Likewise, 3 × 3-hour 1 MAC anaesthetic exposure to the same anaesthetic agents produced no histological changes in the liver, not even in rats which had undergone microsomal enzyme induction.

Exposure of rats to chloroform and fluroxene, however, produced marked systematic depression of hepatic glutathione levels (see Table I and Fig. 2). The reduction in glutathione levels produced by chloroform was significant only when biotransformation of the drug was enhanced by microsomal enzyme induction, while that produced by fluroxene, gross after enzyme induction, was

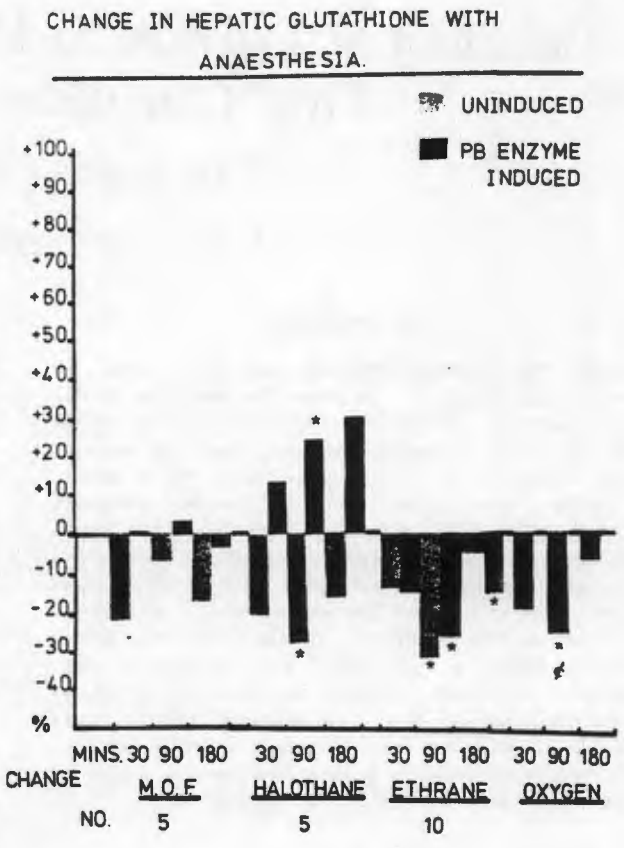

Fig. 1. Hepatic glutathione levels after exposure to methoxyflurane, halothane, ethrane and oxygen expressed as percentage change from control value (* difference from control value $P<0,05$, ϕ 95% confidence limits 7 - 43%).

TABLE I. HEPATIC GLUTATHIONE LEVELS (μg/100 mg WET WEIGHT)

		No enzyme induction				Enzyme induction			
		Anaesthetic time (min)				Anaesthetic time (min)			
Anaesthetic		0	30	90	180	0	30	90	180
Methoxyflurane	Mean	215	169	202	177	186	186	193	180
	SEM	26	10	30	12	24	20	29	32
	N	5	5	5	5	5	5	5	5
Halothane	Mean	264	207	189*	221	167	189	208*	218
	SEM	17	23	11	15	9	13	10	28
	N	5	5	5	5	5	5	5	5
Ethrane	Mean	160	138	104*	158	181	154	132*	152*
	SEM	20	13	11	26	8	12	9	10
	N	10	10	10	10	10	10	10	10
Chloroform	Mean	223	235	201	208	197	128*	69*	27*
	SEM	21	11	7	15	16	19	23	4
	N	5	5	5	5	5	5	5	5
Fluroxene	Mean	311	278	180*	199	144	30*	21*	23*
	SEM	36	69	40	48	16	3	6	2
	N	5	5	5	5	5	5	5	5
Oxygen	Mean	212	174	160*	198				
	SEM	17	18	8	22				
	N	10	10	10	10				

* Difference from control value $P<0,05$.

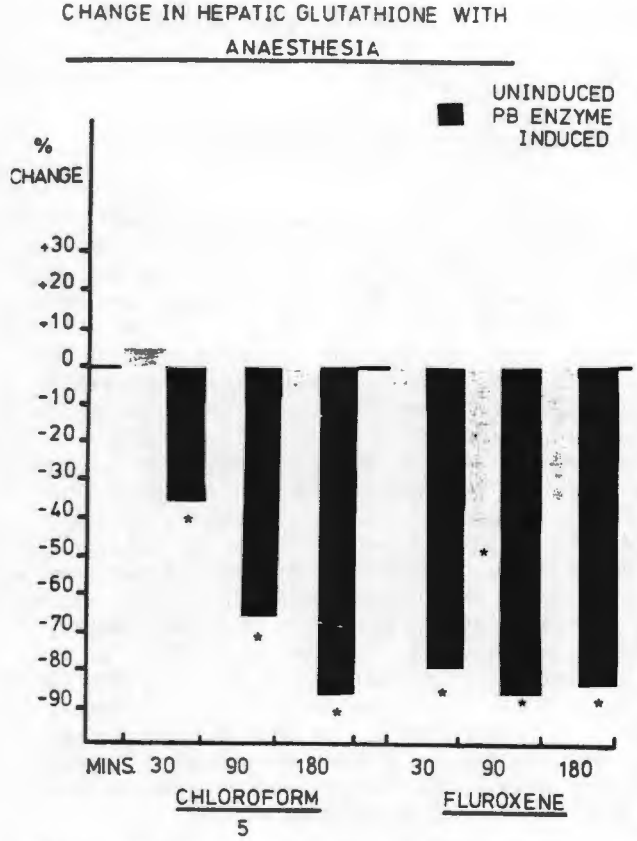

Fig. 2. Hepatic glutathione levels after exposure to chloroform and fluroxene expressed as percentage change from control value (* difference from control value $P<0,05$).

significant even in the absence of such enhancement. Exposure of animals to fluroxene after enzyme induction resulted in all but total depletion of hepatic glutathione.

Exposure of rats to the same anaesthetics, in the protocol described, produced widespread hydropic degeneration in the absence of enzyme induction and gross centrilobular necrosis when microsomal activity was enhanced. Furthermore, in these latter circumstances, all rats died after only one exposure to the anaesthetic.

DISCUSSION

From these experiments, a clear correlation emerges between the potential hepatotoxicity of an anaesthetic as a result of its biotransformation and its ability to destroy liver glutathione.

Two anaesthetics that were found in these experiments to have these properties — chloroform and fluroxene — have been shown in addition to cause destruction of cytochrome P-450 both *in vivo* and *in vitro*.[3,4,8] In the clinical field, the association of both these anaesthetics with liver necrosis is well documented.[6,7]

The association between halothane and clinical post-anaesthetic hepatitis and liver necrosis, although rare, sporadic and unpredictable, is now accepted by most authorities.[5]

In contrast to chloroform and fluroxene, it has not proved possible to reproduce this lesion in a laboratory animal until recently. Certainly, in this study, halothane produced neither histological liver necrosis, death of experimental animals, nor destruction of liver glutathione. However, Ivanetich *et al.*'s[9] *in vitro* demonstration that halothane destroys cytochrome P-450 by an action on its haem moiety does seem to provide evidence that biotransformation can result in reactive metabolites. Their activity appears to be quenched by its reaction with cytochrome P-450, the first protein with which it comes in contact, and liver glutathione levels are not affected, nor is any liver necrosis evident.

Biotransformation of fluroxene and chloroform, on the other hand, especially in the presence of enzyme induction, seems to produce an overwhelming flood of reactive metabolites which, having destroyed cytochrome P-450, require further quenching by glutathione. Once glutathione is depleted, other cell structures are attacked and cell death and necrosis follow.

Recently, Nastaincyk *et al.*[10] showed *in vitro* that in the presence of low oxygen tensions, cytochrome P-450 caused the reductive synthesis of a highly reactive trifluorocarbene complex from halothane, which in turn has been shown to cause liver necrosis in rats *in vivo*[11] — two discoveries which could add much to our understanding of halothane hepatitis.

In the past, screening of an anaesthetic agent's hepatotoxic potential has usually involved *in vivo* animal and subsequently human exposure to the agent, followed by serial observations of various measurements of liver function, most of which reflect only gross change. The experimental evidence presented here suggests that investigation of the interaction of the anaesthetic agent with hepatic glutathione and cytochrome P-450, both *in vivo* and *in vitro* in the laboratory animal, would provide a valuable and relevant screening test for any hepatotoxic potential the drug might have relative to its microsomal biotransformation.

Of the anaesthetics tested here, only halothane and ethrane are still widely used. While halothane is now under suspicion of being a potential cause of hepatitis, ethrane has still to prove itself in prolonged and widespread clinical use.

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Do Methoxyflurane and Enflurane Induce Hepatic Drug-Metabolizing Enzymes?

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SUMMARY

The effect of acute anaesthetic (1,0 MAC) and chronic subanaesthetic (0,1 MAC) exposure to methoxyflurane and enflurane on the levels of several liver enzymes is reported. Methoxyflurane appears to be an enzyme-inducing agent similar to spironolactone, whereas enflurane does not appear to be an inducing agent for any of the enzymes studied.

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A wide variety of drugs and xenobiotics are known to be capable of elevating *in vivo* the levels of hepatic enzymes involved in the metabolism of xenobiotics.[1,2] The enhanced levels of these enzymes *in vivo* result from an induction of enzyme synthesis rather than from a decrease in the rate of enzyme degradation.[1,2] Inducing agents can elevate the levels of the hepatic microsomal enzymes cytochrome P-450, NADPH-cytochrome *c* (P-450) reductase and cytochrome b_5, as well as microsomal and supernatant transferases and other enzymes. Some inducible hepatic enzymes, such as cytochrome P-450 and UDPGA transferase (UDP-glucuronyl transferase), exist in multiple forms, with different forms being induced by different agents, whereas other inducible enzymes such as epoxide hydratase and cytochrome b_5 appear to comprise only a single form.[1-6] Some inducing agents (e.g. phenobarbitone) enhance the proliferation of the endoplasmic reticulum and increase the levels of several hepatic enzymes.[1-3] Other inducing agents (e.g. 3-methylcholanthrene, benzpyrene, β-naphthoflavone) do not enhance the proliferation of the endoplasmic reticulum and elevate the levels of relatively few enzymes, such as aryl hydrocarbon hydroxylase (cytochrome P-448) and UDPGA transferase.[1-3,7,8]

A number of investigations of the ability of methoxyflurane ($CCl_2HCF_2OCH_3$) and enflurane ($CClFHCF_2OCF_2H$) to induce hepatic microsomal drug-metabolizing enzymes have been reported.[9-16] Although mice or rats were utilized in most of these investigations, there is lack of agreement between these studies as to whether methoxyflurane and enflurane are inducing agents for hepatic enzymes involved in the metabolism of xenobiotics. The contrasting conclusions drawn from these studies might be explained by differences in the characteristics and inducibility of drug-metabolizing enzymes between species and between age groups of one species.[1] Alternatively, the differing conclusions may be attributed to the diverse and often indirect methods which were utilized by different laboratories to assess induction by methoxyflurane and enflurane.[9-11,13,16] In particular, it would appear that indirect methods such as measurement of hexobarbital sleeping time, or measurement of the ratios of urinary corticosteroids and cortisols, might not provide an accurate estimation of the extent of induction of hepatic drug-metabolizing enzymes.[1] We have therefore attempted to assess the potential of methoxyflurane and enflurane as inducing agents by investigating the effect of these compounds on the levels of several hepatic enzymes which are involved in the metabolism of xenobiotics. Measurement of the levels of several hepatic enzymes was thought to be advantageous because it would provide a more detailed picture of the abilities of methoxyflurane and enflurane to induce hepatic drug metabolism, and might facilitate identification of these compounds as inducing agents in the event that they only slightly elevate the levels of relatively few drug-metabolizing enzymes.

MATERIALS AND METHODS

Methoxyflurane (2,2-dichloro-1,1-difluoro-ethyl methyl ether) and enflurane (2-chloro-1,1,2-trifluoro-ethyl difluoromethyl ether) were obtained from Abbott Laboratories.

Male Wistar rats weighing between 165 and 195 g were used. Control and anaesthetized rats were paired by weight to within ± 5 g. Except when in the anaesthetic chamber, animals were allowed free access to Epol Laboratory Chow (protein min. 20%, fat 2,5%, fibre max. 6%, calcium 1,4%, phosphorus 0,7%) and water, and were bedded on shredded paper. The temperature of the animal room was maintained at 22° - 24°C, and lighting was controlled to give 12 continuous hours of light per day.

Animals were exposed to methoxyflurane or enflurane at anaesthetic concentrations (1,0 MAC) for 3 hours per day for 1 - 4 days and at subanaesthetic concentrations (0,1 MAC) for 6 hours per day 5 days a week for a period of 4 - 16 days. The animals were anaesthetized in groups of 12 or less in a plexiglass anaesthetic chamber (30 × 30 × 60 cm). Animals were placed on a fenestrated floor raised 8 cm from the base of the chamber, the space below the floor being filled with standard anaesthetic soda lime. The anaesthetic agent was vaporized by a 5 l/min stream of oxygen through a Cyprane temperature flow-compensated Pentec or Enfluratek vaporizer and passed as a continuous stream into the anaesthetic chamber

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through an inlet port sited at one corner beneath the fenestrated floor and exhausted through a port sited at the diagonally opposite upper corner. Concentrations of anaesthetic agents in gas samples taken from the exhaust port of the anaesthetic chamber were determined by gas liquid chromatography on a Pye Unicam GCV chromatograph with a 6-mm × 150-cm column of 10% di-iso-decylphthalate on chromosorb P. The column temperatures were 60° and 40°C for methoxyflurane and enflurane determinations respectively. Control animals were exposed to oxygen alone. Animals were fasted overnight after the final anaesthesia or exposure to oxygen and were sacrificed the following morning (16 - 22 hours after anaesthesia or oxygen exposure). Livers were excised immediately and a portion (50 - 100 mg) removed for determination of glutathione; the remainder of the liver was utilized immediately for the preparation of microsomes and postmicrosomal supernatant.

Preparation of Microsomes

Microsomes were prepared from fresh liver homogenates in 0.02M tris-HCl - 0,15M KCl, pH 7,4, by differential high-speed centrifugation.[17] The final microsomal pellet was resuspended at the required protein concentration in 0.02M tris-HCl, pH 7,4. Protein concentration was determined by the method of Lowry *et al.*[18] as modified by Chaykin,[19] using bovine serum albumin as standard. Microsomal suspensions were prepared separately from each liver.

Assays on Microsomes

Cytochrome b_5 and cytochrome P-450 were determined on microsomal suspensions (2 mg protein per ml) by the method of Omura and Sato.[20] NADPH-cytochrome *c* reductase was determined as described by Omura and Takesue,[21] the concentration of microsomal protein in the assay mixture being 0.08 mg/ml. The *O*-demethylation of *p*-nitroanisole was determined by the method of Netter and Seidel[22] using 1,3 mg microsomal protein per ml and 1,0 mM *p*-nitroanisole. The conversion of 3,4-benzpyrene to 3-hydroxy-3,4-benzpyrene was determined spectrophotometrically by the method of Prough *et al.*[23] at 2,0 mg microsomal protein per ml and 80 μM benzpyrene. UDPGA transferase activity was measured using microsomal suspension (0,8 mg protein per ml), 0,4 mM *p*-nitrophenol and 0,04% Triton X-100, essentially as described by Lueders and Kuff.[24] The activity of glucose-6-phosphatase was determined using a modification of the method of Nordie and Arion.[25] Microsomal suspension (1,3 mg protein per ml) was incubated with glucose-6-phosphate (20 mM) for 20 minutes at 30°C. The mixture was deproteinized by the addition of 10% trichloroacetic acid and subsequent centrifugation. The supernatant was assayed for inorganic phosphorus by the method of King.[26]

All assays except the assay for benzpyrene hydroxylase were performed on a series of microsomal suspensions, with each suspension prepared separately from the liver of a single animal. Standard deviations for these assays therefore reflect differences between identically treated animals. Because of the large volume of microsomal suspension required for the measurement of benzpyrene hydroxylase activity, this assay was performed on a mixture of equal volumes of all anaesthetized or all control animals on a given day. The standard deviations reported for this assay do not therefore reflect variations between identically treated animals.

Assays for Glutathione and Glutathione-S-Transferase

The levels of glutathione in rat liver homogenates were assayed fluorometrically by the method of Cohn and Lyle.[27] The glutathione-S-transferase activity of rat liver postmicrosomal supernatant was assayed using 1-chloro-2,4-dinitrobenzene as substrate according to the method of Habig *et al.*[28] The reaction mixture comprised postmicrosomal supernatant (0.06 - 0.09 mg protein per ml), 1,3 mM 1-chloro-2,4-dinitrobenzene and 2,5 mM glutathione in 0,1M potassium phosphate buffer, pH 6,5.

Statistical Analysis

Statistical analysis of the data was performed using Student's *t* test. Significance was taken as $P<0,01$, probable significance as $P<0.05$.

RESULTS

The effects on the levels of liver components of exposure of animals to methoxyflurane and enflurane at anaesthetic and subanaesthetic concentrations are shown in Tables I - IV. After exposure to methoxyflurane at anaesthetic doses (1,0 MAC, 3 h/d) for 1 - 4 or 8 days, there are in general no changes in the levels of liver enzymes or glutathione relative to unanaesthetized controls (Table I). The only exceptions are the increases in the levels of NADPH-cytochrome *c* reductase relative to controls on days 3 and 8, and the slight decrease in the levels of cytochrome P-450 relative to controls on days 4 and 8.

Exposure of animals to subanaesthetic concentrations of methoxyflurane (0,1 MAC, 6 h/d) for 4 - 16 days does not affect the levels or activities of any of the hepatic enzymes investigated relative to values for unanaesthetized controls (Table II).

The effects of anaesthesia with enflurane (1,0 MAC, 3 h/d) on hepatic components are shown in Table III. After 1 - 4 days of anaesthesia, the levels of all hepatic enzymes and of glutathione were unchanged relative to controls. Exposure to enflurane (0,1 MAC, 6 h/d) for 4 - 16 days did not in general affect the levels or activities of the hepatic components investigated relative to control animals (Table IV). On day 12, however, the slight increases in the levels of cytochrome b_5 and glutathione transferase relative to controls are probably significant. There are no significant differences in the ratios of liver weight to body weight after acute or chronic exposure of animals to methoxyflurane or enflurane (Tables V and VI). In addition, there are no significant differences in the yield of microsomal protein per gram of liver under these conditions (Tables V and VI).

TABLE I. EFFECT OF METHOXYFLURANE ANAESTHESIA ON THE LEVELS AND ACTIVITIES OF HEPATIC COMPONENTS*

Component	Anaesthetized					Controls				
	Day 1	Day 2	Day 3	Day 4	Day 8	Day 1	Day 2	Day 3	Day 4	Day 8
Cytochrome P-450 (nmol/mg)	0,81 ± 0,18	0,86 ± 0,14	0,91 ± 0,16	0,72 ± 0,10‡	0,77 ± 0,10‡	0,81 ± 0,30	0,84 ± 0,08	0,95 ± 0,17	0,91 ± 0,09	0,93 ± 0,08
Cytochrome b_5 (nmol/mg)	0,41 ± 0,09	0,43 ± 0,08	0,47 ± 0,09	0,40 ± 0,04	0,48 ± 0,04	0,36 ± 0,08	0,35 ± 0,03	0,37 ± 0,04	0,47 ± 0,06	0,46 ± 0,03
NADPH-cytochrome *c* reductase (U/mg) × 10^{-1}	0,68 ± 0,22	0,69 ± 0,15	0,87 ± 0,15†	0,72 ± 0,07	0,81 ± 0,01†	0,59 ± 0,09	0,63 ± 0,12	0,56 ± 0,09	0,68 ± 0,12	0,59 ± 0,05
p-NO_2 anisole demethylase (nmol/mg/min)	1,13 ± 0,34	0,96 ± 0,25	1,00 ± 0,30	0,60 ± 0,18	1,18 ± 0,16	0,75 ± 0,17	0,69 ± 0,28	0,73 ± 0,28	0,72 ± 0,30	1,15 ± 0,07
Benzpyrene-3-hydroxylase (nmol/mg/min) × 10^{-1}	0,48 ± 0,08	0,47 ± 0,07	0,33 ± 0,08	0,22 ± 0,04	0,23 ± 0,03	0,35 ± 0,17	0,36 ± 0,07	0,24 ± 0,06	0,23 ± 0,14	0,29 ± 0,04
UDPGA transferase (nmol/mg/min)	19 ± 4	19 ± 1	19 ± 2	21 ± 3	—	16 ± 3	15 ± 5	22 ± 3	17 ± 2	—
Glucose-6-phosphatase (μg P_i/mg/min)	6,5 ± 0,3	8,3 ± 0,7	7,5 ± 0,5	6,1 ± 1,1	5,1 ± 0,8	6,1 ± 0,3	7,6 ± 1,3	8,5 ± 1,5	7,6 ± 1,4	6,5 ± 0,5
Glutathione transferase (nmol/mg/min)	70 ± 10	51 ± 10	54 ± 4	70 ± 5	—	67 ± 5	58 ± 6	55 ± 4	67 ± 8	—
Glutathione (μg/100 mg wet liver)	97 ± 9	120 ± 20	121 ± 14	—	—	120 ± 15	130 ± 27	109 ± 4	—	—

* 1,0 MAC (0,2%) 3 h/d. Levels reported per mg microsomal protein except for glutathione transferase, which is reported per mg protein of postmicrosomal supernatant, and glutathione.
† Differs significantly from controls, $P<0.01$.
‡ Probably differs from controls, $P<0.05$.

TABLE II. EFFECT OF SUBANAESTHETIC DOSES OF METHOXYFLURANE ON THE LEVELS AND ACTIVITIES OF HEPATIC COMPONENTS*

Component	Exposed				Controls			
	Day 4	Day 8	Day 12	Day 16	Day 4	Day 8	Day 12	Day 16
Cytochrome P-450 (nmol/mg)	1,03 ± 0,15	0,86 ± 0,24	0,89 ± 0,11	1,15 ± 0,12	1,07 ± 0,13	0,68 ± 0,06	0,86 ± 0,14	1,01 ± 0,07
Cytochrome b_5 (nmol/mg)	0,44 ± 0,04	0,44 ± 0,07	0,41 ± 0,03	0,50 ± 0,05	0,41 ± 0,07	0,44 ± 0,10	0,39 ± 0,03	0,49 ± 0,04
NADPH-cytochrome *c* reductase (U/mg) × 10^{-1}	0,60 ± 0,07	0,66 ± 0,08	0,64 ± 0,05	0,88 ± 0,02	0,62 ± 0,05	0,60 ± 0,14	0,56 ± 0,11	0,91 ± 0,01
p-NO_2 anisole demethylase (nmol/mg/min)	0,57 ± 0,06	0,59 ± 0,15	0,36 ± 0,08	0,77 ± 0,12	0,57 ± 0,13	—	0,39 ± 0,06	0,75 ± 0,04
Benzpyrene-3-hydroxylase (nmol/mg/min) × 10^{-1}	0,18 ± 0,02	0,22 ± 0,06	0,20 ± 0,02	0,29 ± 0,02	0,22 ± 0,02	0,22 ± 0,02	0,23 ± 0,07	0,32 ± 0,03
UDPGA transferase (nmol/mg/min)	21 ± 4	17 ± 1	19 ± 1	20 ± 1	15 ± 3	19 ± 6	16 ± 4	16 ± 2
Glucose-6-phosphatase (μg P_i/mg/min)	8,7 ± 1,2	7,5 ± 0,5	7,5 ± 0,4	6,9 ± 0,8	8,2 ± 1,0	8,9 ± 0,8	7,1 ± 1,1	6,7 ± 0,3
Glutathione transferase (nmol/mg/min)	71 ± 8	69 ± 5	50 ± 8	70 ± 4	63 ± 5	74 ± 9	63 ± 3	66 ± 13
Glutathione (μg/100 mg wet liver)	129 ± 31	188 ± 13	123 ± 7	172 ± 22	136 ± 4	161 ± 6	125 ± 14	134 ± 22

* 0,1 MAC (0,02%) 6 h/d, 5 d/wk. Levels reported per mg microsomal protein except for glutathione transferase, which is reported per mg protein of postmicrosomal supernatant, and glutathione.

TABLE III. EFFECT OF ENFLURANE ANAESTHESIA ON THE LEVELS AND ACTIVITIES OF HEPATIC COMPONENTS*

Component	Anaesthetized				Controls			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
Cytochrome P-450 (nmol/mg)	1,17 ± 0,13	1,00 ± 0,15	1,09 ± 0,22	1,13 ± 0,20	0,81 ± 0,30	0,84 ± 0,08	0,93 ± 0,13	0,91 ± 0,09
Cytochrome b_5 (nmol/mg)	0,49 ± 0,04	0,41 ± 0,05	0,47 ± 0,09	0,47 ± 0,09	0,36 ± 0,08	0,35 ± 0,03	0,41 ± 0,04	0,47 ± 0,06
NADPH-cytochrome *c* reductase (U/mg) × 10^{-1}	0,71 ± 0,14	0,68 ± 0,08	0,65 ± 0,05	0,79 ± 0,07	0,59 ± 0,09	0,63 ± 0,12	0,56 ± 0,09	0,68 ± 0,12
p-NO_2 anisole demethylase (nmol/mg/min)	0,80 ± 0,36	0,69 ± 0,07	0,69 ± 0,11	1,24 ± 0,13	0,75 ± 0,17	0,69 ± 0,28	0,73 ± 0,28	1,22 ± 0,17
Benzpyrene-3-hydroxylase (nmol/mg/min) × 10^{-1}	0,54 ± 0,04	0,23 ± 0,04	0,27 ± 0,05	0,42 ± 0,04	0,35 ± 0,17	0,30 ± 0,07	0,29 ± 0,04	0,38 ± 0,08
UDPGA transferase (nmol/mg/min)	16 ± 2	11 ± 3	21 ± 5	16 ± 3	16 ± 3	15 ± 5	22 ± 3	17 ± 2
Glucose-6-phosphatase (μg P_i/mg/min)	5,1 ± 1,1	5,4 ± 0,7	7,0 ± 1,2	6,1 ± 0,5	6,1 ± 0,3	6,5 ± 1,2	7,4 ± 0,4	6,3 ± 0,1
Glutathione transferase (nmol/mg/min)	53 ± 6	62 ± 12	56 ± 10	61 ± 9	67 ± 5	58 ± 6	55 ± 4	67 ± 8
Glutathione (μg/100 mg wet liver)	129 ± 30	115 ± 2	124 ± 15	99 ± 17	120 ± 15	130 ± 27	109 ± 4	93 ± 2

* 1.0 MAC (2.0%), 3 h/d. Levels reported per mg microsomal protein except for glutathione transferase, which is reported per mg postmicrosomal supernatant, and glutathione.

TABLE IV. THE EFFECT OF SUBANAESTHETIC DOSES OF ENFLURANE ON THE LEVELS AND ACTIVITIES OF HEPATIC COMPONENTS*

Component	Exposed				Controls			
	Day 4	Day 8	Day 12	Day 16	Day 4	Day 8	Day 12	Day 16
Cytochrome P-450 (nmol/mg)	1,05 ± 0,09	1,19 ± 0,02	1,13 ± 0,07	0,99 ± 0,07	1,07 ± 0,13	1,24 ± 0,04	0,86 ± 0,14	1,01 ± 0,07
Cytochrome b_5 (nmol/mg)	0,38 ± 0,01	0,53 ± 0,06	0,51 ± 0,01†	0,47 ± 0,06	0,41 ± 0,07	0,44 ± 0,10	0,40 ± 0,03	0,49 ± 0,04
NADPH-cytochrome *c* reductase (U/mg) × 10^{-1}	0,62 ± 0,05	0,60 ± 0,14	0,61 ± 0,02	0,64 ± 0,05	0,62 ± 0,11	0,64 ± 0,01	0,65 ± 0,01	0,68 ± 0,08
p-NO_2 anisole demethylase (nmol/mg/min)	1,19 ± 0,15	1,27 ± 0,24	—	0,69 ± 0,09	1,26 ± 0,13	0,99 ± 0,05	—	0,75 ± 0,04
Benzpyrene-3-hydroxylase (nmol/mg/min) × 10^{-1}	0,39 ± 0,05	—	0,30 ± 0,05	0,16 ± 0,06	0,36 ± 0,08	—	0,28 ± 0,09	0,15 ± 0,06
UDPGA transferase (nmol/mg/min)	14 ± 2	17 ± 3	20 ± 5	17 ± 2	15 ± 3	18 ± 6	18 ± 3	16 ± 2
Glucose-6-phosphatase (μg P_i/mg/min)	7,5 ± 0,6	8,1 ± 0,1	9,1 ± 0,5	7,3 ± 0,6	8,2 ± 1,0	8,9 ± 0,8	7,5 ± 0,8	6,7 ± 0,3
Glutathione transferase (nmol/mg/min)	51 ± 11	71 ± 9	74 ± 3†	65 ± 7	63 ± 5	74 ± 9	63 ± 3	66 ± 13
Glutathione (μg/100 mg wet liver)	80 ± 4	127 ± 4	133 ± 9	112 ± 15	97 ± 4	123 ± 18	125 ± 14	134 ± 22

* 0.1 MAC (0.2%), 6 h/d, 5 d/wk. Levels reported per mg microsomal protein except for glutathione transferase, which is reported per mg postmicrosomal supernatant, and glutathione.
† Probably differs from controls, $P<0.05$.

TABLE V. RATIOS OF TOTAL MICROSOMAL PROTEIN TO LIVER WEIGHT AND OF LIVER WEIGHT TO BODY WEIGHT FOR CONTROLS AND RATS EXPOSED TO ANAESTHETIC LEVELS OF METHOXYFLURANE AND ENFLURANE FOR 3 h/d

Treatment of animal	Yield of microsomal protein/liver weight (mg/g)				Liver weight/body weight (g/100 g)				
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4	Day 8
O_2	8,6 ± 1,0	12,8 ± 1,9	11,1 ± 2,9	10,5 ± 2,4	3,56 ± 0,20	3,52 ± 0,27	3,58 ± 0,18	3,46 ± 0,29	3,36 ± 0,23
1,0 MAC methoxyflurane	10,3 ± 0,4*	12,3 ± 1,8	8,1 ± 2,5	8,9 ± 2,0	3,51 ± 0,22	3,63 ± 0,39	3,75 ± 0,22	3,51 ± 0,38	3,85 ± 0,49
1,0 MAC enflurane	10,2 ± 2,3	12,3 ± 2,5	14,7 ± 2,4*	11,2 ± 2,6	3,64 ± 0,09	3,53 ± 0,05	3,67 ± 0,19	3,22 ± 0,38	—

* $P = 0,05$, relative to O_2-treated animals.

TABLE VI. RATIOS OF TOTAL MICROSOMAL PROTEIN TO LIVER WEIGHT AND OF LIVER WEIGHT TO BODY WEIGHT FOR CONTROLS AND RATS EXPOSED TO SUBANAESTHETIC LEVELS OF METHOXYFLURANE AND ENFLURANE FOR 6 h/d, 5 d/wk

Treatment of animal	Yield of microsomal protein/liver weight (mg/g)				Liver weight/body weight (g/100 g)			
	Day 4	Day 8	Day 12	Day 16	Day 4	Day 8	Day 12	Day 16
O_2	9,3 ± 2,2	8,9 ± 1,1	8,8 ± 1,8	10,5 ± 2,2	3,77 ± 0,19	3,65 ± 0,27	3,72 ± 0,25	3,19 ± 0,06
0,1 MAC methoxyflurane	10,1 ± 1,9	7,7 ± 1,0	9,0 ± 1,2	12,6 ± 2,4	3,55 ± 0,25	3,39 ± 0,21	3,75 ± 0,13	3,23 ± 0,16
0,1 MAC enflurane	10,1 ± 0,3	10,8 ± 2,1	9,2 ± 1,2	10,1 ± 2,3	3,81 ± 0,25	3,84 ± 0,33	3,66 ± 0,27	3,30 ± 0,10

DISCUSSION

The results indicate that methoxyflurane and enflurane at anaesthetic or subanaesthetic dosages do not in general increase the levels or activities of hepatic drug-metabolizing enzymes, and that these anaesthetic agents are therefore not inducing agents for most of the hepatic enzymes investigated. Methoxyflurane and enflurane are thus definitely not in the same category of inducing agent as phenobarbitone, which induces several hepatic drug-metabolizing enzymes including microsomal cytochrome P-450, flavoprotein reductases, cytochrome b_5, UDPGA transferase and cytosol glutathione-S-transferase.[1-3] These compounds are also not similar to polycyclic hydrocarbons such as 3-methylcholanthrene[1,29] because they do not elevate aryl hydrocarbon hydroxylase (Tables I and II).

Anaesthesia with methoxyflurane does significantly elevate the levels of NADPH-cytochrome *c* reductase (Table I), suggesting that methoxyflurane may induce the synthesis or decrease the rate of degradation of this enzyme. This confirms a report by Brown and Sagalyn[21] which demonstrated increased levels of this reductase after exposure to 0,03% (0,15 MAC) or 0,1% (0,5 MAC) methoxyflurane. It is possible that methoxyflurane may be acting in a manner similar to the steroid hormone, spironolactone, which induces in male rats the synthesis of NADPH-cytochrome *c* reductase but not other hepatic microsomal drug-metabolizing enzymes such as cytochrome P-450, cytochrome b_5 or NADH-cytochrome *c* reductase.[29,30] The induction of NADPH-cytochrome P-450 reductase in male rats by spironolactone does not enhance the hepatic microsomal metabolism of hexobarbital and 3,4-benzpyrene, presumably because the transfer of reducing equivalents via this reductase is not a rate-limiting step in the cytochrome P-450-dependent metabolism of these drugs.[21] One might therefore anticipate that the increased levels of this reductase brought about by methoxyflurane also would not greatly stimulate hepatic microsomal drug metabolism.

The only other significant consequence of methoxyflurane anaesthesia observed was a decrease in the levels of cytochrome P-450 (Table I) which also occurs with spironolactone and confirms an earlier observation by Brown and Sagalyn.[21] This slight alteration in the levels of cytochrome P-450 was not accompanied by significant alterations in *p*-nitroanisole demethylation, benzpyrene-3 hydroxylation (Table I) or aniline hydroxylation, while hexobarbital oxidation was reportedly decreased.[21] Consequently, it would appear that prior exposure to methoxyflurane at anaesthetic or subanaesthetic doses should not greatly alter the hepatic microsomal metabolism of many xenobiotics. There are reports, however, by Van Dyke[9] and by Berman and Bochantin,[22] that exposure of animals to methoxyflurane induces hepatic microsomal drug metabolism. This conclusion does not rely on direct measurement of the levels of hepatic microsomal enzymes but rests on the observation that exposure to methoxyflurane enhances the defluorination of methoxyflurane and the demethylation of aminopyrene by postmitochondrial supernatant *in vitro*. The discrepancies between the conclusions drawn from the different studies may result from differences in the parameters that were measured, from the different experimental conditions (e.g. postmitochondrial supernatant versus microsomes), or possibly from metabolism of methoxyflurane and aminopyrene by cytosol enzymes.[22] In any case, the direct measurement of hepatic micro-

somal enzyme levels, as reported here and by Brown and Sagalyn,[11] would be expected to provide the definitive experiments.

Methoxyflurane has also been reported to induce hepatic drug metabolism following the observation that the production of urinary metabolites from methoxyflurane *in vivo* was stimulated by prior exposure to methoxyflurane.[9] However, when both bone and urinary fluoride levels were measured, as deemed necessary by Fiserova-Bergerova,[10] prior exposure to methoxyflurane did not alter the extent of metabolism of a subsequent dose of methoxyflurane *in vivo*.

The sole remaining evidence that methoxyflurane is an inducing agent for hepatic microsomal enzymes rests on *in vivo* studies where enzyme induction was assessed indirectly by hexobarbital sleeping time.[12] Historically, the measurement of hexobarbital sleeping time was proposed as being useful because inducing agents decreased hexobarbital sleeping time (although decreased sleeping time did not always indicate enzyme induction), and because this assay provided an easy screening method for identifying compounds as possible inducing agents. However, many researchers and reviewers in the field of anaesthesiology have accepted decreased hexobarbital sleeping time as unequivocal evidence for enzyme induction.[20-26] In the literature, however, one finds an extremely poor correlation between the ability of an anaesthetic agent to decrease barbiturate sleeping time and its ability to induce hepatic microsomal drug-metabolizing enzymes. Decreased hexobarbital sleeping time has been reported to occur after exposure to diethyl ether, halothane, fluroxene, ethyl vinyl ether, chloroform, enflurane, methoxyflurane and other anaesthetic agents.[22-24] Of these compounds, diethyl ether is an established enzyme inducer similar to phenobarbitone.[25] Halothane, according to some reports, induces only hepatic microsomal NADPH oxidase and does not elevate the levels of other drug-metabolizing enzymes or stimulate hepatic microsomal drug metabolism.[27-29] Fluroxene, halothane and chloroform degrade hepatic cytochrome P-450 *in vitro* and/or *in vivo*, which would result in decreased rather than increased levels of this drug-metabolizing enzyme.[30-42] The use of hexobarbital sleeping time as the sole experimental evidence to support the ability of a compound to induce hepatic microsomal drug-metabolizing enzymes therefore appears to be invalid, and as Conney[1] stated as early as 1967: 'In order to find out whether a compound induces drug-metabolizing enzymes, it is ultimately necessary to prepare the liver or its fractions from treated animals and assay enzyme activity . . .'.

The results presented here indicate that enflurane does not significantly elevate the levels of any of the hepatic drug-metabolizing enzymes investigated (Tables III and IV) and therefore does not induce the synthesis of these enzymes. This observation is consistent with the data of Rietbrock[14] which demonstrated that enflurane anaesthesia (5 × 1 h or 5 × 3 h, 2,5%) does not result in elevated levels of hepatic cytochrome P-450 per gram of rat liver. Enflurane has, however, been reported to induce hepatic drug-metabolizing enzymes. In some reports, *in vitro* parameters were monitored to assess induction by enflurane. In one case the enzyme levels were reported per 100 grams of rat, but if enzyme levels are instead considered per gram of rat liver, the data do not appear to support the claim that enflurane induces hepatic microsomal enzymes.[14] In another case, where Hitt *et al.*[15] report that enflurane anaesthesia results in a 2 - 3-fold increase in cytochrome P-450 levels, which is comparable to that observed after phenobarbitone induction, no values for unanaesthetized, oxygen-exposed control animals are reported. The increasing levels of serum inorganic fluoride observed during 1 - 8 days of enflurane anaesthesia by these workers[15] may be attributed to the additive effect on serum fluoride levels of the repeated doses of enflurane rather than to enzyme induction.

Other reports which suggested that enflurane was an inducing agent relied on indirect methods for assessing enzyme induction, e.g. hexobarbital sleeping time (see above) and measurement of the ratio of urinary 6-hydroxycortisol to 17-corticosteroids.[16] The ratio of urinary 6-hydroxycortisol to 17-hydroxycorticosteroids has been shown to vary with a wide variety of factors, including physiological factors[43] which were uncontrolled during attempts to utilize this assay to establish enzyme induction by enflurane.[16]

We conclude that there is no unequivocal evidence which demonstrates that enflurane induces hepatic microsomal drug-metabolizing enzymes and, in view of our own results, suggest that enflurane may in fact not be an inducing agent for the hepatic enzymes investigated in this study. Methoxyflurane, while shown to increase NADPH-cytochrome *c* reductase slightly and to decrease cytochrome P-450 slightly, may be an inducing agent similar to spironolactone, but by virtue of its inducing capabilities would appear to have little effect on hepatic microsomal drug metabolism *in vitro*.

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ENFLURANE AND METHOXYFLURANE: THEIR INTERACTION WITH HEPATIC MICROSOMAL STEARATE DESATURASE*

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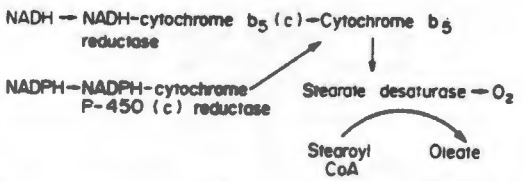
Abstract—The effects of the volatile anesthetic agents enflurane ($CClFHCF_2OCF_2H$) and methoxyflurane ($CCl_2HCF_2OCH_3$) on hepatic microsomal electron transfer components and stearate desaturase are reported. Both enflurane and methoxyflurane stimulated electron flow from NADH and NADPH through hepatic microsomal cytochrome b_5. The stimulation of electron flow from cytochrome b_5 by the anesthetic agents was not inhibited by metyrapone or CO, but was inhibited by 0.5 mM KCN. The effects of enflurane and methoxyflurane were influenced by the diet and pretreatment of the rat prior to death. A high-carbohydrate diet enhanced the effects, while fasting with or without phenobarbitone treatment diminished them. The anesthetic agents did not affect the rate constant for the autoxidation of purified trypsin-cleaved cytochrome b_5 or the activity of hepatic microsomal NADH- and NADPH-cytochrome *c* reductase, except that enflurane slightly increased the activity of NADH-cytochrome *c* reductase. The values of the equilibrium constants (K_{eq}) for the stimulation of the oxidation of hepatic microsomal cytochrome b_5 by enflurane and methoxyflurane were determined to be 1.2 and 0.5 mM, respectively. The K_{eq} for enflurane differed from the K_s and K_m values for the interaction of this anesthetic agent with cytochrome P-450, whereas the K_{eq} for methoxyflurane differed from the K_m for NADPH oxidation by cytochrome P-450, but not from the K_s for binding to cytochrome P-450 or the K_m for fluoride ion production from this anesthetic agent by cytochrome P-450. The K_i values of 0.08 and 0.11 mM obtained for cyanide inhibition of the enhancement of the oxidation of cytochrome b_5 by enflurane and methoxyflurane, respectively, are within experimental error of the K_i for cyanide inhibition of stearate desaturase. Enflurane and methoxyflurane, however, did not inhibit the conversion of stearoyl CoA to oleate by hepatic microsomal stearate desaturase. It is concluded that enflurane and methoxyflurane stimulate hepatic microsomal electron flow from NADH and NADPH through cytochrome b_5 *in vitro*, apparently by interacting with stearate desaturase.

The cytochrome P-450 mixed function oxidase of the hepatic endoplasmic reticulum catalyzes the primary step in the metabolism of many xenobiotics [1]. The first steps in the metabolism of the volatile anesthetic agents enflurane ($CClFHCF_2OCF_2H$) and methoxyflurane ($CCl_2HCF_2OCH_3$) involve dehalogenation and ether cleavage reactions mediated by hepatic microsomal cytochrome P-450 [2–4]. Detailed pathways for metabolism of these anesthetic agents *in vivo* have been proposed, but the pathways have not, as yet, been fully established, particularly for enflurane which is not metabolized extensively *in vivo* [2–6].

Stearate desaturase participates in another major electron transfer pathway of hepatic endoplasmic reticulum membranes. The physiological role of stearate desaturase appears to be to convert stearoyl CoA to oleate. This reaction can be supported by either NADH or NADPH and requires flavoprotein reductases and cytochrome b_5 as obligate intermediate electron carriers [7–11] as shown in Scheme 1.



Scheme 1. Electron transfer to stearate desaturase. Straight arrows indicate electron flow.

Stearate desaturase also exhibits mixed function oxidase activity in the oxidation of methyl sterols [12, 13]. Both the oxidase and desaturase activities are inhibited by cyanide ($K_i = 0.1$ mM) [10, 12]. Stearate desaturase has not been widely investigated with regard to its ability to interact with xenobiotics. It has, however, been reported to interact with phenolic xenobiotics [11] and with the volatile anesthetic agent halothane ($CF_3CHBrCl$) [14]. In an attempt to clarify further the metabolism and the physiological effects of the volatile anesthetic agents, enflurane and methoxyflurane, we have investigated their interaction with hepatic microsomal electron transfer proteins and with stearate desaturase. In the following investigation, in order to alter the levels of stearate desaturase, advantage was taken of the

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fact that the enzyme is under dietary control. The levels of stearate desaturase are negligible in fasted rats, measurable in fed rats, and elevated by feeding semi-purified high-carbonate diets [8, 15, 16].

The data indicate that enflurane and methoxyflurane enhance hepatic microsomal electron flow through cytochrome b_5 at clinically achievable anesthetic concentrations. The observed stimulation of electron transfer appears to arise from the interaction of enflurane and methoxyflurane with hepatic microsomal stearate desaturase.

EXPERIMENTAL

Materials. NADH and NADPH were obtained from Miles Laboratories, Cape Town, South Africa. Stearoyl CoA and [1-^{14}C]stearoyl CoA were obtained from Sigma Chemicals, Poole, England, and from New England Nuclear, Boston, MA, U.S.A., respectively. Dextrin was supplied by Merck Chemicals, Darmstadt, Germany, and by Sigma Chemicals.

The vitamin mixture used in the diet was constituted from vitamins received as a gift from Roche Pty. Ltd., Isando, Transvaal, South Africa. Choline chloride, sodium dithionite and cellulose were obtained from B. D. H. Chemicals Ltd., Poole, England. Casein was obtained from Merck Chemicals. Sodium phenobarbitone was supplied by Maybaker, Port Elizabeth, E. P., South Africa. Halothane (fluothane) was obtained from Halocarbon Laboratories Inc., Hackensack, NJ, U.S.A. Enflurane (ethrane) and methoxyflurane (penthrane) were obtained from Abbott Laboratories, Aeroton, Transvaal, South Africa. Trypsin-cleaved cytochrome b_5 was purified from rat liver microsomes by the method of Omura and Takesue [17].

Treatment of animals. In all experiments, male Long Evans rats weighing 250–300 g were used. Rats were fed on a normal laboratory diet of Epol Laboratory Chow, manufactured by Epol Ltd., Goodwood, Cape Town. This diet is referred to throughout as the normal diet, and is composed of protein (min. 20%), fat (2.5%), fibre (max. 6%), calcium (1.4%), and phosphorus (0.7%). Hepatic microsomal stearate desaturase was routinely induced by feeding rats a high-carbohydrate semi-purified diet of the following composition: dextrin, 126 g; sucrose, 30 g; cellulose, 4 g; casein, 30 g; NaCl, 4 g; KCl, 2 g; vitamin mixture, 6 g; and choline chloride, 0.2 g [8]. The vitamin mixture comprised the following: vitamin A, 2.5 g (325,000 I.U./g); vitamin D, 2.0 g (200,000 I.U./g); vitamin B_2 (Riboflavin), 500 mg; niacin, 7.5 g; and pantothenic acid, 1 g, made up to a total of 500 g with dextrin. This diet is referred to throughout as the high-carbohydrate diet. Rats were fed this diet for 2 days, fasted on day 3 and were refed the diet for 2 days [16]. The rats were killed and experiments were performed on day 6.

Isolation of hepatic microsomes. Rat hepatic microsomes were isolated by differential centrifugation, as described earlier [4]. The protein concentration of the microsomes was determined by the method of Lowry *et al.* [18], as modified by Chaykin [19]. Hepatic microsomes from rats fed a high-carbohydrate diet, suspended at a protein concentration of 1.5 mg/ml in 0.05 M Tris–HCl, pH 7.4, were used for all experiments unless indicated otherwise.

Cytochrome P-450 determination. Cytochrome P-450 concentrations were determined from measurements of the difference spectrum of CO-ferrocytochrome P-450 versus ferrocytochrome P-450, according to the method of Omura and Sato [20]. An extinction coefficient of 91 $cm^{-1}\,mM^{-1}$ for the difference in absorbance between 450 nm and 490 nm was utilized [20].

Re-oxidation of NADH-reduced cytochrome b_5. Hepatic microsomal cytochrome b_5 was reduced with a slight excess of NADH, and the first order reoxidation of ferrocytochrome b_5, which occurs on exhaustion of the NADH, was monitored spectrally at 424 nm and 409 nm, using the method of Oshino *et al.* [8].

Redox steady-state of cytochrome b_5 *in the presence of NADPH.* The steady-state redox status of NADPH-reduced hepatic microsomal cytochrome b_5 was determined from the change in absorbance between 424 nm and 409 nm by the method of Oshino *et al.* [8]. The results are expressed as the percentage reduction of hepatic microsomal cytochrome b_5 by NADPH relative to dithionite. For both the reoxidation of NADH-reduced cytochrome b_5 and the NADPH steady state assays, the anesthetics, when present, were added to 3 ml of microsomal suspension and vortex mixed prior to the addition of NAD(P)H and cyanide.

NAD(P)H-cytochrome c reductase assays. The activities of NADPH-cytochrome *c* reductase and NADH-cytochrome *c* reductase were determined by the method of Omura and Takesue [17]. The anesthetic agent was suspended in 2.10 ml of Tris–HCl, pH 7.4, by vortex mixing, prior to the addition of the NAD(P)H, cytochrome *c* and hepatic microsomes. The increase in the absorbance of ferrocytochrome *c* at 550 nm ($\varepsilon = 21.1\ cm^{-1}mM^{-1}$) was recorded.

Oxidation of purified trypsin-cleaved ferrocytochrome b_5. Purified trypsin-cleaved cytochrome b_5 was reduced by a modification of the method of Smith [21]. Purified ferricytochrome b_5 was bubbled with N_2 for 20 min, and 5% Palladium on asbestos (2% w/v, final concentration) was added to the cytochrome solution. The suspension was then bubbled with H_2 for 1–2 hr to convert the cytochrome b_5 to the ferrous form. Aliquots of ferrocytochrome b_5 were then removed from the reducing suspension, filtered through an 8 μ millipore filter, and 30 μl of the resultant solution (*ca.* 25 μM cytochrome b_5) were added to 1.25 ml of air equilibrated 0.1 M Tris–HCl, pH 7.4, in the presence or absence of the anesthetic agents. The oxidation of purified trypsin-cleaved ferrocytochrome b_5 was monitored spectrally at 424 nm.

Stearate desaturase assay. The activity of stearate desaturase was assayed via the conversion of [^{14}C]stearoyl CoA to [^{14}C]oleate, essentially by the method of Oshino *et al.* [10]. Incubation mixtures contained hepatic microsomes (1.0 mg protein), 1 mM NADH, and 40 mM [^{14}C]stearoyl CoA (0.2 μCi) in 0.5 ml of 0.1 M Tris–HCl, pH 7.25. Incubations were for 4 min with shaking at 37°. At the end of the incubation period, 2 mg each of carrier oleate and stearate were added to the reaction mixture just

prior to methylation. The fatty acids were methylated with $BF_3 \cdot CH_3OH$ by the method of McIntosh *et al.* [22], and the methyl esters were separated by argentation thin-layer chromatography on silica gel plates (25 cm × 25 cm × 0.25 mm). according to Berman *et al.* [14]. Scrapings from the thin-layer chromatograms were assayed for radioactivity in 7 ml of Instafluor scintillation mixture (Packard), using a Beckman model LS 8100 liquid scintillation counter. The results of the assay were expressed as the percentage of radioactivity in oleate/radioactivity in oleate + stearate [10].

Spectrophotometry. All spectral measurements were performed at 25° in a Unicam SP 1800 recording spectrophotometer using the thermostatically controlled compartment which is designed to accommodate turbid samples.

Calculations and statistical analyses. The observed first order rate constants (k_1) for the re-oxidation of NADH-reduced hepatic microsomal cytochrome b_5 in air saturated buffer were calculated from the slopes of plots of ln $(A_t - A_\infty)$ versus time, where A_t and A_∞ are the absorbance changes between 424 nm and 409 nm at time t and at infinity, respectively.

The observed first order rate constants (k_1) for the oxidation of purified trypsin-cleaved cytochrome b_5 were calculated from plots of ln $(A_{424_t} - A_{424_\infty})$ versus time. In all cases, the absorbance at infinite time was determined after approximately ten half-lives. Student's t-test for unpaired data was utilized to assess statistical significance, with $P < 0.05$ being probably significant, $P < 0.01$ being significant, and $P < 0.001$ being highly significant.

RESULTS

NADPH steady-state of hepatic microsomal cytochrome b_5. In the presence of enflurane, halothane and methoxyflurane, the redox status of NADPH-reduced cytochrome b_5 of hepatic microsomes from rats with elevated levels of stearate desaturase was shifted toward the ferric form of the hemoprotein (Table 1).

Table 1. Effects of anesthetic agents on the NADPH steady-state of hepatic microsomal cytochrome b_5*

Additions (mM)	NADPH steady-state (% reduction)
None	56.1 ± 13.5
Enflurane (14)	26.0 ± 4.5†
Halothane (18)	26.9 ± 5.5†
Methoxyflurane (1)	37.1 ± 1.6†

* Values reported are means ± S.D. for assays in duplicate for three to seven separate preparations of hepatic microsomes from rats fed a high-carbohydrate diet. The reaction mixture contained 3 ml of hepatic microsomes (1.5 mg protein/ml), 15 μM NADPH, and additions as indicated, at 25°.

† Differs significantly from no additions, $P < 0.01$.

Effects of anesthetic agents and inhibitors on the re-oxidation of hepatic microsomal ferrocytochrome b_5. For all studies of the re-oxidation of hepatic microsomal ferrocytochrome b_5, unless indicated otherwise, hepatic microsomes were from rats in which the levels of stearate desaturase were elevated by feeding a high-carbohydrate diet (see Experimental). That the levels of stearate desaturase in these microsomal preparations were elevated was demonstrated by the ability of stearoyl CoA to enhance the rate constant for the re-oxidation of cytochrome b_5 [8] (Tables 2–4).

The anesthetic agents, halothane, enflurane and methoxyflurane, stimulated the re-oxidation of microsomal cytochrome b_5 (Table 2), as was reported earlier for halothane [14]. The rate constants for the re-oxidation of cytochrome b_5 in the presence of stearoyl CoA or the anesthetic agents were decreased by 0.5 mM KCN ($P < 0.01$) (Table 2). The inhibitors of cytochrome P-450, namely metyrapone and carbon monoxide, had no effect on the re-oxidation of cytochrome b_5 in the presence or absence of stearoyl CoA or any of the anesthetic agents ($P > 0.1$) (Tables 3 and 4).

NAD(P)H-cytochrome c *reductase activities*. The effects of enflurane and methoxyflurane on the

Table 2. Effect of cyanide on the enhancement of the rate constants for the re-oxidation of cytochrome b_5 by stearoyl CoA and anesthetic agents*

Additions (mM)	First order rate constant for oxidation of ferrocytochrome b_5 $10^2 k_1$ (sec^{-1})
None	1.24 ± 0.35
KCN (0.5)	1.28 ± 0.20
Stearoyl CoA (0.012)	2.76 ± 0.73†
Stearoyl CoA (0.012) + KCN (0.5)	1.55 ± 0.37
Halothane (18)	1.73 ± 0.29†
Halothane (18) + KCN (0.5)	1.23 ± 0.30
Enflurane (14)	2.44 ± 0.63†
Enflurane (14) + KCN (0.5)	1.67 ± 0.54
Methoxyflurane (1)	1.98 ± 0.28†
Methoxyflurane (1) + KCN (0.5)	1.68 ± 0.08

* Values reported are means ± S.D. for assays in duplicate on each of three separate preparations of hepatic microsomes from rats fed a high-carbohydrate diet. Reaction mixtures contained 3 ml of hepatic microsomes (1.5 mg protein/ml), 1–5 μM NADH, and additions as indicated, at 25°.

† Differs significantly from no additions, $P < 0.01$.

Table 3. Effect of metyrapone on the enhancement of the rate constants for the re-oxidation of cytochrome b_5 by stearoyl CoA and anesthetic agents*

Additions (mM)	First order rate constant for the oxidation of ferrocytochrome b_5 $10^2 k_1$ (sec^{-1})
None	1.09 ± 0.22
Metyrapone (2.3)	1.17 ± 0.18
Stearoyl CoA (0.012)	4.19 ± 0.59†
Stearoyl CoA (0.012) + metyrapone (2.3)	4.15 ± 0.33†
Halothane (18)	1.68 ± 0.15†
Halothane (18) + metyrapone (2.3)	2.03 ± 0.48†
Enflurane (14)	2.23 ± 0.36†
Enflurane (14) + metyrapone (2.3)	2.13 ± 0.49†
Methoxyflurane (1)	1.78 ± 0.18†
Methoxyflurane (1) + metyrapone (2.3)	1.70 ± 0.12†

* Values reported are means ± S.D. for assays in duplicate on each of three separate preparations of hepatic microsomes from rats fed a high-carbohydrate diet. Reaction mixtures contained 3 ml of hepatic microsomes (1.5 mg protein/ml), 1–5 μM NADH, and additions as indicated, at 25°.

† Differs significantly from no additions, $P < 0.01$.

Table 4. Effect of carbon monoxide on the enhancement of the rate constants for the re-oxidation of cytochrome b_5 by stearoyl CoA and anesthetic agents*

Additions	First order rate constant for the oxidation of ferrocytochrome b_5 $10^2 k_1$ (sec^{-1})
None	0.97 ± 0.15
$CO–O_2$ (80:20, v/v)	1.04 ± 0.22
Stearoyl CoA (0.012 mM)	3.26 ± 1.05†
Stearoyl CoA (0.012 mM) + $CO–O_2$ (80:20, v/v)	3.31 ± 0.38†
Halothane (18 mM)	1.35 ± 0.28‡
Halothane (18 mM) + $CO–O_2$ (80:20, v/v)	1.10 ± 0.30
Enflurane (14 mM)	1.49 ± 0.08†
Enflurane (14 mM) + $CO–O_2$ (80:20, v/v)	1.62 ± 0.20†
Methoxyflurane (1 mM)	1.69 ± 0.31†
Methoxyflurane (1 mM) + $CO–O_2$ (80:20, v/v)	1.56 ± 0.42†

* Values reported are means ± S.D. for assays in duplicate on each of three separate preparations of hepatic microsomes from rats fed a high-carbohydrate diet. Reaction mixtures contained 3 ml of hepatic microsomes (1.5 mg protein/ml), 1–5 μM NADH, and additions as indicated, at 25°.

† Differs significantly from no additions, $P < 0.01$.

‡ Probably differs from no additions, $P < 0.05$.

Table 5. Effect of anesthetic agents on hepatic microsomal NADPH- and NADH-cytochrome *c* reductase and on the oxidation of purified trypsin-cleaved cytochrome b_5*

Additions (mM)	Autoxidation of purified ferrocytochrome b_5 $10^2 k_1$ (sec^{-1})	NADPH-cytochrome *c* reductase (units/mg protein)	NADH-cytochrome *c* reductase (units/mg protein)
None	0.85 ± 0.15	0.051 ± 0.005	0.95 ± 0.10
Enflurane (14)	0.89 ± 0.16	0.053 ± 0.005	1.34 ± 0.14†
Methoxyflurane (1)	0.97 ± 0.05	0.057 ± 0.001	1.09 ± 0.14

* For the NADPH- and NADH-cytochrome *c* reductase assays, 100 μl and 20 μl of hepatic microsomes (1.5 mg protein/ml) from rats fed a high-carbohydrate diet were added to 2.10 ml of 0.1 M Tris–HCl, pH 7.4, containing 20 μM cytochrome *c*, 0.1 mM of either NADPH or NADH, and additions as indicated. For the oxidation of purified, trypsin-cleaved ferrocytochrome b_5, the reaction mixtures contained 1.25 ml of 0.1 M Tris–HCl, pH 7.4, 30 μl of purified ferrocytochrome b_5, and additions as indicated. Reported values are means ± S.D.

† Differs significantly from no additions, $P < 0.01$.

