

**PATHOPHYSIOLOGICAL EFFECTS OF BRAIN DEATH
ON POTENTIAL DONOR ORGANS, AND THE
INTRODUCTION OF A NEW METHOD OF DONOR
MANAGEMENT**

DIMITRI NOVITZKY

University of Cape Town

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POTENTIAL DONOR ORGANS, AND THE INTRODUCTION OF
A NEW METHOD OF DONOR MANAGEMENT**

BY

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ABSTRACT

Two methods of inducing brain death in experimental animals (the baboon and pig) have been utilized to study the pathophysiological effects of brain death on potential donor organs, with particular emphasis on the heart. When initiated by an acute increase in intracranial pressure, brain death has two major effects on the experimental animal. The first is a series of haemodynamic changes which take place and which can lead to histopathological damage of the major organs, notably the heart. The second is a depletion of certain circulating hormones, notably triiodothyronine, cortisol, and insulin, which result in a change from aerobic to anaerobic metabolism, which in turn leads to depletion of myocardial energy stores, with resulting deterioration in cardiac function. A similar deterioration in function has been documented in the kidney, and it would seem likely that similar changes take place in the other major organs. Similar observations have been made in human brain-dead potential organ donors. From these observations, the concept of replacement hormonal therapy has been developed, and its effect in both the experimental animal and human brain-dead potential organ donors has been assessed.

During the agonal period, marked sympathetic activity occurs, with significant increases in circulating and endogenous catecholamines, resulting in extreme vasoconstriction, leading to significant increases in systemic vascular resistance and mean arterial pressure. A great increase in systemic vascular resistance results in transient acute left ventricular failure, with mean left atrial pressure rising significantly above the mean pulmonary arterial pressure in the majority of cases. A temporary arrest of pulmonary capillary blood flow occurs, leading to blood pooling within the lungs. These changes may lead to disruption of the anatomical integrity of the pulmonary capillaries and pulmonary oedema and/or alveolar septal interstitial haemorrhage. Such tissue damage may lead to lung failure after transplantation, or may be a major factor in the development of the so-called

"reimplantation response" which can occur in lungs following transplantation.

The endogenous catecholamine release within the myocardium leads to a prolonged period of tachycardia, with rhythm abnormalities and electrocardiographic changes of ischaemia. During this period, major structural damage to the myocardium may occur. In various studies, between 73 to 100% of hearts showed myocardial damage in the form of contraction bands, myocytolysis, and coagulative necrosis; injury to the myocardial conduction tissue was seen in 41% and to the coronary artery smooth muscle in 70%. The coronary artery contraction bands indicate a severe spasm which may lead to a transient total cessation of coronary blood flow, particularly to the subendocardial region; this may contribute to the ischaemic injury which occurs in the myocytes and conducting cells, and to the electrocardiographic changes seen.

These histopathological changes, resulting in inadequate myocardial function or heart block, may possibly be the cause of the reported cases of early donor heart failure after transplantation; clinical evidence for this is documented. Certain histopathological changes in the myocardium which result from brain death may lead to confusion in the interpretation of endomyocardial biopsies during the first days after transplantation of the heart.

Myocardial structural damage can be prevented by previous total cardiac sympathectomy or the prior administration of either an adrenergic blocking agent, such as propranolol, or a calcium antagonist, such as verapamil hydrochloride.

The second major effect of brain death is a rapid reduction of circulating triiodothyronine (T_3): in the baboon a reduction of cortisol and insulin is also seen. This hormonal depletion is associated with an increasing inability of the tissues throughout the body to metabolise aerobically, with an associated increase in anaerobic metabolism.

Depletion of myocardial high energy stores (ATP and CP) and glycogen, and accumulation of lactate, occur, resulting in a deterioration of cardiac function. There is also evidence that these significant metabolic changes have a deleterious effect on renal function (and almost certainly on other organs also). The change to anaerobic metabolism, the loss of myocardial energy stores, and the deterioration of cardiac function, can all be reversed by T_3 replacement therapy. T_3 would appear to be essential for satisfactory mitochondrial function.

Similar reductions in circulating T_3 , and, to a lesser extent, cortisol and insulin have been observed in human brain-dead donors, and a similar deterioration of myocardial function in the hours following the diagnosis of brain death has been noted also. In a clinical study, a group of 26 potential donors showed increasing acidosis, necessitating repeated administration of bicarbonate in order to maintain a normal acid-base balance. This was accompanied by progressive haemodynamic deterioration requiring increasing inotropic support to maintain cardiovascular stability. Despite this therapy, 20% of potential donors were considered unsuitable for purposes of transplantation due to a progressive cardiovascular deterioration or sudden ventricular fibrillation.

In comparison, a group of 21 donors treated with hormonal therapy (T_3 , cortisol, and insulin) showed a significant improvement in cardiovascular status, despite reduced needs for inotropic support and bicarbonate. Following the initiation of hormonal therapy, significant reductions in serum lactate and pyruvate were observed. In this group, all donors were suitable for transplantation of either heart or heart and lungs, together with the kidneys, and excellent organ functions was observed following the implantation of all grafts.

Despite the elapsed time, the clinical application of hormonal therapy to brain dead organ donor is expanding. According to UNOS data currently in the US 19.9% of donors receive this therapeutic modality (T_3 , cortisol-methylprednisolone, vasopressin, and insulin as required) for the hemodynamic recovery. Following hormonal therapy, brain dead organ donors become hemodynamically stable, allowing a significant increment of organ procurement and transplant procedures in the recipient. Furthermore recipients of organs from treated donors at one year have shown a significant survival improvement, most probably this benefit may be observed as time elapses. There is no doubt that hormonal therapy is now being used with more frequency, and will expand over the coming years, thus this dissertation despite the elapsed time remains of great value for the management of brain dead organ donors, I think the re-submission of the thesis is timely and only now the scientific transplant community is finding that a rational treatment of the brain dead organ donor yield organs in a removed metabolic state and prolongs the life of the recipient.

An overview on ethical issues related to this dissertation has been done. The research for this presentation was performed in non-human primates and on the pig. The provided care in the 80's is reviewed and compared with current research. There were no differences found in the provided care to the animals, the humane care was the result of the input from the UCT Departments of Anesthesia, Surgery, Neurosurgery and Cardiothoracic Surgery. The chairs and faculty were actively involved in the development of the required analgesia and anesthesia. Thus despite the elapsed time the ethics of the performed animal research, did fulfill current institutional requirements.

In summary, the acute rise in intracranial pressure which occurs in many subjects during the development of brain death leads, therefore, to major haemodynamic

changes which may result in significant injury to the heart and lung, and probably to other organs also. The depletion in various circulating hormones which subsequently occurs may have a profound effect on the metabolism and function of the organs being considered for transplantation.

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SUMMARY OF CONTRIBUTIONS MADE BY THESE STUDIES

This thesis presents details of studies on the physiopathology of the agonal period and the hours which follow brain death, with specific emphasis on its effects on potential donor organs, in particular the heart. The chapters detail the results of, firstly, animal experimentation and, subsequently, observations from human brain-dead potential organ donors. From these original observations, a new form of hormonal therapy is detailed, which restores aerobic metabolism in the brain-dead subject, enabling normal metabolic activities to proceed, and rendering the donor organs in an improved condition before transplantation.

Each chapter provides information which either substantiates previous work in this area, or provides original contributions.

Chapter 1

Reviews the literature regarding the pathophysiology of brain death.

Chapter 2

A new method of inducing brain death in the baboon is described. A detailed analysis of the electrocardiographic changes which occur during and after brain death is presented; no previous study has documented these changes so thoroughly.

The haemodynamic changes which take place during and after brain death are investigated. The extreme rise in systemic vascular resistance, resulting in significant increases in the mean arterial and left atrial pressures, is highlighted. The rise in systemic vascular resistance

leads to a virtual cessation of aortic blood flow, a rise in left atrial pressure above that in the pulmonary artery, with subsequent pooling of blood in the lungs for a short period of time. These observations provide information on the pathophysiology of neurogenic pulmonary oedema, and it is suggested they may be a causative factor in the "reimplantation response", seen in some patients after heart-lung transplantation, and even of lung failure.

The endocrine changes which take place in the body during and after brain death have been investigated. There is an early, but short-lived, rise in circulating catecholamines, but evidence is provided which suggests that endogenous catecholamines are largely responsible for the tissue damage which can result following the induction of brain death.

The rapid depletion of T_4 and T_3 , occurring within 16 and 19 hours respectively following brain death has not been documented previously. The half-life of these hormones in the normal subject is at least 4 and 1 days respectively, and therefore their rapid depletion following brain death suggests that this is not due solely to degradation by the monoiodinases. In the present state of our knowledge, we cannot adequately explain this rapid degradation of T_3 and T_4 . Levels of thyroid stimulating hormone remain normal. Following brain death, when induced by this method, the hypothalamic-pituitary portal system connections are disrupted, suggesting that, in addition to TSH, other humoral or neurogenic factors are required for the synthesis of T_4 .

Rapid depletion of serum cortisol and insulin levels has also been documented following brain death.

The histopathological changes which can occur in the myocardium during the induction of brain death are described and illustrated. A high percentage of hearts suffer significant injury during the agonal period. Damage to the conduction tissue and to the smooth

muscle of the walls of the coronary arteries does not appear to have been described previously in the literature; such injury may lead to early death after heart transplantation from arrhythmia or myocyte damage.

Chapter 3

The mechanism by which myocardial injury occurs during brain death has been investigated, and evidence is provided to suggest that it is a result of endogenous catecholamine release, resulting from extreme sympathetic activity ("sympathetic storm"). Such myocardial damage can be prevented by prior sympathetic or total denervation of the heart. It can also be prevented by prior adrenergic blockade or calcium antagonism, suggesting that the endogenous catecholamine activity is calcium mediated.

Chapter 4

Evidence is provided that brain death leads to depletion of myocardial high energy and glycogen stores, with an increase in tissue lactate, suggesting a change to anaerobic metabolism. These changes are associated with significant functional deterioration of the isolate heart taken from a brain-dead animal.

Storage of the heart for 24 hours by continuous hypothermic perfusion is associated with little deterioration in myocardial function when the heart is taken from an anaesthetized animal. When the heart is excised from a brain-dead animal, however, significant further deterioration in myocardial function has been documented.

Chapter 5

The depletion of myocardial energy stores and increase in tissue lactate (described in

Chapter 4) were found to be reversible by the administration of hormonal therapy, consisting of T_3 , cortisol and insulin, to the brain-dead animal. Evidence is presented to show that this hormonal therapy results in a reversal from anaerobic to aerobic metabolism, and leads to significant improvement in myocardial function on testing of the isolated heart.

Chapter 6

Studies on the kidney confirm that similar changes to those described in Chapter 4 also take place in this organ after brain death. Hormonal therapy (as outlined in Chapter 5) again results in reversal of these changes and normalization of function.

Chapter 7

It is conclusively shown that brain death results in inhibition of aerobic metabolism, with a resulting increase in anaerobic metabolism. The administration of T_3 alone reverses these changes, and leads to a full recovery of oxidative phosphorylation. Evidence is provided to show that T_3 is necessary for mitochondrial activity under the circumstances of brain death. This would appear to be an important observation, possibly with major implications in biochemistry and physiology.

Chapter 8

A Clinical survey of early donor heart failure in patients with heart transplants is presented. It is suggested that early donor heart failure after transplantation can result from myocardial damage occurring during the donor agonal period. Histopathological features seen in the myocardium of certain patients with heart transplants who died from early donor heart

failure show extreme similarities to those seen in hearts taken from baboons subjected to brain death.

Chapter 9

A Clinical trial of hormonal therapy (T_3 , cortisol, and insulin) has been carried out in brain-dead potential organ donors. Certain metabolic and functional parameters have been monitored before excision of the heart for purposes of transplantation. Changes seen in this group of brain-dead donors has been compared with those seen in a previous group of brain-dead donors who did not receive such hormonal therapy. It is concluded that hormonal therapy leads to metabolic and haemodynamic improvement in the donor, rendering the heart (and probably other organs also) in a better state for subsequent transplantation. Such a study has not been undertaken previously, and clearly may have important implications in the field of organ transplantation. Certain data included in this thesis have been published previously, or are in the process of publication at present. The sources of this published data, and my co-authors, are listed below.

Chapter 10

Current management of brain organ donors is presented, in this overview the impact of the use of T_3 , cortisol/methylprednisolone, vasopressin in brain dead organ donors is presented. Hormonal therapy did impact not only in the cardiac recovery, but also on all organs used for transplantation. More organs were harvested from brain dead organ donors whenever were treated with hormonal replacement. One year survival of the recipient was significantly improved following cardiac and renal transplantation. This overview presents the current brain dead organ donor management, the outcomes following standard management and following the introduction of hormonal replacement.

Therefore the organ donor pool was significantly increased, allowing to provide care to sick patients awaiting organ transplantation.

Chapter 11

The ethical aspects of the performed animal research in the 80's is reviewed, and has been comprehensively described, the provided animal care was no different to the care currently provided in institutions with Animal Research Committees. The comparisons are clearly outlined.

PUBLICATIONS AND PRESENTATIONS

1. NOVITZKY, D., WICOMB, W.N., COOPER, D.K.C., ROSE, A.G., FRASER, R.C., BARNARD, C.N. Electrocardiographic, haemodynamic and endocrine changes occurring during experimental brain death in the Chacma baboon. Present to the International Society for Heart Transplantation. New York, 1984. (President's Award). J Heart Transplant. 4, 63-69, 1984.
2. NOVITZKY, D., COOPER, D.K.C., WICOMB, W.N., REICHART, B. Haemodynamic changes, myocardial injury, and pulmonary development of brain death in the baboon. Present to a Symposium on Organ Procurement, Detroit, 1985 Transplant Proc. 18, 609-612, 1986.
3. WICOMB, W.N., COOPER, D.K.C., LANZA, R.P., NOVITZKY, D., ISAACS, S. The effects of brain death and 24 hours storage by hypothermic perfusion on donor heart function in the pig. J Thorac Cardiovasc Surg. 91, 896-909, 1986.

4. NOVITZKY, D., COOPER, D.K.C., WICOMB, W.N., REICHART, B. Improved function of stored hearts following hormonal therapy after brain death in pigs. Presented to the European Society for Organ Transplantation. Munich, 1985. Transplant Proc. 18, 1419-1421, 1986.
5. NOVITZKY, D., WICOMB, W.N., COOPER, D.K.C., ROSE, A.G., FRASER, R.C., REICHART, B. Prevention of myocardial injury during brain death by total cardiac sympathectomy in the Chacma baboon. Ann Thorac Surg. 41, 520-524, 1986.
6. NOVITZKY, D., WICOMB, W.N., COOPER, D.K.C., REICHART, B. Evidence of myocardial and renal functional recovery following hormonal treatment after brain death. Presented to the Southern African Transplantation Society, Ceres, 1985, (Abstract in S Afr J Surg. 24, 50, 1986) and to a Symposium on Organ Procurement, Detroit, 1985. Transplant Proc. 18, 613-615, 1986.
7. NOVITZKY, D., COOPER, D.K.C., WICOMB, W.N., REICHART, B. Brain death-induced haemodynamic changes resulting in cardiac and pulmonary injury in the baboon. Paper presented to the European Society for Organ Transplantation, Munich, 1985. Transplant Proc. 18, 1190-1192, 1986.
8. NOVITZKY, D., WICOMB, W.N., COOPER, D.K.C., TJAALGARD, M.A. Improved cardiac function following hormonal therapy in brain dead pigs: relevance to organ donation. Cryobiology. 24:1-10, 1987.
9. NOVITZKY, D., ROSE, A.G., COOPER, D.K.C., REICHART, B. (Factors potentially confusing to the interpretation of endomyocardial biopsy following heart transplantation. S Afr Med J. 70, 789-792, 1987.

10. NOVITZKY, D., WICOMB, W.N., ROSE, A.G., COOPER, D.K.C., REICHART, B. Pathophysiology of pulmonary oedema following experimental brain death in Chacma baboon. Ann Thorac Surg. 43:288-94, 1987.
11. NOVITZKY, D., COOPER, D.K.C., ROSE, A.G., REICHART, B. Prevention of myocardial injury by pre-treatment with verapamil hydrochloride following experimental brain death in the baboon. Amer J Emerg Med. 15:11-18, 1987.
12. NOVITZKY, D., COOPER, D.K.C., REICHART, B. Haemodynamic and metabolic responses to hormonal therapy in brain dead potential organ donors. Transplantation. 43:852- 854, 1987.
13. NOVITZKY, D., COOPER, D.K.C., REICHART, B. The value of hormonal therapy in improving organ viability in the transplant donor. Present to the Transplantation Society, Helsinki, 1986 and to the Society of Thoracic and Cardiovascular Surgery of Great Britain and Ireland, Edinburgh, 1986. Transplant Proc. 19:2037-2038, 1987.
14. NOVITZKY, D., COOPER, D.K.C., ROSE, A.G., WICOMB, W.N., BECERRA, E., REICHART, B. Early donor heart failure following transplantation - the possible role of myocardial injury sustained during brain death. Transplantation 43:852-854, 1987.
15. NOVITZKY, D., COOPER, D.K.C., WICOMB, W.N., REICHART, B. Improved function of stored hearts following hormonal therapy after brain death in pigs. Presented to the European Society for Organ Transplantation, Munich, 1985. Transplant Proc 18:1419-21, 1986.

16. NOVITZKY, D., COOPER, D.K.C., MORRELL, D., ISAACS, S. Brain death, triiodothyronine (T_3) depletion, and inhibition of oxidative phosphorylation: Relevance to management of organ donors. Transplant Proc 19:4110-1, 1987.
17. NOVITZKY, D., WICOMB, W.N., COOPER, D.K.C., TJAALGARD, M.A. Improved cardiac function following hormonal therapy in brain-dead pigs: Relevance to organ donation. Cryobiology 24:1-10, 1987.
18. NOVITZKY, D., WICOMB, W.N., ROSE, A.G., COOPER, D.K.C., REICHART, B. Pathophysiology of pulmonary edema following experimental brain death in the Chacma baboon. Ann Thorac Surg 43:288-94, 1987.
19. NOVITZKY, D., COOPER, D.K.C., ROSE, A.G., REICHART, B. Prevention of myocardial injury by pre-treatment with verapamil hydrochloride prior to experimental brain death: Efficacy in a baboon model. Amer J Emerg Med 15:11-18, 1987.
20. NOVITZKY, D., COOPER, D.K.C., ROSE, A.G., REICHART, B. Prevention of myocardial injury by pre-treatment with verapamil hydrochloride prior to experimental brain death: Efficacy in a baboon model. Amer J Emerg Med 15:11-18, 1987.
21. NOVITZKY, D., COOPER, D.K.C., MORRELL, D, ISAACS, S. Brain death, triiodothyronine (T_3) depletion, and inhibition of oxidative phosphorylation - relevance to management of organ donors. Presented to the European Society for Organ Transplantation, Gothenburg, 1987. Transplant Proc 19:4068-4069, 1987.
22. NOVITZKY, D., COOPER, D.K.C., REICHART, B. Preservation of organs & acute tubular necrosis: Procurement and preservations of organs: The value of hormonal

therapy in improving organ viability in the transplant donor. Transplant Proc 19:2037-2038, 1987.

23. NOVITZKY, D., COOPER, D.K.C., ZUHDI, N. Triiodothyronine therapy in the cardiac transplant recipients. Presented as a poster at an International Organ Transplant Forum in honor of Dr. T. E. Starzl, Pittsburgh, 1987, Transplant Proc 20:65-8, 1988.
24. NOVITZKY, D., COOPER, D.K.C., WICOMB, W.N. Endocrine changes and metabolic responses. Trans Proc 20:33-8, 1988.
24. WICOMB, W.N., NOVITZKY, D., COOPER, D.K.C., Effects of hormonal therapy on subsequent organ (kidney) storage in the experimental animal. Trans Proc 20:55-8, 1988.
25. NOVITZKY, D., COOPER, D.K.C. Results of hormonal therapy in human brain-dead potential organ donors. Trans Proc 20:59-62, 1988.
26. NOVITZKY, D., COOPER, D.K.C., HUMAN, P.A., REICHART, B., ZUHDI, N. Triiodothyronine therapy for heart donor and recipient. J Heart Transplant 7:370-376, 1988.
27. NOVITZKY, D., HUMAN, P.A., COOPER, D.K.C., Effect of triiodothyronine (T_3) on myocardial high-energy phosphates and lactates following ischemia and cardiopulmonary bypass - An experimental study in baboons. J Thorac Cardiovasc Surg 96:600-607, 1988.
28. NOVITZKY, D., COOPER, D.K.C., The physiological management of cardiac

transplant donors and recipients using triiodothyronine (T₃). Presented as a poster at the International Organ Transplant Forum in honor of Dr. T.E. Starzl, Pittsburgh, 1987. Transplant Proc 20:803-805, 1988.

29. NOVITZKY, D., COOPER, D.K.C., MORRELL, D., ISAACS, S. Change from aerobic to anaerobic metabolism after brain death, and reversal following triiodothyronine (T₃) therapy. Transplantation 45:32-36, 1988.
30. ROSE, A.G., NOVITZKY, D., FACTOR, S.M. Catecholamine-associated smooth muscle contraction bands in the media of coronary arteries of brain-dead baboons. Am J of Cardiovasc Pathol 2:63-67, 1988.
31. NOVITZKY, D., ROSE, A.G., COOPER, D.K.C. Injury of myocardial conduction tissue and coronary artery smoother muscle following brain death in the baboon. Transplantation 45:964-966, 1988.
32. NOVITZKY, D., HUMAN, P.A., COOPER, D.K.C., Inotropic effect of triiodothyronine (T₃) following myocardial ischaemia and cardiopulmonary bypass. 1: An experimental study in pigs. Ann Thorac Surg 45:50-55, 1988.
33. COOPER, D.K.C., NOVITZKY, D., WICOMB, W.N. Pathophysiology of brain death in the experimental animal: Extracranial aspects. Transplantation Proceedings 20:25-28, 1988.
34. NOVITZKY, D., COOPER, D.K.C., HUMAN, P.A., REICHART, B. Triiodothyronine therapy for heart donor and recipient. J Heart Transplant 7:340-6, 1988.

35. NOVITZKY, D., COOPER, D.K.C., SWANEPOEL, A. Inotropic effect of triiodothyronine (T_3) in low cardiac output following cardioplegic arrest and cardiopulmonary bypass: Initial experience in patients undergoing open heart surgery. Eur J Cardiothorac Surg 3:140-145, 1989.
36. NOVITZKY, D., COOPER, D.K.C., BARTON, C., GREER, A., CHAFFIN, J., GRIM, J., ZUHDI, N. Triiodothyronine (T_3) as an inotropic agent after open-heart surgery. Presented at the C. Walton Lillehei Symposium, Minneapolis, 1988. J Thorac Cardiovasc Surg 98:972-977, 1989.
37. NOVITZKY, D., COOPER, D.K.C., MUCHMORE, J.S., ZUHDI, N. Pituitary function in brain-dead patients. (Letter). Transplantation 48:1078-9, 1989.
38. NOVITZKY, D., HORAK, A., COOPER, D.K.C., ROSE, A.G. Electrocardiographic and histopathologic changes developing during experimental brain death in the baboon. Presented as a poster to the Transplantation Society, Sydney, 1988. Transplant Proc 21:2567-2569, 1989.
39. NOVITZKY, D., COOPER, D.K.C., CHAFFIN, JS., GREER, A.E., DEBAULT, L.E., ZUHDI, N. Improved cardiac allograft function following triiodothyronine (T_3) therapy to both donor and recipient. Presented to the American Society of Transplant Surgeons, Chicago, 1989. Transplantation 49:311-6, 1990.
40. NOVITZKY, D. Triiodothyronine replacement, the euthyroid sick syndrome and organ Transplantation. Submitted at the Society for Organ Sharing, First International Congress, June 18-21, 1991, Rome, Italy. Accepted for publication in Transplantation Proceedings, October 1991.

41. NOVITZKY, D. Heart transplantation, euthyroid sick syndrome and Triiodothyronine replacement. Presented at the International Congress on Current Issues in Thoracic Organ Transplantation, September 19-21, 1991, Essen, Federal Republic of Germany. J of Heart and Lung Transplantation 11:S196-S198, 1992.
42. NOVITZKY, D., FONTANET, H., SNYDER, M., COBLIO, N., McKEOWN, P., SMITH, D., PARSONNET, V. The Impact of Triiodothyronine (T₃) Therapy in the Survival of High Risk Patients Undergoing Open Heart Surgery. J Thorac and Cardiovasc Surg 1994.
43. NOVITZKY, D., RHODIN, J., COOPER, D., YE, Y., MIN, K.W., DEBAULT, L. Ultrastructural Changes Associated with brain death in the Human Donor Heart. Transplant International, June 1995.
44. NOVITZKY, D. Selection and management of cardiac allograft donors. [Review] [51 refs]. Current Opinion in Cardiology, 11(2):174-82, 1996 Mar.
45. NOVITZKY D. Novel actions of thyroid hormone: the role of triiodothyronine in cardiac transplantation. [Review] [32 refs]. Thyroid, 6(5):531-6, 1996 Oct.
46. NOVITZKY, D., RHODIN, J., COOPER, D.K.C., YE, Y., MIN, K.W., DEBAULT L. Ultrastructure changes associated with brain death in the human donor heart. Transplant International, 10(1):24-32, 1997.
47. NOVITZKY, D. Early organ injury following experimental brain death. First International Scientific Teleconference "Cardiovascular Surgery and Angiology – 2003" Book of Conference, pgs. 22- 27, December 2003.

48. NOVITZKY, D., Cooper, D.K.C., Rosendale, JD., Kauffman, HM. Hormonal therapy of the brain-dead organ donor: experimental and clinical studies. Transplantation. 2006 Dec 15;82(11):1396-401. Review.
49. David K.C. Cooper, MD, PhD, FRCS; DIMITRI NOVITZKY, MD, FCS(SA); Winston N. Wicomb, PhD; Murali Basker, MD; John D. Rosendale, MS; H. Myron Kauffman, MD A Review of Studies Relating To Thyroid Hormone Therapy in Brain-Dead Organ Donors and Patients Undergoing Cardiopulmonary Bypass.

ABBREVIATIONS

ADH	Antidiuretic hormone
ADP	Adenosine diphosphate
AO	Aorta
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
BDOD	Brain dead organ donors
BF	Best fit
CF	Coronary flow
cm	Centimetre
CO	Cardiac output
CP	Creatine phosphate
DNA	Desoxyribonucleic acid
DPM	Disintegration per minute
ECG	Electrocardiogram

FT	Functional testing
g	Gram
HCl	Hydrochloric acid
i.m.	Intramuscular
i.v.	Intravenous
IVC	Inferior vena cava
kg	Kilogram
LA	Left atrium
LAP	Left atrial appendage
LDH	Lactate dehydrogenase
LV	Left ventricle
LVP	Left ventricular pressure
LVEDP	Left ventricular end diastolic pressure
MAP	Mean arterial pressure
meq	Millequivalent

min	Minute
ml	Millilitre
mmHg	Millimetres of mercury
mmol	Millimol
mRNA	Messenger RNA
mv	Millivolts
n	Number of observations
NAD ⁺	Nicotinamide adenine dinucleotide (reduced)
NADP	Nicotinamide adenine dinucleotide phosphate (oxidized)
P	Probability
PA	Pulmonary artery
PAP	Pulmonary artery pressure
P/O	Phosphate: oxygen ratio
PVR	Pulmonary vascular resistance
Q	Flow

RA	Right atrium
RV	Right ventricle
SEM	Standard error of the mean
SG	Swan-Ganz catheter
SR	Sarcoplasmic reticulum
SV	Stroke volume
SVC	Superior vena cava
SVR	Systemic vascular resistance
TCA	Tricarboxylic acid cycle
T ₃	Triiodothyronine
T ₄	Thyroxine
TSH	Thyroid stimulating hormone
wt	Weight (mass)

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CHAPTER 1

PATHOPHYSIOLOGY OF BRAIN DEATH - ORGAN DONOR, GENERAL CONSIDERATIONS

INTRODUCTION

There is a persistent shortage of donor organs world wide (Slapak 1978, van der Vleit et al. 1982, Cooper et al. 1982, Hertzner et al. 1986), yet, as increasing numbers of heart transplants and other organs (kidneys, liver, pancreas, bone marrow) are performed, the need for such organs is becoming increasingly urgent.

The loss of vasomotor control, development of diabetes insipidus and the large and rapid blood and fluid volume shifts that accompany brain death contribute to haemodynamic instability in potential cardiac donors.

Jorgensen (1973) studied 63 such patients in which the diagnosis of brain death had been established and the patient was maintained on a ventilator; 62% suffered cardiac arrest within 24 hours and 87% by the end of 72 hours. Muhlberg et al. (1986) showed that 10% of referred donors are lost due to progressive cardiovascular deterioration.

At our own centre, supportive therapy of the donor in the past has taken the form of intravenous (i.v.) fluids to replace the large urine output which brain dead patients produce (diabetes insipidus) and to maintain a central venous pressure (CVP) of between 5-10 cmH₂O, inotropic support to maintain a mean arterial pressure (MAP) of above 60 mm Hg, and vasopressin given either by i.v. infusion or by intermittent intramuscular (i.m.) injection, both to reduce urinary output and help maintain an adequate MAP by peripheral

vasoconstriction (Johnston 1981). Despite these measures, in Cape Town approximately 20% of hearts have been lost through deteriorating haemodynamic function before excision for transplantation, or have been deemed unsuitable for transplantation.

Many authors have stressed the importance of stabilizing the cardiovascular system, correcting the acid-base balance in order to prevent further deterioration of the donor. (Slapak 1978, Montefusco et al. 1984, Dickerman et al. 1986). In unstable donors, Rieder (1986) has advocated the use of hypothermic body perfusion for multiple organ removal and, in the past, cardiopulmonary bypass was used, lowering the body temperature, in an attempt to prevent organ injury (Barnard et al. 1969, and Toledo-Pereyra et al. 1980). In spite of many different therapeutic methods, 30% of patients referred to Groote Schuur Hospital for cardiac transplantation die before a donor is available.

The advantages of being able to store a donor heart for a few hours are obvious (Wicomb et al. 1984). There would be time to transport the donor or the isolated graft to the transplant centre, prepare the recipient, and perform tissue typing; a much larger geographical donor pool could be tapped. We therefore developed a technique of continuous hypothermic perfusion storage of the donor heart which resulted in excellent immediate and long-term myocardial function when the heart was excised from a healthy, anaesthetised baboon or pig (Wicomb et al. 1981). Storage periods up to 48 hours were followed by long-term survival of the recipient baboons into whom these hearts were transplanted (Wicomb et al. 1984).

When hearts taken from human brain-dead donors were stored by the same system, however, a delay of several hours in the return of good myocardial function was observed (Wicomb et al. 1981, Cooper et al. 1983). As the storage system utilized in these patients was identical to that used in our animal studies, it appeared that this delayed function must presumably result from a loss of myocardial energy stores and/or other reversible damage

sustained during and following the onset of brain death, this being the only obvious difference between the two groups.

These observations led me to undertake a series of experiments in the baboon and pig to elucidate the effects of brain death and the subsequent management of the brain-dead donor on myocardial function. The additional effect of 24 hours storage by hypothermic perfusion on hearts taken from brain-dead animals was also investigated.

PATHOPHYSIOLOGY OF BRAIN DEATH

The neocortical part of the brain maintains an intricate balance of input and output with lower centres, especially the reticular formation of the mid brain; through this pathway, the cortex is kept alert and the individual awake. The limbic-mid brain circuits maintain haemostatic equilibrium and the relation of instinct-emotional behaviour, together with the perception of specialized sensory reception, e.g., sight, taste, smell, hearing, and balance; Hypothalamic-pituitary-endocrine functions (Baylis 1983) influence the complex mechanisms of cell metabolism. Continuous regulation of the viscera through the autonomic nervous system maintains and regulates functions such as respiratory, cardiovascular, and gastro-intestinal, and modulates the physiology of organs such as the pancreas, kidneys, and liver.

The most recent evolutionary acquired functions of the neocortex are the so-called higher functions of the nervous system, comprising learning, memory, judgement, ideation, language and other psychological functions of the mind. The integration of these higher functions represents the ultimate contribution of the central nervous system to intellectual and conscious life. The irreversible loss of these functions creates a state of "vegetative" existence.

Recent advances in clinical investigation, such as computerized tomography, continuous intraventricular pressure monitoring, measurement of cerebral blood flow, and the new acquisition, scanning by nuclear magnetic resonance, have thrown new light onto the structural and pathophysiological changes which occur during severe brain damage.

BRAIN INJURY

Two types of injury occur when the head is accelerated after a blow or when the moving head undergoes sudden deceleration. The "primary injury", known as impact damage, occurs at the time of injury. Secondary brain damage may occur as a consequence of a complication of the primary injury or may be related to metabolic disorders which are in turn associated with such conditions as cardiac arrest and hypoxia.

PRIMARY BRAIN DAMAGE

Direct injury to the brain produces two types of lesions. Cerebral contusion and diffuse axonal injury.

Contusion

The initial report by Strich (1956) on 5 patients who, following closed head injury, survived for periods of 5-15 months in more or less decerebrated and extremely demented states, drew attention to the effect of these lesions. The most obvious signs of primary brain damage are haemorrhagic contusions on the surface of the cerebral cortex which vary in extent with regard to both surface area and depth. They may coalesce, producing sizeable intracranial lesions, such as haematomas.

Diffuse Axonal Injury in the White Matter

At present it is believed that the most important cause of primary brain damage following head injury is the widespread presence of sub-cortical lesions. Adams (1982), reviewing 45 cases, showed a triad of focal lesions in the corpus callosum and in the dorsolateral quadrant of the rostral brain stem, with microscopic evidence of diffuse damage to axons. Holbourn (1945), observing the mechanical deformation of the brain which occurs, especially during violent rotation of the head, postulated that a swirling motion of the brain within the skull during the impact induces widespread damage to axons throughout the white matter which results directly from sheer or tensile strain. These lesions are often associated with intraventricular bleeding. The histopathological appearance is of the presence of diffuse axonal injury with so-called "retraction balls"; subsequent degeneration of the axonal system leads to a microglial scar.

Clinical Manifestations of Primary Brain Damage

The most common result is alteration in the level of consciousness. The importance of the conscious level as an indicator of brain damage was emphasized by Teasdale and Jennet (1974) from Glasgow who developed a clinical scale which assessed the depth of impaired consciousness and coma based on three major aspects: (a) motor responsiveness, (b) verbal performance, (c) eye opening. This became known as the Glasgow Coma Scale. It now seems likely that the different depths of coma reflect varying degrees of diffuse axonal injury. Deep and persistent coma is usually associated with severe and widespread axonal damage.

Gennarelli et al. (1981) reported histological evidence of diffuse axonal injury in primates unconscious for more than two hours after injury but with subsequent recovery of

consciousness. Lesions of the reticular activating system in the mid brain are well known to be associated with loss of consciousness, leading some to propose that this phenomenon of concussion is largely a brain stem event. A study of experimental head injury by Povlishosk et al. (1978) showed evidence that the endothelium of brain stem vessels may be damaged (and thus the blood-brain barrier disturbed) following any injury which induces a short period of unconsciousness. Mitchell and Adams (1973) suggested that primary brain stem injury does not exist in isolation but is always part of diffuse brain damage.

SECONDARY BRAIN DAMAGE

Reduction in the initial post-injury level of consciousness in patients is likely to be related to secondary brain damage. The ultimate mechanism for this damage to the brain is either hypoxia, or ischaemia, or a shift of the brain with distortion and compression. Graham et al. (1978) showed ischaemic lesions in 91% of 151 patients dying after severe head injury; these lesions were spread widely in the brain, involving mainly cortex, cerebellum, hippocampus, and basal ganglia.

Causes of Secondary Brain Damage

(A) Intracranial Factors

Extradural haematomas may occur between the skull and meninges secondary to damage of the middle meningeal artery (extradural haematomas) (Figure 1-1). This may be associated with little or no primary brain damage.

Intradural haematomas are three times as common as extradural and may be (a)

subdural, (b) intracerebral (Figure 1-2), or a combination of both. They induce a cascade of events leading to decreased perfusion of the brain, with resulting brain cell anoxia, and raised intracranial pressure.

Brain swelling denotes an increase in volume of the brain itself. It may be due to an increase in cerebral blood volume (engorgement) or an increase in intra or extra-cellular fluid (oedema). These conditions are often associated with raised intracranial pressure. An elevated intracranial pressure from engorgement may reduce cerebral venous return and lead to an increase in cerebral blood volume.

The movement of water across the capillary membrane causing oedema is related to the hydrostatic pressure within the blood vessels and to the oncotic pressure in both plasma and tissues. The special characteristics of the permeability of the brain capillaries result in the special properties of the blood-brain barrier. Function of this barrier may fail. Several different mechanisms may produce brain oedema; the cause may be vasogenic, cytotoxic, hydrostatic, or interstitial. All are inter-related with direct brain injury and with the occurrence of hypoxia or other cause of secondary brain damage. Cytotoxic oedema usually takes place as a result of secondary injury, while oedema around an intracranial haematoma (primary injury) is probably vasogenic in origin.

(B) Extracranial Factors

These are usually related to hypotension and/or hypoxia. The major source of energy to the brain is through aerobic glycolysis. The interrupted supply of oxygen and glucose is directly related to the cerebral blood flow and oxygen tension.

Overgaard and Tweed (1974) studied regional cerebral blood flow in brain oedema and

increased intracranial pressure in 43 patients. Patients with flow rates of less than 20 ml/100 gm/min (normal is above 50 ml/100 gm/min) either died or survived in a chronic vegetative state, indicating that perfusion at this level is incompatible with cerebral functional recovery. Autoregulation of cerebral blood flow during the first few hours after head injury was studied by Fieschi et al. (1974); autoregulation was intact initially and impaired 2-3 days afterwards. Eklof and Siesjo (1971), in an experimental model in rats in which ischaemia was induced by carotid occlusion, measured ATP, ADP, and AMP; these were all significantly decreased following bilateral ligation of the carotid arteries. McPherson and Graham (1978) studied 96 patients who died from non-penetrating head injury, and showed that the incidence of vascular spasm was 41%.

Clinical data implicate vasospasm, (McPherson and Graham 1978), a factor of major importance in the overall morbidity associated with subarachnoid haemorrhage, and it may also be responsible for severe ischaemic brain damage. A slow cerebral circulation may be due to a combination of increased intracranial pressure and decreased systemic blood pressure and vascular capillary thrombus formation.

Ames (1968) demonstrated an inability to perfuse portions of rabbit brain following a period of oxygen and glucose deprivation. Chiang et al. (1968), using electron microscopy, showed a decrease of vascular diameter and an increase in cerebrovascular resistance following similar deprivation. Both oxygen and glucose deprivation may, directly or indirectly, produce generalized intracranial hypertension. Hypoxaemia, from whatever cause, will impair glucose utilization by the brain cells. Opitz and Schneider (1967) showed experimentally that the critical oxygen tension is reflected by a venous pO_2 of 19 mmHg; animals lost consciousness and death followed when the venous pO_2 reached a range of 12-14 mmHg; concomitant hypotension greatly potentiates the effects of hypoxia.

RAISED INTRACRANIAL PRESSURE

This is a common observation in severe head injuries. The relationships between the different intracranial components are shown in Figure 1-3. Because the skull is rigid, an increase in volume in any one of the components must lead to a reduction in one or more of the others. Once the compensatory mechanisms are overcome, intracranial hypertension results.

Teasdale et al. (1977) showed that a blood flow reduction of 40-70% (to 30-60% of control) resulted in disturbances of cerebro-electrical function. (Figure 1-4). A flow of less than 16 ml/100 grams brain/minute resulted in slowing of cerebro-electrical activity. Between 12-16 ml there is a reversible increase in the level of potassium in the extracellular space, and a flow between 8-10 ml indicates a failure of the mechanism responsible for maintaining the action potential. If this persists for more than 30 minutes, irreversible damage to neurons will take place. Graham et al. (1978) showed that many patients with intracranial haematomas showed an increase in intracranial pressure resulting from ischaemic damage. (Figure 1-5).

BRAIN SHIFTS

In any of the above-discussed mechanisms (such as haematoma or brain oedema), a shift may occur from one compartment to another, especially in relation to the tentorium, which divides the intracranial spaces into supratentorial and infratentorial. During tentorial herniation, compression of the oculomotor nerve occurs and the parahippocampal gyrus will distort and compress the brain stem, affecting its blood supply; compression of the posterior cerebral artery may lead to infarction of the occipital cortex. (Figure 1-1).

Hypertension within the posterior fossa will affect the cerebellar tonsils and the medulla, extruding them through the foramen magnum. Compression of the medulla leads to vasomotor and respiratory derangement, leading to sudden respiratory arrest and hypoxic death.

During the episodes of endocranial hypertension increased sympathetic activity has been shown by many authors, Clifton (1981) studied plasma noradrenaline and dopamine in 48 patients, correlating the elevation of catecholamines with increase in blood pressure, pulse rate and temperature. Peerles (1981) showed a 12-14 fold catecholamine rise following subarachnoid haemorrhage. Wauchob (1984) discussed the implication of massive sympathetic response, most likely of hypothalamic origin, and its effect on the lungs, inducing neurogenic pulmonary oedema. Nathan (1975) working with rats, induced lesions in the anterior hypothalamus which released adrenal catecholamines. Hunt (1985), pre-treating rats with propranolol, prevented myocardial lesions after stimulation of the hypothalamus, mid brain reticular system, or following catecholamine infusion. Feibel (1981) found a significant fall of catecholamines in pre-terminal patients as soon as the brain stem reflexes were lost, concluding that it is likely that the sympatho-adreno-medullary failure is due to the destruction of the hypothalamic-diencephalic structures and loss of mid brain autonomic function. Hall (1982) studied TSH, prolactin, and cortisol concentrations in brain-dead patients, finding that there were no significant changes in a subsequent 24 hour follow up period, but circadian cortisol variation was absent; he concluded that after the clinical diagnosis of brain death has been made, the hormonal evaluation of such patients does not help further in determining brain death.

CLINICAL DIAGNOSIS OF BRAIN DEATH

A patient in whom life is maintained by a ventilator will be unresponsive and unreactive to external stimuli, with loss of brain stem reflexes. Extensive reviews on this subject and

the implications of death of the brain stem have been contributed by Black (1978), Pallis (1983), and de Villiers (1984), concluding that the clinical diagnosis of brain stem death is reliable in diagnosing irreversible loss of function of the brain.

CONCLUSIONS

The crucial events which lead to the phenomenon of brain death (Figure 1-5) are (i) the failure of the blood-brain barrier, (ii) the breakdown of neuronal membranes so that repolarization and energy transport is impossible, (iii) oxygenation below the level required for basic metabolism of the nerve cells, (iv) the production and utilization of abnormal metabolic substances by the cell, and finally, (v) the decrease or arrest of cerebral blood flow.

LEGENDS

Figure 1-1

The effects of an intracranial haematoma - raising the intracranial pressure, inducing midline shift, midbrain distortion, herniation of the temporal lobe through the tentorium, and compression of the 3rd nerve. (After Jennet B. Introduction to Neurosurgery, 3rd Edition, Heinemann, London, 1977).

Figure 1-2

Effects of an intracerebral mass leading to an elevated intracranial pressure in the supratentorial area, transmitted through to the infratentorial portion, and inducing compression of the midbrain and cerebellum. Cerebellar protrusion through the foramen magnum occurs, resulting in compression of the distal midbrain and proximal spinal cord (coning).

Figure 1-3

Estimated volumes (in mls) of the intracranial contents. (After Jennet B. Introduction to Neurosurgery, 3rd Edition, Heinemann, London, 1977).

Figure 1-4

The effect of reduction of cerebral blood flow on central nervous system function. (After Jennet B., and Teasdale G. Management of Head Injuries, Davis, Philadelphia, 1981).

Figure 1-5

Schematic representation of pathophysiological pathways leading to brain death. (After Mendelow AD, Teasdale GM, 1983, Pathophysiology of head injuries, Brit J Surg, 70, 641).

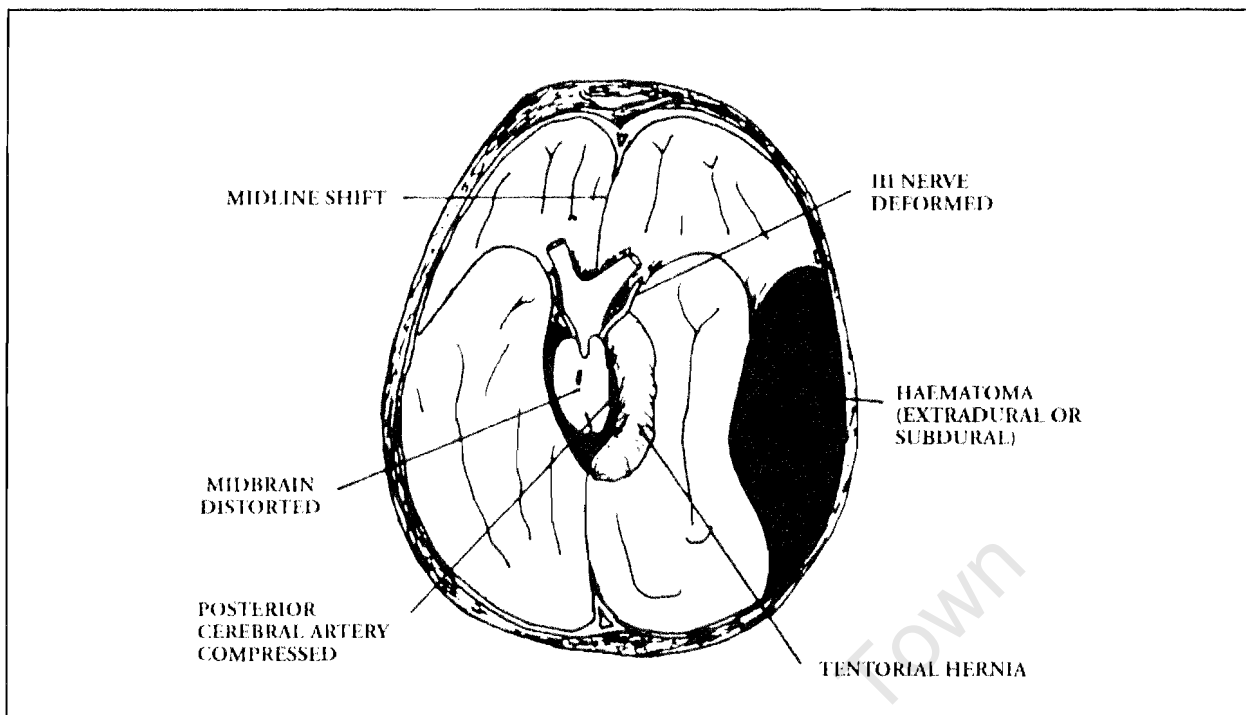


Figure 1-1

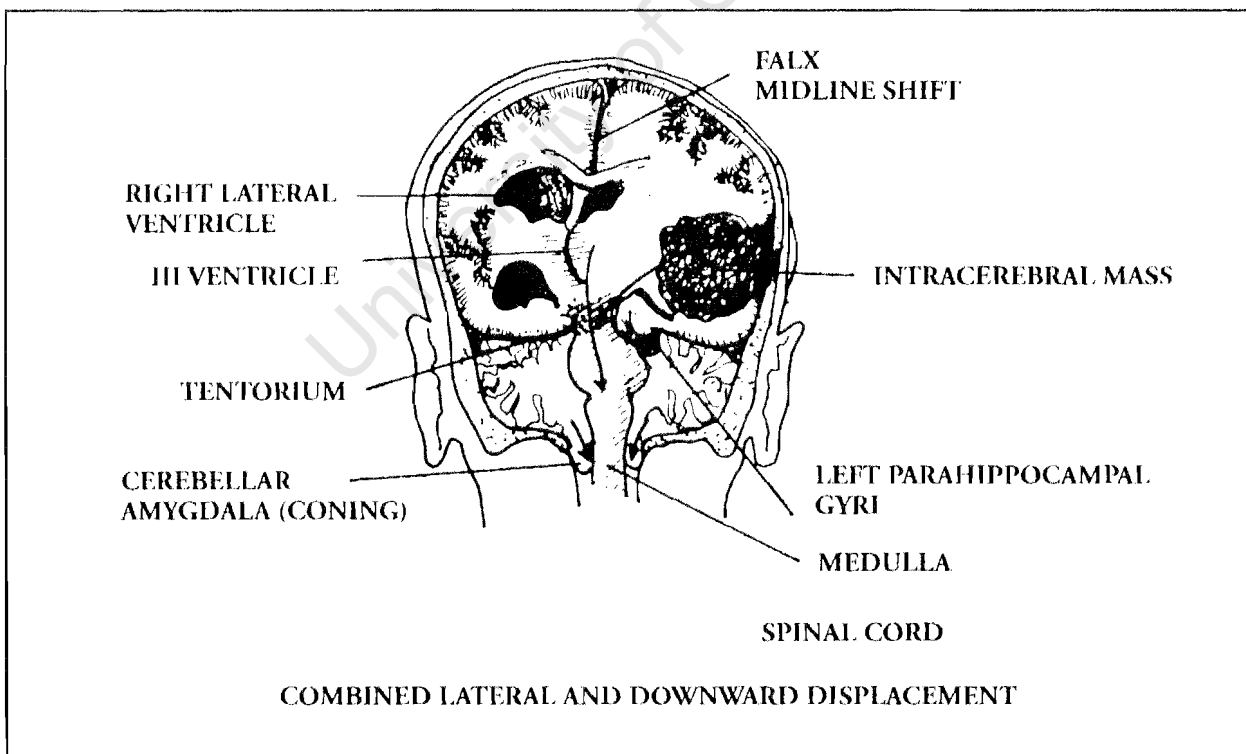


Figure 1-2

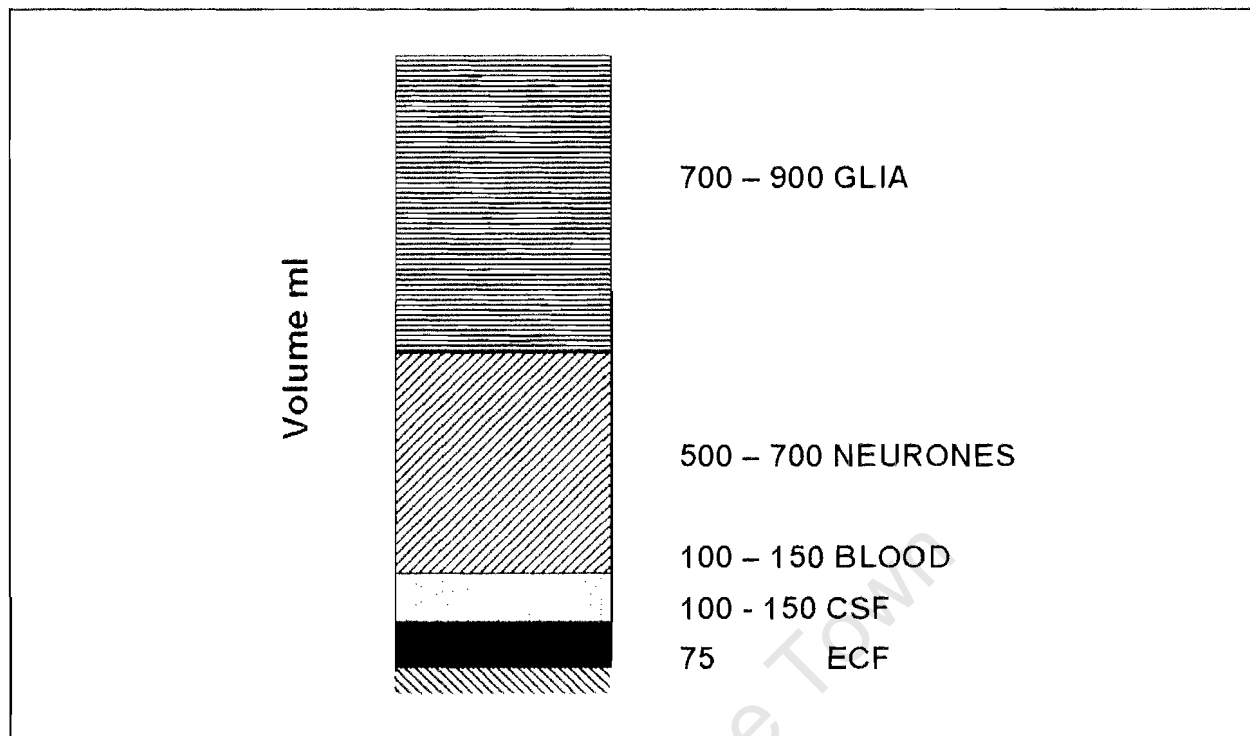


Figure 1-3

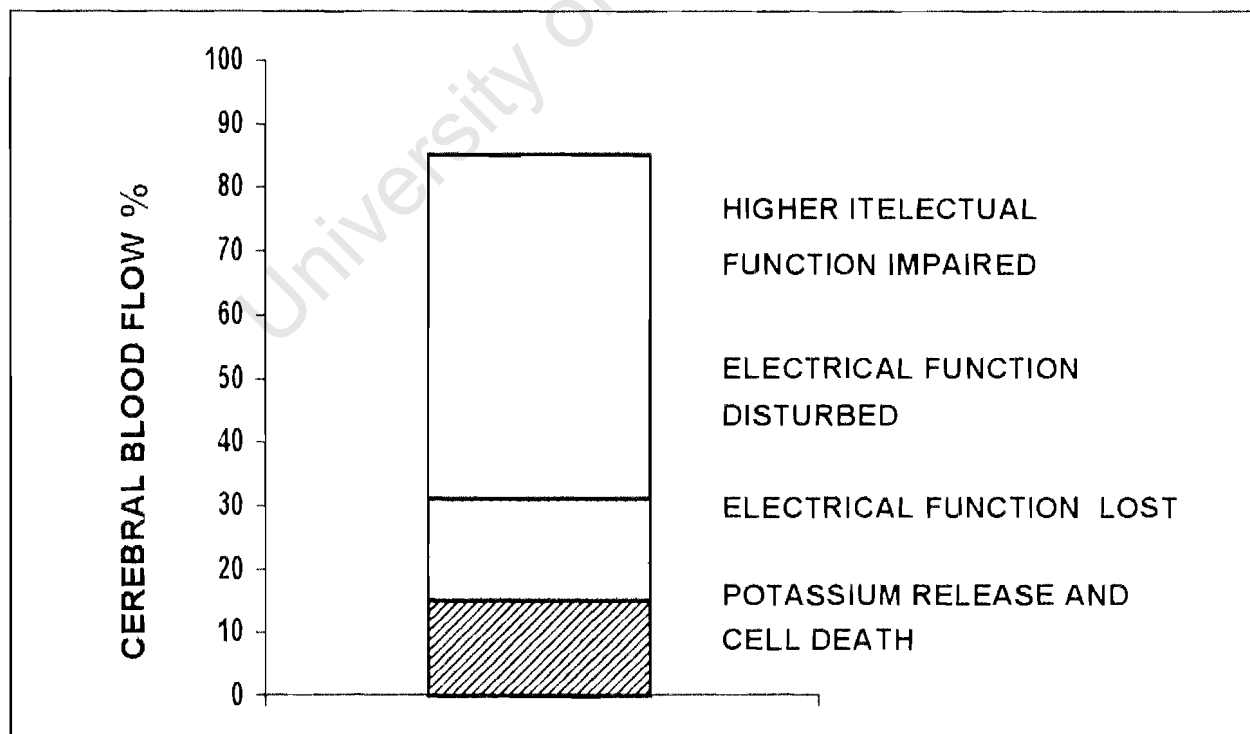


Figure 1-4

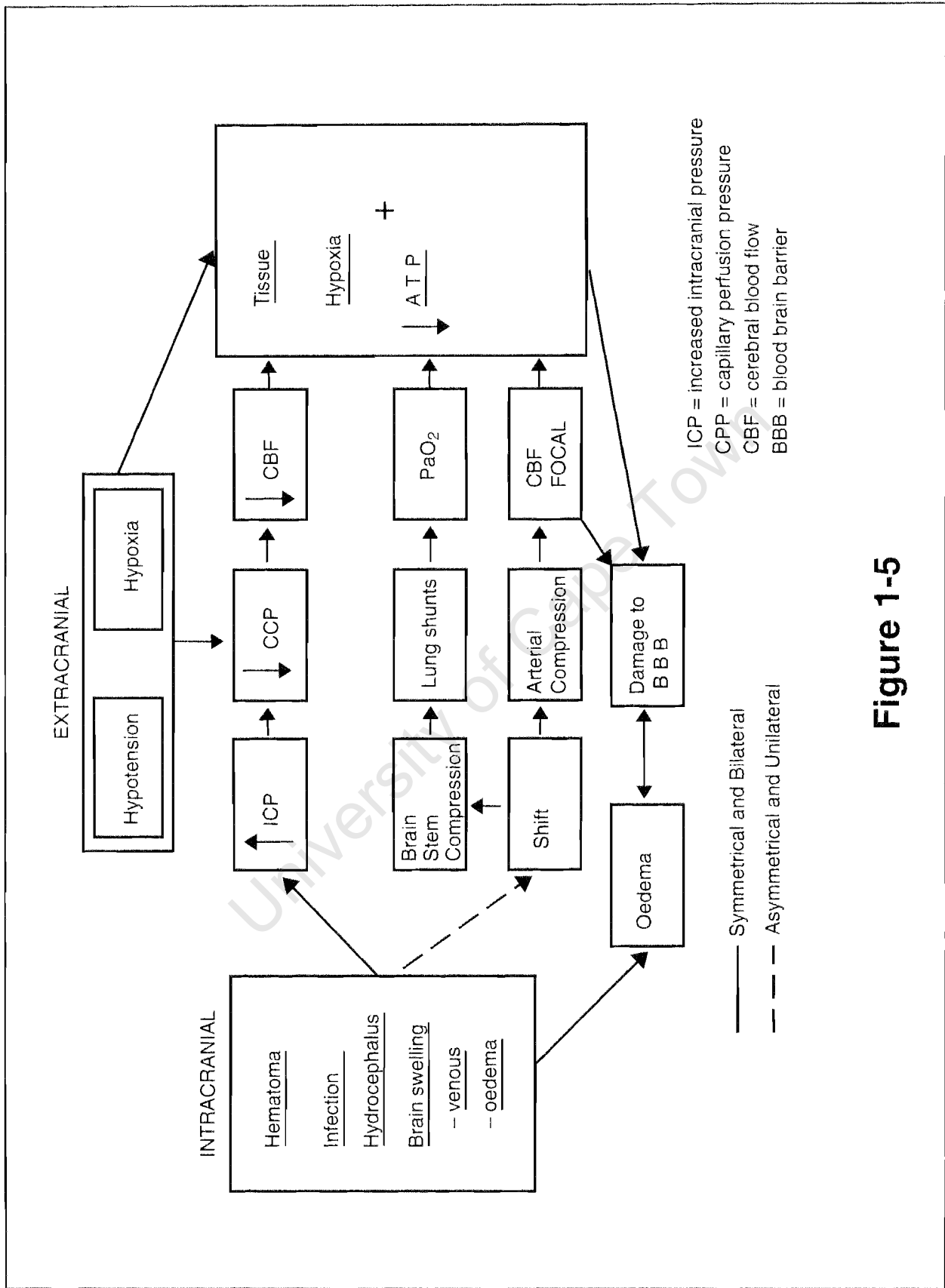


Figure 1-5

CHAPTER 2

ELECTROCARDIOGRAPHIC, HAEMODYNAMIC, ENDOCRINE AND HISTOPATHOLOGICAL RESPONSES TO THE INDUCTION OF BRAIN DEATH IN THE BABOON

Part A

METHODS OF INDUCTION OF BRAIN DEATH IN THE EXPERIMENTAL ANIMAL.