Table 6. Effect of anesthetic agents and KCN on the rate constants for the re-oxidation of NADH-reduced hepatic microsomal cytochrome b_5 in differently pretreated rats*

Additions (mM)	10^2 k_1 (sec^{-1})		
	Fed a normal diet	Fasted	Fasted, phenobarbitone-induced
None	1.56 ± 0.17	1.63 ± 0.41	2.65 ± 0.07
KCN (0.5)	1.48 ± 0.20	1.34 ± 0.13	2.03 ± 0.43
Stearoyl CoA (0.012)	2.09 ± 0.12†	1.83 ± 0.27	2.78 ± 0.17
Stearoyl CoA (0.012) + KCN (0.5)	1.73 ± 0.30	1.55 ± 0.18	2.17 ± 0.25
Enflurane (14)	2.28 ± 0.26†	2.23 ± 0.38‡	3.61 ± 0.94‡
Enflurane (14) + KCN (0.5)	2.08 ± 0.15	2.02 ± 0.39	3.50 ± 0.78
Methoxyflurane (1)	1.90 ± 0.27‡	1.86 ± 0.09	3.00 ± 0.27‡
Methoxyflurane (1) + KCN (0.5)	1.73 ± 0.28	1.75 ± 0.18	2.82 ± 0.47
Cytochrome P-450 (nmoles/mg protein)	0.98 ± 0.05	1.24 ± 0.33	2.49 ± 0.12

* Values reported are means ± S.D. for assays in duplicate with each of three separate preparations of hepatic microsomes. Experimental details are as in Table 2.
† Differs significantly from no additions for similarly pretreated rats, $P < 0.001$.
‡ Probably differs from no additions for similarly pretreated rats, $P < 0.05$.

Table 7. Effect of anesthetic agents and KCN on the re-oxidation of NADH-reduced hepatic microsomal cytochrome b_5 from differently pretreated rats*

Additions	Per cent increase in first order rate constant (k_1)							
	High-carbohydrate diet		Normal diet		Fasted		Phenobarbitone-induced and fasted	
	−KCN	+KCN	−KCN	+KCN	−KCN	+KCN	−KCN	+KCN
Stearoyl CoA	100–800	50–200	34	16	12	17	5	7
Halothane	40	0	58†	10†	19†	22†	22†	30
Enflurane	97	30	46	40	37	24	36	32
Methoxyflurane	60	31	22	11	14	7	13	6

* Percentage increases were calculated from the values presented in Tables 2 and 4. Percentages are relative to the corresponding value for NADH alone
† Calculated from data in Ref. 14.

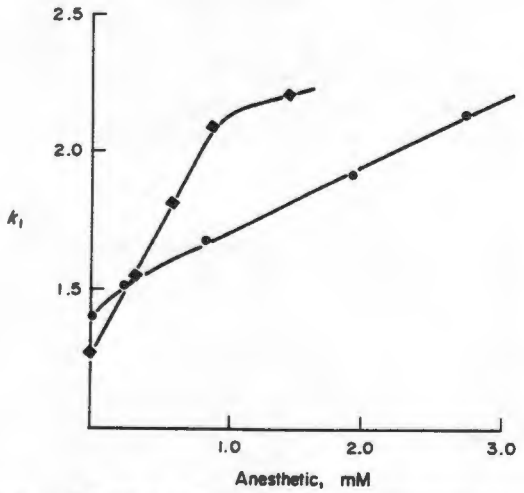

Fig. 1. Effects of enflurane (●) and methoxyflurane (◆) on the rate constants (k_1) for the re-oxidation of NADH-reduced hepatic microsomal cytochrome b_5. The first order rate constant k_1 is in units of 10^{-2} sec^{-1}. Experimental conditions are as given in Table 2.

activities of NADPH- and NADH-cytochrome *c* reductase are shown in Table 5. Neither enflurane nor methoxyflurane had a statistically significant effect on NADPH-cytochrome *c* reductase. Enflurane slightly enhanced the activity of NADH-cytochrome *c* reductase, whereas methoxyflurane did not.

Autoxidation of trypsin-cleaved ferrocytochrome b_5. The rate constants for the autoxidation of purified trypsin-cleaved ferrocytochrome b_5 in the presence of enflurane and methoxyflurane are given in Table 5. As can be seen, the rate constant for the autoxidation of purified ferrocytochrome b_5 is not altered in the presence of enflurane or methoxyflurane ($P > 0.1$).

Re-oxidation of hepatic microsomal ferrocytochrome b_5 *from fasted, fed or phenobarbitone-pretreated rats*. The rate constants for the re-oxidation of NADH-reduced cytochrome b_5 in microsomes from fasted, fed or phenobarbitone-induced rats are shown in Table 6, and the data are summarized in Table 7. Stearoyl CoA and enflurane increased the k_1 in fed rats, but not significantly in fasted rats

Table 8. Equilibrium constants for the interaction of anesthetic agents with hepatic microsomal enzymes

Compound	K_{eq} (mM) for cytochrome b_5 re-oxidation	K_s* (mM) for binding to cytochrome P-450	K_m* (mM) for oxidation of NADPH by cytochrome P-450	K_m* (mM) for fluoride ion production by cytochrome P-450
Enflurane	1.18 ± 0.16	0.46 ± 0.15	0.15 ± 0.10	0.36 ± 0.07
Methoxyflurane	0.48 ± 0.14	0.48 ± 0.13	0.10 ± 0.01	0.40 ± 0.12†
				4.9 ± 0.9

* Data for hepatic microsomes from fasted, uninduced male rats from Ref. 4.
† Two K_m values were calculated from biphasic Eadie–Hofstee plots for this process.

pretreated or not with phenobarbitone. Cyanide inhibited the ability of stearoyl CoA to enhance the re-oxidation of cytochrome b_5 in microsomes from rats fed a normal diet ($P < 0.001$), but not in microsomes from fasted or phenobarbitone-treated rats ($P > 0.1$). Cyanide had no significant effect on the oxidation of cytochrome b_5 in the presence of enflurane or methoxyflurane in hepatic microsomes from fed, fasted or phenobarbitone-treated rats. The activity of stearate desaturase (as assessed by the stimulation of the re-oxidation of cytochrome b_5 by stearoyl CoA [8]) and the abilities of enflurane and methoxyflurane to stimulate the cyanide sensitive re-oxidation of NADH-reduced cytochrome b_5 decreased in the order of rats fed a high-carbohydrate diet > rats fed a normal diet > fasted rats ≅ fasted, phenobarbitone-treated rats (Table 7).

Determination of the equilibrium constants (K_{eq}) for the stimulation of hepatic microsomal electron transfer by enflurane and methoxyflurane. The effects of increasing concentrations of enflurane and methoxyflurane on the rate constants for the re-oxidation of NADH-reduced cytochrome b_5 were utilized to calculate the equilibrium constants (K_{eq}) for the stimulation of microsomal electron transfer by these anesthetic agents (Fig. 1, Table 8). The value of K_{eq} for enflurane differed from the K_s and K_m values for the binding and metabolism of enflurane by hepatic microsomal cytochrome P-450 (Table 8) ($P < 0.01$). The K_{eq} calculated for methoxyflurane differed ($P < 0.01$) from the K_m for NADPH oxidation and the high K_m for fluoride production by cytochrome P-450, but did not differ significantly from the K_s for binding to cytochrome P-450 or the low K_m for the production of fluoride ion from methoxyflurane by cytochrome P-450 ($P > 0.1$).

Inhibition of the re-oxidation of hepatic microsomal cytochrome b_5 *by KCN—Determination of* K_i. The effects of increasing concentrations of cyanide on the stimulation of the rate constants for the re-oxidation of NADH-reduced microsomal cytochrome b_5 by enflurane and methoxyflurane are shown in Fig. 2. The K_i values for cyanide were found to be 0.08 ± 0.01 mM for enflurane and 0.11 ± 0.02 mM for methoxyflurane. These constants compare well with the K_i of 0.14 mM determined for the cyanide inhibition of the stimulation of re-oxidation of hepatic microsomal cytochrome b_5 by halothane reported by Berman *et al.* [14] and with the value of K_i of 0.1 mM for cyanide inhibition of the conversion of stearoyl CoA to oleate by stearate desaturase reported by Oshino *et al.* [10].

Stearate desaturase assay. The effects of halothane, methoxyflurane and enflurane on the activity of stearate desaturase, as assayed via the conversion

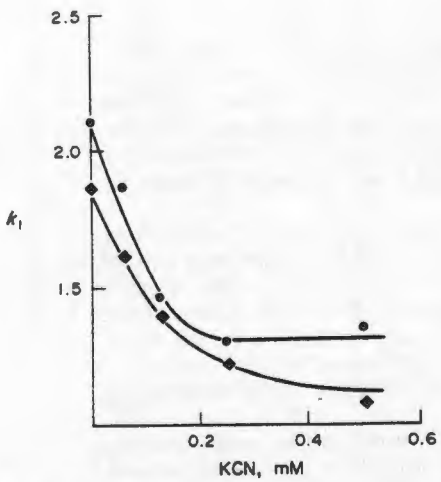

Fig. 2. Effect of cyanide on the rate constants (k_1) for the reoxidation of NADH-reduced hepatic microsomal cytochrome b_5 in the presence of (●) 1.4 mM enflurane and (◆) 0.6 mM methoxyflurane. Experimental conditions are as given in Table 2.

Table 9. Effect of anesthetic agents on the stearate desaturase mediated conversion of stearate to oleate*

Additions (mM)	Oleate (oleate + stearate)
None	0.34 ± 0.16
Halothane (18)	0.38 ± 0.14
Enflurane (14)	0.36 ± 0.12
Methoxyflurane (1)	0.35 ± 0.16

* Values are means ± S.D. for three separate assays using microsomes from rats fed a high-carbohydrate diet. See Experimental Section for details.

of [^{14}C]stearoyl CoA to [^{14}C]oleate, are shown in Table 9. None of the anesthetic agents had any effect on this reaction ($P > 0.1$).

DISCUSSION

Enflurane and methoxyflurane appear to stimulate microsomal electron transfer from NADPH and NADH via cytochrome b_5, as shown by the ability of these anesthetic agents to shift the redox status of NADPH-reduced cytochrome b_5 toward the ferric form of the protein and to increase the rate constants for the reoxidation of NADH-reduced cytochrome b_5 (Tables 1 and 2). Since enflurane and methoxyflurane did not decrease the activity of microsomal NADH- and NADPH-cytochrome *c* reductase (Table 5), it would appear that these anesthetic agents probably do not decrease the rate of reduction of cytochrome b_5 but rather enhance the rate of oxidation of this heme protein.

The oxidation of ferrocytochrome b_5 can proceed via an autoxidation reaction or via the transfer of electrons to other microsomal proteins such as cytochrome P-450 or stearate desaturase. In the absence of added substrates for cytochrome P-450, stearate desaturase, or other microsomal enzymes which accept reducing equivalents from ferrocytochrome b_5, the oxidation of microsomal cytochrome b_5 *in vitro* is thought to arise in large part from the autoxidation of this hemoprotein [14, 23–26]. The autoxidation reaction involves the transfer of reducing equivalents from ferrocytochrome b_5 directly to oxygen to produce ferricytochrome b_5 and superoxide [23, 26].

Purified trypsin-cleaved cytochrome b_5, a heme peptide of approximately ninety residues which differs from the intact hemoprotein only in that the hydrophobic tail which attaches the protein to the membrane is lacking, was used as a model to assess the effects of enflurane and methoxyflurane on the autoxidation of ferrocytochrome b_5. Trypsin-cleaved cytochrome b_5 was chosen as a model system because it does not aggregate in water, as does cytochrome b_5 prepared by detergent solubilization, and the structure of the heme crevice and the rate of autoxidation of the hemoprotein are not altered following tryptic digestion of cytochrome b_5 [14, 27]. The first order rate constant for the autoxidation of purified trypsin-cleaved cytochrome b_5 reported herein (Table 5) is identical to values reported elsewhere [14] and is similar to the first order rate constant for the oxidation of membrane-bound hepatic microsomal ferrocytochrome b_5 in the absence of substrates for cytochrome P-450 and stearate desaturase [14, 23] (Tables 2 and 5). Since the rate constants for the autoxidation of trypsin-cleaved ferrocytochrome b_5 were not affected by enflurane and methoxyflurane, it would appear that these compounds probably do not affect the autoxidation of ferrocytochrome b_5. This conclusion is supported further by the observation that the autoxidation of cytochrome b_5 is not inhibited by KCN [14] (Table 2), while the effects of enflurane and methoxyflurane on hepatic microsomal electron transfer are. It would be anticipated, therefore, that enflurane and methoxyflurane may stimulate electron transfer from ferrocytochrome b_5 to another microsomal protein.

From several lines of evidence, it would appear that the microsomal electron acceptor in question is not cytochrome P-450. NADH, which does not effectively support cytochrome P-450-dependent reactions, supported the phenomenon as effectively as did NADPH (Tables 1 and 2). Neither prior induction of cytochrome P-450 with phenobarbital nor the presence of the inhibitors of cytochrome P-450—metyrapone and CO (see e.g. Refs. [28] and [29])—in the reaction mixture had any effect on the enhanced oxidation of cytochrome b_5 seen in the presence of enflurane and methoxyflurane (Tables 3, 4 and 6). In contrast, cyanide, which is not an effective inhibitor of cytochrome P-450, inhibited the process with a K_i which was well below the range of K_i values of 2.5 to 10 mM reported for the inhibition of cytochrome P-450 by cyanide [30, 31]. Finally, the K_{eq} for the stimulation of the re-oxidation of cytochrome b_5 by enflurane differed significantly from the K_s and K_m values for the interaction of enflurane with cytochrome P-450 [4] (Table 8).

It would appear that the microsomal 6-desaturase is not involved in the stimulation of microsomal electron transfer by enflurane and methoxyflurane, since its levels are not elevated by the feeding of a high-carbohydrate diet [15] which enhances the effects of the anesthetic agents (Tables 6 and 7). It is also possible to exclude catalase—a microsomal contaminant—as having an important role in mediating the effects of enflurane and methoxyflurane, because, although this enzyme is cyanide-sensitive, the K_i for cyanide inhibition of this enzyme (approximately 8 μM [32]) is 10-fold lower than the K_i calculated for cyanide inhibition of the re-oxidation of cytochrome b_5.

The results presented herein are consistent with the proposal that the transfer of electrons to stearate desaturase is responsible for the enflurane- and methoxyflurane-mediated enhancement of microsomal electron transfer through cytochrome b_5. The magnitude of the observed effect parallels the dietary induction of stearate desaturase; for example, the feeding of a high-carbohydrate diet, which induces stearate desaturase, results in maximal enhancement of electron transfer, while fasting, which reduces stearate desaturase to negligible levels [8, 15, 16], eliminates the effects of the anesthetic agents (Tables 6 and 7). In addition, the enhanced re-oxidation of cytochrome b_5 is inhibited by cyanide (Table 2), as is stearate desaturase. The K_i values calculated for cyanide inhibition of the stimulation of electron transfer by enflurane and methoxyflurane are within experimental error of the K_i of 0.1 mM reported for cyanide inhibition of the conversion of stearoyl CoA to oleate by stearate desaturase [10].

The lack of effect of enflurane and methoxyflurane on the conversion of stearoyl CoA to oleate is not inconsistent with the above proposal since the anesthetic agents may not bind to the substrate binding site of the enzyme. Other compounds which have been reported to interact with stearate desaturase, namely halothane and p-cresol, also do not inhibit the conversion of stearoyl CoA to oleate [11, 14].

In conclusion, it would appear that enflurane and methoxyflurane, at concentrations achieved in physiological fluids [33, 34], stimulate hepatic microsomal electron flow from NADH or NADPH by enhancing the oxidation of cytochrome b_5. The autoxidation of cytochrome b_5 and the transfer of electrons from cytochrome b_5 to catalase, to the 6-desaturase or to cytochrome P-450 appear not to be involved in the stimulation by these agents of microsomal electron transfer. That the enhancement of microsomal electron transfer by enflurane and methoxyflurane parallels the dietary induction of stearate desaturase and that the K_i for cyanide inhibition of the effect of enflurane and methoxyflurane equals the K_i for inhibition of stearate desaturation strongly suggest that the effects of enflurane and methoxyflurane are mediated via stearate desaturase. The nature of the interaction of enflurane and methoxflurane with stearate desaturase and the physiological and pathological effects thereof are under investigation.

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INFLUENCE OF TWO HALOALKANES ON THE REDOX BEHAVIOR OF HEPATIC MICROSOMAL CYTOCHROME b_5 AND ITS POSSIBLE RELATIONSHIP TO STEARATE DESATURASE

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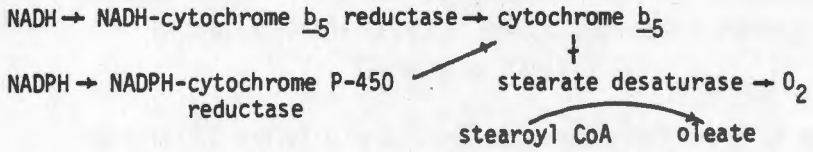
ABSTRACT

The possible interaction of two haloalkanes - bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane - with stearate desaturase was assessed in hepatic microsomes from rats fed a high carbohydrate diet which elevates the levels of stearate desaturase. Both compounds shifted the redox steady state of NADPH reduced hepatic microsomal cytochrome b_5 towards ferricytochrome b_5 and enhanced the re-oxidation of NADH reduced hepatic microsomal cytochrome b_5. The equilibrium constants for the enhancement of microsomal electron transfer by the haloalkanes in these preparations were 2.2 + 0.3 mM and 0.46 + 0.1 mM for bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane, respectively. The haloalkane mediated enhancement of the oxidation of cytochrome b_5 in hepatic microsomes from rats fed a high carbohydrate diet was diminished by KCN and the inhibitors of cytochrome P-450, CO and/or metyrapone, as well as by fasting of the experimental animals. The I_{50} values for KCN inhibition of the effects of the haloalkanes on the re-oxidation of cytochrome b_5 (0.1 mM) were identical to the I_{50} for KCN inhibition of stearate desaturase (Oshino et al., 1966). The haloalkanes did not affect the activity of hepatic microsomal NADH- or NADPH-cytochrome c reductase, the autoxidation of purified trypsin-cleaved ferrocytochrome b_5 or the conversion of stearoyl CoA to oleate. It is concluded that bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane stimulate hepatic microsomal electron transfer from NADH via cytochrome b_5 by interacting with cytochrome P-450 and with stearate desaturase.

INTRODUCTION

The stearoyl CoA desaturase enzyme system catalyzes the cyanide inhibitable conversion of stearoyl CoA and palmitoyl CoA to their Δ9-10 unsaturated analogues (Berman et al., 1975; Ivanetich et al., 1980). This enzyme system comprises the electron carriers - the NADH- and NADPH-dependent flavoprotein reductases and cytochrome b_5 - and a terminal oxidase which is known as stearate desaturase or the cyanide sensitive factor (Scheme 1) (Oshino et al., 1966, 1971; Oshino and Sato, 1971; Jones et al. 1969 ; Shimikata et al., 1972).



Scheme 1: Electron transfer to stearate desaturase
(Straight arrows indicate electron flow)

Stearate desaturase has been reported to interact with a small number of xenobiotics, including phenols and the volatile anaesthetic agents halothane ($CF_3CHBrCl$), enflurane ($CFClHCF_2OCF_2H$) and methoxyflurane ($CCl_2HCF_2OCH_3$) (Oshino and Sato, 1971; Berman et al., 1975; Ivanetich et al., 1980). In as much as none of these compounds affect the conversion of stearoyl CoA to oleate by the hepatic microsomal stearoyl CoA desaturase enzyme system, the evidence for their interaction with stearate desaturase is indirect: These compounds stimulated the cyanide inhibitable re-oxidation of NADH reduced cytochrome b_5 in hepatic microsomes, with the I_{50} for KCN inhibition of this process being identical to that for KCN inhibition of stearate desaturation (0.1 mM), and the magnitude of the enhancement of the re-oxidation of microsomal cytochrome b_5 by these compounds paralleled the dietary induction of stearate desaturase (Oshino and Sato, 1971; Berman et al., 1975; Ivanetich et al., 1980).

We have attempted to establish whether the halogenated alkanes, bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane, are capable of interacting with hepatic microsomal stearate desaturase. The interaction of these compounds with stearate desaturase was of necessity assessed indirectly, viz., by measuring their effects on the oxidation state of cytochrome b_5 in the presence and absence of cyanide (Oshino et al., 1971). In these investigations, advantage was taken of the dietary control of stearate desaturase: The levels of this enzyme were decreased by fasting and were routinely elevated by re-feeding a high carbohydrate diet (Oshino et al., 1971; Shimikata et al., 1971; Oshino and Sato, 1972; Lee and Sprecher, 1971).

MATERIALS AND METHODS

Bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane were from Aldrich Chemical Co., Milwaukee, Wis., and ICN Pharmaceuticals, Plainview N.Y., respectively. The treatment of Long Evans rats (250-300 g) and other experimental details are as reported earlier (Ivanetich et al., 1980).

Experiments were routinely conducted with hepatic microsomes (1.5 mg protein/ml 0.05 M Tris-HCl, pH 7.4) at 25°. Reported values are means ± S.D. for determinations in duplicate to quadruplicate on two to four separate preparations of hepatic microsomes. Final concentrations of compounds added to incubation mixtures were as follows: KCN (0.5 mM), stearoyl CoA (12 μM), NADH (15-45 μM), NADPH (0.15 mM), bromotrichloromethane (3.4 mM), 1,2-dibromo-1,2-dichloroethane (0.6 mM), unless otherwise indicated.

RESULTS

Effects of haloalkanes on the oxidation state of hepatic microsomal cytochrome b_5. In the presence of NADPH and oxygen, microsomal cytochrome b_5 exists in a redox steady state (Oshino et al., 1971). The ability of stearoyl CoA to shift the redox steady state of cytochrome b_5 towards the ferric form confirms that the hepatic microsomes from rats fed a high carbohydrate diet contained appreciable stearate desaturase activity (Data not shown) (Oshino et al., 1971). Both bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane shifted the redox steady state of cytochrome b_5 towards the ferric form. KCN diminished the effect of both compounds on the redox steady state of cytochrome b_5 (Table 1).

For assessing the re-oxidation of hepatic microsomal cytochrome b_5, microsomal suspensions were reduced with limiting amounts of NADH, and the re-oxidation of ferrocytochrome b_5 which occurs on exhaustion of the NADH was monitored. Since NADH reduces ferricytochrome b_5 rapidly, once the NADH is exhausted, the oxidation of ferrocytochrome b_5 follows pseudo first order kinetics (Oshino et al., 1971; Berman et al., 1975). Any increase in the pseudo first order rate constant for the re-oxidation of microsomal ferrocytochrome b_5 above this basal value thus provides a measure of the oxidation of ferrocytochrome b_5 and indirectly

TABLE 1. Effect of haloalkanes on the oxidation state of hepatic microsomal cytochrome b_5

Additions	NADPH steady state (% Reduction of cytochrome b_5)		Re-oxidation of cytochrome b_5 [$10^2 k_{obs}$ (sec^{-1})]			
	Rats fed a high carbohydrate diet		Rats fed a high carbohydrate diet		Fasted rats	
	- KCN	+ KCN (0.5 mM)	- KCN	+ KCN (0.5 mM)	- KCN	+ KCN (0.5 mM)
None	58.6 ± 3.5	62.0 ± 4.2	1.35 ± 0.35	1.21 ± 0.28	1.31 ± 0.26	1.12 ± 0.36
Bromotrichloromethane (3.4 mM)	29.2 ± 0.9*	38.1 ± 1.1*	3.44 ± 0.64*	2.42 ± 0.09*	2.63 ± 0.34*	2.36 ± 0.53*
1,2-Dibromo-1,2-chloroethane (0.6 mM)	34.6 ± 9.0*	47.1 ± 3.3*	1.61 ± 0.15+	1.10 ± 0.20*	1.27 ± 0.07	0.95 ± 0.06

Sample and reference cuvettes, each containing 3 ml of hepatic microsomes (1.5 mg protein/ml) were equilibrated at 25°. The compound to be tested was added with a Hamilton syringe to the sample cuvette below the surface of the microsomal suspension, the cuvette was stoppered and the mixture was vortex mixed for 30 sec. The reaction was initiated by the addition of 0.15 mM NADPH or 15 μM NADH.

* Differs significantly from no additions entry, $p < 0.01$.

+ Probably differs from no additions entry, $p < 0.05$.

provides a measure of microsomal electron transfer from ferrocytochrome $\underline{b}_5$ (Oshino *et al.*, 1971; Berman *et al.*, 1975).

The effects of the two haloalkanes on the pseudo first order rate constant (k_{obs}) for the re-oxidation of NADH-reduced cytochrome $\underline{b}_5$ in hepatic microsomes from rats fed a high carbohydrate diet and from fasted rats are shown in Table 1. Bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane increased the rate constant for the re-oxidation of cytochrome $\underline{b}_5$ in hepatic microsomes from rats fed a high carbohydrate diet, and the magnitude of this increase was diminished by KCN. With hepatic microsomes from fasted rats, the effect of bromotrichloromethane on the re-oxidation of cytochrome $\underline{b}_5$ was diminished ($p < 0.01$) and the cyanide sensitivity of the effect was eliminated. Fasting eliminated the ability of 1,2-dibromo-1,2-dichloroethane to enhance the re-oxidation of microsomal cytochrome $\underline{b}_5$ (Table 1). CO and metyrapone, which are effective inhibitors of cytochrome P-450 (De Bruin, 1976), did not significantly affect the re-oxidation of ferrocytochrome $\underline{b}_5$ in microsomes from rats fed a high carbohydrate diet in the absence of added haloalkane ($p > 0.1$). CO did not significantly ($p > 0.1$) affect and metyrapone slightly (by 20%) diminished ($p < 0.01$) the effects of bromotrichloromethane on the re-oxidation of microsomal ferrocytochrome $\underline{b}_5$. Both inhibitors decreased the effect of 1,2-dibromo-1,2-dichloroethane on the re-oxidation of microsomal ferrocytochrome $\underline{b}_5$ to negligible levels ($p < 0.01$).

The effects of varying concentrations of the two haloalkanes on the rate constants for the re-oxidation of NADH reduced cytochrome $\underline{b}_5$ in microsomes from rats with elevated levels of stearate desaturase are shown in Figures 1 and 2. The concentrations of these compounds for half maximal effect on the re-oxidation of cytochrome $\underline{b}_5$ were calculated from Figure 2 to be 2.2 ± 0.3 mM and 0.46 ± 0.1 mM for bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane, respectively.

The effects of variable amounts of KCN on the rate constant for the re-oxidation of NADH reduced hepatic microsomal cytochrome $\underline{b}_5$ in the presence of bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane are shown in Figure 3. The I_{50} values were calculated to be 0.12 ± 0.03 and 0.07 ± 0.02 mM KCN for the inhibition of the stimulated microsomal

electron transfer in the presence of bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane, respectively.

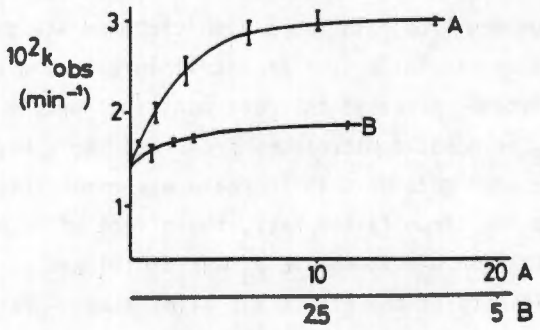


Figure 1. Pseudo first order rate constants for the re-oxidation of cytochrome b_5 in hepatic microsomes from rats fed a high carbohydrate diet in the presence of bromotrichloromethane (A) and 1,2-dibromo-1,2-dichloroethane (B). Experimental details are described in Table 1.

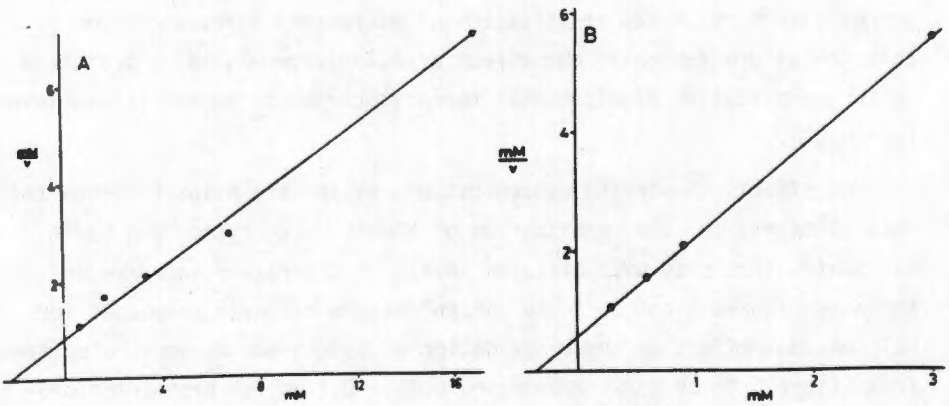


Figure 2. Hanes plots of the enhancement by bromotrichloromethane (A) and 1,2-dibromo-1,2-dichloroethane (B) of the re-oxidation of cytochrome b_5 in hepatic microsomes from rats fed a high carbohydrate diet. Experimental details are described in Table 1. v represents the difference in 10^2k_{obs} in the presence and absence of xenobiotic.

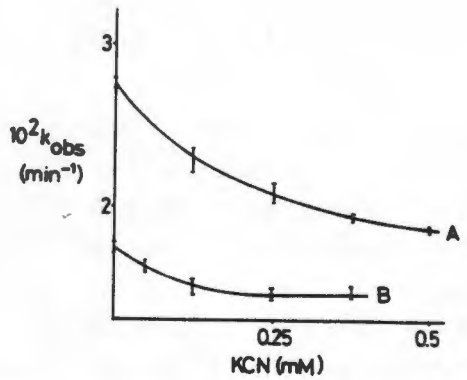


Figure 3. Effect of KCN on the stimulation of the re-oxidation of cytochrome b_5 in hepatic microsomes from rats fed a high carbohydrate diet by 3.4 mM bromotrichloromethane (A) and by 0.6 mM 1,2-dibromo-1,2-dichloroethane (B). Experimental details are described in Table 1.

Effects of compounds on microsomal electron transfer proteins and stearate desaturase. Neither haloalkane had a significant effect on the activity of microsomal NADH- or NADPH-cytochrome c reductase or stearate desaturase or on the rate constant for the autoxidation of purified trypsin cleaved ferrocytochrome b_5 ($p > 0.1$) (Data not shown).

DISCUSSION

Since bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane shifted the redox steady state of cytochrome b_5 towards the ferric form (Table 1) and stimulated the re-oxidation of NADH reduced hepatic microsomal cytochrome b_5, which is a measure of the rate of oxidation of this hemoprotein (Oshino et al., 1971; Berman et al., 1975), but did not affect the activity of the microsomal NADH- and NADPH-cytochrome c reductase[1] (Table 1, See Results), it would appear that they are increasing the rate of oxidation and not affecting the rate of reduction of microsomal cytochrome b_5.

The observed increase in the rate of oxidation of microsomal ferrocytochrome b_5 for the two haloalkanes could reflect an increased rate of

[1] Since electron transfer from ferrocytochrome b_5 to ferricytochrome c is rapid, the cytochrome c reductase activities provide a measure of the rate of reduction of ferricytochrome b_5 (see e.g. Strittmatter and Velick, 1956).

autoxidation[2] of this hemoprotein or an increased rate of electron transfer from ferrocytochrome $\underline{b}_5$ to one or more of the terminal microsomal oxidases.

The effects of bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane on the re-oxidation of hepatic microsomal ferrocytochrome $\underline{b}_5$ would not appear to result from an effect on the autoxidation reaction since the stimulation of the re-oxidation of microsomal ferrocytochrome $\underline{b}_5$ by these compounds was inhibited by cyanide to nearly background levels (Figure 3), while the autoxidation of purified trypsin cleaved ferrocytochrome $\underline{b}_5$ is cyanide insensitive (Berman et al., 1975). In addition, the autoxidation of purified trypsin cleaved ferrocytochrome $\underline{b}_5$ was unaffected by these two halogenated hydrocarbons (See Results).

The enhanced oxidation of hepatic microsomal ferrocytochrome $\underline{b}_5$ by bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane would thus appear to be a consequence of the stimulation by these compounds of electron transfer to one or more of the terminal microsomal oxidases, such as N-hydroxylamine oxidase, the phospholipid desaturase, the acyl CoA Δ5-, Δ6- and Δ9-desaturases or cytochrome P-450 (Oshino, 1978).

Microsomal N-hydroxylamine oxidase would not appear to be involved in the effects of bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane on the re-oxidation of cytochrome $\underline{b}_5$ inasmuch as this oxidase is insensitive to KCN and CO (Kadlubar et al., 1973), while the effects of these compounds on the re-oxidation of microsomal ferrocytochrome $\underline{b}_5$ were sensitive to KCN and in the case of 1,2-dibromo-1,2-dichloroethane also to CO (Table 1, Figure 3, See Results).

The phospholipid desaturase and acyl CoA Δ5- and Δ6-desaturases would appear to play no role in the enhancement of oxidation of microsomal ferrocytochrome $\underline{b}_5$ by the two haloalkanes. The acyl CoA Δ5-desaturase and phospholipid desaturase are less sensitive to KCN [I_{50} of 1-5 mM KCN (Inkpen et al., 1969)] than was the enhancement of microsomal electron flow by the two haloalkanes (I_{50} of 0.1 mM KCN). The activity of the acyl CoA Δ6-desaturase is not enhanced by re-feeding a high carbohydrate diet (Inkpen et al., 1969) which enhanced the effects of bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane on microsomal electron transfer (Table 1).

[2] Autoxidation refers to the direct transfer of an electron from ferrocytochrome $\underline{b}_5$ to molecular oxygen (See e.g. Berman et al., 1976).

It is also possible to exclude the microsomal contaminant catalase as mediating the effects of the halo compounds on the oxidation of microsomal ferrocytochrome $\underline{b}_5$ inasmuch as the K_i for KCN inhibition of catalase (approximately 8 μM) (Chance, 1952) is ten fold lower than the I_{50} for the KCN inhibition of the enhancement of the re-oxidation of cytochrome $\underline{b}_5$ in the presence of the haloalkanes (see Results).

The abilities of bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane to enhance the oxidation of cytochrome $\underline{b}_5$ may be a consequence of the interaction of these compounds with cytochrome P-450. Both compounds appear to be substrates for cytochrome P-450[3] (Sipes et al., 1977). Furthermore, CO and/or metyrapone which are effective inhibitors of cytochrome P-450 (Boveris et al., 1972), but not of stearate desaturase (Ivanetich et al., 1980), inhibited the re-oxidation of microsomal ferrocytochrome $\underline{b}_5$ in the presence of these compounds, and neither KCN nor fasting eliminated the enhancement by bromotrichloromethane of the re-oxidation of microsomal cytochrome $\underline{b}_5$ (see Results, Table 1).

It would appear, however, that bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane may, at least in part, stimulate the oxidation of hepatic microsomal ferrocytochrome $\underline{b}_5$ by interacting with stearate desaturase. The magnitude of the enhancement of the oxidation of microsomal ferrocytochrome $\underline{b}_5$ by bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane and the ability of cyanide to inhibit their effects, paralleled the dietary induction of stearate desaturase: The effect was measurable in rats fed a high carbohydrate diet which elevates the levels of stearate desaturase and the effect or its cyanide sensitivity was decreased or eliminated by fasting the experimental animals which drastically decreases the levels of stearate desaturase (Table 1)(Oshino et al., 1971; Shimikata et al., 1971; Oshino and Sato, 1972; Lee and Sprecher, 1971). Furthermore, the effects of these compounds on the re-oxidation of microsomal ferrocytochrome $\underline{b}_5$ were inhibited by cyanide which is an inhibitor of stearate desaturase (Table 1, Figure 3). The I_{50} values for KCN inhibition of the enhanced re-oxidation of hepatic microsomal ferrocytochrome $\underline{b}_5$ by bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane (see Results) are equivalent to the I_{50} value (ca. 0.1 mM) for KCN inhibition of hepatic microsomal stearate

[3] Both compounds produce type I difference spectra with hepatic microsomal cytochrome P-450. V. Manca, Ph.D. Thesis, University of Cape Town, 1980.

desaturase (Oshino et al., 1966; Berman et al., 1975), but are significantly lower than the K_i values (2.5 - 8 mM) reported for the inhibition of cytochrome P-450 by cyanide (Jefcoate et al., 1969; Correia and Mannering, 1973).

The inability of bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane to inhibit the metabolism of stearoyl CoA by stearate desaturase (see Results) would not appear to mitigate against the proposal that these compounds stimulate the oxidation of ferrocytochrome b_5, at least in part, by interacting with stearate desaturase. Other compounds, including phenols, a haloalkane and haloethers, which appear to stimulate microsomal electron transfer by interacting with stearate desaturase, also have no effect on the hepatic microsomal metabolism of stearoyl CoA, possibly because stearoyl CoA binds extremely tightly to the enzyme or perhaps because the binding site for stearoyl CoA may be distinct from that for the xenobiotics (Oshino and Sato, 1971; Berman et al., 1975; Ivanetich et al., 1980).

The latter proposal is supported by the following consideration: Under the experimental conditions used herein[4], if the haloalkanes and stearoyl CoA were binding at the same site on stearate desaturase, a 35% to 40% decrease in the metabolism of stearoyl CoA would be expected, based on a comparison of the ratios of the experimental concentration to the K (or concentration required for half maximal effect) for stearoyl CoA and the haloalkane. Since no inhibition was observed (S.D. $\pm$ 2% for bromotrichloromethane, S.D. $\pm$ 9% for 1,2-dibromo-1,2-dichloroethane), it would appear that these compounds bind to a site distinct from the binding site for stearoyl CoA.

The apparent abilities of bromotrichloromethane and 1,2-dibromo-1,2-dichloroethane to stimulate microsomal electron transfer via stearate desaturase may reflect the metabolism of these compounds by the stearate desaturase enzyme system. Alternatively, these haloalkanes may be uncouplers of this enzyme system, and stimulate electron transfer without being metabolized. No uncouplers of the stearate desaturase enzyme system

[4] The concentrations of haloalkanes were the highest which would not disrupt the microsomal suspension (assessed visibly) or convert cytochrome P-450 to cytochrome P-420 (Shimikata et al., 1972). Therefore, it was not possible to conduct experiments using higher concentrations of the xenobiotics.

are known, but n-perfluorohexane has been reported to uncouple the hepatic microsomal cytochrome P-450 enzyme system (Ullrich and Diehl, 1971).

In view of the growing list of xenobiotics which stimulate microsomal electron flow apparently by interacting with stearate desaturase, it would appear that the possible interaction of xenobiotics with this enzyme should be considered in experiments conducted with hepatic microsomes, particularly those isolated from fed rats.

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Metabolism and Toxicity of Volatile Anaesthetic Agents

Kathryn M. Ivanetich, Veronica Manca and Gaisford G. Harrison

In 1924 the American physiologist, Howard W. Haggard, concluded *inter alia* from his classical investigation of the 'absorption, distribution and elimination of diethyl ether' that 'ether is not destroyed nor utilised in the body. It is all excreted unchanged.'[1] This statement went unchallenged for 40 years. The same was believed of other volatile anaesthetic agents. This was in spite of the implications of a demonstration by Barrett and Johnson,[2] Powell[3] and Thomas Butler[4] that trichloracetic acid and trichloroethanol were detectable in blood and urine of both dogs and man after exposure to trichlorethylene. From this complacent belief in the inertness of the volatile anaesthetics, we have been rudely awakened in the last 20 years or so, by an explosion of interest in the hepatic biotransformation of anaesthetic drugs, to the realization that *all volatile anaesthetics* are metabolized in the body, the only difference between these compounds being one of extent. An incentive for this interest in the metabolism of anaesthetic agents was provided by the emergence of the rare syndrome of 'unexplained post-anaesthetic hepatitis'. Though mainly the consequence of anaesthesia from halothane, the syndrome has also been displayed in patients exposed to other volatile anaesthetics. Most worrying to clinicians was, and still is, the apparent unpredictability of the syndrome and the serious threat it poses to the life of an afflicted patient.[5]

The main thrust of investigations into hepatic anaesthetic and other xenobiotic metabolism and its relevance to anaesthetic-induced liver dysfunction has centred on interactions of these agents with the microsomal cytochrome P-450 enzyme system (see e.g. refs 6–11). The oxidative metabolism of a variety of anaesthetic agents, including fluroxene, methoxyflurane and chloroform, by this enzyme system has proved to result in the metabolic activation of these agents into reactive and/or toxic species.[6-8,10,11] The oxidative metabolism of halothane by hepatic microsomal cytochrome P-450 did not, however, produce toxic results, in spite of an early, unconfirmed report that trifluoroethanol produced from halothane by this enzyme system was responsible for the deleterious effects of halothane anaesthesia.[12]

With the recent discovery that cytochrome P-450 can reductively* metabolize some xenobiotics, including halothane,[13,14] considerable interest has focused on this pathway. The reductive and

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*The term reductive is used in an unusual sense, and refers to the ability of the reaction to proceed in the absence of oxygen.

oxidative pathways compete with each other, as it were, at the active site of cytochrome P-450. Surprisingly, Ahr *et al.*[14] have shown that the reductive metabolism of halothane and other haloethanes can predominate at the oxygen tensions normally found within the hepatocyte. The products of the reductive metabolism of halothane include CF_3CH_2Cl, $CF_2=CHCl$ and $CF_2=CBrCl$ as well as reactive species which degrade the cytochrome P-450 and bind to cellular constituents.[13-17] The stimulation of the reductive metabolism of halothane by hepatic cytochrome P-450 has been shown by Burnell Brown and coworkers to provide a reproducible experimental animal model for halothane hepatitis.[18,19]

One aspect of our investigations into the mechanisms of toxicity of anaesthetic agents, has centred on their interaction with other microsomal enzymes. The emphasis of our approach was to ascertain whether these microsomal enzymes might mediate or influence in any way the toxic effects of the anaesthetic agents.

Our initial interest in this regard centred on microsomal stearate desaturase, after the demonstration by Berman *et al.*[20] that exposure of microsomes to halothane enhanced electron transfer to this enzyme. It was thought that this interaction might contribute to halothane hepatitis, since the levels of stearate desaturase are controlled by diet, which might in part relate to the unexplained nature of this form of hepatitis. We therefore assessed the effect *in vivo* of alterations in the levels of stearate desaturase on halothane hepatitis. The initial experimental model of Sipes and Brown — halothane anaesthesia of rats exposed to phenobarbital under anoxic conditions[19] — reproducibly and effectively produced halothane-mediated hepatoxicity. However, the induction of stearate desaturase by diet interfered with the induction of cytochrome P-450 by phenobarbital. Consequently, we developed a different experimental model, omitting phenobarbital induction, but retaining halothane anaesthesia under anoxic conditions. This produced toxicity in about half of the experimental animals. The induction of stearate desaturase with a high carbohydrate diet, prior to anaesthesia, fully protected the animals against the toxic effects of halothane[21] as assessed by mortality and by serum enzyme levels (SGOT). These observations suggest the exciting possibility of protecting patients against halothane hepatoxicity by providing them with a high carbohydrate diet for several days before an operation involving anaesthesia.

Another aspect of our investigations centred on elucidating which halogenated anaesthetic agents interact with stearate desaturase, and the mechanism by which these compounds stimulate microsomal electron transfer via this enzyme system. In addition to halothane, enflurane and methoxyflurane appeared to interact with stearate desaturase, whereas fluroxene, trichloroethylene and chloroform did not.[20-22]

The nature and mechanism of the interaction of these anaesthetic agents with stearate desaturase have yet not been fully elucidated. At present, it appears that halothane, enflurane and methoxyflurane stimulate microsomal electron transfer in part by enhancing the rate of reduction of oxygen by the enzyme. The evidence from our laboratory indicates that these agents are not measurably biotransformed by the stearate desaturase enzyme system, but stimulate the uncoupling of this system, in particular the transfer of reducing equivalents directly to oxygen to produce H_2O_2.[21]

$$NAD(P)H + \tfrac{1}{2}O_2 \xrightarrow{\text{stearate desaturase}} NAD(P)^+ + H_2O_2$$

These observations raise a number of points which motivate current research in our laboratories. First, could any deleterious effects arise *in vivo* from the active oxygen species produced? Free radicals are known to cause cellular damage and to produce certain types of tumours. Therefore, is it not possible that the anaesthetic agents could cause deleterious effects without undergoing biotransformation, by a process such as the stimulation of the uncoupling of an enzyme system? Finally, should not these possibilities be considered before a new drug is released for human use?

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HALOTHANE: INHIBITION AND ACTIVATION OF RAT HEPATIC GLUTATHIONE *S*-TRANSFERASES

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Abstract—Multiple halothane anesthesias (1.25 MAC for 1 hr on 3 alternate days) of male Long–Evans rats initially decreased by up to 30% and subsequently increased to up to 185% liver cytosolic glutathione *S*-transferase activity toward 1-chloro-2,4-dinitrobenzene, 3,4-dichloro-1-nitrobenzene and *trans*-4-phenyl-3-buten-2-one and glutathione peroxidase activity. Halothane rapidly and reversibly activated hepatic cytosolic glutathione *S*-transferases and purified isoenzyme 1-2 but not isoenzymes 1-1 and 3-3. At high concentrations of halothane (*ca.* 22 mM), maximal activation was *ca.* 25%. Halothane, enflurane, isoflurane and methoxyflurane, but not the halothane metabolite 1-chloro-2,2-difluoroethylene, inhibited a mixture of liver cytosolic glutathione *S*-transferases with time (*ca.* 30% inhibition/15 min). The inhibition exhibited pseudo-first order kinetics ($k_{obs} = 0.13\ min^{-1}$) and an I_{50} for halothane of ≥15 mM. Halothane inhibited glutathione *S*-transferases 3-3, 3-4, and 4-4 by 50–60%, but did not affect isoenzymes 1-1 and 1-2. The ability of halothane to diminish hepatic glutatione *S*-transferase activity *in vivo* may in part reflect the time-dependent inhibition of glutathione *S*-transferase isoenzymes containing the 3- and 4-subunits.

The GSH* *S*-transferases (EC 2.5.1.18) are a family of ubiquitous, multi-functional proteins found primarily in mammalian liver. The GSH *S*-transferases catalyze the conjugation of a wide range of hydrophobic electrophiles with GSH. CDNB is a universal substrate for all GSH *S*-transferase isoenzymes whereas the activity of isoenzymes toward other substrates is more limited. In rat liver, GSH *S*-transferase activity toward DCNB and BSP is characteristic of the 3-subunit, while activity toward *trans*-4-phenyl-3-buten-2-one is limited to the 4-subunit [1]. Several isoenzymes also function as non-selenium-dependent GSH peroxidases [1, 2]. The GSH *S*-transferases can bind covalently to strong electrophiles and non-covalently to a number of non-substrate ligands, such as BSP and bilirubin [1, 3, 4]. The cytosolic GSH *S*-transferases are dimers, with rat liver containing the 1-1, 1-2, 2-2, 3-3, 3-4, 4-4 and 5-5 isoenzymes [1].

Halothane ($CF_3CHBrCl$) is a widely used volatile anesthetic agent. The oxidative and/or reductive metabolism of halothane by cytochrome P-450 may mediate the hepatic damage occasionally observed following halothane anesthesia [5, 6].

Neither halothane nor the majority of its metabolites appear to be metabolized by the GSH *S*-transferases; one exception is the reductive metabolite 1-chloro-2,2-difluorothylene which may undergo GSH conjugation *in vivo* [7, 8]. However, repeated exposure of rats to halothane vapor reduced the clearance of BSP by isolated perfused liver, GSH dependent conjugation of BSP in liver homogenates, and cytosolic GSH *S*-transferase activity toward CDNB [9, 10]. The structural analogue 1,2-dibromoethane also diminished hepatic GSH *S*-transferase activity *in vivo* perhaps in part via the time-dependent, irreversible inhibition of selected GSH *S*-transferase isoenzymes [11, 12].

The effects of halothane anesthesia of rats on hepatic GSH *S*-transferase activities *in vivo* are reported, as are reversible and irreversible interactions of halothane with rat hepatic GSH *S*-transferases *in vitro*.

EXPERIMENTAL

Materials and animals. Materials were obtained as follows: halothane, Maybaker, R.S.A.; isoflurane, enflurane and methoxyflurane, Abbott Laboratories, Kent, U.K.; GSH, Sigma Chemicals, St Louis, MO; *trans*-4-phenyl-3-buten-2-one, Aldrich Chemicals, Gillingham, Dorset, U.K.; cumene hydroperoxide (70% in cumene), Fluka AG, Buchs, Switzerland; bovine serum albumin, Miles Research Products, Cape Town, R.S.A. 1-Chloro-2,2-difluoroethylene was from PCR Research Chemicals, Gainsville, FL.

All experiments utilized male Long–Evans rats (200 ± 10 g) or preparations derived therefrom. Hepatic cytosol was prepared by differential centrifugation, the mixture of rat liver cytosolic GSH *S*-transferases essentially free of other proteins was isolated by *S*-hexyl GSH affinity chromatography, and rat liver GSH *S*-transferase isoenzymes were purified by chromotofocusing and characterized, as described earlier [12].

Halothane anesthesia. Rats (groups of 9–18) were anesthetized with halothane (1.25 MAC in medical air) or exposed to medical air alone, once for 3 hr or thrice for 1 hr on three alternate days, in a manner previously detailed [13]. Rats were fasted for 24 hr

* Abbreviations used: CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 3,4-dichloro-1-nitrobenzene; GSH, reduced glutathione; BSP, bromsulfophthalein.

prior to each exposure to halothane or medical air. GSH *S*-transferase and GSH peroxidase activities were measured with liver cytosol from anesthetized or control rats.

Assays. Protein concentration was determined by the method of Lowry *et al.* [14] as modified by Chaykin [15] using bovine serum albumin as standard. The purified isoenzymes were precipitated with ammonium sulfate (90% saturation), centrifuged at 2000 *g* for 10 min, and redissolved in water prior to assay.

Initial rates of GSH *S*-transferase activity toward CDNB, DCNB and *trans*-4-phenyl-3-buten-2-one and of GSH peroxidase toward cumene hydroperoxide activity were measured spectrally for one minute at 25° [16–19] on a Beckman 5230 or Unicam SP1800 spectrophotometer. All other experimental details are as given earlier [12, 13].

Metabolism of halothane by GSH S-transferases. The metabolism of halothane by hepatic cytosolic GSH *S*-transferases was assessed by GSH depletion in reaction mixtures containing an ethanolic solution of halothane or 1,2-dibromoethane (20 mM) vortex mixed into Tris–HCl (35 mM), pH 8.2, dialyzed cytosol* (0.5 unit/ml) and GSH (0.2 mM). Incubations were at 25° with shaking at 60 cycles per min. At the end of the incubation period, 50 μl of the reaction mixture was diluted with 10 ml of 10 mM HCl. Two ml thereof was lyophilized, reconstituted with 2 ml of 10 mM HCl, and heated at 60° for 30 min. GSH concentration was analyzed spectrophotometrically with Ellman's reagent by the method of Brehe and Burch [20].

Reversible activation of GSH S-transferases by halothane. Halothane (50 μl of an 0.6–1.3 M solution in ethanol, initial concentration) or ethanol (50 μl) was vortex mixed into 2.60 to 2.85 ml of phosphate buffer, pH 6.5 (0.1 M). GSH (0.13–1.45 mM) and CDNB (0.028–0.27 mM) were added. The reaction was initiated with the addition of 50 μl of enzyme preparation (*ca.* 0.5 unit/ml).

Time-dependent inhibition of the GSH S-transferases. The effect of xenobiotics on the activity of rat liver GSH *S*-transferases *in vitro* was assessed as a function of time in reaction mixtures (total volume 500 μl) constituted as follows: Forty μl of an ethanolic solution of the anesthetic (5–40 mM) or haloethylene (16–57 mM) or an equivalent volume of ethanol was vortex mixed into Tris–HCl buffer (35 mM), pH 8.2, followed by the addition of EDTA (4 mM) and GSH *S*-transferase preparation (normally *ca.* 0.3 unit/ml). Reaction mixtures were incubated at 25° with shaking at 60 cycles per min. The reaction was terminated by chilling to 0°. GSH *S*-transferase activity toward CDNB was assessed with reaction mixtures diluted 60 fold in phosphate buffer (0.1 M), pH 6.5. Slight losses of activity (viz. 10 ± 5%) were noted on incubation of enzyme in buffer for 15 min. Ethanol (3.3%) was without effect on enzyme activity.

Statistical analysis and calculations. Results are given as means ± SD. For *in vivo* studies, N represents the number of determinations each on the pooled cytosols from control or anesthetized rats. Reported N values for *in vitro* experiments asse[illegible] GSH *S*-transferase activity as a function of [illegible] reflect the number of determinations each at [illegible] time or following 15 min incubation. For kinetic [illegible]ies of GSH *S*-transferase activity in the presence or absence of halothane, curves were generated by non-linear regression analysis of the Henri–Michaelis–Menten equation. The apparent K_m and V_{max} values generated by non-linear regression analysis for the rat hepatic cytosolic GSH *S*-transferases and purified isoenzymes in the absence of halothane were consistent with previously reported values [3]. Statistical analyses were performed using Student's *t*-test. A significant difference between means was taken as $P < 0.01$.

* Dialyzed versus 10 mM Tris–HCl, pH 7.8, for 16 hr at 4°.

RESULTS

Effect of halothane anesthesia on [illegible]tic GSH S-transferase activity in vivo

A single halothane anesthesia (1 [illegible] for 3 hr) did not significantly affect the sp[illegible]ctivity of hepatic cytosolic GSH *S*-transferas[illegible]d CDNB at 1, 5, 10, 15, 25 or 30 days after anes[illegible] (data not shown). In contrast, repeated halotha[illegible] anesthesia initially decreased and subsequently increased the specific activity of rat hepatic cytosolic GSH *S*-transferase and of GSH peroxidase (Table 1). The specific activity of hepatic GSH *S*-transferase toward CDNB and DCNB and GSH peroxidase toward cumene hydroperoxide reached a nadir on day 10 and a maximum on day 25 or 30 (Table 1). The specific activity of GSH *S*-transferase toward *trans*-4-phenyl-3-buten-2-one was not strikingly affected by multiple halothane anesthesias. The effect of multiple halothane anesthesias on the GSH *S*-transferase activities per gram of wet weight liver showed patterns similar to the specific activities per mg protein, except for *trans*-4-phenyl-3-buten-2-one (Table 1).

Halothane metabolism by GSH S-transferases in vitro

Halothane was not measurably metabolized (≤0.2 nmol/mg protein/min) by hepatic cytosolic GSH *S*-transferases. Under identical reaction conditions, 1,2-dibromoethane exhibited significant GSH *S*-transferase dependent conjugation (3.8 nmol/min/mg protein).

Reversible activation of hepatic GSH S-transferase by halothane in vitro

Halothane produced a rapid activation of moderate magnitude of certain GSH *S*-transferase preparations under standard assay conditions (1 mM CDNB, 1 mM GSH). The activation was apparent with liver cytosolic GSH *S*-transferases and one pure isoenzyme, and was complete before spectral tracings were begun (i.e. $t_{\frac{1}{2}} \leq 5$ sec). The rapid activation of the cytosolic GSH *S*-transferases by halothane was readily reversible. Dilution of halothane in reaction mixtures from 22.4 mM to 11.2 mM and 7.8 mM, reduced the percentage activity in the presence of halothane relative to controls from 117 ± 1%* to 112 ± 1%* and 104 ± 1%, respectively (N = 4–8).

The activation of the cytosolic GSH *S*-transferases as a function of GSH and CDNB concentration is

Table 1. Effect of multiple halothane anesthesias on hepatic GSH *S*-transferase and GSH peroxidase activity

Assay	Activity per	Percentage activity relative to air exposed controls Time after first halothane anesthesia (days) 5	10	16	20	25	30
CDNB	mg protein	94 ± 3	64 ± 2†	93 ± 3	108 ± 4	153 ± 2†	111 ± 2†
	g liver	94 ± 3	70 ± 2†	89 ± 3*	92 ± 3	114 ± 2†	125 ± 2†
	(N)	(6)	(4)	(6)	(6)	(6)	(6)
DCNB	mg protein	89 ± 2†	66 ± 1†	90 ± 1†	103 ± 2	133 ± 1†	102 ± 2
	g liver	90 ± 2*	72 ± 1†	85 ± 1†	87 ± 2†	99 ± 1	115 ± 3†
	(N)	(4)	(6)	(6)	(6)	(6)	(6)
Trans-4-phenyl-3-buten-2-one	mg protein	95 ± 7	90 ± 1†	115 ± 6*	104 ± 2	109 ± 4*	95 ± 3*
	g liver	95 ± 7	98 ± 2	110 ± 6	88 ± 1†	82 ± 4†	107 ± 3*
	(N)	(6)	(6)	(6)	(8)	(7)	(8)
Cumene hydroperoxide	mg protein	101 ± 3	84 ± 2†	99 ± 1	122 ± 3†	185 ± 7†	107 ± 1†
	g liver	102 ± 3	92 ± 3*	95 ± 1*	103 ± 3	138 ± 5†	121 ± 1†
	(N)	(6)	(6)	(6)	(6)	(6)	(6)

Groups of 9–15 rats were exposed to 1.25 MAC halothane or medical air for 1 hr on days 1, 3 and 5. Results are from one experiment, with each time point representing n determinations each on the pooled cytosols from either 3 contol or 3 anesthetised rats. Similar results for days 5, 10 and 16 were obtained in a separate experiment. Specific activities for control rats in μmol/mg protein/min were as follows: CDNB, 1.0; DCNB, 0.055; *trans*-4-phenyl-3-buten-2-one, 0.013; cumene hydroperoxide, 0.17.

* Differs significantly from corresponding control, $P < 0.01$.

† Differs significantly from corresponding control, $P < 0.001$.

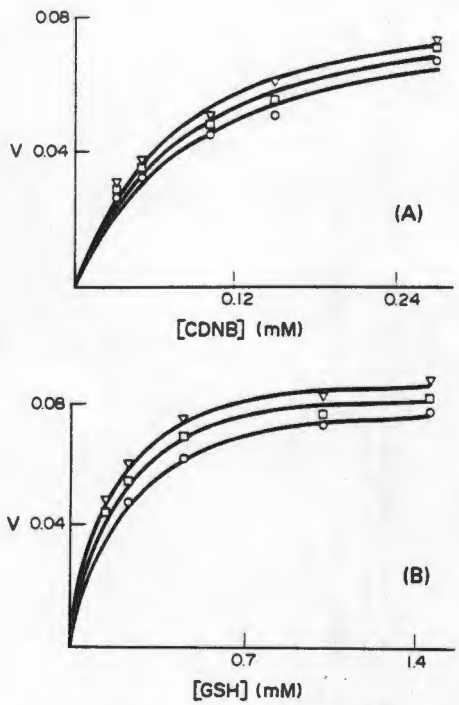

Fig. 1. Reversible activation by halothane of cytosolic GSH *S*-transferases as a function of the concentration of (A) CDNB and (B) GSH. v is given as the change in absorbance at 340 nm per min. For (A), GSH concentration was 1 mM; for (B), CDNB concentration was 0.27 mM. ○, ethanol; □, 11.2 mM halothane; ▽, 22.4 mM halothane.

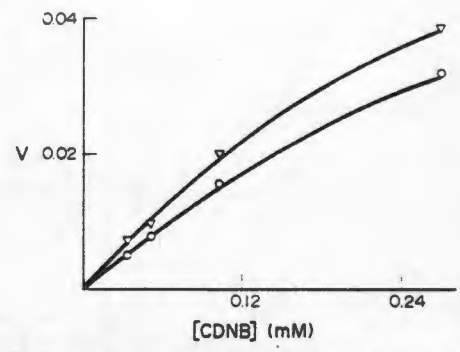

Fig. 2. Reversible activation by halothane of GSH *S*-transferase 1-2. v is given as the change in absorbance at 340 nm per min. The concentration of GSH was 1 mM. ○, Ethanol; ▽, 22.4 mM halothane.

shown in Fig. 1. Halothane significantly activated purified isoenzyme 1-2 (Fig. 2), but did not produce significant, reproducible activation of isoenzymes 1-1 and 3-3 as a function of CDNB concentration (data not shown).