INTRODUCTION

Two different models of the induction of brain death have been employed. Both were followed by almost identical haemodynamic changes, and therefore the differences in each technique of induction of brain death do not appear important. In the majority of our studies in the baboon, brain death was induced by a sudden increase in intracranial pressure brought about by inflation of the balloon of an indwelling Foley catheter (Method 1 below). In the majority of our studies in the pig, and also in a small group of baboons, brain death was induced by clamping or ligation of the two major arteries arising from the arch of the aorta which supply the upper part of the body (Method 2 below); this brought about sudden ischaemia of the brain, as both the carotid and vertebral arterial supplies were interrupted.

ANAESTHESIA AND MONITORING

Both large white Landrace pigs and Chacma baboons (*Papio ursinus*) weighing 10 to 33 kg were sedated with ketamine hydrochloride (5-10 mg/kg/i.m. and i.v. morphine (1.5 mg/kg). (In some animals, muscle relaxation was brought about with alcuronium dichloride (0.5 mg/kg/i.v.) in pigs and pancuronium bromide (4.8 mg/i.v.) in baboons). After endotracheal intubation, a mixture of oxygen (4ℓ/min) and nitrous oxide (6ℓ/min)

was administered, the animal breathing spontaneously. Blood gases and urine output were measured. A neurological examination confirmed the presence of brain stem reflexes. (Table 2A-1). Electrocardiogram (ECG), mean arterial pressure (MAP), and central venous pressure (CVP) were continuously recorded in all animals. Temperature was monitored by rectal thermometer.

In selected groups of animals further monitoring was carried out, namely of left atrial pressure (LAP) or pulmonary capillary wedge pressure (PCWP), and stroke volume (SV). Aortic and pulmonary arterial blood flows (QAo-QPa) were measured simultaneously by electromagnetic flowmeters (Figure 2A-1). Cardiac output (CO) was measured, and systemic (SVR) and pulmonary (PVR) vascular resistances calculated. When flowmeters were placed around the major vessels and when direct LAP was monitored, necessitating median sternotomy, oxygenation was maintained by positive pressure ventilation.

In some groups, blood specimens were taken at intervals both before and after induction of brain death for estimation of catecholamines, metabolites, hormones, and enzymes. At the end of the experiment, an autopsy was performed. The myocardium was biopsied for estimations of high energy phosphates, glycogen and lactate. The heart, and other organs were also biopsied for histological examination.

METHOD 1

After baseline measurements, the anaesthetic level was deepened (ketamine 2 mg/kg/i.v.). A burrhole was drilled in the right fronto-parietal area of the skull, the duramater was incised, and a Foley catheter was introduced into the subdural space. The rapid injection of 10 to 20 ml of saline inflated the balloon of the catheter, producing an acute increase in intracranial pressure with herniation of the para-hippocampal gyri, compression of the

midbrain, and paralysis of brain stem reflexes. Cerebellar herniation (coning) causes interruption of neurological pathways between the midbrain and the spinal cord. (Figure 2A-2). Brain death occurred within 20 minutes in all animals and was established by neurological examination (Table 2A-1).

When spontaneous respiration ceased, positive pressure mechanical ventilation was initiated; it was continued throughout the experiment and adjusted following blood gas measurements. Potassium chloride was administered to maintain the serum level between 3 and 4.5 mmol/l; no inotropic agents were given to any of the brain dead animals in the initial studies, but were administered to later groups (as detailed below).

METHOD 2

Median sternotomy was performed and the pericardium opened longitudinally. The two arteries to the upper part of the body which arise from the aortic arch were mobilized, ligated or clamped, interrupting the arterial blood supply to the central nervous system via the carotid and vertebral arteries. Previous studies in our laboratory showed that this procedure led to irreversible and total brain damage in the pig within a period of 120 - 135 minutes, and in the baboon within a period of 15 - 45 minutes, Lanza et al. (1983). In this model there was no acute increase of the endocranial pressure, but rather an acute ischaemia (see Chapter 1).

During the induction of brain death in the animals using method 1 and method 2, marked electrocardiographic, haemodynamic, endocrine, and histopathological changes took place.

LEGENDS

FIGURES

Figure 2A-1

Diagram showing the haemodynamic monitoring of the systemic and pulmonary circulations. Two electromagnetic flow meters were inserted around the aorta and main pulmonary artery for independent flow measurement. (SG = Swan-Ganz catheter; RA = right atrium; AO = aorta; PA = pulmonary artery; LA = left atrium).

Figure 2A-2

Cast of the endocranial contents measuring 200 ml from a baboon weighing 25 kg. The cast has a great similarity to the macroscopic appearance of a baboon's brain. (F = frontal lobe; T = temporal lobes; O = occipital lobe; Cer = cerebellum; MB = midbrain; FM = position of foramen magnum; Tn = position of tentorium).

TABLE 2A-1 MODIFIED MIDBRAIN REFLEXES IN THE BABOON BY KETAMINE HCl AND N₂O

	Ketamine HCl	Ketamine + 40% N ₂ O	Ketamine + 60% N ₂ O	Ketamine no N ₂ O for 15 minutes
Spontaneous breathing	+++	+++	+++	+++
Respiratory rate	28	16	16	30
Spontaneous limb movements	+++	++	-	+++
Plantar response to stimulation (withdrawal)	++	++	±	+++
Palpebral	-	-	-	±
Corneal	+++	+++	++	+++
Gag	++	-	-	+
Cold caloric	++	+++	+++	+++
Pupils - reaction to light	+++	+++	+++	+++
Spontaneous blinking	++	+	-	++

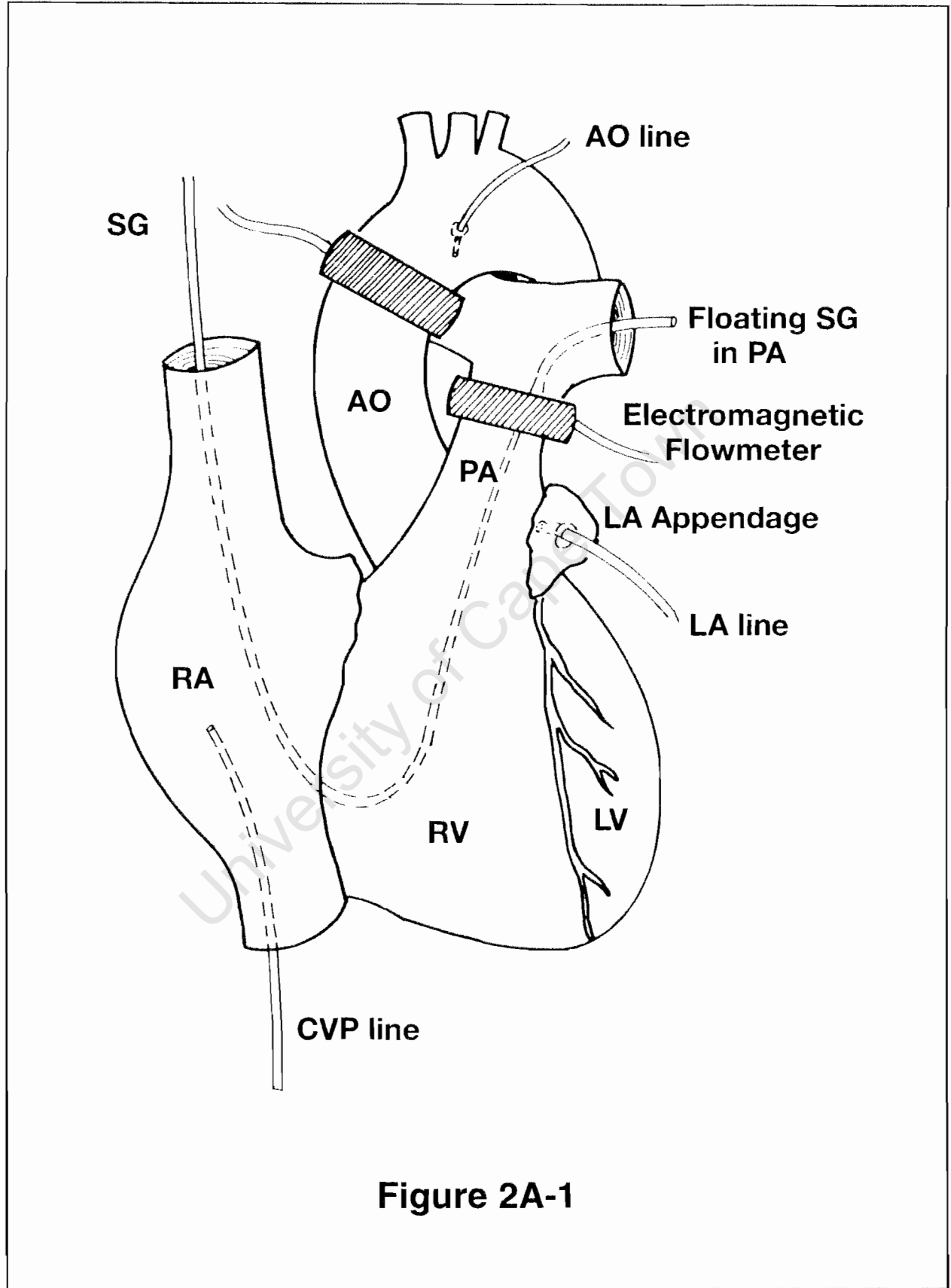


Figure 2A-1



Figure 2A-2

Part B**ELECTROCARDIOGRAPHIC CHANGES****INTRODUCTION**

An unmodified induction of brain death in the baboon (n=17) was studied. The ECG was continuously displayed on a monitor and a 12-lead ECG recorded on a Mingograf (Siemens-Elema) obtaining: (i) a control ECG prior to brain death induction and a continuous recording while brain death was induced and during the next 10 minutes. When significant ECG changes were seen on the monitor these were also recorded for a period of one minute, noting the time from the induction of brain death and the duration of the events. ECG monitoring and recording lasted for a mean period of 17 hours, with a range of 16-24 hours.

The following ECG parameters were studied: (1) rhythm, (2) rate, (3) PR interval, (4) QRS duration, (5) R wave voltage at V5, (6) QT interval, (7) QT corrected for rate (QTC), and (8) T wave and the ST segment configuration.

RESULTS

Five different stages following the induction of brain death were defined based on: 1) rhythm, 2) rate, 3) ventricular ectopics, 4) ventricular tachycardia, and 5) ischaemic changes. (Table 2B-1) (Figures 2B-1 to 2B-4).

Stage 1: Appeared immediately during the increase of endocranial pressure, characterized in all animals by a sinus bradycardia of a mean rate of 44 beats per minute, mean duration

of 4.5 minutes. Eleven animals had third degree A-V block, 10 presented second degree A-V block and 8 first degree A-V block. Six of 17 had sinus pauses, sinus arrest with AV dissociation and nodal escape. The longest pause lasted for 17 seconds. (Table 2B-1) (Figures 2B-1, 2B-5).

Stage II: Characteristics were a sudden recovery to sinus rhythm and a rapid increase in the heart rate to a mean of 144 beats per minute, with no ischaemic changes. This stage lasted for a mean period of 7.3 minutes. (Table 2B-1) (Figures. 2B-1 to 2B-4).

Stage III: Characterized by the presence of ventricular ectopics. Fifteen animals had ventricular tachycardia at a rate of 160 per minute. All animals had unifocal or multifocal ventricular premature contractions and couplets associated with bigeminal rhythm. The longest ventricular tachycardia lasted for 2 minutes 30 seconds and most animals had a recurrence of runs of ventricular tachycardia. The mean time of ventricular tachycardia was 43% of the duration of Stage III; 28% of the remaining beats were of sinus origin. (Table 2B-1) (Figs. 2B-1 to 2B-4).

Stage IV: Started when all ventricular ectopic activity ceased, was of extremely variable duration (mean 51.3 minutes) with a rate of 160 beats per minute with marked ischaemic changes, temporary presence of Q waves in 5 animals. ST elevation or depression and inversion of T wave in all animals. Two of the 17 studied animals had a continuous tachycardia in Stage IV until the end of the experiment. Both animals had a spontaneous cardiac arrest. (Table 2B-1) (Figures 2B-1 to 2B-4).

Stage V: Characterized by a reduction in the heart rate to control levels or below lasting until the experiment was electively terminated.

DISCUSSION

Ten animals had T wave inversion, associated with ST segment elevation in 7 and ST depression in 3. Only 4 animals had T wave inversion with no ST segment changes, and 3 presented with ST depression. Six animals had a more prominent T wave, in some associated with the previous changes. In 4 animals, the ST segment-T waves remained unchanged.

Only one animal developed a Q wave infarct pattern beginning during Stage II in the precordial lateral leads, suggestive of a transmural infarct. Two animals had an ECG pattern of non-transmural myocardial infarct.

In 6 animals, depolarization changes were seen as early as in Stage I (very similar to the classical J wave) persisting throughout the experiment and becoming more evident in Stage V. An example is shown in Figure 2B-4).

The statistical comparisons of the different stages versus control are shown in Table 2B-2.

LEGENDS

FIGURES

Figure 2B-1

Examples of the 5 ECG stages following the induction of brain death (baboon 570) recorded from the precordial leads V3-V6. Control - sinus rhythm, heart rate 120 per minute. Stage I shows a sinus bradycardia with third degree A-V block and junctional escape beats; heart rate 20 per minute. Stage II shows a sinus tachycardia, heart rate of 160 beats per minute, otherwise normal ECG. Stage III, bifocal ventricular ectopics at a rate of approximately 170 per minute. Stage IV, sinus tachycardia at a rate of 160 per minute with marked ischaemic changes, ST elevation suggestive of an acute myocardial infarct. Stage V, a resting ECG with a heart rate similar to control in sinus rhythm, loss of amplitude of the QRS complex, and flattening of the T waves.

Figure 2B-2

Recording of ECG from precordial leads, C: (control) heart rate of 54 beats per minute, sinus rhythm and a normal PR interval with some flattening and inversion of the T wave. Stage I, sinus bradycardia, heart rate of 31 beats per minute, T wave inversion in V2, otherwise no significant changes. Stage II, sinus tachycardia of 150 beats per minute, no evidence of ischaemic changes and a positive T wave in V2. Stage III, ventricular tachycardia of approximately 166 beats per minute of multifocal origin. Stage IV, sinus tachycardia of 165 beats per minute, with marked elevation of the ST segment in V2 and V3, as seen in the early stage of a myocardial infarct. Stage V, sinus rhythm of 100 beats per minute, loss of the R wave amplitude, mainly in lead V3, and more marked T wave

inversion than in the control trace, possibly compatible with ischaemia or a non-transmural myocardial infarct.

Figure 2B-3

ECG recording from the precordial leads. C: (control) sinus rhythm, heart rate of 95 beats per minute. Stage I junctional rhythm, rate of 37 beats per minute, normal QRS configuration and a ventricular extrasystole is seen, suggesting that during this early stage, sympathetic activity is present. Two P waves are shown not followed by the QRS complex. Stage II, sinus tachycardia, rate of 166 per minute, unspecific ST segment and T waves present in leads V5 and V6. Stage III, multifocal ventricular tachycardia with an approximate rate of 200 beats per minute. Stage IV, sinus tachycardia with a heart rate of 166 beats per minute, ischaemic ST segment depression in leads V4, V5 and V6. Stage V, sinus rhythm, heart rate of 80 beats per minute with no specific ST segment elevation in leads V3, V4, V5, and more prominent T waves, no loss of R wave, more prominent S waves and a new S wave in V6.

Figure 2B-4

C: (control) standard leads, heart rate of 107 beats per minute. Stage I, sinus rhythm, heart rate of 59 beats per minute, depolarization changes or incipient appearance of a J wave. The ST segment and T wave do not differ significantly from the control. Stage II, sinus tachycardia, heart rate 150 beats per minute, loss of the QRS wave voltage amplitude in all leads, disappearance of the previously seen J wave; the remaining parameters are not significantly different from control. Stage III, ventricular tachycardia of 115 beats per minute. Stage IV, sinus tachycardia of 136 beats per minute, depolarization changes or J wave at the end of the QRS complex, inversion of T

waves in leads II, III and aVF. Stage V, sinus rhythm, heart rate of 88 beats per minute, more evident QRS complex changes and a J wave are seen, loss of the QRS complex amplitude and early repolarization changes.

Figure 2B-5

Stage I, from 3 different animals, A = sinus arrest, occasional escape junctional beats. B = third degree A-V block, junctional QRS complex, irregular rhythm. C = sinus and atrial standstill with junctional escape beats.

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TABLE 2B-1 ELECTROCARDIOGRAPHIC CHANGES INDUCED DURING BRAIN DEATH

STAGES:	C	I	II	III	IV	V
Rhythm	Sinus	Sinus arrest 2° A-V block 3° A-V block	Sinus	VT, VPBs Coupling Multifocal VPBs	Sinus	Sinus
Heart Rate	94 (12)	44 (17)	144 (22)	VT	160 (29)	88 (19)
PR interval	0.14 (0.01)	See text	0.15 (0.02)	0.12 (0.02)	0.13 (0.04)	0.13 (0.04)
ORS (seconds)	0.062 (0.007)	0.062 (0.005)	0.06 (0.005)	VT	0.061 (0.005)	0.06 (0.006)
R wave V ₅ (voltage)	9 (3)	10 (3)	9 (3)	VT	8.9 (4)	8.7 (3.6)
Q ^T	0.30 (0.03)	0.35 (0.05)	0.28 (0.03)	0.25 (0.04)	0.25 (0.04)	0.32 (0.03)
Q ^T C	0.38 (0.033)	0.283 (0.041)	0.433 (0.036)	0.408 (0.043)	0.381 (0.043)	0.381 (0.033)
Time duration minutes	---	4.5 (1.20)	7.3 (3.13)	11.50 (5.21)	51.3 (9.71)	17 (2.1) hrs

Figures in brackets = standard error of the mean

VT = ventricular tachycardia

TABLE 2B-2
ELECTROCARDIOGRAPHIC STAGES
COMPARED STATISTICALLY TO CONTROL*

C	HR (p)	RW (p)	QT (p)	PR (p)	QTC (p)
I	<.0001	n s	<0.0001	n s	<0.001
II	<.0001	"	n s	"	<0.0005
III	<.0001	"	<0.0001	"	n s
IV	<.0001	"	<0.0001	"	"
V	n s	"	n s	"	"

C = control

HR = heart rate

RW = R wave measured in lead V5

QT = QT interval

PR = PR interval

QTC = QT corrected for heart rate

* = Analysis of variance - Dunnett's t test

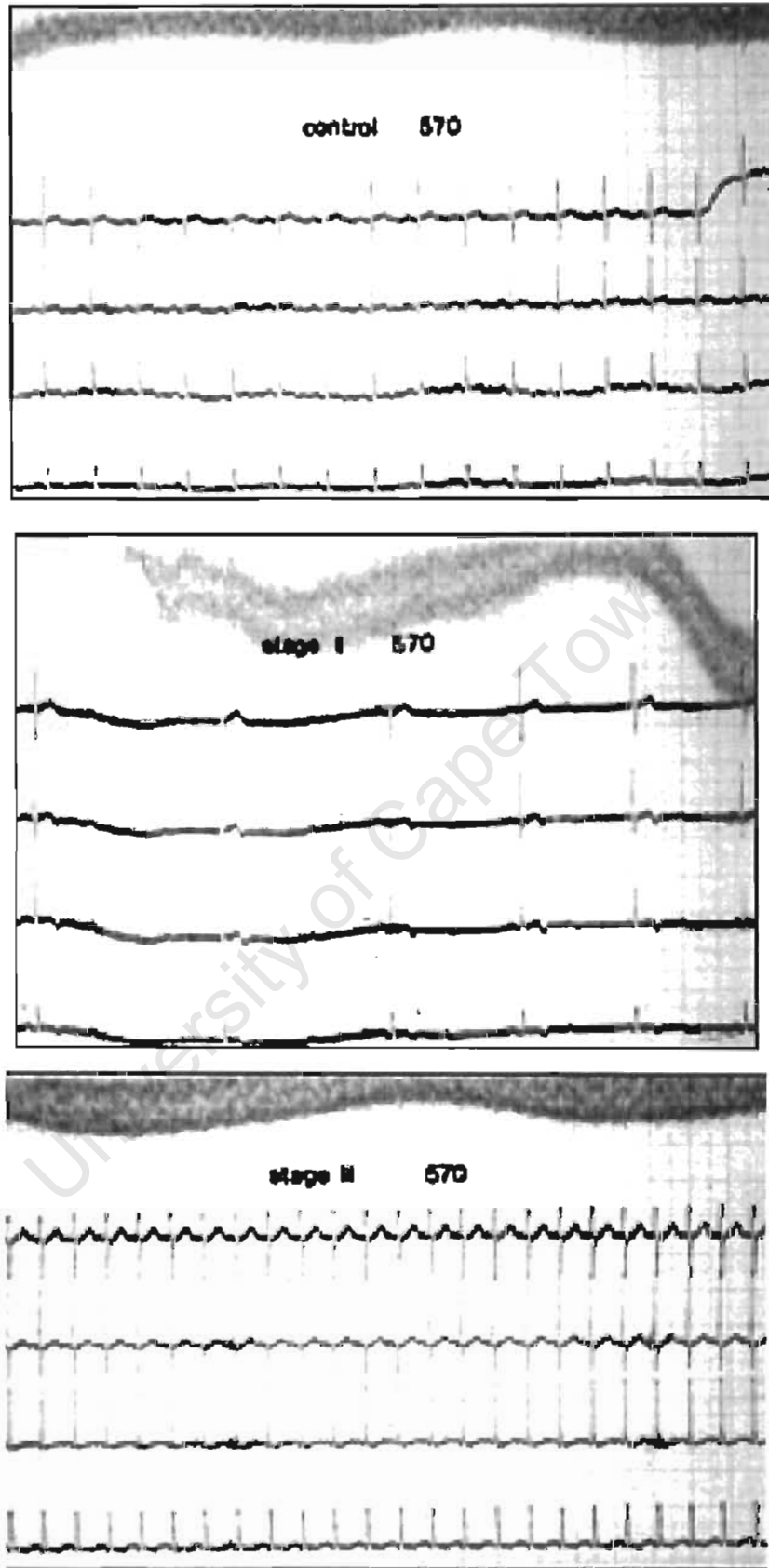


Figure 2A-1

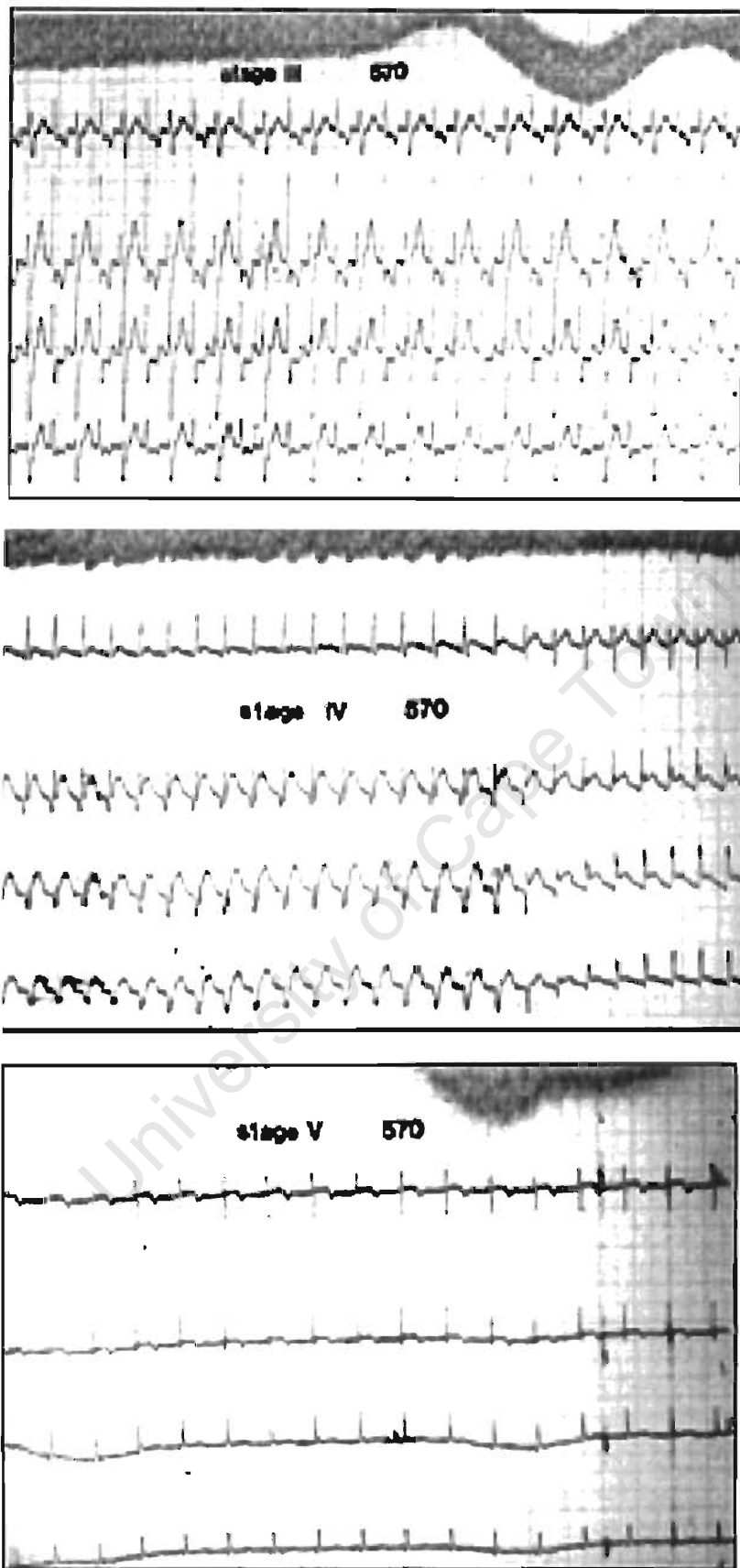


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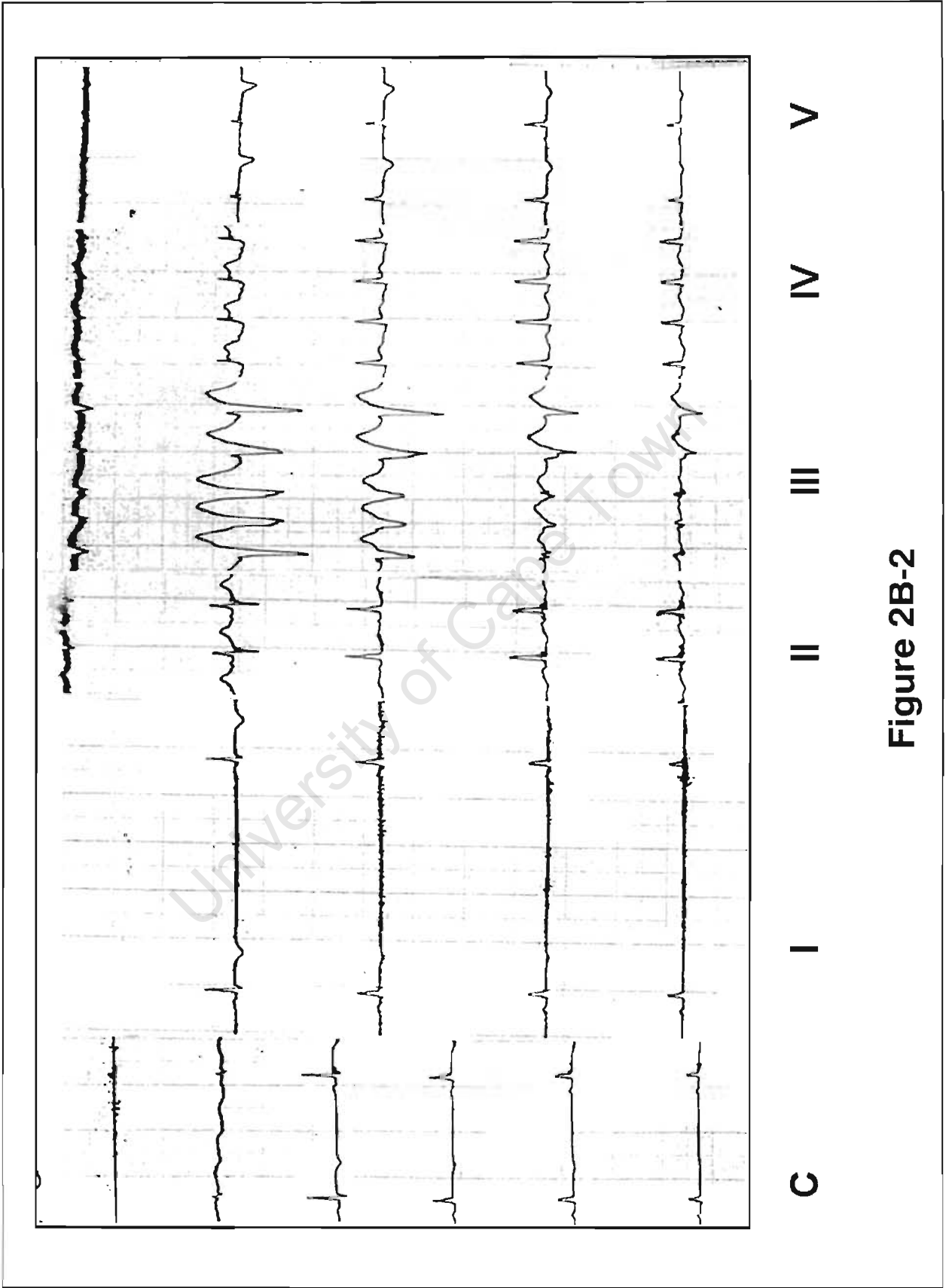


Figure 2B-2

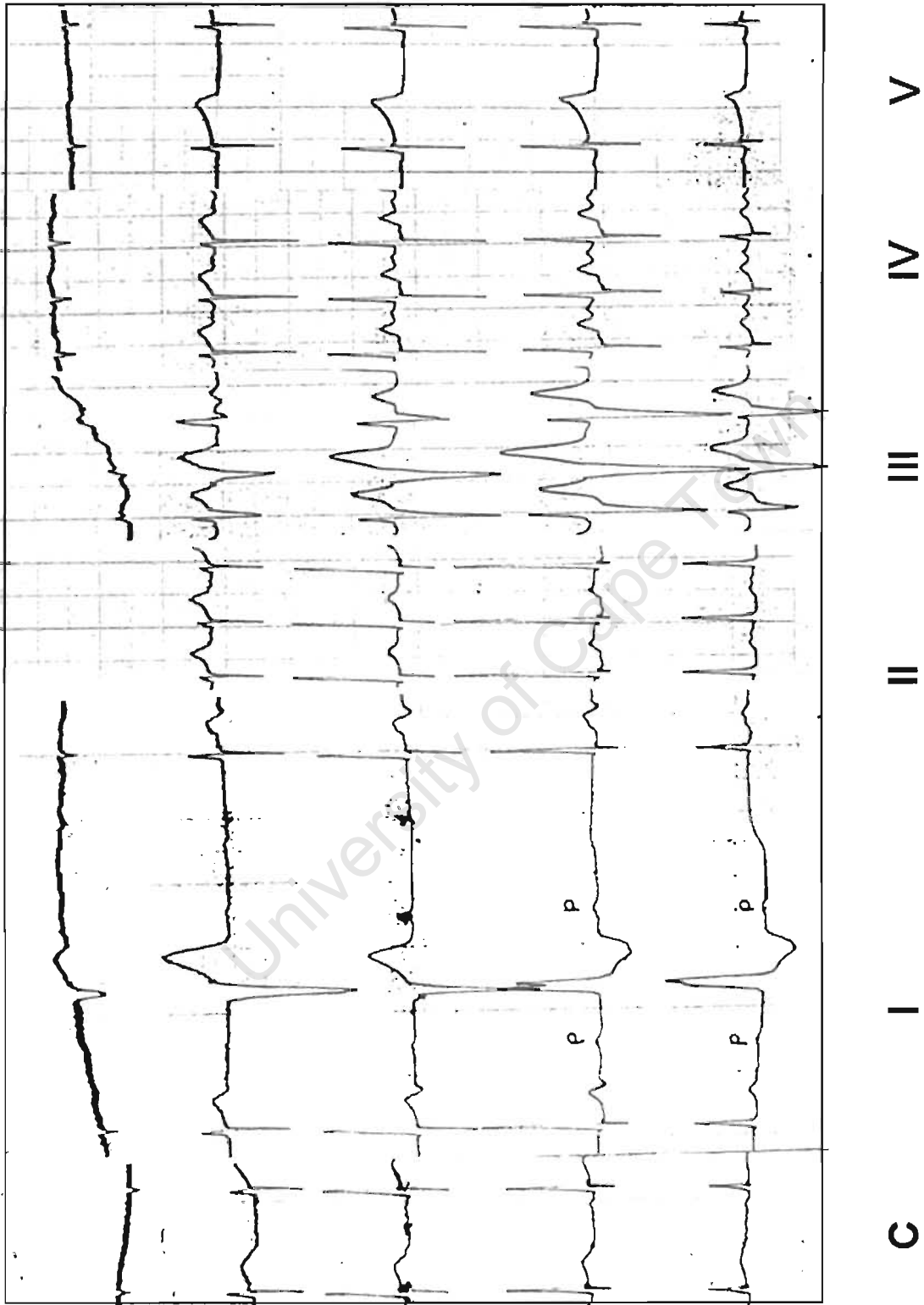


Figure 2B-3

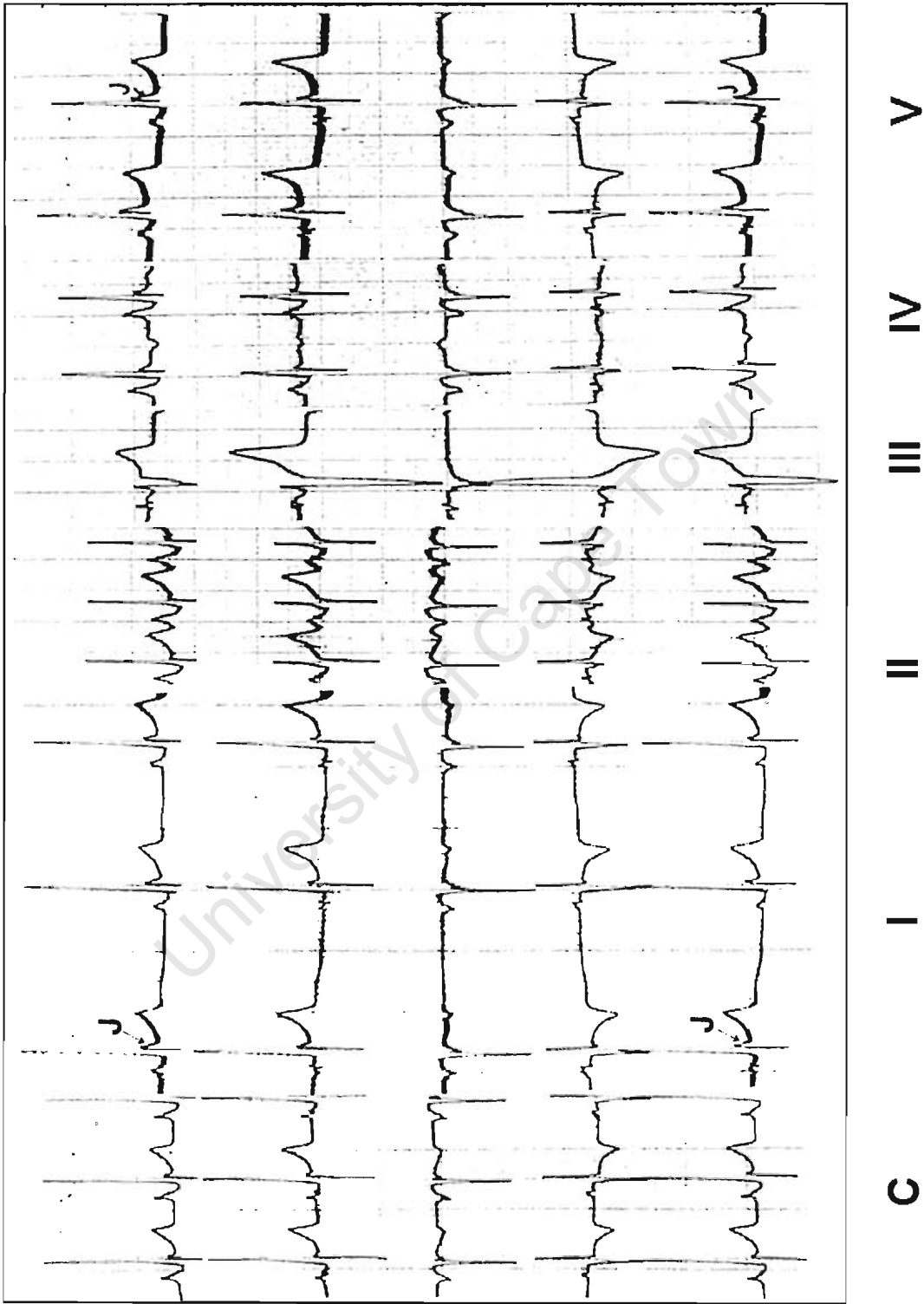
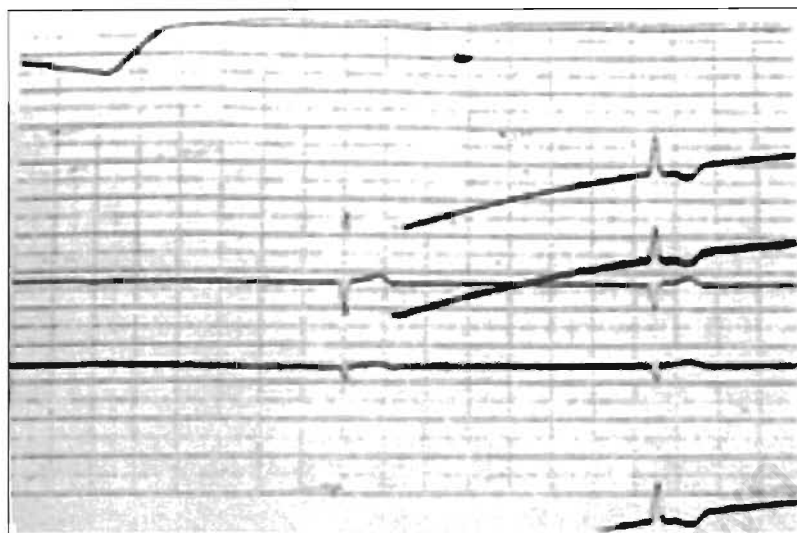
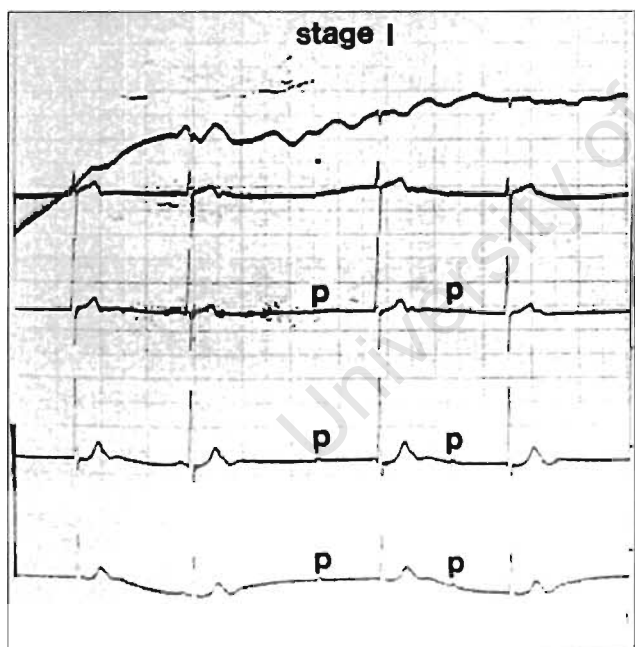


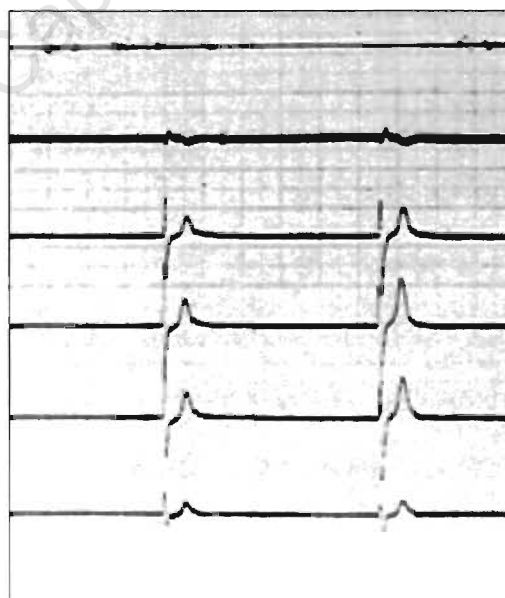
Figure 2B-4



A



B



C

Figure 2B-5

Part C**HAEMODYNAMIC CHANGES**

These were studied in 11 animals. In 8 baboons, a midline splitting sternotomy was performed, the pericardium was opened and the aorta was dissected from the main pulmonary artery. Two electromagnetic flowmeters were placed around the arteries (Figure 2A-1) providing simultaneous recordings of flow through both vessels and hence the cardiac output. A catheter was also introduced through the left atrial appendage for continuous left atrial pressure monitoring. In this group, positive pressure ventilation was maintained throughout the experiment.

All animals had continuous ECG recordings made and MAP and CVP pressures were monitored via catheters introduced through the femoral vessels. A Swan-Ganz catheter was inserted through the opposite femoral vein and positioned under fluoroscopic control within the pulmonary vessels to obtain PAP and PCWP.

In 3 animals sternotomy was not performed; two Swan-Ganz catheters were inserted to monitor pulmonary artery pressure (PAP) and pulmonary capillary wedge pressure (PCWP); in these 3 animals, aortic and pulmonary artery flowmeters were not implanted.

All pressures were recorded on a physiological data recording system (ARG Honeywell) multichannel recorder, allowing simultaneous recording of all haemodynamic parameters; SVR and PVR were calculated.

Prior to induction of brain death (by method 1), control recordings of all the parameters were taken.

Continuous haemodynamic monitoring was maintained for the first five minute period from the moment of the distention of the Foley catheter balloon; thereafter, all parameters were recorded at 15 minute intervals during the first hour, at 30 minute intervals for the next six hours. The experiment was then electively terminated; the heart and lungs were excised and examined histologically.

RESULTS

Systemic Haemodynamic Changes

During the induction of brain death, significant changes took place (Figs 2C-1 to 2C-6). MAP rose from a control of 100 mmHg to a peak of 296 mmHg (range 170-660 mmHg) ($p < 0.002$), an increase of 196%. SVR showed a marked increment at the time of the dramatic increase in MAP from a control level of 45 Wood units to a peak level of 287 Wood units (range 80-800 Wood units) ($p < 0.02$), an increment of 537%. CVP showed no significant changes at the time of the maximal MAP or SVR, but rose slightly afterwards. Though this rise was significant ($p < 0.01$) at a later stage, at peak MAP and SVR it did not achieve significance. Mean aortic blood flow fell from a control level of 2.25 ℓ/min to 1.30 ℓ/min range 0.78-2.40 ℓ/min ($p < 0.0003$), a fall of 42%. When SVR subsequently decreased, the aortic blood flow increased, in some cases back to control levels, though in most animals remaining low. (Figs 2C-1 to 2C-7).

Pulmonary Haemodynamic Changes

The control mean LAP (or PCWP) 8 mmHg rose to a mean of 52 mmHg (range 13 to 90 mmHg) ($p < 0.0001$) (Figs 2C-1 to 2C-6). (On visual observation, the left atrium became markedly distended). The mean PAP of 14 mmHg reached a peak of 34 mmHg (range

10-55 mmHg) ($p < 0.002$). In 9 of 11 baboons in this study, the LAP or (PCWP) exceeded the PAP; this reversal of the normal pressure relationship lasted for a mean period of 57 (14.4) seconds. Mean PVR showed a rise from a control level of 3 to a peak of 7 Wood units (range 1-18 Wood units); this did not reach statistical significance. In 4 cases, the PVR fell below the baseline level, whilst in the remaining baboons it showed a marked increase. Pulmonary artery blood flow, which was equal to the aortic blood flow (2.25 ℓ/min) before the induction of brain death, rose to a peak of 2.82 ℓ/min (range 0.10-4.70 ℓ/min), an increase of 25%; this rise, however, did not reach statistical significance.

Changes in Pulmonary Blood Volume

Under normal haemodynamic conditions, the lung contains approximately 20% of the total blood volume (Ganong 1977); in addition, an extra 4% is contained within the left atrium and left ventricle. The pulmonary and left heart blood volume, therefore, comprises approximately 24% of the total blood volume. (Ganong, 1977).

In the present study, during induction of brain death, when aortic blood flow was markedly reduced following the extreme rise in SVR, the pulmonary and left heart blood volume increased to a mean of 72% of the total blood volume ($p < 0.0001$) (Fig. 2C-4 to 2C-10). As the PVR remained relatively low, right ventricular failure did not occur, as evidenced by no significant changes in the CVP at this time. As the SVR decreased, the left ventricle became capable once again of pumping blood through the aorta. A significant increase in the aortic blood flow occurred, and the blood which had been pooled within the lungs was returned to the systemic circulation. At this stage, aortic blood flow exceeded pulmonary blood flow, until the pulmonary blood volume had returned to normal. Thereafter, aortic and pulmonary flows remained equal once again.

LEGENDS

FIGURES

Figure 2C-1

Mean changes in systemic and pulmonary haemodynamic data during the induction of brain death in 8 baboons. The left hand graph shows changes in systemic haemodynamics between control levels (A) and those recorded at the peak of systemic vascular resistance (B). The SVR was calculated in Wood units. Q = aortic blood flow (l/min). Changes in MAP, SVR and Q_{Ao} reached significance.

The right hand graph shows changes in pulmonary haemodynamics between control levels (A) and those obtained at peak systemic vascular resistance (B). Q = pulmonary arterial blood flow (l/min). The changes in PA and LA reached statistical significance.

Figure 2C-2

Simultaneous pressure recordings from a femoral artery catheter and PCWP obtained from a wedged Swan-Ganz catheter (giving PCWP). Control (pre-induction of brain death) values are shown. The arrow indicates the moment of the induction of brain death (by inflating the balloon of the Foley catheter). There is a rapid response with a drop in the arterial blood pressure and an evident slowing of the heart rate (as seen by the arterial pulse trace) corresponding to the ECG stage I. Following a period of 5 minutes, there is a rapid significant increment of both the systolic and diastolic arterial blood pressures and a rapid increase in heart rate corresponding to the ECG stages II, III, and IV. During stage I, the wedge pressure does not show significant changes. However, at the time of the

arterial pressure increment corresponding to the peak of the SVR, the wedge pressure rises rapidly (off the scale), reaching a peak of 90 mm Hg.

Figure 2C-3

Simultaneous recordings of the MAP, PCWP (wedged) and CVP, showing that at the induction of brain death (arrow), a significant increment of the MAP occurs simultaneously with the PCWP elevation. The CVP does not show significant changes.

Figure 2C-4

Systemic and pulmonary haemodynamic data during induction of brain death in a Chacma baboon. The upper graph shows changes in SVR (Wood units), MAP (mmHg), QPA, and QAO. The discrepancy between flows (shadowed area) represents the period and extent of blood pooling within the lungs; in this case, blood pooling extended for a period 160 seconds.

In the lower graph, changes in mean LAP (mm Hg), mean PAP (mm Hg) and PVR (Wood units) are shown. The shaded area represents the period of 85 seconds during which the LAP exceeded the PAP.

Figure 2C-5

Systemic and pulmonary haemodynamic data during induction of brain death in a second baboon in this series. Legends as for Figure 2C-4. In this case, blood pooling within the lungs extended over 105 seconds. LAP rose above PAP for only 6 seconds.

Figure 2C-6

Systemic and pulmonary haemodynamic data during induction of brain death in a third baboon of this series. In this case, blood pooling within the lungs extended for 140 seconds. LAP rose above PAP for 55 seconds.

Figure 2C-7

Shows control (A) cardiac output, and the QPA-QAO at the time of peak SVR. (B) QAO decreases significantly. A significant difference in flows was recorded at B. Statistical differences are shown.

Figure 2C-8

Central blood volume pooling (QPa-QAo) in litres, in each of the 8 animals in which flow meters were inserted, the shaded areas represent the pooled blood in the lungs and left atrium.

Figure 2C-9

Shows the normal central blood volume (A) contained within the lungs and the left side of the heart, and at the time of peak SVR (B). The increase in the central blood volume is statistically significant.

Figure 2C-10

Simultaneous ECG (lead II) and arterial pulse trace recordings during the control period

and during the first 4 ECG stages. The heart rate and the blood pressure for each stage are shown. During Stage I the MAP remained unchanged in spite of a marked bradycardia, indicating an increment of the SVR, as a result of increased sympathetic activity. ECG stages II, III, and IV, as per 2B-1. The resulting hypertension started settling by the end of stage IV.

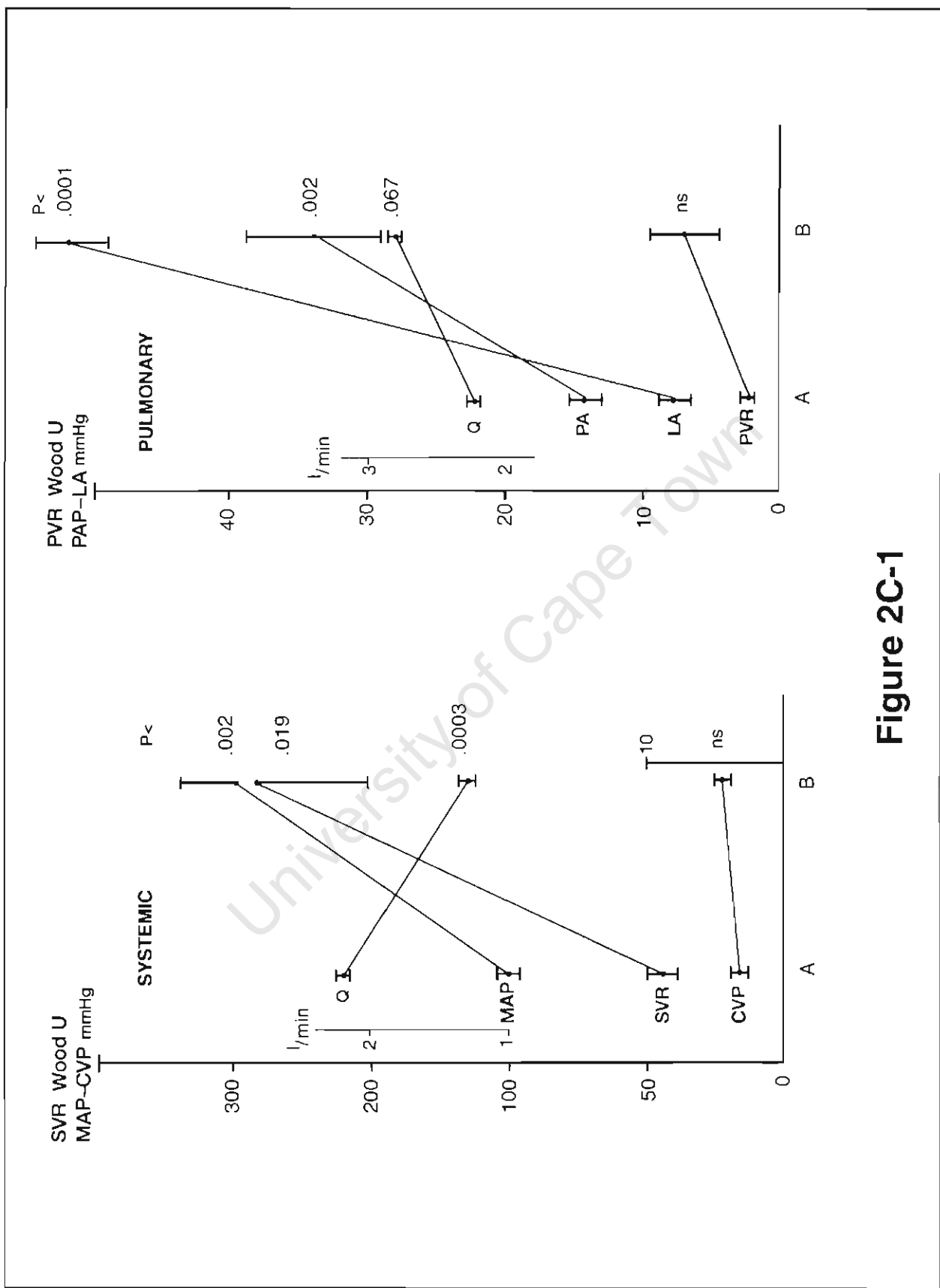


Figure 2C-1

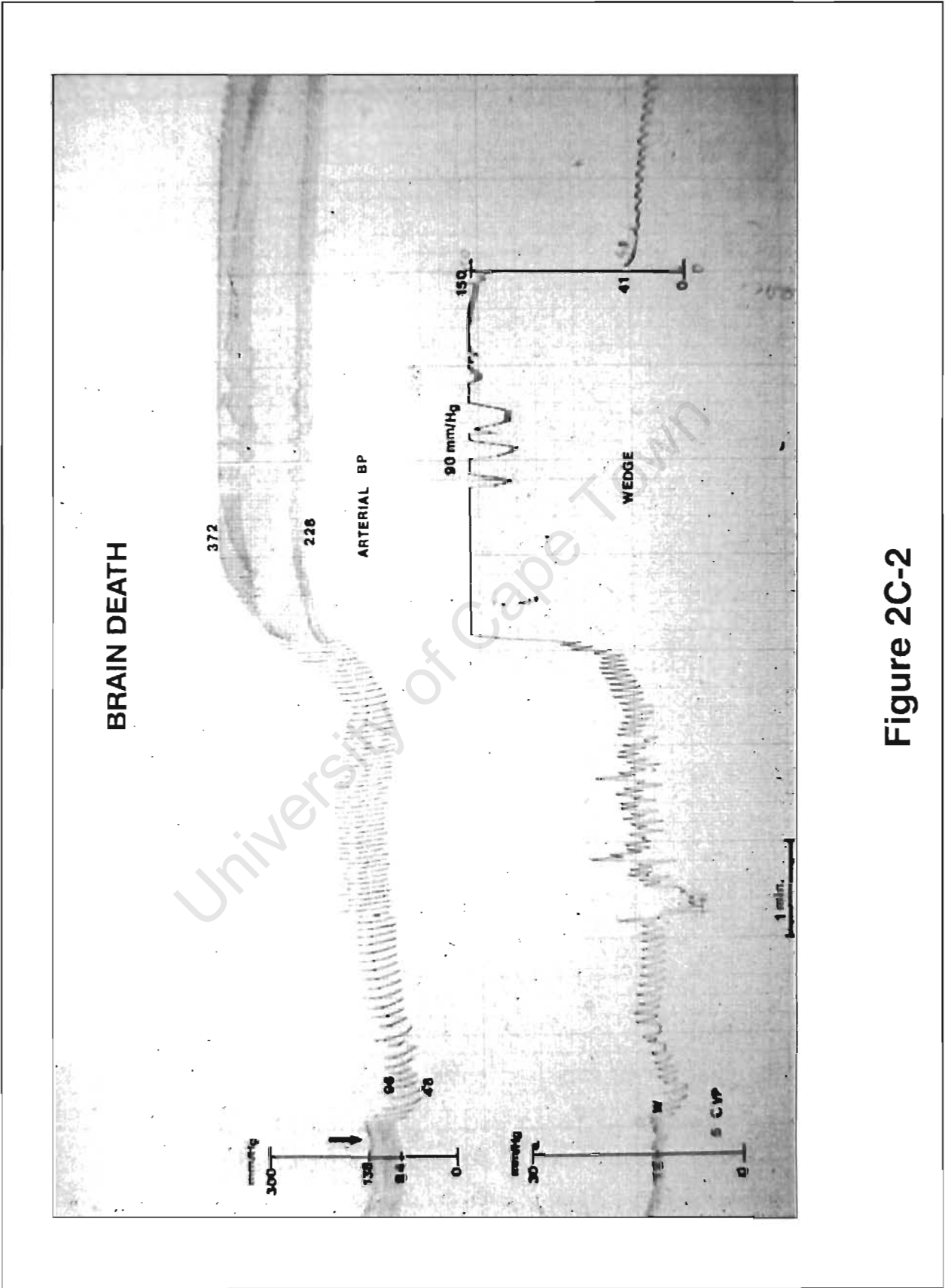


Figure 2C-2

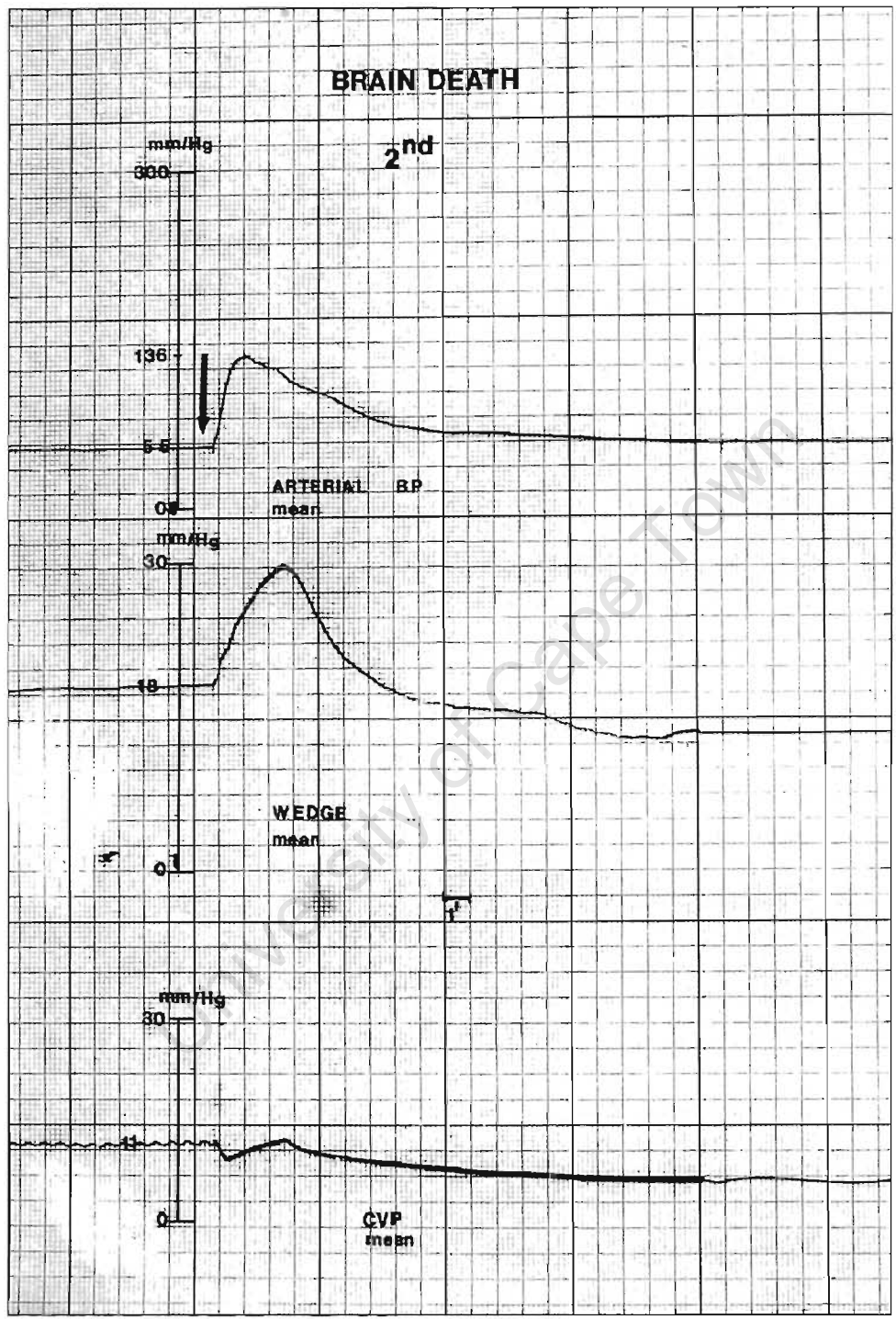


Figure 2C-3

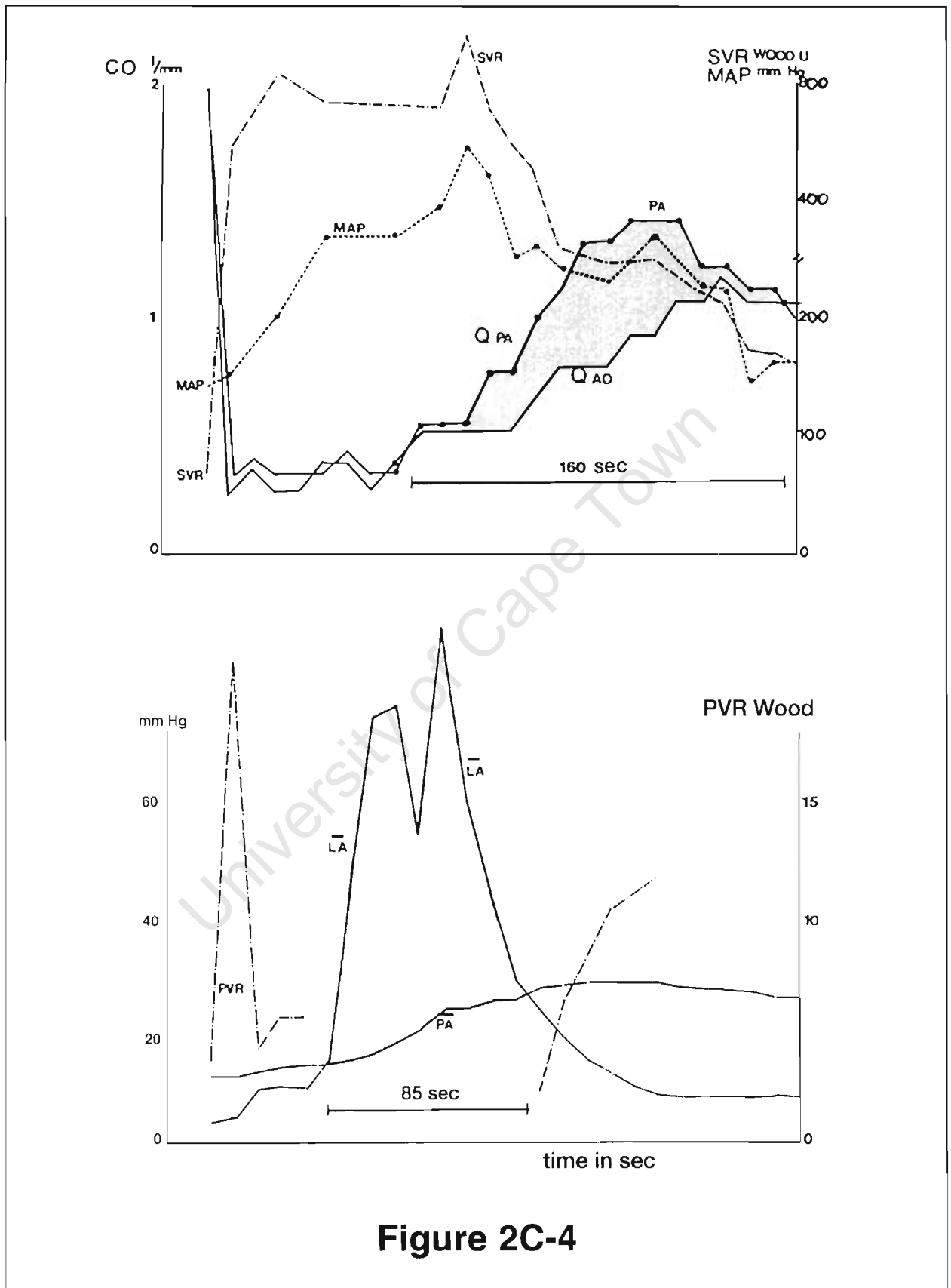


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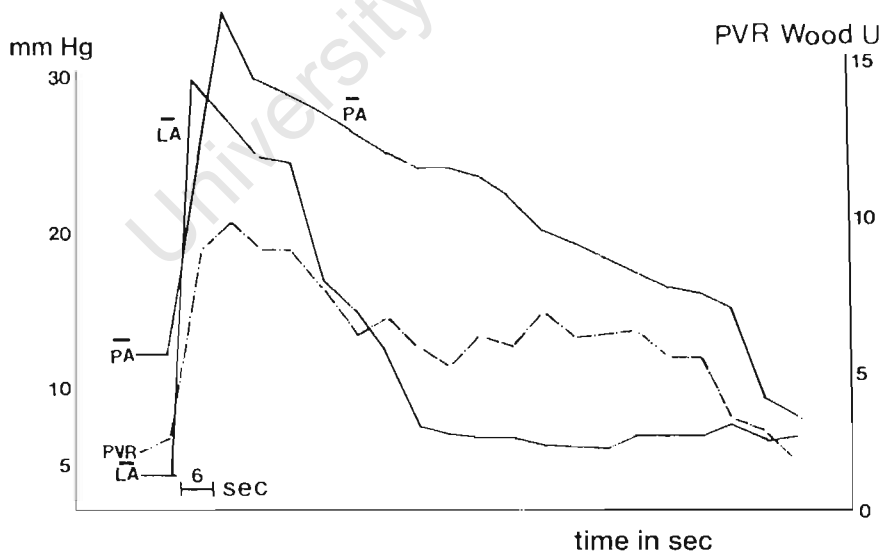
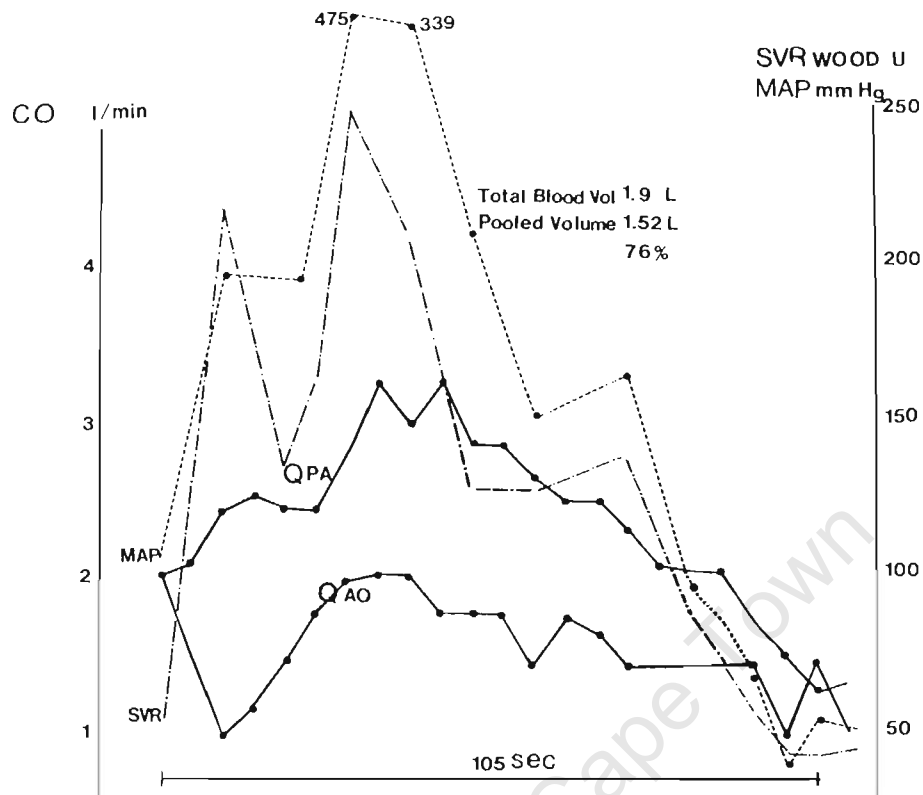


Figure 2C-5

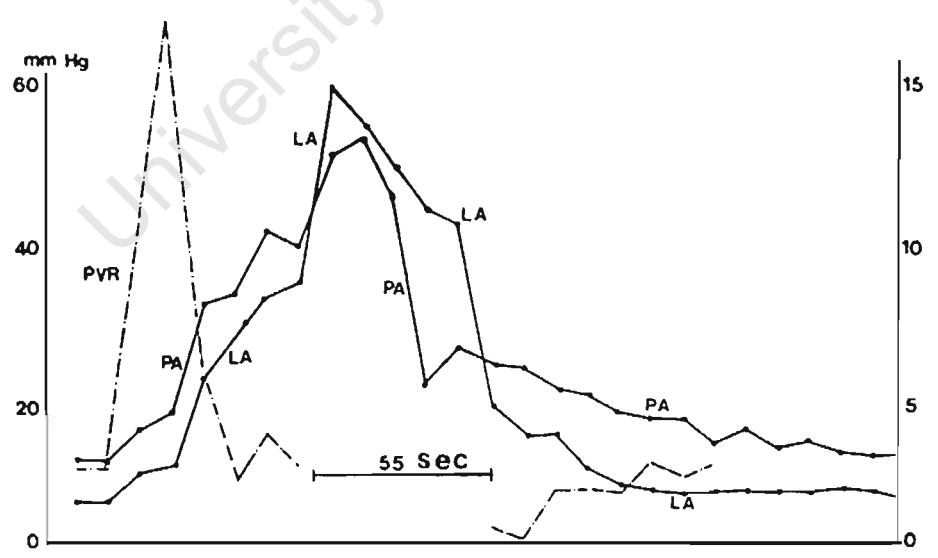
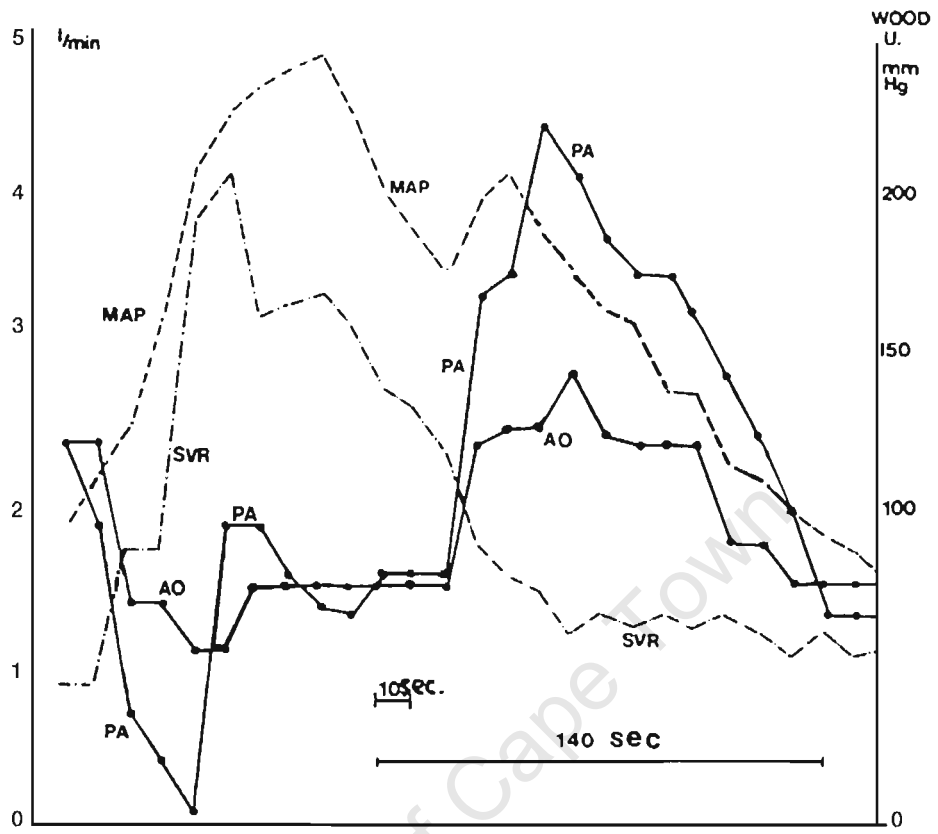


Figure 2C-6

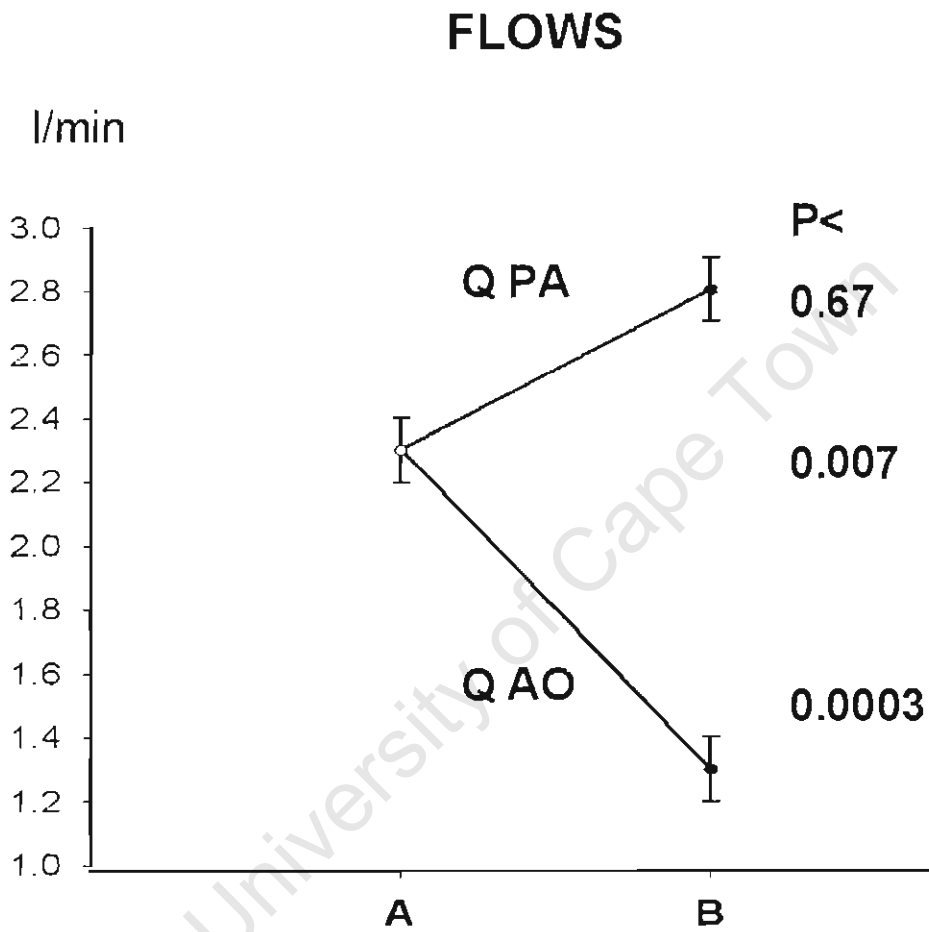
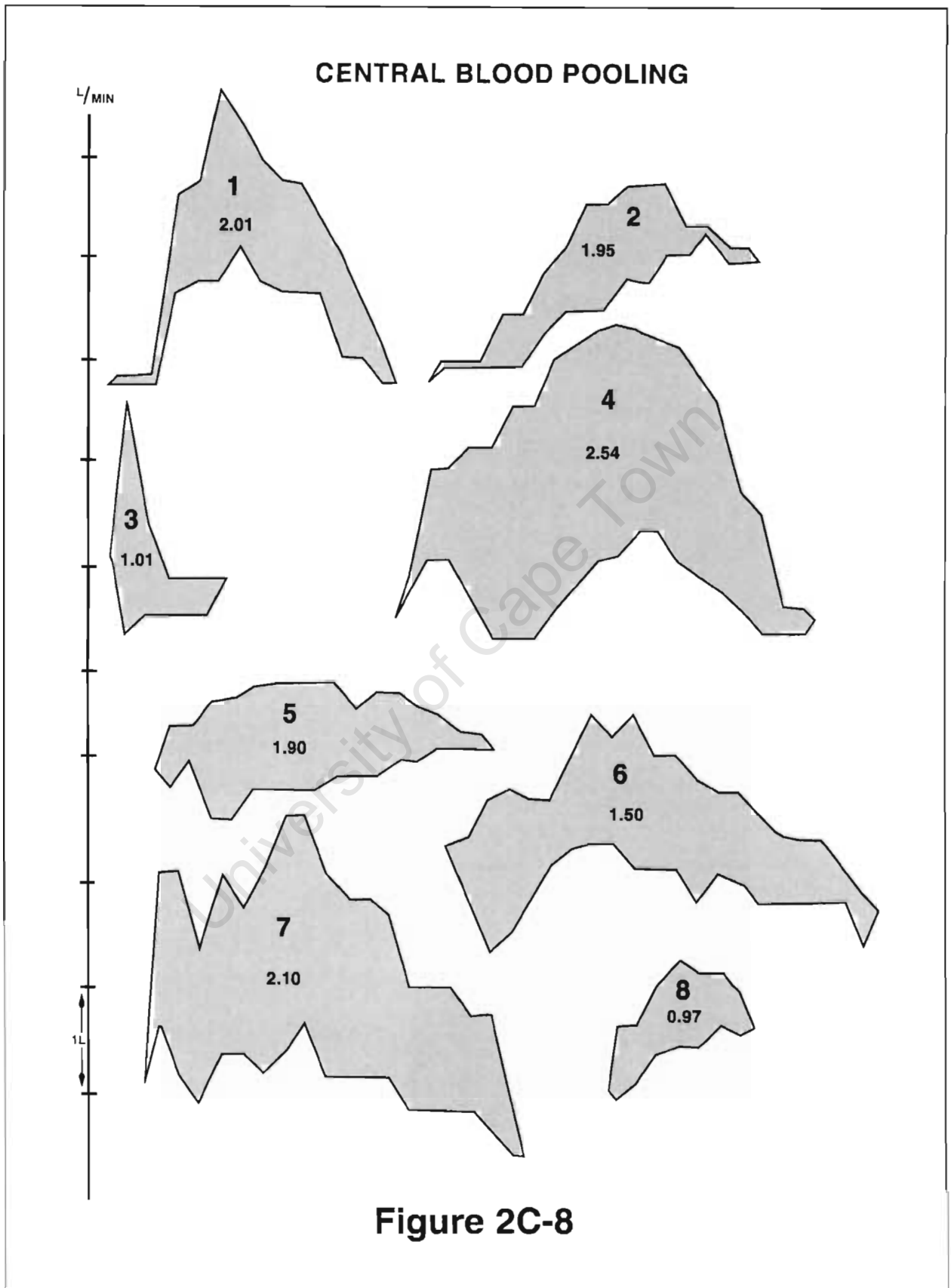


Figure 2C-7



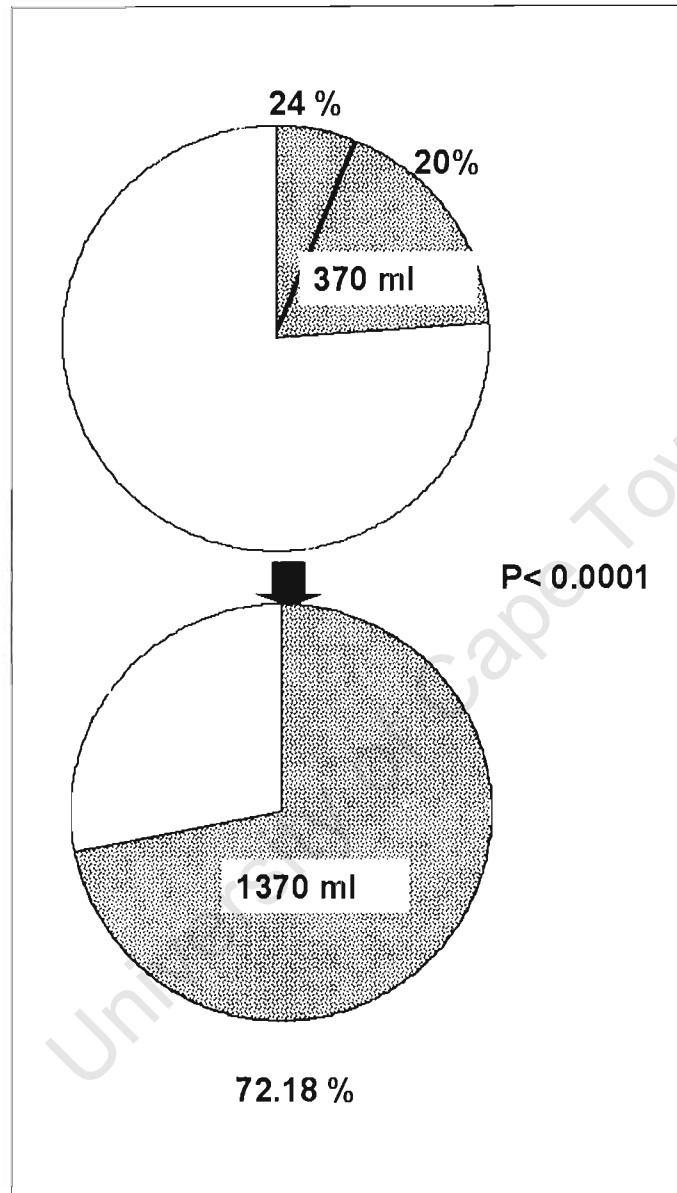


Figure 2C-9

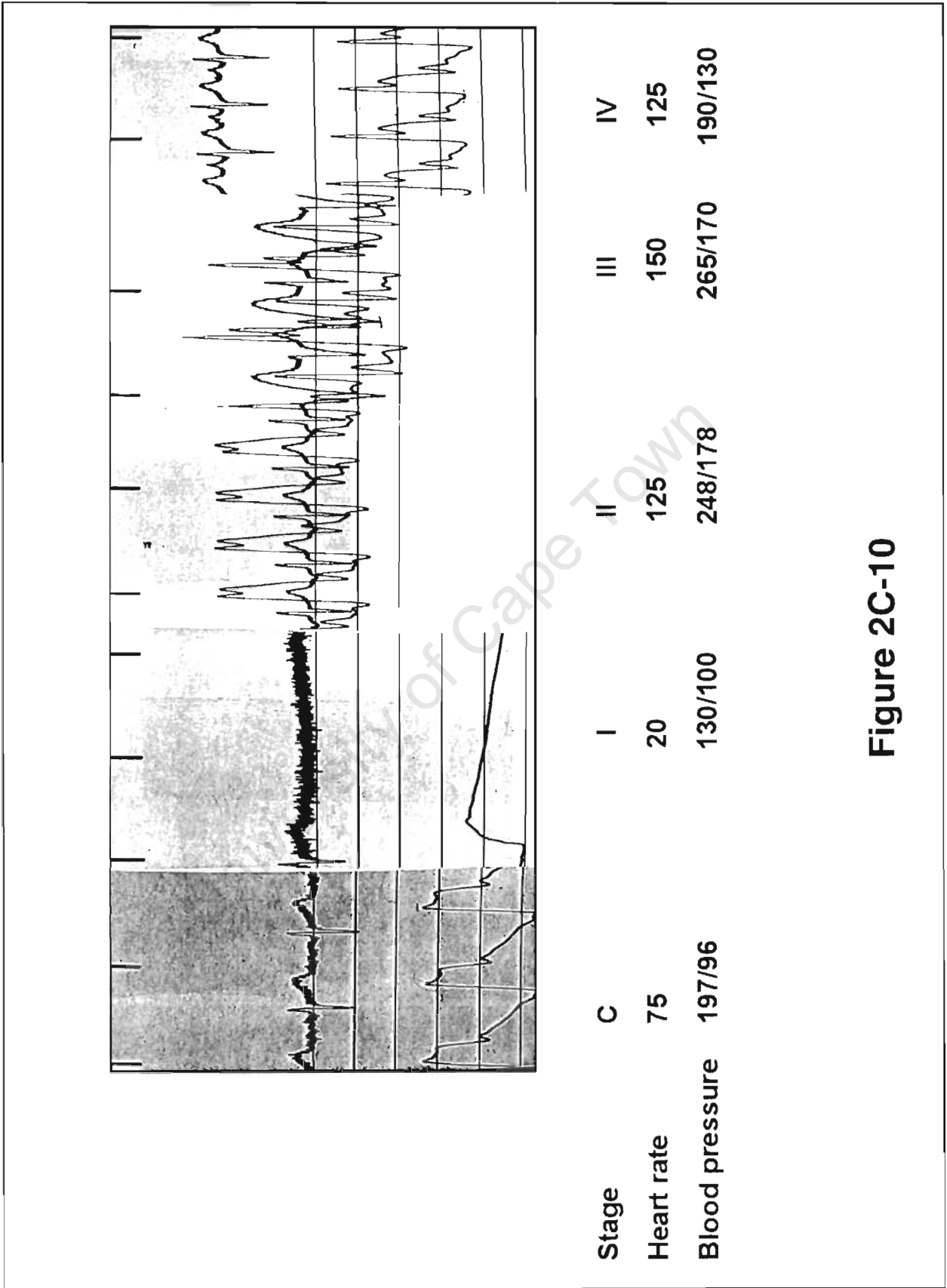


Figure 2C-10

Part D

ENDOCRINE CHANGES

Studies were carried out in two groups of animals. Five animals were included in each group. All underwent brain death as per method 1. Group A received no or minimal amounts of fluids. Group B received a continuous i.v. infusion of a crystalloid solution (Maintelyte) in order to replace fluid losses and maintain MAP of above 60 mmHg. Blood specimens were taken for control measurement and following induction of brain death at 5 and 15 min, and at 1,7,10,13,16 and 19 hours, or until the experiment was electively terminated, or the animal had a cardiac arrest. (For laboratory methods see Appendix I).

Catecholamines

Circulating catecholamines were measured in 5 animals (Fig. 2D-1). A significant increase occurred within 5 minutes of inflation of the Foley catheter balloon. Adrenaline concentration rose elevenfold over baseline levels ($p < 0.001$), noradrenaline threefold ($p < 0.01$), and dopamine twofold ($p < 0.05$). Ten minutes later, these levels returned to control values. By the third hour, catecholamine levels had decreased further, below baseline values. Only the noradrenaline drop, however, was significant ($p < 0.05$). During the later stages of the experiments, catecholamine levels were not measured.

T₄-T₃ - TSH (n=10)

The thyroid hormones (Fig. 2D-2 and 2D-3) T₃ and T₄ fell sharply to 50% of control values ($p < 0.04$) by the end of the first hour; no circulating T₃ or T₄ was detectable 16-19 hours after induction of brain death ($p < 0.0001$). TSH (Fig. 2D-4) showed no significant change from the control level throughout the experiment.

Cortisol (n=10)

Cortisol blood levels rose in all animals during the first 5 minutes, then declined progressively to 50% of baseline values at one hour ($p < 0.05$) (Fig. 2D-5). By 16 hours, a further decline had occurred ($p < 0.0001$).

Insulin (n=10)

Circulating insulin also fell in all baboons during the first 5 minutes (Fig. 2D-6). By one hour, the fall was significant ($p < 0.04$), and at 13 hours the level had reached 37% of the control level ($p < 0.003$).

Antidiuretic Hormone (n=10)

Antidiuretic hormone levels fell significantly in all animals, disappearing from the circulating plasma within 6 hours ($p < 0.0001$). Unless fluid replacement was given actively, urine output ceased between the second and third hour of observation, by which time MAP had fallen to approximately 45 mmHg. When no fluid replacement was given, mean total urine output in baboons was 500 ml. If fluid replacement were given, the baboon continued to pass large quantities of urine throughout the experimental period, the mean total output reaching 8 litres in 24 hours ($p < 0.0001$).

Glucagon (n=10)

Blood levels of glucagon showed no significant changes from baseline values.

Ionized Calcium (n=6)

The blood levels of ionized calcium showed no significant changes from baseline values.

LEGENDS

FIGURES

Figure 2D-1

Changes (mean of 5 animals) in circulating adrenaline (ad), noradrenaline (na), and dopamine (dop), during the first three hours following the induction of brain death. The statistical differences between control levels (C) and levels taken at 5 minutes, 15 minutes, and 3 hours after induction of brain death are shown.

Figure 2D-2

Changes in circulating thyroxine (T_4) following the induction of brain death. Group A received no i.v. fluid support; group B received a continuous i.v. infusion to maintain a CVP of 5-10 cmH₂O and MAP of 60 mmHg. The statistical differences between control levels (C) and levels taken at one hour and 19 hours after induction of brain death are shown.

Figure 2D-3

Changes in circulating triiodothyronine (T_3) following the induction of brain death, (Groups A and B, as in Fig. 2D-2). The statistical differences between control levels (C) and levels taken at one hour and 16 hours after induction of brain death are shown.

Figure 2D-4

Changes in circulating TSH (Groups A and B, as in Fig. 2D-2) following the induction of brain death. No statistical differences were found throughout the experiment.

Figure 2D-5

Changes in circulating cortisol (Groups A and B as in Fig. 2D-2) following the induction of brain death. The statistical differences between control levels (C) and levels taken one hour and 16 hours after induction of brain death are shown.

Figure 2D-6

Changes in circulating insulin (groups A and B, as in Fig. 2D-2) following induction of brain death. The statistical differences between control levels (C) and levels taken one hour and 13 hours after induction of brain death are shown.

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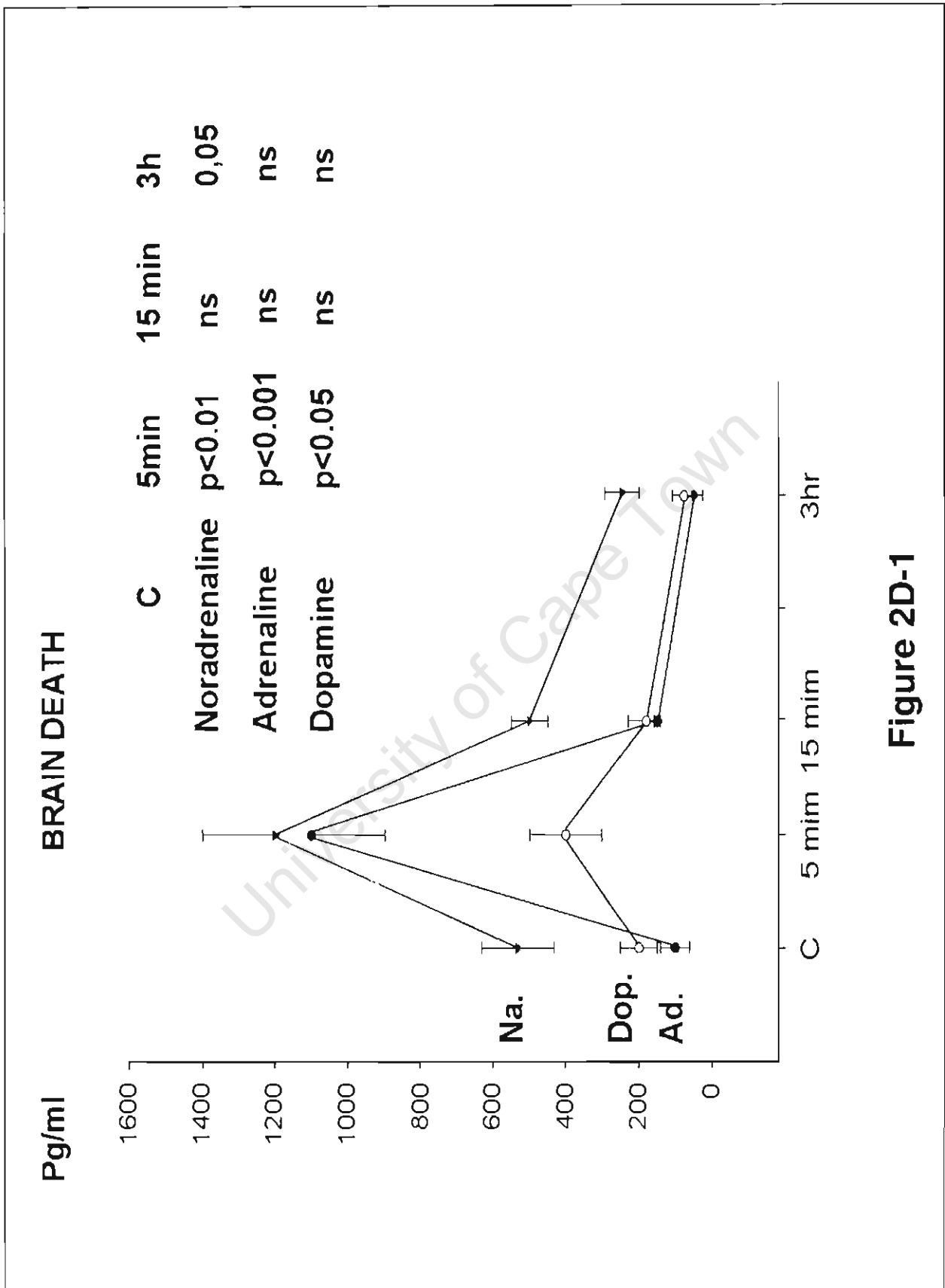


Figure 2D-1

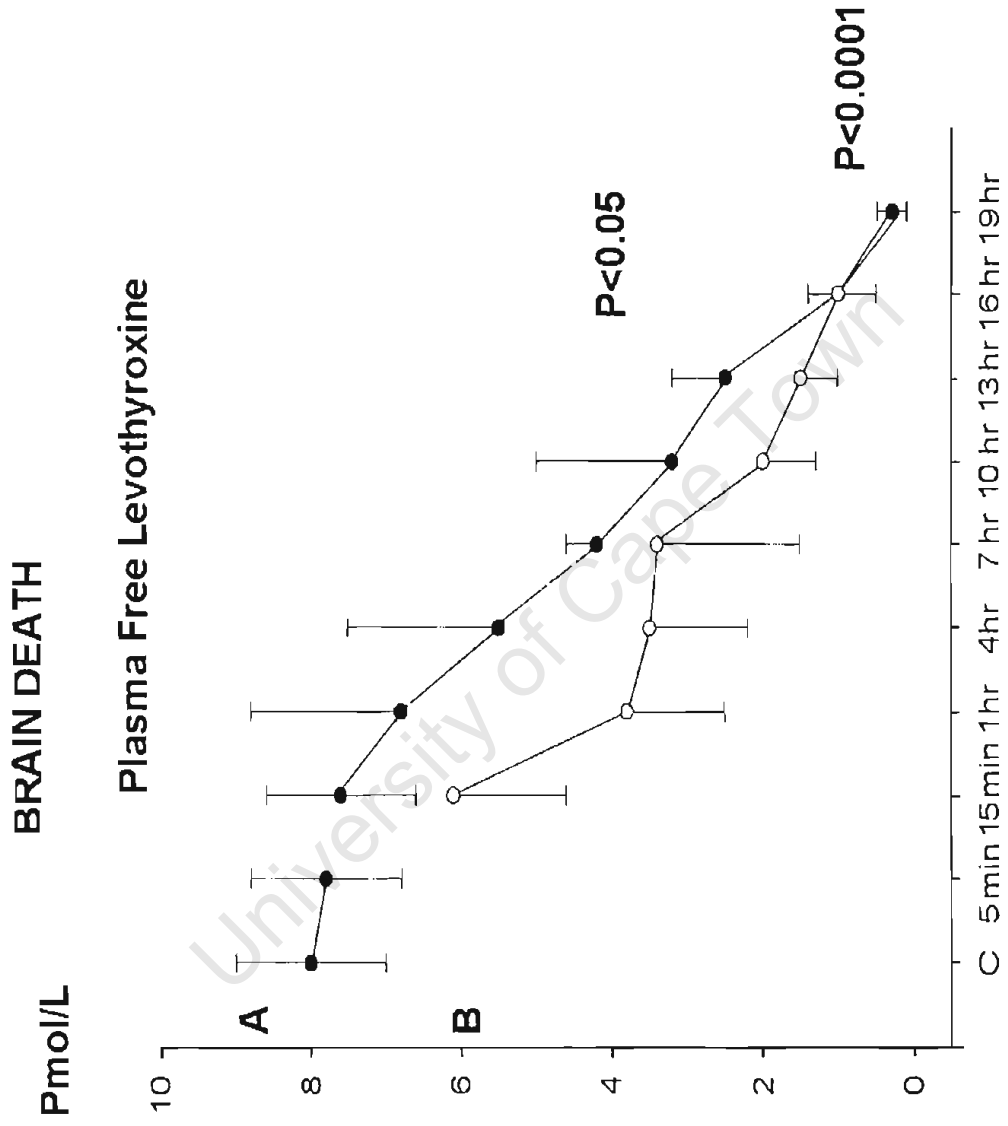


Figure 2D-2

BRAIN DEATH

Plasma Free Triiodothyronine

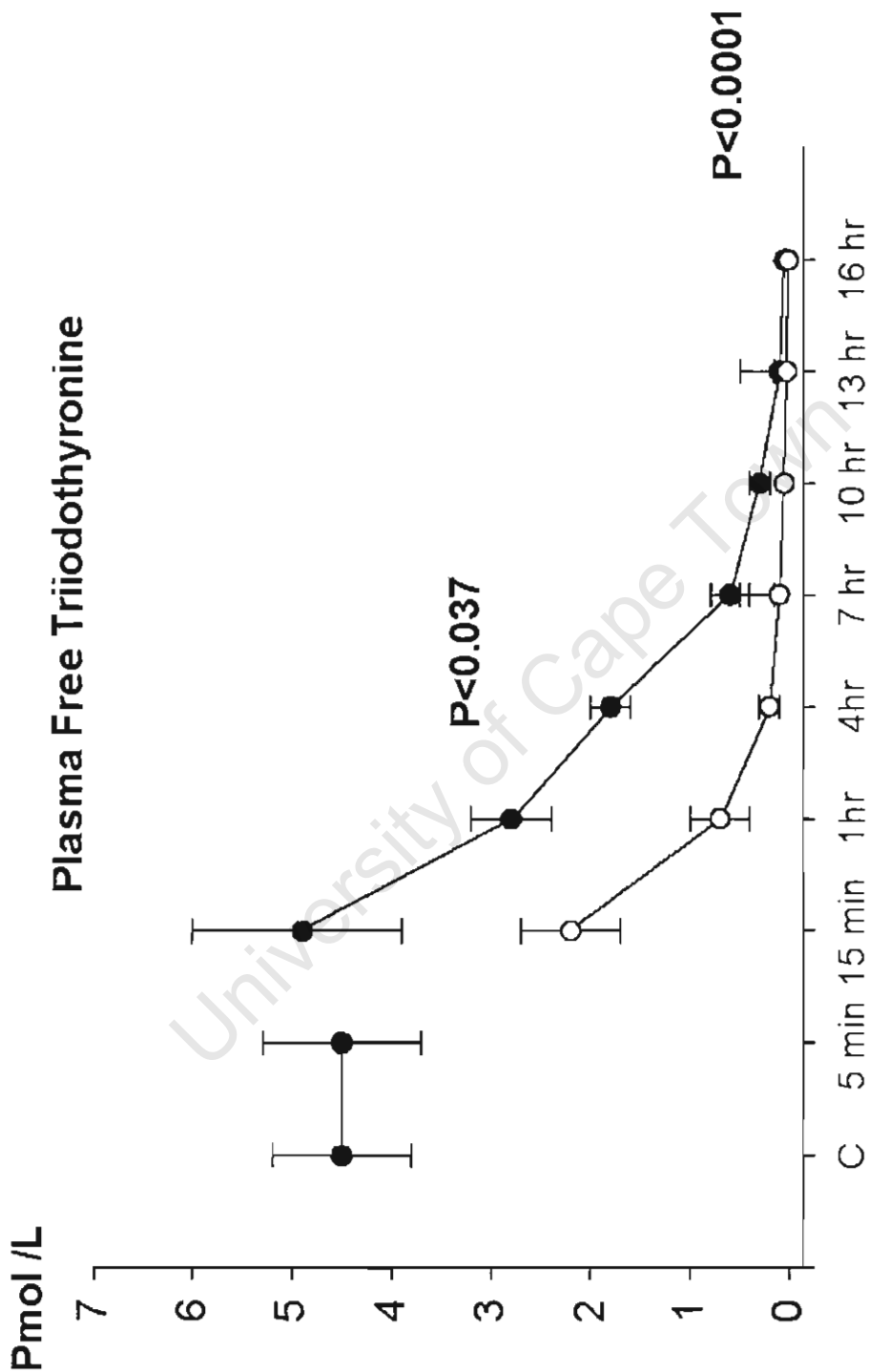


Figure 2D-3

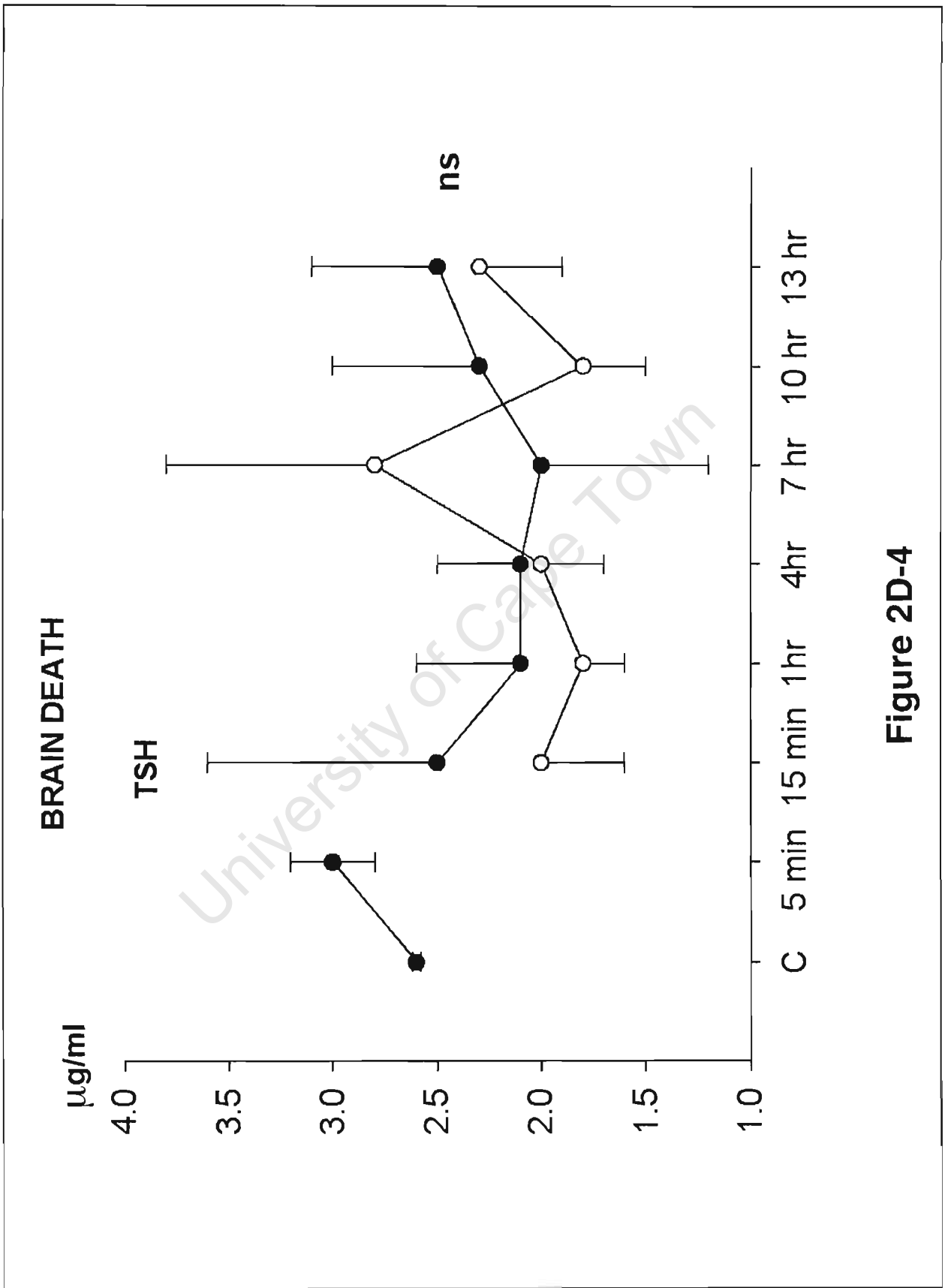


Figure 2D-4

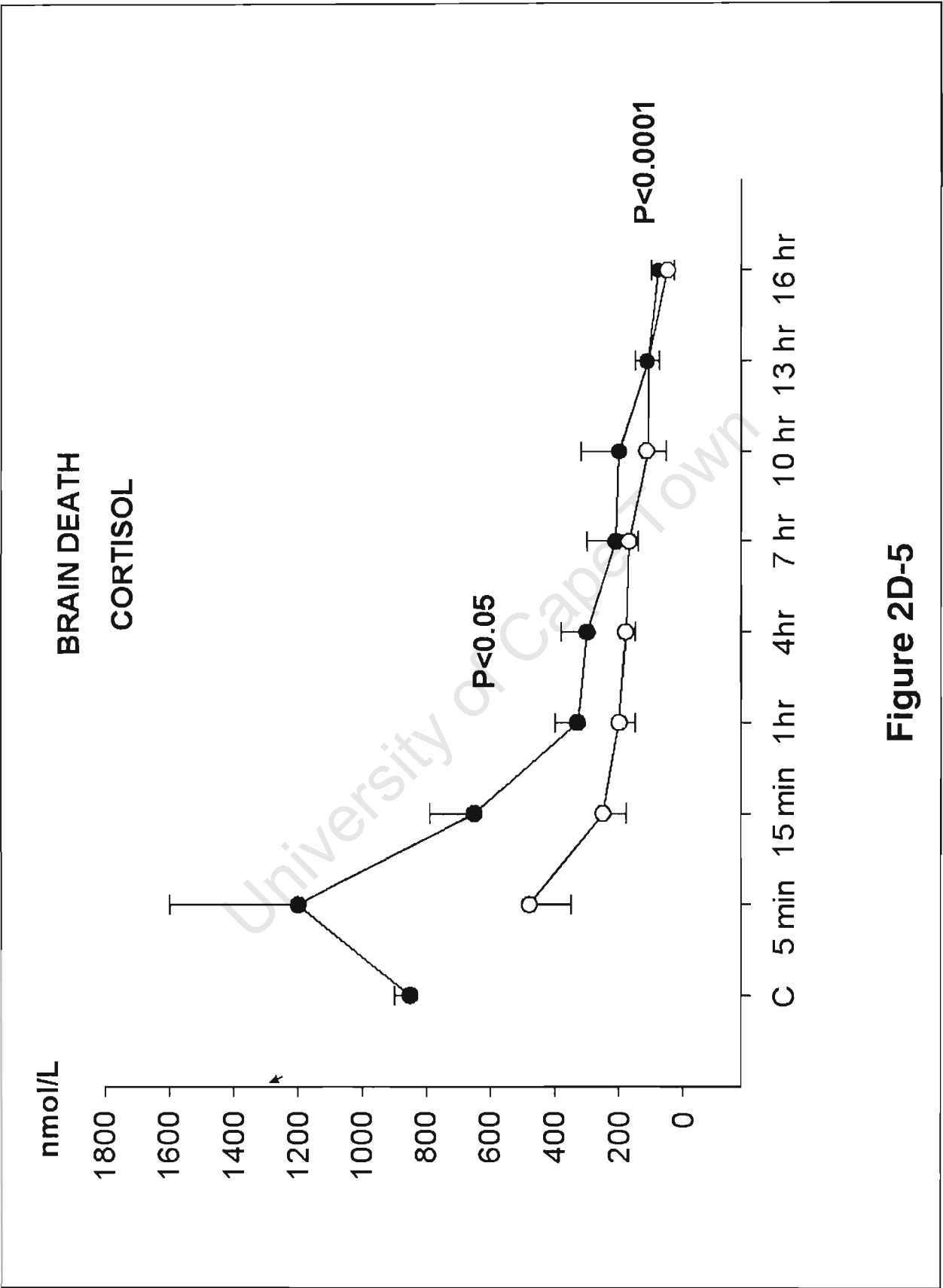


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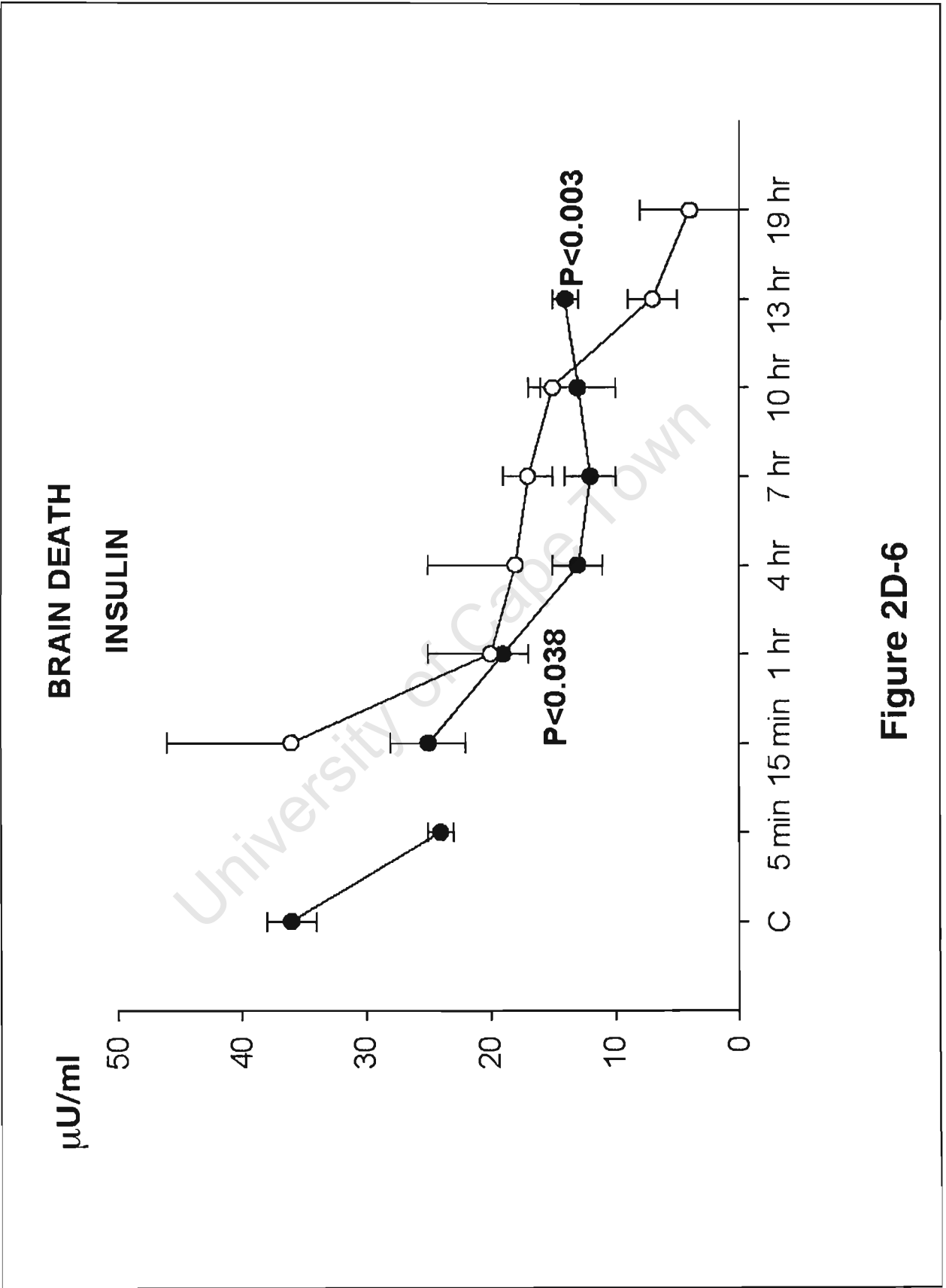


Figure 2D-6

Part E

HISTOPATHOLOGICAL CHANGES (LIGHT MICROSCOPY)

PULMONARY

Of 11 animals studied, 4 (37%) showed pulmonary oedema with an exudate rich in proteins (Figs. 2E-1 to 2E-4). There was also evidence of capillary endothelial damage and, in one animal, diffuse haemorrhage, both of the alveolar walls and into the alveolar space (Fig. 2E-2). The remaining animals showed normal lungs on light microscopy.

CARDIAC

Myocytes

In 15 of 17 animals (88%) histopathological changes could be seen in the left and right ventricular walls, and interventricular septum, and in the atria. These consisted of contraction band necrosis (in 15), focal coagulative necrosis (in 5), and myocytolysis, with oedema formation and various degrees of interstitial mononuclear cell infiltration (Table 2E-1) (Figs. 2E-5 to 2E-10).

Conduction Tissue

The study was performed in 17 baboons. The tissue blocks for examining the atrioventricular node and bundle of His were fashioned according to the method of Hudson. In 7 animals (41%) the hearts showed features of injury to the conduction tissue. Three of the 17 animals showed an abnormal infiltrate of mononuclear cells in the region of the A-V node. Six hearts

demonstrated focal areas of contraction banding, mononuclear cell infiltration and fibrinoid necrosis of the bundle of His. In 2 hearts, both the A-V node and the bundle of His were compromised (Figs. 2E-11, 2E-12 and 2E-18).

Coronary Arteries

Sections of major coronary arteries (right coronary artery, left anterior descending, and circumflex) were performed in the proximal mid and distal third portions.

Of the 17 animals, 12 (70%) presented with contraction banding of the smooth muscle of one or more major coronary artery walls characterized by focal eosinophilic densities with intervening pallor and some interstitial oedema (Figs. 2E-13 and 2E-14). In one further heart the left anterior coronary artery showed focal medial hyalinization and in one heart the circumflex coronary artery demonstrated a mild mononuclear cell infiltrate. These histopathological changes were present in only one coronary artery in 8 animals, in 2 arteries in 3 animals, and all 3 vessels were affected in one animal.

In five animals in which the histopathological sections showed contraction bands of the coronary arteries, specimens were stained with the von Kossa stain technique, demonstrating stippled calcification within the damaged myocytes of the coronary arteries, probably within the mitochondria. (Figs. 2E-15 and 2E-16).

PANCREAS

In one animal haemorrhagic infiltrate was present throughout the organ. (Fig. 2E-17).

LEGENDS

FIGURES

Figure 2E-1

Histopathological section of the lungs showing severe pulmonary oedema- intra-alveolar fluid rich in protein, filling all the alveolar spaces. The haemodynamics of this animal are shown in Fig. 2C-4 (haematoxylin-eosin x 150).

Figure 2E-2

Histopathological section of the lung showing intra-alveolar haemorrhage, thickening of the septum, and erythrocytes seen outside the capillary lumen. The haemodynamics are shown in Fig. 2C-5 (haematoxylin-eosin x 600).

Figure 2E-3

Histopathological section of the lungs belonging to the animal whose haemodynamic changes are shown in Fig. 2C-6, showing air bubbles within oedema fluid in the alveoli (haematoxylin-eosin x 600).

Figure 2E-4

Histopathological section of the lungs. A: Intra-alveolar exudate with air bubbles (haematoxylin-eosin x 150) and B: High power magnification of view A in a different area showing protein-rich alveolar exudate and extravasation of erythrocytes in the alveolar septa (haematoxylin-eosin x 600).

Figure 2E-5

Histopathological view of the wall of the left ventricle showing abundant contraction bands within the myocytes, some interstitial haemorrhage and a mild degree of interstitial oedema. (haematoxylin-eosin x 150).

Figure 2E-6

Histopathological view of the wall of the left ventricle showing extensive contraction band necrosis within the myocytes, associated with moderate interstitial oedema and diffuse mononuclear cell infiltration (haematoxylin-eosin x 150).

Figure 2E-7

Histopathological view of the myocardium showing the presence of two necrotic myocytes in the centre of the picture with abundant mononuclear cells. This view resembles the appearance of severe acute rejection (haematoxylin-eosin x 600).

Figure 2E-8

Histopathological view of the myocardium showing hyper-eosinophilic staining of the myocytes and loss of the normal striation (left hand side of the picture) and central myocytolysis; the whole area is invaded by mononuclear cells. The appearances are impossible to differentiate from those of severe acute rejection following heart transplantation (haematoxylin-eosin x 600).

Figure 2E-9

Histopathological view of the wall of the left ventricle showing myocytolysis with moderate interstitial oedema and mononuclear cell infiltration (haematoxylin-eosin x 150).