Time-dependent inhibition of hepatic GSH S-*transferases* in vitro

Four volatile anesthetic agents produced a significant, time-dependent inhibition of the activity toward CDNB of the mixture of rat hepatic GSH *S*-transferases in the absence of GSH (Table 2). The metabolite of halothane 1-chloro-2,2-difluoroethylene (16 mM–57 mM), did not produce significant, dose-dependent inhibition of the mixture of rat liver isoenzymes with time; percent activities remaining at 15 min varied from 80 to 104% relative to controls (data not shown).

The inhibition of the partially purified mixture of rat liver cytosolic GSH *S*-transferase isoenzymes by halothane in the absence of GSH followed pseudo-first order kinetics (Fig. 3). The inhibition was characterized by a pseudo-first order rate constant of 0.13 min^{-1} (half-life of 5.4 min). Half-maximal inhibition of the activity of the mixture of isoenzymes occurred at concentrations of halothane greater than or equal to 15 mM (Fig. 4).† The addition of albumin (400 mg/ml) or liver cytosol (0.04 mg protein/ml)‡ to incubations containing halothane and the mixture of GSH *S*-transferase isoenzymes eliminated the halothane dependent inhibition (data not shown). With rat liver cytosol as the source of GSH *S*-transferase activity, halothane failed to produce statistically significant time-dependent inhibition. Neither the *in situ* perfusion of the rat liver to blanching with ice cold 10 mM Tris–HCl, pH 7.8, prior to the isolation of cytosol, nor the dialysis of the liver cytosol against the same buffer for 1 to 24 hr at 4° subsequent to isolation altered this result (data not shown).

Halothane produced significant time-dependent inhibition of purified rat liver GSH *S*-transferase isoenzymes 3-3, 3-4 and 4-4 but not of isoenzymes 1-1 and 1-2 as a function of time (Table 3).

DISCUSSION

Multiple halothane anesthesias decreased GSH *S*-transferase activity as reported earlier [9], and produced a previously unreported, subsequent rebound in activity (Table 1). The rebound of GSH *S*-transferase activity may be analogous to the superinduction phenomenon seen following suicide inhibition [21, 22] or may represent enzyme activation. In our study and elsewhere [9], a single halothane anesthesia did not affect the binding or catalytic function of the hepatic GSH *S*-transferases.

Information on the hepatic GSH *S*-transferase isoenzymes affected by multiple halothane anesthesias is available from a comparison of the data in Table 1 with the substrate specificities of GSH *S*-transferase isoenzymes. The loss of DCNB activity indicates that isoenzymes containing the 3-subunit were affected by halothane. Consistent with this observation, multiple halothane anesthesias have been reported to decrease hepatic BSP metabolism [9], a process which is characteristic of GSH *S*-transferases containing the 3-subunit [1].

The slight loss of *trans*-4-phenyl-3-buten-2-one activity following multiple halothane anesthesias

* Differs significantly from activity in the absence of halothane, $P < 0.001$.

† These assays were not performed under conditions where the loss of enzyme activity was directly proportional to time (Fig. 3).

‡ This level of cytosolic protein contributed less than 10% of the total GSH *S*-transferase activity in the incubation mixture.

Table 2. The time-dependent inhibition of GSH *S*-transferases by anesthetic agents *in vitro*

Anesthetic (mM)	% CDNB Activity after 15 min (N)	
	−Anesthetic	+Anesthetic
Halothane (40)	91 ± 5 (18)	70 ± 1 (18)*
Isoflurane (40)	93 ± 7 (12)	76 ± 4 (12)*
Enflurane (40)	93 ± 7 (12)	70 ± 10 (12)*
Methoxyflurane (40)	93 ± 7 (12)	65 ± 5 (12)*

Incubation mixtures contained the mixture of rat liver GSH *S*-transferases (*ca.* 0.3 Unit/ml), and an ethanolic solution of anesthetic or ethanol in 35 mM Tris–HCl, pH 8.2, at 25°. Percentage activity is relative to zero time samples constituted exactly as were those incubated for 15 min.

* Differs significantly from value for ethanol, $P < 0.001$.

(Table 1), suggests that GSH *S*-transferases containing the 4-subunit were affected, but to a lesser extent than isoenzymes containing the 3-subunit. The loss of cumene hydroperoxide activity following multiple halothane anesthesias may indicate decreased activity of the 1-, 2- or 5-subunit containing GSH *S*-transferase and/or decreased selenium-dependent GSH peroxidase activity.

Halothane does not appear to be a GSH *S*-transferase substrate: The anesthetic was not appreciably metabolized by hepatic cytosol plus GSH (see Results) while the positive control, 1,2-dibromoethane, was [23]. However, halothane bound to one or more hepatic GSH *S*-transferases as evidenced by the rapid, reversible activation of enzyme preparations by this drug. Halothane produced a net activation of hepatic cytosolic GSH *S*-transferases and purified isoenzyme 1-2, but not isoenzymes 1-1 and 3-3 (Figs 1 and 2 and Results). The physiological relevance of the reversible activation of hepatic GSH *S*-transferases by halothane appears questionable since the process was readily reversible, and exhibited low extents of activation (≤125% of controls) even at concentrations of halothane 20-fold greater than the blood level of halothane (*ca.* 1 mM) required for anesthesia [24].

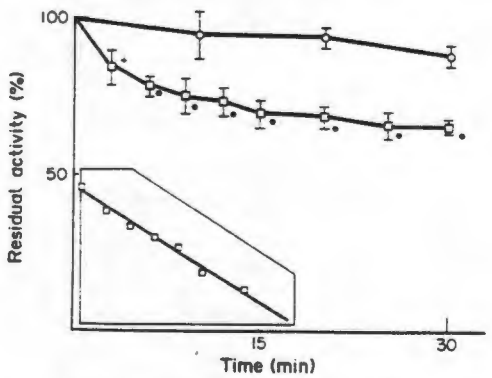

Fig. 3. Time-dependence of halothane inhibition of GSH *S*-transferase activity. Inset: Ln $(A_t - A_\infty)$ vs time. The mixture of rat liver isoenzymes (0.44 units/ml) was incubated at 25° with EDTA (4 mM), Tris–HCl (40 mM), pH 8.2, in the presence (□) and absence (○) of halothane (40 mM). Differs from zero time sample, * $P < 0.01$, † $P < 0.05$.

The time-dependent inhibition of GSH *S*-transferases by halothane *in vitro* occurred on the minute time scale, and exhibited an I_{50} approximately one order of magnitude greater than halothane blood levels [24] (Figs. 3 and 4). The reaction exhibited pseudo first-order kinetics and incomplete inhibition of purified isoenzymes at infinity (Table 3). The incomplete inhibition may reflect that only half the sites are reactive, incomplete reaction, or competing pathways (partitioning) [25]. It appears that the halogenated anesthetics isoflurane, enflurane and methoxyflurane inhibit the GSH *S*-transferases in a manner similar to halothane (Table 2), although the effects of these anesthetic agents were not studied further.

The isoenzyme specificity of halothane inhibition of the GSH *S*-transferases *in vitro* correlated with the effects of halothane anesthesias *in vivo*. Notably, the 3- and 4-subunit containing isoenzymes were affected in both systems. Although both forms appeared to be equally susceptible to inhibition *in vitro*, the activity associated with the 3-subunit was inhibited more effectively by halothane *in vivo* (Tables 1 and 3). A direct correlation of magnitudes of halothane inhibition of GSH *S*-transferase activity *in vitro* and *in vivo* is not possible since GSH, protein and possibly other high molecular weight cytosolic components protected against halothane inhibition *in vitro* (see Results), and the magnitude of these potential effects *in vivo* is unknown.

Table 3. Time-dependent inhibition of GSH *S*-transferase isoenzymes by halothane

Isoenzyme	% CDNB activity after 15 min	
	+Ethanol	+Halothane
1-1	N.D.	94 ± 3
1-2	95 ± 3	89 ± 6
3-3	109 ± 3	43 ± 3*
3-4	85 ± 4	62 ± 4*
4-4	80 ± 1	45 ± 12*

Incubation mixtures contained pure isoenzymes (*ca.* 0.3 Units/ml), halothane (40 mM) or ethanol in 35 mM Tris–HCl, pH 8.2 at 25° and 10 mM GSH. (N = 3–4 for each analysis). N.D. Not determined.

* Differs significantly from activity in the presence of ethanol, $P < 0.01$.

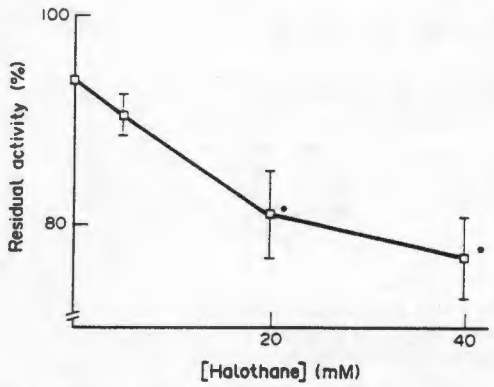

Fig. 4. Effect of halothane concentration on the time-dependent inhibition of GSH *S*-transferase activity. The mixture of rat liver isoenzymes (2.0 units/ml) was incubated with EDTA (4 mM), GSH (10 mM), Tris–HCl (40 mM) and variable concentrations of halothane at 25° for 15 min. Residual activity is given as percentage of the zero time activity of corresponding samples. * Differs significantly from controls, $P < 0.01$.

Several xenobiotics have been shown to inhibit analogous hepatic GSH *S*-transferase isoenzymes *in vitro* and *in vivo*. 1,2-Dibromoethane inhibited isoenzymes 3-3 and 3-4 *in vitro* and *in vivo* [11, 12], and chloroform diminished the activity of isoenzymes 1-2, 2-2, 3-3 and 3-4, but not 4-4 and 5-5 *in vitro* and *in vivo* [26].

The correlation between the selectivity of inhibition of GSH *S*-transferase isoenzymes by halothane and other xenobiotics *in vitro* and *in vivo* suggests that GSH *S*-transferase inhibition may provide one mechanism for the loss of transferase activity *in vivo*. An alternate mechanism may involve the release of hepatic GSH *S*-transferase activity into the serum which is stimulated by several xenobiotics [11, 26, 27]. The effect of halothane on serum GSH *S*-transferase activity was not investigated.

Since halothane did not require metabolic activation *in vitro* to produce time-dependent inhibition, the capability for transferase inactivation appears to reside in the parent molecule, in contrast to chloroform which requires metabolic activation by cytochrome P-450 to inhibit GSH *S*-transferases [26]. This proposal is supported by the relative ineffectiveness of 1-chloro-2,2-difluoroethylene to produce time-dependent inhibition of GSH *S*-transferase activity *in vitro* (see Results). This cytochrome P-450 metabolite of halothane is the only halothane metabolite thought to undergo GSH conjugation *in vivo* [7, 8]. The relative unreactivity of the haloethylene compared to the parent molecule is consistent with the reactivity toward nucleophilic addition to protein side chains. Identical order of reactivity was found for 1,1,2-trichloroethane versus trichloroethylene (unpublished results).

It is concluded that halothane rapidly and reversibly activated one or more GSH *S*-transferase isoenzymes, and by a different mechanism produced a slow inhibition of the 3- and 4-subunit containing GSH *S*-transferase isoenzymes *in vitro*. The latter effect may underlie the loss of hepatic GSH *S*-transferase activity *in vivo* following halothane anesthesias.

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Halothane Decreases Albumin and Transferrin Synthesis: Studies in the Isolated, Perfused Rat Liver and in the Intact Rat

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Isolated perfused rat livers exposed to 1.5% halothane (equivalent to 1.35 MAC) in O_2/CO_2 or to O_2/CO_2 alone produced urea, as well as albumin and transferrin (both measured by immunodiffusion), at constant rates during a 4.25-h perfusion. Urea production did not differ in the two treatment groups, but halothane depressed albumin and transferrin synthesis 43% and 45%, respectively. Intact rats were also exposed to halothane, after which albumin synthesis was measured by the (^{14}C)carbonate technique. The minimum halothane concentration required to insure sufficient relaxation for ventilation was selected and ranged from 1.0 to 1.5%. Measurements were made in control rats not exposed to halothane (group I) and in halothane exposed rats immediately after 1 h of anesthesia (group II), 24 h after the start of 1 h of anesthesia (group III), and immediately after ½ h of anesthesia preceded by a 1-h exposure 24 h earlier (group IV). Single exposures to halothane (groups II and III) resulted in a decrease in albumin synthesis immediately or 24 h later that did not differ significantly from controls (group I). However, halothane given twice to rats at 24-h intervals (group IV) reduced their mean albumin synthesis rate to half that of controls. The early onset and constancy of halothane depression of export protein synthesis by isolated, perfused livers may reflect a response to halothane itself, rather than an effect resulting from the accumulation of halothane metabolites. Similarly, reduction of albumin synthesis in intact rats immediately after a second halothane exposure may indicate a response to halothane, rather than to halothane metabolites. (Key words: Anesthetics, volatile: halothane. Liver: albumin synthesis; transferrin synthesis; urea production. Toxicity: halothane.)

HEPATIC DYSFUNCTION of varying degree has been attributed to exposure to most volatile anesthetics, especially halothane,[1–4] but the only abnormality reported to occur regularly after halothane anesthesia is delayed BSP excretion.[5] Histologic and functional derangements can be produced by hormonal and/or pharmacologic manipulation of intact experimental animals anesthetized with halothane,[6–8] and changes in carbohydrate and protein metabolism in perfused rat livers[9–11] and in isolated rat hepatocytes[12] have been observed when these preparations were exposed to halothane.

One major function of the liver is synthesis of proteins for use in the liver itself or elsewhere in the organism. We have, therefore, examined the effect of a clinically relevant level of halothane (equivalent to 1.35 MAC) on albumin and transferrin synthesis by the isolated, perfused rat liver. We also examined the effect of halothane on the synthesis of albumin in the intact rat.

Materials and Methods

ANIMALS

Animal use was approved by the Ethics and Research Committee of the Faculty of Medicine of the University of Cape Town. Male Black-hooded rats of the Long Evans strain weighing 296–305 g, housed under controlled conditions of temperature and humidity and allowed free access to food and water at all times, were used as liver and red cell donors. Male rabbits of the New Zealand White strain, maintained under the same conditions, were used as plasma donors. Rats used for (^{14}C)carbonate studies were, in addition, given drinking water containing 0.008M NaI for at least 24 h before injection. Animals in this group weighed between 275 and 325 g.

LIVER PERFUSIONS

Considerations and techniques for perfusing isolated rat livers have been reviewed recently.[13] For our experiments, livers were perfused[14–16] with a heterologous mixture[17] consisting of heparinized rabbit plasma and rat erythrocytes washed twice with 0.9% saline. Perfusate volumes ranged from 113 to 144 ml, with hematocrits of 25–29%. The perfusate was exposed to O_2/CO_2 (95/5) by circulation in a warmed (37° C), humidified perfusion cabinet for 0.5 h before insertion of the liver and throughout a 4.25-h period of liver perfusion. Before placement in the perfusion chamber, the liver was flushed with isotonic crystalloid (Plasmalyte A, Tra-

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TABLE 1. Schematic of Protocol

Group	Time of Halothane Exposure		Time of Measurement of Albumin Synthesis
	First (1.0 h)	Second (0.5 h)	
I	—	—	0 h
II	0 h	—	1 h
III	0 h	—	24 h
IV	0 h	24 h	24.5 h

venol, Deerfield, IL) for 2 min at a pressure of no more than 15 cm of H_2O. The perfusion mixture was then pumped through the portal vein in an antegrade direction, with pressure maintained at 12–13 cm of H_2O. In control perfusions, plasma donor rabbits and red cell and liver donor rats were anesthetized briefly with ether in O_2/CO_2, whereas, in halothane experiments, 1.5%, *i.e.*, 1.35 MAC[18] halothane, was used. In addition, in halothane experiments, the perfusate was exposed to 1.5% halothane in O_2/CO_2 throughout the 0.5-h preparatory and 4.25-h study periods. Perfusate flow and bile production were monitored continuously. Perfusate blood gases were sampled distal to the liver in the perfusate circuit, *i.e.*, on the "venous" side, at 0.5-h intervals. p_{O_2} was maintained at >90 mmHg, p_{CO_2} between 34 and 38 mmHg, and *p*H between 7.35 and 7.44, the last by addition, when needed, of 1.0–3.0 ml of 0.5M $NaHCO_3$. Perfusate hematocrit was measured before and at the end of liver perfusion, whereas samples for the measurement of albumin, transferrin, and urea[19] were taken before insertion of the liver and 0.25, 0.75, 1.25, 1.75, 2.25, 3.25, and 4.25 h after the start of hepatic perfusion. Estimates of total albumin, transferrin, and urea production were adjusted for loss due to sampling. The concentrations of albumin and transferrin produced by the liver were measured by radial immunodiffusion.[20] The antisera used (Cooper Diagnostics, Malvern, PA) reacted with rat but not rabbit albumin or transferrin. Urea production and albumin and transferrin synthesis rates were estimated by linear regression[21] of accumulated values, with rates taken as equal to the slopes of the fitted lines. Rates for the 4.25-h perfusion period were expressed as $mg \cdot h^{-1}$ per 300 g rat. Results in the control and halothane treated groups were compared by Student's *t* test.

INTACT RATS

Many of the methods have been described before.[22–24] Usually, two rats were brought from the animal quarters each experimental day at 0700. Table 1 shows a schematic of the experimental protocol, indicating each treatment group, the hour that the rats in each group were anesthetized, and the hour that albumin synthesis was measured in rats in each treatment group. The control animal (group I) was lightly anesthetized with diethyl ether in O_2/CO_2 (95/5) and injected intravenously at the base of the tail with a mixture of 200 μCi of sodium (^{14}C) carbonate[25] and 10 μCi of ^{125}I-albumin. Ether anesthesia was stopped after this injection, with total ether exposure time lasting less than 5 min. The rat was returned to a cage with free access to food and water. Blood samples were collected from the tip of the tail at 0.25, 4, 4.5, 5, and 5.5 h after injection. At 6 h, the rat was anesthetized with ether and bled by cardiac puncture. The experimental rat (group II, III, or IV) was anesthetized with halothane in warmed, humidified air/oxygen ($F_{I_{O_2}}$ = 0.4). Body temperature was maintained with the aid of a heat lamp. EKG monitoring revealed no detectable changes or abnormalities during the anesthetic period. The oxygen/air mixture was obtained from compressed gas sources and delivered *via* a rotameter bank to a halothane plenum vaporizer. The anesthetic mixture was delivered at 4 $l \cdot min^{-1}$ *via* a T-piece circuit to a Tygon cylinder (d = 2.5 cm, h = 5 cm) stoppered at one end. The gases entered through a lateral opening in the cylinder and exited through an opening at the stoppered (distal) end. With the rat in a prone (physiologic) position, the cylinder fit snugly over the muzzle and, when held in place with both hands, thumbs thrusting the mandible down and forward, delivered, under careful observation, an adequate tidal volume at 55–60 b/min. Intermittent positive pressure in the system was developed by occluding gas outflow with a solenoid valve. Occlusion was carried out manually by an assistant or with a foot switch. Between 1.0 and 1.5% halothane was used; the minimum concentration necessary to insure sufficient relaxation for ventilation was selected. (Ventilation was assessed in a pilot experiment in three animals anesthetized with halothane, but otherwise excluded from the study. Thirty and 60 min after the start of anesthesia, femoral arterial blood samples revealed *p*H, Pa_{O_2}, and Pa_{CO_2} in the normal range.) After 1 h of halothane anesthesia, the experimental rat was injected and samples taken (group II) as described for the control rat, with cessation of anesthesia after isotope injection. Alternatively, the experimental rat was anesthetized with halothane for 1 h without injection and sampling, and returned to a cage with access to food and water. Twenty-three hours later, the animal was either anesthetized briefly with ether (group III) and otherwise treated like groups I and II, or anesthetized for 0.5 h with halothane (group IV) and otherwise treated like groups I and II. In all rats, ^{125}I-albumin activities were measured in the 0.25- and 6-h plasma samples for determination of plasma volume and to provide a correction factor for loss of labeled plasma albumin by catabolism and transfer to interstitial fluids. Urea carbon specific activity was measured in the 4–5.5-h samples. The values for each animal were plotted against time, fitted by a single

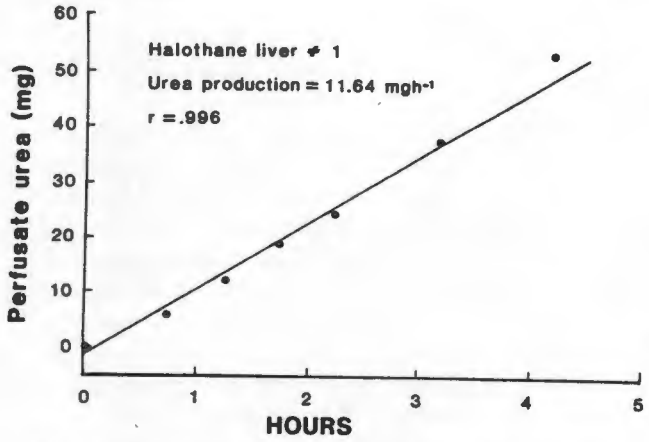

FIG. 1. Urea production by an isolated perfused rat liver exposed to 1.5% (equivalent to 1.35 MAC) halothane. Each data point represents the total amount of perfusate urea less the pre-perfusion value. Urea production rate is derived from the slope of the regression line and expressed per 300 g rat.

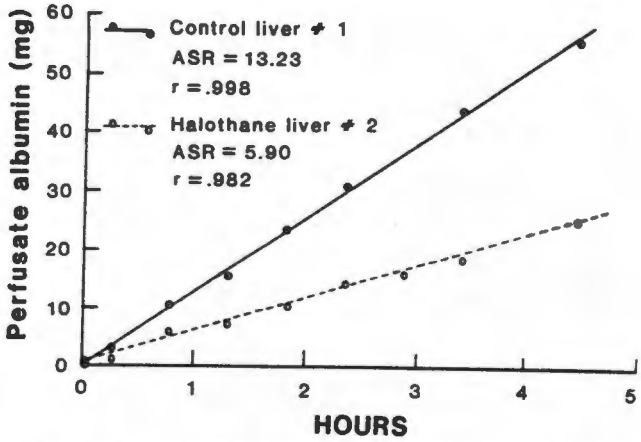

FIG. 2. Albumin synthesis rates per 300 g rat (ASR) by isolated perfused rat livers with and without exposure to 1.5% halothane. Data points and synthesis rates were derived as described in figure 1.

exponential function, and corrected by extrapolation to zero time (t_0). Albumin and urea concentrations and arginine-6-carbon specific activity in albumin were measured in the 6-h sample,[24] and the latter was corrected to t_0 by multiplying by the albumin loss-correction factor. From these data, the albumin synthesis rate at t_0, in $mg \cdot h^{-1}$ per 300 g rat, was calculated for each animal.[25]

Control and treatment groups were compared by one-way analysis of variance, and by the Student-Newman-Keuls multiple comparison procedure.[21] Statistical significance was inferred if $P < 0.05$.

Results

Perfusion Studies

Perfusate flow, in $ml \cdot m^{-1}$ per g liver, ranged from 1.7 to 3.9 in control livers and from 1.6 to 3.5 in halothane-treated livers. Bile flow, in $\mu l \cdot m^{-1}$ per g liver, ranged from 1.5 to 1.9 in control livers and from 1.1 to 2.2 in halothane-treated livers. Halothane perfusate and bile flow rates did not differ significantly from corresponding control values.

Urea production (mean ± SEM) was 11.36 ± 1.17 and 13.26 ± 1.01 $mg \cdot h^{-1}$ per 300 g rat in control and halothane livers, respectively. These means do not differ significantly. Figure 1 shows a plot of urea production from a typical halothane liver perfusion. A constant rate of urea production over 4.25 h was a characteristic finding in all experiments, with and without halothane exposure.

Figure 2 shows plots of albumin synthesis by representative control and halothane-treated livers. Again, a constant rate of production was found throughout the course of each liver perfusion. Similarly, transferrin was synthesized at a constant rate during each perfusion. However, the overall rate of synthesis of each of these export proteins was significantly depressed by halothane exposure. Table 2 shows the mean and standard error for albumin and transferrin synthesis by isolated, perfused rat livers exposed to O_2/CO_2 (control) and to O_2/CO_2 plus 1.5% halothane for 4.25 h. Halothane reduced albumin synthesis by 43% and transferrin synthesis by 45%. Albumin synthesis in control livers approximated that observed in intact rats as measured by the (^{14}C)carbonate method and by extrapolation from catabolic data.[22-24] The transferrin synthesis rate, measured in rats by isotopic techniques, has been reported to be about one-fifth that of albumin,[26-27] but rates measured in our control liver preparations averaged closer to one-third the albumin synthesis rate.

Intact Rat Studies

Table 3 shows the mean and standard error for the albumin synthesis rate in each treatment group. A single halothane exposure did not significantly decrease albumin production either immediately or 24 h later (groups II and III). However, halothane given twice to rats in a 24-h interval (group IV) reduced their mean

TABLE 2. Effect of Halothane on Albumin and Transferrin Synthesis by the Isolated, Perfused Rat Liver

Group (n)	Protein Synthesis ($mg \cdot hr^{-1}$ per 300 g rat)	
	Albumin	Transferrin
Control (6)	12.42 ± 1.15*	4.35 ± 0.75
Halothane (5)	7.10 ± 0.75†	2.40 ± 0.17‡

* Mean ± SEM.
† Differs from control ($P < 0.01$).
‡ Differs from control ($P < 0.02$).

TABLE 3. Effect of Halothane on Albumin Synthesis in the Intact Rat

Group (n)	Treatment	Time of Measurement*	Synthesis Rate (mgh^{-1})†
I (14)	No halothane	—	14.07 ± 0.76‡
II (6)	Halothane × 1	1 h	11.00 ± 1.29§
III (6)	Halothane × 1	24 h	11.81 ± 1.42§
IV (8)	Halothane × 2	24.5 h	7.07 ± 1.06

* After start of initial 1-h anesthesia.
† Per 300 mg rat.
‡ Differs from group IV, $P < 0.01$.
§ Differs from group IV, $P < 0.05$.

rate of albumin synthesis to half that of controls (group I).

Discussion

Our data from the isolated, perfused rat liver show that 1.5% halothane, equivalent to 1.35 MAC, depressed albumin synthesis and transferrin synthesis 43 and 45%. Nearly equivalent suppression of production of these two proteins may be fortuitous or may reflect action on a common pathway of protein synthesis, though at what level is difficult to say. Aune *et al.*,[12] exposing isolated rat hepatocytes to presumably comparable concentrations (2mM) of halothane, found a 65% reduction in uptake of radiocarbon-labeled valine into medium proteins after 2 h. Flaim *et al.* reported a 40% decrease in the rate of incorporation of radiolabeled leucine into albumin by rat livers perfused *in situ* and exposed to 4%, but not to 2%, halothane.[11] A possible explanation for this discrepancy between perfusion studies may be a difference in methodology. In our study, albumin and transferrin production were measured by radial immunodiffusion in a heterologous perfusate. In this way, we avoided the use of radiolabeled amino acids, a technique which may underestimate albumin synthesis in the perfused liver,[17] and possibly obscure differences. Indeed, albumin synthetic rates obtained by us in control perfusions were equivalent to rates accepted for the intact rat. Other reasons for this close approximation to the *in vivo* rate of synthesis may include careful monitoring of perfusate *p*H and oxygen and carbon dioxide partial pressures and the use of perfusates with hematocrits less than 30%. These measures allow for perfusion over a prolonged period without increases in organ vascular resistance and decreases in bile flow, common monitors of functional integrity of perfused livers.[15] Further evidence for functional integrity of the liver during the perfusion period is provided by the steadily maintained rates of urea, albumin, and transferrin production that were observed. Synthetic rates after more than 4 h of perfusion differed little from those during the first hour. Similarly, Flaim *et al.* noted a steady rate of incorporation of tritiated leucine into secreted albumin during a 2-h perfusion.[11] These observations may be particularly noteworthy in halothane-treated livers, because they indicate an early effect that is not cumulative.

Our measurements in intact rats by the (^{14}C)carbonate technique[25] confirmed the inhibition of albumin synthesis by halothane, but it is interesting that two exposures were required to produce a significant effect in this experimental setting. The (^{14}C)carbonate method is based on the concept that "flash" labeling of hepatic protein takes place *via* arginine-6-carbon as albumin is being assembled within the hepatocyte.[25] Incorporation of (^{14}C)carbon occurs during the first few minutes after the injection of sodium (^{14}C)carbonate, which first enters the arginine-ornithine cycle, labeling both urea and the guanidine (six) carbon of arginine. Albumin synthesis is thus measured "instantaneously" and does not reflect an average estimate over a period of hours. This feature of the method is important in evaluating the effect of halothane exposure, because estimates of the rate of synthesis of export proteins are little affected by changes in concentration of halothane or halothane metabolites that occur after isotope injection, while the ^{14}C-urea slope is being determined.

Instantaneous measurement in intact animals may help resolve the issue of whether decreased albumin synthesis is due to halothane metabolite accumulation, and it may allow some speculation regarding the double exposure phenomenon. While fluorinated hydrocarbons may be retained to a significant degree for more than 24 h following a single 1-h halothane anesthetic[28] (though this observation has been questioned[29]), albumin synthesis was not significantly depressed 24 h later (group III). Yet, a substantial (nearly 50%) decrease in the rate of synthesis was observed after two exposures to halothane (group IV). It seems unlikely that a second exposure lasting only 30 min could generate enough halothane derivative(s) to play an important role in reducing albumin synthesis, as measured at the end of that 30-min period. A more tenable hypothesis is enhancement by halothane, itself, during a second anesthetic of a previous effect of halothane or possibly of its metabolites on the protein synthesis apparatus. Thus, although a single exposure to halothane was not sufficient in itself to decrease albumin synthesis significantly, this first anesthetic predisposed the synthetic apparatus to marked inhibition immediately following re-exposure. (In fact, a single exposure *may* decrease albumin synthesis; if groups II and III are combined, multiple comparison testing shows their mean of 11.40 mgh^{-1} does differ significantly from the control mean of 14.07, $P < 0.05$).

The mechanism of halothane-induced depression of hepatic export protein synthesis is unknown. Reduced splanchnic blood flow during anesthesia has been proposed as a cause of hepatic dysfunction, but, in our

isolated liver preparations, perfusion flow rates did not differ in control and halothane-treated livers. General metabolic depression by halothane also seems unlikely, since bile flow by the perfused livers was maintained during halothane exposure and since urea production, a measure of the activity of the arginine-ornithine cycle, occurred at similar rates in control and halothane-treated livers, perhaps reflecting the presence of sufficient amounts of fatty acids[30] and amino acids[17] in the perfusate plasma. In addition, careful ventilation of intact animals during anesthesia appears to have ruled out hypoxia and hypercarbia as contributing causes.

By whatever mechanism halothane caused decreased albumin and transferrin synthesis, its effect appeared early and remained constant during more than 4 h of perfusion. Further, albumin synthesis in the intact rat was swiftly decreased by a short, second exposure to halothane. This speed and constancy of effect may reflect a direct or indirect action of halothane, itself, on the protein synthesis apparatus, rather than a response due to halothane metabolites accumulating during the course of perfusion. Whether this effect is limited to halothane or is associated with other potent inhalational agents remains to be determined.

Our finding that exposure to clinically relevant partial pressures of halothane depresses albumin (and transferrin) synthesis supports the view that prolonged or repeated exposure to halothane should be employed cautiously, if at all, in patients with compromised protein metabolism.

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The liver and anaesthesia

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Historically, when considering the effects of anaesthesia on the liver, attention has usually been focused on the possible harmful effects, often unpredictable, of specific anaesthetic agents. Never was this more the case than with halothane when, soon after its introduction, sporadic reports of fulminant post-anaesthetic hepatitis compromised the clinician's confidence in the safety of what seemed to be the most acceptable inhalational anaesthetic agent yet introduced. Today 'the enigma of halothane hepatitis continues to enslave the enlightened and confound the foolish'.[1] The drug and this condition have probably been the subject of more research and consequent literature than has any other single drug. Yet the incidence of post-halothane hepatitis is such that the average clinical anaesthetist may expect to see but one case in a professional lifetime.[2] Because of its potential for causing harm to the otherwise fit patient for reasons unrelated to a surgical illness, concentration of attention on this aspect of the liver and anaesthesia has been, to a large extent, at the expense of that given to the more relevant and common clinical problem of the anaesthetic management of the patient with liver disease. This problem, by contrast, concerns the non-specific effects of anaesthesia and its associated techniques — whatever the drugs used — on the patient's already compromised liver function and the obverse of that coin, the constraints imposed by the patient's deranged liver function on the anaesthetist's ability to control the physiological homeostasis of the patient during the operative and peri-operative period.

It is this latter aspect of the 'liver and anaesthesia' that will form the major burden of this short review.

In exploring briefly the importance for the clinical anaesthetist of the pathophysiological disturbances which accompany liver disease, the subject is treated in a general, all-embracing sense rather than in the more conventional manner of devoting separate discussion to hepatocellular disease/cirrhosis, obstructive jaundice and liver failure.

The patient with parenchymal liver disease may present to the anaesthetist for surgery related either to his disease or to some incidental condition. In either event the problems presented to the anaesthetist are basically the same, varying only in degree. The many features of deranged liver function and the consequent problems posed for the anaesthetist are dynamically interrelated, but in the interests of clarity these will be presented as separate entities.

Liver blood flow (LBF)

A most important determinant of liver function that is greatly affected by all the circumstances of surgical operation, embracing the effects of anaesthetic drugs and techniques together with surgical manipulation, is LBF.

The bulk of the liver's 25% share of the cardiac output reaches it indirectly via the portal vein, only 30% coming directly via the hepatic artery although each source is responsible for 50% of the organ's oxygen flux. Since vasomotor control in the liver itself is minimal, variations in LBF follow changes in the three variables on which its perfusion pressure depends:

- systemic arterial pressure (hepatic artery)
- splanchnic vascular resistance (portal venous pressure)
- central venous pressure (hepatic venous pressure)

From their effects on these three variables, it seems that all the circumstances of anaesthesia and surgery conspire to reduce LBF and the best we anaesthetists can do is seek to lessen the degree by careful clinical management. In the case of the volatile anaesthetic agents in current use in South Africa — halothane and enflurane (Ethrane; Abbott) as well as the soon-to-be-introduced forane — reduction in LBF follows the dose-related fall in systemic arterial pressure.[3]

The anaesthetic-associated technique of intermittent positive pressure ventilation (IPPV) may cause reduction of LBF for mechanical reasons[4] while the associated blood gas complications of hypoxia, hypercarbia or hypocapnia all cause increases in splanchnic vascular resistance from sympathetic stimulation and thus reduction in LBF,[5] an effect that is neutralized by halothane.[4] (The effects of enflurane and forane in these circumstances are not yet documented).

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While all the above conditions are under the control of the anaesthetist, the factor causing the major depression in LBF during surgery and anaesthesia is not. Gelman's[6] demonstration of the depression in LBF during performance of an intra-abdominal operation to less than 50% of that during controlled conditions of general anaesthesia, clearly identified surgical manipulation and trauma as the major determinant of LBF during anaesthesia and surgery.

A concomitant of general anaesthesia is a reduction in tissue oxygen utilization so that the potential for harm of any reduction in organ blood flow and thus oxygen delivery can only be measured when delivery and demand are correlated. Libonati *et al.*[7] have done this by estimating the ratio of change in LBF to change in liver oxygen utilization for various agents and circumstances (Table I). Although their work reports *inter alia* on drugs and techniques no longer in clinical use, it is of interest to see how halothane emerges as the agent to cause least disturbance of the oxygen supply : demand ratio.

While the reductions in LBF demonstrated to accompany anaesthesia and surgery have not been shown to be associated with evidence of liver hypoxia, anaerobiasis or long-term effects on liver function in the fit and healthy, it is likely that further iatrogenic reduction may well be detrimental to the cirrhotic patient with an already compromised LBF. Certainly, reduction in LBF appears to explain adequately the disastrous fulminant liver necrosis that may follow the inadvertent or unavoidable administration of a general anaesthetic to a patient incubating or suffering from viral hepatitis.[8] Inadvertent administration of a general anaesthetic may be due to misdiagnosis of jaundice as obstructive rather than viral hepatocellular.

It appears that the anaesthetist may best shepherd homeostasis of LBF by seeking to maintain adequate cardiac output and systemic arterial pressure while maintaining normocarbia with correctly patterned IPPV.

Circulatory homeostasis — haemorrhage and other factors

Successful control by the anaesthetist of the circulatory homeostasis of the patient with hepatocellular disease during anaesthesia and operation is severely compromised by sequelae of the disease that promote blood loss as well as certain other factors. Those that promote *blood loss* are:

- mechanical
- deficient haemostasis

Mechanical factors promoting blood loss

Vascularity of the liver

The extreme vascularity of the normal liver is evidenced by its 25% share of the cardiac output. In addition, the anatomy of the vascular bed is such that control of surgical bleeding may be difficult to achieve. This is of particular relevance to the anaesthetist in the anticipation of blood loss in the resection of liver tumours, drainage of the humdrum liver abscess (when inflammatory hyperaemia will compound the problem) as well as in the anaesthetic management of the increasingly common ruptured liver from blunt abdominal trauma, often the result of motor vehicle accidents.

Portal hypertension

Hepatic venous obstruction resulting from the loss of vascular bed in the cirrhotic process leads to the venous hypertension in the portal circulation which opens up a multitude of portosystemic venous shunts round the gastrointestinal tract. During surgery these are the source of much of the increased bleeding in these patients. As much of this blood is lost in undramatic oozing, largely hidden from view under swabs, assistants' hands, etc., 'look/see' judgement of the volume of loss is very inaccurate.

Correct volume replacement is of particular importance in these circumstances, since the patient with severe portal hypertension may be particularly sensitive to hypovolaemia; the latter will have occurred in the high-output circulatory state secondary to the enhanced venous runoff of the multiplicity of portosystemic collaterals.

At worst, portal hypertension may cause life-threatening haemorrhage from oesophageal variceal rupture that is of itself the indication for surgery. Happily for the anaesthetist today, control of this bleeding is now usually achieved by tamponade of the variceal region of the oesophagus with the Sengstaken-Blakemore tube together with initial resuscitation followed by peroesophageal sclerotherapy.[9] Prolonged anaesthesia of these often moribund patients for emergency variceal ligation, gastric transection or shunt procedures is a thing of the past. These procedures, especially the modern distal portosystemic shunts, are now usually reserved for the long-term prevention of rebleeding.

Deficient haemostasis

In the patient with hepatocellular disease the above circumstances may be compounded by defective haemostasis. This defect results from lowered levels of fibrinogen, prothrombin and factors V, VII, IX and X secondary to depression of protein synthesis. In the patient with severe portal hypertension with splenomegaly, this deficiency in haemostosis may be compounded further by thrombocytopenia. Fibrinogen levels are usually depressed only

TABLE I. LIVER OXYGEN SUPPLY/DEMAND RATIO[7]

Anaesthetic	Ratio change LBF : change in oxygen consumption	Respiration
Halothane	0,85	Spontaneous
Halothane	0,82	IPPV normocarbia
N_2O/dTc	0,82	IPPV normocarbia
N_2O/dTc	0,59	IPPV hypocapnia
Cyclopropane	0,79	IPPV normocarbia
Methoxyflurane	0,55	IPPV normocarbia
Spinal	0,73	Spontaneous

when hepatocellular disease is very severe. Obstructive jaundice alone may result in prothrombin deficiency because of malabsorption of vitamin K. This is easily corrected by intravenous administration of the vitamin pre-operatively. However, when associated hepatocellular disease is severe, vitamin K replacement may be rendered ineffective by deficient protein synthesis.

Ascites

Of other factors influencing the management of circulatory homeostasis ascites may be of importance primarily for mechanical reasons. If the ascites is so gross as to cause a tense abdomen, raised central venous pressure (CVP), raised intrapleural pressure with volume restriction of respiration, and distress and discomfort for the patient, it should be tapped some hours pre-operatively. When circumstances are not extreme enough to indicate pre-operative drainage but ascites is gross, the anaesthetist must be aware that sudden release of ascitic fluid by the surgeon on commencement of a laparotomy with a consequent rapid drop in the raised intra-abdominal pressure will cause a sudden — usually transient — fall in venous return with a concomitant profound drop in cardiac output and blood pressure, a circumstance that may well endanger the well-being of the seriously ill patient.

Obstructive jaundice and bradycardia

Lastly, in circulatory homeostasis management a factor to be considered in that common anaesthetic problem, the patient with obstructive jaundice, is the accompanying bradycardia thought to be induced by bile salts. If not appropriately controlled with atropine, this bradycardia and its consequences may be worsened by visceral traction during operation.

Management

To manage the patient's circulatory homeostasis successfully in all these circumstances, certain precautions are necessary. The possibility of coagulopathy should be investigated pre-operatively and corrected whenever possible. When thrombocytopenia is apparent, platelet packs for transfusion should be ordered well in advance. Provision must always be made for *rapid* large-volume transfusion; at least one infusion line should have a 14 gauge cannula *in situ*. Equipment for pressurized augmentation of infusion rate should be available. Fresh blood (less than 48 hours old) should be available for loss replacement and should be warmed. In general blood should be replaced early, bearing in mind the defective liver's reduced ability to metabolize the citrate anticoagulant. When massive transfusion is indicated, calcium gluconate 1 g/5 units of replaced blood will prevent the effects of citrate intoxication. Fresh-frozen plasma supplementation will correct coagulation factor deficiencies that will have been worsened by loss/replacement washout.

There must be high-grade cardiovascular monitoring, always including that of CVP. It should be noted that portal vein obstruction during surgery will result in sequestration of splanchnic blood flow in the gut so that part of the replaced losses may not reach the general systemic circulation or reflect correspondingly on the CVP level.

Blood gas and acid-base state in liver disease

For the anaesthetist, correlated with control of circulatory homeostasis is the maintenance of a normal acid-base state in the blood and ultimately in the tissues. As with the former, portal hypertension, particularly if severe, has also an important influence on the latter. Some of the portosystemic shunts in the mediastinum (lower oesophageal area) contrive to drain through bronchial veins via pulmonary veins into the left atrium, constituting ultimately a veno-arterial shunt which manifests as arterial hypoxaemia. The hyperventilatory response to this hypoxaemia often causes hypocapnic respiratory alkalosis in these patients. The portal diversion/hepatic bypassing of ammonia and certain gut-generated amines and kinins which may directly stimulate the respiratory centre is proposed as a further contributory cause of this hyperventilation. To the anaesthetist accustomed to associate respiratory and metabolic acidosis with the seriously ill patient, the respiratory alkalosis in these seriously ill patients must seem paradoxical. Because of its cardiac arrhythmogenesis, the anaesthetist should also be aware that this alkalosis may contribute to or worsen the hypokalaemia that results from the secondary aldosteronism of liver failure, especially in the presence of ascites.

Blood glucose

As blood gas and acid-base state reflect metabolism and perfusion, it is relevant to note here that among its manifold functions the liver is the body's glucostat. Although this function usually fails late and only in the presence of very severe parenchymal disease, the anaesthetist should always monitor blood glucose levels and correct appropriately.

Renal function and obstructive jaundice

In the past a particularly high incidence of postoperative renal failure was noted to be associated with operations for obstructive jaundice, especially when the bilirubin level exceeded 20 mmol/l. Dawson demonstrated that a mannitol-induced diuresis prevented this complication.[10] Two factors were proposed in the pathogenesis: (*i*) renal pigment load, and (*ii*) anaesthetic/surgery-induced reduction in renal blood flow — and thus glomerular filtration rate — paralleling reduction in LBF.

While induction of diuresis during operation has certainly reduced the incidence of postoperative renal failure, the proposed pathogenesis has not been confirmed. Endotoxaemia secondary to bile-acid depletion in the gut is the hypothesis most favoured today.[11] Whatever the pathogenesis, it is accepted that induction of diuresis before and during surgery does prevent postoperative renal shutdown. A protocol for the induction of diuresis is shown in Table II. Still to be decided is

TABLE II. PROTOCOL FOR DIURESIS IN CASES OF OBSTRUCTIVE JAUNDICE

Serum bilirubin $mmol^{-1}$	Management
> 20	**Urinary catheter: monitor urine output** **If urine output > 50 ml/h^{-1} no action** **If urine output < 50 ml/h^{-1} IV fluid load plus mannitol 5% or 10% until diuresis established**
> 140	**Pre-operative** **Antibiotics** **Restoration of fluid balance** **Urinary catheter** **Peri-operative** **CVP monitoring** **Fluid load** **Mannitol 5-10% to keep urine flow > 50 ml/h^{-1}** **Postoperative** **Continue mannitol 24-36 h to maintain diuresis**

IV = intravenous.

whether mannitol is necessary or if fluid-loading induced diuresis alone is not preferable.

Pharmacokinetics in liver disease

So far what may be considered the anaesthetic environment created by liver disease has been discussed. Perhaps more fundamental for the anaesthetist is the manner in which liver disease modifies the disposition and excretion of the drugs used. When a drug enters the circulation:

- A variable fraction becomes protein bound and is no longer diffusible but is in dynamic equilibrium with
- an unbound free fraction which attaches either to specific drug receptors which mediate its effects or
- to other tissues which play no part in specific drug effects. All of these constitute the *volume of distribution* of the drug at steady state.

The free fraction of the drug is also immediately exposed to various mechanisms of disposal:

- it may be metabolized by hepatocyte endoplasmic reticulum and/or it or its metabolites secreted into the bile
- the drug or its metabolites may be excreted by the kidney, or
- it may be acted on by liver-synthesized plasma esterases

With the exception of unchanged drug renal excretion, the liver plays the major role in these mechanisms so that disease of this organ can materially affect drug clearance by circumstances that lead to:

1. Altered hepatic blood flow
   - altered total hepatic perfusion
   - altered degree of intrahepatic shunt
2. altered intrinsic hepatic drug clearance
   - altered number of hepatocytes
   - altered hepatocyte function
3. altered plasma protein composition and drug binding with consequent alteration in drug volume of distribution.

In the situation under discussion there will be, in addition, the effects on these mechanisms of general anaesthesia.

Intrinsic hepatic clearance for every drug is different but this combined with hepatic blood flow and plasma protein binding defines the independent biological variables that determine the rate of drug elimination. If the drug concerned is one of high intrinsic clearance and extraction ratio, the hepatic clearance will reflect the volume of LBF and will be sensitive to its reduction, whereas if the drug is one of low intrinsic clearance and extraction ratio, excess drug is generally available to the elimination process which will then be little affected by changes in blood flow. The reverse applies for alterations in microsomal enzyme activity, the elimination of a drug with low intrinsic hepatic clearance being most sensitive to change. A common alteration of which the anaesthetist should be aware is microsomal enzyme induction in the alcoholic patient, manifesting as a cross-tolerance to other hypnotic and narcotic drugs.[13]

Protein binding

In the patient with parenchymal liver disease, reduction in plasma albumin levels is perhaps the major determinant of the associated altered pharmacokinetics. Changes in drug disposition and dynamics follow: (*i*) decreased/altered protein drug binding, and (*ii*) an increased volume of distribution.

With regard to drug binding, increase in the free drug fraction depends on whether the drug is an acid or a base. In the former, intensity of drug action will be increased in the presence of hypo-albuminaemia, since such drugs bind predominantly to albumin. Hypo-albuminaemia becomes particularly important when high doses of drugs which exceed the binding capacity of available albumin are given. This phenomenon can occur after bolus intravenous injection when free drug concentration in plasma- and vessel-rich tissues such as heart and brain is high before the drug has been distributed to less well perfused tissues. The result will be a greater pharmacodynamic effect and possible toxicity. In the patient with hepatocellular disease the behaviour of thiopentone (Intraval-sodium; Maybaker) exemplifies this situation.

Basic drugs on the other hand are bound to gammaglobulins and lipoproteins. As the former, being produced extrahepatically, are not reduced in liver disease — and may even be increased — the action of these drugs will not be changed and may even be decreased. The resistance of the patient with liver disease to the action of the relaxant d-tubocurarine (dTc) reported so many years ago by Dundee and Gray[14] was once thought to be caused by gammaglobulin binding of dTc but is now known to be the result of an increase in the volume of distribution of the drug.

Volumes of distribution

Increase in a drug's volume of distribution poses very difficult cir-

cumstances for the anaesthetist. Because of the increase a lower free drug fraction and thus reduced pharmacodynamic effect is achieved from a conventional dose of the drug; hence the patient appears resistant and more drug must be administered to achieve the desired result. However, retention of the drug in the increased volume of distribution slows elimination and thus prolongs its effect. With some non-depolarizing relaxants this may render reversal of their action difficult. The behaviour of pancuronium (Pavulon; Organon) in the patient with liver disease typifies this reaction.[15]

Interestingly, in the patient with obstructive jaundice the elimination half-life of pancuronium is also increased because of the reduction of biliary excretion of the drug.[16] A conventional dose of the drug will suffice initially and the patient will not appear resistant, but prolongation of its action may then occur with consequent reversal difficulties.

Narcotics and benzodiazepines

Other drugs to which the altered drug kinetics of liver disease render the patient unduly sensitive include the narcotics morphine and pethidine because of increased elimination half-lives due to decreased intrinsic hepatic clearance, fentanyl for the same reasons together with an increased volume of distribution, and the benzodiazepines for all these reasons in addition to decreased protein binding. With some members of this group of drugs the phase I metabolites are pharmacologically active. There appears to be a theoretical advantage to the use of those drugs in this group which require conjugation only (phase 2) for excretion, such as lorazepam and oxazepam.

Plasma cholinesterase and succinylcholine

Another casualty of the reduced protein synthesis of liver disease is butyryl or plasma cholinesterase (BuChE). This nonspecific esterase hydrolyses the depolarizing relaxant succinylcholine (Scoline: Glaxo); the normal adult exhibits BuChE activity sufficient to hydrolyse 80 mg succinylcholine/minute. A fall in the plasma level of this enzyme may be an early sign of cirrhosis, and thereafter a fall in plasma level parallels the clinical severity of the disease. Foldes[17] found patients with moderately severe liver disease to have depression of BuChE activity of about 50% with a resultant twofold increase in the duration of apnoea after a standard dose of succinylcholine, and patients with severe liver disease had 20% of normal BuChE activity, resulting in a three-fold increase in apnoea time. However, as this amounts to a time of only 30-45 minutes, it is not of great clinical import and the use of succinylcholine in moderate doses is in general safe in patients with liver disease — even liver failure.

Pre-operative assessment

In assessing the patient with liver disease for anaesthesia, the same principles apply as for any other diseases. The aim is to detect departures from normal and to correct them before anaesthesia as far as is possible within the constraints of the disease state and the urgency of surgery. In the context of liver disease, the pathophysiological changes discussed in this paper must be given special attention. For the convenience of the reader, the normal values of biochemical tests relevant to liver disease and inferences from abnormalities are listed in Table III.

With a composite problem such as this, it is useful if we can quantitate 'hepatic reserve' and have some indication of prognosis. For this pur-

TABLE III. LIVER FUNCTION TESTS AND INFERENCES[18]

Test	Normal value	Comment
Albumin	35-50 g/l	Lowered in chronic liver disease — <25 g/l indicates serious problem
Alpha-l-fetoprotein	2-10 µg/l	Raised in hepatoma
Bilirubin — total	3-20 µg/l	>20 µmol/l — clinical jaundice
Ceruloplasmin	150-600 mg/l	Low in Wilson's disease
Cholinesterase	7-19 kU/l	Low in chronic liver disease
Enzymes		
Aminotransferases (AST, SGOT, SGPT)	0-40 IU/l	Raised in liver disease, after myocardial infarct and surgery
Alkaline phosphatase	30-115 IU/l	Raised in biliary obstruction
Lactate dehydrogenase (LDH)	90-300 IU/l	Raised in liver disease
Hydroxybutyrate dehydrogenase	100-250 IU/l	Iso-enzyme of LDH — more specific for liver disease
Gammaglutamyl transpeptidase	15-85 IU/l (male) 5-55 IU/l (female)	Raised with chronic ingestion of alcohol
Globulin	25-30 g/l	Raised in chronic liver disease
Glucose	3,6-6 mmol/l	Hypoglycaemia may occur in liver disease
Prothrombin time	11-13 s	Prolonged in biliary obstruction and hepatocellular disease

AST = aspartate aminotransferase; SGOT = serum glutamic oxalo-acetic transaminase; SGPT = serum glutamic oxalo-acetic transaminase.

TABLE IV. PATIENT STATUS/HEPATIC RESERVE[19]

	Points scored for increasing abnormalities		
	1	2	3
Serum bilirubin (μmol l^{-1})	25	25 - 40	40
Serum albumin (g l^{-1})	35	28 - 35	28
Prothrombin time (s)	1 - 4		
(increase over control)	1 - 4	4 - 6	6
Encephalopathy (grade)	None	1 & 2	3 & 4
Risk of operation	Good	Moderate	Poor

5 - 6 points = good operative risk (equivalent to Child Grade A)
7 - 9 points = moderate operative risk (equivalent to Child Grade B)
10 - 15 points = poor operative risk (equivalent to Child Grade C)

pose, Pugh's modifications of Child's original criteria are perhaps the most useful[19] (Table IV). These criteria are helpful too in assessing pre-anaesthetic response to treatment aimed at improving the patient's condition and prognosis before surgery.

The HBsAg patient

After the consideration we have given to what the anaesthetist may do to the patient, it is in our own interests to think of what the patient may do to the anaesthetist. Patients who have suffered an attack of viral hepatitis B — identified by HBsAg in the blood — may have become carriers of the virus and pose the hazard of infection to the health-care workers who deal with them.[20] There is an increased incidence of viral hepatitis B among people such as anaesthetists, surgeons, pathologists and renal-dialysis workers. It is important therefore to identify HBsAg positive patients by screening all sufferers from chronic liver disease and to adopt sensible precautions to prevent infection of operating-room personnel. Such precautions include wearing disposable gloves and protective clothing, restricting injections and blood sampling to the absolute minimum, and using disposable anaesthetic equipment. Non-disposable equipment must be sterilized with heat (60°C x 12 hours), ethylene oxide, bleach solutions and glutaraldehyde.[18]

Postoperative liver dysfunction

Changes in liver function as detected by raised serum enzyme levels are relatively common after surgery/anaesthesia and appear to correlate with the site and magnitude of the procedure, being absent or negligible after short surface operations and greater after long abdominal, especially upper abdominal, operations. These changes have little more significance than fluctuations recorded in blood sugar level with or without food intake. Patients with pre-existing liver disease may undergo further deterioration in liver function postoperatively, but in patients without pre-existing liver disease serious liver dysfunction that manifests clinically as jaundice as well as biochemically is not common. While we can identify the major functional defects engendered biochemically, aetiological diagnosis is usually by the unsatisfactory process of exclusion. The usual causes are related to blood transfusion/pigment load, tissue injury and liver blood supply, infection and various drugs. While an anaesthetic contribution to most of these complications may not be identifiable, the effects of anaesthetic drugs and techniques on hepatic blood flow must be borne in mind. Just as surgical manipulation provides the environment for anaesthetic problems, so the coincidental anaesthetic sector problems of hypoxia, hypercarbia, hypocapnia and hypotension mutually provide the environment for the factors that cause postoperative liver dysfunction.

Very, very rarely cases of profound, even life-threatening, liver dysfunction for which no identifiable causes are apparent occur after anaesthesia. In these circumstances, suspicion must fall on the anaesthetic agent involved. We are then faced with the whimsical, unpredictable 'halothane hepatitis'. But that is another story.

Summary and conclusion

To safely anaesthetize the patient suffering from liver disease, the anaesthetist must have a knowledge of the complex pathophysiology associated with liver dysfunction in order to understand and deal with the constraints this imposes on his ability to control the patient's physiological homeostasis during surgery. In general, patients with chronic liver disease who on pre-operative assessment score six points or less on the Pugh criteria have compensated liver function and do not constitute a major anaesthetic risk, whatever the surgery contemplated. By contrast, those who score seven or more constitute a serious anaesthetic and operative risk, the outcome being conditioned to a large extent by the magnitude of the surgical procedure undertaken.

There is no clear evidence that any of the currently used anaesthetic agents or techniques has any definite advantage or detrimental effect, provided the constraints of disturbed liver function are recognized and adapted to appropriately.

In the conduct of anaesthesia, the anaesthetist should pay particular attention to:

- Reducing the dose of premedicant drugs or avoiding their use altogether in patients with chronic liver disease who score seven or more points on Pugh's criteria.
- Cautiously titrating the intravenous induction agent used since there is great variation in the dose required because of changes in protein binding, drug volumes of distribution and, in the alcoholic patient, perhaps microsomal enzyme induction.
- Limiting the dose of narcotics administered in proportion to the degree of liver derangement.
- Minimizing the dose of volatile agent so as not to affect the LBF adversely. Halothane may be safely used in these patients provided there is no history of halothane administration with adverse reaction or, many would add, recent (2 months) administration of the drug.

- Maintaining normal $PaCO_2$ in the interests of minimizing changes in splanchnic vascular resistance and LBF.
- Monitoring the degree of neuromuscular blockade in order to minimize the dose of muscle relaxant drug used.
- Inducing diuresis during surgery (perhaps even before) when the patient's pre-operative bilirubin level exceeds 20 mmol/l.

Lastly, the anaesthetist must beware of the two problems posed for him by viral hepatitis:

- The fulminant liver failure that may follow general anaesthesia of the sufferer from active viral hepatitis identifies the latter as an absolute contraindication to anaesthesia except for immediate life-threatening surgical emergency.
- The risk of infection to the anaesthetist and other operating-room personnel posed by HBsAg carriers.

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CROSS-CIRCULATION BETWEEN MAN AND BABOON

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Summary The circulating blood-volume of a baboon (*Papio ursinus ursinus*) was washed out with Ringer's lactate solution under hypothermia. When all the baboon's blood was judged to have been removed the Ringer's lactate was replaced with human blood. The animal was then used for a successful and uncomplicated cross-circulation with a patient in terminal hepatic coma.

Introduction

PRELIMINARY (unpublished) work in our laboratory has shown that the baboon (*Papio ursinus ursinus*; Roberts 1951) can tolerate complete exchange transfusion with human blood. Although hæmolysis does develop, it is delayed, and immediate transfusion or other reactions have not occurred. Accordingly we decided to cross-circulate a patient in terminal hepatic failure with a baboon, after its blood had been replaced with human blood compatible with that of the patient.

Method

PREPARATION OF THE BABOON

Anæsthesia

Anæsthesia was induced in a healthy male baboon weighing 26 kg. with phencyclidine 40 mg. by intramuscular injection

at 10.15 P.M., and diazepam 10 mg. intramuscularly at 10.45 P.M. Before the operation the animal was carefully shaved and washed with cetrimide 2% and chlorhexidine 2%. At 11.45 P.M., after endotracheal intubation, anæsthesia was commenced with oxygen and nitrous oxide, with intermittent halothane using an intermittent positive-pressure respiration (I.P.P.R.) technique with a non-return circuit. At midnight, atropine 0·4 mg. was given intramuscularly.

Cardiopulmonary Bypass and Hypothermia

The femoral artery and vein were exposed in the right groin. The left femoral vein was cannulated and a polyvinyl catheter inserted for central-venous-pressure recordings and infusion therapy as required.

The chest was opened through a left thoracotomy in the fifth intercostal space and the heart was exposed. After systemic heparinisation (60 mg.) the femoral artery and right atrium were cannulated. The extracorporeal circulation was so arranged to allow for: induction of profound hypothermia, complete washout of the animal's circulating blood-volume with Ringer's lactate solution, and subsequent replacement of the baboon's circulating fluid volume with human blood (fig. 1).