Figure 2E-10

Histopathological section of the subendocardial area showing extensive myocytolysis, interstitial oedema and hyper-eosinophilic staining of the myocytes, indicating severe injury. The endocardium lies on the left hand side of the picture (haematoxylin-eosin x 150).

Figure 2E-11

Histopathological section of the bundle of His showing a necrotic myocyte (arrow) within the sarcolemma. The area is surrounded by moderate interstitial oedema (haematoxylin-eosin x 600).

Figure 2E-12

Histopathological view of the A-V node showing moderate mononuclear cell infiltration, interstitial oedema and probably myocyte necrosis on the left upper quadrant of the picture (arrow) (haematoxylin-eosin x 600).

Figure 2E-13

Histopathological section of the left anterior descending coronary artery showing contraction bands in the outer third of the media at the junction with the adventitia (haematoxylin-eosin x 50).

Figure 2E-14

Histopathological view of the outer third of the media of the left anterior descending coronary artery showing contraction bands of the smooth muscle (arrow). In addition, there are pale-staining vacuolization areas within the myocytes, providing further evidence of cellular damage (haematoxylin-eosin x 600).

Figure 2E-15

Histopathological view of the previous coronary artery showing stippled calcification within the damaged myocytes (arrows) of the outer third of the media (von Kossa stain x 100).

Figure 2E-16

Histopathological view of Figure E2-15 at higher magnification, showing intracellular calcium deposits. (von Kossa stain x 600).

Figure 2E-17

Histopathological view of the pancreas showing extensive interstitial haemorrhage and interstitial oedema throughout the pancreatic parenchyma (haematoxylin-eosin x 150). This is the only animal of a series of 17 in which the pancreas had a macroscopic haemorrhagic appearance.

Figure 2E-18

Histopathological view of the bundle of His showing widespread contraction band necrosis and mild oedema (haematoxylin-eosin x 600).

TABLE 2E-1
MYOCARDIAL HISTOPATHOLOGICAL FEATURES FOLLOWING
INDUCTION OF BRAIN DEATH IN THE NORMAL BABOON (N=17)

Left Ventricle	MYOCYTE NECROSIS		MONONUCLEAR CELL INFILTRATE		OEDEMA
	Conduction Tissue	Coronaries			
N = 15 (88%)	N = 7 (41%)	N = 12 (70%)	N = 17 (100%)	N = 17 (100%)	

Compare with Table 3-1

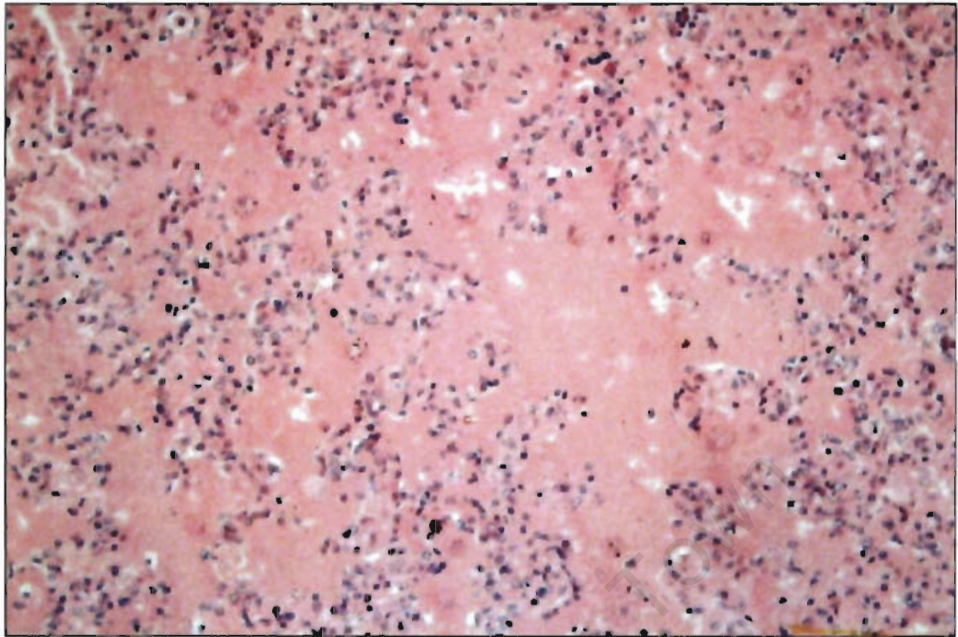


Figure 2E-1

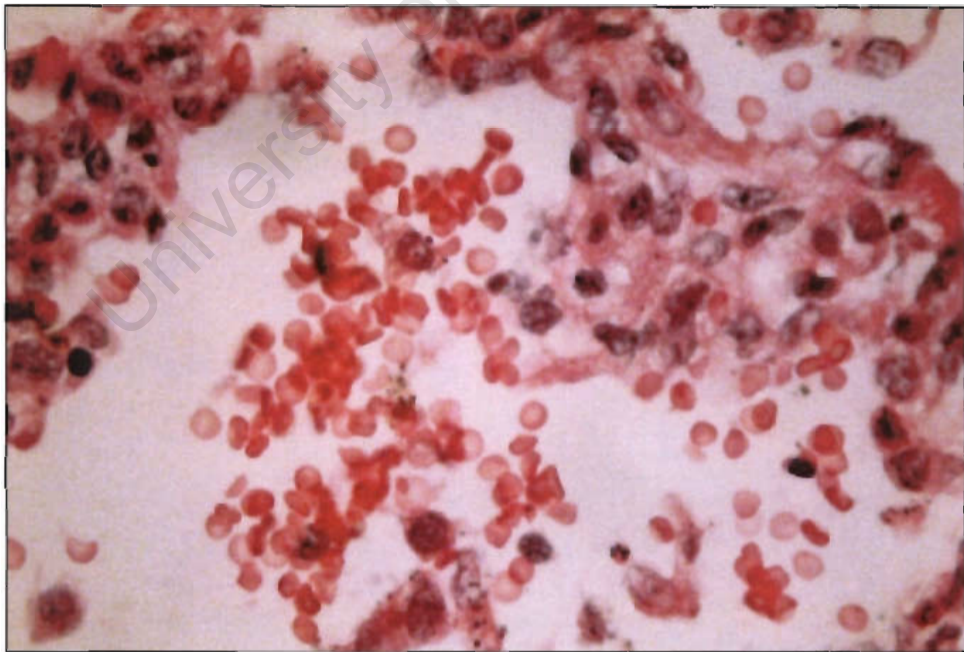


Figure 2E-2

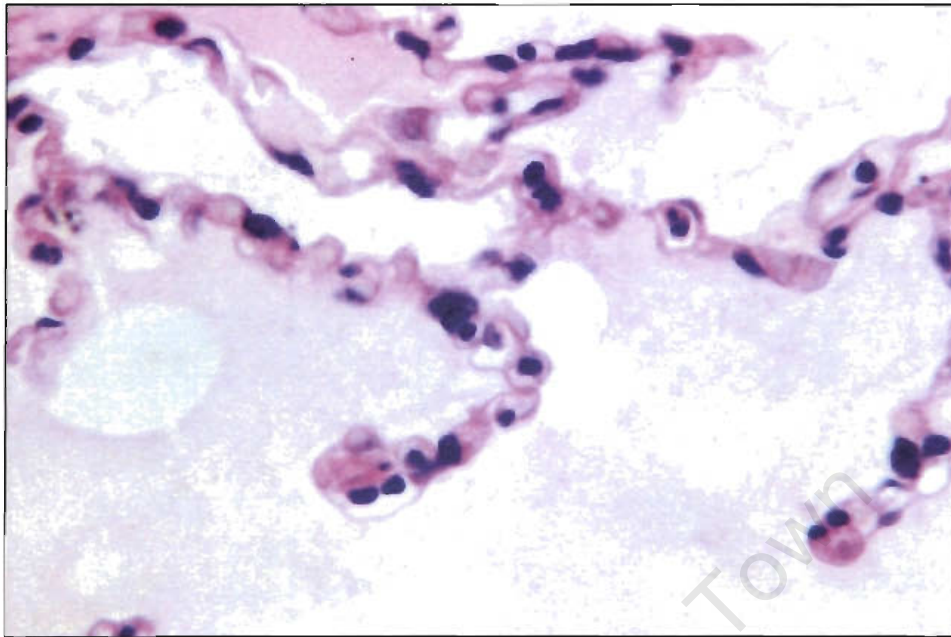


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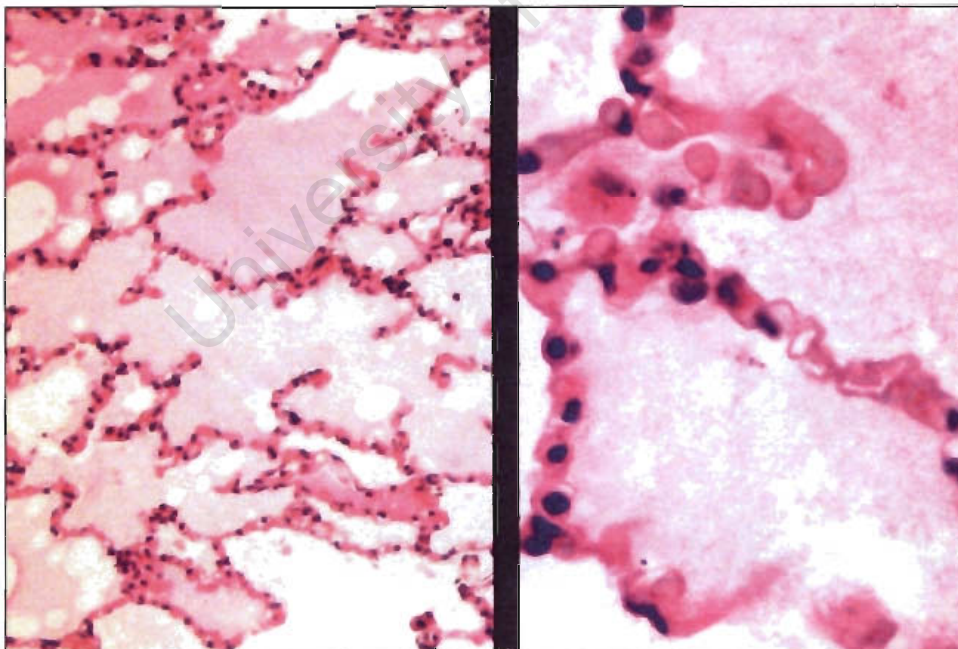


Figure 2E-4

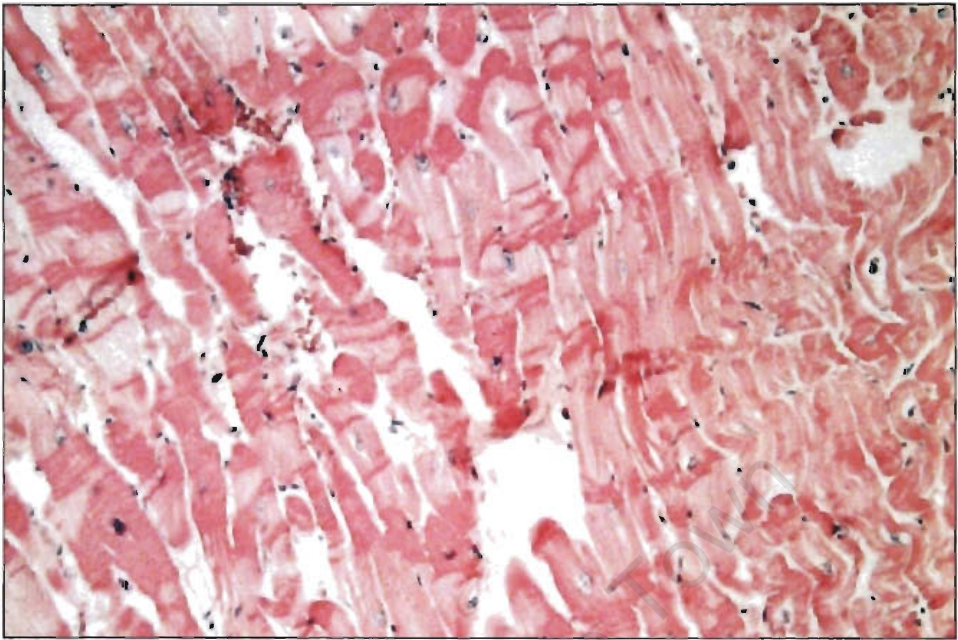


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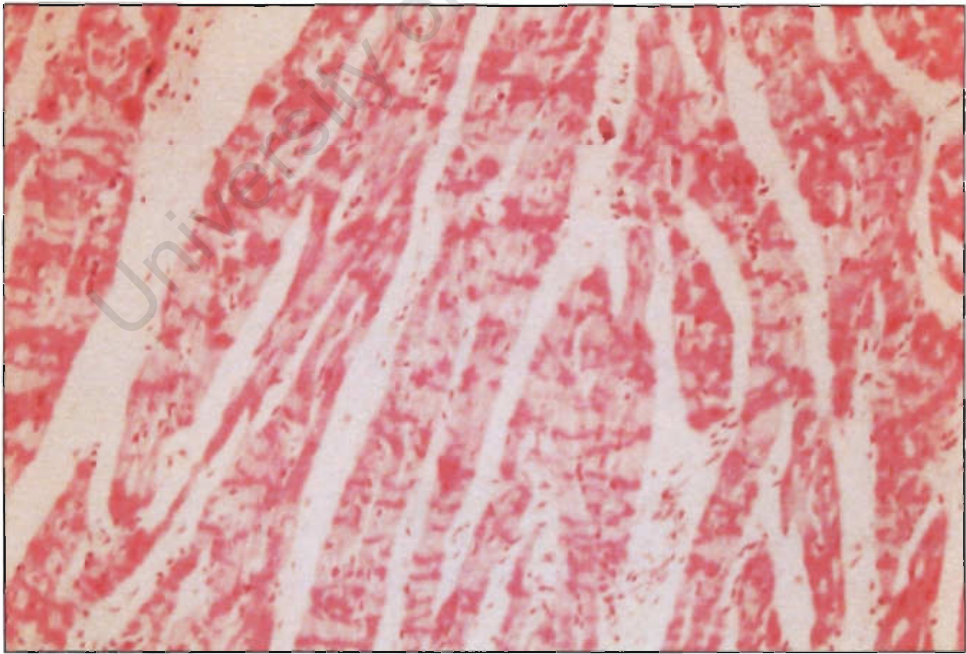


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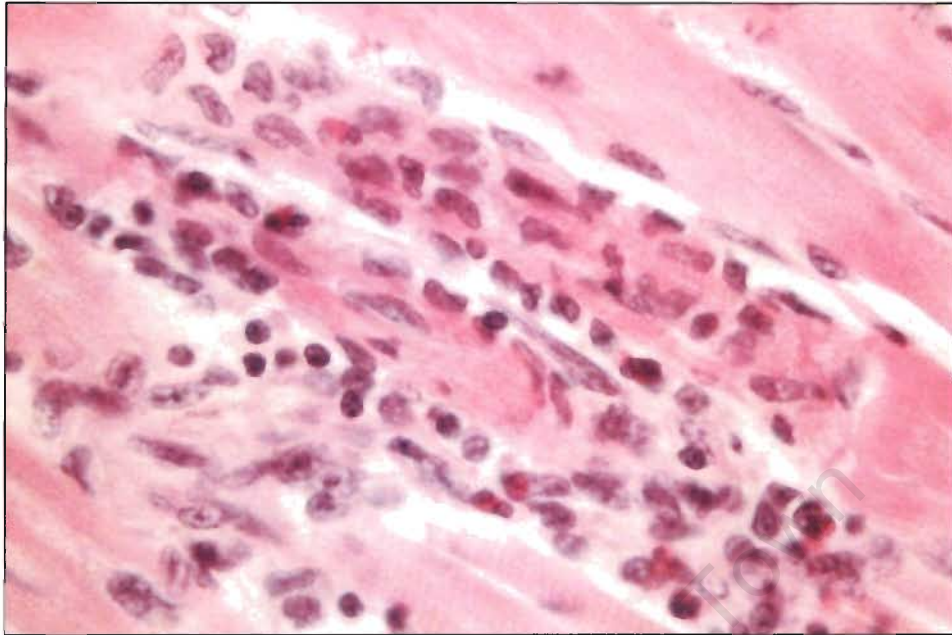


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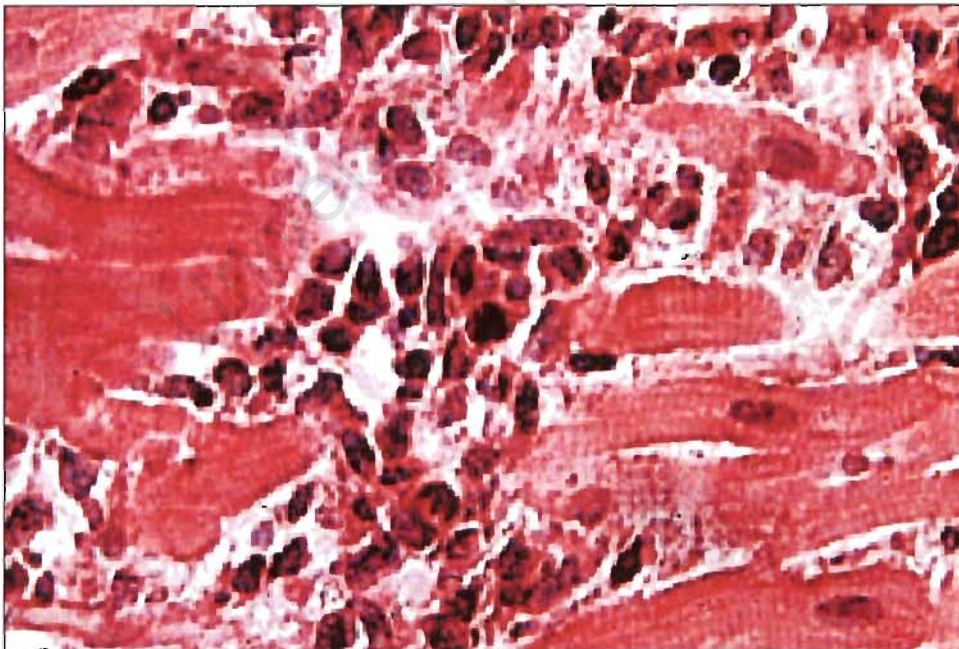


Figure 2E-8

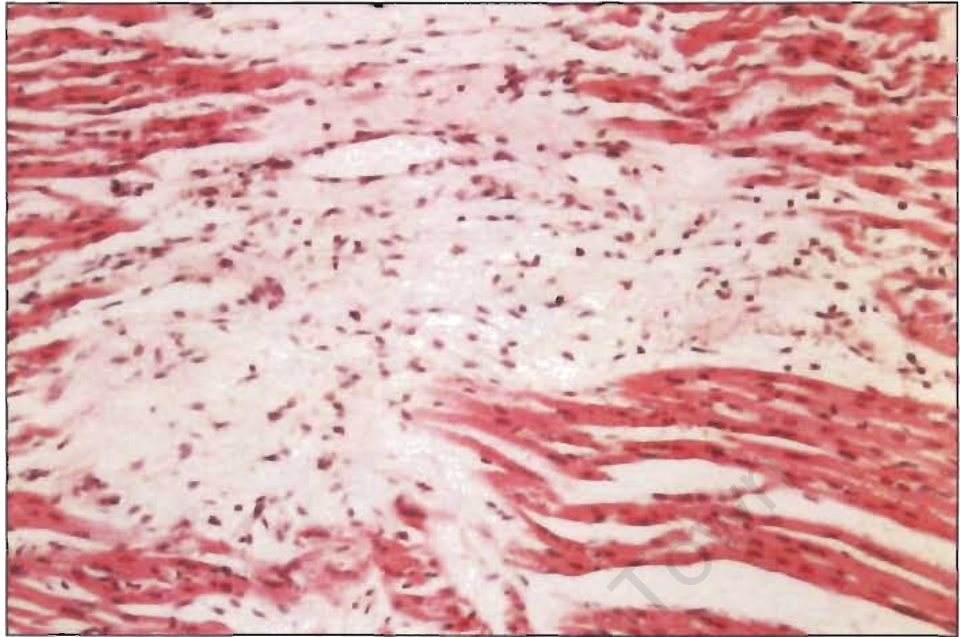


Figure 2E-9

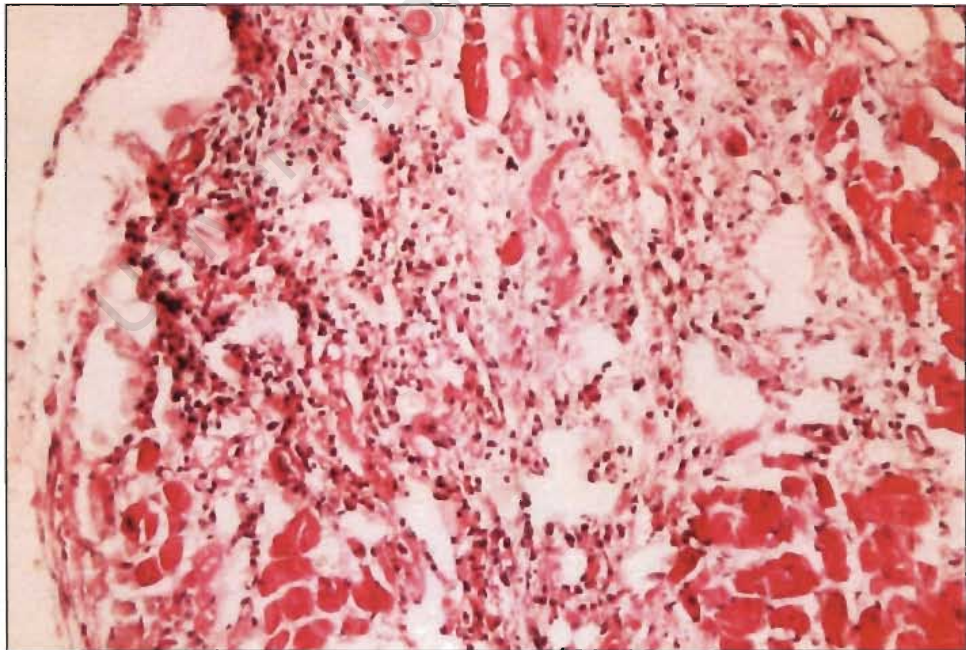


Figure 2E-10

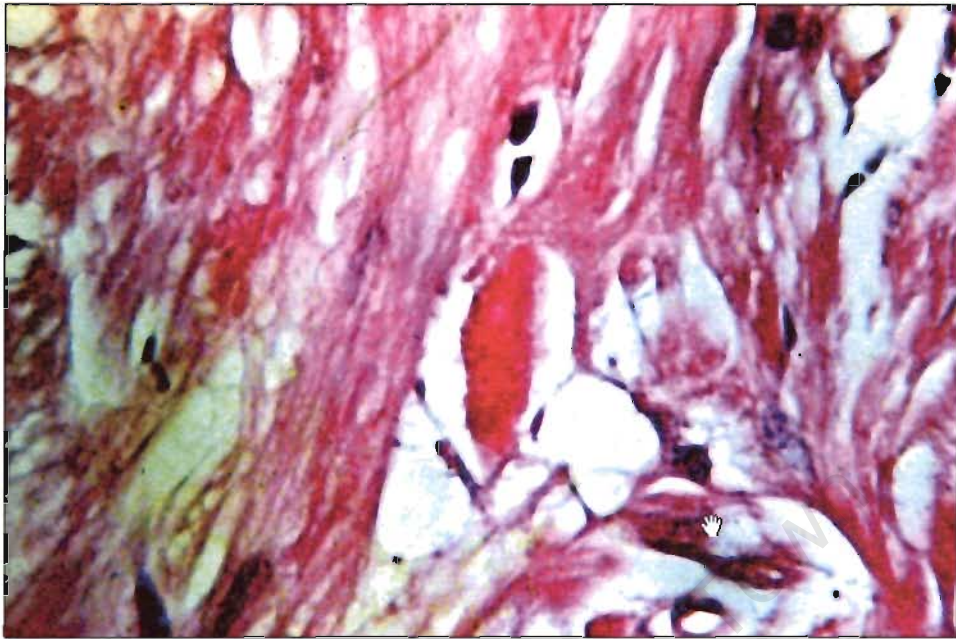


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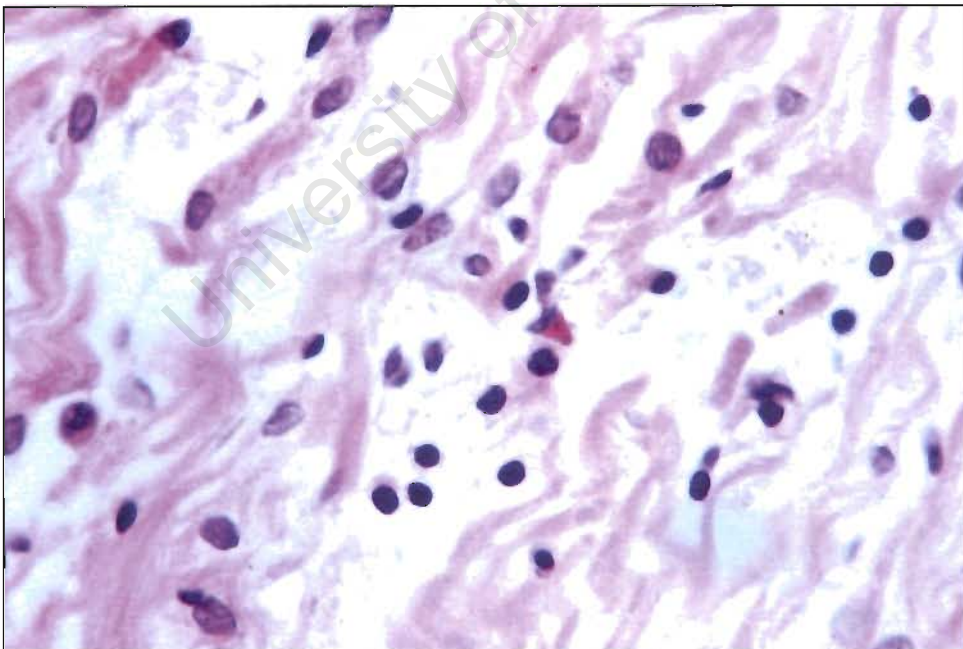


Figure 2E-12



Figure 2E-13

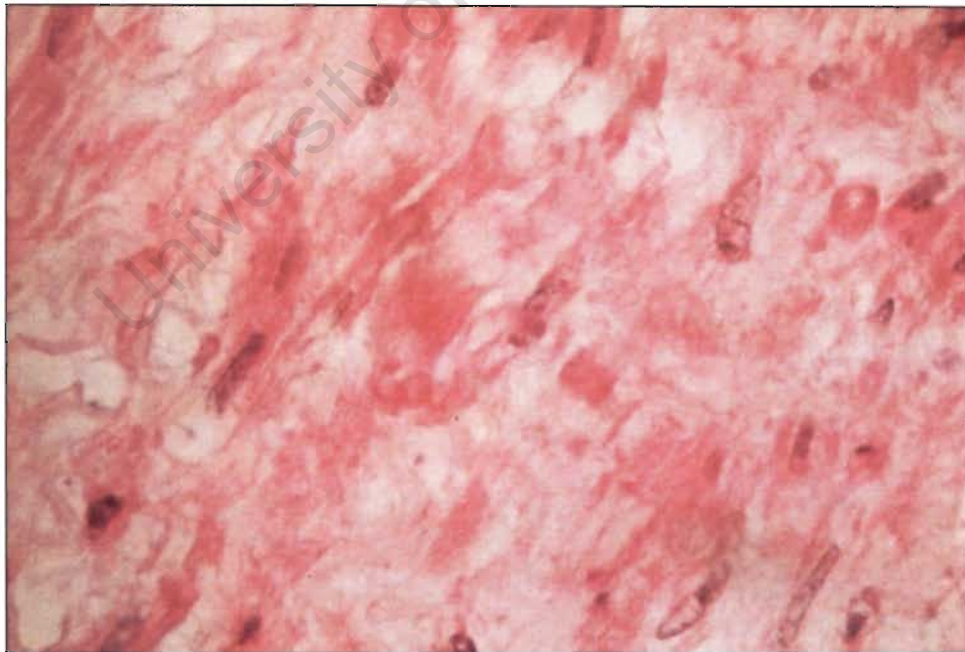


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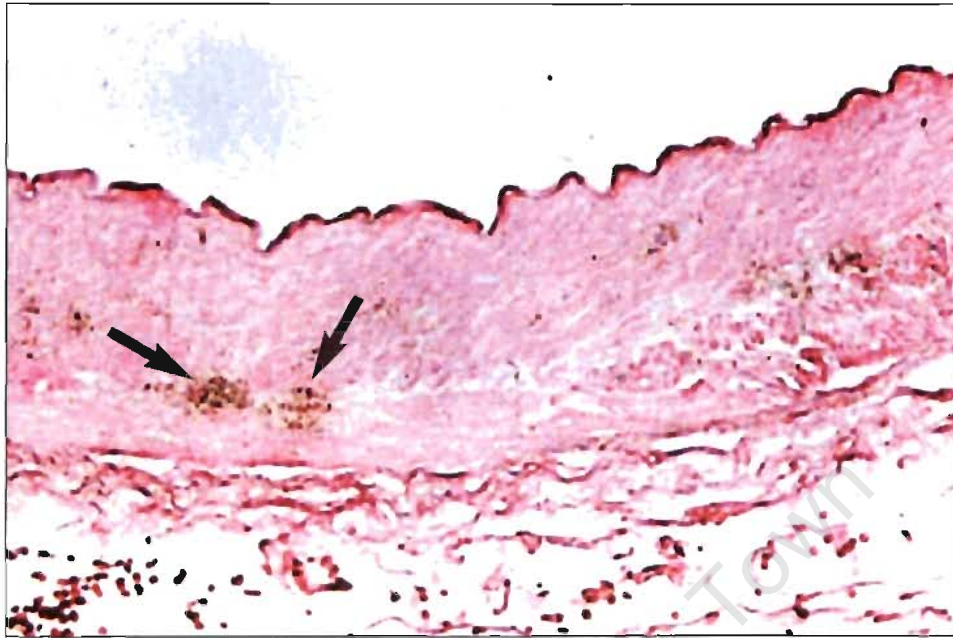


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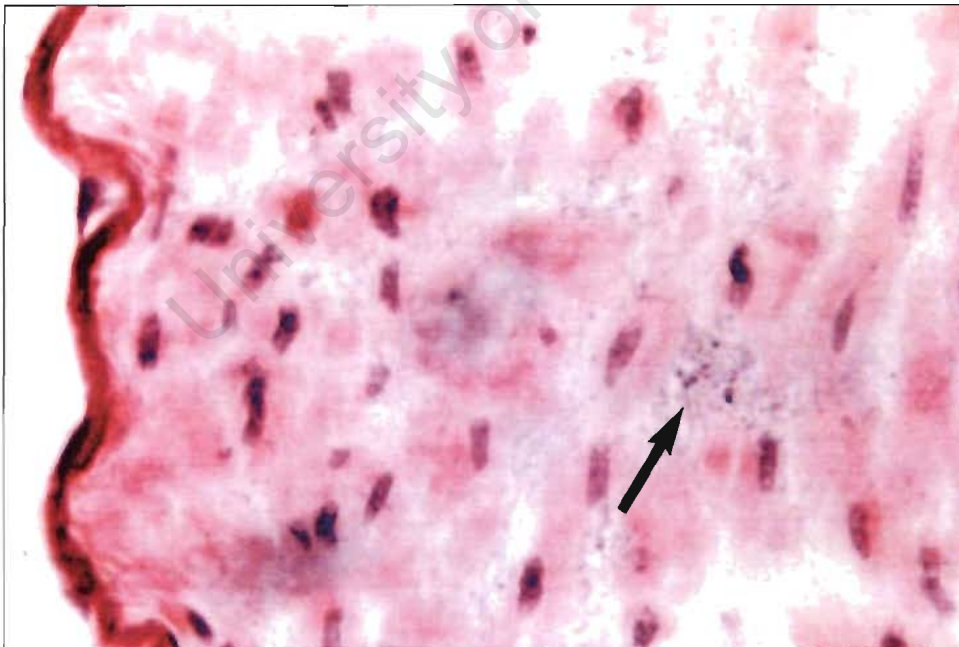


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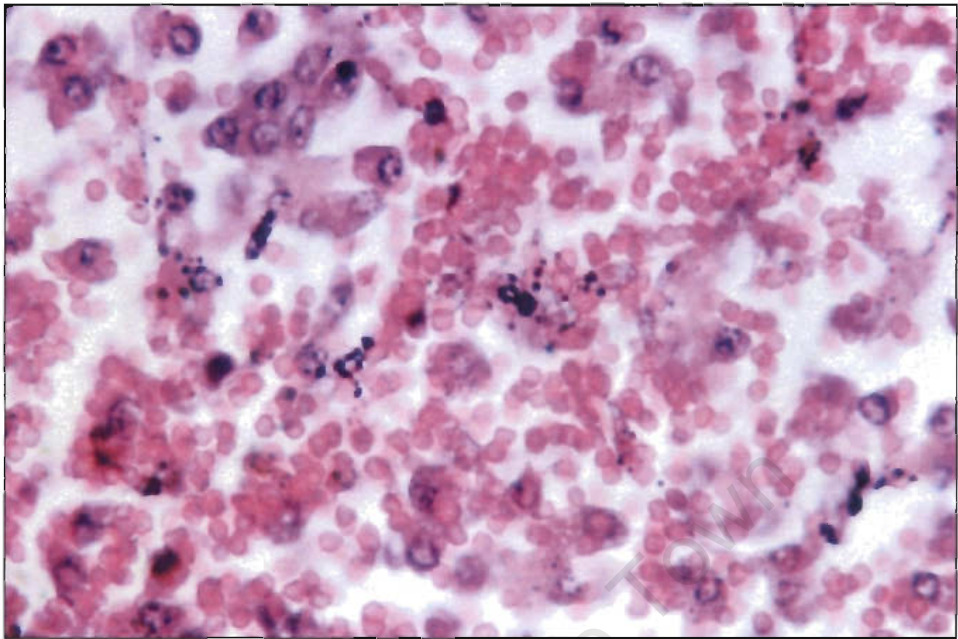


Figure 2E-17

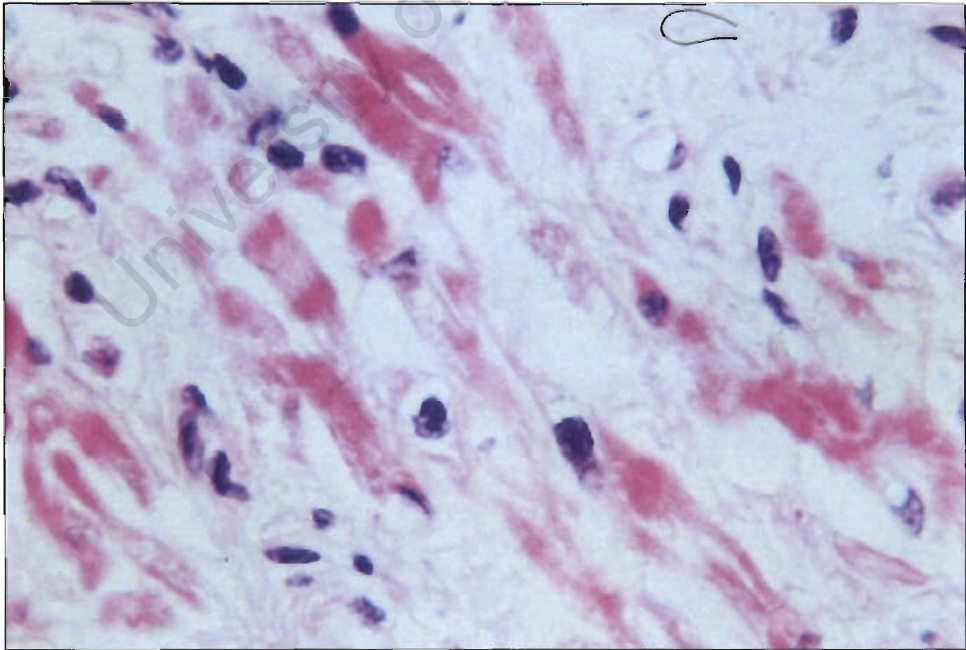


Figure 2E-18

DISCUSSION

Experimental brain death has been performed in chimpanzees by Ducker (1968), in the cat by Hoff (1970), in the rat by Nathan (1975), in cats by Garcia-Uria (1981), and in sheep by Simon (1984). The authors Abildskov (1970), Clifton (1981), and Friedman (1984), consistently showed that sympathetic activity is present in episodes of raised endocranial pressure or during brain anoxia.

The ECG findings in patients following brain death have been comprehensively reviewed by Abildskov (1970), Drory (1975), Jackuck et al. (1975), Yamour (1980), Clusin et al. (1983). ECG changes described in patients with raised endocranial pressure or following brain death, include the presence of prominent T waves, ST segment changes, notched T waves and shortening or prolongation of the QT intervals. The presence of the J wave has been attributed to progressive hypothermia. As the process of brain death advances, a progressive slowing of the heart rate has been observed, the ECG showing progressive slowing of the depolarization and repolarization processes, broadening of the QRS complexes, and finally, as a terminal event, progressive bradycardia, ventricular ectopic activity and ventricular fibrillation Jorgensen (1973). Rajs & Jacobson (1976) showed a correlation between severe head trauma and subsequent cardiac lesions causing heart failure and death.

Though this experimental model provides a sudden and rapid increase in endocranial pressure which may not exactly reproduce the development of brain death in all patients who subsequently become potential organ donors, certain observations that were made are of relevance to the clinical situation, and are worthy of comment.

There is a large increase in circulating catecholamines in the baboon during the early few minutes after induction of brain death, which is associated with an increase in

myocardial activity together with the appearance on the ECG of ventricular arrhythmias. Structural damage occurs in the myocardium at this stage, though the more protracted period of tachycardia, probably related to continuing endogenous catecholamines release within the myocardium, is almost certainly more important, with ischaemic and even infarction patterns seen on the ECG.

The classic interpretation of the appearance of the J wave in the brain dead patient has been attributed to hypothermia. The ECG appearance of the J wave disappears following the transplantation of the heart in one or two days, although the body temperature at the end of the procedure is usually between 36°-37°C. In my own observations J waves can be seen as early as Stage I, during the predominantly parasympathetic-vagus mediated effect although sympathetic activity is already present. (Figures 2B-4, 2C-10). The histological evidence of injury of the conduction tissue at the A-V node and bundle of His may play a role in the appearance of the J wave. In clinical practice (see Chapter 9) the presence of the J wave in hypothermic patients is quite a common phenomenon. After the institution of hormonal therapy, in which T₃ is the most important component (Chapter 9), the J wave disappears in many cases between 60-90 minutes, although the central temperature of the body remains unchanged. Another factor, therefore, must play an important role, possibly a catecholamine effect plus progressive myocardial energy depletion and abnormalities in the depolarization-repolarization process.

The Q-T interval measures the myocardial depolarization and repolarization. Prolongation of the Q-T interval occurs in several states, such as quinidine hyperkalaemia, and other drug intoxication. It may identify cardiac conditions susceptible to serious arrhythmias. Abildskov (1976) reviewed the effects of the Q-T interval on the ECG in dogs, and showed that rapid catecholamine infusion prolongs the Q-T interval. Prolonged sympathetic nerve stimulation and slow infusion of catecholamines induced

shortening of the Q-T interval. Browne (1982) showed that propranolol significantly increases the Q-T interval; in contrast, atropine significantly shortened the mean measured Q-T interval. The simultaneous administration of propranolol plus atropine in patients led to shortening of the mean Q-T interval. Ahnve (1985) showed that changes in the autonomic tone influenced the heart rate and consequently there must be a relationship between heart rate and Q-T interval.

The results presented in Table 2B-2 clearly show a significant prolongation of the Q-T interval by 16% ($p < 0.0001$) during Stage I when the parasympathetic tone has its maximum effect on the myocardium. During sympathetic stimulation (Stages II, III, and IV) it is significantly shortened during the normal sinus beats seen in Stages III and IV, decreasing by 16% ($p < 0.0001$). In Stage V, it is not significantly different from the control ECG.

This clearly correlates in an inverse way with the work done by Browne (1982).

The Q-TC (QT corrected for heart rate) using the Bazett's formula (Bazett 1920) was shown in this study to be unsuitable for extreme bradycardias as in Stage I in which the Q-TC decreased by 23% ($p < 0.001$) and by an increment of 10% during Stage II ($p < 0.0005$) being not significant during Stages III, IV, and V, fully agreeing with the work of Browne (1982) and Abildskov (1976).

The haemodynamic changes observed in these animals reflect the body's attempts to compensate for the intracranial changes taking place during "coning" (Cushing's reflex 1902). The significant increments in MAP and SVR during the agonal period are almost certainly the direct result of a great increase in sympathetic nervous activity, which produces an extreme degree of peripheral arterial vasoconstriction. Blood is therefore redistributed into the capacitance vessels, leading to a rapid accumulation within the

great veins and right atrium. Due to a combination of low PVR, high pulmonary vessel compliance, and pulmonary capillary reserve recruitment (Vaughn et al. 1976), associated with a higher degree of right ventricular compliance compared to the left ventricle, the right ventricle is able to adjust to this increased venous return, and increase its output, demonstrated by a significant increase in pulmonary artery flow compared with aortic flow at this time ($QPA - QAO = 1.31$ ($p < 0.007$)) for a mean period of 157 seconds. As a result of the extreme degree of peripheral vasoconstriction may lead to a transient acute failure of the left ventricle, inducing a change in its geometry, leading to an acute and temporary event of mitral incompetence, which accounts for the LAP rising above the mean PAP.

This would indicate that one of the major events leading to neurogenic pulmonary oedema is a marked rise in LAP, transmitted retrogradely through the pulmonary veins and disrupting the integrity of the pulmonary capillaries. If this is the case, etiology of neurogenic pulmonary oedema should not be classified as a form of pulmonary oedema associated with normal PCWP.

In 9 of 11 animals, the LAP (or PCWP) markedly exceeded the PAP (mean peaks of 52 vs 34 mmHg respectively) for a mean period of 57 seconds. In one animal at the time of peak SVR the LAP had risen from 4 mmHg to 90 mmHg while the mean PAP remained unchanged. This remarkable and surprising observation implies that the pulmonary capillary blood flow temporarily ceased entirely. It would seem likely that it is during this period that disruption of the normal pulmonary capillary anatomy could occur (Staub 1978). As the peak LAP far exceeded the normal hydrostatic pulmonary capillary pressure, capillary integrity within the lungs would be disrupted, resulting in pulmonary oedema with high protein content (Theodore 1976) and interstitial haemorrhage; this, in fact, occurred in 36% of cases. In the remaining animals in which light microscopy did not

show pulmonary oedema, LAP in general rose less markedly, it is possible that capillary injury occurred but was not observed by light microscopy, though changes may have been seen if the lung tissue had been examined by electron microscopy.

The observed pulmonary oedema following capillary damage may contribute towards acute failure of the lungs and death in patients who have undergone transplantation of the heart and both lungs (Siegelman 1973, Staub 1978, Reitz 1980). A mild degree of pulmonary injury (in addition to ischaemic injury and inadequate pulmonary preservation) in patients with heart and lung transplants may result in early post-operative pulmonary oedema, also called re-implantation response, and an extreme degree of injury will result in patient death due to lung failure.

Cardiac muscle contraction (Appendix 2) depends mainly on the ability of the myocyte to raise the level of intra-cellular calcium from 0.1 to 10 mmol/mg (Dhallan 1982, Gevers 1982, Opie 1984). Langer et al. (1982) showed that at this calcium level the troponin C sub unit can react with myosine. Tada (1982) showed the intrinsic control mechanism is closely related to the level of energy stores, (ATP and CP), within the myocardium. Stiles (1984) showed that the above-mentioned mechanisms are sensitive to many pharmacological agents, catecholamines being the most important. Rasmussen (1984) suggested that hydrolysis of the enzyme phosphatidylinositol takes place after the action potential arrives, activating calcium-dependent ATPase, an enzyme related to calcium channel control at the sarcolemma.

β receptors also activate adenylyl-cyclase, increasing the level of cyclic AMP (Wikman and Coffelt 1983). The increased level of cyclic AMP mobilizes calcium from the sarcoplasmic reticulum, mitochondria and extracellular fluid, increasing the cytosolic calcium level and inducing an increased recruitment of actinomysin linkages, leading to a progressive myocardial contraction.

Reichenbach (1975) showed that 80% of patients dying suddenly from causes other than ischaemic heart disease, demonstrate extensive myocardial contraction band necrosis. Szakacs (1958) showed the noxious effect of infused catecholamines on the myocytes. Klein (1961) correlated myocyte injury to the presence of pheochromocytoma, leading to similar myocardial damage. Cooley (1972) described ischaemic contracture (stone heart) following poor myocardial protection during cardiopulmonary bypass, resulting in similar myocardial injuries. French (1952) showed that electrolyte disorders induced similar injury. Connor (1968) showed myocardial injury following brain stem stimulation. Hunt (1985) correlated subarachnoid haemorrhage and myocyte necrosis. Milei (1978) concluded that myocardial necrosis induced by Isoprenaline is primarily related to the stimulation of adenylyl-cyclase and activation of calcium and sodium channels; exaggerated calcium inflow induces an excess of excitation-contraction coupling mechanisms, energy consumption and cellular death. Baroldi (1975) described the various types of myocardial cell necrosis, and Lown (1976) correlated hypothalamic stimulation, peripheral sympathetic stimulation, and psychological stress with myocardial ectopics and fibrillation, all these effects were related to circulating and endogenous catecholamine release.

In the present study, during the agonal phase major structural damage also occurs in the specialized myocytes of the conduction tissue and smooth muscle of the coronary arteries. Evidence from this study correlates with the release of circulating and endogenous catecholamines from sympathetic nerve endings stimulating the myocardial cell receptors, leading to increased calcium uptake within the sarcoplasmic reticulum and mitochondria, these latter confirmed by the presence of intracellular calcium deposits related to a calcium overflow. In addition, there is almost certainly a temporary decrease in coronary blood flow mainly to the subendocardium during the period of tachycardia (Stages II, III and IV) associated with ECG features of ischaemia, in addition to an increase of coronary artery resistance. (Simons 1985).

Almost identical features of myocardial damage have been found in human donor hearts used for transplantation, and will be discussed later (Chapter 8). In the human, such myocardial histopathological changes could possibly be related, at least in part, to the intravenous administration of high doses of inotropic agents. In the present experiments, however, baboons undergoing brain death and showing widespread myocardial cell necrosis received no catecholamine infusion at all.

The endocrine changes brought about by the induction of brain death were mostly surprising, as in many ways they contradict the normal physiological concepts. (The methods used to measure each different hormone are described in Appendix I).

The catecholamine increment in the baboon at 5 minutes following induction of brain death and the subsequent reductions to below control levels by 3 hours indicate that the catecholamine "storm" is of extremely short duration. The rapid disintegration of sympathetic nervous system control over both circulating and endogenous catecholamines, and over the regulation of energy metabolism, occurs. Most probably, by the end of these experiments, no circulating catecholamines were present (Feibel 1981).

The level of circulating T_4 rapidly declined, and reached half control level within 4 to 7 hours. A rapid drop also occurred in T_3 (reduced by half only one hour post-induction of brain death), while TSH remained unchanged throughout the experiment.

In humans, it is well known that the half life of T_4 is approximately 4 to 7 days, and that of T_3 one to 2 days;

TSH half life is only 50 to 55 minutes (Dillon, 1980).

A remarkable finding was the unchanged value of TSH, in the presence of extremely rapid depletion of T_4 and T_3 , equating with an acute hypothyroid crisis. Although the mechanisms of T_4 and T_3 degradation are well known (Ingbar & Woeber 1981), with present physiological knowledge, it is impossible to explain the rapid catabolism and depletion of T_4 and T_3 during the induction of brain death.

Observations made in human brain dead patients by Hall (1983) also revealed normal TSH values. This implies that the adenohypophysis is still able to produce TSH without the influence of hypothalamic thyroxine releasing hormone TRH. It is well known that the major inhibitory mechanism of TSH production is the level of circulating free T_4 , but if TSH is produced, why not T_4 , or is another interrelated hormone to TSH missing?

Cortisol was found to decrease rapidly after an initial increment, being reduced by 50% within 15-60 minutes. In humans, half life of cortisol is approximately 110 minutes. ACTH was not measured in these experiments.

The insulin drop following the induction of brain death was less significant; its reduction occurred rapidly within the first 5 minutes, with a slower fall during the first hour. At this time interval, reached half its control value. In humans, half life of insulin varies between 10-25 minutes (Tepperman 1983). Glucagon remained insignificantly changed throughout the observations; its half life in humans is between 5 and 10 minutes. The significant fall of insulin in the absence of the significant change in glucagon level was another unexpected finding of this study.

ADH rapidly disappeared to unrecordable levels by 6 hours; it is known that in humans the half life is about 18 minutes.

The rapid increment of endocranial pressure at the time of inflation of the Foley catheter induces destruction of the hypothalamic region and its connections both to the anterior hypophysis (via the portal system) and to the neurohypophysis. The anterior and posterior pituitary lobes are no longer influenced by the releasing factors such as thyroxine releasing hormone TRH and corticotrophin releasing hormone (CRH). Loss of ADH, secreted by the supraoptic and paraventricular hypothalamic nuclei, results in diabetes insipidus, which was clearly observed in this study.

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CHAPTER 3

MECHANISM OF MYOCARDIAL INJURY OCCURRING DURING THE AGONAL PERIOD IN THE BABOON

INTRODUCTION

A series of experiments in baboons, using Method 1 to induce brain death, was carried out to try to elucidate the mechanism of myocardial injury occurring during the agonal period following brain death. The theory that the prolonged release of endogenous myocardial catecholamines which was the main factor leading both to the ischaemia seen on ECG and to the histopathological changes, was put to the test. Four groups were studied (Table 3-1).

EXPERIMENTAL GROUPS

Group A (Control) (n = 17)

Brain death was induced with no other surgical or pharmacological intervention (Chapter 2-E). Group B; (n=9), underwent bilateral cardiac sympathectomy (n = 5), auto-transplantation performed 7 days previously (n=2) or bilateral vagotomy plus β blockade (n=2), prior to the induction of brain death. Group C (n=5), calcium blockade was achieved using verapamil hydrochloride prior to the induction of brain death. Group D (n=7), in which the sympathetic nerve supply to the heart was retained, but other interventions were performed. (Table 3-1).

Group B: Cardiac Sympathectomy or Pharmacological Blockade

Surgical Technique

Under standard anaesthesia and muscle relaxation, a midline splitting sternotomy was performed, both pleural cavities were entered and the apical portion of the lung retracted exposing the stellate ganglia and the first four sympathetic thoracic ganglia in the posterior mediastinum. Using electrocautery the sympathetic tissue was removed bilaterally and sent for histological examination to confirm the nature of the tissue.

Groups of baboons underwent either cardiac sympathectomy (as above) or cardiac sympathectomy followed immediately by bilateral vagotomy at the base of the skull (n=3).

In 2 further animals autotransplantation was performed 7 days before induction of brain death. The surgical technique has been described previously by Cooper et al. (1983). Briefly, this involves the institution of cardiopulmonary bypass, cardioplegic arrest and excision by division of the right and left atria, aorta and pulmonary artery, care being taken to avoid damage to the sinus node, which was retained as part of the excised heart. All autonomic nerve supply to the heart was, therefore, severed. Replacement was performed using continuous suturing of each of the divided atria and vessels. Seven days later brain death was induced.

Two further animals underwent bilateral vagotomy at the base of the skull; this was followed by an increase in heart rate. The β adrenergic receptor antagonist propranolol was then administered intravenously in a dose of 10 mg/kg. This was followed by a fall in heart rate to 40-50 beats/minute. Brain death was then induced.

Group C: Calcium blockade with verapamil hydrochloride

A group of 5 animals had a midline splitting sternotomy performed, and flowmeters placed around the aorta and pulmonary artery. An intravenous infusion of the calcium antagonist verapamil hydrochloride in a dosage of 0.25 mg/kg was administered to bring about a reduction in MAP to 60 mmHg, together with some slowing of the heart. After 15-30 minutes, brain death was induced. Verapamil infusion was continued throughout the first 3-4 minutes of the agonal period or until such time as the MAP was seen to be falling once again to control pressures; verapamil was then discontinued.

Group D: Cardiac sympathetic nerve supply remaining fully or partially intact (n=7)

These animals underwent the following procedures before induction of brain death.

In 2 animals both vagi were divided surgically at the base of the skull through bilateral high neck incisions.

In one animal the left stellate ganglion was left in situ and only the right was removed; only a partial sympathectomy was therefore performed.

In 2 animals, autotransplantation was performed (as in Group B) but brain death was not induced until 4 and 5 months later respectively, at which time, there was evidence that reinnervation of the heart had occurred (Novitzky et al. 1986).

In 2 animals bilateral adrenalectomy was performed.

Following the induction of brain death, baboons in Group B, C and D were studied for a period of 6 hours. Basic haemodynamic monitoring was as described in Chapter 2-C for group

A animals in which a midline splitting sternotomy was performed and electromagnetic flowmeters were inserted around the aorta and pulmonary arteries. During the 6 hours of observation fluid replacement was given, but no inotropic or other drugs (other than those mentioned above) were administered. At the end of the experiment the heart was excised, biopsies were taken from the lungs and different tissues were examined histologically.

RESULTS

Electrocardiographic and Haemodynamic Observations

Following the induction of brain death in the animals which had undergone previous bilateral cardiac sympathectomy (Group B), the resulting sinus bradycardia (Stage I) was immediately followed by a tachycardia (Stages II,III,IV), though this was by no means as marked as in the control animals described earlier (Chapter 2). There were only occasional ventricular extrasystoles and no evidence of ischaemic changes during Stage IV (Figures 3-1 and 3-2). Changes in MAP and SVR remained unmodified.

In the animals which underwent cardiac sympathectomy and bilateral vagotomy, the initial sinus bradycardia (Stage I) was completely abolished; otherwise the changes were similar to those seen after bilateral cardiac sympathectomy alone (Figure 3-2). In the animals in which autotransplantation was performed 7 days previously, minimal ECG changes were seen following the induction of brain death - a moderate increment of heart rate and occasional coupling during Stage III. Otherwise, the haemodynamic response remained the same as in those undergoing cardiac sympathectomy and vagotomy. (Figure 3-3).

Following vagotomy and β receptor blockade with propranolol, there was again abolition of Stage I, and a slight increment of heart rate at Stages II-III-IV but no other electrocardiographic or haemodynamic changes were seen following induction of brain death.

In Group C animals after the administration of the calcium blocker verapamil HCl (Table 3-2, 3-3), induction of brain death resulted in only a mild tachycardia; there was occasional ectopic activity and no ischaemic changes on the ECG. The number of ventricular ectopic complexes seen in stage III was significantly less than the control group ($p < 0.005$), similarly, the duration of ectopic activity was significantly reduced ($p < 0.0001$) (Behrens-Fischer T test). The rise in MAP and other haemodynamic changes were not significant except for the increment of the cardiac output (Figures 3-4, 3-5).

In Group D animals which retained sympathetic cardiac innervation showed ECG and haemodynamic changes basically identical to those in the control animals discussed earlier (Figure 3-6).

Histopathology

All of the above animals which had undergone total sympathectomy either surgically or by pharmacological blockade (Group B) or had received a calcium antagonist (Group C) showed normal myocytes on histological examination, though mononuclear cell infiltration and mild interstitial oedema were present (Figures 3-7, 3-8). The animals that retained sympathetic innervation (either complete or incomplete (Group D)), all showed myocardial histopathological changes identical to those seen in the control animals (Group A); contraction band type of myocardial necrosis was the most common. (Table 3-1). (Chapter 2-E).

DISCUSSION

Total denervation of the heart (autotransplantation) or bilateral cardiac sympathectomy led to a greatly modified cardiac haemodynamic response to brain death, with reduction of the tachycardia, and abolition of ventricular arrhythmias and ischaemic features on the ECG, but did not completely abolish the increase in MAP or SVR. This would suggest that the cardiac response to the increased circulating catecholamines is attenuated or abolished, and the output of endogenous myocardial catecholamines inhibited, but the peripheral vascular response remains intact. β adrenergic receptor blockade abolished the entire response. No hearts in which the sympathetic supply had been interrupted showed evidence of myocyte necrosis on light microscopy.

When the sympathetic supply to the heart remained even partially intact, the haemodynamic response to the induction of brain death and the nature and incidence of myocardial structural damage were almost identical to those seen in control animals. The presence or absence of an intact vagal supply to the heart did not play a role in the mechanism of myocyte injury.

The myocardium has a rich sympathetic plexus, with fibres arising from the cervical sympathetic ganglia, giving origin to the cardiac nerves (Bowman 1980). When stimulated, the post-synaptic nerve endings release endogenous catecholamines which directly affect the myocardial cells, β receptors (in the myocytes and conduction tissue) and receptors in the coronary arteries. (To my knowledge, this is the first communication in the literature confirming conduction tissue injury and calcium deposits probably within the mitochondria in the smooth muscle of the coronary arteries associated with brain death).

The early massive increase in circulating catecholamines, brought about partially by

release of catecholamines from the adrenal glands, is relatively short-lived, lasting for only a few minutes. In some control animals, however, the heart rate remained high at 160 bpm for several hours (in 2 animals for up to 15 and 17 hours), which may suggest sympathetic activity on the myocardium and to the release of endogenous catecholamines. The reason for this prolonged activity in animals otherwise clearly brain dead remains unclear. It may be related to lack of inhibition of spinal sympathetic centres from higher mid brain inhibitory centres.

It would seem that the initial short outburst of circulating catecholamines is less likely to have a lasting damaging effect on the myocardium rather than the prolonged activity of endogenous catecholamines in the myocardium itself. This theory is supported by the observation that, following bilateral adrenalectomy, myocardial damage still occurred. The mechanism of myocardial injury, however, is probably multifactorial, with both sources of catecholamines playing an important part, resulting in significant increment of the SVR and MAP and severe coronary spasm leading to an acute myocardial oxygen debt and ECG features of severe ischaemia (Stages III,IV).

As the prior administration of a calcium antagonist verapamil hydrochloride greatly attenuates the haemodynamic response to brain death, and prevents myocardial structural injury (Cheung et al. 1984, Reves 1984, Hamman et al. 1984, Schwartz 1984, Pedrinelli 1984), it is highly suggestive that the myocardial damage (necrosis) which occurs is the result of excessive calcium uptake by the myocytes, coronary arteries and conduction tissue during endogenous catecholamine release. (Reichenbach 1970, Waldenstrom et al. 1978, Baroldi 1975, Ganote 1983).