The Rygg bag in circuit 1 (fig. 1) was primed with two litres of 'Plasmalyte B', and extracorporeal circulation commenced at 12.55 A.M. Hypothermia was effected by the

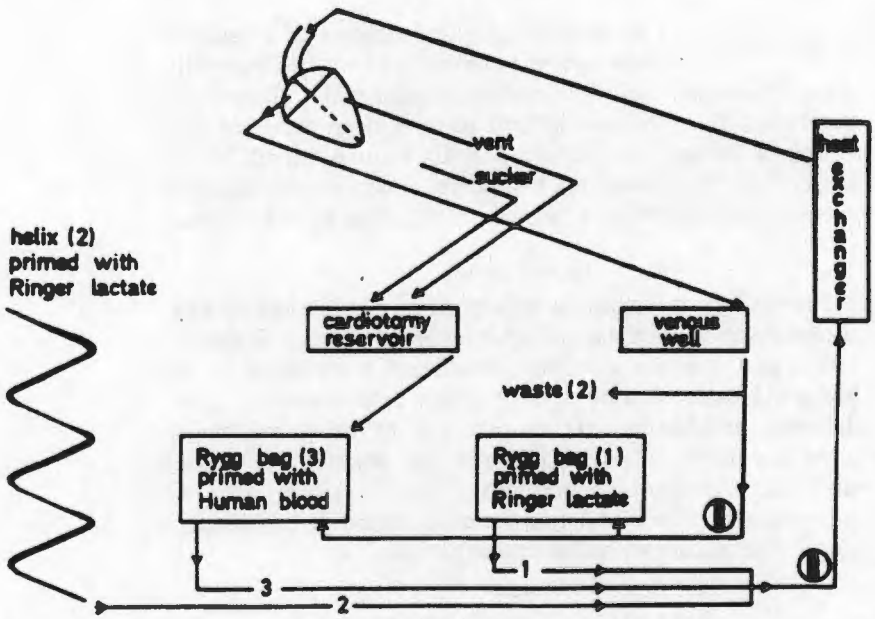

Fig. 1—Circuits 1, 2, and 3 used for the replacement of baboon blood by human blood.

heat exchanger in the circuit (Sarn's 2 litre). At 1 A.M., with the œsophageal and rectal temperatures at 26·0°C and 31·0°C, respectively, ventricular fibrillation commenced. The left ventricle was immediately vented. At 1.10 A.M. with the temperatures, œsophageal 13·5°C, rectal 22·0°C, the bypass was changed to circuit 2 (fig. 1). With the protection of hypothermia, the animal's blood was effectively washed out.

Ringer's lactate, precooled to 4°C, was run into the helix in circuit 2 which acted as a reservoir, and with a flow-rate of 1·2 litres per minute, the animal was washed out, the cardiotomy return running to waste where it was accurately measured. To ensure complete emptying of the pulmonary circulation of baboon blood, the lungs were ventilated and the heart gently massaged. The left ventricular vent ran to waste. At 1.21 A.M. the cardiotomy return from both the right atrium and left ventricle was macroscopically free of red blood-cells. This fluid contained no protein on electrophoresis and very few red blood-cells on microscopy. The third circuit was primed with human blood, prewarmed to 37°C, oxygenated, and returned to the animal (fig. 1). The cardiotomy return was allowed to run to waste for a further 1 litre, at which stage the venous return did not appear to be hæmodiluted macroscopically. At 1.25 A.M. the animal was on routine cardiopulmonary bypass with only human blood in the circuit, with œsophageal and rectal temperatures at 20·4°C and 20·2°C, respectively, and a central venous pressure (C.V.P.) of 2 cm. of water. At 1.27 A.M. the heart defibrillated spontaneously to sinus rhythm with an œsophageal temperature of 27·0°C and a rectal temperature of 21·0°C. The circulation was supported with cardiopulmonary bypass and the baboon was rewarmed.

The left ventricular vent was now removed and the ventriculotomy was repaired with 3/0 black silk. At œsophageal and rectal temperatures of 37°C and 29·8°C, respectively, and a C.V.P. of 11 cm. water, cardiopulmonary bypass was discontinued. The arterial pressure was 120 mm. Hg, packed cell volume (P.C.V.) 25%, arterial blood pH 7·431, Po_2 310·0 mm. Hg, Pco_2 25·1 mm. Hg, and base-excess −7·3 meq. per litre. The base deficit was corrected with intravenous sodium bicarbonate.

After removal of the right atrial catheter, the atriotomy was repaired and the pericardium closed with interrupted sutures. The chest wall was closed in layers over a size 28 Argyle catheter with underwater drainage. The right-femoral-artery cannula was replaced with a size 14 'Teflon' tipped 'Silastic' catheter and the right femoral vein was then cannulated with a size 13 teflon-tipped silastic catheter. The arteriovenous shunt was allowed to circulate while the patient was prepared in an adjoining theatre. At 2.45 A.M. surgery of the animal was completed and 15 mg. of heparin were given intravenously in preparation for cross-circulation with the patient.

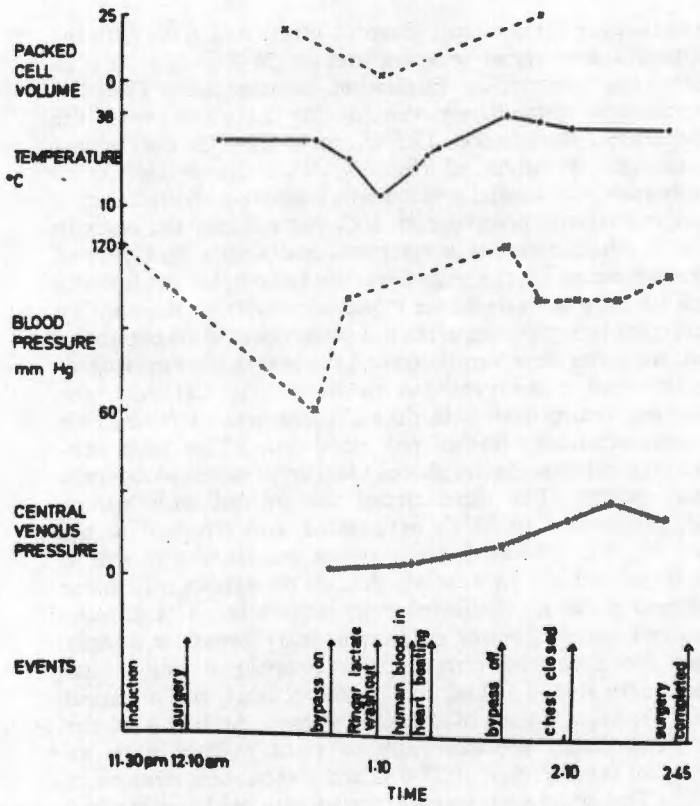

Fig. 2—Changes in the packed-cell volume, temperature, blood-pressure, and central venous pressure during the preparation of the baboon.

Anæsthesia was maintained in the animal via an I.P.P.R. non-return circuit with nitrous oxide, oxygen, and intermittent ether. Isoprenaline ten 0·05 ml. drops per min. (1/450,000 solution) was used to support the animal's circulation.

Fig. 2 shows the changes in the P.C.V., temperature, blood-pressure, and C.V.P. during the preparation of the baboon.

The baboon tolerated cardiopulmonary bypass with hæmodilution and profound hypothermia well, and appeared unaffected by replacement of its blood with fresh human blood.

PREPARATION OF THE PATIENT

A White married female, aged 29 years, was admitted to Groote Schuur Hospital in hepatic coma: details of her case are described by Saunders et al. (1968). To facilitate exchange transfusion a Quinton-Scribner arteriovenous shunt was inserted into the right arm under local anæsthesia. 'Teflon' tips, sizes 14 and 13, were inserted into the radial artery and cephalic vein, respectively.

At 2.40 A.M. on the morning of the cross-circulation a polyvinal catheter was inserted into the inferior vena cava through a branch of the saphenous vein to measure C.V.P. The patient was on I.P.P.R. (Bird's respirator, 40% oxygen).

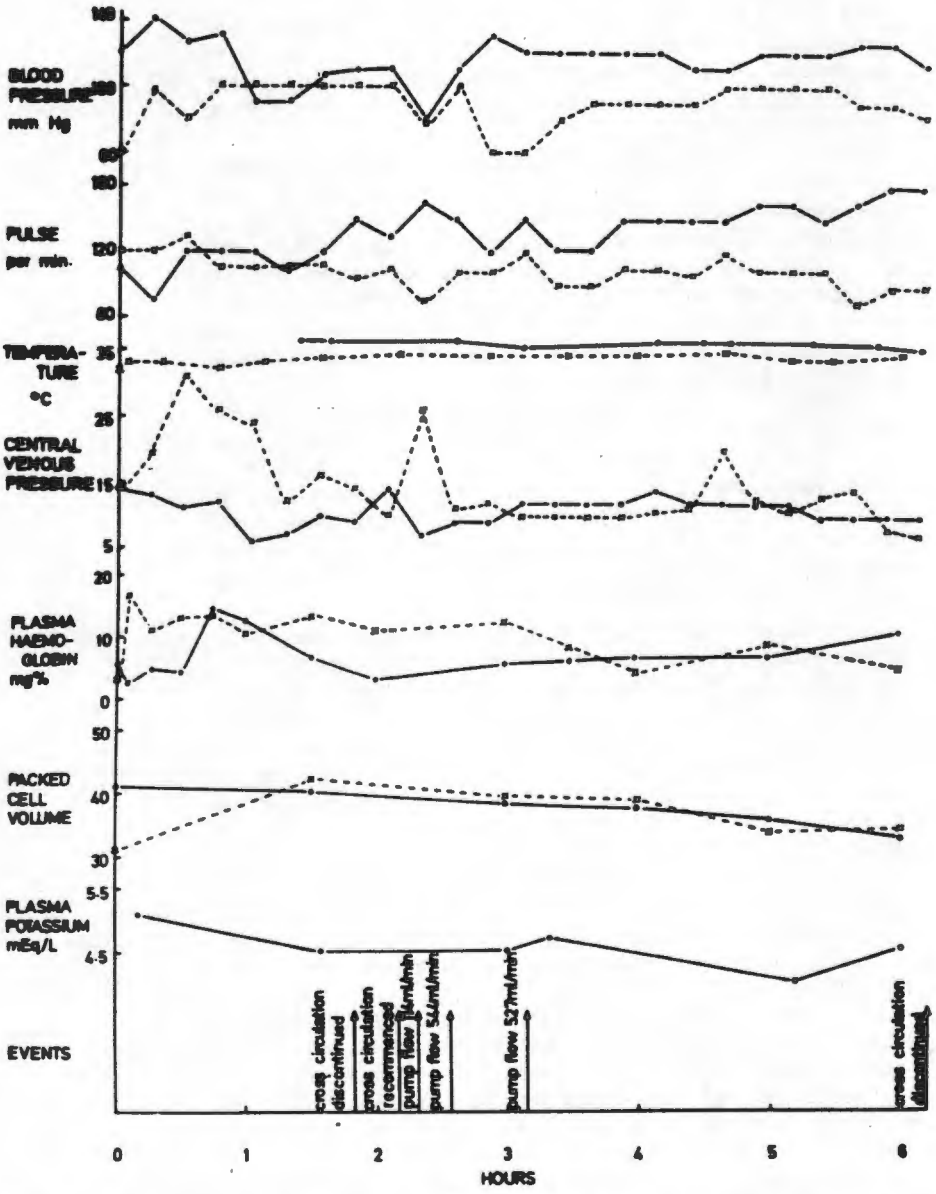

Fig. 3—Blood-pressure, pulse-rate, temperature, central venous pressure, plasma-haemoglobin, packed-cell volume, and plasma-potassium during cross-circulation.
Continuous line is for the patient, broken line for the baboon.

Repeated interruption of the I.P.P.R. did not result in spontaneous breathing. She was in a terminal stage of hepatic coma.

CROSS-CIRCULATION

At 3.10 A.M. (zero hour, fig. 3) the cross-circulation was established by connecting the patient's radial arterial flow to the femoral vein of the baboon and the baboon's femoral artery to the right cephalic vein of the patient. The clamps were released simultaneously.

Direct cross-circulation was maintained between the animal and the patient from 3.10 A.M. to 5 A.M. During this period it was obvious that the patient was bleeding into the baboon as shown by a fall in the C.V.P. and blood-pressure of the patient and a rise in C.V.P. and blood-pressure of the animal (fig. 3). A screw clamp was then applied to the arterial line from the patient to reduce blood-flow. It was felt that although the cross-circulation was adequate, the hazard of exsanguinating the patient had not been eliminated.

At 5.25 A.M. two Sarn's modulator pumps were introduced into the circuit. The arterial blood from both patient and animal was allowed to run into a venous well ($2^1/_2$ in. Mayon tubing) and then returned to the appropriate venous end to effect cross-circulation. The levels in the venous wells were matched, and the pumps set to run at the same speed. It was now easy to adjust the screw clamp on the patient's arterial line to effect even cross-circulation.

After introduction of the pumps there was again an episode of blood-loss from the patient into the baboon as judged by a rise in the C.V.P. of the animal and a corresponding drop in the patient's C.V.P. Flow-rate at this stage was 114 ml. per minute. By increasing the flow-rate to 544 ml. per minute and controlling the radial arterial flow from the patient with a screw clamp, an even cross-circulation was maintained for the remaining 4 hours. The rate of cross-circulation must, of necessity, depend on the lesser volume of arterial flow—in our case the arterial flow from the animal. The arterial flow of a larger volume per unit of time has to be controlled to maintain uneventful cross-circulation. Cross-over flow volume remained at 500 ml. or more per minute for the rest of the cross-circulation-time (4 hours).

Changes in the patient and baboon with regard to hæmoglobin, P.C.V., and plasma-potassium are shown in fig. 3. There was no evidence of hæmolysis.

After 1 hour of cross-circulation, spontaneous respiration was noted for the first time, but the tidal volume was inadequate and I.P.P.R. was recommenced. After 3 hours and 50 minutes of cross-circulation I.P.P.R. was discontinued and she breathed spontaneously thereafter (tidal volume 500–600 ml.). After 4 hours and 45 minutes of cross-circulation she was breathing without assistance and her minute-volume was 11 litres, P_{CO_2}

27 mm. Hg, and Po_2 79 mm. Hg. Cross-circulation was continued for 6 hours, by which time the patient had a sinus tachycardia of 150 per minute, possibly the result of isoprenaline given to the baboon.

Within 60 hours of the cross-circulation the patient showed obvious improvement and within another 12 hours was lucid, orientated and fully conscious.

Conclusion

We have preferred not to use cross-circulation between patients in hepatic coma and human volunteers and feel that the use of the baboon, prepared as described here, avoids exposing human volunteers to a number of hazards. Our preliminary experience suggests that cross-circulation between man and a subhuman primate is feasible and safe, and we believe that this technique may be of value in liver assistance in hepatic failure, not only in massive liver-cell necrosis due to hepatitis but also after liver transplantation.

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ACUTE HEPATIC COMA TREATED BY CROSS-CIRCULATION WITH A BABOON AND BY REPEATED EXCHANGE TRANSFUSIONS

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Summary A patient in terminal hepatic coma with high-brain-stem dysfunction was treated by multiple exchange blood-transfusions and by cross-circulation with a baboon (*Papio ursinus ursinus*) after the animal's blood had been replaced with human blood compatible with that of the patient. Spontaneous respiration was restored by the cross-circulation which was free from complications and the patient subsequently became fully conscious and lost all her abnormal neurological signs.

Introduction

HEPATIC coma due to massive liver-cell necrosis complicating viral hepatitis has been treated by means of exchange blood-transfusion (Trey, Burns, and Saunders 1966, Saunders 1967), heterologous liver perfusion (Eiseman 1967), and by human cross-circulation (Burnell et al. 1967, Swift et al. 1967). These procedures represent attempts to tide the patient over the critical period while

liver cells regenerate. These patients are also treated by the more conventional method of a protein-free, high-carbohydrate diet, neomycin or kanamycin by mouth, and large doses of corticosteroids (Saunders 1967).

We report here the treatment of a patient in severe hepatic coma, due to massive liver-cell necrosis as a result of viral hepatitis, by means of repeated exchange blood-transfusions and cross-circulation through the intact living baboon (*Papio ursinus ursinus*).

Case-report

BEFORE CROSS-CIRCULATION

A White female aged 29 was noted to be jaundiced at a medical examination when she was about 2 months pregnant. She had been feeling ill for about a week, with nausea, high temperature, and dark urine, and she was treated with bed rest. 1 week later she was more deeply jaundiced and her stools were pale, but after 5 weeks she was much better, was no longer jaundiced, and the urine contained a trace of bilirubin. Plasma-bilirubin did not rise above 3 mg. per 100 ml. On July 14, 1968, 4 days before her admission to Groote Schuur Hospital she again became ill, started vomiting, and her jaundice reappeared. A pustular rash developed all over her body, especially on the chest and upper arms, and she was admitted to the Frere Hospital, East London. She had the early signs of hepatic failure, with a flapping tremor, and the liver was small on percussion. On July 15 she aborted and thereafter was very ill and in the afternoon became stuporose, the tendon reflexes becoming brisker. Treatment with a protein-free diet, oral neomycin, and corticosteroids was commenced. Her serum-total-bilirubin was 15 mg. per 100 ml. (conjugated 8·7 mg. per 100 ml.); serum-glutamic-oxaloacetic-transaminase (S.G.O.T.) 400 units; serum-electrolytes (meq. per litre): sodium 130, chloride 100, potassium 3·5; blood-urea 27 mg. per 100 ml.; hæmoglobin 13·6 g. per 100 ml., packed-cell volume (P.C.V.) 45%; white-blood-cell (W.B.C.) count 24,700 per c.mm., with 85% polymorphonuclear cells and 15% lymphocytes. An exchange transfusion of 10 pints of fresh heparinised blood was performed, and she was thought to be slightly improved on July 18 when she was flown to Groote Schuur Hospital. There was no history of previous administration of blood or blood products and she had been taking no drugs, but there was an epidemic of infective hepatitis in East London at the time.

On admission to Groote Schuur Hospital the patient was comatose, and responded very slightly to painful stimuli. She had repeated myoclonic movements of the limbs; pupils equal and reacted well to light; tendon reflexes all very brisk; she had bilateral Hoffmann's signs and bilateral extensor plantar responses. She was deeply jaundiced, and the liver could not be felt or percussed. There was a striking fœtor hepaticus, but no elicitable flapping tremor. Blood-pressure 100/60 mm. Hg;

cardiovascular system and chest normal. Urine contained protein +++ and a large amount of bile, but no urobilin and no formed elements on microscopy. She was given 1 g. neomycin through a stomach tube 6-hourly and 600 mg. of hydrocortisone, 2 g. ampicillin, and 4 g. cloxacillin intravenously daily. On admission she was given an enema with 2 g. of neomycin and this was retained for as long as possible. She received vitamin K_1 10 mg. intramuscularly daily. An arteriovenous shunt was inserted between the radial artery and vein in the forearm and an exchange transfusion of 5 litres of fresh heparinised blood was carried out. The cerebrospinal-fluid glutamine before the exchange transfusion was 33 mg. per 100 ml., serum-total-bilirubin 12·1 mg. per 100 ml. (conjugated 9·7 mg. per 100 ml.), cholesterol 323 mg. per 100 ml., alkaline phosphatase 6·2 units, thymol turbidity 0, zinc turbidity 4, S.G.O.T. 23 units, lactic dehydrogenase (L.D.H.) 245 units, albumin 2·9 g. per 100 ml., and globulin 1·3 g. per 100 ml. The blood pH was 7·2, with a P_{CO_2} of 49 mm. Hg, standard bicarbonate of 16·5 meq. per litre, base excess −10·2 meq. per litre, actual bicarbonate 18·5 meq. per litre, and a total carbon-dioxide of 20 meq. per litre. She was given sodium bicarbonate intravenously to correct the metabolic acidosis. Protamine sulphate was given intravenously in the usual way at the end of this and all subsequent exchange transfusions. After the exchange transfusion the serum-total-bilirubin was 4·9 mg. per 100 ml. (conjugated 4·1 mg. per 100 ml.) (fig. 1). A tracheostomy was performed and she was given 'Trasylol' (aprotinin) 250,000 units intravenously 4-hourly for three doses.

At 9.30 the next morning (July 19) the serum-electrolytes (meq. per litre) were sodium 133, potassium 2·7, and chloride 92, and she was given additional potassium. At 2.30 P.M. a second exchange transfusion was performed and completed at 4.15 P.M., 4·5 litres being exchanged (fig. 1). Before the exchange the patient was deeply unconscious, responding only sluggishly to painful stimuli, and during the procedure she became restless and moved about semipurposively. She became cyanosed and coughed quite a great deal and the exchange was therefore stopped. Her pulse-rate increased and suctioning yielded copious yellowish secretions. Before and during the exchange she had decerebrate movements. Vigorous physiotherapy resulted in a great improvement in the chest signs. There was no acidosis after the exchange and her serum-electrolyes were normal. Culture of the pus in the pustules of the skin grew a coagulase-positive staphyococcus, sensitive to most antibiotics except penicillin. Blood-cultures were consistently negative throughout her illness. By 6 P.M. on July 19 there were no physical signs in the chest and she was more responsive than she had been in the morning. Her temperature was 100·8°F (38·1°C), and the electrocardiograph was normal. She still had notable hyperreflexia and bilateral extensor plantar responses.

At 9 A.M. on July 20 the serum-electrolytes (meq. per litre)

were sodium 121, potassium 4·3, and chloride 98. The blood pH was 7·51, Pco_2 36·5 mm. Hg, standard bicarbonate 29·5 meq. per litre, and base excess + 6·6 meq. per litre. There was considerable bronchospasm and X-ray of the chest showed diffuse opacities which were again greatly improved by physiotherapy. Her eyelids were very œdematous. At 2.30 P.M. she had athetoid movements of the arms and the tendon reflexes were brisk. Her liver was now just palpable under the costal margin. Another exchange transfusion of 5 litres of blood was performed at 3.40 P.M. (fig. 1). She had bilateral extensor spasms with forceful pronation before the exchange, and after the procedure there was no obvious clinical improvement. She was given gentamicin, 80 mg. intramuscularly 12-hourly and a single 60 mg. dose of testosterone proprionate for engorgement of the breasts. After the exchange transfusion she had a respiratory alkalosis (pH 7·5). At 1.30 A.M. on July 21 she responded to minimal painful stimuli, but the extensor spasms were still present. She had bilateral, brisk tendon reflexes and extensor plantars. She was breathing quietly, her chest was clear and her temperature was then normal.

On July 21 she had a 5th exchange transfusion of 5 litres of blood (fig. 1) without any sign of improvement.

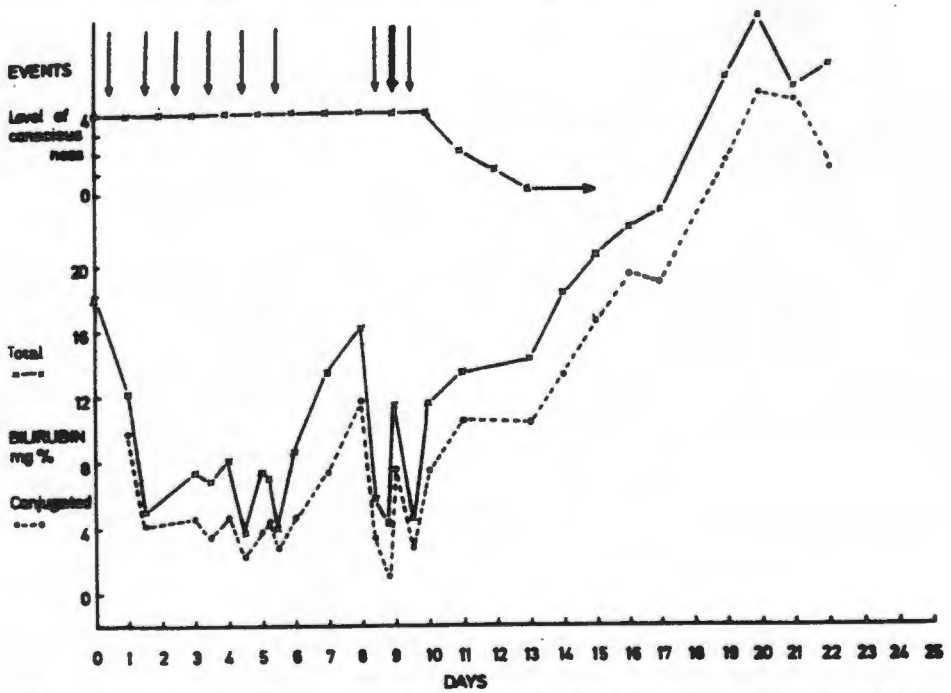

Fig. 1—Changes in level of consciousness and bilirubin during repeated exchange transfusions and cross-circulation with a baboon.

Thin arrows represent exchange transfusion; the thick arrow on day 9 represents cross-circulation. Level of consciousness is graded from 0 (full consciousness) to 4 (deep hepatic coma). Continuous line is for the patient, broken line for the baboon.

On July 22 she was in deep coma, reacting very slightly to painful stimuli. She still had abnormal movements, her plantars were both extensor, but her pupils were equal and reacting well to light. There was clinical evidence of chest infection. At 4 P.M. the 6th exchange transfusion of 5 litres of blood was performed (fig. 1) without any evidence of improvement. Her blood-count at this stage showed a hæmoglobin of 16·1 g. per 100 ml., P.C.V. 45%, platelets 109,000 per c.mm., W.B.C. 9360 per c.mm., with 82% polymorphs, 16% lymphocytes, and 2% monocytes and an occasional polychromatic cell in the peripheral smear, with a slight shift to the left for the neutrophils and some toxic granulation. A number of atypical lymphocytes were present.

On July 23 she responded slightly better to painful stimuli, but the neurological signs were otherwise unchanged. No exchange transfusion was performed on that day. Apart from mild jaundice (fig. 1) there was no detectable biochemical abnormality. Vaginal examination showed the cervix was dilated to one finger and the uterus was about the size of a 6-week pregnancy.

On July 24 her level of consciousness remained about the same, but the bilirubin had risen. Decerebrate spasms of the limbs were present.

On the morning of July 25 she responded to light painful stimuli, the pupils were reacting to light, the tendon reflexes were abnormally brisk, and both plantars were extensor. The chest showed right-upper-lobe consolidation. The liver was just palpable below the costal margin. At 10.45 A.M. blood pH was 7·455, Pco_2 35·5 mm. Hg, standard bicarbonate 26 meq. per litre, base-excess +2·6 meq. per litre, buffer base 63 meq. per litre, actual bicarbonate 23·4 meq. per litre, total carbon-dioxide 24·1 meq. per litre. At 12.30 P.M. the patient's respiration became depressed and she started breathing at a rate of 7 per minute. There were no other changes in her clinical condition, she was not cyanosed and her pupils reacted briskly to light. She was put on intermittent partial-pressure respiration, using a Bird respirator. At 3.30 P.M. the electrolytes and enzymes were normal. The cerebrospinal-fluid glutamine was 43 mg. per 100 ml.; the cerebrospinal-fluid glucose 100 mg. per 100 ml. and it contained 3 lymphocytes and 560 red blood-cells per high-power field. An exchange transfusion was performed and after the exchange of 5 litres of blood the patient breathed spontaneously and normally for about 10 minutes. She then again started breathing at 7 respirations per minute and intermittent positive-pressue respiration was recommenced. The pupils reacted to light and an eyelash reflex was present. She had had a diuresis, with disappearance of much of the periorbital œdema, and she was noted to have doll's-eye movements. She was unconscious and responded to no sensory stimuli except for non-purposive limb movements when she was touched. She had decerebrate movements of the limbs. All agreed that she would not recover with the treat-

ment used up to that stage, and we decided to cross-circulate the patient with a baboon.

The details of the preparation of the animal and the cross-circulation itself are described by Bosman, Terblanche, Saunders, Harrison, and Barnard (1968).

AFTER CROSS-CIRCULATION

She did not breathe spontaneously after 4.45 P.M. The blood pH just before cross-circulation was 7·599 and the Pco_2 21·8 mm. Hg. The cross-circulation was done between 3.10 and 9.15 A.M. on July 26. At 4.10 A.M. (after 60 minutes of cross-circulation) spontaneous respiration recommenced and the respirator was no longer used after 7.00 A.M. During the $11^1/_2$ hours before spontaneous respiration was seen, the respirator was stopped for up to 60 seconds on a large number of occasions and at no time was any spontaneous respiration observed. Throughout this period, and when spontaneous respiration returned and was established, the Pco_2 remained about the same. She was still deeply unconscious and doll's-eye movements persisted. Sinus tachycardia developed and she was given digitalis. It is possible that isoprenaline given to the baboon may have caused the tachycardia. Electroencephalogram (E.E.G.) before the procedure showed diffuse, moderate-to-severe, generalised slowing compatible with hepatic ence-

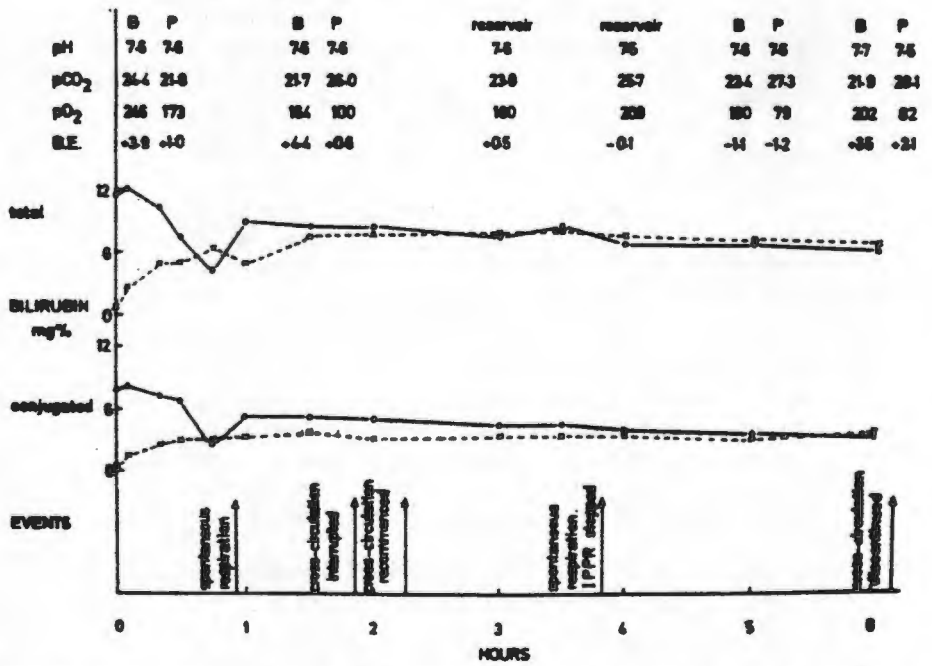

Fig. 2—Changes in pH, Pco_2, Po_2, base-excess (B.E.), and plasma-bilirubin in baboon (B), patient (P), and reservoir during cross-circulation.

The continuous line represents the patient, the broken line represents the baboon.

phalopathy, and after the cross-circulation the E.E.G. showed some improvement, with changes suggestive of brain-stem damage. The changes in blood pH, P_{CO_2}, P_{O_2}, and bilirubin are shown in fig. 2. At 10.45 A.M. on July 26 the cerebrospinal-fluid glutamine was 35 mg. per 100 ml. That afternoon a final exchange of 5 litres of blood was performed in an attempt to remove any antigenic material which may have entered the patient from the baboon. The patient's neurological state remained unchanged throughout that day. It seemed that she had irreversible high-brain-stem dysfunction. At 9 P.M. the patient bled from the site of the femoral cut-down and her coagulation-time was 22 minutes. 250,000 units trasylol were given intravenously 6-hourly for three doses, and vitamin K_1, 10 mg. per day intramuscularly, was continued. At 4.30 P.M. on July 26 her total bilirubin was 4·7 mg. per 100 ml. serum (conjugated 2·8 mg. per 100 ml.). There was no disturbance of acid/base status and her serum-electrolytes were normal.

On July 27 she reacted more readily to mild painful stimuli, but was still deeply unconscious. Her pupils reacted to light, respiratory-rate was 16 per minute and respiratory excursions were normal in depth. Her prothrombin-time was 60% of normal, coagulation-time 12 minutes, blood pH 7·52, P_{CO_2} 23·5 mm. Hg, standard bicarbonate 24·9 meq. per litre, base excess +1 meq. per litre, buffer base 56 meq. per litre, actual bicarbonate 18·2 meq. per litre, total carbon-dioxide 13·8 meq. per litre, P_aO_2 117 mm. Hg; serum-electrolytes (meq. per litre): sodium 135, potassium 4·7, chloride 103; serum-total-bilirubin 11·6 mg. per 100 ml. (conjugated 7·5 mg. per 100 ml.), serum-fibrinogen 83 mg. per 100 ml. At 6 P.M. she was given 6 g. fibrinogen and, over the next 16 hours, 625,000 units of trasylol. At 7 P.M. on July 27 her blood-pressure suddenly became unrecordable and her pulse fell to 40 per minute. Deep sighing respirations developed. Her blood-pressure returned again as the foot of the bed was elevated, the respirations and pulse became normal. She was given 1 pint of blood. There was no disturbance of her acid/base status or electrolytes. Subsequent administration of fibrinogen also caused hypotension and this protein may have accounted for this episode as well.

On July 28 she was unconscious, responding to painful stimulation by limb withdrawal, and showed facial grimacing. Doll's-eye movements were still present. A ciliospinal and corneomandibular reflex were elicited. The eyes moved to the right in response to caloric stimulation of the right ear. She had a cogwheel type of rigidity, poorly elicited tendon reflexes, and extensor plantar responses. Pupillary response to light was normal.

She then made a dramatic recovery. By the evening of July 28 she recognised her husband and by the next morning she was fully conscious and responded purposefully to commands. In retrospect we interpreted the abnormal neurological signs as being due to a metabolic subcortical lesion with

resultant functional decortication or due to direct metabolic depression of high brain stem activity.

Apart from a mild anæmia and a platelet-count of 54,000 per c.mm., the blood-count was normal on July 29. Plasma-fibrinogen was 101 mg. per 100 ml. and she was given 500 ml. of platelet-rich fresh plasma. She bled from the arteriovenous shunt in the forearm and was given 1 litre of blood. Thereafter, she remained alert, lucid and fully orientated, and within a few days no abnormal neurological signs could be detected.

FOLLOW-UP

On Aug. 29 she was still fully conscious and had no abnormal neurological signs. She was on a normal diet and no longer had ascites nor a bleeding tendency, both of which had been present during her recovery. She was still deeply jaundiced and the serum-albumin was 2·8 g. per 100 ml. It is probable that she had two pulmonary emboli during August.

Discussion

This patient had severe hepatic failure as a complication of hepatitis, presumably viral in origin. For 7 days she was in deep coma and at times she responded to varying grades of painful stimuli. During this period six exchange blood-transfusions were performed with, at times, slight improvement. On the eighth day high-brain-stem dysfunction became apparent and " doll's-eye movements " developed in addition to the extensor decerebrate spasms she had shown during most of her illness. Respiratory failure developed and it is of interest that the exchange transfusion done at that time restored normal respiration, albeit for a short while. This is in keeping with our impression that exchange transfusion is of value in hepatic coma due to massive liver-cell necrosis (Trey, Burns, and Saunders 1966, Saunders 1967). She again breathed very slowly and finally stopped breathing spontaneously, and we thought that irreversible upper-brain-stem and medullary lesions were present. All observers agreed that there was no hope for the patient and, as a desperate measure, cross-circulation through a baboon was performed, after its blood had been replaced with human blood compatible with that of the patient. After 60 minutes of cross-circulation, spontaneous breathing recommenced and after 3 hours 50 minutes of cross-circulation intermittent positive-pressure respiration was finally stopped. We were very aware at the time of the possibility of apnœa following on mechanical forced ventilation and analysis of her blood P_{CO_2} levels confirmed our clinical impression that the recommencement of spontaneous respiration was due to the cross-circulation and not to any change in ventilation.

The cross-circulation was performed without mishap and there was no immediate or subsequent evidence of harm to the patient. In particular there has been no evidence of any abnormal immunological response to date. After the procedure, the signs of upper-brain-stem damage persisted and she remained unconscious but breathing well. At this stage we felt that recovery from "irreversible" brain damage was unlikely. She then made a steady dramatic recovery and became fully conscious, and normally orientated, with no abnormal neurological signs. Although Davis et al. (1968) have pointed out that patients in severe hepatic coma can recover with more conventional treatment, we do not think that our patient would have done so.

It would seem from this case-report that cross-circulation between man and the baboon is a safe procedure, and may provide an additional method of treatment of liver failure. Its advantages over human cross-circulation are self-evident. We believe that it will have application not only in the management of massive liver-cell necrosis, but also in the treatment of hepatic failure after liver transplantation.

We thank the Western Province Blood Transfusion Service for their invaluable assistance, the departments of chemical pathology and hæmatology for their help, and Dr. J. G. Burger, medical superintendent of Groote Schuur Hospital, for permission to publish this report. This work was supported in the University of Cape Town by the Council for Scientific and Industrial Research of South Africa and by the C. L. Herman Bequest.

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Pig Liver Perfusion in the Treatment of Fulminant Hepatic Necrosis

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Hickman, R., Saunders, S. J., King, J. B., Harrison, G. G. & Terblanche, J. 1971. Pig Liver Perfusion in the Treatment of Fulminant Hepatic Necrosis. *Scand. J. Gastroent.* 6, 563-568.

This paper describes 4 patients with hepatic coma resulting from presumed viral hepatitis with massive liver cell necrosis, who were treated with pig liver perfusion alternating with exchange transfusion. None of the patients survived, but two showed a response to the program of treatment employed, in that there was an improvement in the level of consciousness. All patients died of haemorrhagic complications. The contribution of the oxygenator used in the circuit to the development of thrombocytopoenia is discussed, with reference to other circuits with and without oxygenators. Thrombocytopoenia may also result from disseminated intravascular coagulation in the patient, and from sequestration of platelets in the liver perfused with heterologous blood.

Key-words: Fulminant hepatic necrosis; porcine liver perfusion; thrombocytopoenia

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Between June, 1963, and February, 1970, 77 patients with fulminant hepatic necrosis were seen at Groote Schuur Hospital and the Red Cross War Memorial Children's Hospital, Cape Town. The majority of these patients were presumed to have viral hepatitis. The overall mortality irrespective of the form of treatment was 80 per cent (10). This paper describes the use of pig liver perfusion in the treatment of four of these patients in terminal hepatic coma.

PERFUSION TECHNIQUE

The circuit utilized for patient perfusion has been described previously (6). It is identical to that used for perfusion of the isolated liver. The circuit is connected to the patient through a Quentin-Scribner shunt, which is routinely inserted into all patients who receive exchange transfusion, an additional procedure used in our patients (10). The arterial end of the shunt is connected by Silastic tubing to a reservoir sited above, and draining into, the oxygenator, thus permitting measurement of flow from the patient. Blood is returned to the cephalic vein from the vena caval reservoir via a bubble trap and roller pump where inflow can be monitored (Fig. I).

The circuit is primed with TisUsol (Saphar Laboratories, Ophirton, Johannesburg) physiological perfusion solution, and fresh heparinized human blood is added to a haematocrit of 30 to 35 per cent. The perfusate is mixed, warmed, and corrected to normal acid base balance.

Simultaneously, dissection of the pig liver occurs in our laboratory half a mile away, according to previously described techniques (6). After removal, the liver is flushed with 3 litres of chilled TisUsol, (4–6° C) and is transferred to the operating theatre submerged in this solution in a basin covered with a

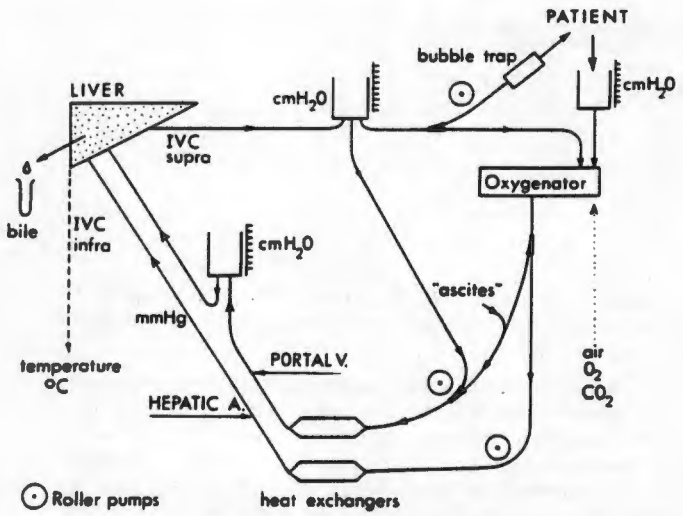

Fig. 1. A diagram of the circuit used to treat patients in hepatic coma by extracorporeal assist with a pig liver.

sterile cloth. The organ is immediately inserted into the circuit and rewarming commences. Half- to three-quarters of an hour of circulation is allowed for total stabilization of the flow and correction of the acid base status before connection with the patient. Once connected to the circuit, the patient's circulation and homeostasis were monitored by observation of the arterial and venous pressures, the electrocardiogram, and the acid-base and electrolyte status.

It was intended that perfusion would continue for at least 4 hours, but in all cases circulation had to be discontinued because of the appearance of bleeding from venepuncture and other sites. This complication will be discussed later.

CASE HISTORIES

General. All patients were isolated and nursed in an intensive care area. The conservative management included administration of systemic hydrocortisone 100 mg 6-hourly, and neomycin 1 g 4-hourly through a naso-gastric tube, which was also used for feeding a protein-free, high calorie diet. Dextrose infusion was given to maintain blood glucose, which was estimated at least 6-hourly, and frequent biochemical analyses were made of the electrolyte and acid-base status. Particular attention was paid to the plasma potassium level. A urinary catheter was inserted to assess urinary output. Antibiotics were given if infection supervened. The volume of blood used for exchange transfusion in all cases was five litres, as had been found effective previously (11). It is our policy to perform tracheostomy after the first exchange transfusion has temporarily and at least partially, corrected coagulation deficits. Three of these patients had this procedure. All personnel in contact with the patient donned gowns, gloves, and masks before entering the room.

Case 1

M. K., an African male of 11 years, with a history of two weeks' mild jaundice, became deeply jaundiced, drowsy, dizzy, and ataxic, and exchange transfusion was commenced. Despite a total of eight exchange transfusions, there was no improvement, and the patient was transferred 800 km to Cape Town for further treatment. On admission, he was assessed to be in deep hepatic coma with doll's eye movements and decerebrate response to painful stimuli. A 2.5 cm liver could be percussed. Before perfusion could be commenced, the patient suffered a respiratory arrest and after tracheostomy, was maintained on intermittent positive pressure respiration. Perfusion was commenced six hours after admission and was

continued for three and a half hours. No haemodynamic difficulties were encountered. Spontaneous respiration resumed one hour after perfusion commenced. Perfusion was discontinued because of oozing of fresh blood from the shunt site and buccal mucosa.

Despite two further exchange transfusions, the patient became hypothermic, then hypotensive, and died following gastrointestinal haemorrhage.

Autopsy revealed gross collapse of most of the liver with only one small regenerative nodule. The remainder of the liver was bile-stained. The general necrosis with minimal regeneration was confirmed histologically.

Case 2

M. A., a Coloured female of 34 years, with a three-week history of malaise and jaundice, became confused two days prior to admission. On admission, she responded to her name and to painful stimuli, but subsequently deteriorated and twelve hours later, despite one exchange transfusion, was hypertonic and deeply comatose, with no percussible liver dullness. Twenty-four hours later, a liver perfusion was commenced and after thirty minutes, the patient recovered consciousness completely and remained lucid and orientated for twenty minutes. Thereafter, she lapsed back into coma. Perfusion was discontinued after one and a half hours, owing to oozing of fresh blood from puncture sites. Three further exchange transfusions were performed at twenty-four hour intervals, but she developed a syndrome of consumptive coagulopathy which was treated with platelet-rich plasma. She remained unconscious except for two further periods of lucidity, the final one lasting three days. Haematemesis and malaena developed, and the patient died following inhalation. Her total stay in hospital was 19 days.

Autopsy showed brightly jaundiced regenerating liver alternating with deep red areas of necrosis. Histology confirmed centri-lobular necrosis. There were two sub-acute ulcers at the cardio-oesophageal junction of the stomach, and a chromophobe adenoma of the pituitary was noted.

In retrospect, a tracheostomy might have prevented the complication of inhalation which contributed to death.

Case 3

V. M., an African female of 26 years, with a five-day history of anorexia and vomiting was admitted jaundiced and uncooperative. Extensor plantar reflexes and confusion were the only neurological features noted. Six hours later she was deeply comatose, and an exchange transfusion was performed. Pig liver perfusion was advocated twelve hours later when a decorticate-type of response to painful stimuli was noted. Perfusion was continued for two hours and was discontinued because of bleeding. Despite another exchange transfusion, she became opisthotonic and another perfusion was performed, also for two hours and was similarly discontinued because of bleeding. This was followed by a further exchange transfusion, and within two days she was conscious although the liver remained impalpable. Ascites and melaena developed.

Another exchange transfusion was performed when the patient lapsed into coma once again; this was perhaps related to gastrointestinal haemorrhage which persisted. The liver edge became just palpable, but the patient developed menorrhagia and massive haematemesis and died.

Autopsy showed inhalation and a lung abscess from which Klebsiella pneumoniae was cultured. Most of the liver showed total necrosis, but in the left lobe, there were three areas of regeneration. The clinical bleeding tendency was confirmed by a massive haemorrhage into the broad ligaments, a subarachnoid haemorrhage, and multiple petechial haemorrhages.

Some biochemical and haematological features of this case are shown in Figure 2. Similar features were seen in the other cases described.

Case 4

J. v. d. M., a Caucasian female of 17 years, suffered jaundice for five weeks, and while being treated at bed-rest, became comatose.

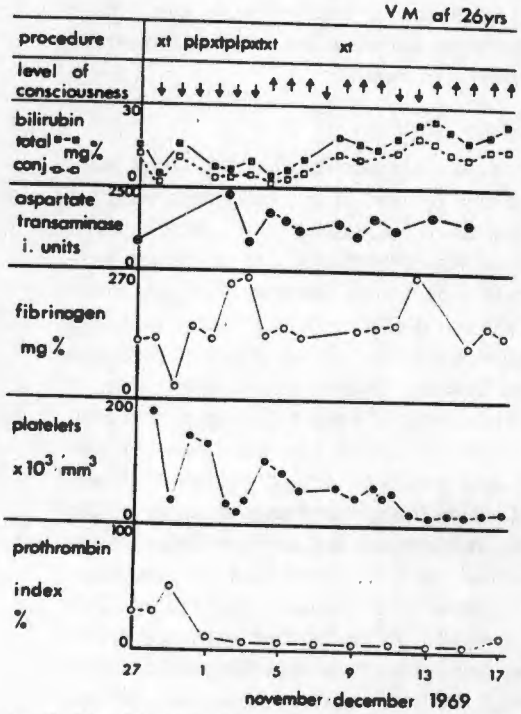

Fig. 2. The biochemical and haematological findings in Case 3. xt refers to exchange transfusion and plp to pig liver perfusion.

Decorticate movements were noted on admission to hospital. After two exchange transfusions, there was no improvement in the level of consciousness, the EEG was iso-electric, and she suffered two generalized convulsions and a respiratory arrest complicated by two cardiac arrests from which she was resuscitated. Pig liver perfusion was performed for two hours and was discontinued because of bleeding from the shunt site. There was no alteration in the level of consciousness or the general condition of the patient, and she died 48 hours later.

Autopsy showed coarse nodularity of the liver representing regeneration, but pale, dead liver stroma predominated. The pituitary showed recent infarction and a chromophobe adenoma.

Pig liver perfusions

During perfusion, the livers functioned the same as the isolated perfused preparation in the laboratory (5). Bile production tended to fall over the course of the perfusion, but bilirubin was seen to be excreted in the ascitic fluid exuding from the liver surface. Owing to the complexity of the procedure, little monitoring of the liver was performed except for the flow rates and pressure recordings, and the maintenance of the acid base balance. Biochemical analyses of the effluent from the liver showed equilibration of the prime with the patient's blood in all cases within the first half-hour of link up. No transhepatic studies were performed.

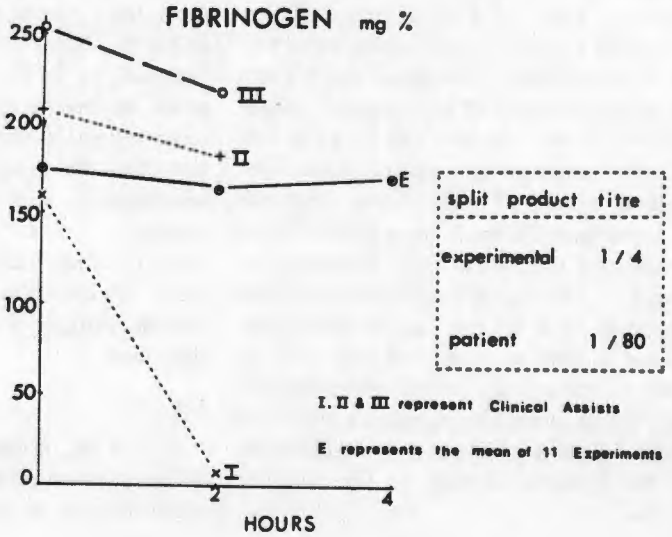

Fig. 3. The levels of fibrinogen and fibrinogen degradation products as obtained from nine experiments.

Figure 3 shows the results in two cases (2 and 3) of heparinization of the patient in addition to the circuit as compared with case 1 where only the circuit was heparinized. In cases 2 and 3, fibrinogen was also given during perfusion. For comparison, the mean results of 9 isolated perfusions of the pig liver with human blood in the laboratory are shown. There is no apparent fall in fibrinogen levels, nor do split products of fibrinogen appear as they do in the clinical situation.

DISCUSSION

The clinical course of the patients presented exemplifies the majority of patients with acute hepatic failure currently being encountered at this hospital. In all four patients, the overall clinical course was one of a steady decline, which appeared to be materially unaffected by intensive care and exchange transfusion. Under such circumstances, pig liver perfusion was offered as an additional therapeutic measure.

In the cases described, only in patient M. A. did there seem to be a direct relationship between the use of pig liver perfusion and a striking temporary improvement in the level of consciousness. Nonetheless, the theoretical advantage of the use of the liver remains – whether the function of the organ be to remove whatever toxins result in hepatic coma, or to provide substrate not manufactured by the necrotic liver.

It should be noted that three of our patients had normal levels of aspartate transaminase at death. This may reflect the severity of necrotic damage, suggesting that no further cells could be destroyed to result in elevation of this enzyme.

It is also of interest that two of the four patients showed a chromophobe adenoma on sectioning of the pituitary, and in one there was evidence of infarction of the gland.

Coagulation disturbances

In the circuit described, an oxygenator acts additonally as a reservoir and ensures adequate blood flow to the liver. Patients treated in this way are often in a terminal state with poor cardiac output, which could result in a poor blood supply to the liver if the circulation were unassisted. However, the oxygenator may cause thrombocytopoenia which results from direct blood/gas contact. This complication is of particular significance in a patient whose levels of prothrombin and fibrinogen are already low. Thrombocytopoenia and low plasma fibrinogen were observed in all four patients. In the longest survivor, V. M., this thrombocytopoenia was striking and progressive. It is possible that in the course of the multiple transfusions with platelet-rich plasma, this patient developed anti-platelet antibodies which would have aggravated the tendency to thrombocytopoenia. It has recently been shown that H-LA compatibility is of great value in ensuring survival of platelets under such conditions (4).

Thrombocytopoenia is a recognized complication of the use of this form of oxygenator in cardiopulmonary bypass (3). However, the duration of such a bypass is shorter than that of perfusion and in addition, the cardiac patient does not usually have other coagulation defects.

Rake et al. (8) describe spontaneous thrombocytopoenia developing in patients with massive liver cell necrosis before any form of therapy is undertaken. This is thought to result from consumptive coagulopathy and fibrinolysis following the liver necrosis. The results of additional heparinization as shown in Figure 3 tend to confirm this hypothesis.

An immunological factor may also be incriminated (7). In any form of rejection phenomenon, platelet deposition may occur on endothelial surfaces, and this form of xenograft of human blood into pig liver in the experimental model might well result in sequestration of platelets in the liver. This could be the explanation of the thrombocytopoenia in the cases of the circuits used by Abouna et al. (1), where no oxygenator was used, and Rö & Flatmark (9), where the level of platelets still declined.

However, the trauma of the oxygenator still remains a challenge for solution of the circuit

described above is to be used. At present we have stopped offering pig liver perfusion for treatment of patients in hepatic coma while studies are under way in the laboratory to attempt to reduce the trauma created by the oxygenator. In addition, the sequestration of platelets in the heterologous liver is under review.

In a recent paper, Abouna et al. (2) have demonstrated conclusively that whilst exchange transfusion may improve the coagulation disturbances, only pig liver perfusion materially influenced the neurological states in their patient, so that the latter may be the preferred treatment in some cases. Once the problems posed by thrombocytopoenia are overcome, we feel it desirable to persist with this form of treatment to evaluate fully its use in acute hepatic failure, a syndrome with a very high mortality.

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BABOON INTO PIG LIVER AND KIDNEY XENOTRANSPLANTATION*

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The possibility of using animal organs in human transplantation has great appeal, as suitable cadaver donors are surprisingly rare despite the analyses of Couch,[1] and Terblanche and Riddell[2] indicating a potentially adequate supply. The need to study xenotransplantation is particularly urgent in organs such as the liver which tolerate poorly the ischaemia that accompanies the procurement of human cadaver organs. To date minimal success has been attained in humans, with the exception of renal xenografts from primate to man.[3,4] There are as yet few published reports on experimental transplantation across a major species barrier, although such studies are essential as it is unlikely that sufficient primates will be available to meet the demand for human organ transplantation.

The pig has been widely used in experimental liver transplantation. It has been[5] demonstrated that a pig can tolerate a liver allograft from an unrelated donor without immunosuppression for as long as 2 years, while untreated pigs frequently demonstrate little clinical or histological evidence of rejection,[6-10] although they reject skin[11] and renal allografts normally.[12] The pig therefore appears to be an important and as yet unevaluated model in which to study liver xenotransplantation. Liver xenotransplantation has been studied by Calne *et al.*[13] who demonstrated that immunosuppressed baboons with pig liver xenografts can survive for up to 3½ days without gross evidence of liver damage, and by Gliedman *et al.*[14] who showed almost immediate cessation of function in heterotopic pig liver xenotransplants in dogs, although immunosuppressed dogs tolerated heterotopic fox liver xenotransplants for up to 22 days. The present study was undertaken to assess the fate of pigs with baboon liver xenografts. No immunosuppression was employed as baseline information on unmodified liver xenografts was not available in the literature. Baboon kidney xenografts in pigs were also studied for comparison.

*Date received: 23 March 1970.

MATERIAL AND METHODS

Orthotopic Liver Xenografts

The donors were adult baboons (*Papio ursinus ursinus*) weighing between 10 and 32 kg with an average of 18 kg. The recipients were either Landrace or crossed Large White/Landrace pigs weighing between 16 and 28 kg with an average of 22 kg.

Seven out of a total of 11 experiments were carried to completion with detailed histological and biochemical studies, and only these experiments will be discussed. The donor baboon liver was transplanted orthotopically into a pig using a technique essentially similar to that described for pig liver allografts,[26] with two differences, viz.: it was found to be unnecessary to bypass the vena cava in a pig, and long lengths of vena cava and portal vein were retained below the donor baboon liver, as a baboon's liver is significantly smaller than that of a pig of the same weight. The donor baboon liver was washed out with 4 - 6 litres of chilled balanced salt solution (Plasmalyte-B, Baxter Laboratories). Various hepatic arterial anastomoses were used including direct anastomosis, Carrel patch to aorta, an aortic segment and anastomosis to the right renal artery. In all cases the liver was observed for varying periods of time after revascularization before closing the animal's abdomen. This allowed the macroscopic changes in the liver to be assessed, and adequate biopsies to be performed at regular intervals. Both the donor and the recipient were heparinized and in the recipient this was usually not neutralized at the end of the operation. In an attempt to prevent an anticipated bleeding tendency Trasylol (Bayer) in doses varying between 50 000 and 250 000 units was administered intravenously to the recipients at the time of revascularization except in experiments 6 and 7. No immunosuppressive agents were employed. During the operation the animals received blood replacement with freshly obtained heparinized abattoir pig blood, and when necessary in the postoperative period they were given fresh citrated abattoir blood which was not cross-matched. Plasmalyte-B was administered intravenously during the operative procedure and in small volumes postoperatively. Glucose was given as indicated by blood sugar determinations with Dextrostix (Ames). Regular blood samples were taken for acid-base studies and sodium bicarbonate was administered as required (see Table I).

Liver-function tests were performed pre-operatively and at 2, 10 and 30 minutes and hourly after revascularization. Biopsy specimens of the donor liver were taken before removal from the baboon, after wash-out during the period of cold ischaemia, and at 2, 10, 20 and 30 minutes and where possible hourly after revascularization. In addition a further specimen was taken as soon after death as possible. Full autopsies were performed with minimal delay and tissues from the donor liver, the donor and recipient lymph nodes, the lungs, the heart and other organs were fixed in 10% formol saline. Paraffin-wax embedded 4-μm tissue sections were stained as a routine with haematoxylin and eosin. Where indicated, special stains for reticulin, fibrin, lipid and haemosiderin were performed.

Heterotopic Kidney Xenografts

Kidney grafts were taken simultaneously from 6 of the baboon donors and were washed out with 100 ml of chilled balanced salt solution (Plasmalyte-B) to simulate the situation in the liver transplants. These were transplanted into other Landrace or Large White/Landrace recipient pigs and placed in the right iliac fossa, using a plug-in technique with polythene tubes. These kidneys were observed for colour change, urine flow and blood flow until no longer functioning. One kidney was left *in situ* for 4 days. Biopsy specimens for light microscopy were taken from the kidney before removal from the baboon, after wash-out during the cold ischaemic period and at 2, 10 and 30 minutes and at 1, 1½, 2, 3 and 3½ hours after vascularization, as the cases permitted. The biopsies were performed in a similar manner to the liver biopsies.

RESULTS

Orthotopic Liver Xenografts

Survival times are best assessed from the time of revascularization, as many of the animals had their abdomens left open for several hours to determine the macroscopic changes in the liver and to enable the removal of specimens for biopsy. The postvascularization survival time varied from 2 to 10 hours (Table I).

One of the most striking findings was that the liver usually regained its normal colour immediately after vascularization, and remained normal during the period of observation. The only exceptions were in the first two experiments, where excessive handling led to traumatic subcapsular haematomata. Even at postmortem examination 4 livers still retained a normal appearance. However, biopsy specimens of these livers tended to disintegrate when placed in formol saline, probably indicating severe damage.

The changes occurring in the serial liver-function tests were similar to the early changes noted in pigs with autotransplanted and allotransplanted livers (unpublished data). A slight rise occurred in SGOT and alkaline phosphatase, and bilirubin remained unchanged. No haemolysis occurred. A striking feature was persisting and increasing metabolic acidosis after vascularization, despite

TABLE I. ORTHOTOPIC LIVER XENOGRAFTS

Experiment No.	*Survival*		*Metabolic acidosis indicated by sodium bicarbonate administered postvascularization*	*Postmortem findings*
	Postvascularization	*Postoperative*		
1	5 h 20 min	3 h 15 min	50 mEq	Moderate peritoneal haemorrhage
2	2 h	30 min	100 mEq	Nil abnormal
3	5 h	1 h 30 min	Not recorded	Right lung collapse
4	10 h	3 h	25 mEq	IVC clotted
5	6 h	4 h	200 mEq	Moderate peritoneal haemorrhage
6	3 h 57 min	3 h 5 min	400 mEq	Nil abnormal
7	4 h	3 h	174 mEq	Nil abnormal

regular correction with sodium bicarbonate, as can be seen by the amounts of sodium bicarbonate required. This was quite unlike our experience in the other groups of pigs already mentioned, where postvascularization acidosis was minimal and not progressive and sodium bicarbonate was not required.

Clinically the xenotransplanted animals developed a shock-like syndrome with blotchy cyanosed skin, poor peripheral circulation and tachycardia which progressed until death. Unlike allografted pigs which wake up promptly and move around in the early postoperative phase, these animals remained inactive and lethargic. Even hepatectomized pigs (unpublished data) and dogs[16] get up and walk around soon after the operative procedure.