LEGENDS**FIGURES****Figure 3-1**

ECG recording from precordial leads (previous sympathectomy - Group B) showing C: (control), sinus rhythm, heart rate of 115. Stage I: sinus bradycardia, heart rate 65. Stage II: sinus tachycardia, heart rate 150. Stage III: sinus rhythm, heart rate 187. Stage IV: sinus rhythm, heart rate 150. Stage V: sinus rhythm, heart rate 88. The ischaemic changes seen in Group A animals were completely abolished and no ventricular ectopic activity was seen during Stage III.

Figure 3-2

ECG recording from precordial leads from an animal which underwent bilateral sympathectomy and vagotomy. C: (control) sinus rhythm, heart rate 105. Following bilateral vagotomy (BV), sinus rhythm, heart rate 136. Following the induction of brain death (BD) the ECG features were similar to Figure 3-1 except that during Stage I the vagus mediated bradycardia was abolished. During Stage III only one ventricular ectopic beat was seen.

Figure 3-3

Precordial ECG recording, from heart autotransplanted 7 days prior to brain death. C: (control) sinus rhythm, heart rate 100. Stage I: probable sinus rhythm, heart rate 100. Stage II: probable sinus rhythm, heart rate 100. Stage III: ventricular ectopic coupling for

a period of 30 seconds. Stage IV: sinus rhythm, heart rate 103. Stage V: sinus rhythm, heart rate 94. The heart rate remained unchanged throughout the experiment; ventricular activity was shown however in Stage III.

Figure 3-4

Haemodynamic response to BD in a baboon treated with verapamil hydrochloride ($\text{Ca}^{++}\beta$). Following the induction of brain death (BD) the aortic and pulmonary flows remained equal, and no blood pooling in the lungs took place. CO went up due to vasodilatation. The MAP and SVR did not rise. The pulmonary haemodynamics showed a normal ratio of PAP to LAP. There was no increment of the PVR. (Compare to Figure 2C-2).

Figure 3-5

Left hand - systemic parameters, right hand - pulmonary parameters.

A: Control haemodynamic parameters prior to induction of brain death.

B: Modified haemodynamic response to induction of brain death after pre-treatment with verapamil hydrochloride in 5 animals. The significant changes seen in the control group (Figure 2C-1) have been abolished.

Figure 3-6

ECGs taken during Stage III in 3 animals, Group D. A, incomplete sympathectomy, B, following bilateral adrenalectomy; and, C, following autotransplantation done 4 months previously. Ventricular ectopic activity is present in the three examples.

Figure 3-7

Histopathological view of the myocardium from a baboon which had been pre-treated with verapamil hydrochloride prior to the induction of brain death. Normal myocyte appearance, a mild interstitial oedema is present, and some mononuclear cell infiltrate. A probable contraction band is seen on the right hand of the picture. (haematoxylin-eosin x 150).

Figure 3-8

Histopathological section of the myocardium following the induction of brain death in the baboon which underwent bilateral sympathectomy, showing normal myocytes with severe interstitial oedema. Other sections of this heart showed only mild oedema (haematoxylin-eosin x 150).

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TABLE 3-1
INCIDENCE OF MYOCYTE NECROSIS, MONONUCLEAR CELL INFILTRATE AND INTERSTITIAL OEDEMA IN LEFT VENTRICULAR MUSCLE, CONDUCTION TISSUE AND CORONARY ARTERIES

GROUP	PROCEDURE BEFORE INDUCTION OF BRAIN DEATH	n	MYOCYTE NECROSIS		MONONUCLEAR CELL INFILTRATION	OEDEMA
			LV	COND. TISS. COR. ARAT.		
A	Control (none)	17	88%*	41%	100%	100%
B	Bilateral cardiac sympathectomy	2				
	Bilateral cardiac sympathectomy and bilateral vagotomy	3				
	Autotransplantation 7 days previously	2	0%	0%	33%	44%
	Bilateral vagotomy + beta blockade	2				
C	Pretreatment with verapamil hydrochloride	5	0%	0%	0%	0%
D	Bilateral vagotomy	2				
	Unilateral sympathectomy	1				
	Autotransplantation 4-5 months previously	2	100%	86%	71%	57%
	Bilateral adrenalectomy	2				

LV = left ventricle

Cond. Tiss. = conduction tissue

Cor. art. = coronary arteries

* = indicates % of hearts injured

TABLE 3-2
VERAPAMIL HYDROCHLORIDE PRETREATMENT, HAEMODYNAMIC RESPONSE TO BRAIN DEATH
SYSTEMIC HAEMODYNAMIC DATA

PARAMETER	GROUP	CONTROL (1)	CONTROL (2)*	AFTER INDUCTION OF BRAIN DEATH (3)**	STATISTICAL DIFFERENCE 2 vs 1	STATISTICAL DIFFERENCE 3 vs 2	STATISTICAL DIFFERENCE A3 vs B3
CVP	A	3.2 (1.16)	3.0 (0.50)	4.8 (1.23)	n s	n s	n s
	B	4.0 (0.89)	4.8 (0.71)	4.6 (0.51)	n s	n s	
MAP	A	100.0 (3.50)	98.2 (4.20)	296.0 (38.50)	n s	<0.01	<0.0005
	B	97.6 (7.83)	76.2 (2.60)	83.6 (15.45)	n s	n s	
SVR	A	45.0 (6.80)	43.2 (7.10)	287.0 (78.50)	n s	<0.01	<0.01
	B	44.9 (2.78)	22.1 (2.45)	23.2 (2.35)	<0.001	n s	
HR	A	120 (5.1)	115 (5.2)	180 (7.2)	n s	<0.001	<0.0001
	B	118 (4.7)	95 (4.6)	120 (3.6)	<0.05	<0.05	
AOQ	A	2.25 (0.06)	2.27 (0.10)	1.30 (0.07)	n s	<0.0003	<0.001
	B	2.41 (0.22)	2.60 (0.82)	3.20 (0.60)	n s	n s	

* = after verapamil infusion in Group B baboons

** = at the time of peak SVR in Group A or equivalent time in Group B

Figures in brackets = standard error of the mean

TABLE 3-3
VERAPAMIL HYDROCHLORIDE PRETREATMENT, HAEMODYNAMIC RESPONSE TO BRAIN DEATH
PULMONARY HAEMODYNAMIC DATA

PARAMETER	GROUP	CONTROL (1)	CONTROL (2)*	AFTER INDUCTION OF BRAIN DEATH (3)**	STATISTICAL DIFFERENCE 2 vs 1	STATISTICAL DIFFERENCE 3 vs 2	STATISTICAL DIFFERENCE A3 vs B3
LAP	A	6.0 (1.30)	8.0 (1.25)	52.0 (2.23)	n s	<0.0001	<0.0001
	B	4.6 (1.63)	6.2 (0.93)	8.1 (3.11)	n s	n s	
PAP	A	14.0 (0.22)	15.1 (0.61)	34.0 (5.10)	n s	<0.002	<0.025
	B	13.0 (1.55)	14.0 (0.73)	17.4 (2.31)	n s	n s	
PVR	A	3.00 (0.45)	3.20 (0.12)	7.1 (2.30)	n s	n s	n s
	B	6.2 (0.54)	4.1 (1.30)	5.8 (0.99)	n s	n s	
PAQ	A	2.25 (0.06)	2.27 (0.10)	2.82 (0.13)	n s	<0.01	n s
	B	2.41 (0.22)	2.60 (0.82)	3.17 (0.59)	n s	n s	

* = after verapamil infusion in Group B baboons

** = at the time of peak SVR in Group A or equivalent time in Group B

Figures in brackets = standard error of the mean

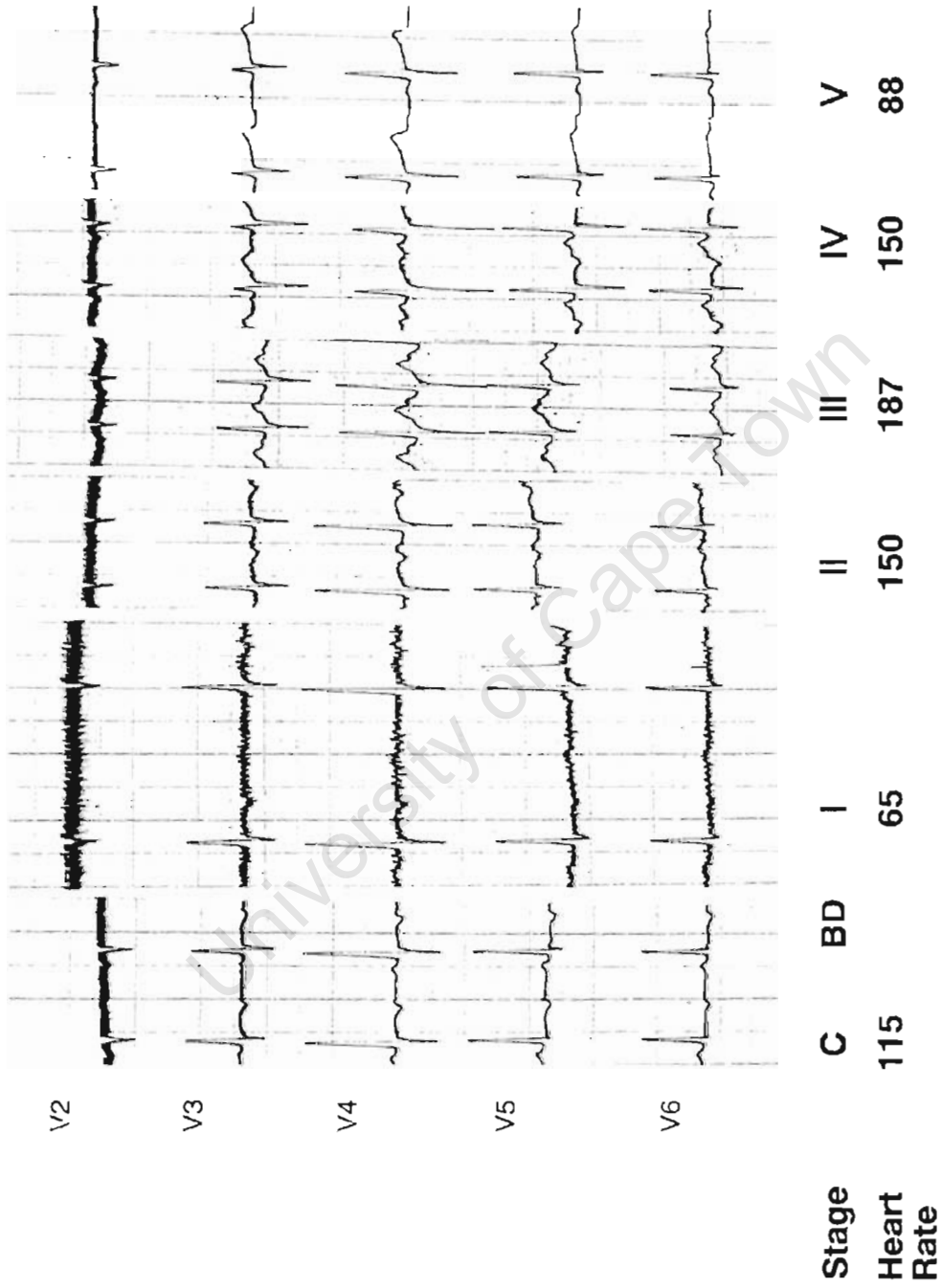


Figure 3-1

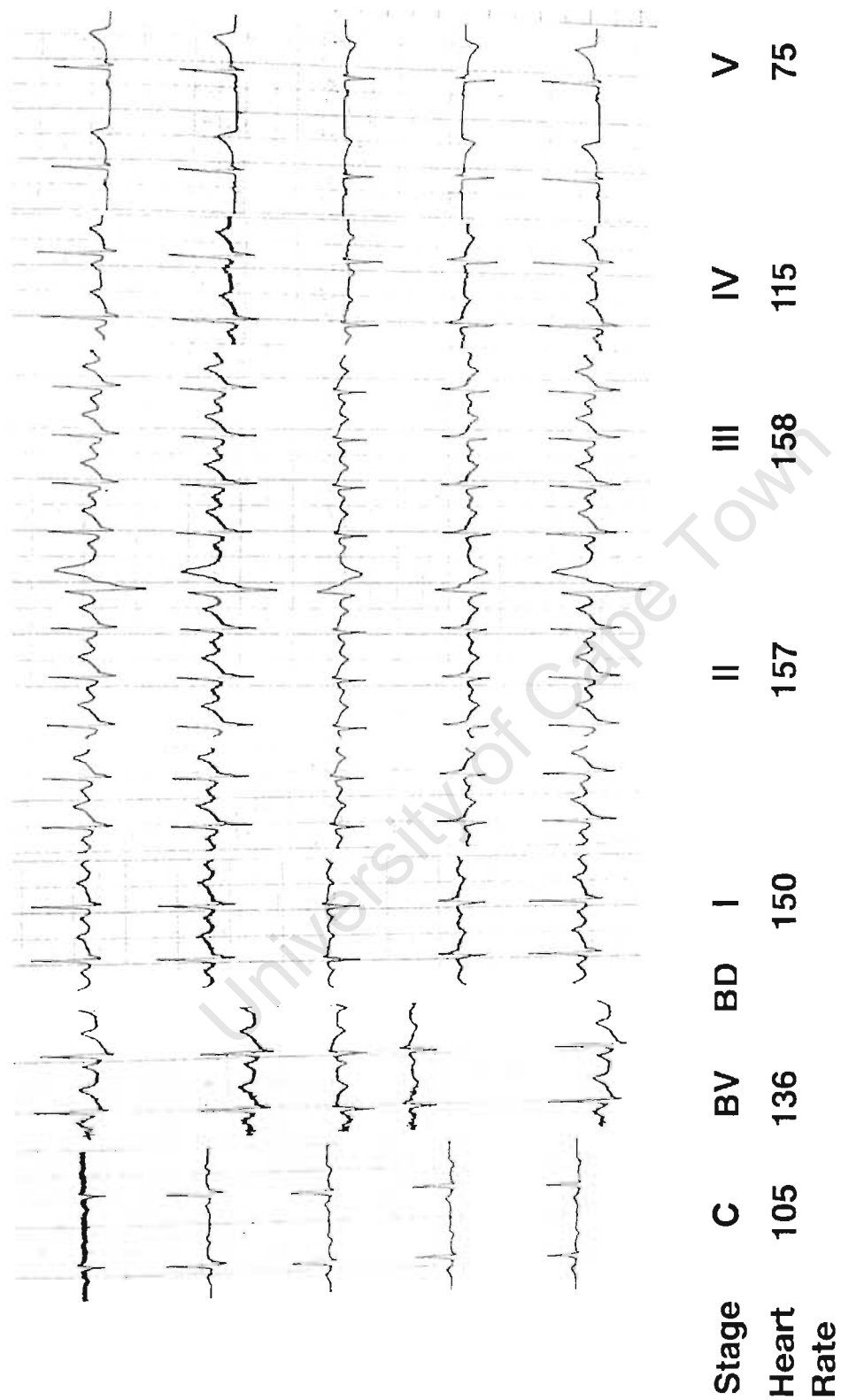


Figure 3-2

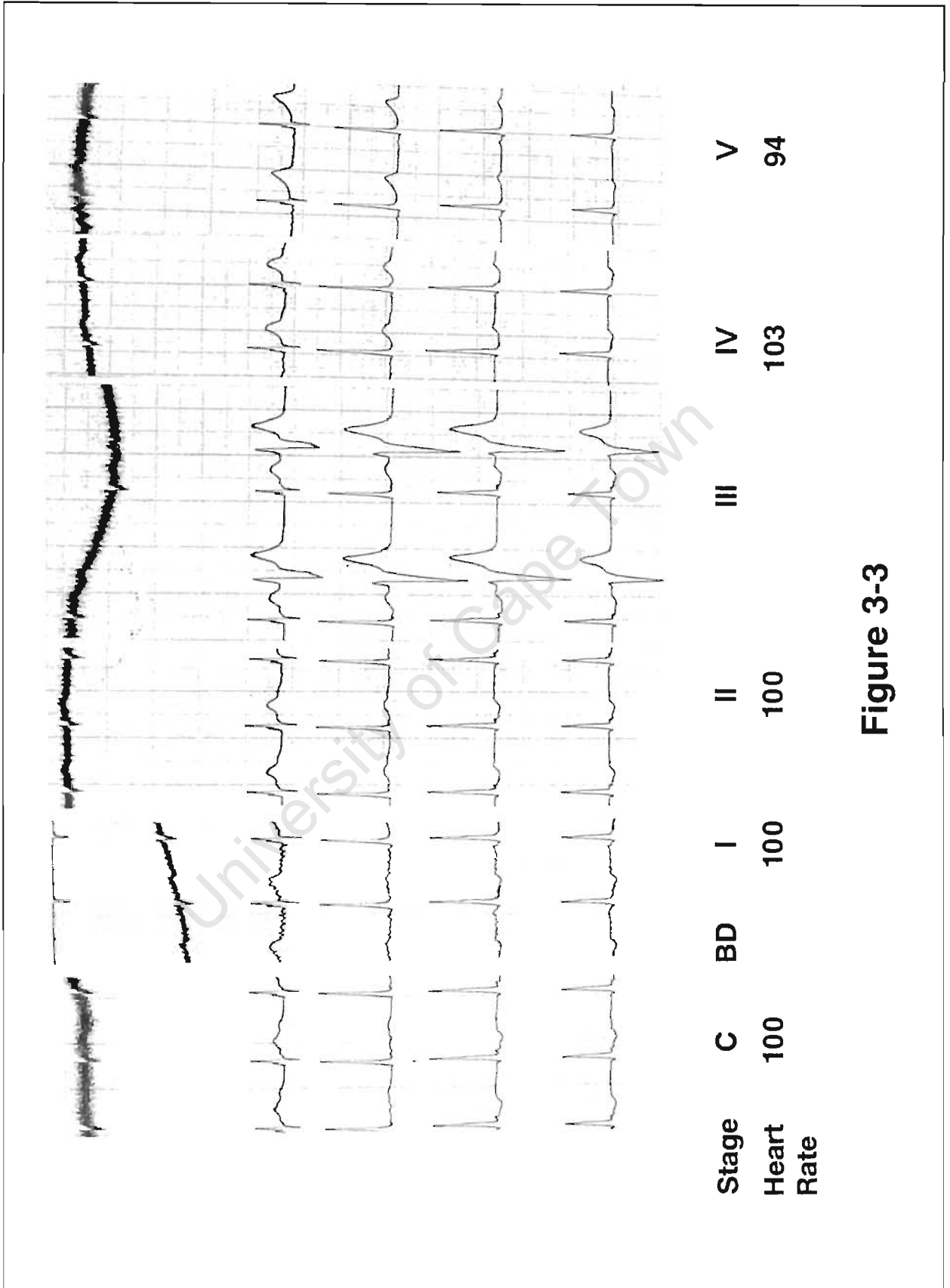


Figure 3-3

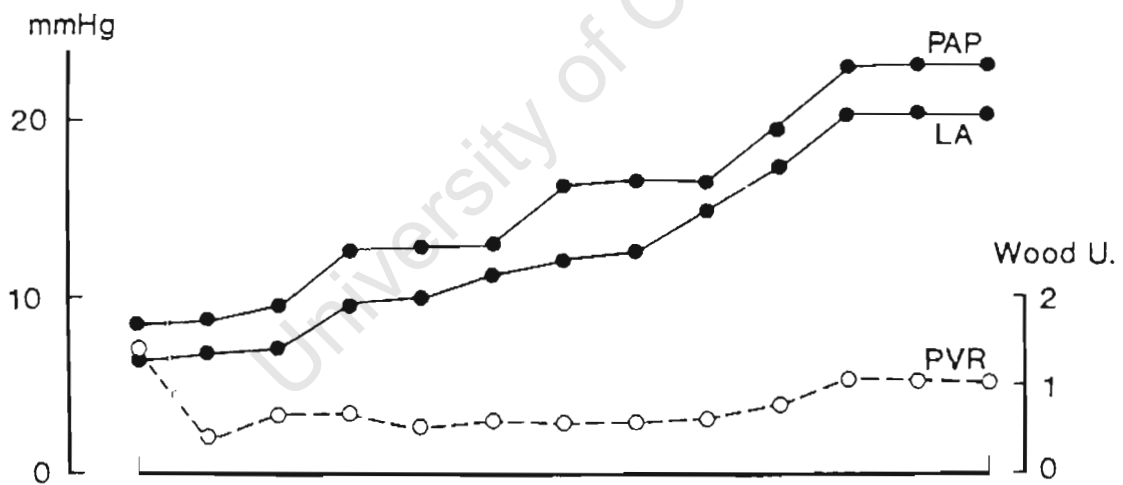
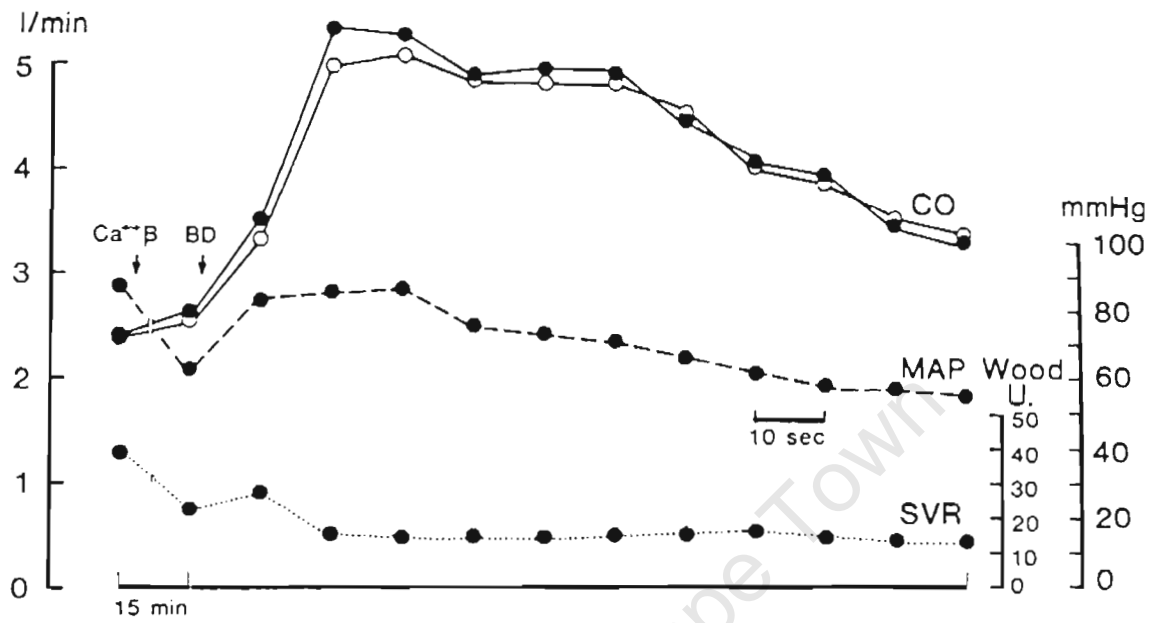


Figure 3-4

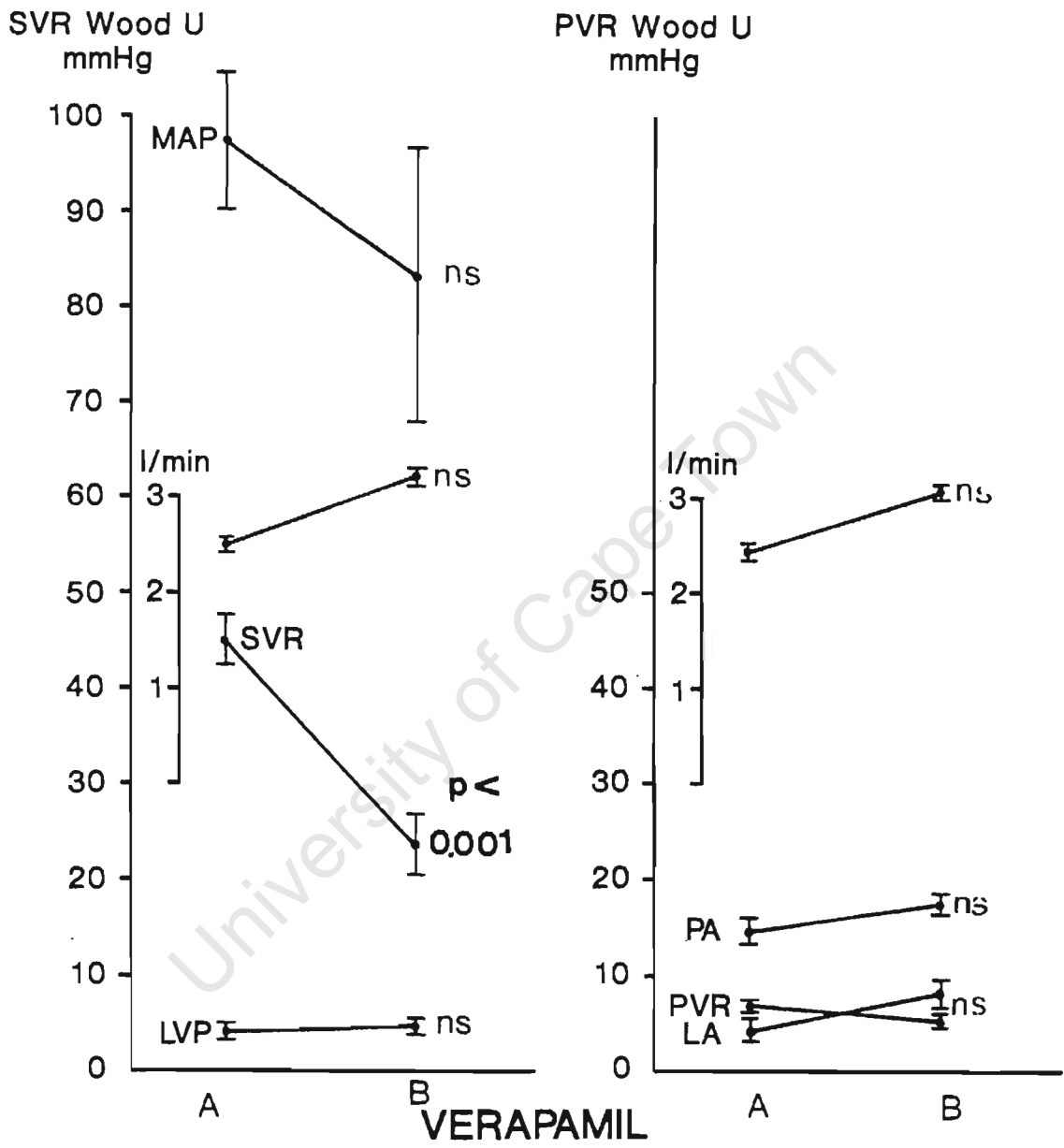
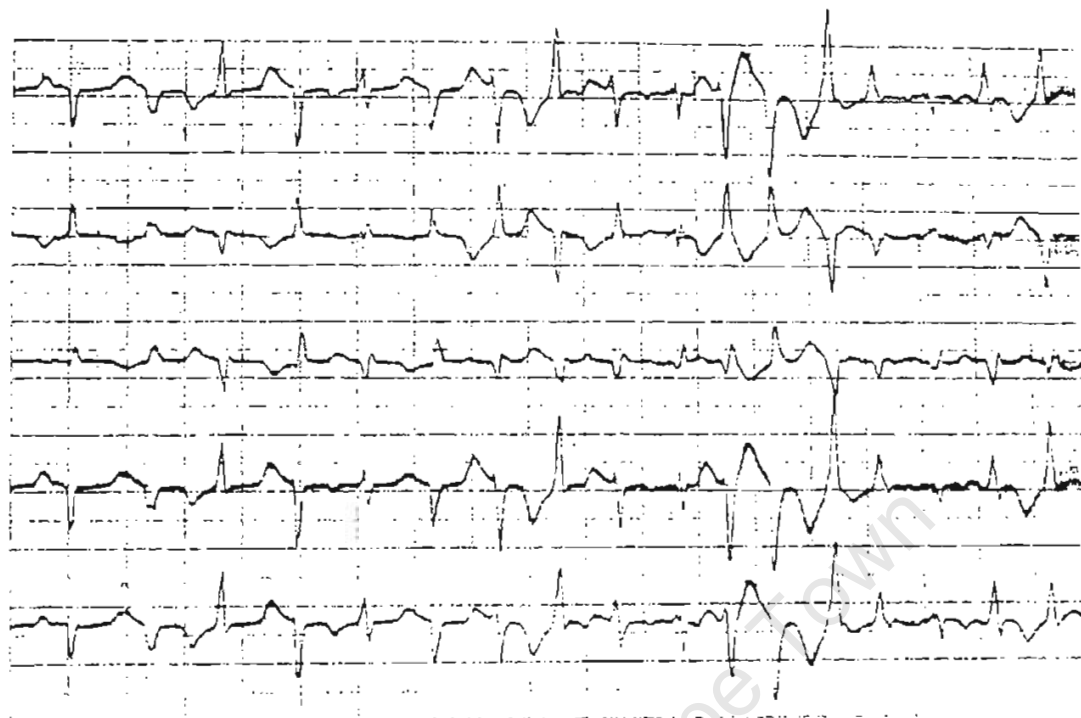
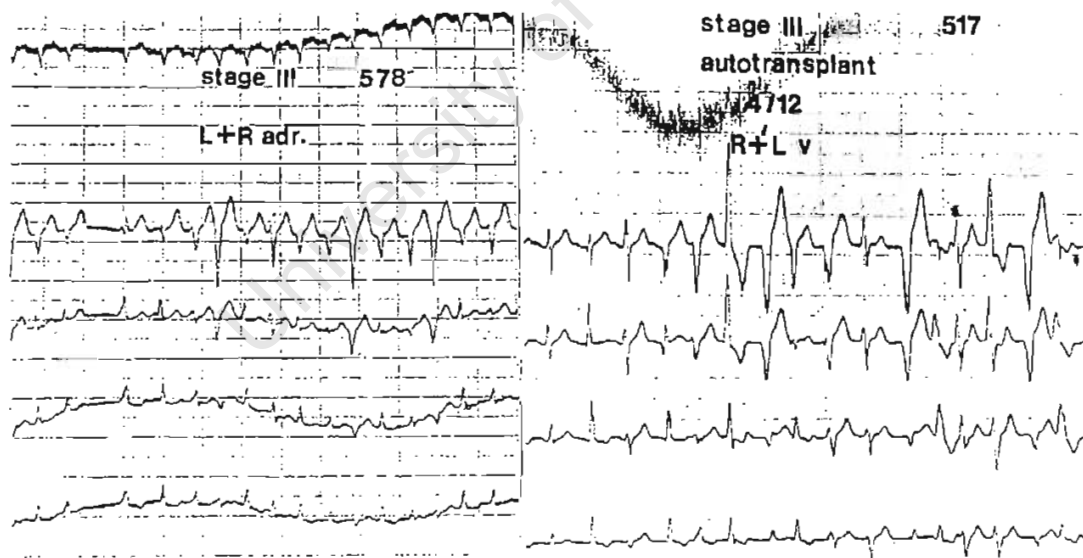


Figure 3-5



A Unilateral sympathectomy



B

C

Figure 3-6

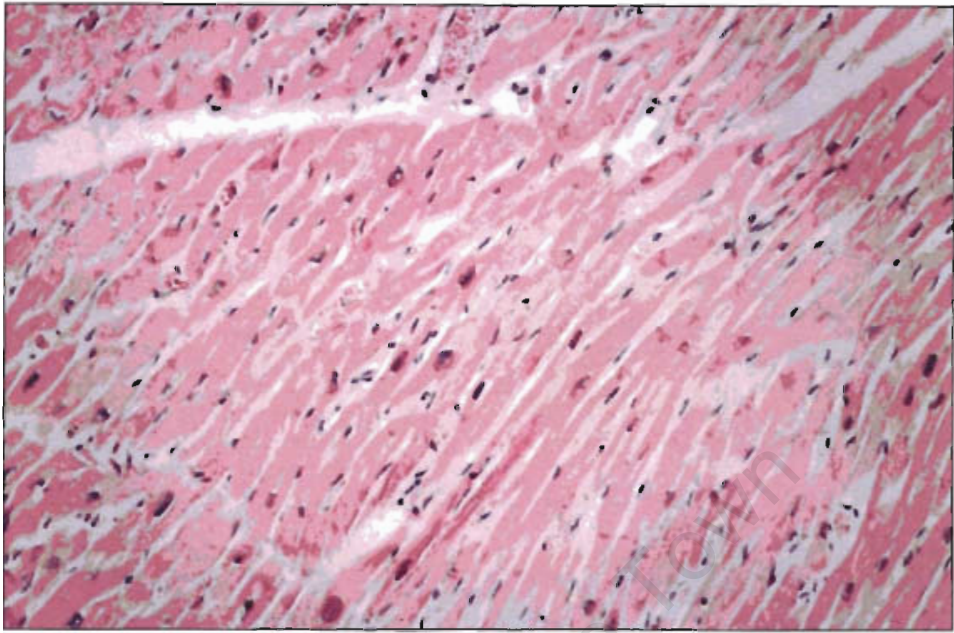


Figure 3-7

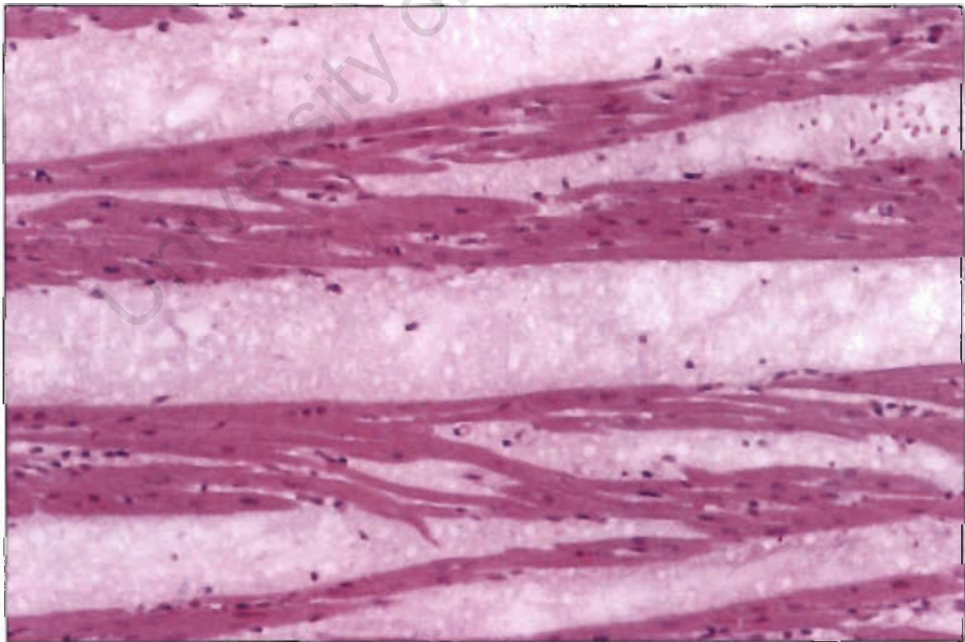


Figure 3-8

CHAPTER 4

THE EFFECTS OF THE AGONAL PERIOD ON THE MYOCARDIUM - FUNCTIONAL AND BIOCHEMICAL STUDIES IN THE PIG

Part A

INTRODUCTION

These studies were carried out in the pig using Method 2 for the induction of brain death. After the experimental procedure (outlined below), all hearts underwent functional testing by a system which has been described in detail previously (Wicomb et al. 1981).

SYSTEM OF EX VIVO FUNCTIONAL TESTING

The functional testing apparatus is shown in Figure 4-1. Oxygenated blood, matched for group, was supplied by a perfusor pig to which deoxygenated blood was returned. Initially, the aorta was retrogradely perfused as a Langendorff system supplying blood to the coronary arteries of the isolated heart. This allowed a period of recovery, usually continued for 5 - 15 minutes. When the myocardial temperature reached 37°C the heart was defibrillated (if spontaneous contractions had not occurred) and the model converted into the working heart mode. Blood was then directed into the left atrium of the isolated heart, converting the role of the heart from a passive to an active one by "challenging" the left ventricle with blood from the left atrial reservoir (preload). If myocardial function was good, the left ventricle pumped blood through the aortic balloon past the non-linear resistance to a height of 100 cmH₂O (afterload), after which the blood was returned to the left atrial reservoir.

Preload and afterload could be adjusted, but were kept constant at 11 cmH₂O and 80 mm Hg respectively during the period of testing. The left ventricular (LVP) and left ventricular end-diastolic (LVEDP) pressures were measured using a Statham P23H transducer. Cardiac output (CO) was obtained by a timed collection of aortic output plus coronary venous return; stroke volume (SV) was calculated. Coronary flow (CF) was similarly measured by a timed collection of coronary venous return. All hearts were tested for one hour during which time these parameters were recorded.

The results obtained by this system of ex vivo functional testing of isolated hearts have been shown to reflect accurately the performance of the heart following orthotopic transplantation (Wicomb et al. 1981).

ESTIMATION OF MYOCARDIAL HIGH ENERGY PHOSPHATES, LACTATE, AND GLYCOGEN (See also Appendix 1)

In selected hearts, the myocardium was biopsied, immediately before undergoing functional testing, for estimation of adenosine triphosphate (ATP) and creatine phosphate (CP), as well as of lactate and glycogen, using standard techniques (Opie 1971).

STATISTICAL ANALYSES

Statistical analyses were carried out using either a one or two way analysis of variance. Paired comparisons were made using the Dunnett's t test. Statistical analyses for multiple comparisons were made using the Bonferroni inequality.

EXPERIMENTAL GROUPS

Four groups of pigs were studied.

Group A1 - Freshly excised (control)

The chest was opened by median sternotomy and the heart excised after infusion of cardioplegic solution (Table 4A-1). The heart was then immediately transferred to the functional testing apparatus and its myocardial function tested for one hour.

Group B1 - Induction of brain ischaemia/death

The pigs sustained a period of brain ischaemia by the technique described earlier (Method 2). Once the MAP returned to an approximate control level, the heart was rapidly excised, and immediately functionally tested for one hour. The period of brain ischaemia varied from one to 2 hours. From evidence from previous experiments in our laboratory (Lanza et al. 1985), some of these pigs almost certainly suffered irreversible cerebral damage, if not complete brain death; others, however, underwent only reversible cerebral ischaemia.

Group C1 - Brain death + hypotension (minimal haemodynamic support).

The heart was retained in the pig for a period of 4 hours following induction of brain death. No intravenous fluid or inotropic support was given unless the MAP fell below a mean of 40 mmHg (irrespective of the CVP). At this MAP, i.v. fluid (a balanced solution of electrolytes-plasmalyte B, supplemented with potassium chloride (30 mmol KCl/l)) was infused in order to maintain the MAP at approximately 40 mmHg. If i.v. fluids were not successful in maintaining this pressure, then inotropic support in the form of an infusion of dobutamine hydrochloride was given, regulated to maintain the pressure at this relatively low level. Most of these hearts did not require any support until the final hour of the test period. In general, less than 500 ml of fluid was administered.

Four hours after clamping of the head and neck vessels, the heart was excised and functionally tested.

Group D1 - Brain death + normotension (fluid and dobutamine support).

This group underwent identical management to Group C1 except that efforts were made to maintain a CVP between 5-10 cmH₂O and an MAP above 60 mmHg. In nearly all animals i.v. fluid support was begun within the first hour; the amount of fluid given varied between one and 3 litres. Where necessary, a dobutamine infusion was commenced.

RESULTS

Functional Testing (Table 4A-2).

Measurements of CO, SV, LVP, LVEDP and CF are shown in Table 4A-2; the statistical differences between the control Group A1 and the experimental groups B1, C1 and D1 are also shown. Functional testing immediately after a period of brain ischaemia (Group B1) did not lead to any significant deterioration of myocardial function when compared with freshly excised hearts (Group A1). The addition of a further period of function of the heart within the brain dead animal, however, led to a deterioration of myocardial function, as evidenced by significant reductions in CO, SV, and LVP in Groups C1 and D1; this deterioration was irrespective of whether the pig had been maintained in a normotensive (Group D1) state; the differences in observed function between hearts in Group C1 and D1 were not statistically significant.

Myocardial High Energy, Lactate and Glycogen (Table 4A-3)

High energy phosphates (ATP and CP), glycogen and lactate were measured in 3 groups only (A1, B1, and C1). A period of brain ischaemia (Group B1) was associated with falls

in ATP, CP, glycogen, and lactate measurement, though in no case did the changes reach significance. When brain ischaemia was allowed to progress to brain death and little or no haemodynamic support given (Group C1), significant falls in CP and glycogen were observed. Measurement of tissue lactate levels in these two groups showed a significant increment.

DISCUSSION

When the haemodynamic status of the pig was maintained for 4 hours from the time of onset of brain ischaemia (C1), deterioration of subsequent myocardial haemodynamic testing function occurred. This was associated with no significant ATP reduction but a significant CP reduction in which creatine phosphate shuttle may play a role (McClellan et al. 1983) and in glycogen, with a significant lactate increment, suggesting that there is a preferential anaerobic metabolism.

It would appear that the attempts (inotropic support and i.v. fluids) to maintain adequate CO, CVP and MAP led to consumption of myocardial energy stores which, during anaerobic glycolysis, the brain dead animal is unable to replenish. Similar studies in the baboon, where post-brain death support was maintained for 24 hours, lend support to this point (Table 4A-4). This would suggest that the inability of the animal to replenish these stores may be related to a rapid tissue reduction in circulating T_3 , insulin and cortisol.

Efforts to maintain the circulation with a CVP greater than 5 cmH₂O and a MAP of more than 40 mmHg may, therefore, result in greater myocardial damage by placing greater demands on the myocardium. This is clearly important if the heart is to be transplanted subsequently. The effects of a low MAP for several hours on the viability of kidneys, however, remains uncertain, and this must be considered if the kidneys are also to be transplanted.

From these studies we can conclude that (i) brain ischaemia leads to no significant reduction in subsequent myocardial function nor in myocardial energy reserves or glycogen, but an initial rise of lactate was observed, (ii) brain death with or without subsequent haemodynamic support of the circulation for 4 hours leads to a significant reduction in subsequent myocardial function, and also results in depletion of myocardial high energy phosphates and glycogen reserves, though the rate of high energy phosphate depletion is reduced, mainly CP, to a certain extent by inhibition of aerobic pathways; (iii) attempts to maintain a normal haemodynamic status over longer periods of time (24 hours in the baboon) results in a significantly greater loss of myocardial energy stores and increase in tissue lactate.

The electrocardiographic and haemodynamic changes observed in the pig following the clamping of the aortic vessels leads to the same changes observed in the baboon (Chapter 2B-2). The same histological features can be observed (myocardial contraction bands, coagulative necrosis and myocytolysis), as a result of the catecholamine injury as previously described.

Most of the hearts removed at the end of brain ischaemia (Group B1) were excised between 1-2 hours following the clamping of the aortic vessels, and by that stage the catecholamine "storm" had settled, and the tachycardia subsided. Most likely by this time the tissue (cellular) T_3 and other hormones remained in sufficient concentration to maintain full activity of the mitochondria; the cells were therefore able to replenish high energy nucleotides aerobically.

Possibly, at a later stage, tissue T_3 becomes depleted and progressive aerobic metabolism within the mitochondria is inhibited (see Chapter 7), leading to energy stores and glycogen depletion and accumulation of tissue lactate, as seen in Groups C1 and D1.

LEGENDS

FIGURES

Figure 4A-1

System for functional testing of the isolated heart. A = the apparatus in the laboratory. B = its diagrammatic representation (HE = heat exchanger; F = filter; AoO = aortic output (for monitoring cardiac output); WK = Windkessel; PR = non-linear resistance device; AoB = aortic balloon; AoP = aortic pressure monitoring transducer (mmHg); ECG = electrocardiographic needle electrodes; LAR = left atrial reservoir; LAP = left atrial pressure (cm H₂O); P = roller pump; LVP = left ventricular pressure monitoring transducer (mmHg); CVR = coronary venous return).

TABLE 4A-1
CONSTITUTION OF CARDIOPLEGIC SOLUTION

	<i>g/l</i>	<i>mM</i>
1. NaCl	6.00	102.0
2. NaHCO ₃	0.38	4.0
3. KCl	0.75	10.0
4. CaCl ₂ ·2H ₂ O	0.15	1.1
5. MgSO ₄ ·7H ₂ O	3.50	14.0
6. Procaine HCl	0.27	1.0
7. Insulin	20 U/l	–
8. Dextrose	50	278
9. Verapamil HCl	0.0015	3/μM
Osmolality	320 Mosm/l	
pH at 4° C	7.4	

TABLE 4A-2
HAEMODYNAMIC PERFORMANCE ON FUNCTIONAL TESTING OF HEARTS NOT SUBJECTED TO STORAGE (GROUPS A1 – D1 AND D3) (Bonferroni inequality level of significance $p < 0.017$).

GROUP	n	CO (ml/min)	(p)	SV (ml)	(p)	LVP (mmHg)	(p)	LVEDP (mmHg)	(p)	CF (ml/min/100g)	(p)
A1	10	2820(220)*		17(1.3)		131(4.4)		5.3(1.03)		258(33)	
B1	6	2738(385)	NS	19(2.5)	NS	124(6.4)	NS	6.4(1.77)	NS	339(67)	NS
C1	6	1537(263)	<0.0025	13(1.5)	<0.05	109(4.2)	<0.005	6.6(1.08)	NS	296(46)	NS
D1	6	1860(329)	<0.02	13(1.9)	<0.05	102(5.3)	<0.0006	4.3(0.59)	NS	378(53)	NS

* Figures in brackets represent standard error of the mean

(p) = at the time of peak SVR in Group A or equivalent time in Group B

NS = not statistically significant.

TABLE 4A-3
MEASUREMENT OF HIGH ENERGY PHOSPHATES, LACTATE AND GLYCOGEN IN
NON-STORED HEARTS (GROUPS A1, B1, C1)

GROUP	n	ATP ($\mu\text{mol/g}$)** (p)	CP ($\mu\text{mol/g}$) (p)	Lactate ($\mu\text{mol/g}$) (p)	Glycogen ($\mu\text{mol/g}$) (p)
A1	10	3.73(0.253)*	6.61(0.720)	3.9(0.91)	29.6(3.14)
B1	8	2.93(0.375)	5.57(0.900)	4.50(0.680)	22.6(4.39)
C1	6	3.29(0.227)	3.69(0.850)	12.9(3.39)	12.7(2.95)

** Fresh weight

* Figures in brackets represent standard error of the mean

(p) = Statistical difference from A1

NS = not statistically significant

TABLE 4A-4
MYOCARDIAL ENERGY STORES AND LACTATE MEASURED 24 HOURS
AFTER BRAIN DEATH IN BABOONS

	ATP (mmol/g)	(p)	CP (mmol/g)	(p)	Lactate (mmol/g)	(p)	Glycogen (mmol/g)	(p)
Control values	33.61 (0.29)		5.02 (0.87)		30.0 (2.9)		6.0 (2.0)	
Group B (brain death + normo- tension for 24 hours)	0.87 (0.56)	<0.01	1.17 (1.03)	<0.01	20.4 (5.1)	<0.01	7.94 (5.1)	<0.01

ATP = adenosine triphosphate

CP = creatine phosphate

Figures in brackets = standard error of the mean

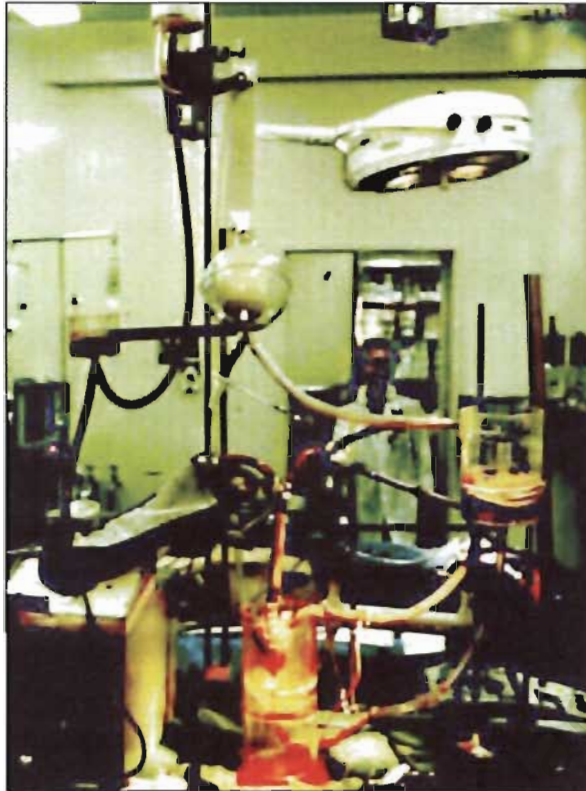


Figure 4A-1

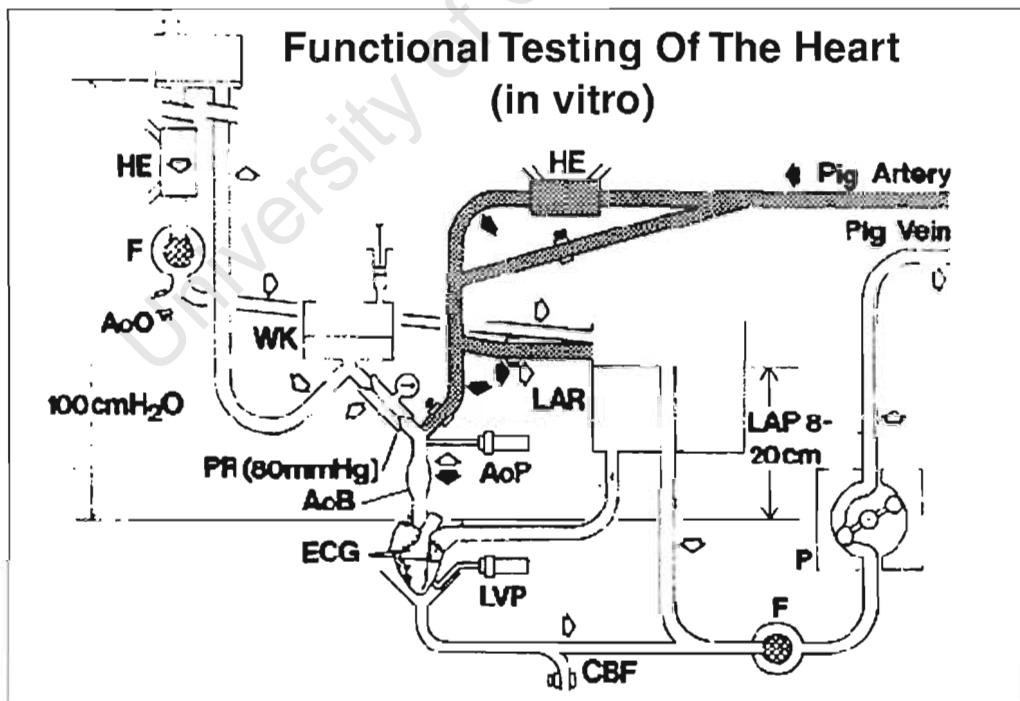


Figure 4B-1

Part B

THE ADDITIONAL EFFECT OF STORAGE OF THE HEART FOLLOWING BRAIN DEATH IN THE PIG

INTRODUCTION

The additional effect of 24 hours storage by hypothermic perfusion in hearts taken from brain dead pigs (Method 2) was also investigated.

SYSTEM OF HYPOTHERMIC PERFUSION STORAGE

Storage of the heart by continuous 24 hours hypothermic (4-10°C) perfusion was carried out using a system previously described in detail (Wicomb et al. 1981), (Wicomb et al. 1984). The perfusion circuit is shown in Figure 4B-1. The perfusate (Table 4B-1) was sterilized by being passed through a 0.8 micron filter, the pH being adjusted to an initial value of 7.2 by bubbling a 97% O₂ and 3% CO₂ mixture through it. The perfusate was both oxygenated and circulated throughout this period of perfusion by the airlift pump principle (Dumbleton 1953). A mixture of 97% O₂ and 3% CO₂ was bubbled through a sterile gas filter into the perfusate in the lower reservoir through an air ejector port inserted into the delivery tube, by which system the fluid was transported to the upper chamber (reservoir) through a Cobe 20 micron filter. At a gas flow of approximately 500 ml/min this gas solution maintained perfusate pH at 7.0 - 7.8 and maintained perfusate flow into the upper chamber at approximately 60 - 120 ml/min. Perfusate pO₂ has been measured at a partial pressure of between 1000 - 2000 mmHg determined at 30°C.

From the upper chamber, perfusate flowed by gravity into the ascending aorta of the suspended heart, perfusing the coronary arteries at a pressure of approximately 8-10 cmH₂O depending on aortic length. Coronary venous effluent to the right atrium returned to the lower reservoir by gravity drainage via the inferior vena cava or pulmonary artery orifices. From the lower chamber, the perfusate was recirculated. An overflow pipe between the upper and lower reservoirs prevented the upper chamber from filling completely with fluid, and thus limited the maximum hydrostatic pressure at which the myocardium could be perfused.

Three litres of perfusate were initially added to the lower chamber. The perfusion apparatus was then placed in a stainless steel container, insulated with polystyrene and perspex, and packed with ice to maintain the desired temperature of 4-10°C throughout the preservation period.

Extensive previous studies in the pig and baboon have shown this hypothermic perfusion system to be consistently successful in storing freshly excised isolated hearts for periods of 24 hours (Wicomb et al. 1981, Wicomb et al. 1983). An extension of this period to 48 hours has been achieved (Wicomb et al. 1985).

The coronary flow (CF) of the perfused heart and the coronary sinus effluent perfusate lactate dehydrogenase (LDH) were measured both at the onset and end of the period of storage.

Functional testing and myocardial biochemical studies were as in the previous group of experiments (Chapter 4A), with which the results may be compared to show the added effect of storage on those of brain death alone.

EXPERIMENTAL GROUPS

Five groups of pigs were studied.

Group A2 - Freshly excised + 24 hours storage

Fresh hearts were excised under identical conditions to those described in Group A1 but were then stored by hypothermic perfusion for 20-24 hours before being functionally tested.

Group B2 - Induction of brain ischaemia/death + 24 hours storage

These hearts underwent brain ischaemia/death and excision (as did the hearts in Group B1), but were then stored for 20-24 hours before being functionally tested.

Group C2 - Brain death + hypotension (minimal haemodynamic support) + 24 hours storage

These hearts underwent identical procedures to those in Group C1, but were then stored for 20-24 hours before being functionally tested.

Group D2 - Brain death + normotension (fluid and dobutamine support) + 24 hours storage

These hearts underwent identical procedures to those in Group D1, but were then stored for 20-24 hours before being functionally tested.

Group E2 - Brain death + normotension (vasopressin support) + 24 hours storage

These hearts underwent identical procedures to those in Group D1, but instead of dobutamine being given as support of the circulation, intermittent vasopressin (0.1 - 0.25 u/kg/i.m.) was administered. After 4 hours the hearts were excised, stored for 20-24 hours, and functionally tested.

Observations during 20-24 hours Storage by Continuous Hypothermic Perfusion (Table 4B-2)

Measurements of CF at the onset and end of the 20-24 hours period of storage are compared in Table 4B-2; changes in perfusate LDH are also shown. There was no statistical difference in CF at the onset of storage between any of the groups, suggesting that brain ischaemia or brain death with or without haemodynamic support did not immediately alter CF. In all groups, however, there was a significant fall in CF during the period of storage. The induction of a period of brain ischaemia alone (Group B2) did not cause a more marked percentage fall in CF during storage when compared with freshly excised hearts (Group A2). At the end of the storage period, CF was significantly lower in Group E2 when compared with group A2, and in Groups C2, D2, and E2 when compared with Group B2. The percentage reduction in CF was, therefore, greater in those animals in which the heart had remained in the donor under brain dead conditions for 4 hours before excision (Groups C2, D2, and E2), particularly in those which had received i.v. fluid and dobutamine or vasopressin support (Groups D2 and E2).

Perfusate LDH was significantly raised at the onset of the storage period in Group B2, which pigs had undergone a period of brain ischaemia only. LDH also rose significantly

during storage in all groups. At the end of the storage period, LDH levels were significantly higher in Groups C2 and E2 when compared with Group A2. The percentage change in LDH between the onset and end of the storage period was again much more marked in those groups where the heart had remained in the brain dead animal for 4 hours (Groups C2, D2 and particularly E2) when compared with Groups A2 and B2.

RESULTS

Functional Testing (Table 4B-3)

A period of brain ischaemia or death led to a reduction in CO in all groups (B2-E2) when compared with the fresh, stored hearts of Group A2 (Table 4B-3). The fall in CO was greater in two of those groups (D2 and E2) which had been allowed to continue functioning in the brain dead animal for 4 hours. There was similarly a significant decrease in SV in Groups C2, D2, and E2 when compared with Group A2. LVP was also lower in Groups B2, D2, and E2, though in Group C2 the fall did not reach statistical significance. LVEDP was higher in Groups D2 and E2. CF remained within control levels in all groups.

Myocardial High Energy Phosphates, Lactate, and Glycogen (Table 4B-4)

ATP and CP were measured in hearts in Groups A2, C2 and D2 (Table 4B-4). ATP was significantly reduced after storage in hearts taken from brain dead pigs (Groups C2 and D2) compared with freshly excised stored hearts (Group A2). CP was not reduced in Group D2. Tissue lactate was measured only in Groups C2 and D2, but fell within the normal range, suggesting lactate diffusion into the perfusate and a decreased rate of anaerobic metabolism was taking place in these hearts as a result of low temperature (4-10°C). Glycogen levels in all 3 groups were within the normal range.

DISCUSSION

I would conclude, therefore, that storage of fresh hearts for 24 hours by continuous hypothermic perfusion leads to a minimal reduction in subsequent myocardial function and no reduction in myocardial high energy phosphates. Haemodynamic support of the brain dead animal by i.v. fluids, dobutamine and/or vasopressin may have a deleterious effect on myocardial function if the heart is to be stored subsequently. Storage, however, would appear to diminish the rate of anaerobic metabolism seen in hearts functioning in brain dead pigs and leads to replenishment of the glycogen stores, though these changes did not appear to be sufficient to result in a return to normal myocardial function.

LEGENDS**FIGURES****Figure 4B-1**

Schematic representation of the portable hypothermic perfusion system.