Histology provided dramatic evidence of early liver cell death. The pretransplant biopsy specimens and those taken during the period of cold ischaemia showed no significant histological differences (Fig. 1). During the first 20 minutes after vascularization the histological

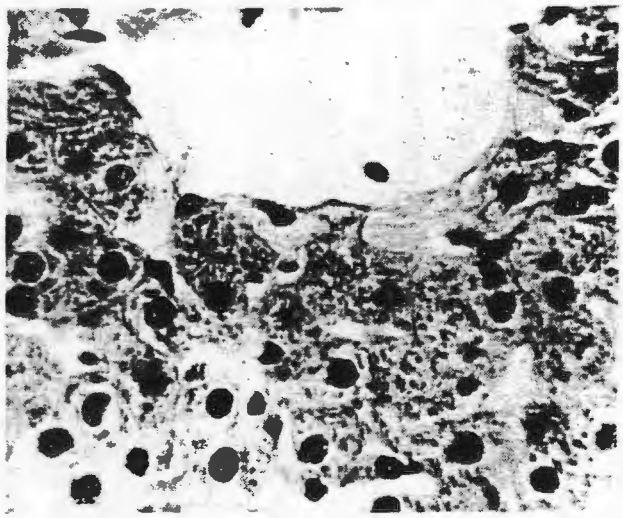

Fig. 1. Centrilobular region of normal baboon donor liver before transplantation (H & E × 670). Experiment 4.

changes were minimal and consisted of prominence of Kupffer cells due to acquisition of cytoplasm and a slight increase of polymorphonuclear leucocytes in the intralobular sinusoidal capillaries. These changes were noted in pigs with autotransplanted livers as well (unpublished data), and no specific significance can be attached to them. However, during the following 10 minutes sinusoidal congestion became evident, and intrasinusoidal polymorphonuclears were more frequent though they could not be regarded as numerous. Signs of liver cell damage also became manifest at this stage, presenting as fine non-lipid vacuolation of the cytoplasm of some liver cells, and as hyaline intracytoplasmic inclusions. Occasionally liver cells also showed nuclear pyknosis and cytoplasmic shrinkage which characterizes death of parenchymal cells (Fig. 2). Whereas cells showing cytological features of necrosis were barely present after 30 minutes, they were easily demonstrable after 1 hour. From 1 to 2 hours after vascularization, a definite pattern emerged which did not vary in nature but increased in intensity with the prolongation

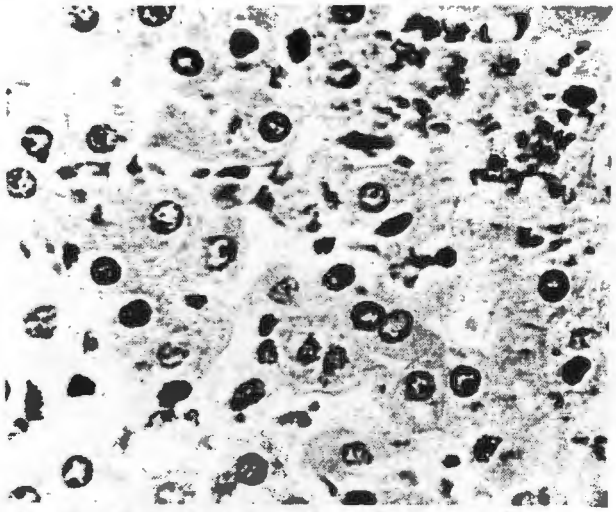

Fig. 2. Baboon donor liver biopsy 30 minutes after transplantation. Sinusoidal congestion is evident and occasional liver cells manifest pyknotic nuclei and shrunken cytoplasm (H & E × 670). Experiment 4.

of survival time, being most noticeable in the pigs that died between 5 and 10 hours after vascularization. The ultimate picture consisted of: (*a*) a notable degree of sinusoidal congestion and associated disintegration of capillary walls; (*b*) mild to moderate polymorphonuclear leucocyte response confined to the sinusoidal lumina; (*c*) liver cell damage in its mildest form manifesting as cytoplasmic vacuolation and in its severest form as the nuclear pyknosis and cytoplasmic shrinkage of cellular death; (*d*) dissociation of liver cells which became free-lying and no longer confined to the columns and trabeculae of the normal lobule; and (*e*) disintegration of liver cells appearing as faintly stained cytoplasmic remnants lying within disrupted sinusoids or between degenerate and dead liver cells (Figs. 3 and 4). None of these changes affected the liver uniformly, but they were patchy in distribution with no constant lobular relationship, and varied in intensity from one area to another. While altered liver cells were easily recognizable in the final stages, it was also clear that the majority of cells showed no morphological abnormalities. In two livers there was a minimal degree of liver and Kupffer cell siderosis which had obviously anteceded the operation. Notably platelet or fibrin thrombi were not evident in the vascular channels. Classical signs of cellular rejection were not seen in any of the sections.

Detailed postmortem examinations (see Table I) revealed little of note with the exception of the liver findings already mentioned. Moderate haemorrhage occurred in experiments 1 and 5 only, and in the former it arose from subcapsular haematomata in the traumatized liver. Even when Trasylol was omitted in the last two experiments no haemorrhage occurred. Additional factors contributing to death were collapse of the right lung in experiment 3, and thrombosis of the inferior vena cava in experiment 4. The regional lymph nodes all showed histological evidence of haemorrhage into the peripheral subcapsular sinuses; activation of the sinusoidal lining cells; intense polymorphonuclear leucocyte response in the sinuses; and evidence

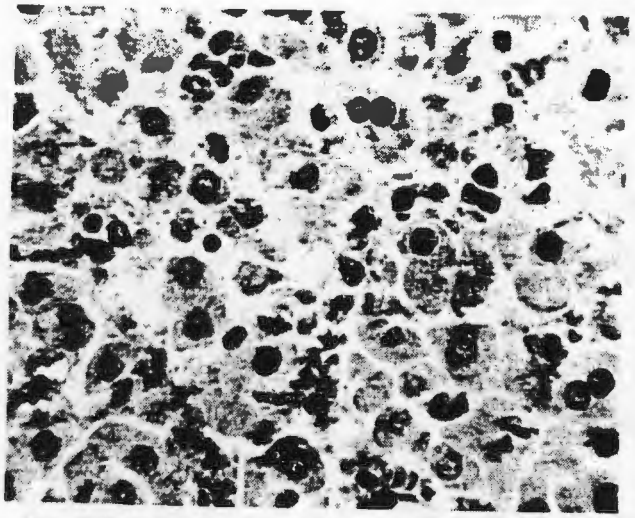

Fig. 3. Baboon donor liver biopsy 3 hours after transplantation. Advanced changes are present which are characterized by nuclear pyknosis and disintegration of liver cells, sinusoidal disruption and polymorphonuclear leucocyte infiltration (H & E × 670). Experiment 4.

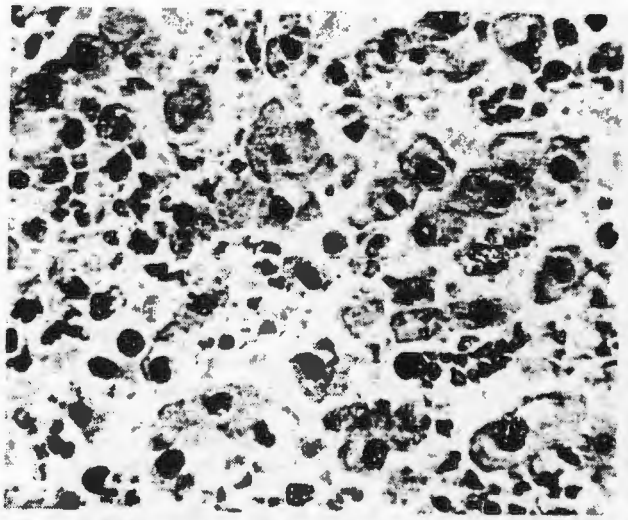

Fig. 4. Baboon donor liver at autopsy 10 hours after transplantation. Signs of liver cell death are more obtrusive than in Fig. 3. In addition there is complete disruption of liver cell trabeculae with necrotic and degenerate liver cells lying free among red blood cells and polymorphonuclear leucocytes (H & E × 670). Experiment 4.

of necrosis of lymphoid and reticular cells. Histological changes in other organs, such as the heart, lungs, kidneys and skeletal muscles were insignificant.

Heterotopic Kidney Xenografts

Five of the 6 kidney xenografts were observed for prolonged periods after vascularization and the sixth was left *in situ* and the pig's abdomen closed. On the fourth post-transplant day the kidney was removed for histology and was found to be autolysed.

Unlike the liver, which remained macroscopically normal in most instances until the animal's death, the kidneys became dark and blotchy in 8 - 12 minutes, and all the kidneys became pitch-black between 30 and 60 minutes. In only 1 of the 6 was there any urine flow, which was short-lived and contained a large amount of protein. Blood flow continued for about 30 minutes and stopped thereafter.

As with the liver xenografts, the regular biopsies provided a clear picture of the morphological changes that occur in this kidney xenograft model. The pre-transplant biopsy specimen and that taken during the period of cold ischaemia showed no significant histological differences. After revascularization little change was noted for the first 10 minutes. At the end of this time amorphous, eosinophilic, protein material became noticeable in the tubular lumina. After 30 minutes this material became more abundant and small numbers of polymorphonuclear leucocytes were noted in the peritubular capillaries and in those of the glomerular tufts. After 1 hour capillary congestion was pronounced, moderate numbers of polymorphonuclears occurred in the capillary lumina, proteinaceous material was abundant in the tubular lumina and the tubular epithelial cells showed commencing evidence of nuclear pyknosis indicative of cell death (Fig. 5). Terminally these changes became more severe. While evidence of tubular epithelial necrosis was observed, the most obtrusive and dominant changes were glomerular and peritubular capillary engorgement and protein precipitation into the tubular lumina. No platelet or fibrin thrombi were noted in the vessels. As in liver xenografts, classical signs of cellular rejection were not seen in any of the sections.

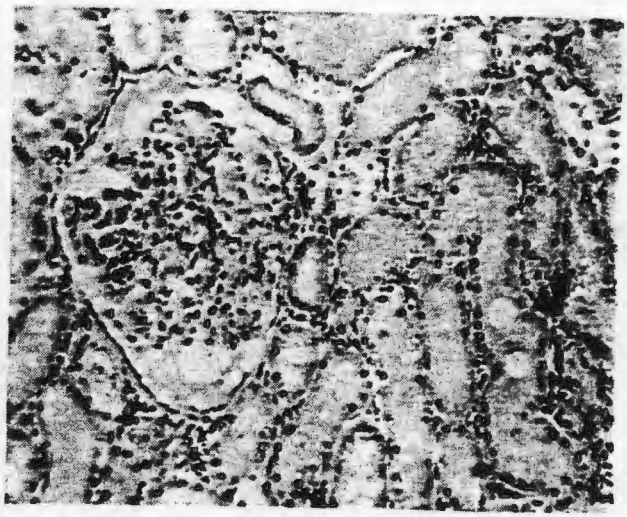

Fig. 5. Baboon donor kidney 1 hour after transplantation. The glomerular and peritubular capillaries are engorged with blood and the tubular lumina are filled with protein (H & E × 160). Experiment 5.

DISCUSSION

Evidence is accumulating that early acute rejection occurs in kidneys transplanted across a major species barrier, and that this rejection is probably due to the presence of naturally-occurring heterospecific antibodies in the recipient,[17-19] and that both humoral antibodies and complement are required as immunological effectors.[17] Thus it is

not surprising that standard immunosuppression appears to be ineffective in preventing this acute rejection,[19] but some prolongation of survival has been obtained by repeated kidney grafts and by antigen pretreatment.[27,28] The present study provides a clear picture of the morphological changes that occur in the xenograft model of a baboon kidney transplanted into an unmodified pig, and will serve as a baseline for our future studies.

Liver xenotransplantation, on the other hand, is a virtually unstudied field and the main aim of the kidney xenografts was to provide a control for the liver xenograft experiments. Pig livers used for extracorporeal hepatic assist in humans with liver failure have been demonstrated to function satisfactorily for several hours in this xenograft model.[20-22] Calne *et al.*[23] reported on 7 baboons with orthotopic pig liver xenografts, some of which were on immunosuppressive regimens. These baboons survived for surprisingly long periods of time with pig liver xenografts and did not show the gross evidence of early organ death that would have been expected in a xenograft across a major species barrier. Three of these baboons survived for 30 hours or longer and one for 3½ days. The main histological finding was centrilobular necrosis of hepatocytes, but in the long-term survivor the hepatocytes were well preserved and there was mononuclear cell infiltration of the portal tracts. Gliedman *et al.*[24] have demonstrated that blood flow through heterotopic pig liver xenografts in dogs rapidly ceases, whereas when the liver xenograft is obtained from the closely related fox, it functions for periods of up to 22 days.

In the present study survival varied between 2 and 10 hours after revascularization of the liver graft. The normal appearance of the livers was in marked contrast to the kidneys which became dull and blotchy after 8 - 12 minutes and pitch-black between 30 and 60 minutes. Histological changes in the liver bore little relation to the macroscopic appearance. Early signs of liver cell damage were manifest by 30 minutes. By 1 hour cytological evidence of liver cell necrosis was easily demonstrable, and increased in intensity with prolongation of survival. Classical signs of cellular rejection were not seen.

Thus early acute rejection occurred in unmodified pigs with orthotopic baboon liver xenografts. The nature of this rejection suggests that it might be mediated by naturally occurring heterospecific antibodies, although this aspect still remains to be investigated. The lack of early acute rejection in Calne's baboons with pig liver xenografts is somewhat surprising and one wonders whether the pig liver, which is so well tolerated as an allograft in the pig[6,7-10,12] and as an extracorporeal xenograft hepatic assist in man,[20-22] has some peculiar properties. This should provide a fruitful line for future research.

All the animals in the present study were inactive and lethargic until death and demonstrated a progressive and marked metabolic acidosis, unlike the baboons reported by Calne *et al.*[23] which woke up rapidly after the procedure, and 4 of which were active and alert the day after operation. This inactivity and acidosis probably reflected liver cell death, rather than a non-functioning liver *per se*, as hepatectomized pigs wake up and walk around soon after the operative procedure (unpublished data).

Trasylol was administered in the first 5 experiments because of the fear of a bleeding tendency in animals that were likely to have early acute rejection of their livers. However, bleeding was not a major problem, and so Trasylol was omitted in the last 2 experiments without a bleeding tendency developing. It is interesting that haemorrhage was a problem in the xenografts performed by Calne except where human fibrinogen was administered.[23] Once again, this discrepancy is difficult to explain.

The universal lack of sufficient suitable human liver donors to meet the increasing demand for liver transplantation makes a study of xenotransplantation imperative. The present study indicates the magnitude of the problem, and provides baseline information on liver xenotransplantation across a major species barrier. In this model it will be necessary to study means of overcoming early acute xenograft rejection to determine if long-term xenograft function is feasible.

SUMMARY

Liver xenotransplantation is a virtually unstudied field, despite its potential importance, as there appear to be insufficient human liver donors to meet the demand for liver transplantation. A detailed study of 7 unmodified pigs with orthotopic baboon liver xenotransplants is presented. The animals all died within hours and this has been attributed to acute xenograft rejection. Although most of the livers appeared normal macroscopically, repeated biopsies revealed commencing morphological evidence of liver cell death within 30 minutes after revascularization. This increased in intensity in the ensuing hours and was associated with a progressive metabolic acidosis and failing circulation until death. A simultaneous study of unmodified pigs with baboon kidney xenografts which showed acute xenograft rejection is presented for comparison.

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PIONEER 28081

PART 4.

HEAT HOMEOSTASIS DURING ANAESTHESIA.

Inadvertent Hypothermia during Anaesthesia.

Induced Hypothermia during Anaesthesia.

Brit. J. Anaesth. (1960), 32, 60

TEMPERATURE CHANGES IN CHILDREN DURING GENERAL ANAESTHESIA

BY

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THE state of anaesthesia depresses and renders inefficient the temperature regulating mechanisms of the body. Placed in conditions of high ambient temperature with which its deranged compensatory mechanisms cannot adequately cope, the body temperature of the anaesthetized patient has been shown to rise. This is especially so in children in whom there are the additional factors of small body mass, high internal heat production and immature heat regulatory mechanisms. The foregoing and the evil effects of hyperpyrexia in the anaesthetic state—notably convulsions and death—are well documented (Knight, 1942; Bigler and McQuiston, 1951; Searles and Lenahan, 1952; Clarke, Orkin and Rovenstine, 1954).

The air-conditioning of operating theatres, as originally recommended by Huntington in 1920 (Searles and Lenahan, 1952), is a logical solution to the problem of heat accumulation during anaesthesia and is now widely accepted in the normal design of operating theatres. There are, however, no published observations on the body temperatures of subjects anaesthetized in such surroundings.

The advent of halothane which appears to cause a more profound disturbance of the body heat regulatory mechanism than does ether, the agent most commonly used in the papers referred to, provided a further stimulus to this study.

We report here on a statistical analysis of observations of body temperature made on 248 infants and children undergoing general anaesthesia and surgery. The rectal temperature, which was recorded with a low reading mercury thermometer, was taken as the most reliable indication of body temperature (Machle and Hatch, 1947) and is used exclusively in this study.

CONDITIONS AND METHODS

The operating theatre.

The operating theatre in which these observations were made is air-conditioned. Air is passed into the theatre from central overhead louvres at a rate of 900 cubic feet per minute, passing out through louvres in the wall of the theatre. Humidity is controlled by "dewpoint" control. The conditioning was set at 75°F with relative humidity of 75 per cent. The actual theatre temperature was recorded in every case with a mercury dry bulb thermometer fixed to the theatre wall. Relative humidity was calculated from this and that recorded by a mercury wet bulb thermometer. The actual temperature and humidity did not vary widely from the limits of setting of the air condition automatic control. Observations extended over both winter and summer.

The drapes.

The standard draping of patients for operations was as follows:

Lower part of body	1 macintosh, 1 sheet.
Chest and arm	2 sheets.
Neonates:	In addition to the above, neonates were insulated with rolls of cotton wool placed along their sides.

The observations made.

The following observations were made on each patient:

Weight and length:

From these the *surface area* was calculated, using the tables of Du Bois and Hannon (Du Bois, 1936).

Age.

Pre-operative and postoperative temperatures: From these the change in temperature was calculated.
Nature of the operation.
Duration of operation and anaesthesia.
Volume of blood replaced.

Anaesthesia.

(1) Halothane group: 166 cases were anaesthetized with halothane, nitrous oxide and oxygen.
(2) Miscellaneous group: 82 cases were anaesthetized with nitrous oxide and oxygen and ether and/or cyclopropane or trichloroethylene. Of these the majority were given ether. In this group 17 cases had anaesthesia induced with halothane, administered for up to 5 minutes.

The agents were administered by means of a modified T-piece system with mask or endotracheal tube, utilizing a high flow of fresh gases, 6 litres per minute. For thoracic and abdominal operations an I.P.P.R. technique was used; the adjuvant use of relaxant drugs, though necessary in the miscellaneous group, was seldom necessary in the halothane group. Thiopentone sodium was used for induction of anaesthesia in very few cases.

Premedication.

All cases with the exception of half of those in the miscellaneous group, who were given scopolamine, were given atropine sulphate on a dose/weight basis. In addition to this some of the patients were given pethidine or papaveretum also on a dose/weight basis.

RESULTS

The observations of change in the body temperature were submitted to statistical analysis in relation to the various factors recorded that would influence the body temperature. The results of such analysis are submitted as histograms.

General results.

The range of age in our cases was from premature newborn to 11 years. The weight range in these cases was from 3 lb. 12 oz. to 96 lb. The range of duration of anaesthesia was from 20 minutes to 360 minutes.

Temperature.

There was a mean fall of temperature in all cases as follows (see figure 1 and table I).

Halothane group: Mean fall 2.1°F (range: −8.4°F to +2°F).

Miscellaneous group: Mean fall 1°F (range: −10°F. to +3°F).

The difference is statistically highly significant.

Detailed Analysis of Temperature Change with Relation to Various Influential Factors.

Theatre temperature.

The theatre temperature and humidity are the most important factors in causing a rise or fall of the temperature of the anaesthetized patient. We stress again that our theatre was air-conditioned and, though the temperature recorded ranged from 63°F to 81°F, the temperature in the majority of cases was between 71°F and 75°F. This is well within the range of ambient

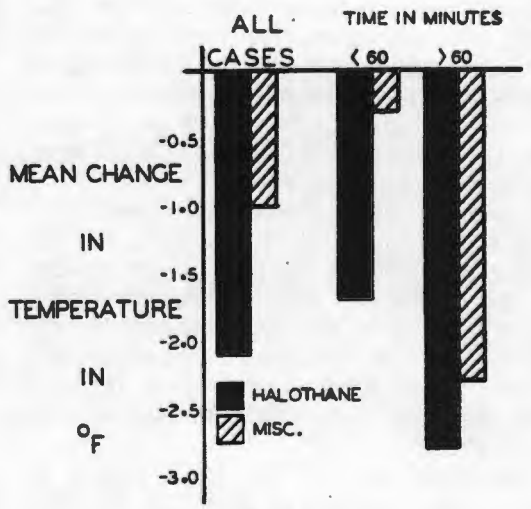

FIG. 1
General histogram.
Mean fall in body temperature in 166 patients anaesthetized with halothane, compared with 82 patients anaesthetized by other methods.

TABLE I (A)

Anaesthetic	Mean change in temp. degrees Fah.	S.D.	S.E.	Number of cases
Halothane	−2·1	1·90	0·15	166
Miscellaneous	−1·1	2·58	0·28	82

TABLE I (B)

Time in minutes	Mean change in temp. degrees Fah.		S.D.		S.E.		Number of cases	
	Halothane	Misc.	Halothane	Misc.	Halothane	Misc.	Halothane	Misc.
<60	−1·7	−0·3	1·58	1·34	0·15	0·18	107	53
>60	−2·8	−2·3	2·30	3·65	0·30	0·68	59	29

temperature at which loss of heat by radiation is highly efficient—i.e. loss of heat by evaporation is of less importance to the body, and is well below the temperatures recorded by other observers at which heat accumulation with all its unpleasant sequelae occurs (Knight, 1942; Bigler and McQuiston, 1951; Searles and Lenahan, 1952; Clarke et al., 1954). The results of an analysis of temperature change in the patient in relation to theatre temperature are reflected in figure 2 and table II. There is an increased fall in patient temperature the lower the theatre temperature. The differences are significant only in the halothane group in our observations. A histogram is submitted for this group only. Calculation of the correlation coefficient (0.4) revealed no linear relationship.

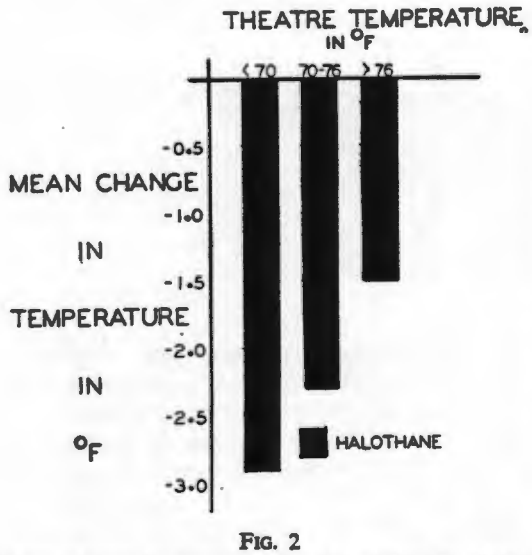

FIG. 2
Mean change in temperature with relation to theatre temperature.

TABLE II

Theatre temp. degrees Fah.	Mean change in temp. degrees Fah.	S.D.	S.E.	Number of cases
<70	−2·9	2·10	0·51	17
71–75	−2·3	1·87	0·19	96
>76	−1·5	1·89	0·26	53

Humidity.

Because of the air conditioning, the range of humidity recorded in this series was relatively narrow, 65–87 per cent, with the majority falling in the range 71–75 per cent. In no case was the actual wet bulb temperature above 75°F, the temperature stressed by Clarke et al. (1954) above which they most consistently observed heat accumulation. Bearing in mind the theatre temperatures recorded in our cases it is hardly surprising that analysis of our cases revealed no relation between the temperature change in the patient and the relative humidity.

Duration of anaesthesia.

Analysis of temperature change in the patient in relation to duration of anaesthesia is illustrated in figures 1 and 3 and tables I and III. There is an increasing fall in temperature with the passage of time. This correlation is not linear, correlation coefficient −0.4. With halothane especially there is a rapid initial drop in temperature whereupon the temperature achieves a plateau level, there being no statistical difference between the mean temperature change in the three arbitrary time groups up to 120 minutes. In operations extending beyond 120 minutes a further significant drop in temperature is evident. This latter group, however, consists largely of "smaller" babies to which reference is made in the sections following. The difference in fall in temperature between the halothane

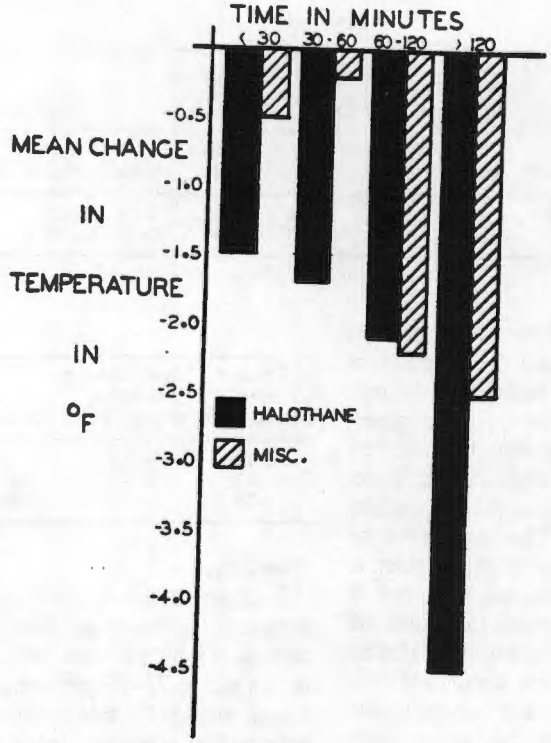

FIG. 3
Mean change in temperature with relation to time.

TABLE III

Time in minutes	Mean change in temp. degrees Fah.		S.D.		S.E.		Number of cases	
	Halothane	Misc.	Halothane	Misc.	Halothane	Misc.	Halothane	Misc.
<30	−1·5	−0·5	1·5	1·66	0·27	0·5	32	11
30—60	−1·7	−0·2	1·6	1·29	0·18	0·20	75	42
60—120	−2·1	−2·2	1·7	1·53	0·26	1·8	41	23
>120	−4·5	−2·5	2·7	4·2	0·64	0·28	18	6

group and that of the miscellaneous group is significant only for the period up to 60 minutes.

Weight.

Analysis of the change in temperature with relation to the weight of the patients (fig. 4 and table IV) reveals that there is a significantly greater fall in temperature in the infants and children who weigh less than 20 lb. than in those who exceed this weight. Whereas with these latter, the fall in temperature is significantly greater with halothane than in the miscellaneous group, there is no significant difference in the fall in temperature between the two groups in the infants who weigh less than 20 lb. Calculation of the correlation coefficient (0.4) reveals no linear relationship between fall in temperature and weight.

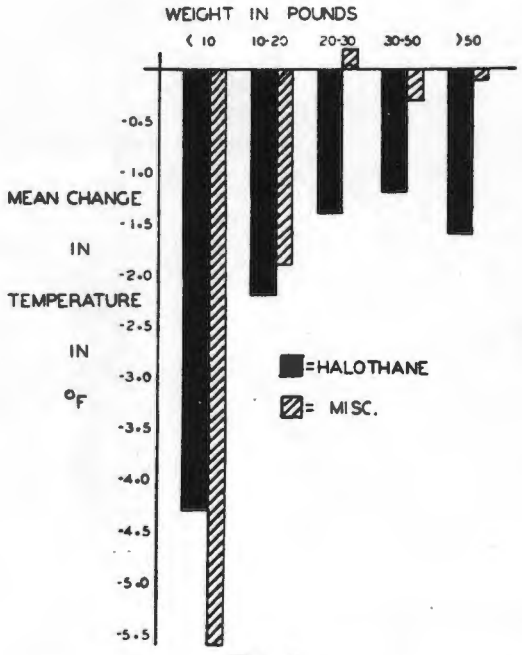

FIG. 4
Mean change in temperature with relation to weight.

TABLE IV

Weight in lbs.	Mean change in temp. degrees Fah.		S.D.		S.E.		Number of cases	
	Halothane	Misc.	Halothane	Misc.	Halothane	Misc.	Halothane	Misc.
<10	−4·3	−5·6	2·19	3·18	0·39	1·13	32	8
10—20	−2·2	−1·9	1·72	3·50	0·30	1·05	32	11
20—30	−1·4	+0·2	1·50	2·27	0·25	0·58	38	15
30—50	−1·2	−0·3	1·28	1·17	0·20	0·21	42	29
>50	−1·6	−0·1	0·81	1·12	0·17	0·25	22	19

Surface area.

The greater cooling of the smaller infants is again borne out by calculation of the mean fall in temperature in relation to surface area (see fig. 5 and table V). In this case the infants with a surface area of less than 0.3 sq.m cool to a significantly greater extent than those with a larger surface area. Again, in these latter, a plateau effect is evident. This lack of linear correlation is confirmed by a correlation coefficient of 0.4.

As it is thought that the degree of temperature change is influenced more by the ratio the mass of the child bears to its surface area than to these two factors separately, the cases were analyzed for temperature change in relation to the ratio surface area/weight. This analysis, however, revealed nothing not apparent in the foregoing analysis and is consequently not submitted.

Duration of anaesthesia and weight of infant.

The relation of the temperature change to the duration of the anaesthetic and to the size of the infant elicited in the foregoing analyses is further amplified when the observations of change in

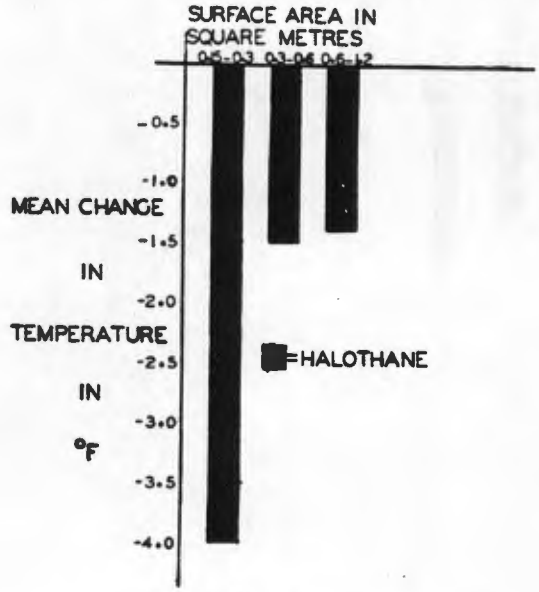

FIG. 5
Mean change in temperature with relation to surface area.

TABLE V

Surface area in sq. metres	Mean change in temp. degrees Fah.	S.D.	S.E.	Number of cases
0·15—0·3	—4·0	2·09	0·32	43
0·3 —0·6	—1·5	1·60	0·22	54
0·6 —1·2	—1·4	1·14	0·16	69

temperature are broken down to reflect both these factors simultaneously (see fig. 6 and table VI). In this analysis the cases are divided as follows:

(1) Children weighing less than 20 lb.
(2) Children weighing more than 20 lb.

These groups are then subdivided on the basis of the duration of anaesthesia: (*a*) anaesthesia of a duration less than 60 minutes; (*b*) anaesthesia of a duration longer than 60 minutes. It is

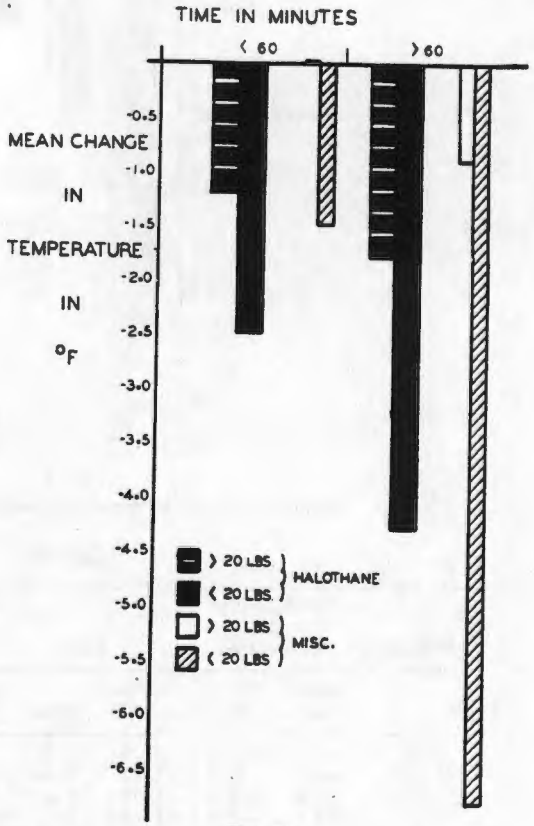

FIG. 6
Mean change in temperature with relation to time and weight.

TABLE VI

Time in minutes		Mean change in temp. degrees Fah.		S.D.		S.E.		Number of cases	
		<20 lbs.	>20 lbs.	<20 lbs.	>20 lbs.	<20 lbs.	>20 lbs.	<20 lbs.	>20 lbs.
<60	Halothane	—2·5	—1·2	1·9	1·30	0·27	0·16	39	68
	Misc.	—1·5	+0·1	1·7	0·98	0·50	0·15	12	42
>60	Halothane	—4·3	—1·8	2·6	1·24	0·49	0·22	28	31
	Misc.	—6·8	—0·9	4·12	2·04	1·60	0·45	7	21

apparent that, regardless of the anaesthetic, not only do the smaller infants have a greater fall in temperature than the larger, but this fall is progressively greater with increased duration of anaesthesia. The use of halothane produces a significantly greater fall in temperature in the larger children than occurs in these children in the miscellaneous group.

Site of operation.

On the site of operation depends the extent to which the patient is exposed. Analysis of our observations of change of temperature with relation to the site of operation reveals an increased fall in temperature with increased exposure of the child particularly of viscera as occurs in abdominal surgery (see fig. 7 and table VII).

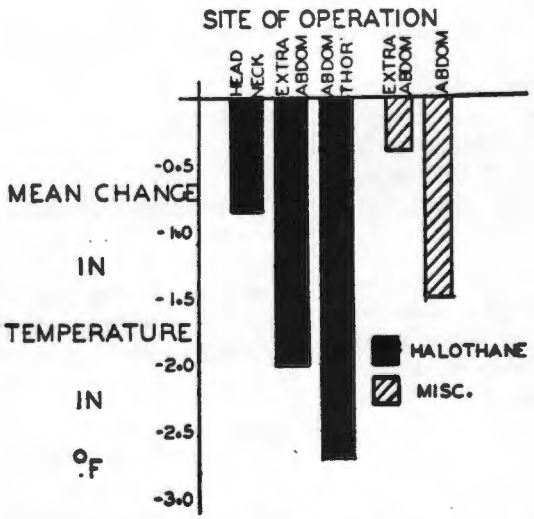

FIG. 7

Mean change in temperature with relation to site of operation.

TABLE VII

	Site of operation	Mean change in temp. degrees Fah.	S.D.	S.E.	Number of cases
HALOTHANE	Head and neck	−0·8	1·13	0·18	38
	Extra abdominal	−2·0	1·17	0·18	42
	Thoracic and abdominal	−2·7	1·97	0·21	85
MISC	Extra abdominal	−0·4	2·29	0·37	38
	Abdominal	−1·5	2·69	0·40	44

Pre-operative temperature.

Bearing in mind the possible part pre-operative pyrexia plays in the production of hyperthermia and "ether convulsions" we analyzed our observations of change in temperature during operation in relation to the patient's pre-operative temperature. This latter we arbitrarily divided into three groups as follows:

(1) Less than 98°F—hypothermic.
(2) 98° and 99°F—normothermic.
(3) 100°F and over—pyrexial.

Figure 8 and table VIII show that, under the conditions of theatre temperature and humidity described, the pre-operative temperature of the patient had no effect on the mean change in temperature which was nevertheless a fall. (Though there is a difference of 1°F between the hypothermic group and the others, this is not quite statistically significant.)

Blood replacement.

The transfusion into the patient of cold blood can be expected to lower the patient's body temperature. Analysis of our cases on this basis confirms this (see fig. 9 and table IX). The mean fall in temperature is 1.6°F greater in those cases that had a blood transfusion than in those that did not. It must be pointed out, however, that those patients who received blood were, by and large, the smaller babies having prolonged operations—two factors which of themselves cause a fall in temperature in our cases.

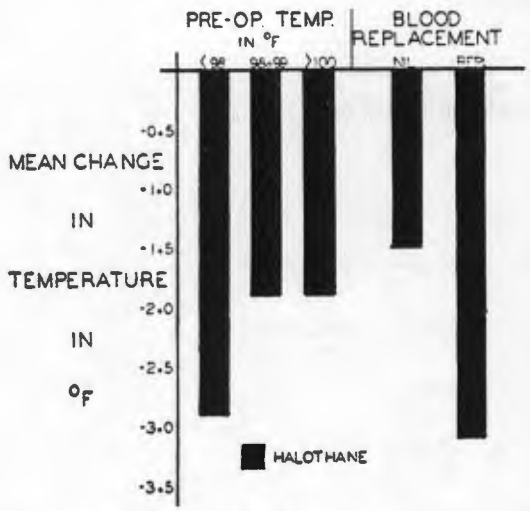

FIG. 8
Mean change in temperature with relation to pre-operative temperature.

FIG. 9
Mean change in temperature with relation to blood replacement.

TABLE VIII

Pre-operation temp. degrees Fah.	Mean change in temp. degrees Fah.	S.D.	S.E.	Number of cases
<98	−2·9	2·78	0·50	31
98 & 99	−1·9	1·76	0·17	103
>100	−1·9	1·13	0·20	32

TABLE IX

Blood replacement				
Nil	−1·5	1·70	0·17	105
Blood replaced	−3·1	2·18	0·29	56

Neonates.

In this series there were 16 neonates. All except one were anaesthetized with halothane, nitrous oxide and oxygen. Duration of anaesthesia in 11 of these cases was longer than 120 minutes, and in the remaining 5 duration of anaesthesia was from 60 to 120 minutes.

In addition to the standard draping described, 5 of these little patients were placed on the operating table on hot-water bottles containing water at 101°F. Whereas in the 11 not so treated the mean fall in temperature was 5.9°F (S.D. 2.3, S.E. 0.69), that in the 5 placed on hot-water bottles was 3.2°F (S.D. 2.9, S.E. 1.6). The mean end anaesthetic temperature of the two groups was 89.6°F and 93.5°F respectively. We are of the opinion that though some fall in temperature is in all probability beneficial during anaesthesia, neonatal temperatures ought to be monitored as the fall is often precipitous and falls of 6°F to 8°F are not uncommon. The temperature should not be permitted to fall below 90°F. One of our cases in whom the temperature dropped to 84°F developed hypothermic apnoea, respiration only being resumed on warming. A further danger

of extreme falls in temperature is the possible onset of sclerema (Rees, 1958).

SUMMARY

We report here on a statistical analysis of observations of body temperature made on 248 infants and children undergoing general anaesthesia and surgery. These observations were made in an air-conditioned operating theatre.

There was a mean fall in temperature during general anaesthesia. This was 2.1°F in the group of cases anaesthetized with halothane and 1°F in a group anaesthetized with other anaesthetics.

The benefit of the air conditioning of the operating theatre is stressed. Over the observed range of theatre temperatures there was a positive relation between this and the patient's change in temperature. In this range of theatre temperature the comparatively narrow range of relative humidity observed had no bearing on the change in temperature of the patient.

Small infants (less than 20 lb.) cool to a significantly greater extent than the larger and this fall is progressive with time. The larger infants (more than 20 lb.) after an initial rapid fall in temperature (especially with the use of halothane), attain a relatively static temperature. Similar conclusions follow an analysis of the fall in temperature in relation to the patient's surface area.

The fall in temperature is related to the site of operation: the greater the exposure, especially of viscera, the greater the fall in temperature.

The presence of pyrexia before operation had no bearing on the change in temperature during anaesthesia.

The transfusion of blood led to an increased fall in temperature.

The mean fall in temperature in neonates was 5.9°F. When hot-water bottles at 101°F were placed under the patient, the mean fall was 3.2°F.

ACKNOWLEDGMENTS

We wish to express our gratitude to Drs. Weinreich and Malherbe who collected much of the data, and to Professor Pollard of the University of Cape Town for statistical advice. We also thank Dr. J. Mostert, Superintendent of the Red Cross War Memorial Children's Hospital, for permission to publish these observations, and Professor J. H. Louw and the surgical staff for their patience and co-operation.

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Method for the Safe and Rapid Pretransfusion Warming of Stored Blood: An In Vitro and In Vivo Evaluation of a Radiofrequency (RF) Instrument

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Inadvertent hypothermia due to massive infusion of stored blood can be prevented by pretransfusion warming. One approach is the heating of individual packs by means of electromagnetic conduction, which is a method safely used over the last 25 years. The prototype instrument, which has now been re-engineered, can effectively raise the temperature of a unit of blood to approximately 33°C in less than 3 minutes. Using this new model, we found, in vitro, a modest increase in free plasma haemoglobin, but this was not accompanied by any change in potassium or lactic dehydrogenase levels and the mean red cell fragility was unaltered. In vivo, the survival of autologous red cells that had been stored for 33 days and then infused as a concentrate, having a mean haematocrit of 0.60, was measured at 24 hours and 21 days. Each donor acted as his own control. In paired studies, pretransfusion radiofrequency heating was shown to have no deleterious effect when compared to measurements using the unwarmed blood pack. It is concluded that this method can be recommended as safe.

Key words: hypothermia, large volumes, red cell survival, transfusion

INTRODUCTION

Inadvertent hypothermia, a major hazard of rapid or massive transfusion defined as greater than 1.5 mL/kg/minute, can be prevented by pretransfusion warming of the blood [1,2]. This can be achieved by direct heat exchange systems applied to the transfusion line or, alternatively, by warming the unit using electromagnetic radiation in both the radio (RF) and microwave frequencies [3,4]. The original Taurus 300 (Plessey, Cape Town, South Africa) utilised a 27 MHz RF energy source [5]. Initially designed to heat blood that was stored in bottles, subsequent modifications accommodated polyvinylchloride (PVC) bags [6,7] and these instruments have been used safely for a quarter of a century in South African hospitals. Advancing technology and employment of red cell concentrates rather than whole blood, on which the safety of RF heating and its influence on erythrocyte integrity had originally been tested [5,7,8], have led to the introduction of a re-engineered system designated the Taurus 301 (CMEI, Cape Town, South Africa). Concern has been expressed that when individual units of blood are warmed by energy transference from an electromagnetic beam, erythrocyte damage may result from either radiation or hot spot formation due to uneven heating of the PVC containers, resulting in haemolysis [9–11], or less obvious lesions, demonstrable only by shortening of erythrocyte survival following transfusion [8,12].

We report details of the new design and operation of this instrument, together with in vitro and in vivo comparisons between RF heated and unwarmed autologous red cells in paired studies and where each donor acts as his own control in the in vivo part of the study.

INSTRUMENT DESIGN AND OPERATION

The individual PVC bags of blood are heated by 350 watts generated by a 27 MHz current from a power oscillator in a tuned circuit. The resistance of the PVC bagged blood, evenly compressed between heavy copper plates, is 2–3 ohms. With approximately 80 watts dielectric loss in the PVC bag wall, a uniform temperature of 33°C is reached in less than 3 minutes.

Technically, the bag of blood is suspended on the inside of the door (Fig. 1) which, when closed, simulta-

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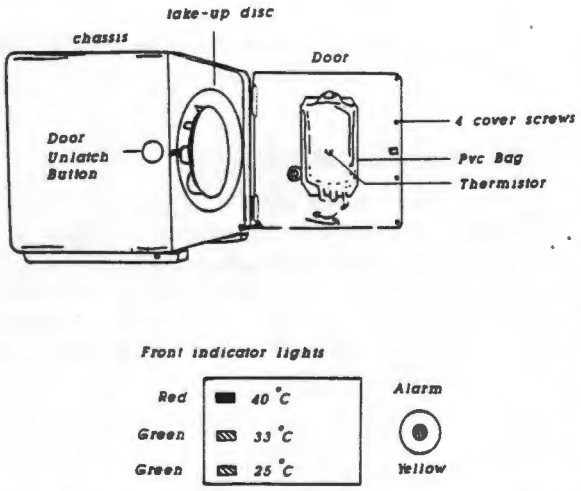

Fig. 1. Diagram of blood warmer. The instrument weighs 30 kg, has external dimensions of 360 × 360 × 460 mm, and requires 800 watts power for operation.

neously compresses the contents between two copper plates and activates the heating cycle. Hot spot formation during heating is prevented by a rocking action of the copper plates at 0.5 Hz, so that the compressed blood unit rotates slowly in the forward direction but rapidly on return. Studies using a floccular suspension in 0.5% saline have shown that this mode of agitation ensures unidirectional flow of the contents within the bag rather than simple oscillation.

A thermistor, which indents the bag, is set in the middle of the doorside plate (Fig. 1) and monitors the temperature of the contents, while a transverse ridge which is raised on the same plate indents the bag, thereby ensuring that the centre of particulate rotation is not on the same axis as the thermistor.

A comparator detects a bridge circuit point, set at 33°C, and terminates heating simultaneously with activation of auditory and visual alarms, which are cancelled only when the door is opened to remove the warmed unit of blood. Fail-safe devices (see Appendix A) inactivate the warmer if agitation ceases, the thermistor breaks, or the temperature exceeds 40°C. In addition, LEDs are mounted on the outside aspect of the door, showing green at 25°C, green at 33°C, and red at 40°C.

BLOOD COLLECTION AND INSTRUMENT PERFORMANCE

Thirty-nine units of whole blood anticoagulated with citrate phosphate dextrose (CPD), each having a volume between 475 and 550 mL, and 21 units of red cells suspended in ADSOL® (Baxter Travenol Laboratories, Deerfield, IL), having a volume of 360 mL, a haematocrit of 0.6 L/L, and stored in PVC bags under standard blood bank conditions [13] for varying periods, were allocated to this study and not subsequently transfused into patients. These conditions accurately reflected standard practice, thereby permitting extrapolation of all measurements to the clinical situation.

Characterisation of the physical performance of the blood warmer included, first, a manual check on the speed of warming, which was recorded from the time of door closure to electronic switch-off and, second, the temperature of the contents using a mercury-in-glass thermometer enfolded in the unit for 1 minute immediately after its removal from the warmer [8]. It was established that this technique exactly matched direct measurement of the temperature by immersion in the contents over a relevant range.

In Vitro Assessment of Red Cell Damage

A series of tests was selected to reflect possible RF damage to cells. Thus, erythrocytic integrity was determined by comparing pre- and post-warming changes in free plasma haemoglobin [14], plasma lactic dehydrogenase [15], plasma potassium (Beckman Astra TM Sodium Potassium Chemistry Module), and saline osmotic corpuscular fragility, including a measurement 24 hours after incubation at 37°C [16].

In Vivo Red Cell Survival Studies

The effect of radiofrequency heating was determined in 12 healthy male volunteers, with a mean age of 36 years (range 29–54). In a randomised design, each subject acted as his own control for comparison of red cell survival at 24 hours and 21 days, using ^{51}Cr as the label. In expressing the results the 100% survival value was calculated from a simultaneous estimate of red cell volume, using ^{99m}Tc. The comparison was made between unwarmed and heated red cells which had been stored under identical conditions.

Participants fulfilled the donor standards of the American Association of Blood Banks [13], and had normal full blood count and body iron stores, as defined by serum iron, percentage saturation of transferrin and ferritin levels [17]. The protocol was approved by the University of Cape Town and Groote Schuur Hospital Ethics and Research Committee and informed consent was a prerequisite.

In phase 1 of the study 480 mL of blood was collected into a triple PVC bag system (Baxter Travenol Laboratories, Deerfield, IL). The collection bag contained 70 mL citrate-phosphate-dextrose anticoagulant and the first satellite bag contained 111 mL of an adenine-containing red blood cell preservative solution (ADSOL®; Baxter Travenol Laboratories, Deerfield, IL). Each unit was

processed within 6 hours into a red blood cell concentrate, with a mean final haematocrit of 0.6L/L ± 5%, which was then stored at 4°C ± 2°C for 31 to 35 days without mixing. These storage times were selected to lie near the end of the permissible limits so that any degree of damage, however small, would be evident.

In phase 2 of the study half of the stored units were randomly assigned to warming in the Taurus 301 unit and the remaining 6 were not warmed. A 10 mL aliquot of blood was carefully removed from each unit, labeled with 1.1 MBq (30 μCi) ^{51}Cr, and then injected into the donor to determine red cell survival. Simultaneously, 5 mL of fresh blood was taken from the donor and labeled with 11.1 MBq (300 μCi) ^{99m}Tc, using a pretinning method [18] (Cadema Medical Products, Inc., New York). The cells were washed twice in saline to ensure that free radiolabel was less than 1% and then reinjected to establish red cell mass.

Four samples were drawn from an indwelling catheter in the contralateral arm at elapsed times of 5, 8, 12, and 15 minutes; further specimens were collected at 24 hours, 7, 14, and 21 days.

Radioactivity was detected in a gamma spectrometer (Minaxi Autogamma®5000 Series Gamma Counter; Packard Instruments Co., Downers Grove, IL). There was automatic correction for ^{99m}Tc decay during counting, based on a physical half-life of 6.03 hours. Since the spill-over from the Compton peak of the ^{51}Cr into the ^{99m}Tc window was less than 0.3%, this was ignored. Technetium blood activity, expressed in counts/minute/mL, was corrected to a reference haematocrit derived from the mean of the original four samples collected in the 5 to 15 minute interval after re-injection, and red cell mass was determined according to recommendations of the International Committee for Standardization in Hematology [19]. The whole blood zero-time activity was obtained by linear extrapolation of a semi-log plot of this blood radioactivity against time in minutes, using a least squares regression method.

Red cell survival at 24 hours and 21 days was calculated according to the International Committee for Standardization in Hematology [20], using a similar extrapolation of radiochromium activity in whole blood to zero time. Elution correction factors were applied to both the 24-hour and 21-day survival studies [21]. Immediate loss was estimated by establishing apparent ^{51}Cr red cell mass and comparing this with the ^{99m}Tc as follows:

$$\text{Immediate loss (\% } ^{51}\text{Cr red cell activity injected)} = \left[1 - \frac{^{99m}\text{Tc RCV}}{^{51}\text{Cr RCV}}\right] \times 100.$$

In phase 3 of the study the donors were retested shortly after 90 days following the first infusion; those whose units were prewarmed now received unheated autologous blood, and vice versa.

STATISTICAL ANALYSIS

The statistical significance of differences between control and experimental observations was tested by Student's t test—that for unpaired variables for the in vitro tests and for paired variables for the in vivo tests. For the latter, because of the small numbers, differences were also tested by the Wilcoxon matched-pairs signed-ranks test.

RESULTS

No electronic or mechanical malfunction occurred during these studies. The physical response to RF heating of whole blood and red cell concentrates differed. The mean switch-off temperatures of 31.5°C (± SEM 0.29°C) and 30.5°C (± SEM 0.39°C) ($P < .05$) were associated with corresponding warming times of 2 minutes 32 seconds (± SEM 31″ as opposed to 1 minute 44 seconds (± SEM 4″) ($P < .001$). No attempt was made to readjust the set comparator end-point once the trial had commenced.

In Vitro Indices of Red Blood Cell Damage (Table I)

For both whole blood and ADSOL® red cell concentrates the only significant abnormality following warming was an increase in plasma free haemoglobin of 17.5 mg/dL and 44.8 mg/dL, respectively. Proportionate to their prewarmed levels, these increases in free haemoglobin—whole blood 40%, RBC concentrate 44%—are similar ($P = .74$). There was no correlation between the length of storage and the degree of haemolysis induced by RF warming, either in whole blood ($r = -0.15$; $P > .1$) or RBC concentrate ($r = -0.18$; $P > .1$).

These changes were not accompanied by increase in plasma potassium, LDH, or mean red cell fragility.

In Vivo Post-Transfusion Red Blood Cell Survival (Table II)

Two donors were excluded from the 24-hour in vivo study; in one there was an incomplete re-injection and in the other a 24-hour sample was inadvertently not collected. Two exclusions also occurred in the 21-day study for similar reasons. No adverse reactions to the transfusions were experienced. The mean storage time of 32 days for unwarmed blood was not significantly different from the 33 days for the bags that were subsequently heated ($P > .05$). Following autologous transfusion there was no statistically significant difference in red cell

TABLE I. In Vitro Indices of Red Blood Cell Damage*

	Storage times ± SEM (days)					
	Whole blood (n = 39); 10 ± 0.96 (range 1–19)			Red cell concentrate (ADSOL®) (n = 21); 15 ± 3.26 (range 1–30)		
Index of red cell damage	Before warming	After warming	Change	Before warming	After warming	Change
Free plasma haemoglobin (mg%)	53.8	71.4	$P < .01$	103.3	148.8	$P < .05$
± SEM	3.27	4.97		10.1	18.9	
K+ (mmol/L)	11.7	11.7	NS	24.4	24.8	NS
± SEM	0.75	0.75		2.9	2.9	
Lactic dehydrogenase (IU/L)	497	518	NS	1,819	1,081	NS
± SEM	36	39		489	208	
MCF % saline						
Immediate	0.52	0.52	NS			
± SEM	0.007	0.007				
Post 24-hour storage	0.55	0.55	NS			
± SEM	0.004	0.004				

*MCF = mean corpuscular fragility; NS = nonsignificant.

survival after 24 hours or at 21 days between the RF heated units and their unwarmed controls.

DISCUSSION

No mechanical or electronic malfunction of the re-engineered RF blood warmer was observed during these studies. The marginally shorter warming time displayed by the RBC concentrate compared to whole blood in response to RF warming has been documented previously and is considered to reflect differences in haematocrit [7] and electrical conductivity [22] between whole blood and RBC suspensions rather than any differences in bag volume. The discrepancy observed between the end-warming temperature and that set for termination of the machine cycle resulted from the temperature sensing thermistor being influenced by heat retention in the copper plates, which is an effect exacerbated by serial use. Subsequent to the conclusion of the trial this deficiency was corrected by modifying the design to include an additional thermistor dedicated to sensing and then activating mechanisms to compensate for the ambient plate temperature.

TABLE II. In Vivo Post-Transfusion Red Blood Cell Survival†

Red blood cell survival	Unwarmed, %	Warmed, %	Change
Immediate loss (n = 12)	4.3	5.5	NS*
± SEM	0.9	1.0	
24 hour (n = 10)	75.7	71.1	NS*
± SEM	2.5	3.5	
21 day (n = 10)	62.2	58.8	NS*
± SEM	2.6	2.7	

†Crossover trial: 12 volunteers. Storage time: 30–33 days.
*Student's 't' and Wilcoxon matched-pairs signed-ranks tests. NS = nonsignificant.

Comparison of in vitro indices for red cell damage in these two products showed no difference. In both, the modest rise in supernatant free haemoglobin was the only evidence of in vitro red cell damage that followed warming. Surprisingly, this was not accompanied by increase in serum potassium or lactic dehydrogenase nor by changes in the mean corpuscular fragility, and no explanation is evident for these discrepancies. In the context of rapid and massive blood transfusion, the magnitude of this increase in free haemoglobin was no greater than that observed following pressurised infusion [23] or in association with cardiopulmonary bypass [24,25].

In addition, the radiofrequency heating of red cell concentrate caused no significant change in red cell survival following autologous transfusion at 24 hours or 21 days (Table II). As pretransfusion warming of blood is essentially undertaken in an emergency situation, the mean red cell recovery of greater than 70% at 24 hours is acceptable since this is the level above which red cells are conventionally regarded as fit for transfusion [26].

The validity of these studies depends upon reliable methodology for determining in vivo red cell survival [27–30]. Although the use of double radiolabeling with indirect calculation of red cell mass has been proposed [30–31], direct quantitation of red cell mass using ^{99m}Tc is probably preferable since it eliminates inaccuracies inherent in making this estimation indirectly from calculations on the plasma volume [29]. Our experience is that the direct quantitation method is only slightly more technically complex than the indirect approach.

The temperature setting of the warmer's switchoff at 33°C resulted in mean and ranges of temperature within

individual blood packs well below the known heat tolerance for blood [32,33], yet achieved a level which would be compatible with the maintenance of safe body temperature [3].

It is concluded that this re-engineered radiofrequency blood warmer is an efficient and safe means of raising the temperature of stored blood to levels where massive or rapid transfusion can be safely undertaken. The availability of this instrument provides a practical alternative to in-line warmers, most of which are recognised as being inadequate for the type of rapid transfusion systems available, although counter-current warmers can be employed in a rather more complex system for rapid massive transfusions [34].

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APPENDIX A

Safeguards and Failsafe Devices

1. A damaged thermistor temperature sensor can be an open circuit or a short circuit. These extremes are detected and the unit is automatically switched off.

2. Apart from the detection of the 33°C end-point, a back-up detection will be activated at 40°C, terminate the heating, and bring on a red LED and an alarm.

3. The presence of an acceptable bag is detected mechanically by a microswitch which turns on the heater supply of the oscillator valve, the detection electronics, and agitation motor power supply.

4. The final door closure is detected by a second microswitch, which allows the high voltage supply to be applied to the oscillator valve *provided* the temperature detection circuits and shake detector agree.

5. The "shake detector" is a magnetic reed switch activated by the magnet swinging up to it if the agitation is *normal*. If more than three successive pulses are missed, the heating is switching off and the alarm is activated.

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Single-dose relative biological effectiveness and toxicity studies under conditions of hypothermia and hyperbaric oxygen

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ABSTRACT

An approach to using hyperbaric oxygen with radiation in a clinical situation has been described in the preceding paper in this issue. To ascertain whether there might be a change in the relative biological effectiveness of radiation on normal mammalian tissue treated under conditions of hypothermia and hyperbaric oxygen, the acute reaction to radiation of pig skin was studied. A single dose enhancement ratio at the erythema reaction level of 1.4 ± 0.08 was obtained when compared with irradiation at normal body temperature in air. We studied also a series of antioxidant enzymes in rat liver and lung after exposure to hypothermia and hyperbaric oxygen. Enzyme changes were such as to combat oxygen toxicity which might develop as a result of the pre-treatment.

In order to ascertain whether there is a change in the radiobiological effectiveness of radiation on normal mammalian tissue treated under hypothermia and hyperbaric oxygen (Sealy et al, 1986), we studied the acute radiation reaction on pig skin. This was chosen as our biological test system since it has been extensively used in RBE determinations (*e.g.* Fowler, 1981) and results can be extrapolated to the human with a high degree of confidence (Hall, 1978). In addition, drug administration regimes similar to those that were envisaged to be used on humans could be put into effect (Sealy et al, 1986).

Secondly, concern for the possibility of oxygen toxicity developing in patients as a result of the hypothermic hyperbaric oxygenation (Sealy et al, 1986) prompted a search for biochemical evidence of oxygen toxicity.

The biochemical probes chosen were the activities of rat lung and rat liver superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and also glucose-6-phosphate dehydrogenase, since the activity of this enzyme has been shown to be increased on exposure to hyperoxia (Tierney et al, 1973). Glutathione levels were also investigated.

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METHODS

Pigs

Pigs of the Large White/Landrace cross variety were used, weighing between 50 kg and 60 kg. In total, nine pigs (three sets of three litter mates) were used in the investigation. Every pig was irradiated to one or more dose levels under each of the following conditions:

(a) in air at normal body temperature (range 34.5–36.5°C);
(b) in hyperbaric oxygen (3 atmospheres absolute) at normal body temperature (range 34.5–36.5°C);
(c) in air at hypothermic body temperature (range 28–31°C);
(d) in hyperbaric oxygen at a hypothermic body temperature (range 28–31°C).