University of Cape Town

TABLE 4B-1
CONSTITUTION OF THE PERFUSATE USED FOR CONTINUOUS
24 HOURS HYPOTHERMIC PERFUSION (GROUPS A2 – E2 + D4)

	g/l	mM
NaCl	7.98	143.80
MgSO ₄ ·7H ₂ O	3.48	14.40
CaCl ₂ ·2H ₂ O	0.16	1.10
KH ₂ PO ₄	0.24	1.73
K ₂ HPO ₄	1.11	6.34
Procaine hydrochloride	0.27	1.10
Chlorpromazine	0.0031	–
Phenoxybenzamine	0.0025	–
Glucose	2.00	11.0
Sucrose	2.50	7.00
Glycerol	12.60	136.00
Taurine	0.50	4.00
Osmolality	410mOsm	
ph	6.85-6.95	

TABLE 4B-2 OBSERVATIONS ON CORONARY FLOW AND PERFUSATE LACTATE DEHYDROGENASE DURING 20 - 24 HOURS OF HYPOTHERMIC PERFUSION STORAGE (GROUPS A2-E2) (Bonferroni inequality level of significance $p < 0.005$).

GROUP	n	CORONARY FLOW ml/min/100g				PERFUSATE LDH (U/L)			
		Beginning of storage	End of storage	Statistical difference between onset and storage (p)	Percentage change	Beginning of storage	End of storage	Statistical difference between onset and storage (p)	Percentage change
A2	10	15.2(1.65)*	10.8(1.22)	<0.02	-29	15(4.1)	25(4.1)	<0.03	+67
B2	6	21.0(3.39)	15.0(1.17)	<0.05	-29	64(11.2)	87(22.8)	<0.05	+36
C2	6	15.4(1.59)	6.8(1.46)	<0.002	-56	28(4.9)	76(7.7)	<0.0003	+171
D2	6	19.5(3.11)	5.4(1.02)	<0.006	-72	30(2.0)	70(14.4)	<0.03	+133
E2	6	15.6(1.97)	4.9(0.40)	<0.002	-69	18(2.8)	63(6.6)	<0.002	+250

*Figures in brackets represent standard error of the mean

TABLE 4B-3
HAEMODYNAMIC PERFORMANCE ON FUNCTIONAL
TESTING OF STORED HEARTS (GROUPS A2 – E2
(Bonferroni inequality level of significance $p < 0.0008$).

GROUP	n	CO (mL/min)	SV (mL)	LVP (mmHg)	LVEDP (mmHg)	CF (mL/min)	(p)
A2	10	2500(90)*	18.3(0.74)	114(3.1)	4.8(1.05)	362(31)	
B2	6	1728(262)	14.3(1.47)	100(6)	5.77(0.80)	278(47)	NS
C2	7	1242(248)	11.3(2.61)	108(7)	8.8(2.64)	352(56)	NS
D2	9	742(65)	8.7(1.53)	97(3)	16.0(1.98)	309(24)	<0.005
E2	10	961(143)	8.1(1.49)	94(3)	14.7(3.53)	288(15)	<0.05

*Figures in brackets represent standard error of the mean

(p) = Statistical difference from A2

NS = Not statistically significant

TABLE 4B-4 MEASUREMENT OF HIGH ENERGY PHOSPHATES, LACTATE AND GLYCOGEN IN STORED HEARTS (GROUPS A2, C2, D2 + D4).

GROUP	n	ATP ($\mu\text{mol/g}$)** (p)	CP ($\mu\text{mol/g}$) (p)	Lactate ($\mu\text{mol/g}$) (p)	Glycogen ($\mu\text{mol/g}$) (p)
A2	10	3.50(0.201)*	5.30(0.520)	Not measured (normal range = 0-5)	26.1(2.42)
C2	4	2.49(0.370)	5.07(0.169)	2.80(1.190)	25.4(4.57)
D2	7	2.22(0.365)	5.01(1.20)	1.34(0.09)	26.8(0.55)

* Figures in brackets represent standard error of the mean

** Fresh weight

(p) = Statistical difference from A2

NS = Not statistically significant

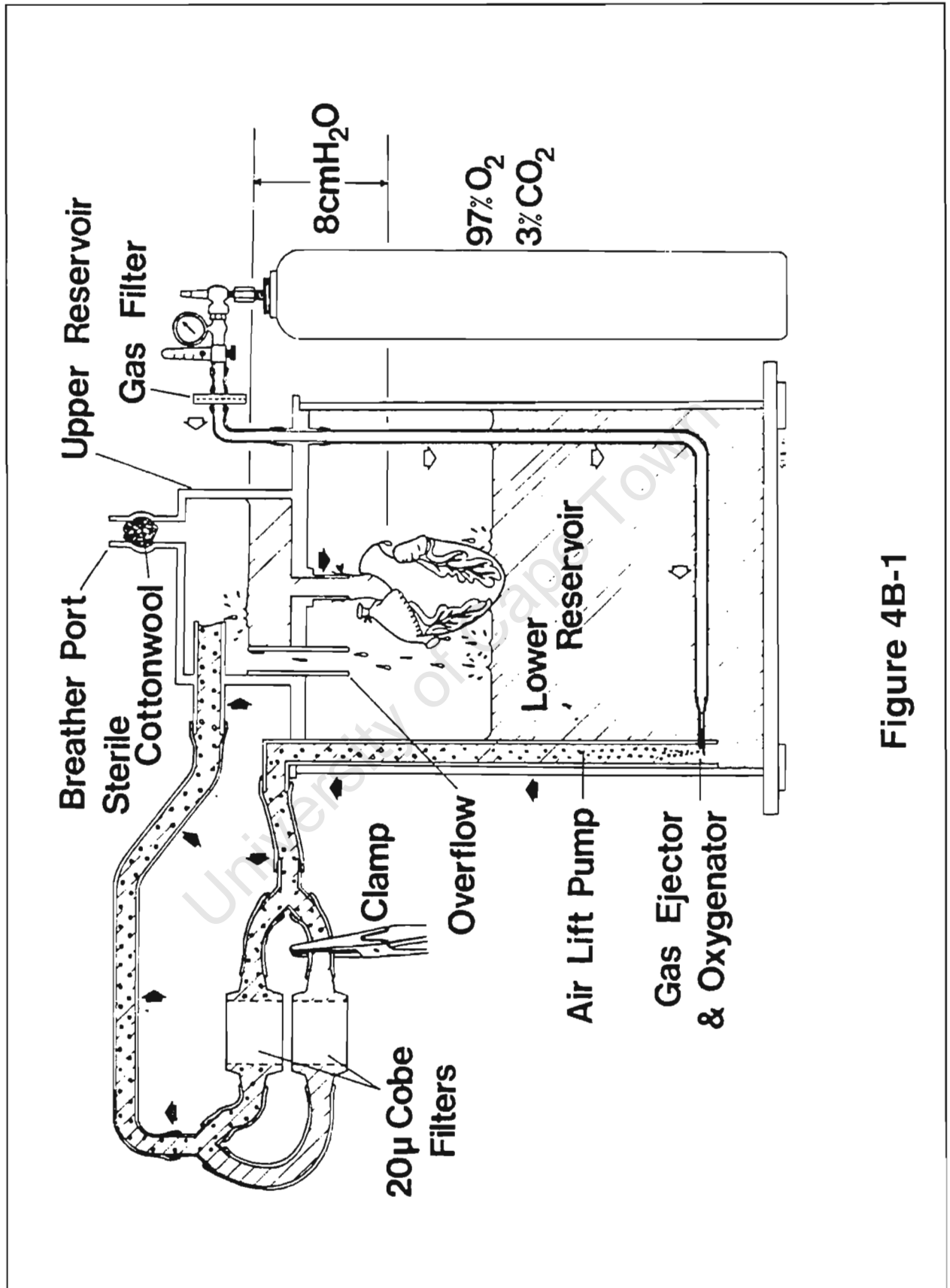


Figure 4B-1

CHAPTER 5

THE EFFECT OF HORMONAL THERAPY ON MYOCARDIAL FUNCTION AND ENERGY STORES IN THE BRAIN-DEAD PIG

INTRODUCTION

The deterioration of function in brain-dead baboons was clearly associated with depletion of both myocardial energy stores (Chapter 4) and certain circulating hormones, notably T_3 , cortisol, and insulin (Chapter 2D-1). An investigation, therefore, was carried out of the effects on both myocardial function and energy reserves of the administration of these three hormones to brain-dead pigs; the value of such hormonal therapy was assessed with and without the addition of a 24 hour period of storage.

Group D3 - Brain death + normotension (fluid and dobutamine support) + hormonal therapy

This group underwent identical procedures to those in the previous Group D1 for 4 hours following arterial ligations; at this time, hormonal therapy was instituted. This consisted of the i.v. administration of triiodothyronine (T_3) (2 μ g), cortisol (100 mg), and insulin (5-10 International Units according to the blood glucose as estimated by the dextrostix test); after one hour this therapy was repeated. After a further hour, the heart was excised and functionally tested.

Group D4 - Brain death + normotension (fluid and Dobutamine support) + hormonal therapy + 24 hours storage.

These hearts underwent identical procedures to those in Group D3, but were then stored for 20-24 hours before being functionally tested.

RESULTS (Tables 5-1, 5-2, 5-3, 5-4, 5-5)

Group D3 hearts showed a return towards control levels in respect of CO (Figure 5-1) and SV (Figure 5-2), but LVP remained reduced. Measured levels of CP (Figure 5-3), glycogen (Figure 5-4) and lactate (Figure 5-5) did not differ significantly from the control Group A1 (Chapter 4).

During the period of hypothermic perfusion storage (in hearts of Group D4) there was a significant fall in CF (as in all the previous stored Groups A2 - E2). Perfusate LDH was significantly raised at the onset of storage, and rose more markedly in Group D4 than in the previous hearts stored following brain death in pigs (Groups C2 - E2).

Myocardial function was not significantly different in Group D4 when compared with freshly excised stored hearts (Group A2), though CF was significantly increased (Figure 5-6 to 5-9).

ATP was not significantly different in Group D4 from the control stored hearts (A2), suggesting replenishment of the energy stores (Figure 5-10); CP was significantly increased in Group D4 (Figure 5-11). Tissue lactate, however, was raised in the hormonally treated hearts of Group D4, compared with the normal levels measured in similar brain dead stored hearts taken from pigs not receiving hormonal therapy (D2). Glycogen levels were similar in D4 to other groups (C2 - E2) (Figure 5-12).

COMMENT

Hormonally treated stored hearts (D4) showed no significant difference from non-stored hearts (D3), with the exception that coronary flow was markedly increased after storage.

Storage led to a significant increase in CP, but ATP and glycogen remained insignificantly changed. Lactate levels remained high, but storage did not significantly alter this increase. Both aerobic and anaerobic metabolism would therefore appear to be increased in hormonally treated stored hearts.

In this study, the administration of the hormonal mixture of T_3 , insulin, and cortisol led to a remarkable recovery of myocardial function and high energy phosphates (D3 vs C1). Myocardial function in the hormonally treated group (D3), however, was not significantly different from that in the freshly excised hearts of Group A1 except for a reduced LVP. The CO of functionally tested pig hearts has been found to be a most sensitive parameter of function in previous studies (Wicomb et al. 1981); it is therefore, particularly notable that CO should be normal in brain dead pig hearts which had been treated with hormones in this way.

Following the administration of hormones, myocardial high energy stores and glycogen returned to normal levels, and lactate levels were correspondingly reduced; hormonal therapy would therefore appear to reduce the rate of anaerobic metabolism occurring in hearts in brain dead subjects.

Hormonal therapy reversed the deterioration in myocardial function which occurred following storage. Myocardial function in these hearts was indistinguishable from that in the control Group A2. Similarly, the significant reduction in ATP which occurred following storage of hearts taken from brain dead pigs (Groups C2 and D2) was reversed by hormonal therapy, which also resulted in increases in CP and lactate production.

It would appear that brain death in pigs is followed by a consumption of myocardial energy

stores which, despite anaerobic glycolysis, the brain dead animal is unable to replenish. This is associated with reduced myocardial function. The administration of hormonal therapy to the brain dead pig for a period as short as only 2 hours leads to replenishment of myocardial energy and glycogen reserves and reduction in lactate, with associated improvement in haemodynamic function.

The myocardial tissue damage associated with brain death as a result of the sympathetic "storm" is manifest by an increased perfusate LDH level observed at the onset of the storage period. The raised LDH in the hormonally treated hearts (Group D4) was unexpected and does not initially appear to correlate with the other findings in this study, which suggest that hormonal therapy is beneficial to the heart. The marked increase in perfusate LDH during the storage period in this group is also contrary to the observations made on well-preserved hearts taken from non-brain dead animals (Group A2), where there was little increase in LDH during storage. The opinion arrived at was that a perfusate LDH greater than 80-100 μl in stored hearts taken from brain-viable pigs strongly suggested myocardial damage, which would result in poor subsequent function. This present study does not confirm that finding.

The observations that both better function and an increase in myocardial energy stores occurred in the D4 hearts (when compared with the C2 and D2 hearts), even though perfusate LDH rose to significantly higher levels during hypothermic perfusion storage, and tissue lactate levels remained high, suggests that T_3 may promote both aerobic and anaerobic metabolism in brain-dead animals.

A possible explanation for these observed high perfusate LDH and tissue lactate levels might be that T_3 therapy increased, or at least maintained the normal rate of protein synthesis during the agonal period. When the heart is subjected to a period of low temperature perfusion, membrane structural changes occur, which influence the

transport of molecules across the membrane. Increased LDH synthesis, resulting from T_3 therapy, would result in a higher release of LDH into the perfusate during the period of storage. The higher levels of tissue lactate seen in the hormonally treated brain-dead animals would also indicate increased LDH activity or concentration in the tissues.

The exact mechanisms by which T_3 , cortisol, and insulin bring about their effects in the brain-dead animal remain uncertain. The role of T_3 will be discussed later (Chapter 7). Its functions and mode of action in the normal subject have been extensively reviewed by Hoch (1962), Horowitz (1977), Limas (1978), Sterling (1979), Oppenheimer (1979), Sestoft (1980), and Dreyer (1984). Cortisol has a large number of activities, and has been the subject of numerous reviews (Labhart 1974, Liddle 1981, Sherman 1984, Davies 1984), as has the role of insulin (Constance 1974, Porte & Halter 1981, Danforth 1983), Watanabe 1984, Kahn 1985.

LEGENDS

FIGURES

Figure 5-1

Mean cardiac output on functional testing of isolated hearts taken from 3 groups of pigs (A1 = freshly excised hearts; D1 = hearts taken from brain-dead pigs after 4 hours supportive therapy with i.v. fluids and dobutamine; D3 = hearts taken from brain-dead pigs after 4 hours supportive therapy but with the addition of two hours hormonal therapy with T₃, cortisol, and insulin. Statistical significances (p) between test and control groups are indicated. There is no significant difference between Groups A1 and D3.

Figure 5-2

Mean stroke volumes on functional testing of isolated hearts taken from 3 groups of pigs as per Figure 5-1.

Figure 5-3

Mean myocardial creatine phosphate (CP) levels from isolated hearts taken from 3 groups of pigs as per Figure 5-1.

Figure 5-4

Mean myocardial glycogen levels from isolated hearts taken from 3 groups of pigs as per Figure 5-1.

Figure 5-5

Mean myocardial lactate levels from isolated hearts taken from 3 groups of pigs as per Figure 5-1.

Figure 5-6

Mean cardiac output on functional testing of isolated stored hearts taken from 3 groups of pigs and compared with data from 3 non-stored groups (A1, D1, and D3) shown previously in Figure 5-1. (A2 = freshly excised hearts stored for 24 hours; D2 = hearts taken from brain-dead pigs after 4 hours supportive therapy with i.v. fluids and dobutamine and subsequently stored for 24 hours; D4 = hearts taken from brain-dead pigs after 4 hours supportive therapy as in Group D2 but with the addition of 2 hours hormonal therapy with T_3 , cortisol, and insulin, and subsequently stored for 24 hours). The statistical differences between Groups D2 and D4 compared with Group A 2 are shown.

Figure 5-7

Mean stroke volume on functional testing of isolated stored hearts taken from 3 groups of pigs (Legend as in Figure 5-5).

Figure 5-8

Mean left ventricular end diastolic pressure on functional testing of isolated stored hearts taken from 3 groups of pigs (Legend as in Figure 5-6).

5-8

Figure 5-9

Mean coronary flow on functional testing of isolated stored hearts taken from 3 groups of pigs (Legend as in Figure 5-6).

Figure 5-10

Mean myocardial ATP levels from isolated stored hearts taken from 3 groups of pigs (Legend as in Figure 5-6).

Figure 5-11

Mean myocardial CP levels from isolated stored hearts taken from 3 groups of pigs (Legend as in Figure 5-5).

Figure 5-12

Mean myocardial glycogen levels from isolated stored hearts taken from 3 groups of pigs (Legend as in Figure 5-6).

TABLE 5-1 HAEMODYNAMIC PERFORMANCE ON FUNCTIONAL TESTING OF HEARTS NOT SUBJECTED TO STORAGE (GROUPS A1 - D1 AND D3)
(Bonferroni inequality level of significance $p < 0.017$).

GROUP	n	CO (ml/min)	SV (ml)	LVP (mmHg)	LVEDP (mmHg)	CF (ml/min/100G)	(p)
A1	10	2820(220)*	17(1.3)	131(4.4)	5.3(1.03)	258(33)	
B1	6	2738(385)	19(2.5)	124(6.4)	6.4(1.77)	339(67)	NS
C1	6	1537(263)	13(1.5)	109(4.2)	6.6(1.08)	296(46)	NS
D1	6	1830(329)	13(1.9)	102(5.3)	4.3(0.59)	378(53)	NS
D3	7	2357(406)	17(3.0)	108(4.8)	3.8(1.68)	335(23)	NS

*Figures in brackets represent standard error of the mean

(p) = Statistical difference from A1

NS = Not statistically significant

TABLE 5-2 MEASUREMENT OF HIGH ENERGY PHOSPHATES, LACTATE AND GLYCOGEN IN NON-STORED HEARTS (GROUPS A1, B1, C1 and D3)

GROUP	n	ATP ($\mu\text{mol/g}$)** (p)	CP ($\mu\text{mol/g}$) (p)	Lactate ($\mu\text{mol/g}$) (p)	Glycogen ($\mu\text{mol/g}$) (p)
A1	10	3.73(0.253)*	6.61(0.720)	3.9(0.91)	29.6(3.14)
B1	8	2.93(0.375)	5.57(0.900)	4.50(0.680)	22.6(4.39)
C1	6	3.29(0.227)	3.69(0.850)	12.9(3.39)	12.7(2.95)
D3	7	3.75(0.380)	6.80(1.140)	7.07(1.56)	30.5(4.21)

** Fresh weight

* Figures in brackets represent standard error of the mean

(p) = Statistical difference from A1

NS = not statistically significant

TABLE 5-3 OBSERVATIONS ON CORONARY FLOW AND PERFUSATE LACTATE DEHYDROGENASE DURING 20 - 24 HOURS OF HYPOTHERMIC PERFUSION STORAGE (GROUPS A2-E2 + D4) (Bonferroni inequality level of significance $p < 0.005$).

GROUP	n	CORONARY FLOW mℓ/min/100g				PERFUSATE LDH (Uℓ)			
		Beginning of storage	End of storage	Statistical difference between onset and end of storage (p)	Percentage change	Beginning of storage	End of storage	Statistical difference between onset and end of storage (p)	Percentage change
A2	10	15.2(1.65)*	10.8(1.22)	<0.02	-29	15(4.1)	25(4.1)	<0.03	+67
B2	6	21.0(3.39)	15.0(1.17)	<0.05	-29	64(11.2)	87(22.8)	<0.05	+36
C2	6	15.4(1.59)	6.8(1.46)	<0.002	-56	28(4.9)	76(7.7)	<0.0003	+171
D2	6	19.5(3.11)	5.4(1.02)	<0.006	-72	30(2.0)	70(14.4)	<0.03	+133
E2	6	15.6(1.97)	4.9(0.40)	<0.002	-69	18(2.8)	63(6.6)	<0.002	+250
D4	6	22.9(1.35)	7.5(1.04)	<0.0001	-67	31(6.7)	140(13.2)	<0.002	+352

*Figures in brackets represent standard error.

TABLE 5-4 HAEMODYNAMIC PERFORMANCE ON FUNCTIONAL TESTING OF STORED HEARTS (GROUPS A2 - E2 + D4) (Bonferroni inequality level of significance $p < 0.008$).

GROUP	n	CO (mL/min)	(p)	SV (mL)	(p)	LVP (mmHg)	(p)	LVEDP (mmHg)	(p)	CF (mL/min/100G)	(p)
A2	10	2500(90)*		18.3(0.74)		114(3.1)		4.8(1.05)		362(31)	
B2	6	1728(262)	<0.05	14.3(1.47)	NS	100(6)	<0.05	5.77(0.80)	NS	278(47)	NS
C2	7	1242(248)	<0.005	11.3(2.61)	<0.005	108(7)	NS	8.8(2.64)	NS	352(56)	NS
D2	9	742(65)	<0.0001	8.7(1.53)	<0.0002	97(3)	<0.01	16.0(1.98)	<0.005	309(24)	NS
E2	10	961(143)	<0.0001	8.1(1.49)	<0.0001	94(3)	<0.005	14.7(3.53)	<0.05	288(15)	NS
D4	6	2074(276)	NS	15.2(1.94)	NS	103(4.5)	NS	4.6(1.8)	NS	607(99)	>0.05

*Figures in brackets represent standard error of the mean

(p) = Statistical difference from A2

NS = Not statistically significant

TABLE 5-5 MEASUREMENT OF HIGH ENERGY PHOSPHATES, LACTATE AND GLYCOGEN IN STORED HEARTS (GROUPS A2, C2, D2 + D4).

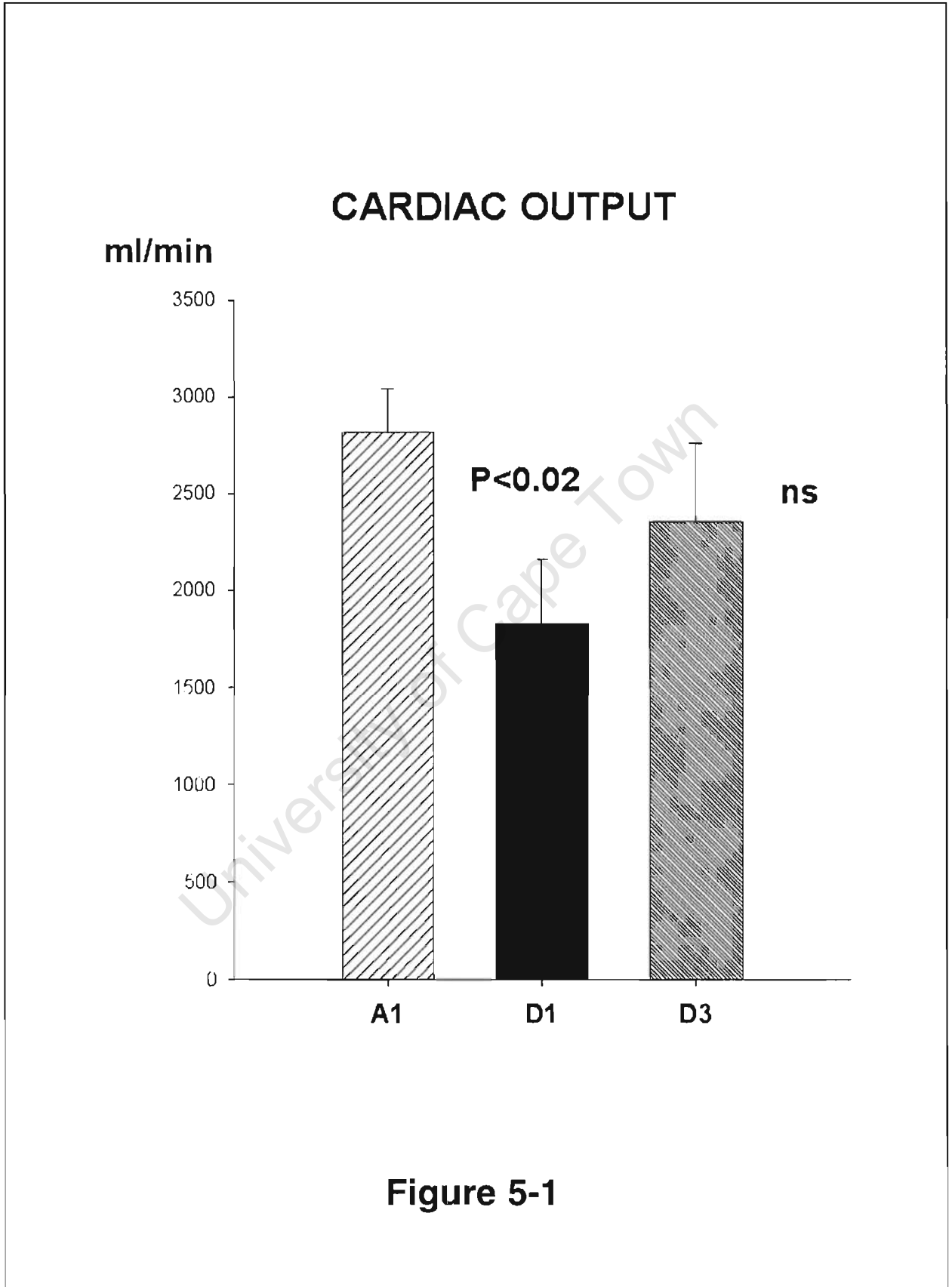
GROUP	n	ATP ($\mu\text{mol/g}$)** (p)	CP ($\mu\text{mol/g}$) (p)	Lactate ($\mu\text{mol/g}$) (p)	Glycogen ($\mu\text{mol/g}$) (p)
A2	10	3.50(0.201)*	5.30(0.520)	Not measured (normal range = 0-5)	26.1(2.42)
C2	4	2.49(0.370)	5.07(0.169)	2.80(1.190)	25.4(4.57)
D2	7	2.22(0.365)	5.01(1.20)	1.34(0.09)	26.8(0.55)
D4	6	3.10(0.186)	9.66(0.388)	9.64(1.668)	21.1(3.92)

* Figures in brackets represent standard error of the mean

** Fresh weight

(p) = Statistical difference from A2

NS = Not statistically significant



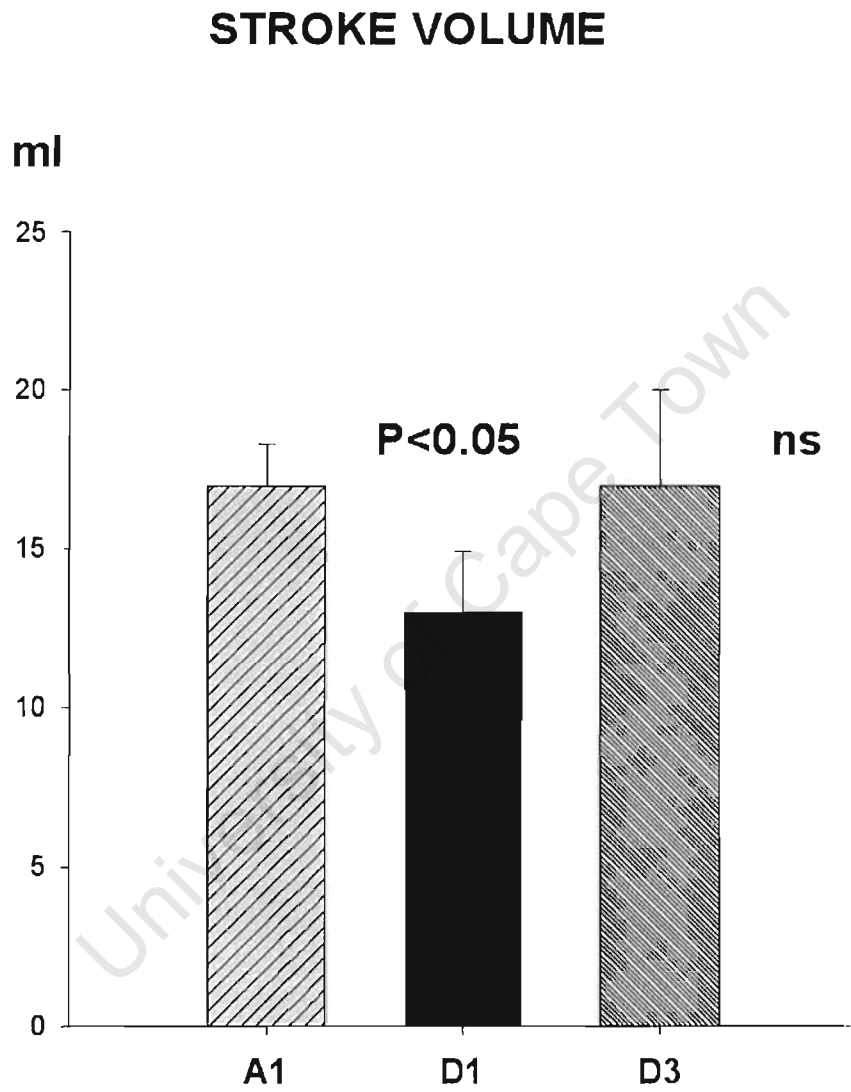


Figure 5-2

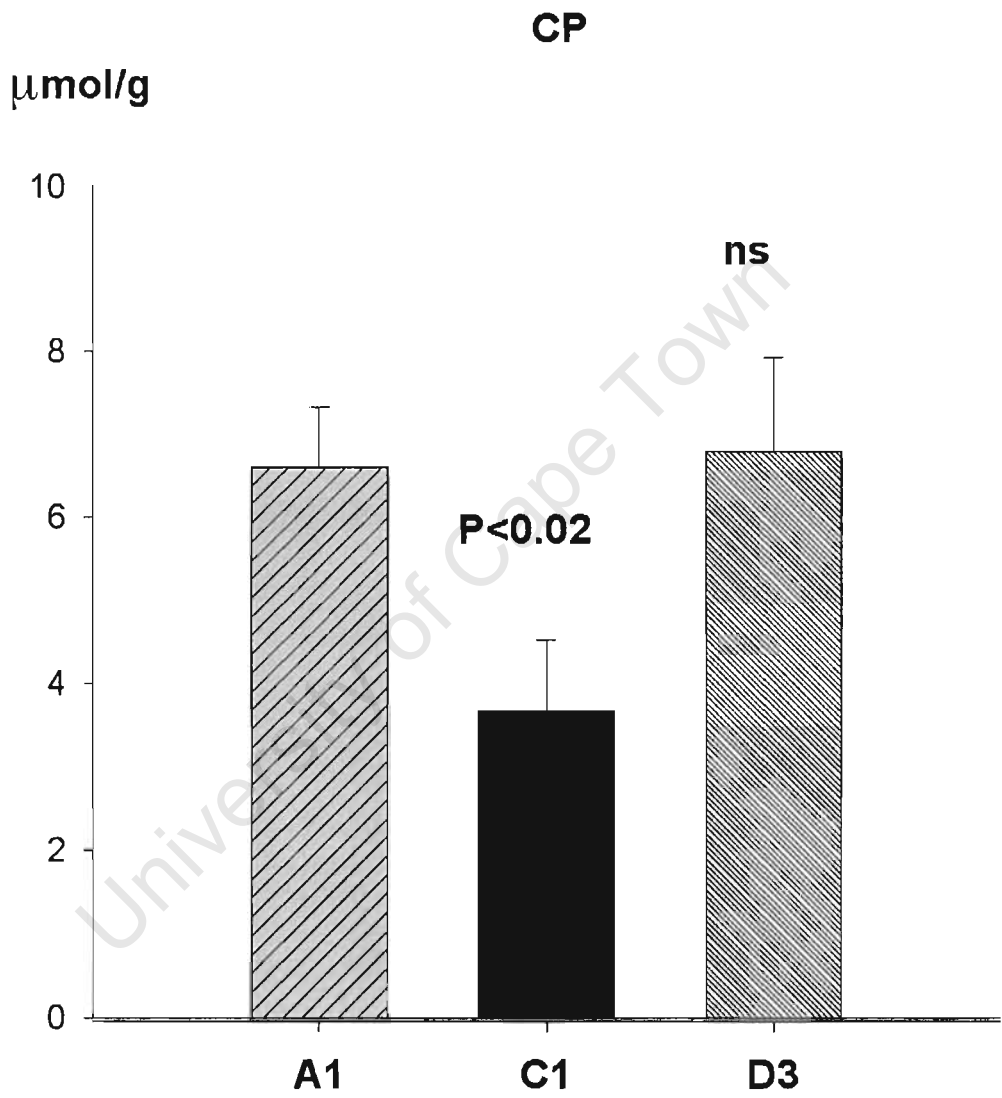


Figure 5-3

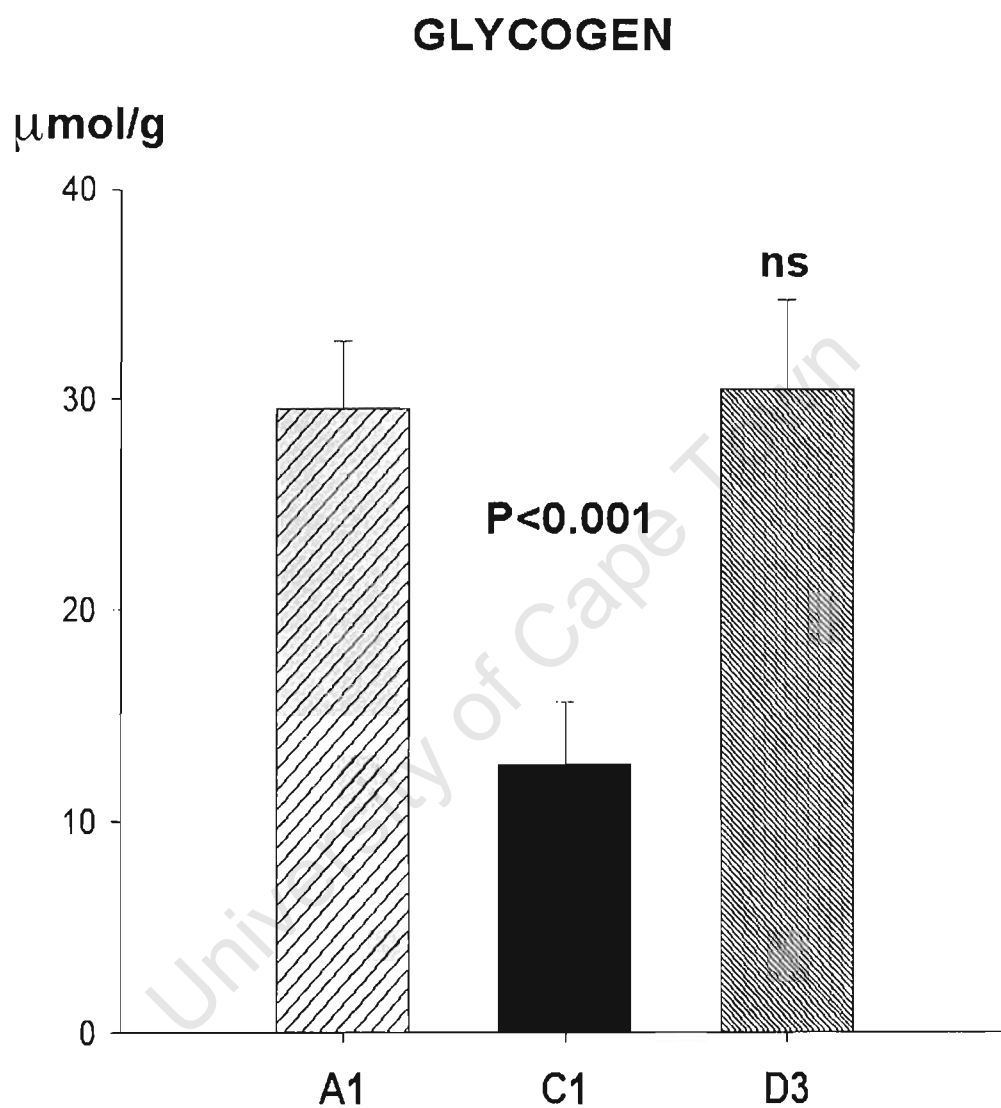
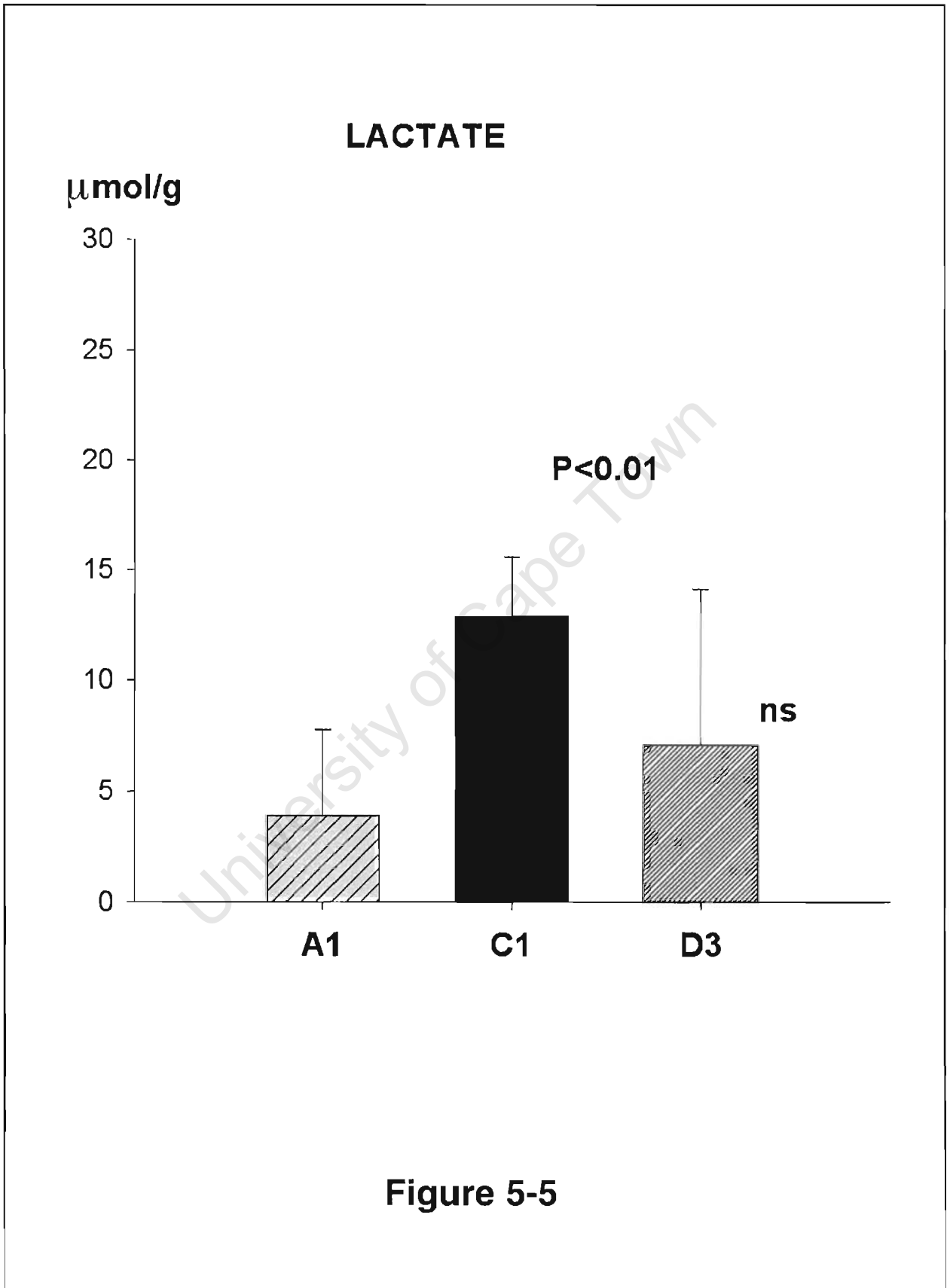


Figure 5-4



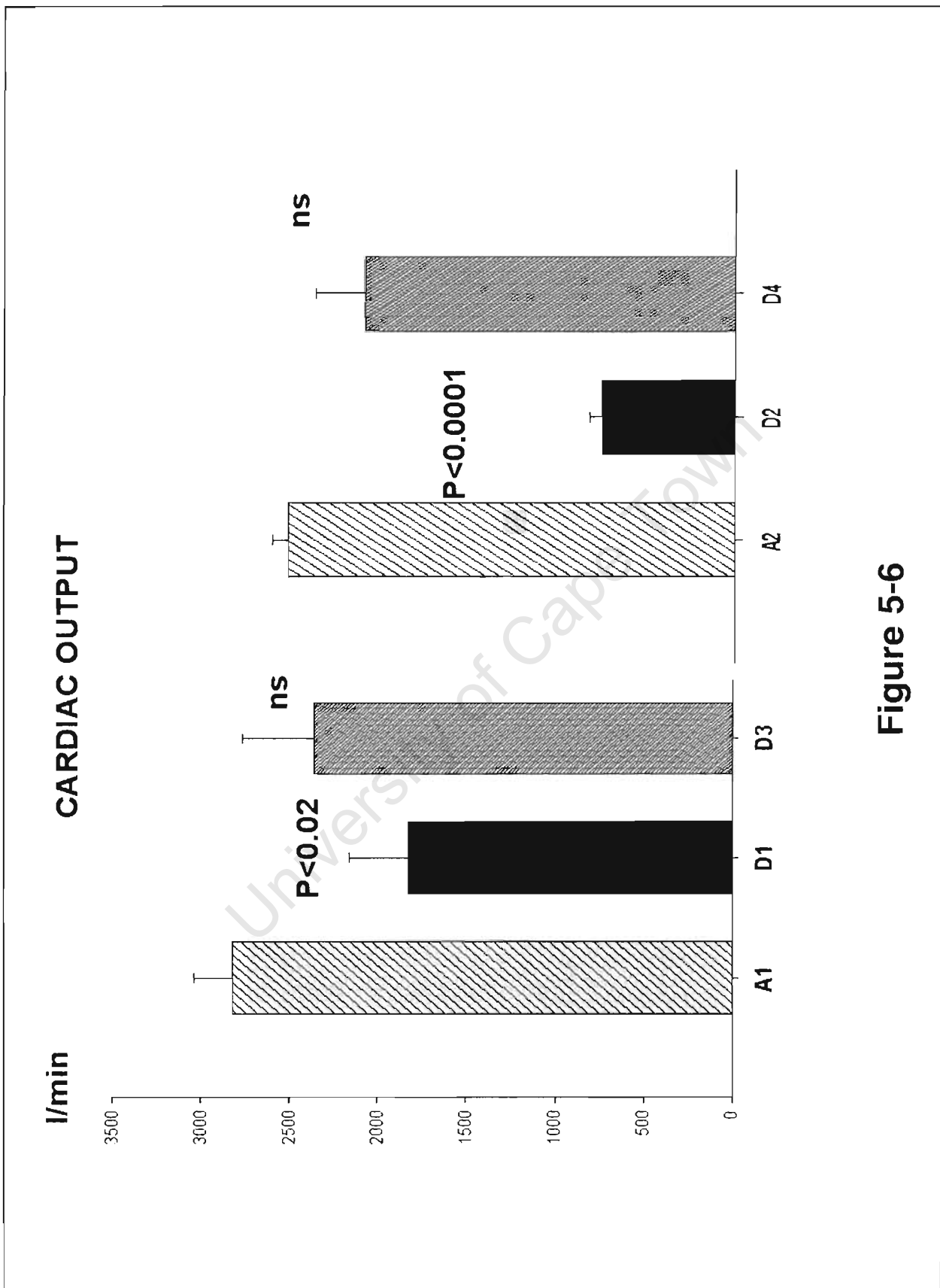


Figure 5-6

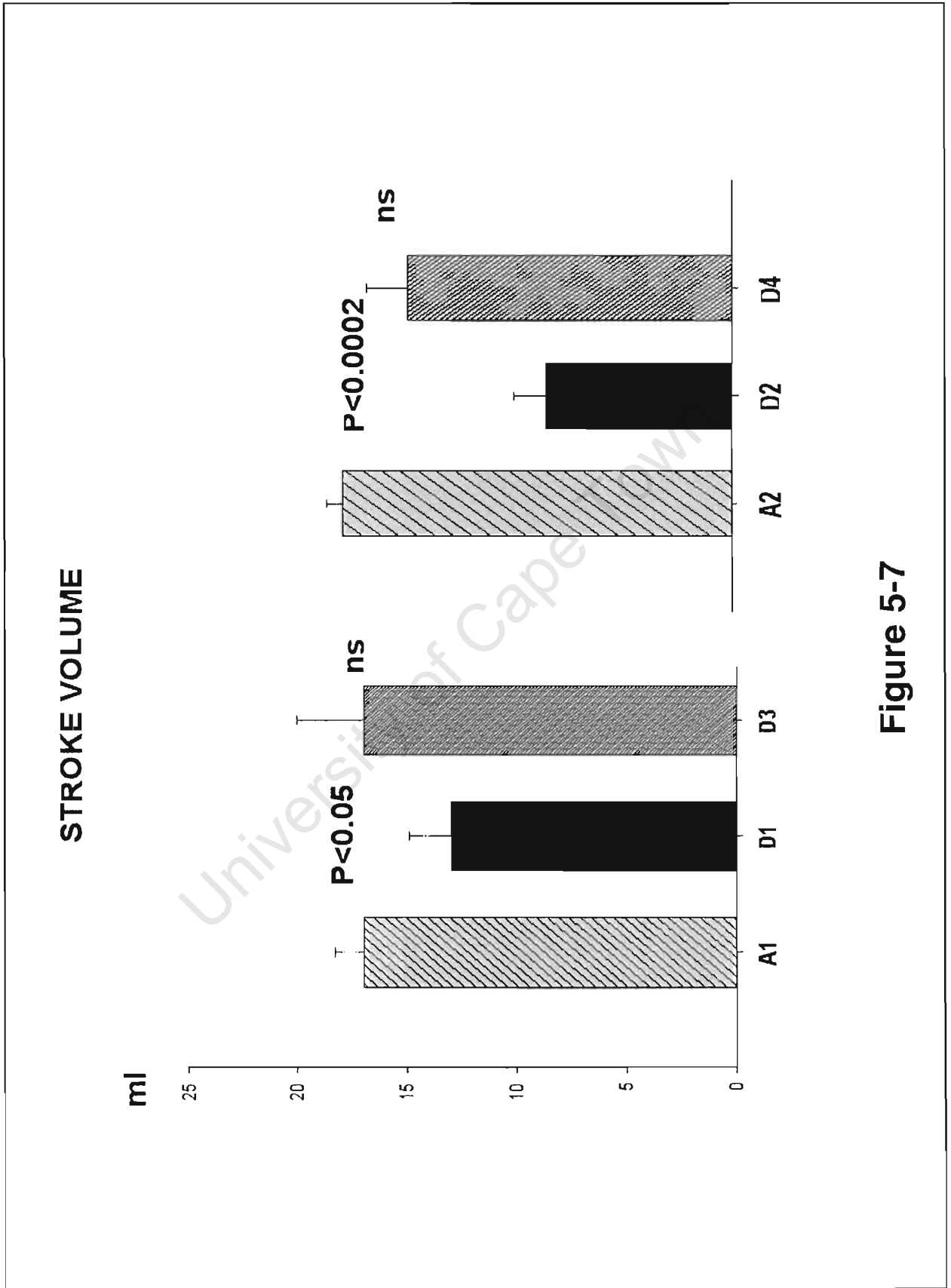


Figure 5-7

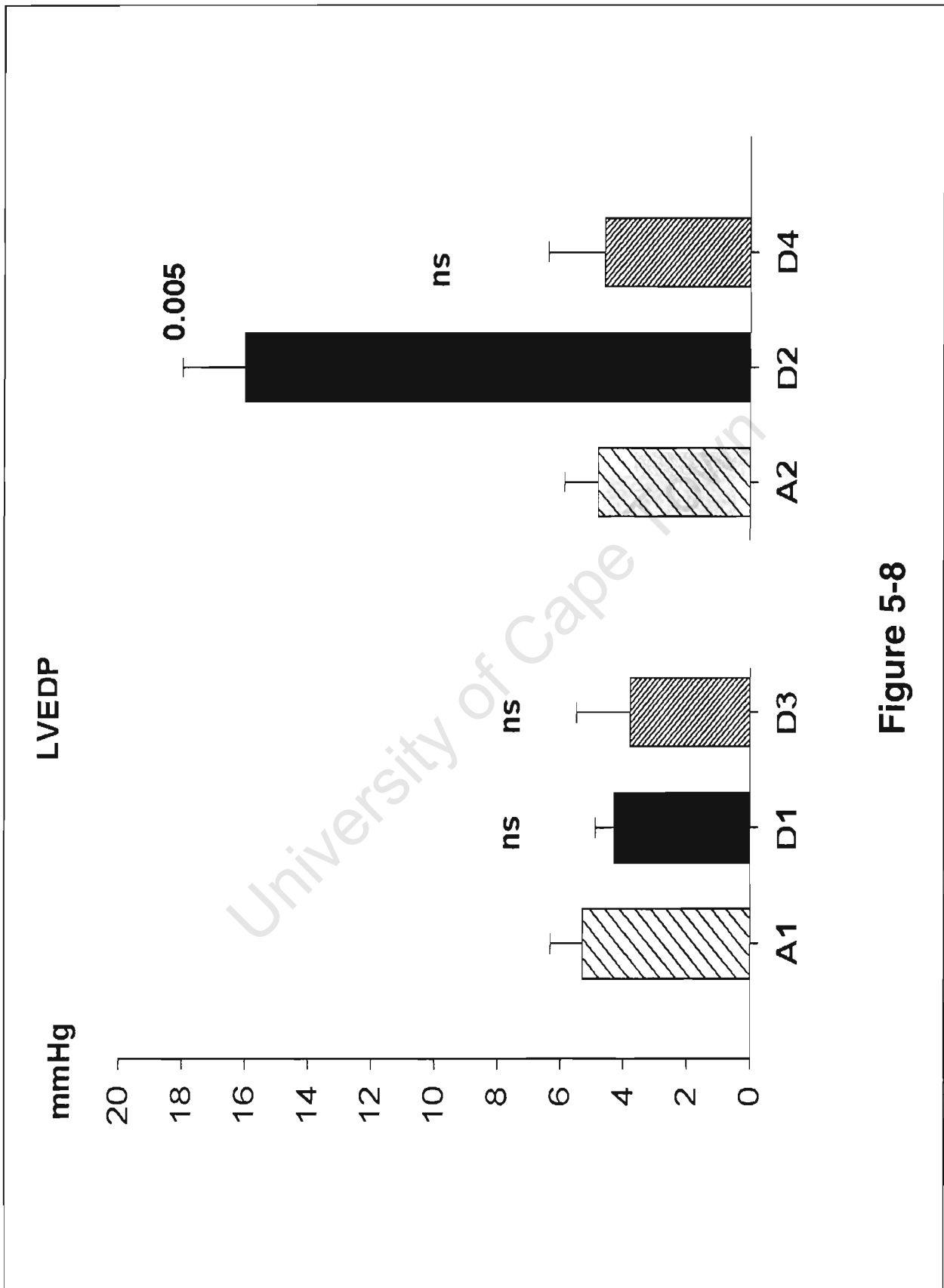


Figure 5-8

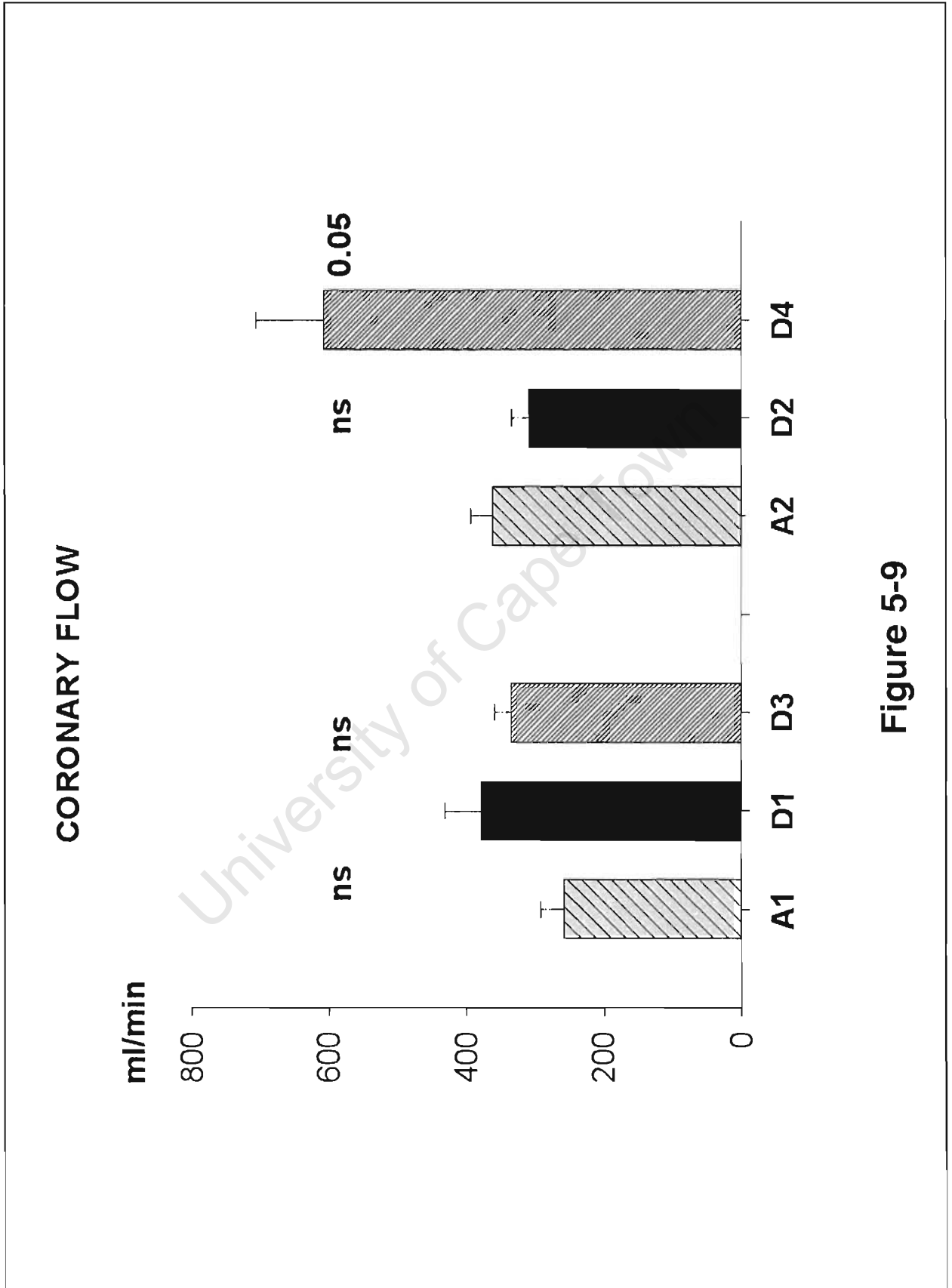


Figure 5-9

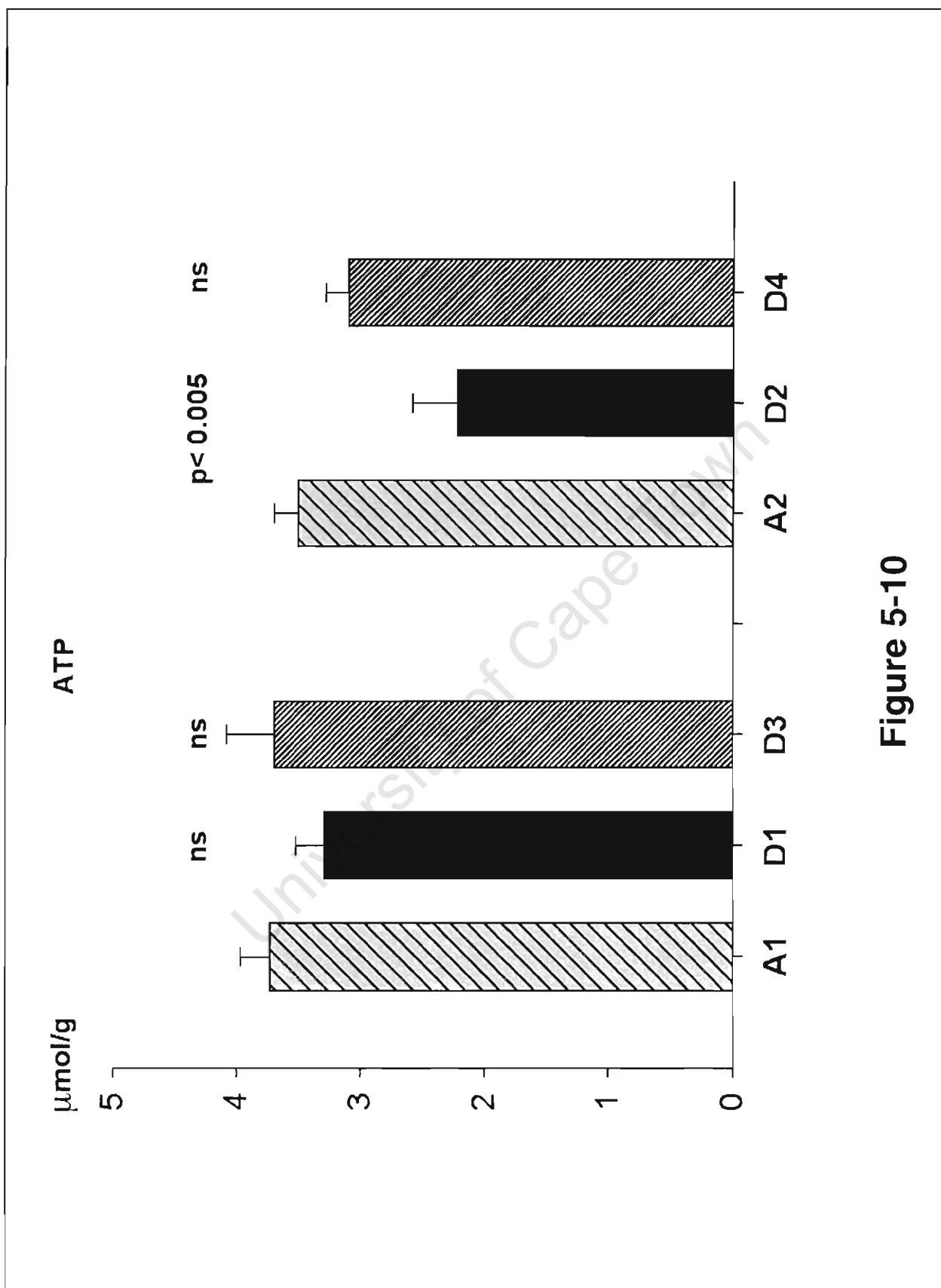


Figure 5-10

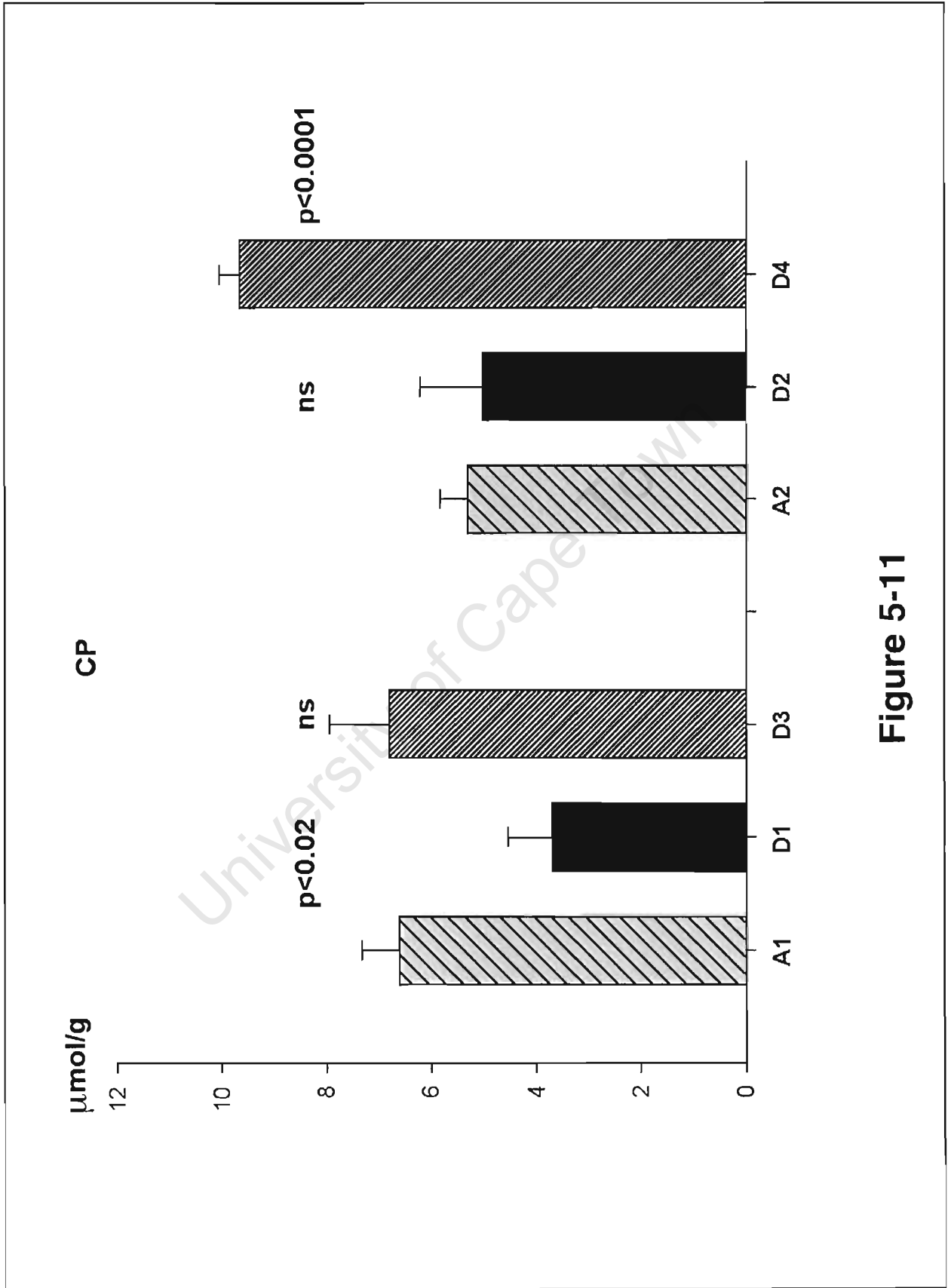


Figure 5-11

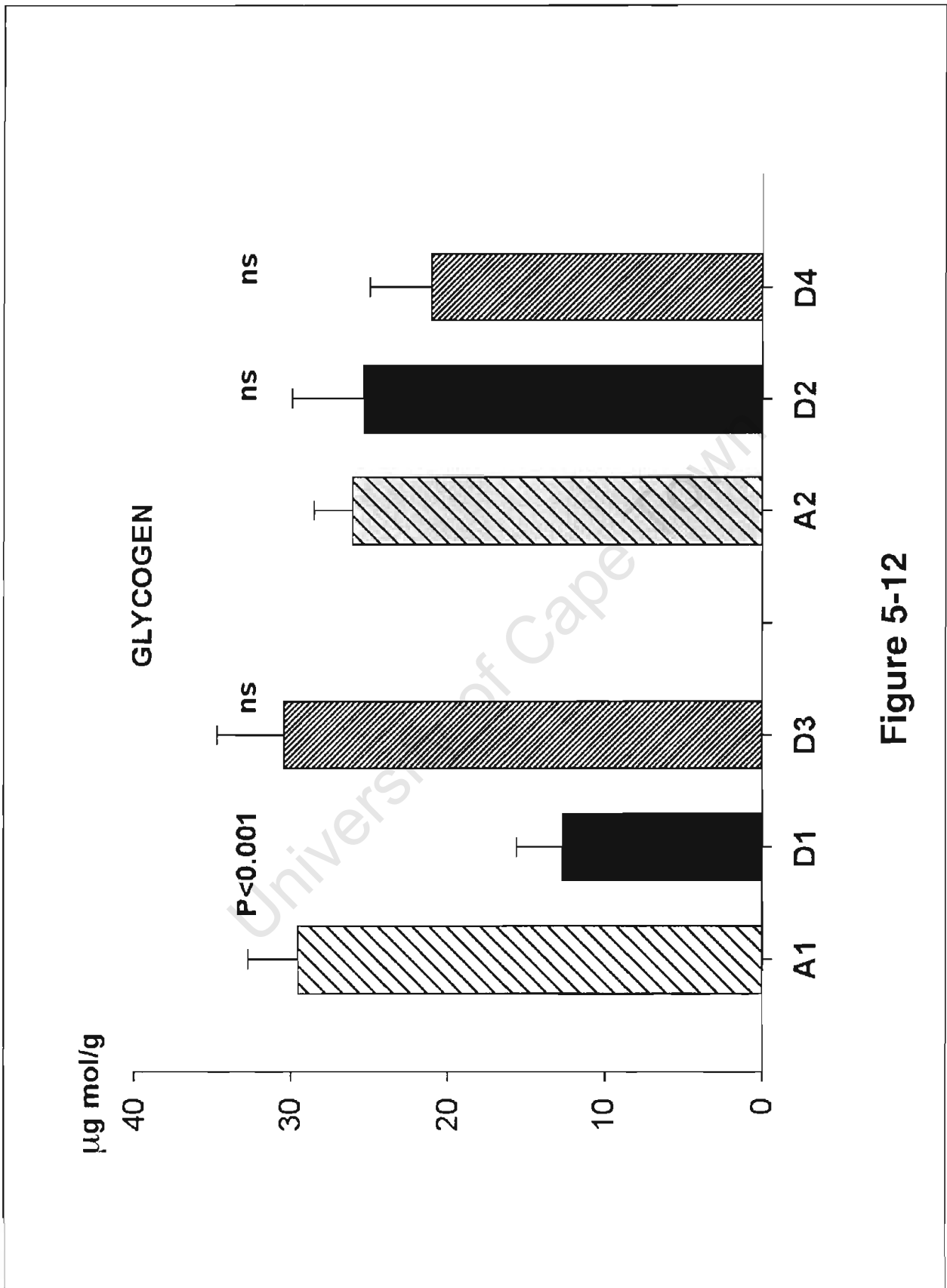


Figure 5-12

CHAPTER 6

THE EFFECTS OF THE AGONAL PERIOD AND HORMONAL THERAPY ON THE DONOR PIG KIDNEY

INTRODUCTION

Having confirmed that the agonal period leads to depletion of myocardial energy stores with diminished function, consideration was given to whether similar changes might occur in other organs, particularly those which might subsequently be transplanted, such as the kidney.

Kidneys were excised from various groups of pigs, some of which had undergone induction of brain death as described earlier. The cellular potassium/sodium ratio (K^+/Na^+) was calculated; there is good correlation between this ratio and kidney function (Collins 1982).

EXCISION OF THE KIDNEYS AND PREPARATION OF KIDNEY SLICES

Through a midline abdominal incision, both kidneys were mobilized, excised, and immersed in iced saline slush ($2^{\circ}C$). The warm ischaemic time never exceeded 90 seconds. After thermal equilibration, the renal arterial system was flushed at a pressure of 100 mm Hg with 100 ml of a low ionic strength intracellular solution (LIC) at $4^{\circ}C$ consisting of $NaHCO_3$, 0.94g/l (10 mM); $K_2 HPO_4 \cdot 3H_2O$, 4.20g/l (18.4 mM); KH_2PO_4 , 0.70g/l (5.1 mM); $MgCl_2 \cdot 6H_2O$, 0.4g/l (2.0 mM); glucose, 42g/l (233 mM) (24) (Collins and Halasz 1982). The time taken to perfuse each kidney was recorded in some animals. Each kidney was frontally divided, the capsule removed, and each half was dissected into six 2 x 2 cm

cubes; slices were cut from the cortical surface of each cube using a Stadie-Riggs microtome. Each cube yielded one slice. A total of 6 slices was therefore obtained from each kidney (3 from each half). Preparation of the slices was carried out at 4°C (Clark et al. 1984). The slices were either tested immediately (see below) or stored for 24 hours in LIC in ice before testing.

VIABILITY TESTING

Slices were placed in scintillation vials containing 3 ml Cross and Taggart solution, and gassed for 20 seconds with pure oxygen, and then quickly sealed (Cross 1950). The vials were placed in a shaker waterbath at 26°C (140 to 160 cycles/min) for one hour. Each slice was then removed from the incubation vial, rinsed four times in 300 mM mannitol to remove extracellular ions, and placed in 3 ml 3% trichloroacetic acid. After a minimum of one hour the supernatant potassium and sodium concentrations were determined by atomic absorption spectrophotometry, and the potassium-sodium (K^+/Na^+) ratio calculated.

EXPERIMENTAL GROUPS (Table 6-1)

Nine groups of kidneys were studied.

Groups A1, B1, C1, and D1 correlated exactly with the Groups A1-D1 of the myocardial studies (Chapters 4, 5), and Groups A2, B2, and D2 correlated with those in which heart storage had been carried out (Groups A2-D2). Groups D3 and D4 correlated with the similar groups in which the effect of hormonal therapy on myocardial function and biochemistry was investigated (Chapter 5).

RESULTS

Kidney Flush Infusion Time (Table 6-2)

In Groups A1, D1, and D3 the time taken to flush 100 ml of LIC using a pressure of 100 mmHg, was recorded (Table 6-2). Kidneys in Group D1 (brain death and normotension) flushed slowly, some regions being inadequately perfused; flush infusion time differed significantly in this group from the control Group A1. Mean flush infusion time of kidneys from hormonally treated pigs (Group D3) was not significantly different from that of the controls.

VIABILITY TESTING

1. Freshly excised kidney slices

In the control Group A1, the slice K^+/Na^+ ratio was 5.17 (Table 6-1). Animals that were subjected to the agonal (brain ischaemia) period only (Group B1) experienced a marked depression in slice ratio. In both groups C1 (brain death and hypotension) and D1 (brain death and normotension), slice functions were improved in comparison with B1, though these differences were not significant but they remained significantly depressed compared to A-1. Slice function of kidneys in Group D3 (brain death, normotension and hormonal therapy) was excellent and did not differ from the control value. (Figure 6-1).

2. Kidney slices stored for 24 hours

Storage resulted in a significant reduction in slice function in all groups except D1 when compared with the freshly excised kidney slices (Table 6-1). Slice function of Group B2

kidneys (brain ischaemia/death) differed significantly from the other groups (A2, D2, and D4), suggesting severe injury.

DISCUSSION

In this study, the viability of the kidneys was assessed by in vitro evaluation of renal slice function. Although kidney slice function was originally used to determine cellular p-amino hippurate accumulation, there is good correlation between the cellular K^+Na^+ ratio, measured by this method, and kidney viability (Fahy et al. 1979, Fahy et al. 1984). Mitochondrial integrity measures maintenance of the K^+Na^+ ratio by supplying sufficient energy to the potassium-sodium ATPase to actively maintain the ionic gradient; an imbalance in the gradient leads to failure of cell volume regulation. Thus the maintenance of an ionic gradient implies an intact energy-producing system within the cell. The ratio does not give information about the vascular system within the organ, but is purely a test of the integrity of the renal parenchyma. In previous studies on kidneys stored for 48 hours at -4°C kidney slice function showed a good correlation with whole organ function (Wicomb et al. 1984).

The results of this study clearly indicate that the agonal period influences renal parenchyma function, and that a subsequent period of storage causes further functional deterioration. Significant injury to the kidney also resulted from ice storage, which also exacerbated the damage which occurred during the agonal period. Ice storage using LIC would, therefore, appear to provide inadequate protection from injury of kidney cells over a period of 24 hours. Subsequent work in the laboratory of fresh kidney slice storage in ice, using a newly developed storage solution, has been followed by a normal K^+Na^+ ratio (Wicomb et al. unpublished data).

The significantly reduced slice ratio seen in Group B1 kidneys almost certainly resulted from poor renal perfusion resulting from the marked rise in peripheral resistance which accompanies the dramatic increases in sympathetic activity and circulating catecholamines which occur immediately after the onset of brain ischaemia. The injury caused to the kidneys during this period was increased when these slices were stored for 24 hours (Group B2). When a 4 hour period was allowed to elapse from the onset of brain ischaemia before kidney excision, the renal slice function showed some recovery, whether the pig had been maintained in a hypotensive (Group C1) or normotensive (Group D1) state. Slice function remained significantly reduced in these kidneys, however, and Group D1 kidneys demonstrated a prolonged flush perfusion time compared with control kidneys (Group A1) suggesting tissue damage. Catecholamines have disappeared from the circulation within this time, and therefore probably do not significantly contribute to an increased renal vascular resistance at this stage. This injury would appear to be reversed following a period of hormonal therapy. The long flush perfusion time may be associated with endothelial cell swelling resulting in increased red blood cell trapping. Previous studies have shown that approximately 45% of red blood cells remain trapped even in healthy fresh kidneys following flushing (Collins et al. 1984).

Administration of the hormones T_3 , cortisol, and insulin to the brain dead pig at hourly intervals resulted in significantly improved renal slice function. The relevance of hormonal therapy will be discussed in Chapter 2, 5, 7, and 9.

LEGENDS**FIGURES****Figure 6-1**

Mean slice potassium-sodium ratios in freshly excised kidneys taken from 3 groups of pigs (A1 - freshly excised; D1 - kidneys taken from brain-dead pigs after 4 hours supportive with i.v. fluids and dobutamine; D3 - kidneys taken from brain-dead pigs after 4 hours supportive therapy but with the addition of 2 hours hormonal therapy with T₃, cortisol and insulin. There is no significant difference between Groups A1 and D3.

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TABLE 6-1
KIDNEY SLICE POTASSIUM-SODIUM RATIOS
IN FRESHLY EXCISED AND STORED KIDNEYS

GROUP	n	(1) Freshly excised kidney slices K/Na ratio (p)	n	(2) 24 hrs stored kidney slices K/Na ratio (p)	STATISTICAL COMPARISON (1) vs (2)
A1/2	8	5.17(0.268)	6	4.21(0.302)	p<0.0002
B1/2	5	4.19(0.379)	4	2.20(0.626)	p<0.04
C1	8	4.58(0.289)	0	-	-
D1/2	8	4.41(0.361)	8	4.01(0.416)	NS
D3/4	8	4.97(0.447)	8	4.07(0.507)	p<0.0004

Figures in brackets refer to standard error

NS = not statistically significant.

(p) = Statistical difference from A1 or A2

TABLE 6-2 KIDNEY FLUSH INFUSION TIMES
IN GROUPS A1, D1 AND D3

GROUP	N	KIDNEY FLUSH INFUSION TIME (seconds)	STATISTICAL COMPARISON	
			vs A1	D1 vs D3
A1	8	104 (9)	-	
D1	8	309 (41)	p<0.001	p<0.002
D3	8	117 (9)	NS	

NS = not statistically significant

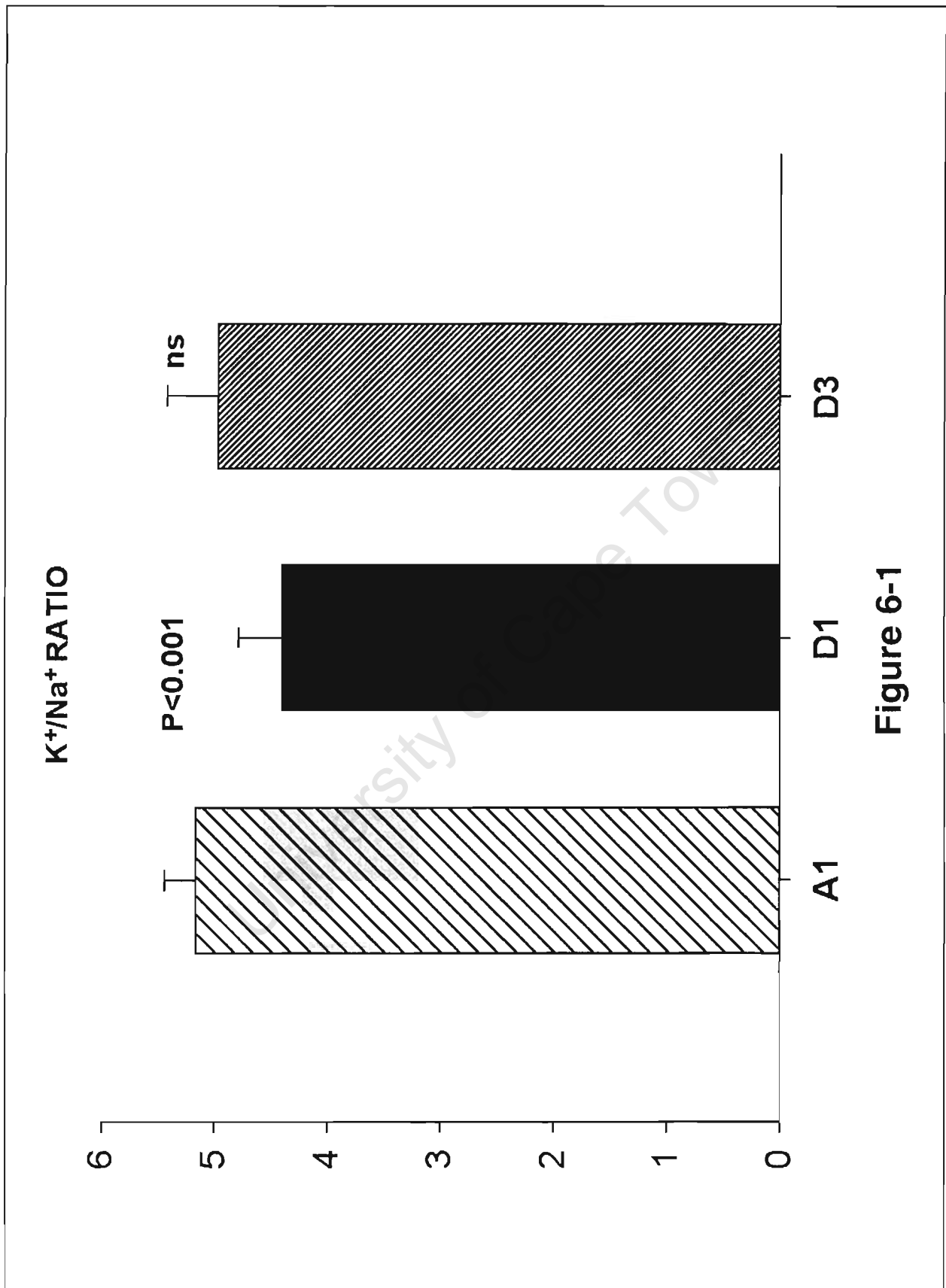


Figure 6-1

CHAPTER 7

CHANGE FROM AEROBIC TO ANAEROBIC METABOLISM AFTER BRAIN DEATH, AND REVERSAL FOLLOWING TRIIODOTHYRONINE (T₃) THERAPY

INTRODUCTION

In the previous chapters it has been documented that a significant reduction in circulating hormones T₃, T₄, cortisol and insulin in baboons and T₃ in humans takes place after brain death. This leads to a progressive haemodynamic deterioration shown by the *ex vivo* testing, and a progressive depletion of ATP, CP and glycogen with accumulation of lactate. These observations suggest a change from aerobic to anaerobic metabolism within the myocytes (and also kidney cells).

The administration of T₃, cortisol and insulin to brain dead pigs leads to replacement of myocardial energy stores, reduction in tissue lactate, stabilization of haemodynamic function, and also K⁺/Na⁺ ratio recovery in the kidneys.

These two essential organs therefore undergo major metabolic disorders resulting from inhibition of aerobic metabolism.

These observations have led to consideration of the metabolic changes which take place in the entire body after brain death. A series of experiments was undertaken employing the principle of single bolus intravenous kinetics in the plasma and ¹⁴C₂ production (following oxidation in tissues) from expired air using ¹⁴C-R in the baboon. Lactate and free fatty acids in blood were also monitored.

EXPERIMENTAL METHODS

Chacma baboons (*Papio ursinus*) weighing 17 to 29 kg were used in all studies. Each study involving $^{14}\text{U-C}$ -glucose, $^{14}\text{C-1}$ -pyruvate, or $^{14}\text{C-1}$ - palmitate utilization was divided into three parts and performed sequentially in the same animal. Study 1 was made on the sedated baboon (control), study 2 followed brain death, and study 3 followed T_3 therapy after brain death.

Anaesthesia and Monitoring

The baboon was sedated with ketamine hydrochloride (5-10 mg/kg/i.m.) After endotracheal intubation, sedation was maintained with a mixture of oxygen (4l/min) and nitrous oxide (6l/min), the animal breathing spontaneously. The ECG, MAP, and CVP were continuously recorded. Blood gases were maintained within normal limits. Body temperature was maintained at 37°C by surface warming.

GLUCOSE UTILIZATION

The principle of single bolus kinetics with labelled carbon ($^{14}\text{C-R}$) with subsequent measurement of both plasma activity (Figure 7-1) (Searle 1976, Roberts 1981) and of exhaled $^{14}\text{CO}_2$ (Figure 7-2), (Bircher 1981, Gordon 1964) was used to study glucose utilization under conditions of (i) sedation, (ii) brain death, and (iii) brain death with T_3 therapy.

Study 1 (Control)

An initial infusion of 30 to 40 grams of glucose was administered intravenously (300-

400 ml of Maintelyte containing 10% dextrose). Universally labelled carbon 14 glucose (^{14}C -U-glucose) (20 μCi) was administered i.v. as a bolus to the sedated baboon. Arterial blood samples were taken at 5 minute intervals for 30 minutes, at 10 minute intervals for a further 30 minutes, at 15 minute intervals for the next 60 minutes, and at 30 minute intervals for the next 4 hours. Following centrifugation, 0.1 ml lactic acid (0.1 molar concentration) was added to 1 ml of plasma to remove the dissolved bicarbonate. The sample was agitated slowly for one minute, following which 10 ml of scintillation fluid were added (Insta-gel, United Technologists, Packard). Finally, 0.1 ml hydrochloric acid (0.1 molar solution) was added to precipitate the proteins.

Radioactivity in the scintillation vial was measured as disintegration per minute (DPM) in a β counter.

Expired CO_2 was measured at the same time intervals as follows. Expired gas was dried by bubbling through absolute alcohol, and drawn into a 3 ml solution of hyamine containing phenolphthalein. This provided an end point for the collection of 1 mMole of CO_2 . The sample was then diluted with 10 ml of scintillation fluid and counted (as for plasma) to determine the proportion of labelled CO_2 in the sample.

After the 6 hour study period, the indwelling arterial and venous lines were removed, the baboon was extubated, and returned to its cage.

Study 2

This study was carried out on the following day, beginning 24 hours after the initiation of study 1. Sedation and monitoring were as in study 1, but, in addition, a urinary catheter was inserted for measurement of urine output. With the animal breathing spontaneously,

baseline haemodynamic parameters were obtained. Samples of blood and expired air were taken, as in study 1, for measurement of control levels of glucose and CO₂ production.

Sedation was then deepened by the intravenous administration of 1-2 mg/kg ketamine hydrochloride, following which brain death was introduced. The technique of induction of brain death has been described in detail previously (Chapter 2A, Method 1). When spontaneous respiration ceased, positive pressure mechanical ventilation was initiated using a Bird Mark 2 ventilator supplying a mixture of air and 40% oxygen. Blood gases, acid base balance, and serum electrolytes were maintained within the normal range. Intravenous fluids (Maintelyte) were administered to replace volume loss from the high urinary output which occurred as diabetes insipidus developed. The CVP was maintained between 2 and 5 cmH₂O with a MAP of a minimum of 50 mmHg.

Twelve to 16 hours (mean 13.8 hours) after the induction of brain death the injection of ¹⁴C-R was repeated and a glucose utilization study (as described in study 1) was carried out over a 6 hour period.

Study 3

This study followed immediately after study 2. An intravenous injection of T₃ (2 micrograms) was given to the baboon, and this dosage was repeated at hourly intervals thereafter. Thirty minutes after the initial T₃ injection, the administration of ¹⁴C-R was repeated, and a glucose utilization study (as described in study 1) was repeated exactly as before over a 4 to 6 hour period (mean 4.6 hours). Since some ¹⁴C-U-glucose remained in the baboon from Study 2, this amount was calculated as 1.8 to 5.1 (mean 2.9) uCi, equal to 14% of the initial dose. A further 20 uCi were administered after initial dose of Study 3. Thereafter a total dose of approximately 22.9 uCi ¹⁴C-U-glucose (and its metabolites) was available at the onset of Study 3.

PYRUVATE UTILIZATION (n=4)

Pyruvate utilization was studied exactly as for glucose utilization (above), except that twelve uCi of ^{14}C -l- pyruvate were injected in each of studies 1, 2 and 3.

PALMITATE UTILIZATION (n=2)

Palmitate utilization was studied exactly as for glucose utilization. ^{14}C -1-palmitate (5 uCi) was injected in each of the three studies.

SERUM LACTATE LEVELS (n=5)

Blood was taken for measurement of serum lactate at three time intervals: Control (before inducing brain death), at 19 hours after induction of brain death (end of study 2), and 4 hours after the administration of T_3 (end of study 3).

PLASMA FREE FATTY ACIDS (n=5)

Blood was taken for measurement of plasma free fatty acids at five time intervals: Control (before induction of brain death), at 1, 3 and 19 hours after the induction of brain death, and finally 4 hours after the administration of T_3 .

STATISTICAL METHODS

For plasma counts (DPM) the data were submitted to the computer programme AUTOAN (Sedman et al. 1972) to provide the best fit function for the measured points. The coefficients and exponents for this function were then used to calculate the half-life

(Roberts 1981, Greenblatt 1985) and plasma clearance of the labelled glucose in each study (Wagner 1976).

The DPM obtained from the CO_2 were plotted in such a way to incorporate absorption kinetics with negative coefficients with a lag time in order to obtain a measure of the rate of appearance of labelled ^{14}C in expired breath. The total number of expired counts from the time of injection of the ^{14}C to peak CO_2 production was calculated by integrating the area under the curve of each studied metabolite (Bircher 1981, Gordon 1964).

Peak CO_2 production in study 1 (control) was found to take place at 2 hours in the case of glucose, 20 minutes in the case of pyruvate, and one hour in the case of palmitate, each time interval corresponding to the point of peak of oxidation (Gordon 1964). The total accumulated counts under the curve from the time of injection of ^{14}C -R until the peak CO_2 production (e.g. 2 hours for glucose) was calculated. The peak production time taken from study 1 (e.g. 2 hours for glucose) was also chosen for calculations of the areas under the curves in studies 2 and 3.

The data obtained from studies 1 and 2, with regard to plasma half-life and clearance and CO_2 production, were compared using the Mann Whitney U test. Study 3 vs 2 were compared using the Wilcoxon matched paired rank test. Considering the complexity of these studies, a p value of less than 0.07 was considered to be significant.

RESULTS

The results are shown in Table 7-1. The information concerning the data is shown in Appendix 3.

Glucose Utilization (n=6) (Table 7-1) (Figures 7-3, 7-4)

The half life of glucose increased by 46% in brain dead animals (Study 2) when compared with controls (Study 1) ($p < 0.04$). The administration of T_3 to the brain dead baboon (Study 3) resulted in a significant reduction of half life by 60% ($p < 0.02$). After T_3 administration, the half life was significantly shorter than the control level ($p < 0.07$).

Plasma glucose clearance fell by 45% in brain dead animals compared with controls ($p < 0.06$), and increased by 122% following the administration of T_3 ($p < 0.02$), returning to a level not significantly different from control values.

Following brain death, $^{14}CO_2$ production fell by 82% ($p < 0.002$). T_3 administration was followed by a 324% increase $^{14}CO_2$ production ($p < 0.02$), by which time it was not significantly different from control.

PYRUVATE UTILIZATION (n=4) (Table 7-1),(Figures 7-5, 7-6)

Following the induction of brain death, the half life of pyruvate increased by 79% ($p < 0.05$), but showed a 68% reduction when brain-dead animals were treated with T_3 ($p < 0.05$). After T_3 therapy the half-life was not significantly shorter than control.

The plasma clearance of pyruvate in brain dead animals dropped by 25% when compared with control ($p < 0.06$), while T_3 therapy increased plasma clearance by 123% ($p < 0.05$); clearance was then significantly greater than in the control studies ($p < 0.04$). CO_2 production fell by 51% ($p < 0.03$) following brain death, but after the administration of T_3 improved by 42% ($p < 0.05$), returning to the control range.

PALMITATE UTILIZATION (n=2) (Table 7-1),(Figures 7-7, 7-8)

(Statistical analyses were not performed in this small group). Palmitate half life increased by 105% following brain death, with a recovery of 46% after T₃ therapy. Clearance progressively increased by 36% (Study 1 vs 2) and 31% (Study 2 vs 3).

¹⁴CO₂ production decreased by 57% following brain death, with complete recovery (increase of 110%) following T₃ administration.

Serum Lactate Levels (n=5) (Table 7-2), Figures 7-9A

After brain death, serum lactate showed an increase of 257% (p<0.0001). Four hours after T₃ administration, the lactate level had fallen by 63% (p<0.0001), not being significantly different from the control level.

Plasma Free Fatty Acid Levels (n=5) (Table 7-2),(Figure 7-9B)

Within one hour of the induction of brain death, the level of free fatty acids had increased by 91% (p<0.0001); By 3 hours normalization of free fatty acids to control levels occurred; by 18 hours the mean free fatty acids increased by 315% above the control level (p<0.0001). Following 4 hours of T₃ therapy a fall of 72% had occurred (p<0.0001), the level no longer being significantly different from the control.

DISCUSSION

Study 1 represents a different physiological and anatomical preparation when compared with studies 2 and 3. After brain death there is a reduction in certain hormone levels, a

loss of vasomotor control, development of diabetes insipidus, and an almost total absence of blood flow to the brain; glucose is therefore not utilized in the brain following brain death. An argument could be made, therefore, that the data obtained in Study 1 should not be compared statistically with those obtained in Studies 2 and 3. Statistical comparison has been made, however, using the Mann Whitney U test which considers Study 1 and Studies 2 and 3 as different preparations.

Studies 2 and 3, however, were made in similar models, the only significant difference (apart from the administration of T_3) being that the animal had been brain-dead for a longer period of time in Study 3; more metabolic derangement may have occurred.

The greater variance in the measured parameters seen in Study 2 may be related to individual pathophysiological responses to brain death - for example, the rate at which T_3 is lost from the circulation. The time interval which had elapsed from induction of brain death may also be a factor. The reductions in plasma clearance of pyruvate and in $^{14}CO_2$ production following pyruvate administration (seen after brain death), did not reach statistical significance (Mann Whitney U test) when compared with control observations. This probably reflects the small number of experiments performed, as the trend closely followed those of glucose utilization. The significant differences seen between Study 3 and Study 1 indicating, for example, that the half life of glucose was less after T_3 therapy than under control circumstances, are most likely due to an excessive dosage of T_3 .

The findings demonstrate that there is a major change in metabolic oxidative processes following brain death. The rate of glucose, pyruvate, and palmitate utilization is markedly reduced, and there is an accumulation of lactate and free fatty acids in the plasma. The findings indicate a change from aerobic to anaerobic metabolism affecting the body as a

whole, and correlate well with the previous findings which concentrated on metabolism in the heart alone (Chapter 4). Body energy stores will be rapidly depleted (presumably from all major organs) under this changed metabolic environment, almost certainly leading to deterioration in function of all organs.

The administration of 2 μg of T_3 at hourly intervals to the brain dead baboon resulted in a dramatic reversal in the rate of metabolite utilization and CO_2 production, and reductions in plasma lactate and free fatty acids, indirectly indicating a reversal from anaerobic to aerobic metabolism. This finding again correlates well with previous studies which shows replacement of myocardial energy stores and improvement in myocardial function following T_3 therapy.

Tissue and plasma lactic acidosis in the presence of adequate perfusion pressure and normal pO_2 , again indicate that pyruvate is converted into lactate and the pyruvate does not enter the mitochondria, Huckabee (1959). Type A and B lactic acidosis has been reviewed by Cohen (1976) and Kriesberg (1984). However, the acidosis seen following brain death does not fit into any of the described forms as no intoxication takes place but rather an acute metabolic derangement induced by hormonal depletion occurs.

These findings from the brain-dead baboon and from human potential organ donors (Chapter 9) suggest that inhibition of a common metabolic pathway for carbohydrates and free fatty acids takes place in the mitochondria, which are the main site of CO_2 production. In the absence of T_3 , the mitochondria cease functioning effectively, and anaerobic metabolism takes place. Following T_3 therapy, the rapid increase in glucose, pyruvate, and palmitate utilization and in CO_2 production, and the normalization of lactate and free fatty acid metabolism, indicate reactivation of the mitochondria, resulting in aerobic oxidative phosphorylation.

T₃ therapy would appear to lead to reactivation of mitochondrial activity, resulting in oxidation of tricarboxylic acid and electron transfer coupling, stimulating ATP production and activation of ATPases at different levels. This is probably from a direct effect of T₃ mediated through calcium (Nayler 1971, Fabiato and Fabatio 1979, Holland 1979), activating key enzymes (Gevers 1981, Nichols 1982, Newsholme-Leech 1983), such as the phosphorylase complex, pyruvate dehydrogenase complex, isocitrate dehydrogenase, oxoglutarate and NAD⁺-linked isocitrate dehydrogenase (Figure 18 Appendix 4-1). The short period of time required following the administration of T₃ to obtain this response would suggest that DNA T₃ receptors and mRNA do not play an initial role. The role of Ca⁺⁺ in the activation of the above-mentioned enzymes is shown in Appendix 4.

LEGENDS**FIGURES****Figure 7-1**

The laboratory method of trapping $^{14}\text{CO}_2$. (A) Actual laboratory set up, and (B) schematic representation. (1) Tubes connected to the ventilator. (2) Endotracheal tube with a T connection for suction of exhaled air. (3) Foley catheter in situ. (4) Flask containing absolute alcohol through which the exhaled air is bubbled. (5) Roller pump, and (6) vial containing hyamine in which CO_2 is collected.

Figure 7-2

Single bolus kinetics - a combined figure from "A Textbook of Clinical Pharmacology", by Rogers HJ, Spector RG, Trounce JR. (Chapter 1: Introduction to Pharmacokinetics). Hodder and Stoughton, London, 1981, pg 14 and 16.

Figures 7-3

A: Best fit data curves plotted for plasma ^{14}C -U-glucose kinetics, Studies 1, 2 and 3.

B: Best fit data curve plotted for $^{14}\text{CO}_2$ produced from ^{14}C -U-glucose. C: (Control) = Study 1; BD (brain-dead animal) = Study 2; BD+ T_3 (brain-dead animal plus T_3 therapy) = Study 3. Comparisons were made from the accumulated counts under the curve up to 2 hours.

Figure 7-4

Histograms for ^{14}C -U-glucose half life, plasma clearance and accumulated $^{14}\text{CO}_2$ studies.

Figure 7-5

- A. Best fit data curves plotted for plasma ^{14}C -1-pyruvate kinetics.
- B. Accumulated $^{14}\text{CO}_2$ counts were compared at 20 minutes. Abbreviations as per Figure 7-3.

Figure 7-6

Histograms for ^{14}C -1-pyruvate, half-life, plasma clearance and $^{14}\text{CO}_2$ accumulated counts for 20 minutes.

Figure 7-7

- A: Best fit data curves plotted for plasma ^{14}C -1-palmitate kinetics.
- B: Expired $^{14}\text{CO}_2$. Comparisons were done for CO_2 accumulated counts up to one hour. Abbreviations as per Figure 7-3.

Figure 7-8

Histograms for ^{14}C -1-palmitate plasma half-life, clearance and accumulated $^{14}\text{CO}_2$ during the first hour.

Figure 7-9

A: Plasma lactate levels C (control) before induction of brain death, at 19 hours and at 23 hours. Statistical comparisons as shown.

B: Plasma free fatty acid levels C (control), at 1, 3, 19 and 23 hours post brain death induction. T₃ therapy initiated showing rapid plasma decrease by 23 hours. Statistical comparisons are shown.

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TABLE 7-1 CHANGES IN GLUCOSE, PYRUVATE AND PALMITATE UTILIZATION

	STUDY 1 (Control)	STUDY 2 (Brain Death)	STUDY 3 (Brain Death + T ₃)	STATISTICAL DIFFERENCE (p) 1 vs 2* 2 vs 3** 1 vs 3
GLUCOSE (n=6)				
Half life	2.84 (0.75)+	4.22 (1.11)	1.66 (0.23)	<0.04 <0.02 <0.07
Plasma Clearance	1.11 (0.22)	0.65 (0.26)	1.47 (0.16)	<0.06 <0.02 <0.6
Acc- ¹⁴ C ₂	16560 (3382)	2971 (746)	12616 (2551)	<0.002 <0.02 <0.4
PYRUVATE (n=4)				
Half life	2.11 (0.26)	3.77 (0.72)	1.72 (0.161)	<0.05 <0.05 <0.03
Clearance	4.13 (0.87)	3.08 (0.74)	6.86 (0.82)	<0.06 <0.05 <0.04
Acc- ¹⁴ C ₂	26250 (1996)	12919 (2290)	18341 (3329)	<0.03 <0.05 <0.1
PALMITATE (n=2)				
Half life	1.74 (0.59)	3.58 (0.52)	1.92 (0.6)	
Clearance	9.49 (2.66)	12.94 (4.91)	16.91 (1.36)	
Acc- ¹⁴ C ₂	768 (394)	333 (255)	698 (126)	

* = Mann Whitney U test

** = Wilcoxon matched paired rank test

+ = Figures in parentheses refer to standard error of the mean

Acc-¹⁴C₂ = Accumulated counts of ¹⁴C labeled carbon dioxide

TABLE 7-2 CHANGES IN SERUM LACTATE AND PLASMA FREE FATTY ACIDS

	STUDY 1 (Control)	STUDY 2 (Brain Death)	STUDY 3 (Brain Death + T ₃)	STATISTICAL DIFFERENCE 1 vs 2 2 vs 3 1 vs 3	(p)*	
LACTATE mmol/l (n = 5)	1.57 (0.23)+	5.61 (0.33)	2.10 (0.17)	<0.0001	<0.0001	NS
FREE FATTY ACIDS umol/l (n = 5)	291 (19)	1209 (40)	341 (23)	<0.001	<0.0001	NS

+ = Figures in parentheses refer to standard error of the mean

* = Analysis of variance

NS = not significant

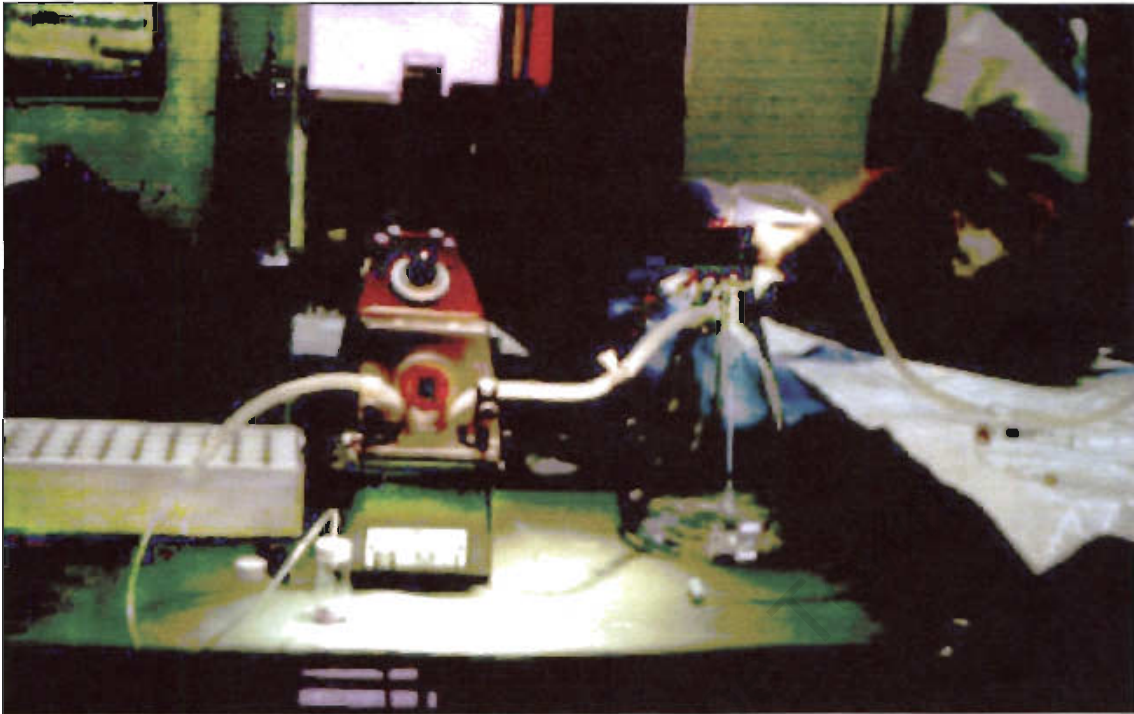


Figure 7-1A

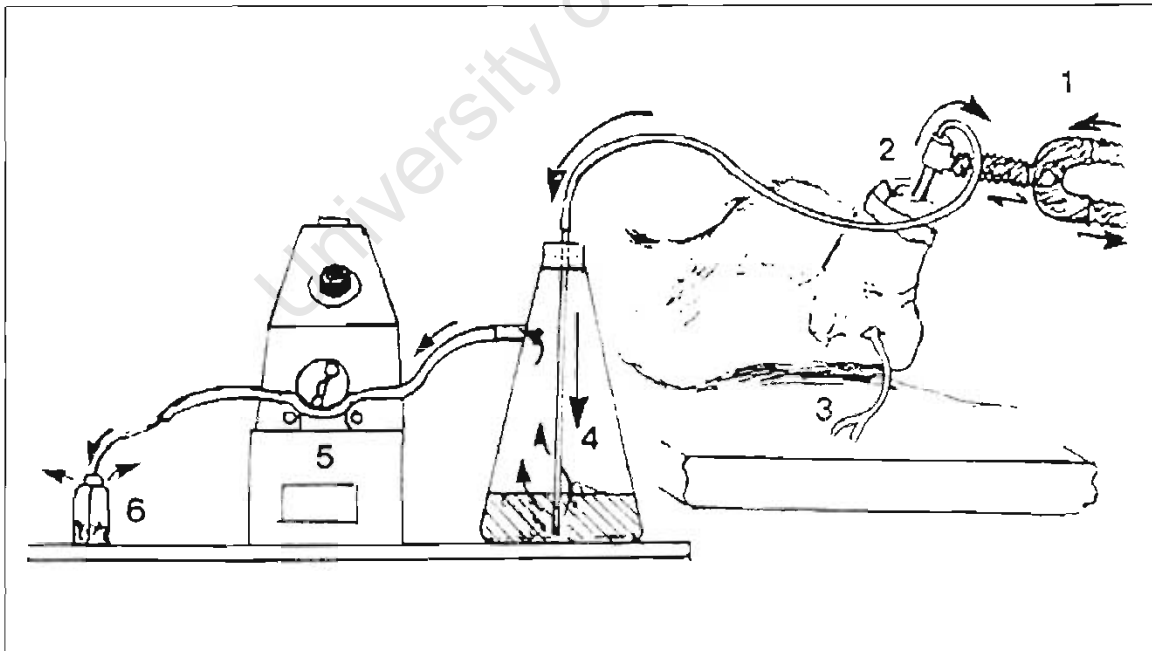
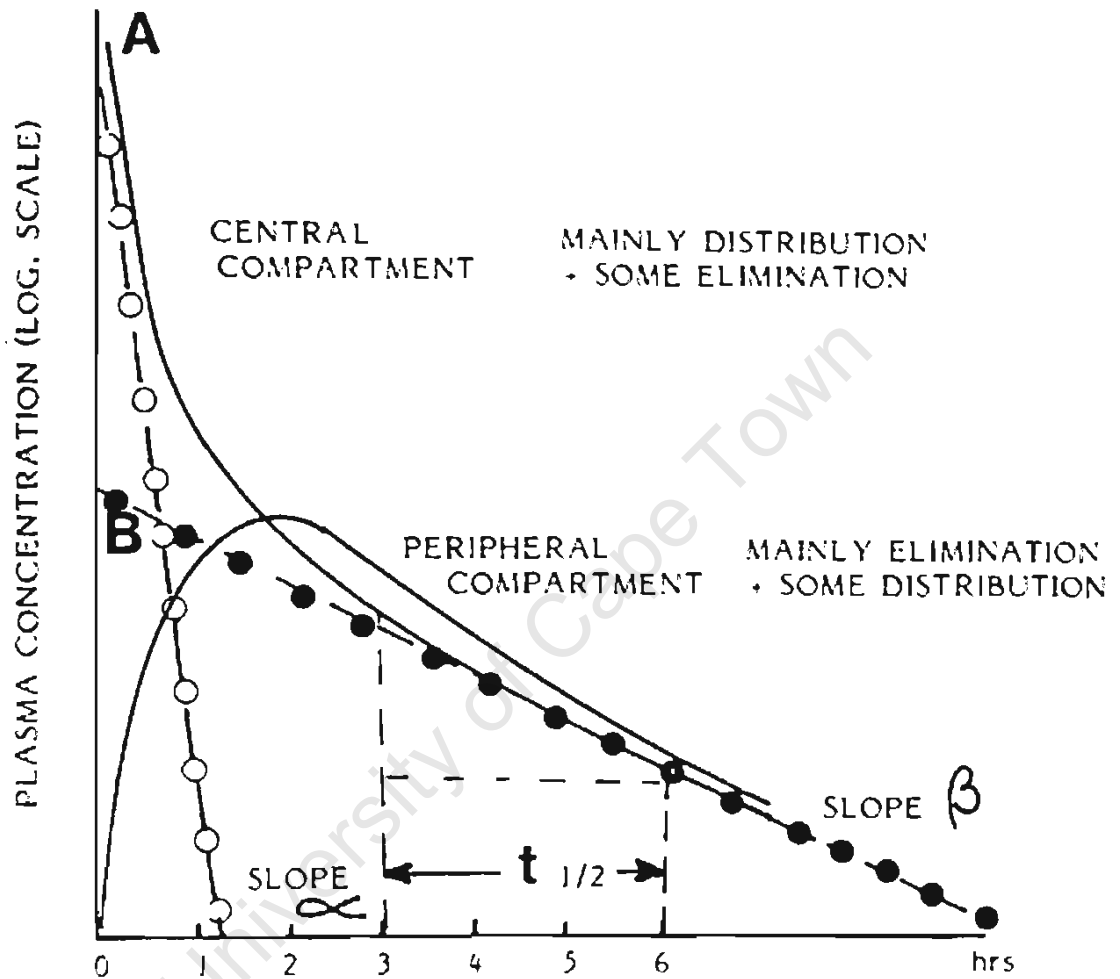


Figure 7-1B



Time course of hypothetical drug distribution in central and peripheral compartments following rapid i.v. injection in a bi-phasic decline in plasma concentration in a two-compartmental system of the body; 1/2 life is shown.