Anaesthesia, cooling and profound vasodilatation in the pigs was achieved in essentially the same manner as that described by Sealy et al (1986) for patients. Experimental animals, to which balanced salt solution and drugs were administered via jugular venous cannulation, were anaesthetised by Alfathesin (alphazalone and alphadalone compound, Glaxo) or Hypnomidate (etomidate, Janssen) by continuous infusion, curarised with Alloferin (alcuronium chloride, Roche) and intubated, ventilated by means of a Vickers fluid logic ventilator and subjected to alpha-adrenergic blockade with Regitine (phentolomine, Ciba-Geigy). Hypothermia was induced by surface cooling with ice packs, care being taken not to cover the areas to be irradiated. Rewarming was achieved by immersion of the animal in water at approximately 40°C. The temperature of the animal was measured on the skin near the irradiated area and in the oesophagus using thermistor probes with a Vickers thermometer.

Irradiation of pig skin. Irradiations were carried out inside a single-patient hyperbaric oxygen chamber (Vickers Mark IV). Eight fields, each 5 cm × 5 cm, were marked by tattooing, four on the right hindquarter and four on the right forequarter of each pig with at least 3 cm between each field. Each field was treated to a dose ranging from 15 Gy to 29 Gy on the surface of the skin.

TABLE I

DEGREE OF RADIATION REACTION OF PIG SKIN

Score	Reaction
0.5	No reaction
1.0	Faint erythema
1.5	Erythema
2.0	Marked erythema
2.5	Moist desquamation of less than half of the irradiated area
3.0	Moist desquamation of more than half of the irradiated area

Eight-megavolt X rays from a Philips SL 75/20 linear accelerator were used with 2 cm tissue-equivalent polystyrene build-up. In order to prevent any hypoxia occurring due to pressure, the polystyrene plate was suspended immediately above the surface of the skin. The animals were irradiated with a vertical beam whilst lying on the left side inside the hyperbaric oxygen cylinder. The doses were measured using a Baldwin-Farmer ionisation chamber and LiF thermoluminescent dosemeters. The dose rate at the skin surface was 1.736 Gy/min.

Skin scoring. The method of estimating the skin reactions was that of visual observations carried out on Mondays, Wednesdays and Fridays by three to five observers who were unaware of how each area had been irradiated. An arbitrary scale was adopted based on that used by Fowler et al (1963) (Table I).

Rats

Male Long-Evans rats, weighing between 250 g and 270 g, were used. Rats were maintained on a standard laboratory diet and allowed food and water *ad libitum*. Animals were divided randomly into four groups of six rats. One group of rats served as controls and received no treatment. The second group of rats (the "hyperbaric" group) was exposed for 4 h to 100% oxygen at 303 kPa. The third group was treated intraperitoneally with 70 mg/kg sodium pentobarbital (Sagatal, May and Baker) to induce hypothermia (Pallavicini & Hill, 1983). The fourth group of rats (the hypothermic hyperbaric oxygen group) received 70 mg/kg sodium pentobarbital intraperitoneally, immediately following which they were placed in 100% oxygen at 303 kPa for 4 h. Exposures to hyperbaric oxygen were conducted in a Vickers Mark IV single-patient hyperbaric oxygen chamber. Temperature was measured in both the hypothermic group and the hypothermic hyperbaric oxygen group with an Electrolaboratoriet monitor by means of thermocouples placed under the skin in the abdominal area. The animals in the hypothermic group and in the hypothermic hyperbaric oxygen group were placed in cages positioned on plastic bags containing melting ice immediately after injection of the sodium pentobarbital. It was found that the temperature in both groups treated with sodium pentobarbital decreased from 36°C to a minimum of 29°C.

Tissue preparation and measurement of biochemical parameters. Rats were sacrificed by cervical dislocation 24 h after commencement of the treatments above, the lungs and livers being removed and used immediately. Cytosol Cu/Zn superoxide dismutase was extracted by the method of McCord and Fridovich (1969) as modified by Sykes et al (1978), and assayed by following the auto-oxidation of epinephrine to adrenochrome (Misra & Fridovich, 1972). Determination of glucose-6-phosphate dehydrogenase was by the method of Löhr and Waller (1963). Catalase was determined by the method of Holmes and Masters (1970), glutathione by the method of Saville (1958), glutathione peroxidase by the method of Lawrence and Burk (1976), glutathione reductase by the method of Worthington and Rosemeyer (1974) and protein as described by Lowry et al (1951).

The significance of differences between mean values for the groups was assessed by Student's *t*-test.

RESULTS

Figure 1 shows the variation of the average skin reaction with time for a single pig irradiated to 23 Gy

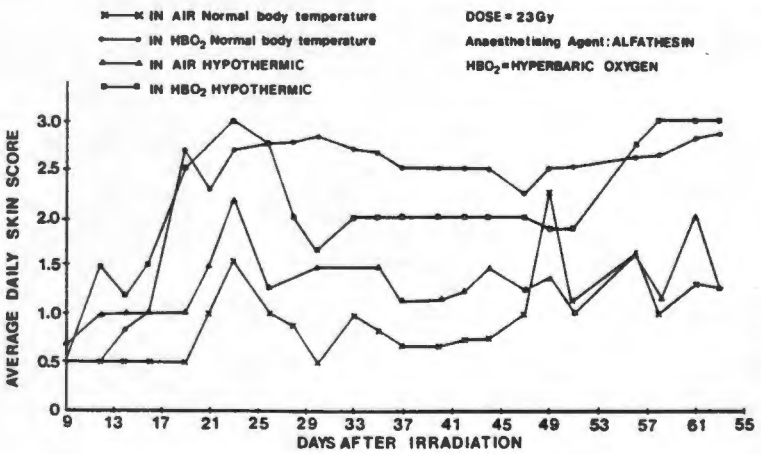

FIG. 1.

Acute radiation responses for pig skin under the four conditions. End-point represents the average score for all scorers for two fields. Note the increased reaction for those areas irradiated in oxygen and the curve suggesting a triphasic response.

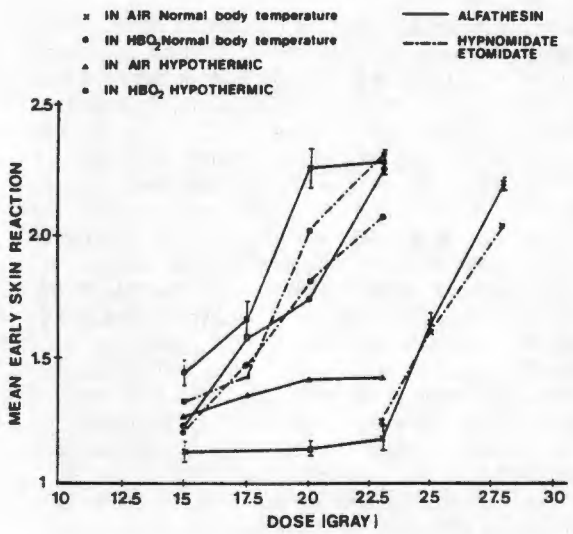

FIG. 2.

The mean early skin reaction for various doses in nine pigs. Data points are given together with standard errors of the means. Not all error bars are shown in order to preserve clarity.

under the various conditions. In general, an early wave of reaction reaching a peak at about 25 days after irradiation was observed, followed by a slight decrease in reaction to a more or less constant, slightly lower level. At about 50 days after irradiation the reaction increased again in intensity. This reaction pattern is similar to that observed by Fowler et al (1963).

The dose–response curves (Fig. 2) were obtained by determining the average skin reaction between the time when the reaction first became apparent (lower limit) and when it started to increase to the second peak (upper limit). This is the early reaction stage referred to by Hall (1978). The size of the animals and maintenance limited us to this region.

TABLE II

STUDY OF PIG SKIN REACTION USING HYPOTHERMIA IN CONJUNCTION WITH HYPERBARIC OXYGEN

Anaesthetising agent	Conditions at irradiation	Enhancement ratio at 1.5 reaction level
Alfathesin	In Air Warm	Standard
	In HBO_2 Warm	1.4
	In HBO_2 Cold	1.5
Hypnomidate	In Air Warm	Standard
	In HBO_2 Warm	1.4
	In HBO_2 Cold	1.4

HBO_2 hyperbaric oxygen.
An error estimate of about ±0.08 on the values for the enhancement ratio was obtained by a simple "envelope of errors" analysis on the dose–response curves.

The shape of the dose–response curves obtained (Fig. 2) resemble those of Fowler et al (1963). From these curves the values for the radiobiological effectiveness relative to the treatment at normal body temperature in air (RBE) were determined, and are listed in Table II. The radiobiological effectiveness was determined at the 1.5 reaction level because patients would not be treated to skin doses resulting in a greater level of damage.

It was noted that the skin damage is increased only slightly under hypothermic conditions in air compared with normal body temperature in air, whereas there was a significant increase in effect for hypothermic/normothermic treatments in hyperbaric oxygen (Fig. 2) compared with irradiation in air.

Results of the hepatic and pulmonary measurements in rats are given in Tables III and IV.

TABLE III

THE EFFECT OF HYPERBARIA, HYPOTHERMIA AND HYPERBARIC HYPOTHERMIA ON BIOCHEMICAL PARAMETERS CHOSEN AS PROBES FOR OXYGEN TOXICITY IN RAT LIVER. THE MEAN RESULTS ±SEM FOR DETERMINATIONS IN SIX ANIMALS ARE GIVEN

Parameter	Controls	100% O_2 at 303 kPa for 4 h	70 mg/kg sodium pentobarbital	70 mg/kg sodium pentobarbital, 100% O_2 at 303 kPa for 4 h
Superoxide dismutase (units/mg protein)	1570±245	1779±223	1674±505	5000±879§
Catalase (μmol H_2O_2 degraded/min/mg protein)	135.0±8.7	106.6±14.8	119.6±29.9	178.2±13.4*
Glutathione (nmol glutathione/mg protein)	167.7±6.5	218.0±24.9	161.0±25.8	244.5±28.8*
Glutathione peroxidase (nmol NADPH oxidised/min/mg protein)	3.8±0.6	6.0± 0.9	7.6± 1.6	11.3± 1.5§
Glutathione reductase (nmol NADPH oxidised/min/mg protein)	48.0±1.7	69.7±6.2†	101.4±8.9§	65.9±3.8‡
Glucose-6-phosphate dehydrogenase (nmol NADP reduced/min/mg protein)	17.7±1.3	31.0±5.0*	27.7±3.9	20.3±2.9

Significantly different from control: *$p < 0.05$: †$p < 0.01$: ‡$p < 0.002$: §$p < 0.001$.

TABLE IV

THE EFFECT OF HYPERBARIA, HYPOTHERMIA AND HYPERBARIC HYPOTHERMIA ON BIOCHEMICAL PARAMETERS CHOSEN AS PROBES FOR OXYGEN TOXICITY IN RAT LUNG. THE MEAN RESULTS ±SEM FOR DETERMINATIONS IN SIX ANIMALS ARE GIVEN

Parameter	Controls	100% O_2 at 303 kPa for 4 h	70 mg/kg sodium pentobarbital	70 mg/kg sodium pentobarbital. 100% O_2 at 303 kPa for 4 h
Superoxide dismutase (units/mg protein)	149±11	131±25	381±111.5‡	474±220*
Catalase (μmol H_2O_2 degraded/min/mg protein)	19.9±0.5	29.7±2.6‡	17.5±4.9	39.9±5.0‡
Glutathione (nmol glutathione/mg protein)	282.7±8.5	332.0±14.3†	105.1±15.4§	165.8±11.4§
Glutathione peroxidase (nmol NADPH oxidised/min/mg protein)	5.5±0.4	7.8±1.1	12.2±2.6*	11.3±1.9†
Glutathione reductase (nmol NADPH oxidised/min/mg protein)	8.0±0.3	12.6±0.9§	12.1±1.4*	9.1±1.6
Glucose-6-phosphate dehydrogenase (nmol NADP reduced/min/mg protein)	11.6±0.7	18.0±1.4‡	17.9±2.2*	14.6±2.5

Significantly different from control: *$p < 0.05$: †$p < 0.02$; ‡$p < 0.01$; §$p < 0.001$.

DISCUSSION

The pig skin studies have shown that, when irradiated in hyperbaric oxygen, the skin reaction was always greater than when treated in air, irrespective of body temperature. The RBE values listed in Table II indicate that, while there is an increased effect in those areas irradiated under conditions of hypothermic hyperbaric oxygenation compared with normal temperature in air, the differences were not statistically significant when compared with those irradiated at normal temperature in hyperbaric oxygen.

It has been demonstrated by Stewart et al (1982) that normal rodent skin is mildly hypoxic. Our findings indicate that, even under the influence of alpha-adrenergic blockade in air, there are some hypoxic cells in normal pig skin and that the protective effect of the hypoxia is partially reversed by hypothermia alone, but more efficiently by hyperbaric oxygenation. The modest effect of hypothermic hyperbaric oxygenation compared with hyperbaric oxygenation at normal temperatures could be explained by the latter overcoming the protective effect of nearly all the hypoxic cells. If our hypothesis is correct, a much greater margin might be expected if large tumours were irradiated, since the proportion of hypoxic cells would be expected to be much greater than in normal skin or smaller tumours.

The lung and liver play major roles in the defence of the body against oxygen toxicity. In the clinical situation, where patients are treated radiotherapeutically under conditions of hyperbaric oxygenation and hypothermia, the duration of the hyperbaric oxygen is about 40 min (Sealy et al, 1986). The antioxidant enzymes are inducible (Tierney et al, 1973; Crapo and Tierney, 1974; Kimball et al, 1976; Rister & Baehner, 1976; Frank et al, 1978) and can provide protection against hyperoxic challenges. In support of this, Deneke and Fanburg (1982) reported that significant increases in the activities of superoxide dismutase, glucose-6-phosphate dehydrogenase, glutathione peroxidase and reductase and, in some instances, catalase occur within 24–48 h in young animals exposed to 100% oxygen. This enabled the animals to resist the toxic effects of 100% oxygen (Frank et al, 1978). It can, thus, be expected that if acute oxygen toxicity in rats was to develop as a result of hypothermia or hypothermic hyperbaric oxygenation, the activities of these enzymes would, in fact, not be increased.

Superoxide dismutase was not induced in the lung by hyperbaric oxygen alone, but was increased almost twofold after sodium pentobarbital and threefold after the combination of pentobarbital and hyperbaric oxygen. We noted also a more than threefold increase in hepatic superoxide dismutase activity following sodium pentobarbital-induced hypothermia and hyperbaric oxygen. These increases in superoxide dismutase activity may afford protection against the increase in free superoxide radicals that might be produced under these conditions.

Glutathione reductase in the lung was induced in response to hyperbaric oxygenation, thus implying a continued supply of reduced glutathione which protects cells under oxidative stress by scavenging free radicals and peroxides (Meister & Tate, 1976). The observed depletion of pulmonary glutathione after sodium pentobarbital or hyperbaric oxygen together with sodium pentobarbital may be partly due to the increase in the activity of glutathione peroxidase in both of these situations.

Increases in activity of antioxidant enzymes have generally only been shown to occur following several days of exposure to hyperoxia (Crapo & Tierney, 1974; Kimball et al, 1976; Rister & Baehner, 1976; Frank et al, 1978). The observed changes in this study after a 4 h exposure to hypothermia and hyperbaric oxygen were, in general, of such a nature that potential hepatic and pulmonary oxygen toxicity or the effects thereof would be combated. Our biochemical evidence is such that oxygen toxicity will probably not develop during

the course of or during hypothermic hyperbaric oxygen irradiation of patients.

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A feasibility study of a new approach to clinical radiosensitisation: hypothermia and hyperbaric oxygen in combination with pharmacological vasodilatation

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ABSTRACT

It is proposed that hyperbaric oxygen fails in the clinical situation due to a high proportion (greater than 33%) of hypoxic cells in human tumours. The means of overcoming this problem are reviewed. Additional to hyperbaric oxygenation, moderate hypothermia (30°C) to allow redistribution of oxygen in the tumour is proposed. A system of externally controlled intravenous anaesthesia has been developed for the single-subject hyperbaric cylinder. Pharmacological vasodilatation is induced in the anaesthetised patient who is then fluid loaded and cooled. Initial single-sensitising treatments are advocated. Twenty-nine patients with advanced mouth cancer have completed a course of this treatment, of whom five of nine were free of disease after 2 years and 10 of 21 at 1 year, with three intercurrent deaths. Fifteen have experienced local failure. This approach would appear to be practical, safe and promising.

Hyperbaric oxygen in clinical radiotherapy has not fulfilled its early promise (Churchill-Davidson et al, 1957). It would appear that large tumour size adversely influences response (Van den Brenk, 1968; Chang et al, 1973; Henk et al, 1977; Henk & Smith, 1977; Sealy, 1979), even when subject to prior shrinkage with chemotherapy (Sealy et al, 1982). Similar results are seen in animal tumours (Suit & Maeda, 1967).

We postulate, therefore, that the poor response to irradiation in hyperbaric oxygen is a function of incomplete radiosensitisation due to an increased percentage of hypoxic cells present in tumours of a more advanced stage. Even small human tumour nodules may have 20–40% hypoxic cells (Denekamp et al, 1977). Murine tumours may have size-dependent hypoxic fractions (for a review, see Moulder & Rockwell, 1984), while Mueller-Klieser et al (1984) have shown that oxyhaemoglobin saturation by hyperbaric oxygen in tumour capillaries also depends upon tumour size. Our hypothesis is supported by the view of Adams (1978) in that a highly significant gain factor for a hypoxic radiation sensitiser treatment will only be seen when less than 0.001% of the tumour cells remain biologically hypoxic when so exposed. Hyperbaric oxygen at 3 atmospheres absolute increased the oxygen-carrying capacity of the blood by about 33% over that in air; thus, it would not be possible, under the most favourable conditions, to oxygenate completely tumours with a very high proportion of hypoxic cells by this means alone. Morgan (1967) has calculated, for a large tumour, that even 1% of hypoxic cells would increase the necessary radiation dose for cure to impossibly high levels for clinical tolerance. It is, therefore, appropriate to explore methods of achieving a several-fold improvement in the oxygen supply of tumours over that which is obtained in conventional hyperbaric oxygen at 3 atmospheres absolute. Increasing oxygen pressure of from 3 to 4 atmospheres raises the oxygen-carrying capacity of the blood by a further 12%. This would not be adequate if more than 45% of the cells were to be hypoxic in even a small area of a tumour.

Peripheral vasoconstriction (Whalen et al, 1965; Bachofen et al, 1971; Johnson, 1971; Johnson et al, 1972) may be an important unwanted side-effect of oxygen therapy which should be avoided in the clinical situation (Sealy, 1979) since it may be protective (Milne et al, 1973; Pallavicini & Hill, 1983). However, its abolition would not improve the supply of oxygen to the tumour more than about 33% above that seen in air (Whalen et al, 1965) and, thus, as a sole additional measure would not be expected to increase the radiation sensitivity of a cell population with greater than 33% hypoxic cells.

Another option is the use of hyperbaric oxygen together with a concomitant reduction in the oxygen utilisation rate of the tumour. This could lead to a centrifugal redistribution of oxygen within the tumour cord (Thomlinson & Gray, 1955) or layers (Scott, 1957). This condition can be achieved pharmacologically (Crabtree & Cramer, 1934; Hall et al, 1952; Durand & Biaglow, 1974; Haynes & Inch, 1976), but systemic doses of drugs in excess of those which can be given under ordinary clinical conditions would probably be required (Durand et al, 1976).

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Induced hypothermia provides an alternative approach. Crabtree and Cramer (1934) and Hall et al (1952) suggested that the radiation sensitivity of mouse tumours might be increased at 0°C. In an attempt to improve radiation sensitivity by causing a reduction in oxygen utilisation and, hence, a redistribution of available oxygen, Bloch et al (1961) irradiated moderately hypothermic patients for glioblastoma multiforme, but found radiation-induced brain oedema. In less critical anatomical situations this might not be a limiting factor. Surface physico-pharmacological cooling to about 30°C is well known in anaesthetic practice and cardio-pulmonary bypass cooling to about 14°C is frequently undertaken in cardiac surgery. As an initial study, we decided to investigate the former.

Two widely differing rates of change of oxygen utilisation with change in temperature are quoted in the literature (Bigelow et al, 1950; Ross, 1954; Horvath & Spurr, 1956; Wright, 1971; Ganong, 1983). Utilising the lower rate quoted (7%/1°C), it can be calculated that the whole-body oxygen consumption at 30°C will be of the order of 50% of what it is at 37°C. This figure seems to have fairly wide acceptance (Bigelow, 1958; Nunn, 1980).

Cardiac output falls with drop in temperature and figures of 50% of normal at 28°C are quoted (Ross, 1954), but even were this to be so and peripheral perfusion decreased proportionately, 3 atmospheres absolute oxygen at 30°C would still effectively provide an excess of additional available oxygen.

If a tumour was fully oxygenated, and the dose for 37% cell survival was about 1.40 Gy, then a single dose of 6.00 Gy would leave a surviving fraction of less than 2%. It would be expected that the redistribution of oxygen following such massive cell death would allow effective reoxygenation of the surviving tumour cells, provided that the significant factor was not destruction of critical elements of the tumour vasculature.

We describe here our experience in 31 patients in whom radiosensitisation was achieved by hypothermia and hyperbaric oxygen (3 atmospheres absolute) in combination with pharmacological vasodilatation.

THE LOGISTICS OF PROVIDING ANAESTHESIA

The provision of an environment for radiotherapy of hyperbaric oxygenation and hypothermia poses pathophysiological and logistic problems, of which the former are the most serious. Of all the pathophysiological accompaniments of induced hypothermia, the most dangerous is cardiac arrhythmia, especially ventricular fibrillation, the threshold for which is usually below 30°C, the target temperature accordingly chosen.

Before treatment, all patients have myringotomy needles inserted. For the induction of hypothermia a standard technique of surface cooling under general anaesthesia is used. Because of the constraints of the hyperbaric cylinder, we have opted for total intravenous anaesthesia with a continuous infusion of etomidate (1–2 mg/kg/h) combined with fentanyl (300–400 μg during the period of cooling), paralysis with d-tubocurarine (approximately 45 mg during total period of anaesthesia) and ventilation with nitrous oxide with 40% oxygen during cooling and oxygen alone during the hyperbaric phase.

After induction of anaesthesia and immediately before commencement of cooling, vasodilatation and control of shivering is achieved by the administration of 20–50 mg of Largactil (May & Baker Ltd) administered with appropriate fluid loading (balanced salt solution and plasma). The patient's physiological homeostasis is controlled by appropriate reactions to monitoring of multiple vital functions. These include electrocardiography and arterial and venous pressure transduced from percutaneous radial arterial and right atrial cannulation. These allow also for serial monitoring of acid–base, blood–gas and electrolyte status. A separate intravenous line is established for fluid and drug infusion. End-tidal CO_2 concentration is continuously monitored by capnography, the volume of ventilation being adjusted to maintain end-tidal CO_2 concentration between 4% and 5% during the fall in temperature and metabolic rate. If necessary, 2–4% CO_2 is added to the inspired gas mixture during cooling to prevent alkalosis.

Temperature is monitored in the middle third of the oesophagus and in the rectum with thermistor probes.

A cooling rate of 1°C/15 min has been found to be optimal in terms of accurate control of afterdrop. Once the temperature has reached its nadir following afterdrop—which approximates 2°C—and oesophageal and rectal temperature have equilibrated, core temperature remains stable for a prolonged period.

Hyperbaric phase

Major logistical problems follow from the spatial constraints of our single-patient pressure vessel (Vickers Mark IV), the spark and explosion danger inherent in the hyperbaric oxygen environment and the enforced remoteness of the anaesthetist from the patient for the period of pressurisation and irradiation. Modifications of the cylinder door were made to permit airtight passage of intravenous lines for the administration of intravenous anaesthetics and other drugs from without by means of constant infusion pumps (IVAC), and cables from all the monitoring devices to appropriate external displays to allow continuous monitoring.

The period for which the patient is remote from the anaesthetist is of the order of 50 min.

Ventilation

Ventilation with pure oxygen in the tank is provided by a compact Vickers fluid logic ventilator. It has no moving parts and so obviates any danger of spark generation. Airway pressure generated during the inspiratory phase is registered on a pressure gauge inside the tank visible through the perspex wall and, together with the capnograph oscillation, provides a visible means of monitoring respiration.

Depressurisation, rewarming and reversal of neuromuscular block

At the conclusion of therapy, the patient is depressurised, removed from the tank and rewarmed in a bath of warm water.

Once rectal and oesophageal temperatures exceed 34°C, the action of muscle relaxant is reversed with glycopyrrolate/neostigmine, spontaneous respiration is re-established, anaesthesia discontinued and the patient allowed to awaken.

Complications

We have applied this technique on 42 occasions to 34 patients, the number of treatments per patient being dictated by radiotherapeutic criteria. All patients have regained consciousness rapidly after treatment and have been fit for discharge from hospital the next morning.

The most dangerous complication associated with induced hypothermia is the onset of various cold-induced cardiac arrhythmias, the incidence increasing sharply at temperatures below 30°C. Fifteen of the 42 treatments have been so complicated. These were, in order of frequency: atrial fibrillation (12), ventricular tachycardia (one), multiple ventricular premature systoles (one), ventricular fibrillation (one). Initially, aggressive attempts at increasing cooling rates resulted in poor control of afterdrop with a high incidence of arrhythmia, but with better control of cooling rates; only four instances of atrial fibrillation have occurred in the last 21 patients treated. Atrial fibrillation has reverted to sinus rhythm in all cases on rewarming, combined with Verapamil (Knoll, Ltd) 5 mg in all but three. The episodes of ventricular tachycardia and multiple ventricular premature systoles were controlled with lignocaine.

Cold-induced hypertension

A consistent reaction has been a hypertensive response of varying degree to the movement and handling of the cooled, vasodilated and fluid-loaded patient and his insertion into, and pressurisation in, the pressure vessel. We have found the continuous infusion of sodium nitroprusside of use in controlling this reaction. The subsequent warming hypotension secondary to vasodilatation is then relatively easily handled by discontinuing the drug and volume loading of the patient.

Cardiac output

The relative cardiac output was determined in six patients, using a radionuclide technique as previously described (Novitsky et al, 1984). Technetium-99m labelling of red blood cells was carried out *in vitro* using the Brookhaven National Laboratory Kit according to the method of Smith and Richards (1976). Activities of about 1500 MBq were used because of the necessity of accumulating a sufficient number of counts in as short a time as possible. A high-resolution, low-energy collimator on a Siemens gamma camera linked to a Medical Data Systems computer was employed, scanning in the anterior and left anterior oblique views.

The mean cardiac output in the conscious patient was 4.1 l/min (range 2.6–5.3 l/min) and in the anaesthetised, cooled, vasodilated, fluid-loaded patient was 4.7 l/min (range 3.5–6.2 l/min). These findings indicate that, under these conditions, decrease in the cardiac output did not occur.

Acid–base and fluid balance

No difficulties have resulted in relation to blood–gas and acid–base homeostasis, but the cold-induced diuresis, usually commencing at a core temperature of 31–32°C and which commonly leads to the passage of 2–3 l of dilute urine during the procedure, does require catheterisation and careful management, especially with regard to its effect on serum K^+ level and control of circulatory volume homeostasis.

CLINICAL RADIOTHERAPEUTIC EXPERIENCE

Patient selection

Since August 1982 the treatment has been offered to patients with Cape Town T_3 (4–6 cm), T_4 (in excess of 6 cm diameter) or N_3 (fixed nodes) histologically confirmed inoperable squamous carcinoma of the mouth. Such tumours thus tend to be larger than those staged as T_3 or T_4 in the International Union against Cancer (UICC, 1978) classification, since invasion of muscle or bone does not advance the Cape Town stage, which is calculated purely on size. It is our experience that about 25% of such patients are free of disease 1 year after conventional radiation treatment. Whilst being prepared for treatment, most patients received methotrexate intravenously (80 mg/m^2 body surface per week) to prevent extension of the tumour. It is our experience that such chemotherapy administered until about 1 week before irradiation does not affect subsequent radiotherapeutic control of the disease (Sealy et al, 1982).

The protocol was passed by our local ethical review committee and informed patient consent obtained.

Irradiation technique

An individual perspex cast was made for each patient and the known tumour and spinal cord localised within it. Individually drawn conventional multifield treatment plans were prepared and special attention given to avoidance of the spinal cord. The cast was made to fasten in a known position on the table top within the hyperbaric cylinder. To allow for refraction of the light beam, the set-up was rehearsed with the cast only, with a simulator of the hyperbaric cylinder on the treatment couch. This enabled the beam entry points and angles and the source–skin distances to be marked on the cast. Ellis-type compensators and a 1 cm build-up where the peak dose was required to be on the skin surface were made. Eight MeV X rays and the Vickers Mark IV hyperbaric cylinder at 3 atmospheres absolute on a modified hand-operated commercial hydraulic ram were

used. The time taken to place the patient into the chamber averaged about 30 min; thus, patient placement took place when the core temperature was about 32°C. Afterdrop cooling continued during this placement period, as well as the compression and the soaking times (15 min). The intratumour treatment levels, as measured by interstitial thermistor probes, closely followed those of the core and were about 30°C in all cases, before and after irradiation. All patients, except the first, also received prophylactic neck nodal irradiation given in air at ambient temperature. The usual nodal dose was about 42 Gy in 14 fractions given three times per week.

Radiation fractionation

Large-fraction hypothermic hyperbaric radiotherapy to the known disease only was given at the beginning of the treatment course. Anaesthetic considerations prevented more than one such treatment per week and the original aim was, therefore, to deliver two fractions of 6.00 Gy tumour dose to produce a large cell kill on Treatment Days 1 and 7, with two fractions of 2.00 Gy on Days 3 and 4. However, it became clear that two hypothermic treatments were difficult to schedule and later only one initial treatment was given. Thereafter, five times weekly treatments in air at ambient temperature were given to clinical tolerance, usually 20×2.00 Gy with two hypothermic fractions (total dose 56.00 Gy in 37 days) and 26×2.00 Gy with one (total dose 58.00 Gy in 38 days). With a single hypothermic treatment, two booster daily doses of 3.00 Gy were usually given to any residual mass at the end of treatment. The respective TDF values were estimated to be about 95 and 100, and corrected for the hypothermic RBE to about 114 and 119, using the data obtained in the accompanying paper (Hering et al, 1986). Two patients, because of machine breakdown or the necessity to control hypertension, received hypothermic irradiation at the end of their course of radiation.

Radiation reactions

Our experience of acute post-irradiation oedema was similar to that of Bloch et al (1961). This appeared some 2–4 h after treatment and in the first patient was of such a degree that tracheotomy was performed as a prophylactic measure before the second treatment. All other patients were then controlled by a single dose of 100 mg of dexamethasone given intravenously at the time the anaesthetic was discontinued. The acute radiation reactions developing during the course of treatment were scored for erythema and membrane formation (Sealy et al, 1982) and did not differ from those experienced during conventional courses of treatment.

With the exception of one patient with mandibular necrosis, marked late effects have not so far been seen. The large tumours treated have necessitated large fields which have resulted in mild to moderate xerostomia. Mild subcutaneous oedema has been seen in four patients but this has regressed in two. One patient has subcutaneous fibrosis at 28 months and another at 24 months.

In five patients who received two initial hypothermic treatments, the mean time to clinical tumour regression was 14.6 (range 7–21) days, whereas in 19 patients who received one such treatment the mean time was 39.3 (range 11–60) days.

Results of treatment

Thirty-one patients have accepted and completed a course of treatment. In two early cases hypothermic treatment was abandoned. Twenty-seven of 29 patients treated hypothermically experienced complete initial regression of the tumour. Five of nine hypothermically treated patients were free of disease after 2 years, whilst 10 of 21 patients at risk at 1 year remain free of disease. In addition, a further three patients have died free of disease in the treated area, of a cerebrovascular accident, lung metastases or pneumonia, possibly associated with lung metastases, less than 12 months after completing treatment. Eight patients completed hypothermic irradiation less than 1 year ago. Compared with a series of 64 consecutive patients with T_3 or T_4 oral cancer treated about the same time with conventional fractions in air (63.00 Gy TD in 30 fractions in 38 days) where only 18 had locally controlled disease at 1 year, the results for control of the disease are significant at the 10% level ($\chi^2 = 2.7$) at 1 year. When the results are compared at 2 years (5/9 and 5/58), although the numbers are very small, the results are significant at less than the 1% level ($\chi^2 = 13.5$). A similar trend is seen when the results are compared with a retrospective series of 47 patients with T_3 mouth cancer (Sealy et al, 1982) treated with large fractions (36.00 Gy TD in six twice-weekly fractions in 18 days) where 11 patients were free of disease at 1 year ($\chi^2 = 3.9$ and 6% level of significance). The results also compare favourably with our past experience of irradiating patients with squamous carcinoma of the mouth in hyperbaric oxygen (45.00 Gy TD in nine twice-weekly fractions in 29 days) with T_3 (4–6 cm diameter), T_4 (greater than 6 cm diameter) or N_3 (fixed nodes) stage disease where one out of 13 patients was free of disease at 1 year. The difference is significant at less than the 5% level ($\chi^2 = 5.8$).

Nodal recurrence caused death in two patients in this series where the individual nodes were very large with diffuse disease in the neck and with estimated volumes of 39 cm^3 and 48 cm^3, respectively, whereas smaller nodes usually seem to have been controlled. In another two, nodal and primary recurrent disease was observed. Two of four patients who had disease involving bone, one with extensive destruction of the anterior mandibular arch, remain well with the primary growth area healed.

One remarkable feature of this method of treatment is the excellent patient tolerance to the prolonged

hypothermic anaesthetic, which makes post-treatment care very easy.

DISCUSSION

We have formulated a theory for the clinical failure of hyperbaric oxygen radiotherapy and laid the groundwork for its application.

The clinical course of events so far supports the original thesis in that 27 of 29 patients in whom treatment was successfully completed, had complete regression of disease. These findings suggest that large primary growths in the mouth may have over 33% hypoxic cells and that large nodal metastases may have even more.

The very rapid regression rate seen with two hypothermic treatments, which seems to involve the whole tumour at once, is of interest since it does not have the same pattern as the classical centripetal regression of Coutard (1932). On the contrary, this is more in keeping with a large random and widespread tumour cell death at the initial treatment rather than that which might be seen with gradual reoxygenation. The slower rate of regression seen after one hypothermic treatment suggests that massive cell kill takes place with a second treatment and that a pattern of regression is induced with a single treatment more akin to that found with successful irradiation in air. Thus, it is possible that, even under the circumstances reported here, oxygen does not easily gain access to the tumour cells because of a poor and perhaps partially destroyed vasculature, as in a large, partially necrotic lymph node. This need only happen in one small area involving no more than about 10^7 cells for local recurrence to take place. It is known that human tumour oxygen supply can vary greatly between regions of only a few millimetres in volume (Mueller-Klieser et al, 1981). Jung et al (1984) have recently shown that haemodilution increases tumour blood flow and the availability of oxygen to a rat tumour. After induction of the alpha adrenergic blockade, our patients received 2–3 l of fluid to maintain blood pressure which, thus, led to a fall in the haematocrit to about 30%. Moreover, our cardiac output studies show that a decrease did not occur due to the hypothermia, presumably due to a high cardiac output state induced by the fluid loading and alpha adrenergic blockade.

Others have found supporting evidence in a mouse tumour for the theory proposed here. Tozer et al (1984), in a search for anaesthetic agents to improve the results of irradiation in hyperbaric oxygen, found that a combination of ketamine and diazepam lowered the body temperature and improved the $TCD_{37/40}$ for a mouse mammary tumour in hyperbaric oxygen, but not in air. It is possible that the anaesthesia used in the early clinical experiences with irradiation, under hyperbaric oxygen, chlorpromazine and pentobarbitone sodium (Churchill-Davidson et al, 1957), acted in a similar manner.

The spinal cord has been rigorously avoided for those treatments given in hypothermic hyperbaric oxygen, but we are at present examining the late effects of single doses on pig spinal cord.

Although this approach is far from trivial and is labour intensive, it is no more so than major head and neck surgery. If a high success rate is maintained, it would be more cost effective than conventional methods of treatment with higher failure and all the attendant late care problems, quite apart from the overriding consideration of failure to cure.

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(The Editors do not hold themselves responsible for opinions expressed by correspondents)

Hypothermic hyperbaric irradiation

THE EDITOR SIR,

Last year you were kind enough to publish an account of our experiences with hypothermic hyperbaric irradiation of advanced head and neck cancer (Sealy et al, 1986b).

Since we have had a number of enquiries about our previous report, we would now like briefly to provide further results. Thirty-nine patients were to have had hypothermic hyperbaric irradiation, but in two early patients, treatment was abandoned because of reversible hypotension or cardiac arrhythmia. Thirty-seven patients, therefore, received this treatment. The initial aim was to give two hypothermic treatments at weekly intervals at the beginning of treatment (seven patients) but this proved to be impracticable. The second group (21 patients), therefore, received one initial hypothermic irradiation and, lastly, a third group (nine patients) received a single hypothermic irradiation after four fractions in air and then completed their irradiation in air. The results are shown in Table I.

Although the results with an initial single hypothermic treatment are perhaps less satisfactory than with the other two regimes, the disease-free results at 1 year (18/37 patients or 48%) of the group as a whole appear to be significantly better than those of a similar series of patients treated with conventional fractionation (63.00 Gy in 30 fractions), where 28% (18/64) of patients were free of disease at 1 year ($\chi^2 = 4.3$) (Sealy et al. 1986a). The whole group has not yet gone to 2 years, but at the present time the disease-free survival rate at 2 years of the first two groups is not significantly better than those patients treated by conventional means (Sealy et al, 1986a). There has been another major toxicity with one patient having a cardiac arrest at 27.2°C following too-rapid cooling. This patient is, however, free of disease and working 14 months after completing hypothermic hyperbaric irradiation. Because of these complications, we are investigating the alternative method of extracorporeal cooling to 16–18°C through a femoral-to-femoral bypass. This is a lesser procedure, is far better controlled and lends itself to standard radiation techniques in air.

Yours, etc.,

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Table I. Hypothermic hyperbaric oxygen in advanced head and neck cancer

Treatment regime tumour dose (Gy)	Fractions per week	Total no. of patients	Alive and well at 1 year	Later local recurrences	Total local failures	Cause of intercurrent deaths (months)
6.00* 2.00 × 2	5	7	5	2	4	
6.00* 2.00 × 20	5	21	8	4	14	Metastasis 7 Stroke 1 Pneumonia 7
2.40 × 4						
6.00* 2.40 × 18–19	4	9	5	1	3	?Cause 3 Pneumonia 6
Totals	14	37	18	7	21	5

*Hypothermic hyperbaric treatments.

MEDICAL RESEARCH COUNCIL CLINICAL RESEARCH CENTRE
DIVISION OF ANAESTHESIA

REPORT: PROF. G.G. HARRISON - VISITING SCIENTIST RESEARCH PROJECT.

JULY-AUGUST 1986.

THE HYPERTENSIVE RESPONSE TO HYPERBARIA, HYPEROXIA & HYPERTHERMIA

THE PROBLEM

Exposure of the conscious individual to an environment of hyperbaric oxygen (3 ATA, Fi 0_2 1) causes no observable changes, other than trivial and transient, in his cardiovascular dynamics.
However, when in the interests of increasing tumour radiosensitivity, patients first rendered hypothermic (core temperature 30° C) - the achievement of which necessitates the induction of anaesthesia, neuromuscular blockade and vasodilatation (α–adrenergic blockade) with fluid loading and surface cooling - were exposed to the self same environment, elevations in mean arterial pressure were observed in virtually all patients[1]. This rise in blood pressure which usually commenced towards the end of the period of cooling and was sometimes associated with manhandling of the patient into the pressure vessel, was always enhanced by pressurization. In some patients the degree of induced hypertension was severe. Further active pharmacologically induced vasodilatation with SNP was found necessary in every case (these numbered 45) to limit this rise in blood pressure to clinically safe limits. Thereafter, fall in blood pressure occurred pari passu with depressurization of the patient at the termination of the radiotherapy. Observations of cardiac output (measured by means of a Technetium/MUGA scan technique) made during the period of cooling, have shown that the rise in cardiac output that follows induction of vasodilatation and fluid loading, is maintained throughout the period of cooling down to a core temperature of 30°C. Other than for ECG monitoring and observations of blood pressure, it has not been possible to continue observation of cardiovascular dynamics under the conditions of hyperbaria within the pressure vessel.

HYPOTHESIS

As a working hypothesis to explain this reaction it was proposed that the gross vasodilatation and fluid loading induced in the patient effectively rendered the vascular bed non complaint. Increase in ambient pressure would then result in an effective increase in peripheral vascular resistance, as has been described in scuba divers[2]. This, in the face of the normal or raised cardiac output previously measured, would lead to the rise in blood pressure observed.

Alternative hypotheses and/or contributory factors that need to be considered include:-

1) Hyperoxic vasoconstriction.
2) A response to an increase in circulating endogenous catecholamines as a reaction to
i) Hypothermia
ii) Hyperbaria
iii) Inadequate depth of anaesthesia/inappropriate anaesthethic agent.

It was proposed to investigate this reaction in swine, utilising the CRC HPNS* pressure facility at Northwick Park Hospital.

* High Pressure Nervous Syndrome

OBJECTIVES

1) To validate the pig as a suitable animal model.
2) To characterise the components of the reaction through measurement of the appropriate parameters of circulatory dynamics.
These would include:-

Systolic and diastolic arterial pressure, rt. atrial, pulmonary artery and pulmonary capillary wedge pressure, together with estimation of cardiac output by a thermodilution technique. From these parameters estimates of pulmonary and peripheral vascular resistance would be derived. These latter parameters would all be measured utilising Swan-Ganz rt. heart catheterisation.
3) To investigate the effect on these components of the reaction to identified variables.

METHODS

Initially objectives 1) and 2) were to be addressed simultaneously. Regrettably, logistic difficulties were experienced with Swan-Ganz catheterisation in the pig and it became rapidly apparent that appropriate adaption of the technique for use in the pressure vessel would prove difficult and time consuming. Accordingly, as all the objectives of the investigation were dependent on the validity of the proposed animal model, subsequently this aspect alone was addressed.

The investigation was conducted on 25kg large white pigs. These were anaesthetised with i.m. ketalar 10mg/kg, thiopentone Na i.v. followed by oral intubation and maintenance of anaethesia with $N_20/0_2$ (30%)+ Halothane administered by IPPV utilising a Harvard animal ventilator for the period of preparation and cooling. Thereafter for the time the animal was in the pressure vessel, halothane and nitrous oxide were discontinued and anaesthesia was maintained by means of a continuous infusion of etomidate 1-2 mg/kg per hour, the animal being ventilated with oxygen alone by means of a Vickers fluid logic ventilator.

Jugular venous and caritod arterial cannulation, which allowed venous access for fluid and drug administration and for arterial and venous pressure recording, was established surgically.

Experiment 1: Effects of vasodilatation, fluid loading and cooling (core temperature 29^0c).
Vasodilatation was achieved by administration of Largactil 50mg followed by a fluid load of 2 1. of Hartmanns solution. The animal was then surface cooled to a temperature of 29^0C - anaesthesia being changed at this stage to continuous etomidate infusion and ventilation with oxygen (FI 0_2 1) at 1 ATA. Thereafter, the animal was rewarmed in a bath of warm water, spontaneous respiration re-established, anaesthesia discontinued and the animal allowed to recover.

Experiment 2: Effects of vasodilation, fluid loading, hyperbaria (5 ATA) and hyperoxia (FI 0_2 1) at normothermia. Following anaesthesia, establishment of monitoring, vasodilatation and fluid loading (2 1. Hartmanns solution) the pig, having been placed in a pressure vessel, was:-

1) Exposed to a pressure of 5 ATA (FI 0_2 1)
2) Depressurised to normal ambient pressure (1 ATA)
3) Fluid loading with a further 1 L. of Hartmanns solution
4) Re-pressurised to 5 ATA with FI 0_2 1.
5) Depressurized to normal ambient pressure (1 ATA).

As both major vessels on each size of the neck had been utilised, this pig was sacrificed.

Experiment 3

Effects of vasodilatation, fluid loading, hypothermia, (29°C) hyperbaria (5 ATA) and hyperoxia (FI 0_2 1). Following anaesthesia, establishment of monitoring, vasodilatation and fluid loading of a degree that raised the central venous pressure (CVP) from 4-12cm H_20 (3 1. Hartmanns solution) the test animal was surface cooled to 29°C. Therafter, it was subjected to a pressure of 5 ATA at an FI 0_2 of 1 for 15 minutes. This was followed by depressurization over 5 minutes.

RESULTS

Experiment 1

Effect of hypothermia.
Cooling caused a steady and gradual 20% fall in blood pressure from a steady state condition following vasodilatation and fluid loading, falling from 100/60 to 80/55 mgHg.

Experiment 2

Effect of hyperbaria and hyperoxia - repeated.
Other than for a transient 5 mmHg rise in systolic and diastolic pressure at commencement of pressurisation on the first occasion, the blood pressure remained stable at a level of 100/90 mmHg throughout both episodes of presurisation, depressurisation and extra fluid loading.

Experiment 3

Combined effects of hypothermia, hyperbaria and hyperoxia.
Vasodilatation, fluid loading (CVP 4-12 cm H_20) and cooling caused a gradual decline in blood pressure from 110/95 to 75/50 mmHg. Thereafter, other than for a transient 5 mmHg rise in systolic and diastolic pressure at the start of pressurisation, no change was observed in blood pressure. It remained stable at 70-75/50-55 mmHg throughout the pressurisation to 5 ATA. Decompression over 5 minutes at termination of the experiment was accompanied by an abrupt rise in blood pressure to 100/70 mmKg.

SUMMARY OF RESULTS.

Cooling of the vasodilated, fluid loaded experimental animal to a core temperature of 29°C caused only the well documented 10-30% fall in systolic and diastolic blood pressure. There was no hint at all of the whimsical rise in blood pressure that we have observed sometimes to accompany cooling of the similarly treated human[1].

Exposure of the test animal to hyperbaria (5 ATA) and hyperoxia (FI 0_2 1) at both normothermia (38°C) and hypothermia (29°C) was accompanied by no significant alteration in blood pressure. All experiments were characterised by an unvarying stability of the blood pressure and circulatory state.

This experimental model failed to reproduce the rise in blood pressure that has been consistently observed to accompany treatment in humans[1].

Ironically, rapid decompression of the experimental animal did cause a 30% rise in systolic and diastolic pressure (70.50 - 100/70 mmHg).

DISCUSSION & CONCLUSION.

While it was our original intention to investigate the changes in blood pressure, cardiac output and systemic and pulmonary vascular resistance in the vasodilated, fluid loaded pig in response to changes

in core temperature, ambient pressure FI 0_2, initial attemps at Swan-Ganz catheterisation in the pig failed. Not only did these attemps fail but they showed us also that the further adaptation and control of this technique for use in the pressure vessel would require considerable time consuming development before it could be successfully applied. As the application of this technique would only become relevant once it was shown that the hypertensive response of the human patient to the induction of hypothermia and exposure to hyperbaria could be reproduce in our animal model, further development in this direction was postponed dependent on such demonstration.

Subsequent experimentation failed to reproduce any semblance of the blood pressure response manifest by humans in reaction to vasodilatation, fluid loading, cooling, compression and hyperoxia, we failed to attain the very first objective of this investigation, i.e. to validate our choice of the pig as an appropriate animal model.

Further, though experimentation was limited, it is fair to infer that those observations we did make serve to refute the working hypothesis proposed, that the changes observed in humans subjected to these conditions follow an increase in peripheral vascular resistence due to the physical effects of hyperbaria on a peripheral vascular bed effectively rendered non-compliant by pharmacological paresis accompanied by excessive fluid loading, cardiac output at the same time being maintained or possibly increased.

The same can be said for the first alternative hypothesis - hyperoxic vasoconstriction.

In this light the most likely alternative hypothesis to explain the observed human phenomenon is that of a cardiovascular response to an increase in circulating endogenous catecholamines engendered by:-

1. Hypothermia.
2. Inadequate depth of anaesthesia or the use of an inappropriate anaesthetic.
3. Hyperbaria.

The most appropriate experimental model for further investigation of this hypothesis is obviously the human patient himself who manifests this phenomenon.

Paradoxically we are finally left to explain the 30% increase in blood pressure induced in the vasodilted, cooled and fluid loaded animal by rapid decompression from 5 ATA. This can only be due to:-

a. A sudden increase in venous return with concomitant rise in cardiac output.
b. A sudden increase in peripheral vascular resistance due to:-
    i. physical reasons
    ii. increase in endogenous catecholamines in response to eardrum pain from decompression.
    iii. oxygen bends.

Of all, perhaps the latter two are the most likely. If the former, it is as odd as the phenomenon we set out to investigate. It is perhaps worth seeing if the reaction is reproducible.

It remains for me to thank most sincerely Drs. J.F.Nunn and M.J.Halsey - Directors respectively of the CRC Division of Anaesthesia and the HPNS Research Group - for inviting me to undertake this investigation at Northwick Park and subventing it, and those CRC/HPNS staff* who were involved in the investigation for their help, expertise and time so willingly given. I am only sorry that the outcome was not more positive.

Gaisford G. Harrison

* Peter Pearce
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PART 5.

MISCELLANEOUS.

Brit. J. Anaesth. (1950), 32, 164

THE CLINICAL USE OF HALOTHANE ANAESTHESIA DURING CARDIOPULMONARY BYPASS FOR OPEN-HEART SURGERY

BY

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DURING open heart surgery with cardiopulmonary bypass, whilst extracorporeal circulation is in progress, the anaesthetist is faced with the problem of maintaining anaesthesia without access to the lungs. In addition, markedly altered blood circulation raises problems with regard to the intravenous route of anaesthetic administration. At the commencement of perfusion, he is faced with the mixing of two blood volumes—that of the anaesthetized patient and that of the donor blood used to prime the pump oxygenator. This mixing inevitably causes marked alteration in the level of anaesthetic agents in the patient's circulation, unless some means of equilibrating anaesthetic levels in both blood volumes can be achieved. In the lightly anaesthetized patient, the dilution of anaesthetic concentration occurring may have the undesirable effect of allowing excessive movement on the part of the patient: even return of consciousness is not unknown. The addition of intravenous anaesthetic agents and relaxants to maintain an even level of anaesthesia is extremely difficult to judge and, as these agents are eliminated relatively slowly from the body, control may be far from flexible. This makes the desired rapid postoperative recovery from the influence of anaesthetic agents and relaxants difficult to achieve, unless extremely fine judgment is exercised in their use.

Certain properties of halothane led us to believe that this agent might prove useful in overcoming these difficulties. Firstly, the clinical use of halothane for cardiac and thoracic surgery, not requiring cardiopulmonary bypass, has shown us that it is capable of providing adequate and safe anaesthesia for these procedures when it is used as the principal anaesthetic agent with nitrous oxide and oxygen without the use of relaxants or of intravenous anaesthetic agents. Secondly, the easy control of the depth of anaesthesia obtained using small changes in the inhaled concentration seems to indicate that its concentration in the blood is readily alterable in either direction.

Following the successful experimental use of halothane anaesthesia for cardiopulmonary bypass procedures in dogs (Bull et al., 1959) it was decided to extend its use to clinical practice for patients undergoing open heart surgery using the DeWall-Lillehei pump oxygenator, with halothane as the principal anaesthetic agent both before and during extracorporeal circulation.

Twenty-eight consecutive cases have been anaesthetized in this way.

METHOD OF ANAESTHESIA

Premedication.

Eighteen cases in the series received pentobarbitone 3 mg/kg by mouth 2 hours before induction and pethidine 1 mg/kg together with atropine 0.6 mg (1/100 grain) by intramuscular injection 1 hour before anaesthesia. In the remaining ten cases pethidine was omitted and pentobarbitone alone was used.

Induction.

In all patients in this series anaesthesia was induced using nitrous oxide, oxygen and halothane. Nitrous oxide and oxygen in the proportion of 70 per cent nitrous oxide to 30 per cent oxygen were delivered through a "Fluotec" vaporizer. At the commencement of induction the halothane concentration was increased fairly rapidly during the first 1 or 2 minutes to 1 per cent and thereafter more slowly to 1.5 per cent. After about 5 minutes, the larynx and trachea were sprayed with 2 or 4 per cent lignocaine,

the total dose of lignocaine never exceeding 2 mg/kg body weight. Intubation was performed after a further 2 or 3 minutes of inhalation of 1.5 per cent halothane, nitrous oxide and oxygen mixture.

Maintenance.

Anaesthesia was maintained with nitrous oxide 70 per cent, oxygen 30 per cent, and halothane 1 per cent using a high flowrate via a semiclosed system with carbon dioxide absorption.

Spontaneous or assisted respiration was allowed up to the time of thoracotomy. Just prior to opening of the chest a very brief period of overventilation invariably allowed full control of respiration to be achieved and in no case was any relaxant used. Controlled respiration was continued—the halothane concentration still at 1 per cent—up to the time of commencing extracorporeal circulation. During extracorporeal circulation anaesthesia was maintained in the following manner. A Fluotec vaporizer was placed on the oxygen supply line to the DeWall bubble oxygenator, which was set up as described by McKenzie and Barnard (1958), but included a stainless steel canister debubbler. During priming of the oxygenator and the helix reservoir with blood, this vaporizer was set at 1 per cent. However, oxygen flow during priming was of the order of 1 l./min and as the Fluotec vaporizer used in this series was the Mark I model, it is likely that the halothane concentration delivered with the oxygen was less than 1 per cent. During perfusion, oxygen flow through the Fluotec was always in excess of 4 l./min and so the concentration of halothane delivered to the oxygenator was assumed to correspond closely to the vaporizer setting (Mackay, 1957; Hill, 1958). In eighteen cases the Fluotec setting was maintained at 1 per cent throughout perfusion and in the remaining ten cases the setting was deliberately altered from 1 per cent to 0 per cent. The oxygen to blood flow ratio through the oxygenator was in all cases approximately 2.5 or 3 to 1. Some variation of perfusion rate without corresponding adjustment of oxygen flow during individual perfusions makes it impossible to give this figure more accurately.

At the conclusion of extracorporeal circulation, pulmonary ventilation was recommenced using nitrous oxide and oxygen only. In all but three cases this sufficed for maintenance during closure. In the three cases mentioned halothane 1 per cent was added for short periods as required.

RESULTS

The natures of the operations, the ages and the weights of the patients, the durations of extracorporeal circulation, the rates of perfusion and the mean perfusion pressures in each patient are given in table I.

General.

The premedication indicated proved satisfactory in all cases and the patients were either asleep or extremely drowsy. The omission of pethidine from premedication in some patients was not governed by any contraindication to its use and both types of premedication provided equally satisfactory sedation.

During induction and intubation no difficulty was experienced. Anaesthesia was rapidly and smoothly achieved. Short-lived coughing during spraying of the larynx and trachea was not uncommon but laryngeal spasm was never encountered. During the period of surgery preliminary to extracorporeal circulation it was necessary to increase halothane concentrations to 1.5 per cent for short periods in two cases during the first 30 minutes of anaesthesia. In all the other cases, 1 per cent halothane provided adequate anaesthesia and allowed easy control of respiration during both extrathoracic and intrathoracic preliminary surgery, prior to cardiopulmonary bypass.

During bypass, no clinically detectable variation in the depth of anaesthesia occurred unless the halothane concentration supplied to the oxygenator was altered. In the ten cases where such alteration was made, it appeared that 0.7 per cent was the critical level below which respiratory movements in the patient commenced. When a change in the depth of anaesthesia was brought about by an alteration of the halothane concentration to the oxygenator, a period of five to ten minutes elapsed before this became clinically obvious. By maintaining 1 per cent halothane with the oxygen to the bubble oxygenator no respiratory or other movement on the part of the patients occurred except in two cases. In these

TABLE I

Type of defect	Age (years)	Weight (kg)	Duration of perfusion (min)	Perfusion rates (ml/kg)	Mean perfusion pressure (mm/Hg)	Pot. citrate arrest (duration in min.)
A.S.D.	8	21	25	80	–	–
P.S.	9	44	23	60	70	–
V.S.D.	6	19	69	85	60	–
V.S.D.	10	31	44	80	60	21
Traumatic fistula	29	53	39	65	90	–
E.C.D.	9	22	120	75	75	–
P.S.	10	24	71	90	70	–
M.S.	37	43	66	60	90	–
F.	13	32	109	75	50	40
M.I.	30	60	70	55	95	–
E.C.D.	10	23	54	75	55	–
P.S. + A.S.D.	23	43	66	65	70	–
P.S.	7	19	32	80	65	8
A.S.D.	16	47	42	60	60	–
P.S.	4	13	60	90	65	–
P.S.	8	26	36	75	60	–
V.S.D.+P.D.A.	4	14	48	85	55	19
F.	7	19	73	100	60	53
E.C.D.	8	19	81	75	75	–
V.S.D. + A.I. + P.I. +T.I.	20	70	83	50	60	48
V.S.D.	7	17	66	80	50	27
V.S.D.	4	13	68	90	40	44
F.	5	14	92	113	40	55
M.I.	44	45	82	70	90	–
A.S.D.	8	19	27	85	50	–
A.S.D.	10	33	33	80	50	–
F.	16	51	79	60	70	25
P.S.	9	26	24	80	55	–

A.S.D.=Atrial septal defect;
V.S.D.=Ventricular septal defect;
M.I.=Mitral incompetence;
E.C.D.=Endocardial cushion defect;
P.I.=Pulmonary incompetence;
P.S.=Pulmonary stenosis;
M.S.=Mitral stenosis;
F=Fallot's tetralogy;
A.I.=Aortic incompetence;
P.D.A.=Patent ductus arteriosus.

two cases three or four gentle diaphragmatic contractions occurred after five minutes of perfusion and then ceased. This was thought to be due to low halothane concentration achieved during priming of the pump oxygenator in these cases. In one other case diaphragmatic contractions occurred after twenty minutes of perfusion. This coincided with a temporary obstruction of the superior vena caval catheter and was corrected as soon as the obstruction was relieved.

Halothane levels in the blood.

In four cases samples of arterial blood were taken for estimation of the halothane content. All samples were taken from a cannula placed in the right internal mammary artery. Halothane estimations were performed by the method of W. A. M. Duncan (1959), modified by J. E. Kench and E. J. Duncan (in press). The results of analysis are given in figure 1.

Blood gas values and pH.

Arterial and venous samples taken before perfusion, during perfusion, at 20 minutes and 10 minutes after perfusion, were subjected to analysis for oxygen saturation and carbon dioxide content. The arterial samples were all taken from the right internal mammary artery. Venous samples were obtained either from a catheter placed in the inferior vena cava via the saphenous vein, or from an arm vein. The result of these estimations is given in figure 2. The pH values of arterial blood samples taken at the same times as above in 12 of these cases are given in table II. The pH estimations were carried out on fresh arterial blood using the Beckman model "G" pH meter

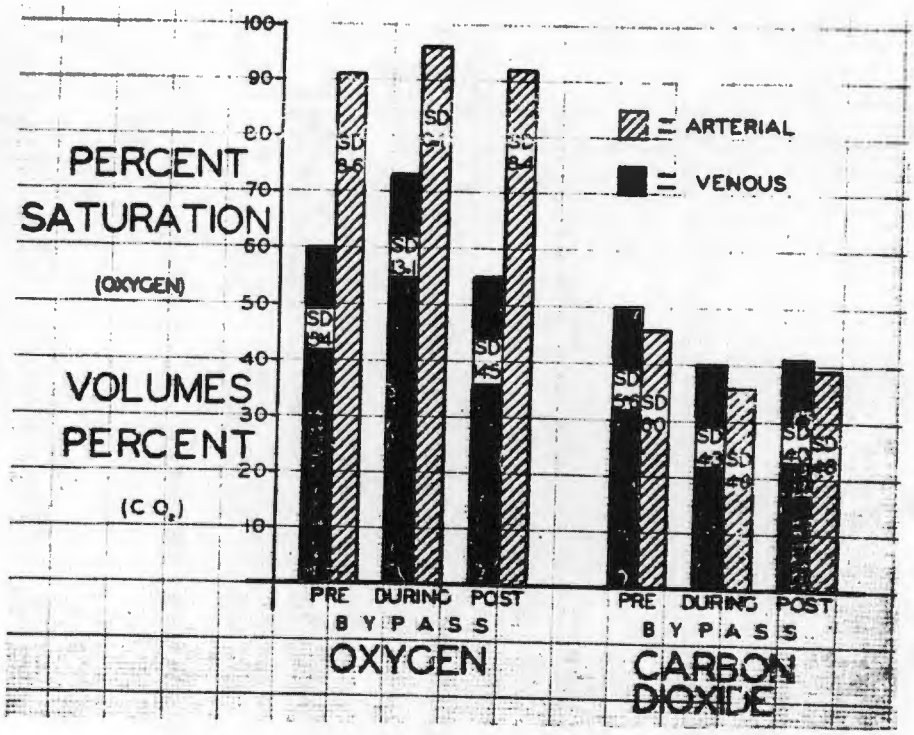

FIG. 1
Mean oxygen saturations and carbon dioxide contents of venous and arterial blood in 28 patients presented as a histogram.

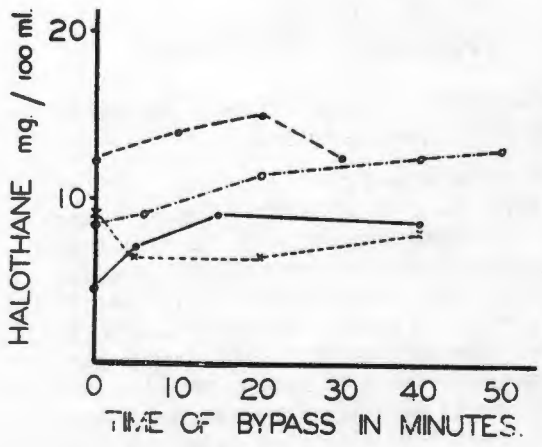

FIG. 2
Halothane values at 0 minutes on graph represent the level in arterial blood before the start of cardiopulmonary bypass. Other points represent the levels at times indicated during bypass in four cases.

by a simplified method (Turner, 1959). In three cases, simultaneous arterial samples and mixed venous samples from the venous well were obtained at known perfusion rates just after closure of the cardiac defect. Using the Fick principle, oxygen uptake was calculated from these values and gave the results indicated in table III.

Perfusion pressures.

Perfusion rates and mean perfusion pressures were as indicated in table I and in all except one case the perfusion pressures were monitored by a strain gauge attached to a catheter in the right internal mammary artery.

Electroencephalograph.

Electroencephalographic monitoring was available in all adult patients. No undue suppression of cortical activity attributable to the anaesthetic agent or technique was evident. This will be the subject of a further communication.

TABLE II

Nature of defect	pH			Total CO_2 m.equiv/l.		
	Before	During	After	Before	During	After
V.S.D.	7.35	7.44	—	23.2	13.2	—
V.S.D.	—	7.36	7.32	—	17.4	24.2
A.S.D.	7.39	7.42	7.32	—	—	—
P.S.	7.36	7.43	7.41	13.52	21.24	14.35
Endo. Cush.	7.41	7.33	7.36	—	—	—
Fallot	7.48	7.53	7.34	—	—	—
Endo. Cush.	7.28	7.41	7.26	27.4	19.0	29.2
Fallot	7.41	7.41	7.38	22.6	16.1	24.6
V.S.D.	7.41	7.43	7.39	23.5	16.0	22.3
P.S.	7.35	7.40	7.34	25.0	15.0	22.0
A.S.D.	7.42	7.38	7.38	22.2	13.9	19.1
A.S.D.	7.43	7.39	7.44	25.4	17.8	25.2

pH values and carbon dioxide determinations are obtained from the same blood sample taken from the internal mammary artery in each case. Samples were taken just before bypass, during bypass, at 20 minutes and 10 minutes after bypass.

TABLE III

Weight of patient (kg)	Pump output (ml/min)	Arterial oxygen saturation per cent	Venous oxygen saturation per cent	Hb. G. per cent ml	Oxygen uptake (ml/kg/min)
33	2800	96	63	13	4.8
19	1980	96	64	13	5.8
26	2080	95	60	13	4.8

The oxygen uptake was calculated, using the Fick principle, from known pump output and arterial and venous oxygen content determined from percentage saturation and haemoglobin value.

The heart.

In the twenty-eight patients operated upon using halothane anaesthesia, the heart tolerated handling during various surgical procedures extremely well. Apart from occasional extrasystoles and short bouts of atrial fibrillation, no serious arrhythmias were noticed.

The arterial pressures were continuously recorded by means of a polyethylene tube in the internal mammary artery and the inferior vena caval pressures by means of a polyethylene tube in this vessel. In none of the cases in this series were any unusual changes noticed which could suggest inadequate cardiac function.

During total cardiopulmonary bypass the heart behaved normally. It tolerated potassium citrate asystole extremely well and took over adequately after the discontinuation of asystole and total bypass.

Postperfusion.

At the end of perfusion, ventilation of the lungs was recommenced using only nitrous oxide and oxygen. The blood pressure in all cases returned to near the level recorded before perfusion, provided that the patient's blood volume had been satisfactorily maintained. In this connection it must be emphasized that accurate assessment of blood loss throughout the operation must be energetically striven for and any deficiency rapidly made good. Nitrous oxide and oxygen provided adequate anaesthesia at this stage and permitted controlled respiration until the closure of the chest, except in the three cases already referred to. All the patients were awake and able to answer questions intelligibly within ten minutes of the end of anaesthesia.

DISCUSSION

The state of anaesthesia obtained with halothane, when used as described, has been entirely satisfactory, both from the point of view of surgeon and of the anaesthetist. A degree of stability and control was achieved more easily than has been possible in our hands using thiopentone and relaxants in a series of thirty cases reported by

one of us (Ozinsky, 1959). There was no death in the series and no morbidity which could be attributed to the anaesthesia. As halothane is eliminated by the lungs there is the advantage that there need be no fears regarding circulatory, renal or hepatic elimination of this agent.

Hypotension before or after perfusion has not been of such a degree as to cause anxiety. A fall in systolic pressure of some 10 to 20 mm Hg from pre-anaesthetic levels is usual. Pressures during perfusion may remain somewhat lower than those which have been encountered with comparable perfusion rates using thiopentone and relaxants. This is probably due to the action of halothane on peripheral vessels described by Burn and Epstein (1959). If so, it is felt that this may be an advantage as it would indicate a lesser degree of peripheral vasoconstriction from sympathomimetic stimuli induced by reflexes brought about by the altered haemodynamics of total body perfusion under light anaesthesia. Such sympathetic stimuli may be a potent cause of metabolic acidosis (Griffiths et al., 1939; DeWall et al., 1956). It is felt that some degree of vasodilatation is also beneficial in the immediate postperfusion stage because it allows the traumatized heart to take over initially against a diminished vascular resistance.

The postoperative recovery and the electroencephalographic recordings have given no indication that cerebral hypoxia occurred in any case. Clinical observation also confirmed the existence of a good peripheral circulation as evidenced by good colour of conjunctivae and extremities throughout perfusion.

Encephalocardiographic recordings and the observation of an experienced cardiac surgeon have given no indication of cardiac arrhythmias or increased myocardial instability which could be attributed to the anaesthesia. In cases in which elective cardiac arrest was produced with potassium citrate (see table I) the resumption of normal rhythm was not delayed. In four cases in which ventricular fibrillation accompanied the termination of potassium citrate arrest, defibrillation was not difficult to achieve.

There is no indication of any interference with gas exchange in the oxygenator. The three figures obtained for oxygen uptake, although too few to provide conclusive evidence, appear to be satisfactory. There is no notable depression in oxygen consumption, but it is interesting to speculate on the possible results of using higher blood anaesthetic concentrations. In one experiment on a dog, one of us (A.B.B.) has deliberately produced cardiac arrest during perfusion with blood containing a high halothane content. This arrest was reversible on eliminating the halothane via the pump oxygenator system, and the possibility remains that a general depression of all tissue metabolism may be attainable, thus permitting the use of lower perfusion rates when this state has been achieved.

The blood halothane levels obtained show a satisfactory state of consistency throughout bypass. Clinical observation and e.e.g. monitoring have led us to the conclusion that 1 per cent halothane provides adequate and safe anaesthetic conditions.

SUMMARY

A technique of anaesthesia for open heart surgery using the Lillehei-DeWall pump oxygenator is described. Halothane, without intravenous agents, is used as the principal anaesthestic agent and is administered via the lungs prior to, and during, extracorporeal circulation and via the bubble oxygenator during cardiopulmonary bypass. The results obtained in twenty-eight unselected patients, in whom most types of open heart surgery were undertaken, when anaesthetized in this way are discussed. There was no mortality or morbidity in this series.

ACKNOWLEDGMENTS

In work such as this which involves a large team, it is impractical to acknowledge individually all those concerned. We would, however, particularly like to thank Dr. C. N. Barnard and Mr. W. Phillips for their encouragement and co-operation in developing this technique for use on their patients. Prof. J. E Kench, Dr. D. MacKenzie, and their technical staff estimated blood halothane levels and pH values for us. Mr. J. Rees of Western Province Blood Transfusion Service provided blood gas values and we also thank Mr. C. Goosen, senior technician in the Department of Surgery for his valuable assistance in providing sampling facilities.

Our thanks are also due to Drs. J. F. W. Mostert and J. G. Burger, Medical Superintendents of Red Cross War Memorial Children's Hospital and Groote Schuur Hospital, for their permission to publish this report.

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RADIOTHERAPY WITHOUT TEARS

BY

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SUMMARY

A method of anaesthesia which has been used on 100 occasions for radiotherapy in infants and unco-operative small children is described. The method is applicable when the anaesthetist must remain outside the treatment room, and consists of the insufflation of nitrous oxide, oxygen and halothane through the side-arm of an oropharyngeal airway. This method overcomes the disadvantages of tracheal intubation or heavy sedation in cases in which therapy must be frequently repeated.

Radiotherapy combined with surgery and cytotoxic drugs is currently accepted as the best treatment of certain malignant tumours of childhood. Such tumours include Wilms tumour, retinoblastoma, lymphoma, neuroblastoma and cerebral tumours. Radiotherapy is prolonged, consisting of sessions of irradiation of about 5 minutes, administered on alternate days for 21 to 28 days or more, depending on total dose desired.

Considering the awesome aspect of the therapy machines together with the fact that no one may be with the patient during the period of irradiation, it is surprising that the great majority submit to the complete course of therapy with little or no restraint and no sedation.

Over the past three years 96 children have been so treated in this Unit. However, in a small number of patients in the infant-to-toddler age group of 1½ to 5 years of age, patient co-operation may be impossible to obtain. In treating some lesions involving small fields such as retinoblastoma, for which small beams are used, complete immobility of the patient is absolutely essential for the accuracy and success of treatment. Further, as these children have usually already suffered enucleation of one eye they do not take kindly to the proximity of a therapy machine to the other. Sedation of the patient becomes virtually a sine qua non. The provision of adequate sedation on such a regular basis poses many problems. If the infant is sedated sufficiently to secure certain immobility during the period of therapy, it will be extremely drowsy and subsequently irritable for the best part of the day—no matter which of the conventional sedatives or tranquillizers is used. This would not matter much over a short period, but over a period of daily treatment as protracted as that needed for the radiotherapy the infant's nutrition may suffer greatly. Further, the infant often develops tolerance to the drug used. This results after a while in uncertainty of dose response.

Basal narcosis with thiopentone sodium, or bromethol given rectally, though theoretically attractive as a method, has been found to be unsatisfactory in practice for several reasons. The children are drowsy and irritable for a long period after bromethol. This is less so with thiopentone. Precision in dosage is difficult to achieve. Chronic usage of these drugs per rectum appears to cause proctitis and colitis with much secretion of mucus resulting in great uncertainty in the absorption of the drug. Accuracy of dosage becomes impossible after as few as five anaesthetics.

Halothane anaesthesia (Bryce-Smith and O'Brien, 1956; Bull, du Plessis and Pretorius, 1958) has certain characteristics which it appeared to us would help solve this problem.

(1) Anaesthesia is rapidly and smoothly induced, especially in infants.

(2) Recovery is rapid and complete, especially after a short period of anaesthesia. Nausea and vomiting are rare accompaniments of halothane anaesthesia. A child may be fed soon after recovery of consciousness.

(3) The activity of pharyngeal and laryngeal reflexes is markedly reduced.

(4) The drug is of sufficient potency to allow of easy maintenance of anaesthesia by an insufflation technique, thus obviating the need of an anaesthetic facepiece or endotracheal tube. The former is undesirable from the aspect of the observation of the patient at a distance and the latter because of the possibility of laryngeal sequelae considering the frequent repetition of the procedure.

(5) Halothane is non-explosive.

We evolved the following simple method of anaesthesia for radiotherapy of infants, which we have now used successfully for the course of radiotherapy of six patients, involving 100 treatment sessions (table I). If therapy is planned for early in the morning, breakfast is postponed until after therapy. If therapy is planned for early in the afternoon, breakfast is given but lunch is postponed till after therapy so that a period of 4 hours is allowed to elapse from the last meal. Forty-five minutes before therapy, premedication of atropine 0.2–0.3 mg is given intramuscularly, depending on

TABLE I

Patients anaesthetized for radiotherapy, 1959–1962.

Patient	Age in years	Tumour	No. of treatment sessions
MB	5	Retinoblastoma	19
VN	4	Retinoblastoma	21
BC	1½	Retinoblastoma	20
FA	1½	Retinoblastoma	12
HO	3	Rhabdomyosarcoma of nasopharynx	12
LB	3	Wilms	16
6			100

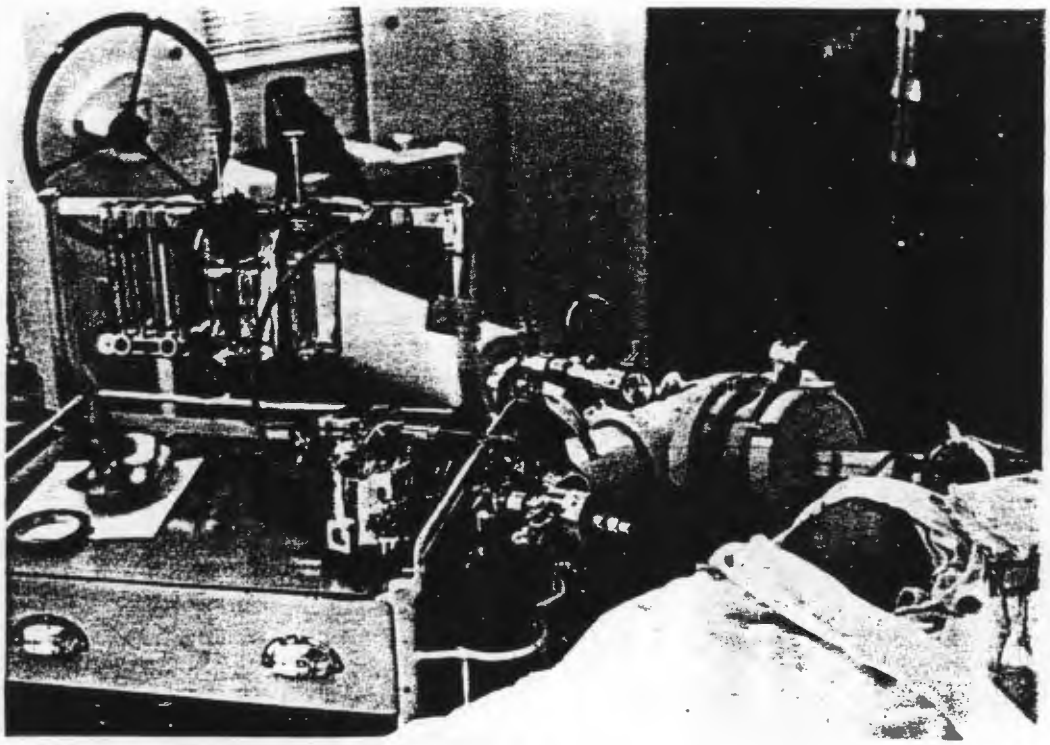

FIG. 1

The patient during therapy (for retinoblastoma) as observed from control bay. Note abdomen, lower thorax and cotton wool "butterfly" on airway clearly visible, as also Boyle machine Rotameters.

the weight of the child. The infant is placed on the therapy table and anaesthesia is induced with nitrous oxide, oxygen and halothane from a standard Boyle machine and Fluotec vaporizer, delivered by means of an infant-modified T-piece bag and facepiece (Cape Town System; Voss, 1963). The halothane concentration is rapidly increased to 2 per cent. Anaesthesia of adequate depth, judged by absence of eyeball movement, small pupils, quiet respiration, absence of pharyngeal reflexes and loss of tone in the limbs (analogous to Guedel stage 3, plane II) is usually achieved in from 5 to 8 minutes. At this stage a Waters oropharyngeal airway (with side-arm) is introduced and the delivery tubing from the Fluotec and Boyle machine, formerly attached to the bag assembly, is now attached to the Waters airway. When radiation of the pharyngeal or mouth region is required, the airway should be non-metal, in order to avoid radiation scatter. The mouth is shut on the airway by means of adhesive tape. The child is now positioned with the head extended and the chest and abdomen uncovered to facilitate observations (figs. 1 and 2). A small cotton wool "butterfly" is fixed over the Waters airway. If desired, a stethoscope with extended tubing may be fixed to the chest wall. This was done on the first ten occasions on which this method was used. However, as the method proved so trouble-free, we deemed it safe to omit. In the presence of continual visual observation of thorax and abdomen we have found the "butterfly" sufficient corroborative evidence of the state of ventilation. The halothane concentration is now reduced to 1 per cent. The radiotherapy machine is positioned and the radiotherapist and anaesthetist retire to the observation bay. Radiotherapy is commenced.

FIG. 2

Close-up of head assembly showing Waters airway, tube for anaesthetic gases and cotton wool "butterfly".

(*Note*: For therapy to pharyngeal growths, this airway should be non-metal to prevent radiation scatter.)

Throughout the period of therapy—usually only 5 minutes—the anaesthetist observes the patient and the anaesthetic machine closely through the glass panel of the control bay. The child's respiration is easily observed by correlating observations of the cotton wool butterfly with movements of the thorax and abdomen. If anything amiss is observed, the radiation is interrupted and the anaesthetist is able to reach the patient within a matter of seconds. In the 100 treatments here reported, such interruptions of therapy have not been necessary.

At the conclusion of the treatment session anaesthesia is discontinued. The child regains consciousness within 5 minutes and may be safely fed an hour later.

ACKNOWLEDGMENTS

We wish to thank Mr. B. Todt of the Clinical Photography Department for the photograph, and the Superintendent of Groote Schuur Hospital, Dr. J. G. Burger, for permission to publish these results.

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RADIOTHÉRAPIE "SANS PLEURS"

SOMMAIRE

Les auteurs signalent une méthode d'anesthésie utilisée dans 100 cas de radiothérapie de bébés et d'enfants en bas âge crispés et causant des difficultés. La méthode peut être employée lorsque l'anesthésiste a la possibilité de rester hors de la pièce de traitement. Ce dernier consiste dans l'insufflation de protoxyde d'azote, d'oxygène et d'halothane par une entrée latérale d'une canalisation de gaz placée dans la région oropharyngée. Cette méthode vient à bout des inconvénients de l'intubation trachéale et évite une sédation trop brutale dans les cas dans lesquels le traitement doit être répété plusieurs fois.

STRAHLENTHERAPIE OHNE TRÄNEN

ZUSAMMENFASSUNG

Es wird eine Narkosemethode, die bei 100 strahlentherapeutischen Eingriffen an Säuglingen und unruhigen Kleinkindern angewendet wurde, beschrieben. Die Methode ist brauchbar, wenn der Anästhesist außerhalb des Behandlungsraumes bleiben muß. Sie besteht in einer Beatmung mit Lachgas, Sauerstoff und Halothane durch ein rechtwinkliges Verbindungsstück eines oropharyngealen Tubus. Diese Methode überwindet die Nachteile der trachealen Intubation oder starken Sedierung bei Fällen, bei denen die Therapie häufig wiederholt werden muß.

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The effect of cardiac lesions on the action of suxamethonium

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A paralysing dose of suxamethonium when injected rapidly intravenously, almost invariably produces marked muscle fasciculation, followed immediately by profound paralysis. However, the author formed the impression that when used to facilitate endotracheal intubation during the induction of general anæsthesia in patients presenting for open heart surgery, a greater number of cases failed to fasciculate than is generally the case. To establish the validity or otherwise of this impression, the following simple investigation was carried out.

METHODS

In all adult patients presenting for open heart surgery, suxamethonium chloride in a standard dose of 50mg was used to facilitate endotracheal intubation following the induction of general anæsthesia with thiopentone sodium. This dose was injected rapidly intravenously into an ante-brachial vein. The subsequent presence or absence of muscle fasciculation was recorded. When present, the quality of the fasciculation was noted as being (a) good to moderate or (b) poor. The time elapsing from injection of suxamethonium to onset of fasciculation was recorded. All these patients had previously been investigated by cardiac catheterization, including the use of dye dilution curves, by the Cardiac Clinic of Groote Schuur Hospital. Details of cardiac output and the characteristics of the dye dilution curves recorded at these investigations were examined in each case. These cardiac laboratory investigations had been done at periods varying from days to months before the patients presented for surgery.

In another randomly selected group of patients presenting for general and ENT surgery, in whom the cardiovascular system

*Based on a paper presented at the 45th South African Medical Congress, 1965

appeared clinically normal, similar observations were made as to the presence or absence and quality of muscle fasciculation following the injection of a standard dose of 50mg suxamethonium chloride. These patients constituted a control group.

RESULTS

The patients included in this investigation fell into three broad groups:

1 23 patients who had atrio–septal defects.
2 39 patients who had mitral valve disease which had resulted in incompetence or mixed incompetence and stenosis. Twelve of these patients had associated tricuspid incompetence while 4 had aortic incompetence. Patients suffering from pure mitral stenosis who were treated by closed heart valvotomy were not included in this investigation.
3 19 patients who had aortic valve disease which had resulted in stenosis and/or incompetence. One of these patients had associated mitral stenosis.

Patients suffering from other lesions such as Fallot's Tetralogy and pulmonary stenosis presented for cardiac surgery during this investigation, but were in insufficient numbers to warrant comment.

Incidence of muscle fasciculation

Observations relating to the incidence and quality of muscle fasciculation in the patients in these 4 groups are presented in table 1.

TABLE 1

Quality of muscle fasciculation following IV suxamethonium 50mg

% incidence in parenthesis

QUALITY OF MUSCLE FASCICULATION	ATRIO SEPTAL DEFECT	MITRAL VALVE DISEASE	AORTIC VALVE DISEASE	CONTROL GROUP
Total No. in Group	23	39	19	39
None	2 (8.7%)	*13 (33.3%)	2 (10.5%)	2 (5.1%)
Poor	2 (8.7%)	†13 (33.3%)	5 (26.3%)	1 (2.5%)
Good-moderate	19 (82.6%)	13 (33.3%)	12 (63.2%)	36 (92.4%)

$X^2 = 34.89$ on 6 d.f. $P < 0.001$

*5 assoc TI, 1 assoc AI

†6 assoc TI, 2 assoc AI

The differences in the incidence and quality of fasciculation shown here are statistically highly significant. From this table it will be seen that as many as one third of the patients with mitral incompetence failed to show muscle fasciculation following the administration of a standard dose of 50mg suxamethonium, while a further one third showed only poor muscle fasciculation.

It was noted further, that in 9 of the 13 patients with mitral incompetence who failed to fasciculate and in 7 of the 13 who showed only poor fasciculation, inadequate relaxation of the larynx and coughing

on intubation necessitated a further dose of suxamethonium. Of these 16 patients 6 had associated tricuspid incompetence. A repeat dose of suxamethonium was necessary in only 1 case in a group of patients suffering from aortic valve disease and in none of those with atrio–septal defects. (See table 2).

TABLE 2

Incidence of repeat dose of suxamethonium
Total No. in each group in parenthesis

TYPE OF FASCICULATION	ATRIO SEPTAL DEFECT	MITRAL VALVE DISEASE	AORTIC VALVE DISEASE	CONTROL GROUP
None	0 (2)	*9 (13)	0 (2)	0 (2)
Poor	0 (2)	†7 (13)	1 (5)	0 (36)
Good–moderate	0 (19)	0 (13)	0 (12)	0 (36)
		*3 assoc TI 1 assoc AI	†3 assoc TI 1 assoc AI	

Dye Dilution Curves

Looked at in another way dye dilution curves, used in the first instance for diagnostic purposes, could be regarded as illustrating graphically the manner in terms of time and concentration in which any drug injected IV would reach the tissues. Certain parameters of the dye dilution curves of these patients were examined from this aspect.

In recording dye dilution curves the site of injection of the dye used—in this case cardio green—and the distal site of sampling are varied to meet the diagnostic need. The one combination of injection site and sampling site, common to all the dye dilution curves examined, was injection of the dye into the main pulmonary artery with sampling via the brachial artery. Only the parameters of this type of dye dilution curve were examined. The following features were noted:

1 General shape of the curve
2 Presence or absence of recirculation curve
3 Peak concentration of dye
4 'Build up' time

While the dye curves of those patients with atrio–septal defects differed from the normal only in the distortion of the disappearance slope, the curves of those patients with mitral incompetence showed marked deviations from the normal. In general, these showed:

(*a*) prolonged build up times
(*b*) low peak concentrations of dye
(*c*) prolonged disappearance slope and
(*d*) absence of recirculation curve

These differences are illustrated in comparison with a normal curve in Figure 1. In the patients with mitral incompetence, the effects of valve incompetence were usually associated with those of low cardiac output. The dye curves of those patients with aortic incompetence showed changes similar qualitatively to those with mitral incompetence but of a much lesser degree.

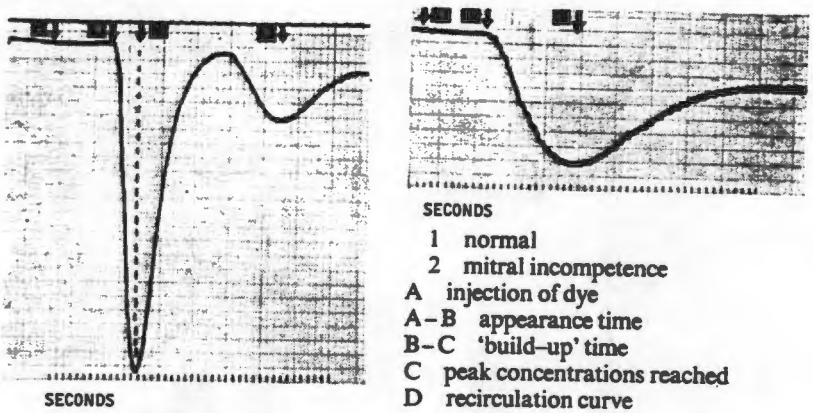

FIGURE 1 Specimen pulmonary artery – brachial artery dye dilution curves

Build-Up Times

Measurement of 'build–up' times alone revealed the differences shown in table 3. (It should be noted that the numbers in the three groups are not the same as in the previous tables, as dye curves were not available for all cases.)

TABLE 3
Dye dilution curve "build-up" time

	ATRIO SEPTAL DEFECT	MITRAL VALVE DISEASE	AORTIC VALVE DISEASE
Mean time seconds	2.7	9.1	6.3
SD ±	0.64	3.61	1.82
Number of curves examined	13	32	10
	F=34	P<0.001	

In the group of patients with mitral incompetence, analysis of the incidence and quality of muscle fasciculation against the dye curve 'build–up' time revealed a grossly prolonged mean 'build–up' time in those patients who failed to fasciculate as compared to those that did. (table 4).

TABLE 4
Dye dilution curve "build-up" time. Mitral valve disease only

	MUSCLE FASCICULATION		
	None	Poor	Good–moderate
Mean time seconds	12.3	8.7	6.8
SD ±	3.87	2.34	1.26
Number of curves examined	12	9	11
	F=11.25	P<0.001	

These differences in 'build–up' time, shown graphically in Figure 2, are, in each case, statistically highly significant on a one–way analysis of variance. The mean 'build–up' time in the patients with mitral incompetence who failed to show muscle fasciculation was 12.3 seconds. Computation of the 5% limits of this mean time would lead

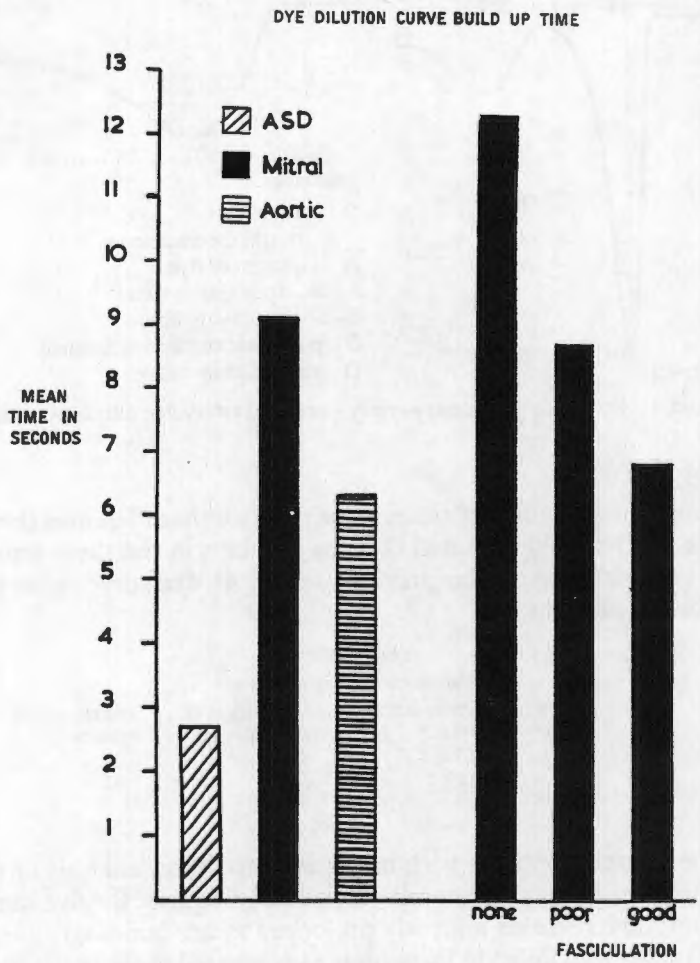

FIGURE 2 Histogram of dye curve mean 'build-up' time

us to expect that 95% of such patients with dye curve 'build-up' times in excess of 10.6 seconds would fail to show fasciculation following injection of a 50mg dose of suxamethonium.

In the case of the group of patients with aortic valve disease, though there is a difference in mean 'build-up' time between poor and good 'fasciculaters', this difference is not significant in the numbers in this investigation.

Circulation time

At the time of induction of anæsthesia, the time taken from injection of suxamethonium to the onset of muscle fasciculation was recorded.

This was regarded as a measure of the arm vein–muscle mass circulation time. Results obtained in those patients who showed muscle fasciculation are recorded in table 5.

TABLE 5

Arm vein—muscle mass circulation time

TIME IN SECONDS	ATRIO SEPTAL DEFECT	MITRAL VALVE DISEASE	AORTIC VALVE DISEASE	CONTROL GROUP NO CARDIAC LESION
Mean circulation time	26	64	39	24
SD ±	11	28	20	8
Range	13–58	27–130	14–88	16–42
No. in group	21	26	17	37

F=31.27 P<0.001

Tested on a one way analysis of variance, the differences shown are statistically highly significant.

As circulation time in general depends on cardiac output, we may perhaps conclude from the excessively slow mean circulation time displayed by patients who had mitral incompetence–over twice as long as that in patients with atrio–septal defects or the control group–that this group of patients had the lowest cardiac output at the time of surgery. Though no direct measurement of cardiac output is made at the time of surgery, this parameter had been measured at the time of catheterization. As the time interval between cardiac catheterization and surgery varied widely between a few days and many months and the conditions under which the two measurements were made were different, it was not surprising that no good correlation could be established between cardiac output as measured during catheterization and the suxamethonium circulation time at the time of surgery. However, the patients with mitral incompetence as a group, showed the lowest mean cardiac index (this difference being statistically significant) at the time of catheterization (table 6).

TABLE 6

Mean cardiac index of patients who showed fasciculation

	ATRIO SEPTAL DEFECT	MITRAL VALVE DISEASE	AORTIC VALVE DISEASE
Mean cardiac index 1/min/m²	3.0	2.1	3.2
SD ±	1.09	0.67	1.57
Number in group	14	23	15

F=6.47 P<0.01

Table 6 includes only those patients who showed muscle fasciculation. If the patients who failed to show muscle fasciculation were included, the mean cardiac index of the patients with mitral incompetence would be lower still.

DISCUSSION

The lesion of mitral incompetence with the possible association of tricuspid incompetence appears to modify the response to the injection of a standard dose of 50mg of suxamethonium, a paralysing dose in the majority of adults. Of a group of patients with mitral incompetence, one third failed to show muscle fasciculation following the injection of suxamethonium. Further, in a high proportion of these the relaxation produced for intubation could be classed as inadequate, necessitating a repeat dose.

The most probable reason for these observations lies in the effect the circulatory dynamics of this lesion have on the manner in terms of time and concentration in which the injected drug reaches the muscle mass. It has been established that the vigour of muscle fasciculation following the injection of suxamethonium appears to be determined by the rate of onset of endplate depolarisation[1]. In general, the slower the injection, the less marked the intensity of the muscle fasciculation[2]. Taking the dye curves displayed as a graphic illustration of the manner in terms of time and concentration in which an intravenously injected drug would reach the tissues, it is shown here that 'the build-up' time to peak concentration of injected dye is much longer in patients with mitral incompetence than in the other groups of lesions mentioned-and much longer than that pertaining in the normal patient. The mean build-up time in the group of patients who failed to fasciculate was 12 seconds, six times longer than the normal. Also, the peak concentration of drug reached is lower. In addition, it must be remembered that the dye curves examined here were main pulmonary artery injections with brachial artery sampling, the injection therefore, being distal to the tricuspid valve. The 'build-up' time must be much longer if the injection site in a peripheral vein is associated with tricuspid incompetence. We also know that the enzymatic hydrolysis of suxamethonium by plasma cholinesterase is extremely rapid[3], particularly in the vascular compartment. The circulatory dynamics in severe mitral incompetence, with its associated large left atrium, congested pulomonary vascular bed and lower cardiac output, would seem to permit prolonged contact of intravenously injected suxamethonium with plasma in the vascular compartment before the drug finally reaches the muscle mass and the presence of tricuspid incompetence would compound this effect. The drug not only reaches the tissues more slowly with a lower peak concentration than normal, but it may well be that the actual quantity of active drug reaching the muscle mass is less. This effect might be anticipated to some extent in patients with severe aortic incompetence. However, in the group of patients presented here, the drug appeared to reach the tissues more rapidly, cardiac output was

better, 'build-up times' were more rapid and so by inference contact of the drug with plasma was less in time.

Implications

The implications that follow from these observations are:

1 That if it is intended to use suxamethonium in patients with severe mitral incompetence – or perhaps severe incompetence of any heart valve – to ensure adequate relaxation and cough-free intubation, a large dose, say 100mg should be given ab initio, rather than the more usual 50mg.

2 Following injection of suxamethonium, the anæsthetist must be prepared to wait much longer than is usual – up to 2 minutes – before conditions for intubation will be adequate. Where a cardiac catheterization investigation and dye dilution curve (pulmonary artery – brachial artery) is available, if the build-up time on such a curve is in excess of 10 seconds, one may venture the prediction that the patient will fail to show muscle fasciculation following a dose of 50mg suxamethonium and relaxation will be inadequate.

3 When the anæsthetist uses thiopentone sodium in a divided dose technique for induction of anæsthesia in such patients, he must bear in mind the excessively slow circulation time demonstrated, allowing adequate time to judge effects between individual doses, if relative overdose and grave cardiovascular depression are to be avoided.

SUMMARY

Severe cardiac valvular lesions modify the clinical response to intravenously injected suxamethonium.

1 The time from injection to muscle fasciculation and subsequent relaxation in patients with such lesions is much longer than in controls. This is probably because of the slower circulation time consequent on reduced cardiac output in these patients.

2 In the presence of severe valve incompetence, the degree of muscle fasciculation produced by suxamethonium is reduced, being entirely absent in many cases. This is particularly so in patients suffering from mitral incompetence. In this group of patients, as many as one third failed to fasciculate and in a further one third, extremely little muscle fasciculation was evident following administration of a standard dose of 50mg suxamethonium.

3 An explanation of this reaction, based on the circulatory dynamics of these lesions is suggested and the clinical implications discussed.

Acknowledgements

This investigation was conducted on patients presenting for cardiac surgery in the Cardio–Thoracic Department. I wish to thank Professor C. N. Barnard for the facilities afforded me. I wish to acknowledge help received from Dr J. Ozinsky in the collection of clinical data and Professor V. Schrire of the Cardiac Clinic, Groote Schuur Hospital, for access to records of the Clinic's investigations in these patients. This paper is presented with the permission of Dr J. G. Burger, Superintendent, Groote Schuur Hospital.

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LIGNOCAINE KINETICS DURING CARDIOPULMONARY BYPASS
Optimum Dosage and the Effects of Haemodilution

D. F. MORRELL AND G. G. HARRISON

SUMMARY

Lignocaine was administered to patients undergoing cardiopulmonary bypass at 28–29 °C in bolus doses of 1.5, 2.5 and 3.5 mg kg^{-1}. Plasma concentrations greater than 1.5 μg ml^{-1} were found briefly and inconsistently in patients receiving the usually recommended dose (1.5 mg kg^{-1}), but reliably for 14 min in those receiving 2.5 mg kg^{-1}. The 3.5 mg kg^{-1} dose produced statistically and clinically significant decreases in mean arterial pressure. Examination of calculated kinetic parameters showed a two-fold decrease in $T_{\frac{1}{2}}^{\alpha}$, two-fold increases in $T_{\frac{1}{2}}^{\beta}$ and V^{ss} and unaltered Cl_P and V_P when compared with those of unanaesthetized, normothermic patients. The alteration in pharmacokinetics may be attributed largely to decreased binding to albumin following haemodilution.

With the use of present methods of myocardial preservation during cardiopulmonary bypass (CPB), ventricular arrhythmias at the end of the procedure pose less of a problem than previously. Antiarrhythmic agents are seldom indicated, but on those occasions when they are required, lignocaine is selected most frequently. In normal subjects the effective plasma concentration of the drug against ventricular arrhythmias is greater than 1.5 μg ml^{-1} (Grossman, Cooper and Frieden, 1969). This study was undertaken to determine the dose required to achieve this concentration during CPB.

PATIENTS AND METHODS

Twenty-four patients undergoing surgery for valve replacement or coronary artery bypass grafts were studied.

Anaesthesia included the use of morphine, halothane and nitrous oxide in oxygen, following the induction of anaesthesia with sufficient thiopentone to abolish the eyelash reflex. Neuromuscular blockade was produced (pancuronium) and ventilation controlled mechanically. The extracorporeal circulation was powered by Sarns Moduler roller pumps and used bubble oxygenators (Polystan Venotherm VT 5000). The bypass technique included haemodilution and cardioplegia with moderate hypothermia (28–29 °C). The pump priming volume was 3 litre of balanced salt solution (Plasmalyte B), and pump flow was calculated on the basis of 2.4 litre/m^2 body surface area.

Blood-gas tensions, pH and plasma potassium concentration were kept constant throughout CPB.

Once cardiovascular conditions had stabilized on CPB at 28–29 °C, lignocaine was injected over 30 s to a port immediately below the bubble oxygenator. The bolus doses of lignocaine administered were (1) 1.5 mg kg^{-1} in 10 patients, (2) 2.5 mg kg^{-1} in nine patients, and (3) 3.5 mg kg^{-1} in five patients. The mean (± SD) doses for the three groups were 105.5 (± 28.9), 163 (± 40.6), and 218.5 (± 50) mg, respectively. The largest dose was abandoned after use in five patients because of unacceptable decreases in mean arterial pressure (MAP).

Blood samples were aspirated from the oxygenator debubbler at 2, 4, 6, 8, 10, 15, 20 and 30 min after injection. Although we recognize the sampling period to be shorter than ideal for a pharmacokinetic study, this limitation was required because of the duration of CPB. Plasma concentrations of lignocaine were assayed by gas–liquid chromatography (Morrell, Chappell and White, 1982). Other biochemical measurements included total protein and albumin concentrations.

From the plasma lignocaine assay data, coefficients and exponents for the concentration curve for each patient were calculated using the Fortran IV program CSTRIP (Sedman and Wagner, 1976), and pharmacokinetic parameters (table I) were calculated using formulae described by Wagner (1976). The R^2 value obtained from CSTRIP was always better than 0.98.

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TABLE I. *Pharmacokinetic symbols*

$T_{\frac{1}{2}}^{\alpha}$	= α phase half-life (min)
$T_{\frac{1}{2}}^{\beta}$	= β phase half-life (min)
Cl_P	= plasma clearance (ml min^{-1} kg^{-1})
V_P	= volume of plasma compartment (ml kg^{-1})
V^{ss}	= apparent steady state distribution volume (ml kg^{-1})
V^{β}	= apparent volume of distribution during β phase (ml kg^{-1})

RESULTS

The mean plasma lignocaine decay curves following the three different doses are shown in figure 1. A decrease to 1.5 μg ml^{-1} occurred within 3, 14 and more than 30 min following bolus injections of 1.5, 2.5 and 3.5 mg kg^{-1}, respectively. Only 60% of patients receiving the usually recommended dose of 1.5 mg kg^{-1} were found to have plasma concentrations above 1.5 μg ml^{-1} at 2 min, declining to 10% at 4 min. While the concentration of 1.5 μg ml^{-1} was achieved for 4 min in all patients receiving the intermediate-sized dose of 2.5 mg kg^{-1}, it was maintained at 30 min in 10% of this group. Following the 3.5-mg kg^{-1} bolus, lignocaine concentrations were greater than 1.5 μg ml^{-1} in all patients for up to 20 min, and in 60% of patients remained so at 30 min.

MAP did not change significantly in those patients receiving the two smaller doses but decreased on average by 20.5% from the pre-injection pressure of 66 mm Hg in those receiving 3.5 mg kg^{-1}. The maximum individual decrease was 45% of the pre-injection pressure.

Haemodilution resulted in a decrease in mean serum albumin concentration to 65% of its pre-bypass value, which was itself at the lower limit of the normal range (table II). Serum protein concentration did not alter during the experimental period.

Pharmacokinetic parameters are recorded in table III. These represent the means of the values as calculated for each patient. The clinically stable sampling period was limited to 30 min and, as a result, our calculated coefficients and exponents are liable to error since a limited number of data points was obtained in the metabolic phase. Therefore, for the purpose of comparison with published work, we recalculated the data of Rowland and colleagues (1971) as if their sampling was discontinued at 30 min. The method used was to calculate from their data expected plasma concentrations at our sampling times using the coefficients and exponents provided, and then to recalculate (using CSTRIP) the exponents and coefficients for the initial 30-min period. From these, pharmacokinetic parameters were derived. The original and recalculated data are provided in table III, rows A and B. Comparison of the data from our 1.5-mg kg^{-1} bolus group (mean dose = 105.5 mg) with theirs (dose = 100 mg) reveals a halving in $T_{\frac{1}{2}}^{\alpha}$ and a doubling in $T_{\frac{1}{2}}^{\beta}$, V^{ss} and V^{β} in our data relative to theirs. Cl_P and V_P were the same.

The extent of the error induced by the shortened sampling period is evident when the original data from Rowland's work are compared with the recalculated data (table III, row A *v.* row B), incurring an underestimate of half-lives and volumes of distribution and overestimate of clearance.

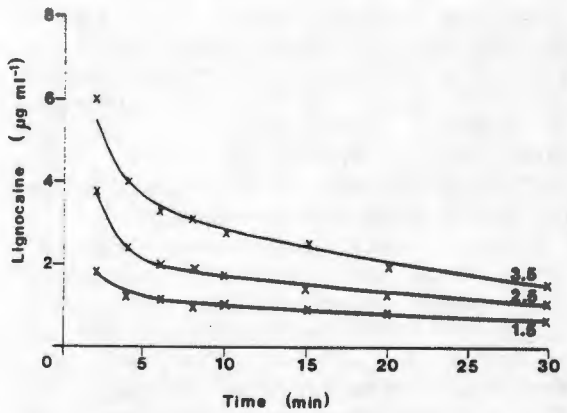

FIG. 1. Computed plasma lignocaine decay curves following 3.5-, 2.5- and 1.5-mg kg^{-1} bolus doses. *x* = mean values for each group at each sampling time.

DISCUSSION

Although the pharmacokinetic behaviour of lignocaine and its relevance to the development of effective dosage schedules have been well reviewed in patients with normal haemodynamics, heart failure and liver or renal disease (Benowitz and Meister, 1978), we are not aware of similar studies having been undertaken during CPB. Many factors which would influence pharmacokinetics are introduced such as anaesthetic and vasoactive drugs,

TABLE II. *Serum protein concentrations (g litre^{-1}) before and after haemodilution (mean ± SD)*

	Before	After
Total protein	62.68 ± 7.9	36.25 ± 6.2
Albumin	34.88 ± 4.23	22.57 ± 4.35

TABLE III. *Pharmacokinetic parameters. Mean ± SD of parameters as calculated for each individual. A = Original data from Rowland and colleagues (1971) for 100-mg bolus; B = A recalculated for a 30-min sampling period. 1 = Parameter in 1.5-mg kg^{-1} bolus group differs significantly from that in 3.5-mg kg^{-1} bolus group ($P < 0.05$; Student's t test); 2 = Parameter in 2.5-mg kg^{-1} bolus group differs significantly from that in 3.5-mg kg^{-1} bolus group ($P < 0.005$; Student's t test)*

Dose (mg kg^{-1})		$T_{\frac{1}{2}}^{\alpha}$	$T_{\frac{1}{2}}^{\beta}$	Cl_P	V_P	V^{ss}	V^{β}
1.5		1.74 ± 0.78	44.4 ± 21	21.7 ± 7.2	535 ± 221	1158 ± 279	1231 ± 263
2.5		1.38 ± 0.6	31.8 ± 12	26.7 ± 8.1	371 ± 220	945 ± 219	1063 ± 208
3.5		1.02 ± 0.3	21.4 ± 6.6	25.1 ± 5.3	243 ± 61	666 ± 114	740 ± 124
	A	8.8	92	9.94	480	1080	1447
	B	3.7	22.6	20.5	493	629	667
Statistics			1		1	1, 2	1, 2

hypothermia, dilution of red cell and protein concentrations, non-pulsatile flow, decreased blood viscosity and exclusion of the lung as a first-pass organ. Furthermore, whereas a total drug concentration of 1.5 μg ml^{-1} has been established as necessary for antiarrhythmic activity in the unanaesthetized patient, it is not axiomatic that this concentration applies following CPB.

The main results of this study were the low initial and rapid decrease in plasma concentrations of lignocaine during CPB. This is in keeping with the finding of altered distribution kinetics which were the two-fold decrease in $T_{\frac{1}{2}}^{\alpha}$ and two-fold increase in V^{ss} and V^{β}. The metabolic handling of lignocaine as reflected by Cl_P is unaltered, the increased $T_{\frac{1}{2}}^{\beta}$ caused by the increased volumes of distribution (Hull, 1981). The addition of an oxygenator prime on a volume basis would not significantly increase volume of distribution as it would form part of V_P which, for a 70-kg man receiving a 1.5-mg kg^{-1} bolus, is of the order of 37.5 litre. The additional 3 litre prime volume is thus less than 10% of V_P and would require only an additional 4.5 mg of lignocaine over and above the 105-mg bolus to achieve a concentration of 1.5 μg ml^{-1}.

We feel that the explanation for the altered distribution kinetics can be attributed largely to an increase in the unbound pharmacodynamic fraction

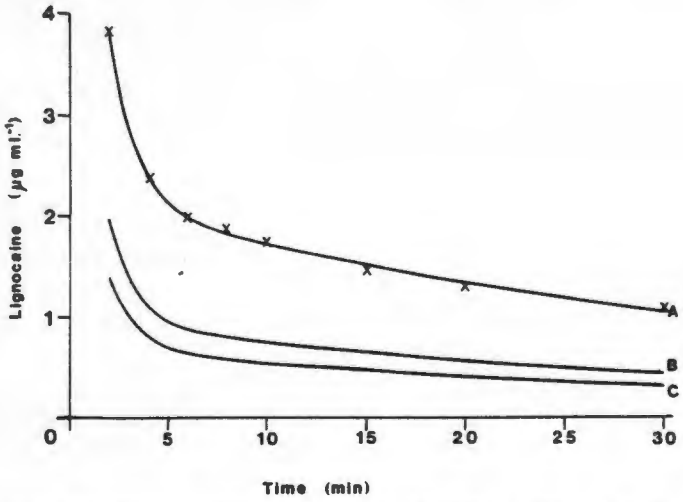

FIG. 2. Curve A = Total lignocaine plasma concentration following 2.5-mg kg^{-1} bolus. Curves B and C = Calculated unbound lignocaine concentrations at albumin concentrations of 23 (B) and 35 g litre^{-1} (C). x = mean value for the group at each sampling time.

of lignocaine caused by the decrease in concentration of binding sites resulting from dilution of plasma albumin. Actual measurements of this unbound fraction were not performed in our study, but estimates may be made using published information. Drug binding to macromolecules (Koch-Weser and Sellers, 1976) is dependent on the total drug concentration, the macromolecular concentration, the number of binding sites per macromolecule and the dissociation constant for the drug–macromolecule complex. Applying the constants derived by Tucker and co-workers (1970) for lignocaine–albumin binding, the calculated change in bound drug concentration following the injection of a 2.5-mg kg^{-1} bolus of lignocaine in our patients is shown in figure 2. This records the decay curves for total and unbound drug at albumin concentrations of 35 and 23 g $litre^{-1}$, representing the pre-CPB and CPB concentrations in our patients. The 35% decrease in albumin concentration following haemodilution produced an almost constant 37% increase in unbound drug concentration over the whole range of total lignocaine concentrations encountered. Furthermore, an anti-arrhythmic total drug concentration of 1.5 $\mu g\ ml^{-1}$ corresponds to an unbound drug concentration of 0.45 $\mu g\ ml^{-1}$ at an albumin concentration of 35 g $litre^{-1}$. Figure 2 demonstrates also that haemodilution to the extent of decreasing albumin concentration to 23 g $litre^{-1}$ extends the time for which the unbound drug fraction is greater than 0.45 $\mu g\ ml^{-1}$ from 14 to 26 min.

Our results indicate that, during CPB with haemodilution, the bolus loading dose of lignocaine with which to precede a continuous infusion is 2.5 mg kg^{-1} rather than the more usually recommended 1.5 mg kg^{-1}. The larger dose produces greater and more sustained plasma concentrations without incurring clinical changes in vascular resistance and MAP.

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PHARMACOCINETIQUE DE LA LIGNOCAINE AU COURS DE LA CEC

Posologie optimum et effets de l'hemodilution

RESUME

De la lignocaine a été administrée à des patients soumis à une CEC à 28–29 °C, sous forme d'injections directes de 1,5, 2,5 et 3,5 mg kg^{-1}. Des concentrations plasmatiques supérieure à 1,5 $\mu g\ ml^{-1}$ ont été retrouvées de façon fugitive et inconstante chez les patients qui recevaient la posologie préconisée habituellement (1,5 mg kg^{-1}), mais de façon fiable pendant 14 min chez ceux qui recevaient 2,5 mg kg^{-1}. La posologie de 3,5 mg kg^{-1} entrainait des diminutions statistiquement et cliniquement significatives de la pression artérielle moyenne. L'examen de paramètres cinétiques calculés montrait une diminution de moitié de $T_{\frac{1}{2}}^{\alpha}$ et des doublements de $T_{\frac{1}{2}}^{\beta}$ et de V^{ss}, sans changement de la Cl_P ni du V_P par rapport à des patients normothermiques non anesthésiés. Les modifications des paramètres pharmacocinétiques peuvent être largement imputées à une diminution de la liaison à l'albumine dûe à l'hémodilution.

LIGNOCAIN: KINETIK WÄHREND KARDIOPULMONALEM BYPASS

Optimale Dosierung und Effekte auf Hämodilution

ZUSAMMENFASSUNG

Patienten während kardiopulmonalem Bypass bei 28–29 °C erhielten Bolusdosen von 1,5, 2,5 und 3,5 mg kg^{-1} Lignocain. Bei Patienten, die die üblicherweise empfohlene Dosis von 1,5 mg kg^{-1} erhalten hatten, wurden nur kurz und nicht übereinstimmend Plasmakonzentrationen über 1,5 $\mu g\ ml^{-1}$ gefunden, bei Patienten mit 2,5 mg kg^{-1} jedoch zuverlässig über 14 Minuten. Die Dosis von 3,5 mg kg^{-1} verursachte einen statistisch und klinisch signifikanten Abfall des mittleren arteriellen Drucks. Die Prüfung der berechneten kinetischen Parameter zeigte einen zweifachen Abfall von $T_{\frac{1}{2}}^{\alpha}$, zweifache Anstiege von $T_{\frac{1}{2}}^{\beta}$ und V^{ss} und unveränderte Cl_P und V_P im Vergleich mit nicht nar-

kotisierten, normothermischen Patienten. Die veränderte Pharmakokinetik ist wahrscheinlich zum Großteil auf die verringerte Albuminbindung durch Hämodilution zurückzuführen.

CINETICAS DE LA LIGNOCAINA DURANTE LA DESVIACION CARDIOPULMONAR

Dosis óptima y efectos de la hemodilución

SUMARIO

Se administró lignocaína a pacientes sometidos a desviación cardiopulmonar, a temperaturas de 28 a 29 °C y en dosis de 1,5, 2,5 y 3,5 mg kg^{-1}. Se encontraron concentraciones de plasma superiores a 1,5 $\mu g\,ml^{-1}$ muy brevemente y de forma irregular en pacientes que recibieron la dosis de 1,5 mg kg^{-1} normalmente recomendada, pero de forma fiable por espacio de 14 min en aquellos que recibieron 2,5 mg kg^{-1}. La dosis de 3,5 mg kg^{-1} produjo disminuciones de la presión arterial media que fueron significativas tanto desde el punto de vista estadístico como desde el clínico. El examen de los parámetros cinéticos calculados mostró una doble disminución del $T_{\frac{1}{2}}^{\alpha}$, un doble incremento del $T_{\frac{1}{2}}^{\beta}$ y V^{ss}, y Cl_P y V_P sin alteración alguna cuando se compararon con los de los pacientes normotérmicos anestesiados. La variación de los aspectos farmacocinéticos pueden atribuirse en gran medida a la disminución de la ligazón a la albúmina a raiz de la hemodilución.

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EFFECT OF DURATION OF ANAESTHESIA ON APNOEA NEONATORUM AFTER CAESAREAN SECTION

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In cases of caesarean section, many authorities (Clifford, 1940; Gillies, 1950; Lull and Hingson, 1948; Ten Teachers, 1955; *Queen Charlotte's Textbook of Obstetrics*, 1952; Wylie, 1953) have emphasized the importance of delivering the child as soon after the induction of anaesthesia as possible, thereby minimizing the amount of anaesthetic agents passing to the foetus and there interfering with the normal onset of respiration. We had gained the impression in clinical practice that the often unseemly haste was not justified. Therefore infants delivered by caesarean section were investigated with special emphasis upon the relationship between the duration of the anaesthesia to which they might have been exposed and the delay in the onset of respiration.

Method

One hundred and two infants, the product of 100 consecutive caesarean sections performed on mothers anaesthetized by one or other of us over a period of six months, were studied. Indications for the sections were varied, and are listed in Tables I and II.

TABLE I.—*Indications for Caesarean Sections*

Indication	No. of Cases	Code for Table II
Incoordinate uterine action ..	11	A
Pre-eclamptic toxaemia	11	B
Disproportion or obstructed labour	35	C
Placenta praevia	8	D
Elective	18	E
Eclampsia	2	F
Oblique lie	1	G
Diabetic elective	3	H
Foetal distress	6	I
Prolapsed cord	5	J
Strong labour in previous rupture of uterus	1	K
Carcinoma of cervix	1	L

TABLE II.—*Data of Investigation*

Baby No.	Anaesthetic	Exposure to Anaesthetic (Min.)	Delay in Respiration (Min.)	Indication for Section (See Table I)	Baby No.	Anaesthetic	Exposure to Anaesthetic (Min.)	Delay in Respiration (Min.)	Indication for Section (See Table I)
1	GOE	23	½	C	52	GOEF	17	0	C
2	GOE	15	5	C	53	GOEF	18	0	C
3	GOE	10	3½	E	54	GOEF	14	4	C
4	GOE	14	1	C	55	GOE	13½	0	C
5	PGOE	18	0	E	56	GOE	12	0	C
6	GOE	8	6	F	57	GOE	15	14	C
7	GOE	15	9	C	58	GOEF	22	0	A
8	GOE	11	3	B	59	GOE	15	15	C
9	PGOEF	14	0	C	60	GOE	19	14	E
10	GOEF	15	0	E	61	GOE	13	14	C
11	PGOEF	10	15	A	62	GOE	14½	4	F
12	PGOEF	16	4	C	63	GOEF	12	SBc	C
13	PGOEF	20	5	A	64	GOE	15	0	C
14	PGOEF	19	½	A	65	GOEF	14	14	A
15	PGOEF	14	7	D	66	GOE	21	7	A
16	GOE	11½	2½	G	67	GOE	20	0	D
17	GOE	16	70	C	68	GOE	14	0	H
18	GOE	13	0	B	69	GOE	19	0	E
19	GOE	9	0	H	70	GOEF	18	0	A
20	GOE	16	2	A	71	GOEF	8	1	C
21	PGOEF	10	½	A	72	GOEF	17	0	C
22	PGOEF	6	12	B	73	GOE	10	7½	E
23	CyGOE	16	13	I	74	GOE	11	0	A
24	PGOEF	15	SBa	D	75	GOE	9	0	B
25	GOE	10	1	L	76	GOEF	22	5	C
26	GOE	12½	2½	I	77	GOE	12½	0	E
27	GOE	20	½	C	78	PGOEF	16	14	A
28	GOE	14½	½	C	79	GOEF	19	7	B
29	PGOEF	5	7	H	80	PGOEF	18	8	E
30	PGOEF	4	90	C	81	GOE	10	½	C
31	GOE	15	0	C	82	GOE	18	0	E
32	GOE	11½	½	I	83	GOE	13	17	C
33	GOEF	19	0	D	84	GOEF	18	0	B
34	GOE	11½	7½	D	85	PGOEF	17	12	C
35	GOE	17	5	D	86	GOE	12½	9½	B
36	GOEF	25	6	C	87	PGOEF	6	0	E
37	GOE	14	0	E	88	GOE	12	5	E
38	GOE	13	3	C	89	GOE	12	5	E
39	GOEF	13	0	J	90	GOE	15½	2	B
40	GOEF	15	6	C	91	GOE	15½	4	I
41	PGOEF	13	13	C	92	GOE	16	0	E
42	GOE	18	12	C	93	GOE	19	SBd	E
43	GOE	9	2	C	94	GOE	16	4	J
44	GOE	28	3	D	95	GOE	18	1	E
45	GOE	19	8	C	96	GOE	8	½	J
46	GOEF	12	SBb	I	97	ChGOE	33½	5½	B
47	GOE	6	0	J	98	GOE	11	0	K
48	GOE	7	0	J	99	GOE	10½	0	B
49	GOE	18½	5½	I	100	GOE	23½	4	C
50	GOEF	19	20	E	101	GOE	14	0	C
51	GOE	10	6	D	102	GOE	15	0	E

Ch=chloroform. Cy=cyclopropane. E=ether. F=gallamine triethiodide. G=nitrous oxide. O=oxygen. P=thiopentone. SB=stillborn: (a) case of placenta praevia; (b) case of foetal distress; (c) case of "failed forceps"; (d) case of prolapsed cord.

TABLE III.—*Anaesthesia*

Anaesthetic Agents	Induction	Gallamine Triethiodide	Maintenance
N_2O, O_2, ether	81	16	N_2O, O_2, and ether in all cases
Chloroform	1	—	
Cyclopropane	1	—	
Thiopentone	17	16	

Premedication in all the cases was confined to atropine, 1 100 gr. (0.65 mg.). However, several of the mothers had had sedatives within the accepted dangerous time limit—for example, morphine within four hours of delivery. In all cases the baby was alive at the time of induction of anaesthesia.

Anaesthesia (Tables II and III).—Anaesthesia was induced in 81 cases with nitrous oxide, oxygen, and ether: in 1 with cyclopropane; in 1 with chloroform; and in 17 with thiopentone sodium. Sixteen of the 17 were also given gallamine triethiodide, and so too were 16 of those who had nitrous oxide, oxygen, and ether. In all cases maintenance was carried out with nitrous oxide, oxygen, and ether.

Timing.—Two periods were measured (Table II): (1) from start of induction of anaesthesia to delivery of the baby from the uterus, and (2) from delivery of the baby from the uterus to establishment of continuing respirations. This end-point —the establishment of continuing respirations—was chosen instead of the more usual "crying-time" or "breathing-time," because many of the infants would take one or two gasps or even cry once or twice and then cease breathing for a varying period before either dying (Baby 93) or beginning to breathe again.

All infants were subjected to some degree of resuscitation. The measures adopted varied from simple wiping of the mouth to the administration of intragastric oxygen. We would commend the method of intragastric oxygenation (Åkerrén and Fürstenberg, 1950), which we feel is what permitted Babies 17 and 30 to survive.

Results

The results are listed in Table II and presented graphically in Figs. 1 and 2.

Two sets of twins were born: Babies 47 and 48, and Babies 52 and 53. Four babies are listed as never having breathed —Babies 24, 46, 63, and 93—though, in fact, Baby 93 gasped several times but never breathed continuously before dying.

The results, *excluding* the four babies that died without breathing, were subjected to statistical analysis. When anaesthetic time was related to the delay in onset of continuing respirations, a correlation coefficient (r) of 0.12 was obtained: $t = 1.158$, and with this value $0.25 < P < 0.30$. This value of r does not differ significantly from zero, and therefore the evidence for the correlation is inadequate.

The babies were divided into three groups according to whether they were exposed to anaesthesia lasting up to 10 minutes and under, 10 to 15 minutes, or over 15 minutes (Fig. 2). The variability in the behaviour in delay in onset of continuing respirations of these three groups was similar: $\chi^2 = 27.94$, $n = 34$, $\sqrt{2\chi^2} - \sqrt{2n - 1} = -0.713$.

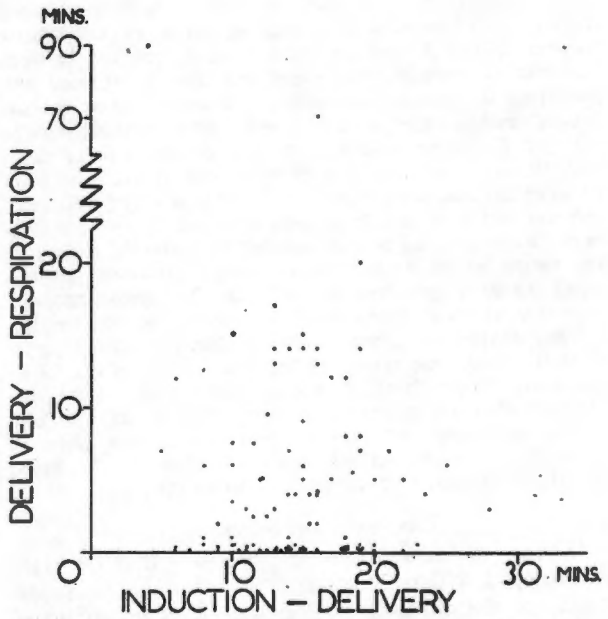

FIG. 1.—Scatterplot of time of exposure to anaesthetic related to delay in onset of " continuing " respirations.

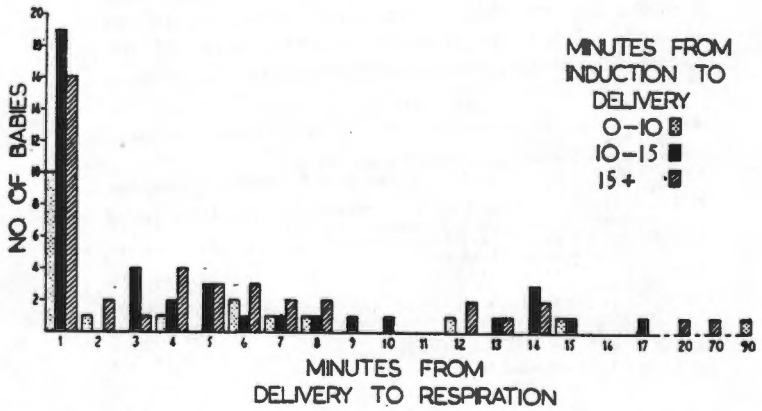

FIG. 2.—Histogram showing time of exposure to anaesthetic related to delay in onset of " continuing " respirations. The three groups are similarly distributed throughout the range.

Discussion

Our results are apparently in conflict with established teaching. It appears that with duration of anaesthesia between 5 and 30 minutes there is no correlation between duration of anaesthesia to which the child is exposed and the delay in onset of respiration. However, closer investigation reveals: (1) Our cases were almost entirely limited (93 out of 102) to those exposed to anaesthesia for a duration of 5 to 20 minutes. No comment can be made on what happens outside these limits. (2) One hundred caesarean sections is not a very large sample on which to base dogmatic statements, as a large number of variables enter into the causation of apnœa neonatorum. However, even a trend towards prolongation of delay in breathing with increased time of exposure to anaesthesia is not evident. (3) All anaesthetics given to the mother may delay somewhat the initial respirations of the infant (Cole *et al.*, 1939). However, if the level of anaesthesia be kept light, the amount of anaesthetic passing to the infant may be kept to the minimum, and thus dangerous—from the point of view of respiratory activity—levels of ether in the blood (Smith and Barker, 1942) of the foetus be avoided.

Conclusion and Summary

In cases of caesarean section, provided that the child is extracted within 20 minutes of induction of anaesthesia of the mother, we feel that a calm unhurried approach by the operating team will not adversely affect the child. 102 infants born by caesarean section were investigated regarding the correlation between the duration of anaesthesia to which their mothers were exposed and the delay in breathing which the infants showed. When the duration of anaesthesia did not exceed 20 minutes, no such correlation was observed.

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THE EFFECT OF INTRA-UTERINE ENVIRONMENT AND ANAESTHETIC FACTORS ON THE CONDITION OF THE BABY AFTER CAESAREAN SECTION*

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Much has been written about the effect of general anaesthesia on the depression of the baby after caesarean section. Although general anaesthesia and its conduct must influence the condition of the baby at birth, the importance of the pre-operative intra-uterine environment has often been disregarded. In an attempt to evaluate the relative importance of certain aspects of general anaesthesia, as opposed to that of the intra-uterine environment on the condition of the baby at birth following caesarean section, we undertook a survey of 1,011 caesarean sections performed in the Maternity Unit of the University of Cape Town Teaching Hospitals. These included emergency and elective caesarean sections. All patients who had received opiate sedation within 6 hours of delivery, or avertin, were excluded from this survey. We also excluded 11 cases which resulted in stillbirth. The surgeons and anaesthetists involved were of both registrar and consultant status. The selection of the surgeon and anaesthetist was completely random, depending upon departmental assignments at the time.

The status of the baby was assessed by means of the Apgar score[1] determined at 1 min. and 5 min. after delivery. The Apgar score was reckoned out of a total of 8 points, the points awarded for colour in Apgar's original description of this method being omitted.[2] This scoring was done by the paediatric registrar in attendance, or, when he was not present, by the anaesthetist.

The incidence of the use of intubation and IPPR required during resuscitation of the baby was used as a separate additional factor in the evaluation of the condition of the baby after delivery. The need for intubation and IPPR was assessed by the person directing the resuscitation of the baby and was instituted when the respiratory function of the baby appeared grossly inadequate, or when the heart rate dropped below 100 beats/min.

Anaesthetic Technique

Certain aspects of the anaesthetic technique were standardized (Table I). All patients received 0·65 mg. of atropine intramuscularly or intravenously. Endotracheal intubation was performed following the injection of succinylcholine. Anaesthesia was maintained with nitrous oxide and oxygen (25% or greater) delivered by an IPPR technique with either carbon dioxide absorption or the use of a non-return system. Muscular relaxation was obtained with d-tubocurarine.

TABLE I. ANAESTHETIC TECHNIQUE

Induction:	Thiopentone sodium	<250 mg.
	or	
	Methohexital	<100 mg.
	or	
	N_2O/O_2 (minimum O_2 25%)	
Maintenance:	N_2O/O_2	
	dTc	
	IPPR	

The induction agent used constituted the only variable in the anaesthetic technique. Induction agents used were:

1. Thiopentone in doses not exceeding 250 mg.
2. Nitrous oxide with 25% oxygen.
3. Methohexital in doses not in excess of 100 mg.

The use of methohexital was a later introduction to this investigation.

Assessment of Foetal Risk

The foetal risk was based on an assessment of foetal maturity, the duration of labour, and the presence of signs of foetal distress. The pre-delivery intra-uterine environment was judged as resulting in high, moderate or low foetal risk as follows:

Group 1 — low risk:
Foetal maturity >36 weeks
Duration of labour <18 hours
No foetal distress

Group 2 — moderate risk:
Foetal maturity >36 weeks
Duration of labour >18<30 hours
No foetal distress

Group 3 — high risk:
Foetal maturity <36 weeks
Duration of labour >30 hours
Foetal distress
Placental insufficiency

For the purpose of this study, the presence of meconium in any amount in the liquor and a foetal heart-rate below 120 beats/min. were regarded as signs of foetal distress.

A special anaesthetic card was devised to facilitate the collection of all the data required. The relevant details

*Based on a paper presented at the 46th South African Medical Congress (M.A.S.A.), Durban, July 1967.

were then transferred to computer cards and the results were subjected to statistical analysis. One-way analysis of variance, Student's t-test or chi-square technique were used where appropriate.

RESULTS

Induction-Delivery Interval and Mean Apgar Score

The time interval between induction of anaesthesia and delivery of the child is a variable — in this series a random one — which may affect the condition of the baby following caesarean section. Dividing our cases into 2 groups on a time basis, namely those in which the induction delivery interval was less than 15 min. and those in which the induction delivery interval was in excess of 15 min., we examined the effect that the length of this interval might have on the condition of the baby after delivery (Table II).

TABLE II. EFFECT OF INDUCTION-DELIVERY INTERVAL ON MEAN APGAR SCORE

	Induction-delivery interval	
	<15 minutes	*>15 minutes*
No. of cases	412	456
Mean Apgar score 1 minute	5·61	5·56
Difference	0·05 (not sig.)	
Mean Apgar score 5 minutes	6·99	6·92
Difference	0·07 (not sig.)	

In the past a similar investigation in our department had failed to show any correlation between the induction-delivery interval and the time required to establish sustained breathing of the baby after caesarean section.[3] This finding was confirmed in this study by a lack of difference between the mean Apgar scores in these 2 groups of cases, both at 1 minute from delivery and at 5 minutes.

Because the induction-delivery interval could not be shown to have any influence on the condition of the baby after caesarean section, this factor was ignored in subsequent analyses, both groups being summed.

Influence of Foetal Risk on Mean Apgar Score

The effect that the pre-operative condition of the foetus (assessed in accordance with the clinical criteria described) had on the condition of the baby after caesarean section is reflected in Table III. It will be noted that a high proportion of cases (61%) fell into the group classed as of high foetal risk. This may reflect the type of case met in hospital practice, as opposed to that in private practice; but we have no comparative information on this point.

TABLE III. EFFECT OF FOETAL RISK ON MEAN APGAR SCORE

Foetal risk	*1*	*2*	*3*
No. of cases	363	29	619
Mean Apgar score 1 min.	6·67	6·17	4·93
Difference	0·5 (1, 2) (not sig.)	1·24 (2, 3) (p<0·001)	
		1·74 (1, 3) (p<0·001)	
Mean Apgar score 5 min.	7·59	7·55	6·60
Difference	0·04 (1, 2) (not sig.)	0·95 (2, 3) (p<0·001)	
		0·99 (1, 3) (p<0·001)	

At 1 min. the babies classed as of high foetal risk achieved the poorest Apgar score. The differences in mean score between this group and those in both low and moderate foetal risk groups, are statistically significant. The small difference between these latter 2 groups is not significant.

At 5 min. the mean Apgar scores had improved, but the differences, though less, are still obvious and the pattern the same. The difference between the high risk foetus and those in the low and moderate risk groups is statistically significant, while the small difference between the last 2 groups is not.

We may infer from the above observations that the condition of the foetus *in utero* is of importance in determining the condition of the baby after delivery. This factor must be taken into account before a valid assessment of the effect of anaesthetic factors may be made.

Effect of Induction Agent on Mean Apgar Score

As no statistically significant difference was apparent between those babies we had classed as of low or of moderate foetal risk, these 2 groups were summed in the subsequent analyses in which we examined the mean Apgar score achieved by the babies in relation to the agent used for the induction of anaesthesia, and in relation to the pre-operative assessment of foetal risk.

In the group of babies regarded as of low and moderate foetal risk (Table IV), those whose mothers received thiopentone achieved the lowest mean Apgar score at 1 min., and those whose mothers received nitrous oxide and oxygen for the induction of anaesthesia the highest, while those whose mothers received methohexital fell between these two. Only the difference in score between those whose mothers received thiopentone and those whose mothers received nitrous oxide and oxygen, achieved statistical significance.

TABLE IV. EFFECT OF INDUCTION AGENT ON MEAN APGAR SCORE (GROUPS 1 AND 2 COMBINED)

Induction agent	*Thiopentone*	*Methohexital*	*N_2O/O_2*
No. of cases	196	44	123
Mean Apgar 1 min.	6·40	6·60	7·14
Difference	0·20 (not sig.)	0·54 (not sig.)	
	0·74 (p<0·01)		
Mean Apgar 5 min.	7·37	7·91	7·83
Difference	0·54 (not sig.)	0·08 (not sig.)	
	0·46 (not sig.)		

At 5 min. all the babies had improved to such an extent that no statistically significant difference in mean Apgar score was apparent in relation to the agent used to induce anaesthesia.

In babies of high foetal risk (Table V) this pattern of response of the mean Apgar score to the anaesthetic induction agent is accentuated. Not only do the 'thiopentone babies' achieve the lowest score at 1 min., but the

difference between this and that achieved by the 'methohexital babies' now achieves significance. As in the previous analysis, the difference in Apgar score between the 'thiopentone babies' and the 'nitrous oxide babies' is statistically significant. Again no difference is apparent in the mean Apgar score of those babies whose mothers had received methohexital and those whose mothers had received nitrous oxide and oxygen for induction of anaesthesia.

TABLE V. EFFECT OF INDUCTION AGENT ON MEAN APGAR SCORE (GROUP 3)

Induction agent	*Thiopentone*	*Methohexital*	*N_2O/O_2*
No. of cases	228	99	292
Mean Apgar 1 min.	4·43	5·19	5·24
Difference	0·76 ($p<0·02$)		0·05 (not sig.)
		0·81 ($p<0·001$)	
Mean Apgar 5 min.	6·43	6·90	6·65
Difference	0·47 (not sig.)		0·25 (not sig.)
		0·22 (not sig.)	

At 5 min. the condition of the babies had again improved to such an extent that no statistically significant difference was apparent between the mean Apgar score in relation to induction agent.

As this improvement in Apgar score in the first 5 min. is, to some extent, a reflection of the efficacy of resuscitation of the baby, we then examined the incidence of the need in resuscitation for endotracheal intubation and IPPR in relation to our assessment of foetal risk and thereafter in relation to the induction agent used.

Need for Intubation and IPPR in Relation to Foetal Risk and Induction Agent

This analysis mirrored, to some extent, the results of the previous analyses. When analysed in relation to foetal risk (Table VI), it can be seen that more than 3 times as

TABLE VI. EFFECT OF FOETAL RISK GRADING ON NEED FOR RESUSCITATION

Foetal risk grading	*Foetal risk 1 + 2*	*Foetal risk 3*
No IPPR	95% (373)*	81% (502)
IPPR	5% (19)	19% (117)
Total cases: 1,011		($p<0·001$)

*Actual No. of cases in brackets.

many babies who were graded pre-operatively as of high foetal risk needed resuscitation by intubation and IPPR than babies graded as of low or moderate foetal risk.

When analysed in relation to the anaesthetic induction agent without consideration of the foetal risk (Table VII), no statistically significant difference is apparent in the proportion of babies needing resuscitation by intubation and IPPR.

When, however, the mean Apgar scores in relation to induction agents are examined in those babies previously graded as of high foetal risk only (Table VIII), it appears that a higher proportion of those whose mothers received thiopentone, subsequently needed resuscitation by intubation and IPPR. The differences in these proportions just

TABLE VII. EFFECT OF INDUCTION AGENT ON NEED FOR RESUSCITATION

Induction agent	*Inhalational*	*Methohexital*	*Thiopentone*
No IPPR	88% (375)*	87% (125)	85% (375)
IPPR	12% (52)	13% (18)	15% (66)
Total cases: 1,011			not sig.

*Actual No. of cases in brackets.

failed to achieve an over-all statistical significance. But if those cases whose mothers received methohexital are excluded, it can be shown that the proportion of babies needing resuscitation after thiopentone is significantly greater than of those whose mothers received nitrous oxide and oxygen only for the induction of anaesthesia.

TABLE VIII. EFFECT OF INDUCTION AGENT ON NEED FOR RESUSCITATION IN FOETAL RISK (GROUP 3)

Induction agent	*Inhalational*	*Methohexital*	*Thiopentone*
No IPPR	84% (244)*	84% (83)	77% (175)
IPPR	16% (48)	16% (16)	23% (53)
Total cases: 619			not sig.

*Actual No. of cases in brackets.

CONCLUSION

From this study we may infer that the most important single factor which determines the condition of the baby at birth after caesarean section, under well-conducted general anaesthesia, is the previous intra-uterine environment of the foetus.

Any conclusions as to the comparative value of different anaesthetic agents or techniques must take this factor into consideration.

A second inference that may be made from this study is that in those babies who may be graded pre-operatively as of high foetal risk, the use of thiopentone as an induction agent may be deleterious. It would appear that where an intravenous method of induction of anaesthesia is desired, methohexital could be substituted with advantage.

SUMMARY

The effects of the intra-uterine environment and agents used to induce general anaesthesia for caesarean section, on the condition of the newborn baby as reflected by the Apgar score, were investigated in 1,011 babies. It was found that the intra-uterine environment was of the greatest importance in influencing the Apgar score of the baby.

We wish to thank our colleagues in the Departments of Anaesthetics and Gynaecology and Obstetrics for their willing cooperation which made the collection of this data possible. We should also like to thank the heads of these departments, Profs. A. B. Bull and D. Davey, for their encouragement and advice; and Dr. J. G. Burger, Medical Superintendent of Groote Schuur Hospital, for permission to publish these findings.

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CARBON DIOXIDE CONCENTRATIONS IN THE BOYLE'S TYPE ANAESTHETIC MACHINE*

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THE elimination of carbon dioxide and the concomitant regulation of the pH of the blood is a respiratory function no less important than the uptake of oxygen. The elimination of carbon dioxide from anaesthetic atmospheres and the concentrations of this gas normally reached in such atmospheres are problems which are engaging a considerable amount of attention at the present time.

Schwartz, Alexander and Adriani (1953) have studied this problem for varying gas flows in the American type of anaesthetic machine. We were unable to trace any work of a similar nature using the standard British Boyle's pattern anaesthetic machine, nor could the manufacturers of our machines supply us with experimental data. Molyneux and Pask (1951) have estimated the volume of backflow of expired gases to the reservoir bag in a semi-closed circuit under certain theoretically deduced conditions, and we have therefore investigated the actual rise in carbon dioxide concentrations in the same type of semi-closed circuit.

Ringrose, Rowling and Harbord (1950) have developed a carbon dioxide indicator designed to make quantitative measurements of this gas in anaesthetic atmospheres. Extensive trials with this R.R.H. Indicator failed to give us reproducible analyses, and we were unable to demonstrate any correlation between the values determined by this instrument and the values determined for the same gas samples when they were analyzed in the Haldane apparatus. Since no calibration curve could be constructed, use of the R.R.H. Indicator had to be abandoned.

EXPERIMENT

Using a standard Boyle's type anaesthetic machine, oxygen was delivered to conscious adult male volunteers at a flow rate of 8 l./minute *via* a corrugated tube of capacity ± 650 ml and a Connell type mask fitting accurately to the subject's face (fig. 1). The reservoir bag of the circuit had a capacity of half a gallon (2,273 ml). The same machine was used in each experiment and gas sampling points were provided proximally at a point just distal to the reservoir bag (A) and distally at the mask itself (B). These sampling points are indicated in figure 1.

The subjects were allowed to breathe from this apparatus for several minutes. During the sixth minute of breathing, two 10-ml gas samples were drawn from each of the two sampling points. Sampl-

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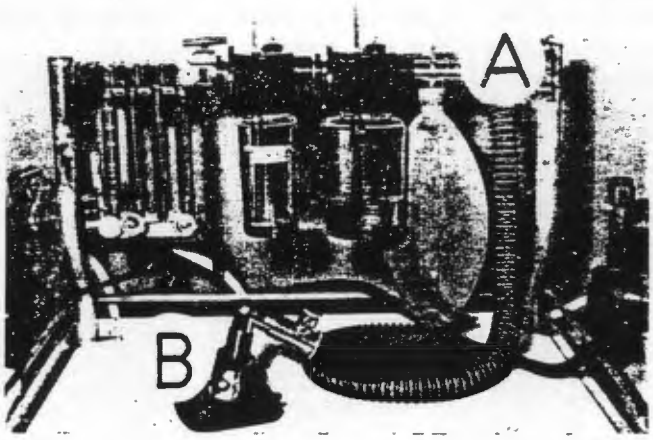

Fig. 1
The type of anaesthetic machine which was used in this investigation. The two sampling points are indicated by the letters.

ing was achieved during the expiratory phase of several breaths, the samples being drawn into all-glass syringes lubricated with glycerine. The syringes were then fitted with gas-tight caps until their contents could be analyzed in duplicate on a standard Haldane apparatus by the usual technique (Lamb, 1930). The values given in table I represent the arithmetical means of duplicate analyses.

TABLE I

Experiment number	Volumes per cent carbon dioxide	
	At mask	At reservoir bag
1	5.12	1.78
2	4.63	0.11
3	4.70	0.33
4	5.11	0.22
5	5.21	0.22
6	5.07	0.17
7	5.39	1.21
8	4.78	0.49
9	5.27	0.38
10	5.00	0.16
11	5.16	0.74
Mean	5.04	0.53
S.E. of Mean	±0.05	±0.02
P	<0.01	<0.01

RESULTS

The concentration of carbon dioxide in atmospheric air is of the order of 0.03 vol. per cent (NTP) (pCO_2=0.23 mm Hg) while the concentrations in alveolar air are accepted as being of the order of ±6.0 vol. per cent (pCO_2=45.6 mm Hg) under normal conditions. We performed 13 experiments on 10 subjects but in 2 experiments the subjects began hyperventilating. This hyperventilation, which may of itself be significant, produced values, at the reservoir bag, which were 8 times greater than the mean values for the CO_2 concentration for the whole series. The results of these two experiments have been discarded and the results of the remaining 11 are set out in table I.

In view of the increased dead-space produced by the mask, the values for the CO_2 concentrations at the mask (mean: 5.04 vol. per cent=38.3 mm Hg pCO_2) are to be expected.

Of more significance are the values for the CO_2 concentrations at the proximal end of the corrugated tubing (capacity ± 650 ml). The mean of 0.53 vol. per cent CO_2 (pCO_2 = 4.03 mm Hg) is 17 times the value for room air. At this sampling point the value depends not only on the gas flow rate from the machine but also on the respiratory minute volume. It is apparent that under the conditions of these experiments a considerable degree of carbon dioxide accumulation occurs in this type of anaesthetic machine.

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CHOICE OF AN ANAESTHETIC FACEPIECE

BY

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THOUGH much time and attention has been devoted to the design of the anaesthetic facepiece, with regard to the requirements of simplicity of construction, ruggedness, ease of cleaning, comfort to the patient and goodness of fit, there are two other requirements which, although important, are seldom considered. These are (*a*) the mechanical deadspace which the facepiece adds to the anatomical deadspace of the patient and (*b*) the cost of the facepiece.

DEADSPACE

The average anatomical deadspace of a normal adult is approximately 150 ml. In calculating the effective alveolar ventilation this anatomical deadspace volume must be subtracted from the tidal volume. The ratio between deadspace volume and tidal volume is very important for it governs both the uptake of inspired gases and the excretion of carbon dioxide. The closer this ratio approaches to unity the less effective is the alveolar ventilation. The conditions pertaining to clinical inhalational anaesthesia tend always to reduce the volume of effective alveolar ventilation. The respiratory centre is depressed by narcotics and anaesthetics and the tidal volume is consequently reduced (the patient breathing spontaneously). The functional deadspace (anatomical + apparatus deadspace) is invariably increased by the apparatus used.

In any apparatus involving its use, the facepiece contributes the major proportion of this increased deadspace, assuming flows of fresh gases are used which are adequate to eliminate rebreathing from the corrugated tubing (Molyneux and Pask, 1951). With the oxygen-rich gas mixtures currently used in clinical anaesthetic practice normal oxygenation may be maintained with subnormal volumes of effective alveolar ventilation; but the excretion of carbon dioxide, which is critically dependent on the volume of effective alveolar ventilation, will be decreased, and thus the carbon dioxide content of the blood will rise. The clinical signs of this, certainly in the early stages, may be masked by anaesthesia. It is thus evident that the deadspace of the facepiece is a very important factor in inhalational anaesthesia, and especially so in systems, such as the Magill attachment, where carbon dioxide elimination depends on volume displacement, and in subjects such as children whose tidal volumes are small and approach the functional deadspace.

It is rather surprising that so little effort appears to have been made, up to the present, to measure the mechanical deadspace of anaesthetic facepieces. Gillespie (1948) suggested that the approximate content of most anaesthetic masks was 150 ml. Stephen (1952) reported that a child's facepiece has a capacity of 250 ml and drew attention to the possibility of rebreathing. Schwartz et al. (1957) emphasized that an adult's medium-sized anaesthetic mask has a volume of 120 ml and a child's mask has a volume of 40 ml. Leigh and Bolton (1948) have pointed out that in children and infants the mask and equipment doubles or trebles the deadspace. Vale (1958) re-emphasizes this important fact by drawing attention to the capacity of an infant's anaesthetic mask and also to the measurements of anatomical deadspace in children reported by Hall (1955). All these workers were content to measure or calculate the capacity of the facepiece itself, although it is obvious that when fitted to the face of a subject some of that capacity will be occupied by the subject's physiognomy. This has been taken into account by Clarke (1958) who fitted under standard conditions a single no. 4 McKesson facepiece to 25 male and 20 female cadavers, and measured the deadspace with the mask in position. He found that the mechanical deadspace

of the facepiece varied between 76 and 107 ml for the males and between 87 and 110 ml for the females. To these figures he added 28–30 ml for the mechanical deadspace of the angled connector and expiratory valve usually found on the Magill attachment.

METHOD

The same measurements as described by Clarke (1958) were carried out. The terminology "mechanical deadspace" is preferred to that of "potential deadspace" for the space measured. The use of the latter terminology by Clarke is based on the possibility of channelling within the mask in the same way as occurs within the bronchi, but until this is proven it should be assumed that the whole of the "mechanical deadspace" is operative with each respiration. The mechanical deadspace of nine adult size anaesthetic facepieces when applied to the faces of 21 adult subjects with full dentition—11 males and 10 females aged between 25 and 35 years—was measured, using a modification of a method suggested by Woolmer (1955). The nine masks comprised seven different types. These are illustrated in figure 1.

The volume measured was that of the mask,

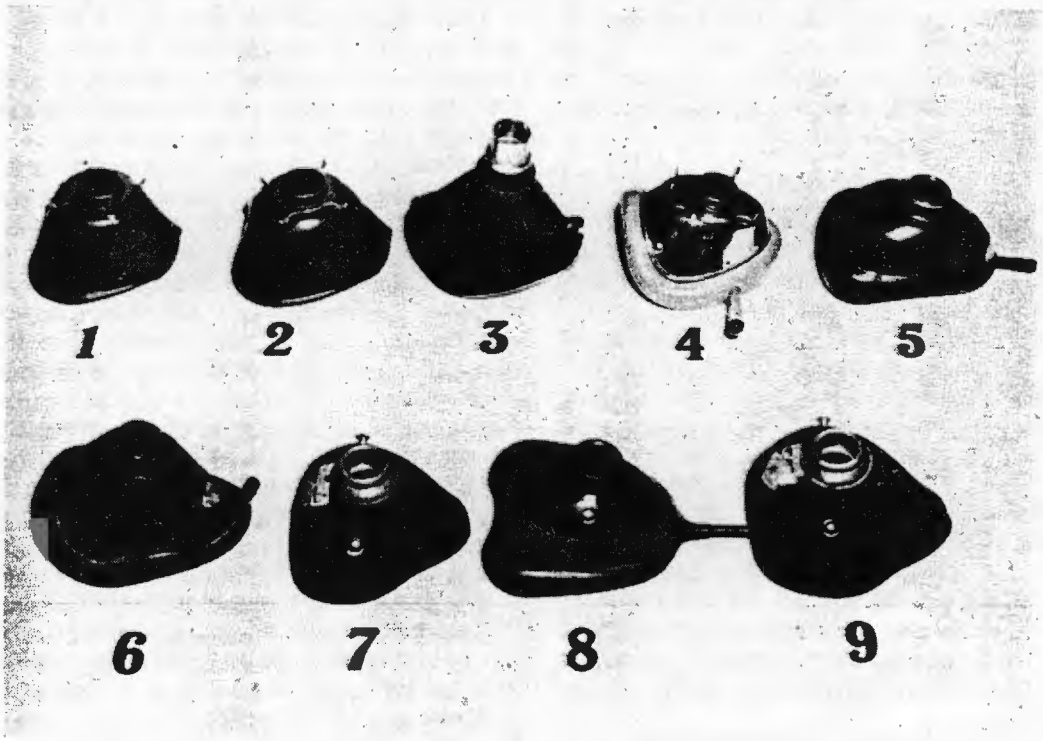

FIG. 1

The anaesthetic facepieces used in the measurements reported.

1. Draeger, Foregger type, size 3, M.9503.
2. Draeger, Foregger type, size 4, M.9504.
3. Foregger, Connell type, adult size, MCS 22.
4. Draeger, Draeger type, chrome base with detachable pad, size 4, M8155 and M6202.
5. Franklin, Rubber Co., London; anaesthetic facepiece with detachable inflatable rubber pad, size 4.
6. B.O.C. Connell type, size 5, MS207.
7. M.I.E. Everseal anaesthetic facepiece, size 3.
8. B.O.C. Anaesthetic facepiece body with detachable inflatable pad, size 5, 22/502 and 22/506.
9. M.I.E. Everseal anaesthetic facepiece, size 4.

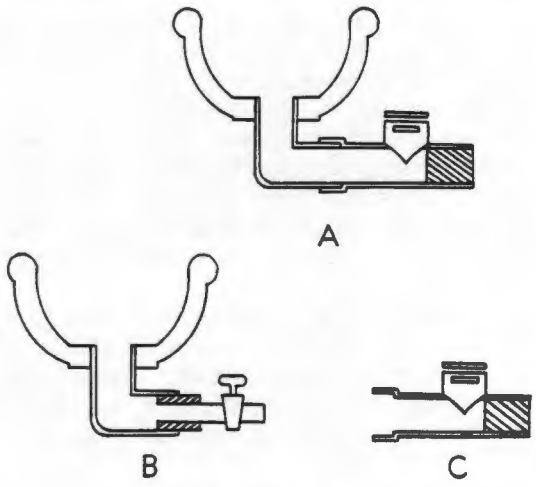

FIG. 2
Diagram of the mask, angle piece and valve piece assembly.

angle piece and valve piece up to the expiratory valve of a standard Magill assembly (fig. 2A).

The mask to be tested was assembled with a standard angle piece. The angle piece was occluded by a cork at the point to which the valve piece of the Magill assembly would overlap. Through this cork a glass tube with burette tap was led. The mask and angle piece as illustrated in figure 2B were now completely submerged in water in a large basin, the burette tap being open. When all the air was displaced from the system the subject, holding his breath, lowered his face into the water and fitted the mask firmly to his submerged face, excess water being displaced through the burette tap. The tap was then closed and the subject removed the mask assembly, still fitted to his face, from the water. Holding the mask horizontally, the subject now gently disengaged his face from the mask, taking care not to spill any water. The water remaining in the facepiece was now carefully decanted through the burette tap into a measuring cylinder. The mean of two readings per mask per subject was recorded. The volume of the valve piece—the proximal end occluded by plasticine up to the expiratory valve (fig. 2C)—was measured separately; this volume then being added to the mean individual mask volume. The volume of the glass tube and tap assembly was subtracted from this

TABLE I

Volume of mask deadspace in ml—individual readings.

Mask	1	2	3	4	5	6	7	8	9
Males									
G.R. 165 lb.	95	100	145	147	150	167	152	212	190
W.L. 165 lb.	90	95	157	162	170	174	190	257	270
H.duT. 172 lb.	81	89	155	142	159	157	185	257	235
P.H. 163 lb.	82	87	125	140	135	115	142	177	205
T.V. 155 lb.	80	89	144	140	155	165	165	207	192
B.S. 150 lb.	62	77	127	132	135	155	142	185	195
D.J. 135 lb.	77	95	152	132	160	167	155	197	217
L.B. 150 lb.	62	77	115	140	135	160	135	180	167
A.B. 150 lb.	70	77	130	125	125	150	155	165	185
G.H. 176 lb.	74	86	148	121	135	105	156	162	175
J.O. 149 lb.	81	86	101	124	134	94	166	187	203
Mean	78	87	136	137	145	146	158	198	203
S.D.	10	7	17	11	14	27	17	31	28
Females									
V.D.M. 135 lb.	88	106	146	143	131	161	138	188	218
D.P. 115 lb.	86	88	146	148	148	161	178	211	248
J.M. 115 lb.	93	108	151	163	161	161	188	148	246
M.G. 130 lb.	68	73	108	133	131	153	148	158	173
V.B. 116 lb.	86	91	136	148	141	168	161	203	166
E.W. 126 lb.	71	86	138	141	133	151	138	186	166
R.B. 137 lb.	86	91	146	126	148	166	176	211	226
V.D.M. 110 lb.	73	91	133	141	136	166	136	183	146
S.V.H. 130 lb.	71	86	136	136	123	156	148	181	173
M.H. 98 lb.	81	91	138	136	133	161	143	173	186
Mean	80	91	137	141	138	160	155	184	194
S.D.	8	10	11	10	11	5	18	20	35
Overall Mean	78	89	137	139	141	153	157	191	198
S.D.	9	9	15	11	13	21	17	27	32

total to give the volume of the complete assembly (fig. 2A).

RESULTS

The results of measuring the mechanical deadspace and the cost analysis are set out in tables I and II. The deadspace of the nine masks tested ranged from 62 to 270 ml with the mean value showing a range of from 78 (S.D. = 9) to 198 (S.D. = 32) ml.

The tables are arranged for convenience so that they read from least to greatest from left to right in terms of mean values. The ratio that these volumes bear to that of the anatomical deadspace —taken as 150 ml—is also appended. Note that masks 1 and 2 are different adult sizes of the same type of mask, as are masks 7 and 9.

DISCUSSION

It will be seen from table II that of the masks tested, in all but one type—of which there were two sizes—the mechanical deadspace observed ranged from just less than to considerably more than the anatomical deadspace. This additional deadspace will lead to a fall in the volume of effective alveolar ventilation and consequently to inefficient elimination of CO_2. It must therefore contribute materially to the respiratory acidosis that often accompanies inhalational anaesthesia (Cullen, 1954; Watrous et al., 1950; Dripps and Severinghaus, 1955). In choosing an anaesthetic facepiece, one with a low deadspace volume should ideally be chosen.

Though all the subjects tested show a wide range of deadspace volumes over the nine masks tested (table I), only two sets of measurements consistently parallel the mean figures in progression from lowest volume to greatest. This is no doubt due to variation in facial features. We regret that we have no satisfactory method of typing faces by measurement of their parameters, though the type of face is undoubtedly of importance in determining the volume of mechanical deadspace in any particular mask, e.g. a small angular face will fit right into a large mask, leaving much less deadspace than when the same mask is fitted to a large flat or concave type of face.

Cost is another factor that must be considered in choosing an anaesthetic facepiece. This is especially so when equipping a large hospital. The costs of the facepieces tested ranged from that of the cheapest up to nearly seven times that price (table II).

In the work here reported we were able to take advantage of a request to test several types of anaesthetic facepieces—adult sizes only were supplied—with a view to recommending one that should be adopted as a standard item of equipment for an extensive hospital system. Since actual local costs are of no value to others, we have introduced them here by taking the lowest price of a mask as unity and expressing the prices of the other masks as multiples of this cost unit.

It must be remembered that the final choice should also take into consideration the other requirements mentioned earlier, viz. simplicity, ruggedness, ease of cleaning, comfort to the patient and goodness of fit. In this respect it is of interest to note that our own tests of these masks included daily clinical use for a period of three months in a very busy general hospital. In this period one of the most expensive facepieces became worn and finally torn at the point of contact with the nasal bridge.

TABLE II

General table of results.

Mask	1	2	3	4	5	6	7	8	9
Deadspace overall mean ml ...	78	89	137	139	141	153	157	191	198
S.D.	9	9	15	11	13	21	17	27	32
Mask deadspace/*anatomical deadspace	0.5	0.6	0.9	0.9	0.9	1.0	1.0	1.3	1.3
Cost factor	6.52	6.9	—	5.2	1.0	3.46	4.64	2.06	4.64

*Taken as 150 ml.

SUMMARY

Observations on the mechanical deadspace of seven types of mask assembled with angle piece and valve stock, reveal that in all but one of these this volume is approximately equal to or greater than the anatomical deadspace volume of an average adult. Patients anaesthetized with such masks might be expected to experience difficulty in the adequate elimination of carbon dioxide. This should be borne in mind when choosing an anaesthetic facepiece.

It is also noted that the costs of facepieces vary greatly from each other.

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MECHANICAL PULMONARY VENTILATION: INDICATIONS FOR ITS USES IN SURGICAL CASES*

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In the widest sense we may say that mechanical ventilation in the lungs is indicated by respiratory failure—the condition where the respiratory gas exchange between the lungs and the blood falls below that between the tissues and the blood.

PHYSIOLOGICAL CONSIDERATIONS

The manner in which respiratory function may fail is best considered in terms of normal respiratory physiology. The processes concerned with the uptake of oxygen and the elimination of carbon dioxide by the lungs—and certain aspects of acid-base balance—may be considered[1] as being (1) ventilation (the mass movement of air in and out of the lungs), (2) distribution (the distribution of the inspired air and the pulmonary blood flow within the lungs—these bear an optimal relationship to each other), (3) diffusion (the process by which O_2 and CO_2 are exchanged between alveolar air and the pulmonary capillary blood), (4) pulmonary blood flow (the amount of blood brought into contact with the respiratory epithelium in unit time), and (5) blood gas transport.

An understanding of the effects that derangements of these factors have on the arterial oxygen saturation and carbon-dioxide tension is essential for a clear understanding of respiratory failure. The first three are the mechanisms most relevant to clinical respiratory failure.

(1) *Ventilation*

If ventilation is impaired the amount of O_2 that can be taken up by the blood, and CO_2 excreted, is limited.

If the shape of the dissociation curves of oxygen and carbon dioxide, and the consequent effect of alveolar ventilation on the blood content of these gases is considered (Fig. 1), it will be realized that the CO_2 tension in the arterial blood will be the first to reflect any change in ventilation, rising or falling proportionately with change in ventilation. Reduced tension of O_2 in the alveolar air only leads to arterial oxygen desaturation of any moment where it has fallen quite markedly. But when it does reach this point where significant desaturation of arterial blood results it is on the slippery slope of the Eiger. Any further reduction in alveolar O_2 tension, as would result from a further decrease in ventilation, will result in gross and catastrophic arterial oxygen desaturation. This should serve to remind us that cyanosis is a sign of severe ventilatory impairment.

It must be noted also that hyperventilation while breathing air cannot increase the arterial oxygen saturation beyond 96 - 98%, its normal level. Remember, too, that with underventilation not only will there be the obvious respiratory acidosis, but the resultant tissue anoxia, leading to an increase in the fixed acids, will result also in a metabolic acidosis, thus adding fuel to the acidotic flame.

(2) and (3) *Distribution and Diffusion*

These two factors may be grouped together. Derangements often occur together, and the changes produced in the arterial oxygen saturation and CO_2 tension are in the same direction. Derangements in distribution result in disturbance of the over-all ventilation/perfusion ratios in the lung. The effects such changes have on the blood gas content are again dependent on the shape of the dissociation curves of the respective gases. Whereas the rise in CO_2 tension resulting from underventilation of a normally perfused lobule of lung may be compensated for by overventilation of another normally perfused lobule—thus preserving a normal total arterial CO_2 tension—the reduction in arterial oxygen saturation so resulting cannot be compensated for by this mechanism. It can be corrected only by raising the inspired, and so alveolar, O_2 tension. Derangements of any moment, therefore, in the ventilation/perfusion ratios in the lung always result in a lowered arterial oxygen saturation, possibly with a normal, not raised, arterial CO_2 tension, certainly in the early stages.

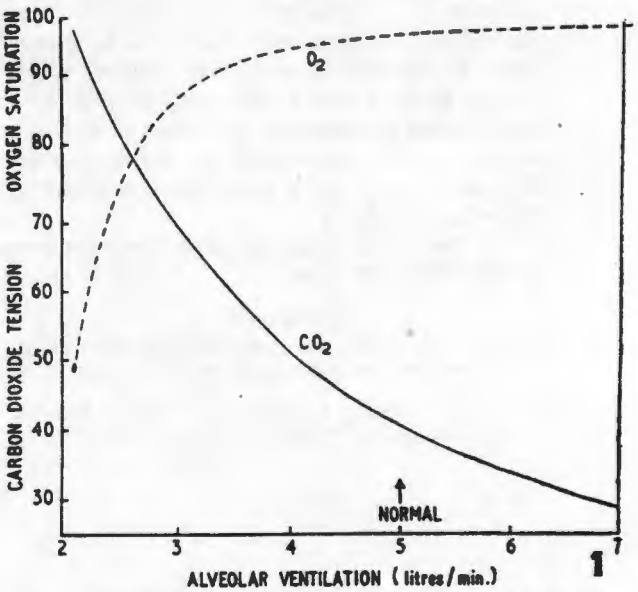

Fig. 1. Effect of the volume of pulmonary ventilation on arterial CO_2 tension (mm.Hg) and oxygen saturation (%).

With disturbances of diffusion, the differences in the relative solubilities of O_2 and CO_2 lead to an impairment of the O_2 uptake of the blood with no significant effect on the excretion of CO_2, and may thus lead to a similar blood gas picture of reduced arterial oxygen saturation with a normal CO_2 tension.

As recently shown by Nunn and Payne,[2] derangement of distribution produces detectable arterial oxygen desaturation in the immediate postanaesthetic phase more often than we realize.

The Work of Breathing

Another aspect of pulmonary physiology that must be

*Based on a paper presented at the 44th South African Medical Congress, Johannesburg, July 1963.

considered is that of the work of breathing.[9, 15] The total mechanical work of breathing in normal subjects at rest is about 0·6 kg.-m. per min.,[1] with a metabolic cost to the patient of 1 - 3% of the total oxygen intake (i.e. about 0·5 ml. O_2 per litre of ventilation, or 4 ml. O_2 per min). In patients with chronic lung or heart disease, the mechanical work of breathing, and consequently the metabolic cost to the patient, may be increased 5 - 10fold. As the rate of oxygen intake is also limited in these patients, the consumption of oxygen by the respiratory muscles may severely restrict the amount of oxygen available to the rest of the body. If the compliance of the lungs is reduced, as it is for instance by postoperative pulmonary complications, the work involved in taking a normal breath is increased. It may therefore become more economical to the patient, in terms of energy expenditure, to take shallower and more frequent breaths. However, the smaller the tidal volume, the larger must be the total pulmonary ventilation to flush the dead space and maintain the level of effective alveolar ventilation.

Changes Produced by Surgical Operations

Another factor to be considered is the reduction in total lung capacity that has been shown to follow immediately after certain operations, even in the absence of recognizable pulmonary complications. This change, most marked after abdominal operations, is due to reduced efficiency of the respiratory musculature and pain in the operative wound. Anscombe and Buxton[3] found a mean reduction in total lung capacity after abdominal operation of 2·4 litres, with a mean reduction in vital capacity of 1·2 litres. These changes, for which a patient with normal pulmonary function compensates easily, may tip an emphysematous patient into respiratory insufficiency and failure.

OBSERVATIONS AND SPECIAL INVESTIGATIONS FOR ASSESSMENT AND MANAGEMENT OF MECHANICAL PULMONARY VENTILATION

Ideally, for assessing a patient as a candidate for mechanical pulmonary ventilation and for correct management of that treatment once it is instituted, there should be on the spot special facilities for measuring (1) volume ventilation as regards (a) rate, (b) minute volume, and (c) tidal volume; (2) arterial CO_2 tension; (3) arterial oxygen saturation; and (4) pH. Though it is possible to conduct the management of patients on ventilators without these facilities, if they are available they remove much of the guesswork and empiricism that result from their absence.

INDICATIONS FOR MECHANICAL PULMONARY VENTILATION

Correlating with the physiological factors mentioned above, two distinct types of respiratory failure may be recognized —distinct certainly in their early stages, though in later stages features of the first type mentioned are predominant. These two types are failure of respiratory function due to (1) a failure of ventilation, or (2) abnormalities of ventilation/perfusion and diffusion.

1. *Failure of Ventilation*

The cases that most often and most obviously call for the use of mechanical pulmonary ventilation are those in which the primary cause of respiratory insufficiency is a failure to move an adequate volume of air in and out of the lungs (i.e. failure of ventilation).

Clinically, this group of cases displays dyspnoea, sweating, and tachycardia, with an initially raised blood pressure. There is often skin vasodilation. Besides appearing shallower than normal, the pattern of respiration is often abnormal. For example, it may manifest a tracheal tug or paralysis of intercostal muscles or diaphragm. Later, there will be cyanosis, mental confusion, large pupils, twitching, low blood pressure, cardiac arrhythmia, and in the end coma and death.

The four special investigations mentioned at the beginning of this section will reveal:

(*a*) A reduced volume of effective pulmonary ventilation.
(*b*) A raised arterial CO_2 tension, usually very high.
(*c*) Arterial oxygen desaturation on breathing air, which reverts to normal on breathing 100% O_2.
(*d*) In severe cases the pH will be lowered because of (i) respiratory acidosis and later (ii) metabolic acidosis from tissue anoxia.

In surgical practice this group of cases would include:

(1) Patients in whom there is a *functional disturbance of the mechanism of ventilation*, viz.:

(*a*) Depression of the *respiratory centre itself*. This may be due to cerebral trauma or to the effects of centrally depressant drugs such as the opiates and other analgesics or the barbiturates.

(*b*) Impairment of the *peripheral transmission mechanism* of the respiratory impulses. The prime example of this circumstance, and the bogey of anaesthetists, is prolonged postoperative curarization from whatever cause. Postoperative ventilatory impairment may also happen in that *rara avis* the myasthenic patient and in patients suffering from certain myopathies and neuropathies. It is seen in the patient with a fractured spine causing paralysis of the diaphragm and/or the intercostal muscles, according to the level of the fracture. Metabolic acidosis[4] is becoming more obtrusive in surgical practice today as the cause of functional ventilatory impairment.

(2) Patients in whom there is a *mechanical or anatomical disturbance of ventilatory capacity*, viz.:

(*a*) One of the commonest conditions that we must consider in this group is chronic obstructive lung disease or emphysema and chronic bronchitis. This is a composite disease that ultimately includes ventilatory/perfusion disturbances, but the basic pathology is bronchiolar obstruction. What so often finally renders the ventilatory capacity of the emphysematous patient inadequate is the association of one of the functional disturbances I have mentioned with emphysema. (Remember that the assessment of adequacy of ventilation from the measurement of gross volume of ventilation alone may be fallacious in view of the increased physiological dead space in patients suffering from emphysema.)

(*b*) A less common anatomical restriction of ventilatory capacity is that which may follow pulmonary resection,[5, 6] where the remaining functioning lung tissue would be just adequate, but is rendered inadequate in the immediate postoperative phase.

In groups (*a*) and (*b*) the possibility of postoperative respiratory difficulty may be anticipated by pre-operative

studies of respiratory function.

(*c*) Patients suffering from a critically crushed chest with multiple fractured ribs—an increasingly common phenomenon—are an important example of mechanical restriction of ventilatory capacity. In these cases there is gross disturbance of the mechanics of the thoracic cage together with pulmonary contusion, leading to a marked ventilatory impairment. Many methods are described of achieving surgical fixation of the unstable flail chest wall. However, in many centres, surgical methods of stabilizing crushed chests have been abandoned in favour of the use of mechanical pulmonary ventilation.[7-9]

2. *Abnormalities of Ventilation/Perfusion and Diffusion*

In this second group of cases, in which respiratory failure is due to ventilation/perfusion disturbances and diffusion abnormalities, the indications for the use of mechanical pulmonary ventilation are not as clear cut, nor are they as common, as those in the first group. Together with the ventilation/perfusion abnormalities and disturbances of diffusion, the increased work load of respiration that results from the lowered pulmonary compliance becomes an important aspect in these cases. An example of this type of respiratory failure is that which sometimes occurs after operations on the heart with cardiopulmonary bypass, particularly in patients who have had pulmonary hypertension. These patients manifest a respiratory distress[10, 11, 14] of which the most obvious sign is tachypnoea and mild cyanosis. There is an obvious increase in the respiratory work needed, and performed, by the patient. The four special investigations will show the following changes, in contrast with those characteristic of cases in which the primary cause of respiratory insufficiency is failure of ventilation:

(*a*) The volume of ventilation is increased.[10, 11] Though tidal volume is decreased, the physiological dead space is also decreased[12] and the frequency of respiration is markedly increased, with a resultant net increase in the total alveolar ventilation.[10, 13]

(*b*) The arterial CO_2 tension may be normal. It is often not raised.[10, 11, 15]

(*c*) The arterial oxygen saturation is reduced on breathing air. This will be increased on breathing 100% O_2, the degree depending on the amount of pulmonary veno-arterial shunting due to atelectasis.

(*d*) The pH may be normal.

Changes of this type are illustrated in Fig. 2. This illustrates daily estimations of ventilatory parameters and arterial oxygen saturation on breathing air and pure O_2 in the immediate postoperative period after operations on the heart with total body perfusion. This investigation was carried out at the British Postgraduate Medical School, Hammersmith Hospital, London.[10, 13] These changes are interesting in that they illustrate the failure of respiratory function without the failure, at least initially, of ventilation *per se*. Later, if the condition worsens, ventilation will begin to fail and the conditions of the common first type of respiratory failure will supervene.

In these cases, raising the inspired oxygen concentration, e.g. by use of an oxygen tent, is usually sufficient to increase the arterial oxygen saturation to adequate levels,

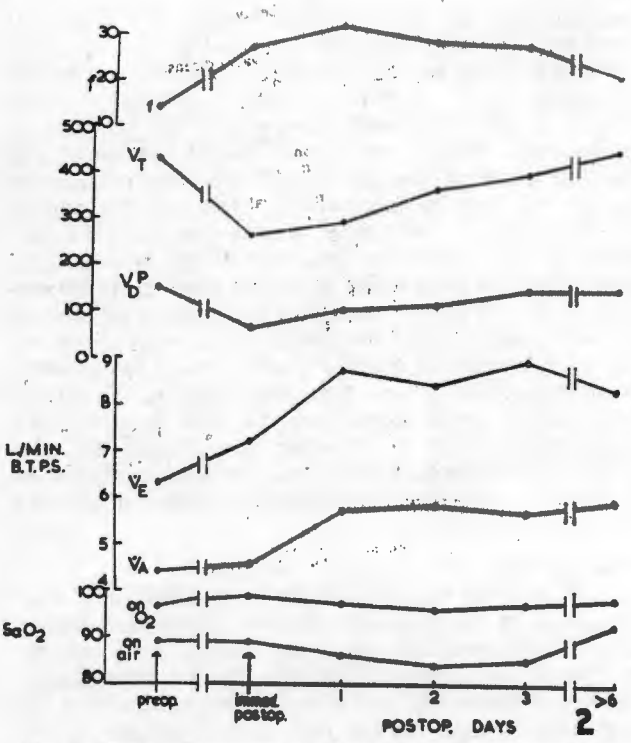

Fig. 2. Daily measurements of ventilatory parameters in the postoperative period after open heart surgery (means of 8 cases). These patients did not require mechanical pulmonary ventilation. (British Postgraduate Medical School, Hammersmith Hospital, London.[10, 13])
f = frequency (breaths per minute). V_T = tidal volume and V_{DP} = physiological dead space (both in ml. BTPS). $\dot{V}_E$ = minute volume and $\dot{V}_A$ = alveolar ventilation (both in litres per minute BTPS). SaO_2 = arterial oxygen saturation (%).

and the tachypnoea, with the increased work it demands, will decrease. However, in the severe cases where the patient is becoming exhausted, the institution of mechanical pulmonary ventilation with high inspired oxygen concentration will benefit the patient. Such benefit results not only from the improved ventilation and diffusion but also, as the work of breathing is taken over by the pump, from the diversion to the general body economy of the oxygen formerly utilized by the overworked respiratory muscles.

MANAGEMENT OF MECHANICAL PULMONARY VENTILATION

Recent advances in the design, sensitivity and response time of triggering mechanisms of some of the mechanical ventilators now available, make 'patient-triggered augmented ventilation' the method of choice in many cases requiring mechanical pulmonary ventilation today.

Patient triggered augmented (PTA) ventilation has the advantage of being virtually 'self-monitored'. The maintenance of the patient's spontaneous respiratory effort serves as a short-term safety mechanism for the emergencies that occur from time to time with this form of therapy. Further, the patient can communicate with his medical attendants by means of speech if the tracheotomy cuff is deflated. We regard PTA ventilation as indicated in all circumstances in which mechanical pulmonary ventilation is required in patients in whom some respiratory muscular activity is

TABLE I. PATIENTS TREATED WITH MECHANICAL PULMONARY VENTILATION

Patients	*Lesion*	*Operation*	*Indications for MPV*	*Type of MPV and make of ventilator*	*Duration of treatment (days)*	*Result*
			Open heart operations			
1	Aortic and mitral valve disease	Aortic and mitral valve prosthesis	Hypoventilation. Cold injury to phrenic	PTA. Bird	7	S
2	Mitral regurgitation	Mitral cusp extension	Pulmonary oedema	IPPR. Radcliffe	4	S
3	Atrial septal defect	Repair of atrial septal defect	Thoracic scoliosis. Hypoventilation	PTA. Bird	14	S
4	Mitral valve disease. Pulmonary hypertension	Mitral valve prosthesis	Respiratory distress	PTA. Bird	14	S
5	Mitral valve disease. Pulmonary hypertension	Mitral valve prosthesis	Respiratory distress	PTA. Bird	3	D
6	Mitral and tricuspid valve disease	Mitral valvotomy. Tricuspid valve prosthesis	Pulmonary oedema	IPPR followed by PTA. Bird	6	D
7	Aortic and mitral valve disease	Aortic valve prosthesis	Respiratory distress	PTA. Bird	5	D
8	Mitral valve disease. Pulmonary hypertension	Mitral valve prosthesis	Prolonged low output. Cerebral damage. Hypoventilation	PTA. Bird	1	D
9	Mitral and tricuspid valve disease	Mitral prosthesis. Tricuspid amuloplasty	Hypoventilation	PTA. Bird	4	S
10	Mitral valvotomy — acute mitral regurgitation	Mitral prosthesis (after 4 days)	Pulmonary oedema	IPPR. Bird	5	S
11	Left ventricular aneurysm	Excision	Pulmonary oedema	IPPR. Bird	4	S
			Closed heart operation			
12	Mitral stenosis	Mitral valvotomy	Pulmonary hypertension and emphysema. Hypoventilation	IPPR. Cyclator	7	S
			Crushed chest			
13	Multiple fractured ribs. Head injury	None	Flail chest	IPPR. Cyclator	5	D
14	Fractured ribs. Fractured sternum. Coronary thrombosis	None	Flail chest	IPPR. Cyclator	6	D
15	Neglected fractured ribs	None	Flail chest. Gross bronchopneumonia	IPPR. Bird	10 hrs.	D
			General surgery			
16	Ruptured abdominal aneurysm	Resection and grafting	Emphysema. Prolonged curarization	PTA. Bird	10	S
17	Gunshot wound	Laparotomy	Emphysema. Prolonged curarization	IPPR. Cyclator	6 hrs.	S
18	Pyelonephritis	Nephrectomy	Prolonged curarization	IPPR. Cyclator	2 hrs.	S
19	Abdominal aneurysm	Resection and grafting	Emphysema. Cardiac failure	IPPR. Cyclator	9	S
20	Bronchopleural fistula, pneumonia, and septicaemia	Insertion of thoracic under-water drain	Tachypnoea + +. Respiratory distress	PTA. Bird	2	D

MPV = mechanical pulmonary ventilation. PTA = patient-triggered augmented ventilation. IPPR = intermittent-positive-pressure ventilation. S = survived (see text). D = died.

present, provided that a negative-pressure phase (such as is necessary for triggering the ventilator) is not contraindicated by such conditions as pulmonary oedema or flail chest. For the patients with respiratory distress characterized by tachypnoea, to match the patient's respiratory efforts it is essential to have a ventilator that has a very rapid response time and is capable of delivering very high flow rates of gas. (For this we use the Bird Mk.8 ventilator.)

In the past 24 months we have had occasion to treat 20 surgical patients with mechanical ventilation for varying periods postoperatively at Groote Schuur Hospital (Table I). From this it may be noted that of these 20 patients, 9 were treated with PTA ventilation. Of the 11 treated by IPPR, 3 were so treated only because a ventilator considered adequate for PTA ventilation was not available at the time. In the column *Result*, 'survived' (S) means that the patient survived beyond the period for which respiratory assistance was necessary, while 'died' (D) means that the patient died while still having mechanical pulmonary ventilation.

As this paper is devoted to 'Indications', discussion of the results and complications encountered in these patients will be the subject of a future communication.

SUMMARY

Mechanical pulmonary ventilation is indicated by respiratory failure, viz.:

(1) That in which the prime failure is of *pulmonary ventilation*. In this type there is:

(a) Reduced pulmonary ventilation.
(b) Increased arterial CO_2 tension.
(c) Decreased arterial oxygen saturation on breathing air, which reverts to normal on breathing 100% O_2.
(d) In late cases a decrease in pH.

(2) Less commonly, that in which the primary failure is one of ventilation/perfusion ratios and diffusion abnormalities. This type may paradoxically show:

(a) Increased pulmonary ventilation.
(b) Normal arterial CO_2 tension.
(c) Arterial oxygen desaturation on breathing air, which may not necessarily be restored entirely to normal on breathing 100% O_2.
(d) The pH is usually normal.

Special reference is made in this type to the increased work demand of respiration.

Given a ventilator with the necessary sensitivity and rapid response time, patient-triggered augmented ventilation is considered the method of choice for providing mechanical pulmonary ventilation.

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