Figure 7-2

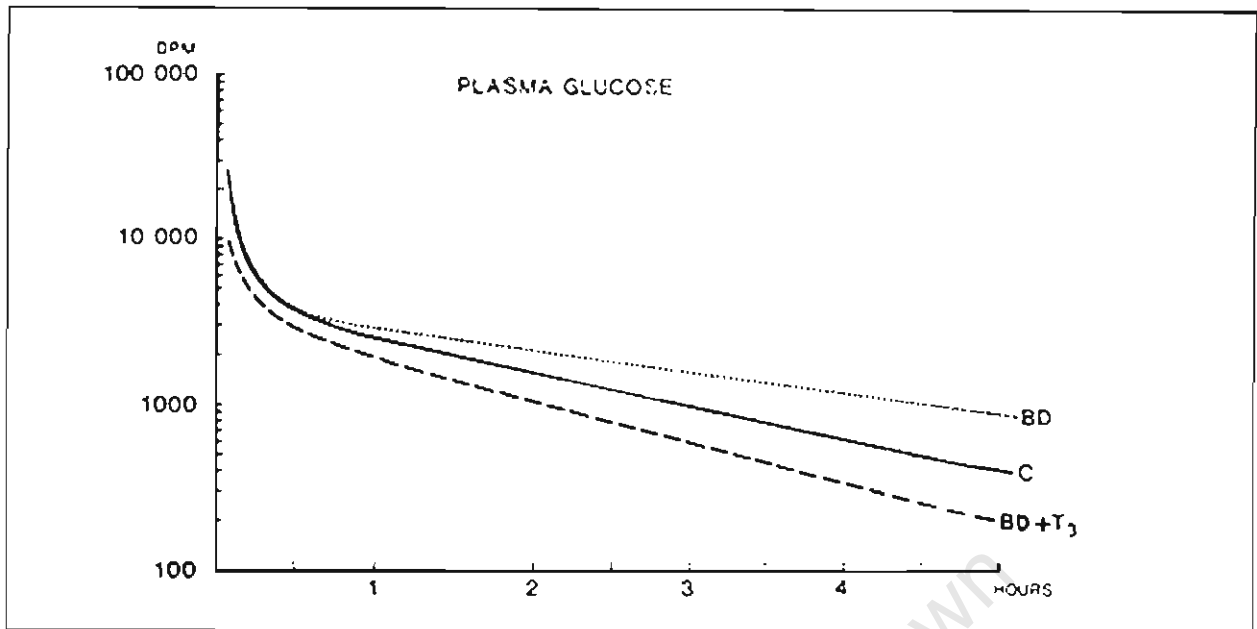


Figure 7-3A

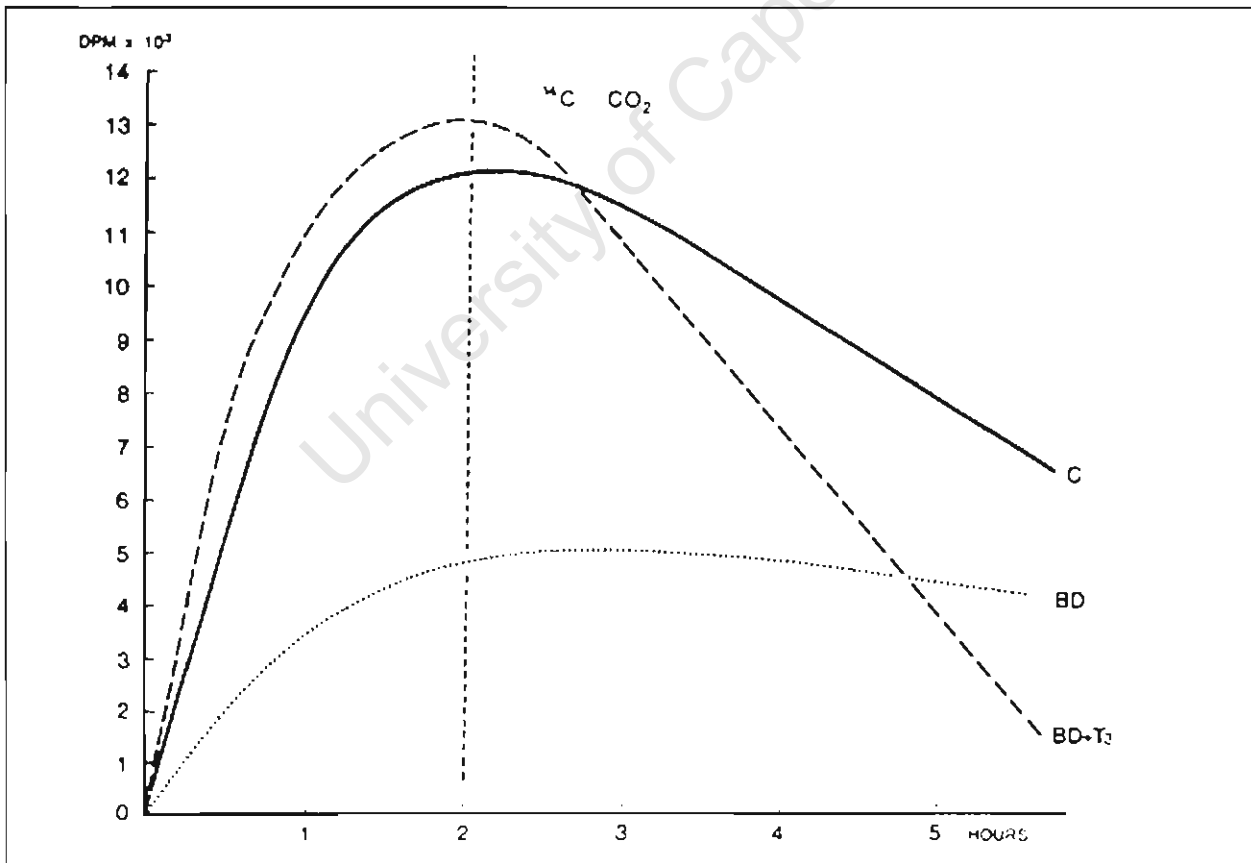


Figure 7-3B

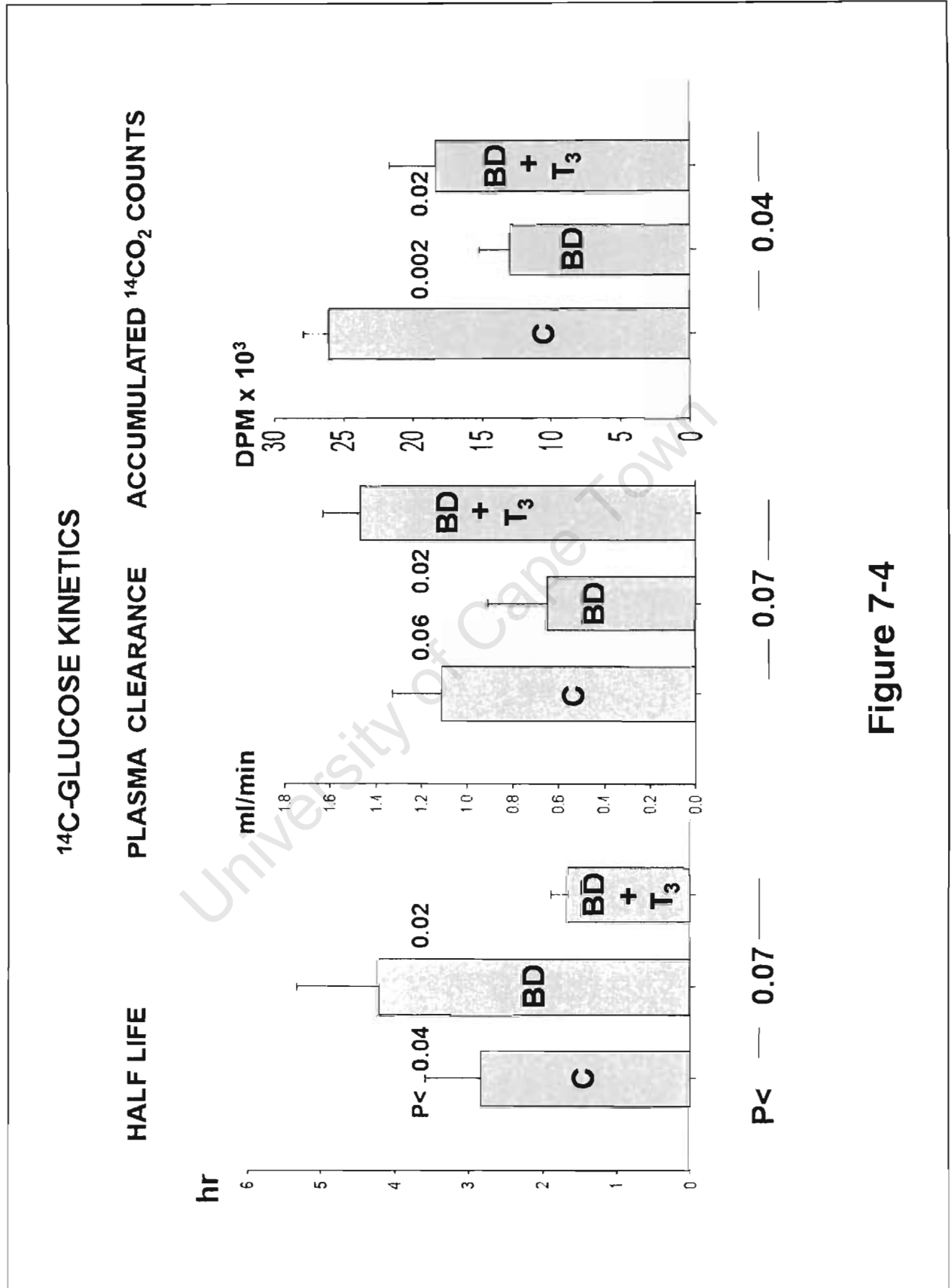


Figure 7-4

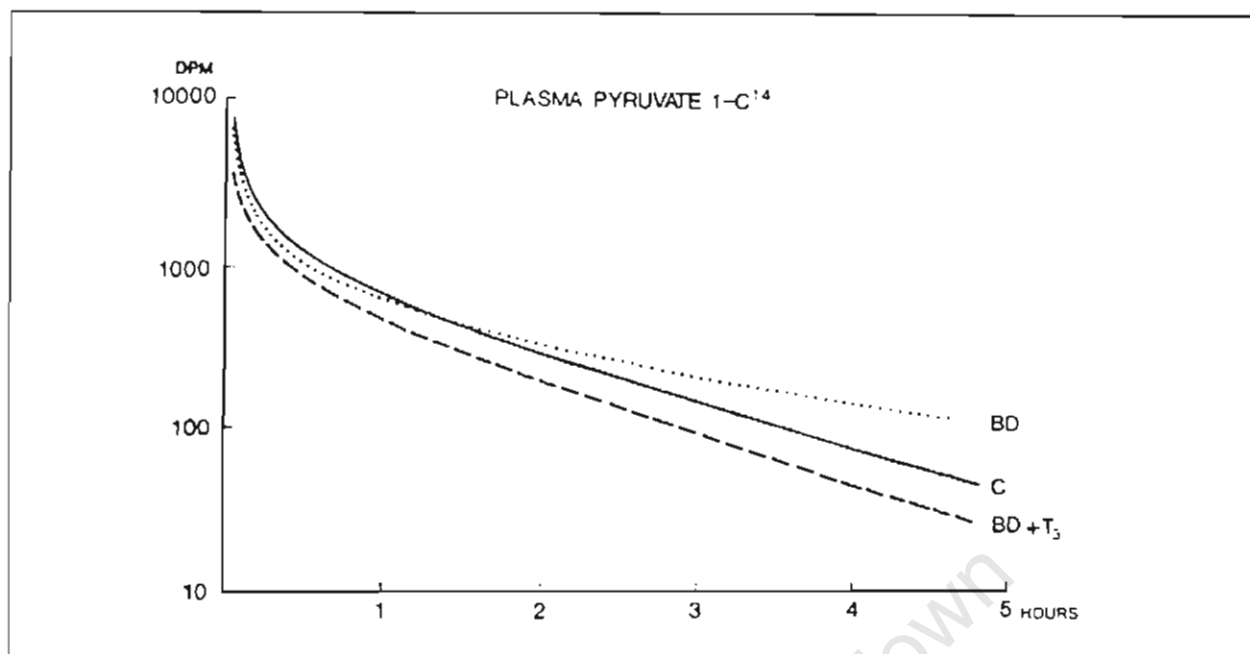


Figure 7-5A

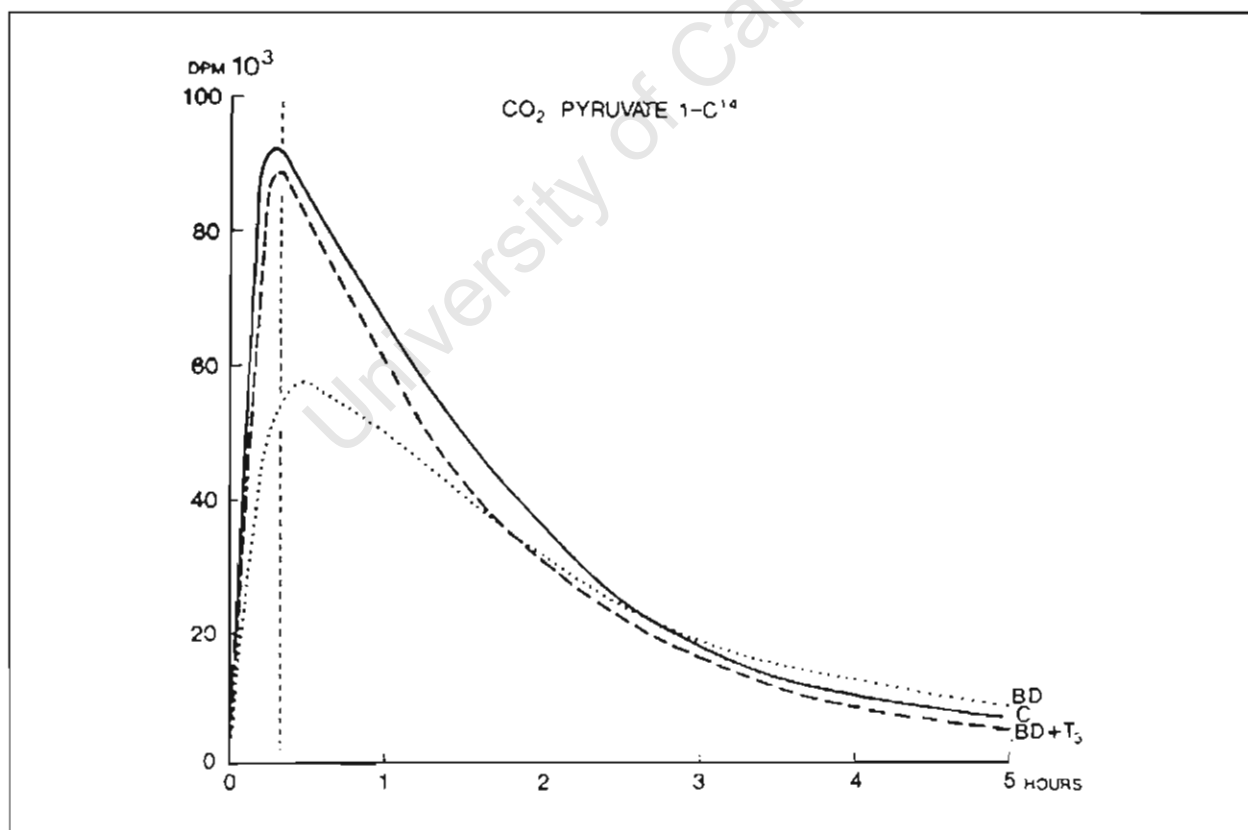


Figure 7-5B

¹⁴C-1-PYRUVATE METABOLIC KINETICS

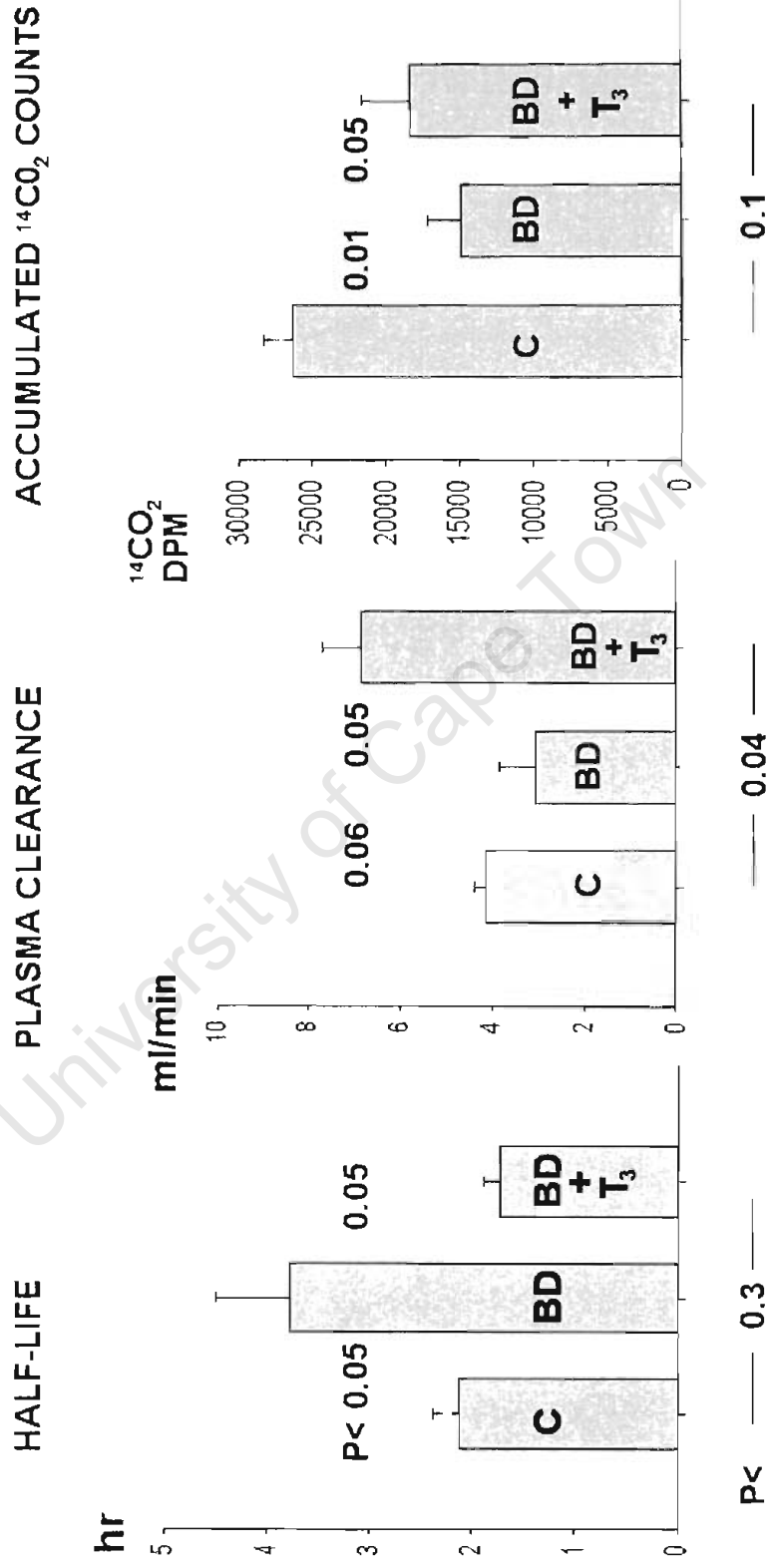
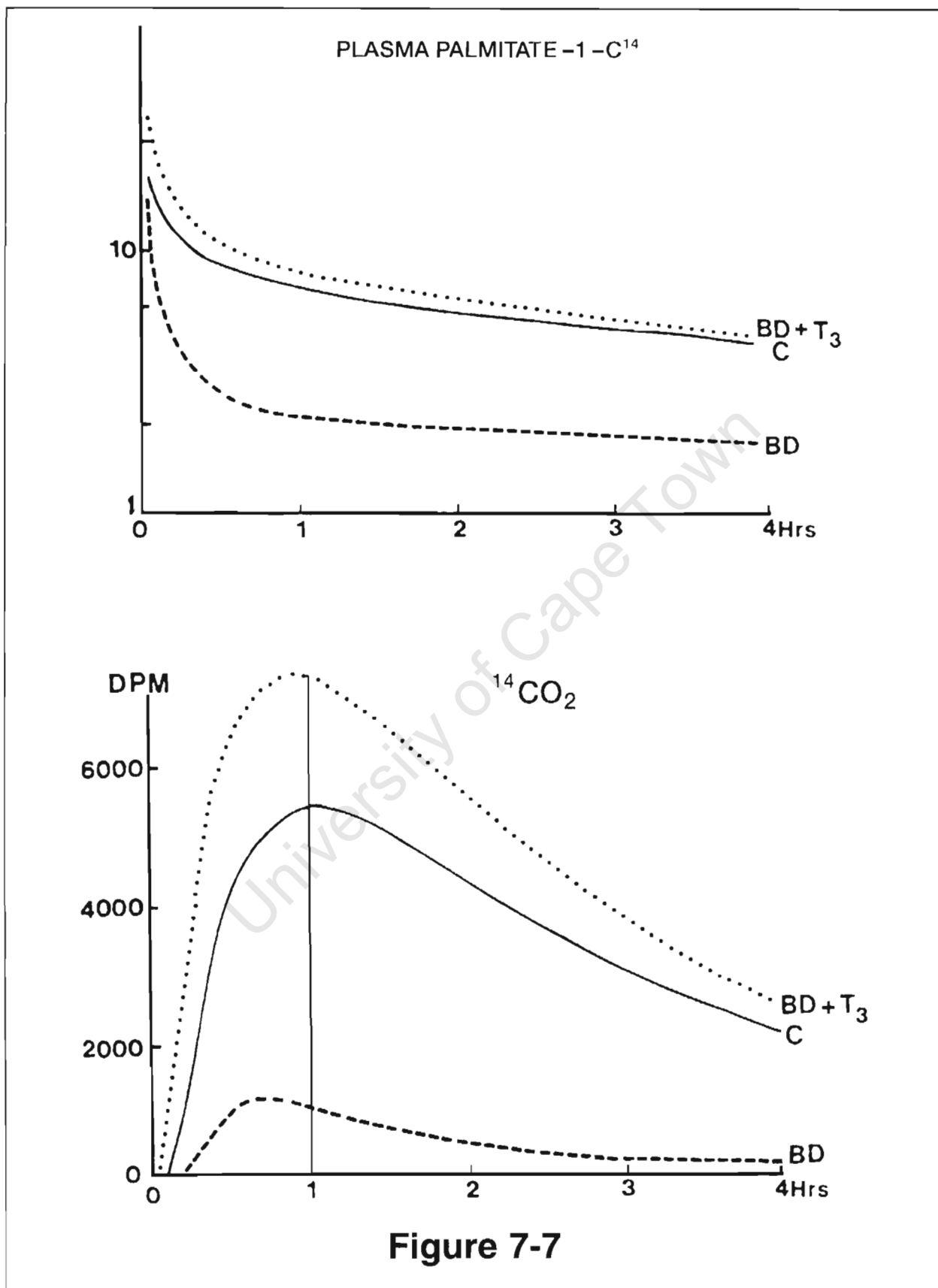


Figure 7-6



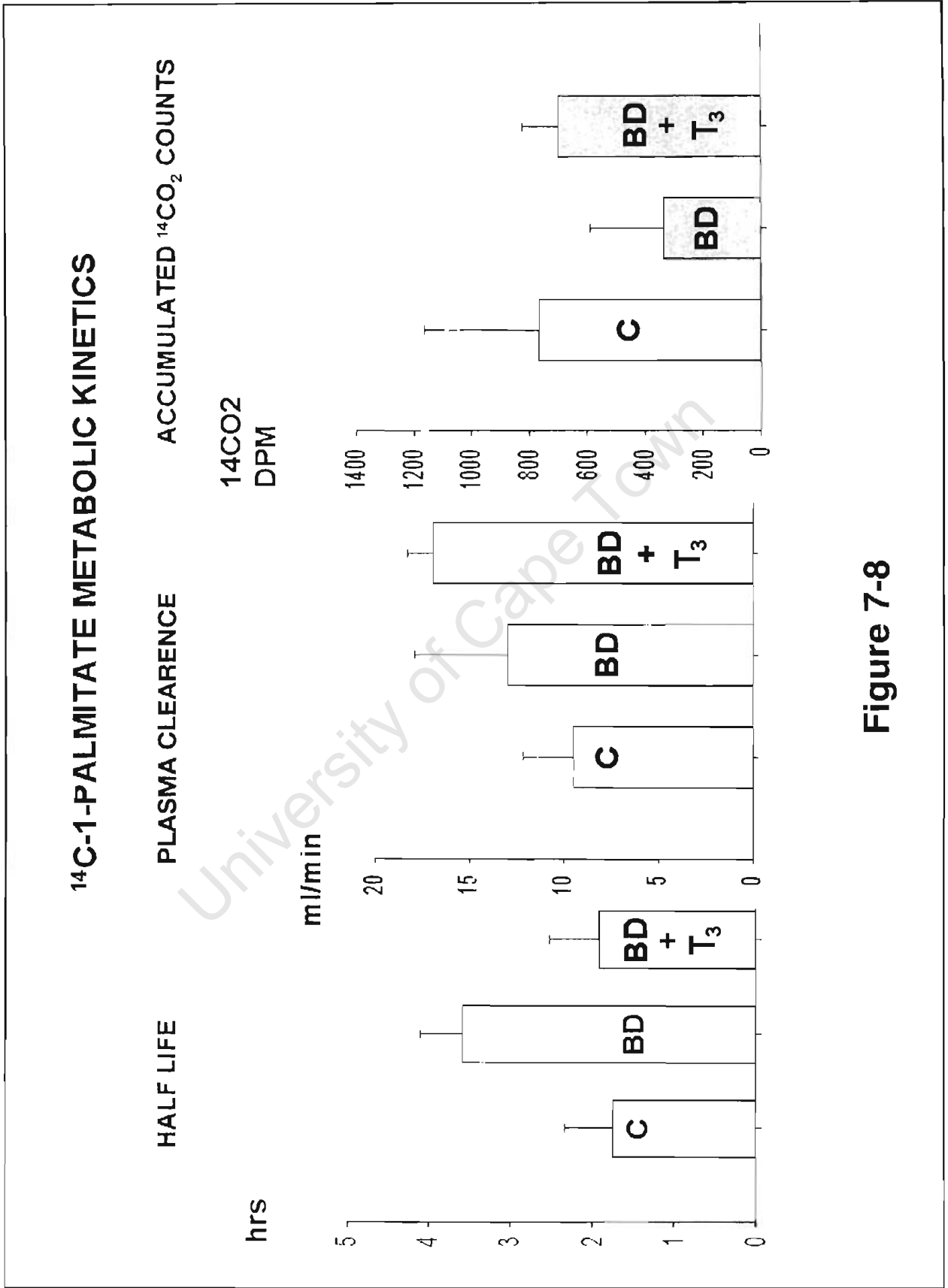


Figure 7-8

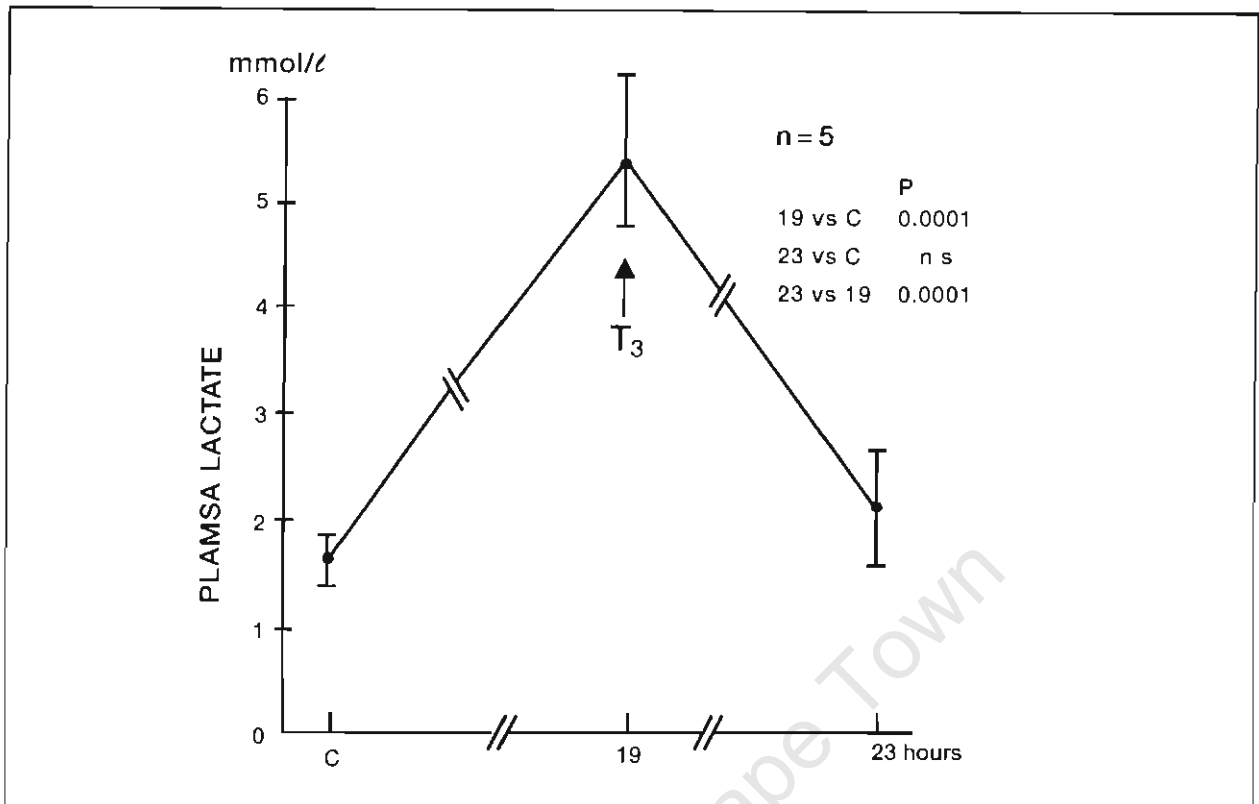


Figure 7-9A

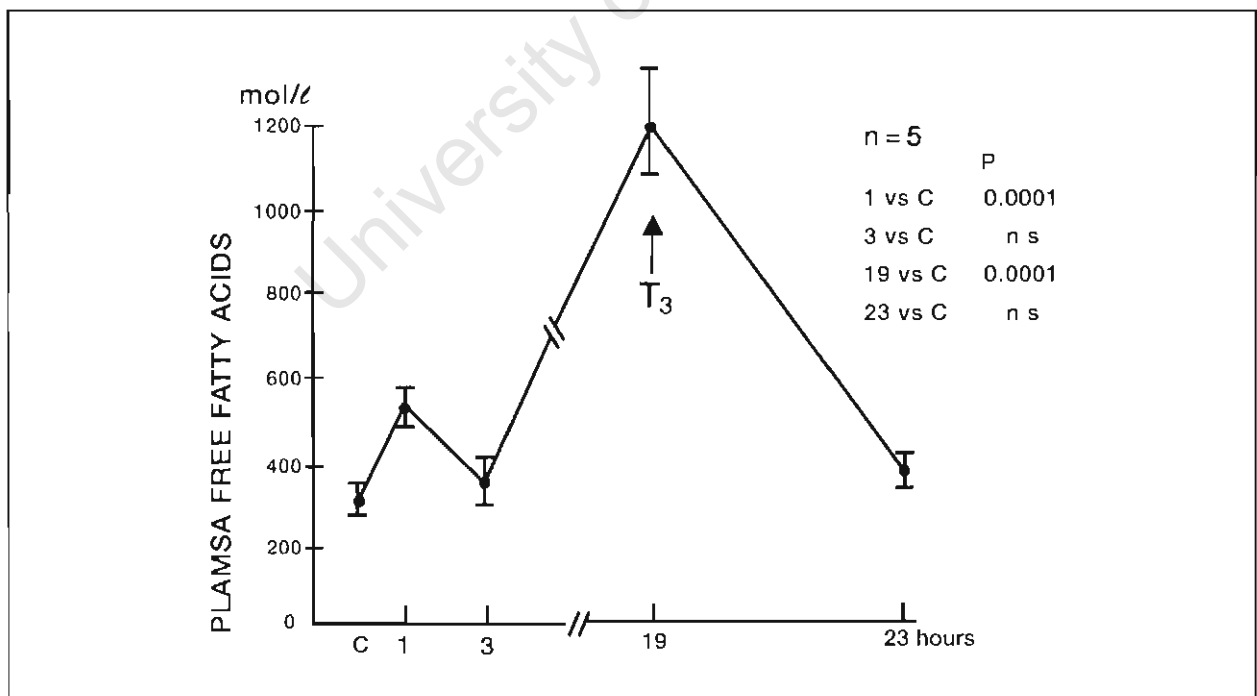


Figure 7-9B

CHAPTER 8

CLINICAL OBSERVATIONS - EARLY DONOR HEART FAILURE

(FOLLOWING TRANSPLANTATION) FROM MYOCARDIAL INJURY SUSTAINED DURING BRAIN DEATH

INTRODUCTION

We have reviewed those patients who died following heart transplantation at the Universities of Cape Town and Munich from hitherto unexplained causes either at operation or in the early post-transplant period. The donors of these hearts did not undergo hormonal therapy.

CLINICAL EXPERIENCE

Five out of 106 patients (4.7%) showed donor heart histopathological features almost identical to those seen in the experimental study outlined above (Chapter 2-E). In the University of Cape Town series, of 76 transplants in 67 patients (8 undergoing a second transplant procedure, and one a third transplant) death from unexplained donor heart failure occurred in 4 patients (5.3%) in the operating room or in the early post-operative period. In the series of 30 transplants performed at the University of Munich, there was one similar early death (3.3%).

Similar early donor heart failure from uncertain cause (sometimes cited as "unspecified death") has also occurred at other centres involved in this form of surgical therapy. (Billingham and Jamieson - personal communication).

In the Cape Town series, the donor heart in one patient undergoing orthotopic

transplantation, never functioned satisfactorily and death occurred on the operating table. The remaining 3 patients, all undergoing heterotopic heart transplantation, died at 2 hours and 7 and 9 days respectively (the last 2 patients haemodynamics were supported by the recipient's heart during this period) after cardiac transplantation. The donor heart never had functioned entirely satisfactorily. There was no histological evidence of acute rejection in these hearts. At the University of Munich, one patient died on the operating table from donor heart inadequacy. The clinical data of the recipient and donor are shown in Tables 8-1 and 8-2.

HISTOPATHOLOGICAL FEATURES OF THE DONOR HEART

In all 5 patients, at autopsy, the donor heart showed a grossly normal macroscopic appearance, except for occasional subendocardial haemorrhage. When examined by light microscopy, all 5 hearts revealed various combinations of 3 forms of acute myocardial necrosis, namely contraction bands, coagulative myocytolysis and coagulative necrosis (coagulative myocytolysis) (Figures 8-1 and 8-2). Focal mononuclear cellular infiltration was also seen in areas with mild to moderate interstitial oedema.

DISCUSSION

The similarity between the histopathological features seen in these donor hearts which failed at or soon after transplantation, with those in the experimental series detailed above (Chapter 2-E) is striking.

While the damaged heart remains within the donor, it may be able to support the circulation for several hours, though in a number of cases we have abandoned a

rapidly failing donor heart before transplantation in spite of significant inotropic support. The circulation may continue to be supported in some cases, however, by a significantly damaged heart, as the brain dead potential donor remains grossly vasodilated, resulting in an extremely low systemic vascular resistance.

The period of ischaemic cardioplegic arrest during transportation and transplantation may provide an additional insult in spite of efforts to keep the organ cool; the heart temperature may rise to 20-25°C, depleting further the energy stores, and progression of the myocyte injury may be unavoidable.

The transplanted heart must function against a normal systemic vascular resistance, and it may be this factor plus the further metabolic deterioration which leads to final inadequacy.

The possibility exists that the myocardial structural changes seen in the patients reported here may, at least in part, have resulted from a massive infusion of inotropic agents administered to the recipient in an endeavour to stimulate donor heart function. In two of the patients, however, donor heart function was clearly totally inadequate before any inotropic support was given (heterotopic heart transplants), and a third received only minimal post-operative inotropic support before sudden donor cardiac arrest. It therefore seems unlikely that this could be the major contributing factor towards the myocardial injury seen.

The above evidence suggests that myocardial structural damage can occur during the development of brain death in potential organ donors. However, progressive hormonal depletion in the donor (mainly T_3 , as shown in Chapters 2C and 5) may also play a major role in the myocardial performance of the heart following transplantation.

The hearts may fail immediately after the discontinuation of cardiopulmonary bypass support or early during the post-operative course. It is possible that besides the myocyte injury in the myocardium, the specialized conduction tissue may be involved in the process inducing unexpected cardiac arrhythmias leading to ventricular fibrillation.

During heterotopic heart transplantation (from untreated donors at Groote Schuur Hospital) poor donor heart function occurred in 10% of the transplanted hearts. After the hearts were perfused in the recipients with normal blood constituents (hormones), a period of functional recovery took place within the first 24-48 hours (Figure 8-3). If these hearts had been transplanted in the orthotopic position, most probably death would have occurred on discontinuation of cardiopulmonary bypass or in the early post-operative hours. This is clearly an advantage of heterotopic heart transplantation.

Endomyocardial biopsies from the donor heart taken from the left ventricle at the time of excision of the donor heart, have shown various degrees of myocyte injury, interstitial haemorrhage, and oedema; cardiac performance following transplantation has been satisfactory (all patients received hormonal therapy, Chapter 9). In 10 biopsied hearts, 8 showed subendocardial lesions (Figures 8-4 and 8-5).

LEGENDS

FIGURES

Figure 8-1

Histopathological section of a human donor heart which failed at the end of the procedure, showing myocytolysis with stromal collapse (haematoxylin-eosin x 450).

Figure 8-2

Histopathological section of the left ventricle belonging to patient N^o. 2 showing myocyte necrosis with stromal collapse and interstitial oedema (haematoxylin-eosin x 600).

Figure 8-3

Simultaneous ECG and pulse wave trace recording following heterotopic heart transplant. The donor heart had been stored ischaemically by continuous hypothermic perfusion for over 12 hours. (D = donor heart ECG QRS complex and its respective pulse wave trace. R = recipient heart ECG QRS complex and its respective pulse wave trace). By 12 hours post-transplantation the R heart performed 70-80% of the cardiac output while the D heart contributed only 20-30%. By 24 hours the D-R ratio was 50% and by 36 hours the D heart maintained the cardiac output close to 100% while the R heart contribution was minimal.

Figure 8-4

Histopathological section of the endomyocardial biopsy taken from the left ventricle from a donor heart before implantation, showing subendocardial haemorrhage, interstitial oedema and some areas of contraction band necrosis (haematoxylin-eosin x 100).

Figure 8-5

Histopathological section of the left ventricle from an unused donor heart because of rapid haemodynamic deterioration showing extensive contraction band necrosis involving practically all the myocytes, interstitial oedema and an area of interstitial haemorrhage (bottom right) (haematoxylin-eosin x 150).

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TABLE 8-1
CLINICAL FEATURES OF CARDIAC RECIPIENTS AND
HISTOLOGICAL FEATURES OF THE DONOR HEART

Patient	Age (years)	Sex	Underlying Cardiac Pathology	Pulmonary Vascular Resistance (Wood units)	Type of Heart Transplant Operation	Time of Death	Post Mortem Donor Heart Pathology
1	52	M	IHD + RHD	4.5	Orthotopic	At operation	Scattered myocytolysis throughout the ventricles. (Figure 8-1)
2	24	F	RHD	2.5	Heterotopic	2 hours post-transplant	Extensive contraction bands throughout the ventricles. (Figure 8-2)
3	45	M	CM	7.1	Heterotopic	7 days	Diffuse focal areas of coagulative necrosis, occasional contraction bands, and mononuclear cells
4	47	F	CM	1.3	Heterotopic	9 days	Moderate diffuse contraction bands, occasional myocytolysis, and scanty mononuclear cells.
5	43	M	IHD	4.5	Orthotopic	At operation	Diffuse contraction bands with focal coagulative necrosis.

IHD = Ischaemic heart disease

RHD = Rheumatic heart disease

CM = Cardiomyopathy

M = Male

F = Female

TABLE 8-2
CLINICAL FEATURES OF CARDIAC DONORS, MYOCARDIAL PROTECTION AND TOTAL ISCHAEMIC TIME

Donor for Patient	Age (years)	Sex	Cause of Brain Death	Period of Brain Death before Excision of Heart	Method of Myocardial Protection	Total cold Ischaemic Time
1	23	M	Motor vehicle accident	Uncertain	Total body hypothermia on cardiopulmonary bypass to 18° C. No cardioplegic agent used.	50 min
2	46	M	Subarachnoid haemorrhage	12 hr 30 min	Cardioplegic arrest	55 min
3	17	M	Motor vehicle accident	12 hr	Cardioplegic arrest, followed by continuous hypothermic perfusion storage for 6 hours.	7 hr 5 min
4	40	F	Subarachnoid haemorrhage	8 hr	Cardioplegic arrest	1 hr 5 min
5	21	M	Motorcycle accident	6 hr	Cardioplegic arrest. Heart transported in ice.	2 hr 18 min

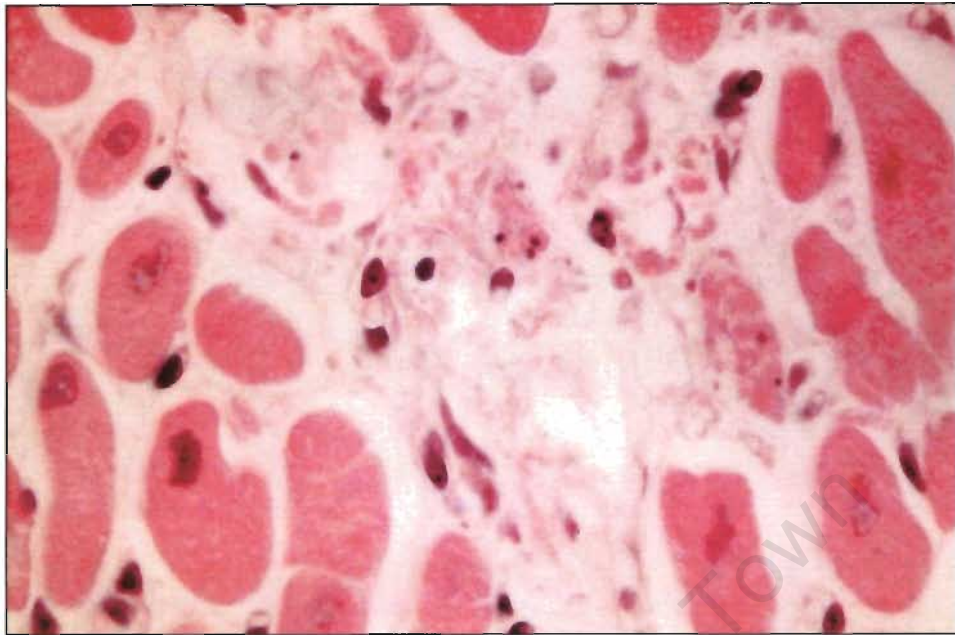


Figure 8-1

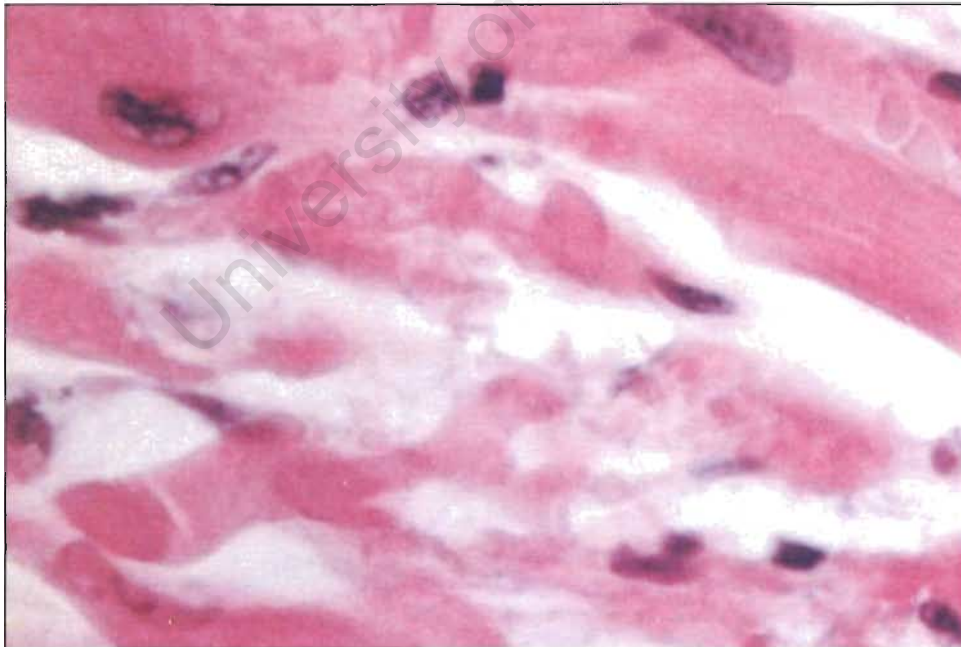


Figure 8-2

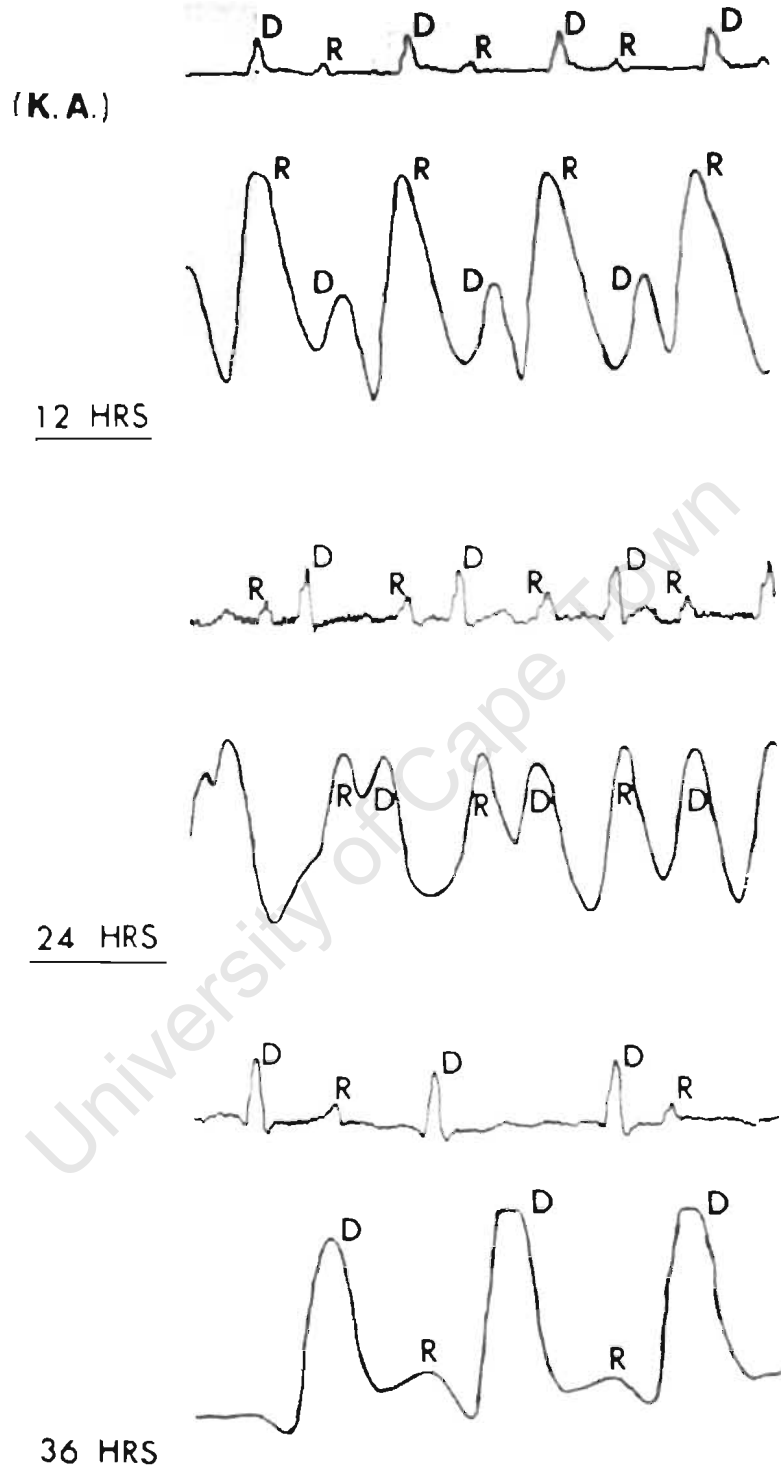


Figure 8-3

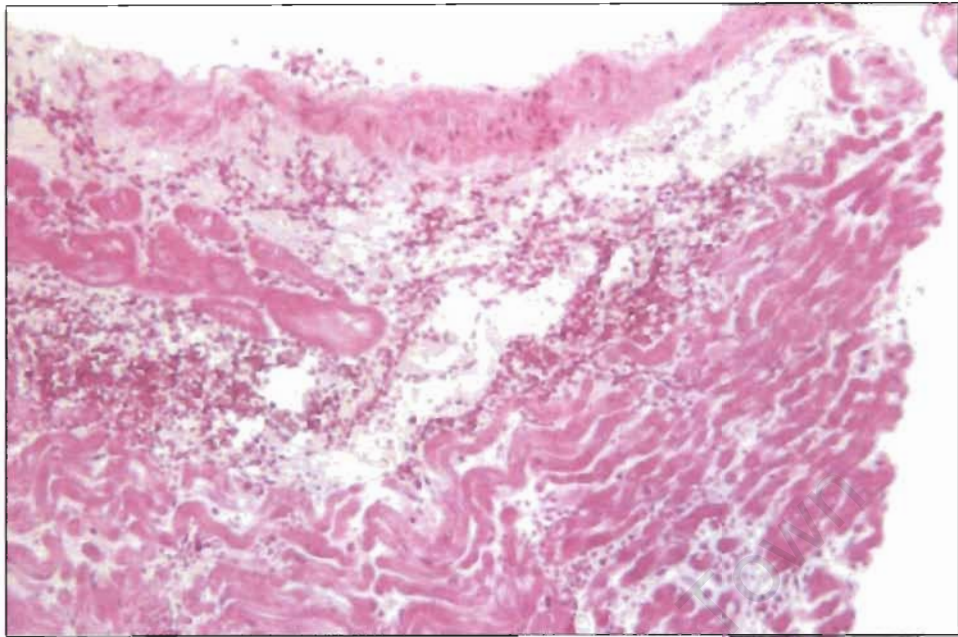


Figure 8-4

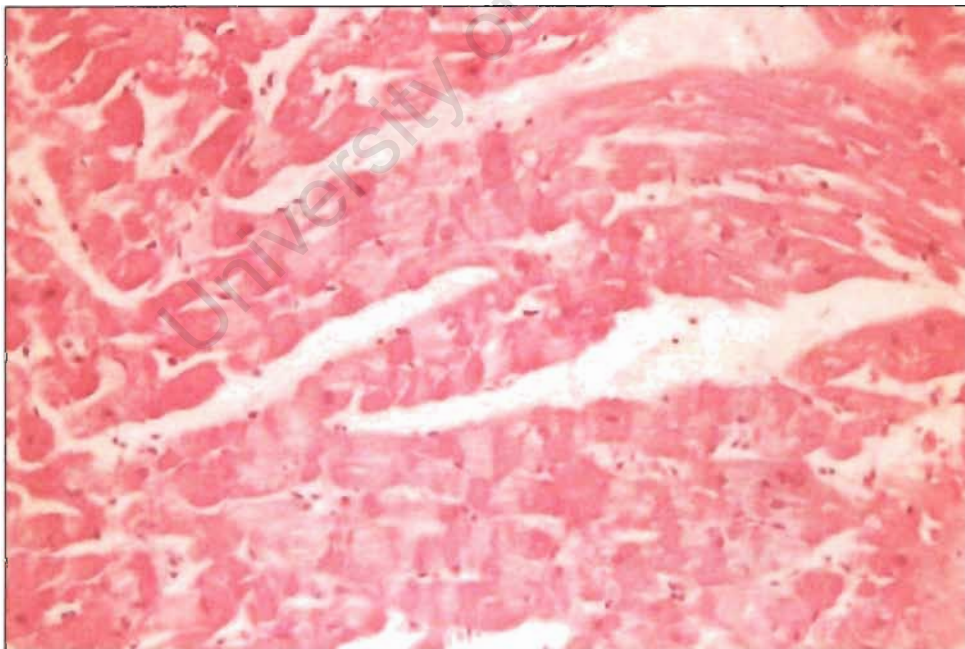


Figure 8-5

CHAPTER 9

CLINICAL OBSERVATIONS - HAEMODYNAMIC AND METABOLIC CHANGES FOLLOWING BRAIN DEATH AND RESPONSES TO HORMONAL THERAPY

INTRODUCTION

Studies in the pig and baboon demonstrated a deterioration in cardiac function after brain death, associated with the fall in circulating hormones and the increasing metabolic change to anaerobic metabolism. This myocardial deterioration was reversed by hormonal therapy, which also brought about a return to aerobic metabolism.

Following these experimental observations, all brain-dead potential donors referred to our centre for heart transplantation have subsequently been treated with hormonal therapy. Our observations in these patients are outlined below. Haemodynamic and metabolic observations on 21 brain-dead potential donors who received hormonal therapy have been compared with observations in 26 who did not.

EXPERIMENTAL GROUPS

Group A

Twenty-six patients continued to be treated conventionally with intravenous fluid and inotropic support.

The majority of potential donors referred to us are already receiving (i) intravenous fluids to replace the large urine output which brain-dead patients produce and to maintain a

CVP of between 5-10 cmH₂O, (ii) inotropic support to maintain a MAP of above 60 mmHg, and (iii) vasopressin given either by i.v. infusion or by intermittent i.m. injection, both to reduce urine output and help maintain an adequate MAP by peripheral vasoconstriction. In the past, despite these measures, approximately 20% of hearts have been lost through deteriorating haemodynamic function before excision for transplantation, or have been deemed unsuitable for transplantation.

Many of these patients were already showing features of metabolic acidosis (despite adequate ventilation) with high serum lactate and were requiring large and frequent infusions of sodium bicarbonate. Their temperatures had also fallen to low levels below 34°C. They required constant potassium infusion to replace loss in the urine.

Group B

In 21 donors (Group B), no changes were made in ventilation, but all were immediately administered i.v. triiodothyronine (2ug), cortisol (100 mg) and insulin (10 International Units irrespective of the blood glucose). All were receiving a 10% dextrose-cristaloid solution (Maintelyte). This therapy was repeated at hourly intervals until the heart was excised. If myocardial function improved, as evidenced by a sustained MAP of 60 mm Hg or more, and an increase in cardiac output (when measured), then inotropic and vasopressin support was steadily reduced and, when possible, discontinued completely. Intravenous fluid administration was similarly reduced where possible, maintaining a CVP of 5-10 cmH₂O.

In some, electrocardiographic changes showing ischaemia were already taking place.

The cause of death in all cases was head injury. Donors were monitored for a mean period of 4.5 hours (range 3 to 8 hours).

HAEMODYNAMIC AND METABOLIC PARAMETERS MONITORED

The following parameters were measured, both when the donor was first seen (control) and immediately before heart excision (approximately 4 hours post-initiation of hormonal therapy): mean arterial pressure (MAP), central venous pressure (CVP), heart rate (HR), bicarbonate requirement to maintain pH and base excess at normal levels, and inotropic support requirements. In 6 donors cardiac output was measured using a thermodilution technique from an inserted Swan-Ganz catheter; serum lactate-pyruvate was measured in 21 patients and plasma free fatty acids in 6 patients.

RESULTS

The following significant changes were noted during the course of management of the donor (Table 9-1). In Group A, the bicarbonate requirement progressively increased up to 100% ($p < 0.0003$) (Figure 9-1) in an attempt to reduce the base deficit, and there was a 35% increase in the level of inotropic support ($p < 0.05$) (Figure 9-2). In addition, 30% of these donors required extra support with isoproterenol, calcium chloride and/or vasopressin. Despite these measures, no significant improvements in haemodynamic function were measured. Five of the 26 donor hearts were eventually considered unsuitable for subsequent transplantation on the basis of poor or deteriorating haemodynamic performance ($p < 0.04$) (exact Fisher test).

In **Group B**, the bicarbonate requirement fell by 95% ($p < 0.0001$) (Figure 9-1), and the level of inotropic support was reduced by 88% ($p < 0.005$) (Figure 9-2). There was a significant increase in body temperature ($p < 0.05$). Cardiac performance significantly improved (MAP increased by 53% ($p < 0.02$) (Figure 9-3), CVP fell by 35% ($p < 0.02$), HR

increased by 35% ($p < 0.003$). A rapid improvement in cardiac output took place within five minutes following hormonal therapy (Figure 9-4, Table 9-2).

Serum lactate-pyruvate ($n=21$) and plasma free fatty acids ($n=6$) were measured in Group B only (Figure 9-5); at the initial measurement, all were significantly raised from normal levels ($p < 0.0001$), but following hormonal therapy, lactate decreased by 52% ($p < 0.02$), pyruvate by 45% ($p < 0.005$), and free fatty acids normalized within control levels ($p < 0.001$) (Figure 9-5).

All 21 of these donor hearts were considered suitable for transplantation, and all showed immediate good function following heart implantation.

In all patients but 5 in Group B, the ECG already showed significant abnormalities (J waves, ST elevation or depression, T wave changes) suggesting ischaemia at the time hormonal therapy was introduced. In all cases, these abnormalities disappeared or improved significantly during the period of hormonal therapy (Figure 9-6, 9-7, 9-8).

In Group B donors, T_3 levels were initially low in all cases (Figure 9-9), but rose to normal or high levels during the period of therapy. Cortisol and insulin levels also rose to above normal levels.

DISCUSSION

These observations, made in potential donors referred to Groote Schuur Hospital, provide further evidence for the markedly impaired aerobic metabolism which occurs in brain-dead donors, which leads to a condition similar to the sickle cell syndrome. The increasing anaerobic metabolism (as evidenced by a low pH, large base deficit,

rise in serum lactate, pyruvate and free fatty acids, repeated and increasing need for bicarbonate administration), is associated with diminished myocardial function (as evidenced by low CO₂, low MAP, high CVP, appearance of abnormalities on ECG, and need for inotropic support).

Hormonal therapy for even as short a period as 3 hours was followed by evidence of decreasing anaerobic metabolism and improving myocardial function.

The dosage of T₃ would appear to be critical, and is almost certainly in the range of 2-4 µg/hour to an adult brain-dead subject. Early in our experience, a dosage of 20 µg was administered to one such brain dead potential donor. As a result of this excessive dosage, the patient's temperature rose to 40°C, pCO₂ rose to 15 kPa, and myocardial function deteriorated though the kidney viability remained excellent.

The great improvement in acid-base balance, and the associated improvement in myocardial function and haemodynamic status of the potential donor following hormonal therapy, has enabled us to salvage some donor hearts which would previously have been unsuitable, and also to transplant all hearts in as good a condition as possible. Immediate and long term function of these hearts after transplantation has been excellent.

The ECG pattern seen at the time of referral of the donor showed ST depression, T wave inversion, junctional rhythm, broadening of the QRS interval, and J waves. Although many of the changes have been attributed in the past to hypothermia and sympathetic activity over the myocardium, from this study (Chapters 2 and 5), it is now clear that the sequence of events leading to abnormal ECG in donors is multifactorial.

The stability of the donor has enabled us to perform heart transplants electively the

following morning when the donor became available the previous afternoon or evening. Four heart transplants and 3 heart and lung transplants have been performed as a scheduled procedure in the morning rather than as an emergency operation during night hours.

This initial clinical experience correlates well with, and confirms, many of the experimental findings described earlier. The present evidence strongly supports the thesis that hormonal therapy of the brain dead donor adds considerably to improving the state of the donor heart (and kidneys), and will become a universal routine therapy in the management of brain dead potential heart and kidney donors in the future.

Furthermore, the experimental evidence presented in Chapter 4B suggests that, if periods of storage in excess of 4 hours are to be successfully employed, then pretreatment of the donor with hormones would appear to be essential if immediate good donor heart function is to be obtained after transplantation.

LEGENDS

FIGURES

Figure 9-1

Bicarbonate requirement in Group A and B potential donors from the time of referral after 4 hours. Note the significant decrease of bicarbonate administered to Group B donors, and increase in Group A. Statistical comparisons are shown at the end of 4 hours of hormonal therapy. Bars represent the SEM. Upper graph shows actual means and standard errors. Normal graph shows percentage changes.

Figure 9-2

Dopamine requirements in Groups A and B, at the time of referral and after 4 hours of treatment. Statistical comparisons are shown.

Bars represent the SEM.

Figure 9-3

Mean arterial pressure in Groups A and B, at the time of referral and after 4 hours of treatment. Statistical comparisons are shown.

Bars represent the SEM.

Figure 9-4

Cardiac output in 6 patients (Group B). C: (control) before initiation of hormonal therapy.

Significant increases by 5 minutes (A), and 3 hours (B) are shown. Notice rapid haemodynamic improvement within a short period. Statistical comparisons are shown. Bars represent the SEM.

Figure 9-5

Metabolic parameters (plasma lactate, pyruvate, and free fatty acids) at time of referral and initiation of hormonal therapy in Group B donors, and response by 4 hours (lactate and pyruvate) and by 9 hours for free fatty acids. Statistical comparisons are shown. Bars represent the SEM.

Figure 9-6

ECG recordings from 3 different donors C: (control) recording taken at the time of donor referral prior to the institution of hormonal therapy. The intervals (in minutes) after beginning hormonal therapy are indicated.

A: Recording from precordial leads V1 and V6. C shows sinus rhythm, heart rate 65, V1 normal ST segment, T wave inversion, V6 same as V1 but biphasic T wave. At 22 minutes - junctional rhythm, 100 per minute, similar features to C. Thirty-nine minutes - junctional rhythm, heart rate 88 per minute and inversion of T wave in V6. At 55 minutes - sinus rhythm, heart rate 115 per minute, normalization of the T wave in V1 and V6. By 81 minutes - sinus rhythm, heart rate 103 per minute, normal ECG. Note gain in amplitude of the QRS complex after hormonal therapy.

B: ECG from precordial leads V4, V5 and V6. C: (control) -sinus rhythm, heart rate 75 per minute, marked ST depression and inversion of T wave. By 20 minutes - sinus rhythm,

heart rate 115 per minute, less evident ST depression, inversion of T waves. By 45 minutes - sinus rhythm, heart rate 115 per minute, mild ST depression in V4, normalization of ST segment in V5 and V6, T waves remain inverted. By 75 minutes - sinus rhythm, heart rate 107 per minute, features similar to 45 minutes ECG.

C: ECG recording (V1 and V6), unstable donor, receiving high inotropic support, C: (control) - junctional rhythm, heart rate 136 per minute, ST depression and presence of J wave. By 15 minutes - sinus rhythm, heart rate 88 per minute, no ST segment depression, less evident J wave. By 45 minutes - sinus rhythm, heart rate 100 per minute, normal ST segment, incipient J wave. By 70 minutes - inotropic support was discontinued, sinus rhythm, heart rate 75 per minute, stable donor, normal ST segment, disappearance of J wave and inversion of T wave in V1.

Figure 9-7

ECG strip recording of unstable donor O = (control) at time of referral bizarre QRS complex, abnormal ST segment and large T wave, heart rate 75 per minute. By 35 seconds heart goes into ventricular fibrillation requiring defibrillation, converting to irregular ventricular rhythm. At this stage, hormonal therapy commenced. By 1.8 minutes irregular ventricular ECG. By 3.4 minutes junctional rhythm, heart rate 30 per minute. By 4.6 minutes appearance of normal QRS complexes, irregular rhythm remains with ventricular ectopics. By 22 minutes remarkable recovery of ECG, sinus rhythm, heart rate 88 per minute, normal QRS complexes, inverted T wave. Stable haemodynamics.

Figure 9-8

Rhythm strip C = (control), recording before initiation of hormonal therapy - junctional rhythm, heart rate 68 per minute, biphasic T wave. By 58 minutes - junctional rhythm, heart rate 75 per minute, normalization of T wave. By 75 minutes - incipient appearance of a P wave with prolonged PR interval, normal QRS and T waves. By 92 minutes - sinus rhythm, heart rate 79 per minute, normal ECG complexes.

Figure 9-9

Free plasma T_3 values in 21 potential donors (Group B) plotted at the estimated time of brain death to the time since had occurred.

University of Cape Town

TABLE 9-1 INITIAL AND FINAL HAEMODYNAMIC AND METABOLIC OBSERVATIONS IN 47 BRAIN DEAD DONORS AT GROOTE SCHUUR HOSPITAL

Group	n	Timing of Observations	Temperature (°C)	MAP (mm Hg)	CVP (cm H ₂ O)	Heart Rate (beats/min)	Dopamine Requirement (ug/kg/min)	Base Deficit (mmol/L)	Bicarbonate Requirement (mEq (8.4%))	Serum Lactate (mmol/L)	Serum Pyruvate (mmol/L)
A	26	INITIAL	33.7 (0.64)	79.9 (6.81)	9.1 (2.70)	72.5 (6.30)	14.4 (3.71)	-12.0 (2.03)	113 (31)		
		FINAL	33.3 (0.70)	82.4 (3.26)	11.8 (2.24)	83.6 (7.40)	19.3 (2.90)	-7.4 (1.40)	225 (33)		
		P	NS	NS	NS	NS	<0.05	<0.005	<0.0003		
B	21	INITIAL	32.5 (0.71)	56.1 (14.4)	11.3 (6.36)	67.4 (10.61)	27.0 (10.36)	-10.6 (3.60)	191 (77)	5.1 (0.97)	0.29 (0.030)
		FINAL	35.6 (0.80)	86.1 (3.60)	7.3 (3.77)	35.6 (0.80)	13.0 (2.73)	-2.4 (1.64)	100 (7)	2.4 (0.32)	0.16 (0.020)
		P	<0.05	<0.02	<0.02	<0.003	<0.005	<0.0002	<0.0001	<0.02	<0.005

P = statistical difference between initial and final observation.

Figures in parentheses refer to standard error.

MAP = Mean Arterial Pressure.

CVP = Central Venous Pressure.

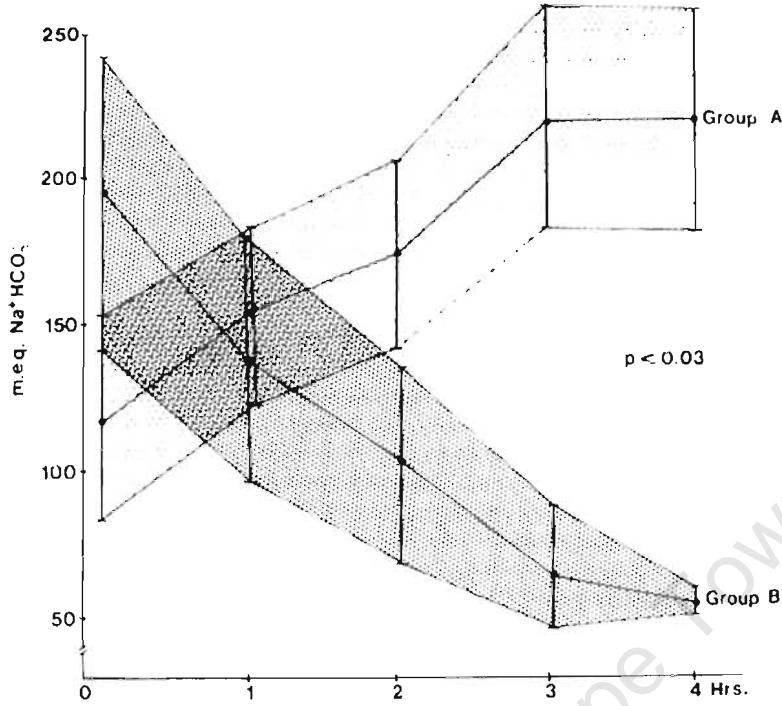
TABLE 9-2
CARDIAC OUTPUT
RESPONSE TO HORMONAL THERAPY (l/min) (GROUP B)

Pt	Control	Time Intervals		
		5 min	3 hrs	6 hrs
1	5.2	11.1	9.9	10.9
2	3.5	7.3	8.0	-
3	2.5	3.7	5.1	-
4	3.5	5.7	7.2	-
5	3.5	5.2	7.3	-
6	2.3	5.7	6.1	5.9
Mean	3.42	6.45	7.26	8.40
SEM	(0.42)	(1.04)	(0.67)	(2.50)
		p<0.01*		
		p<0.005***		

* comparison between 5 minutes and control

** comparison between 3 hours and control

BICARBONATE REQUIREMENT



BICARBONATE REQUIREMENT

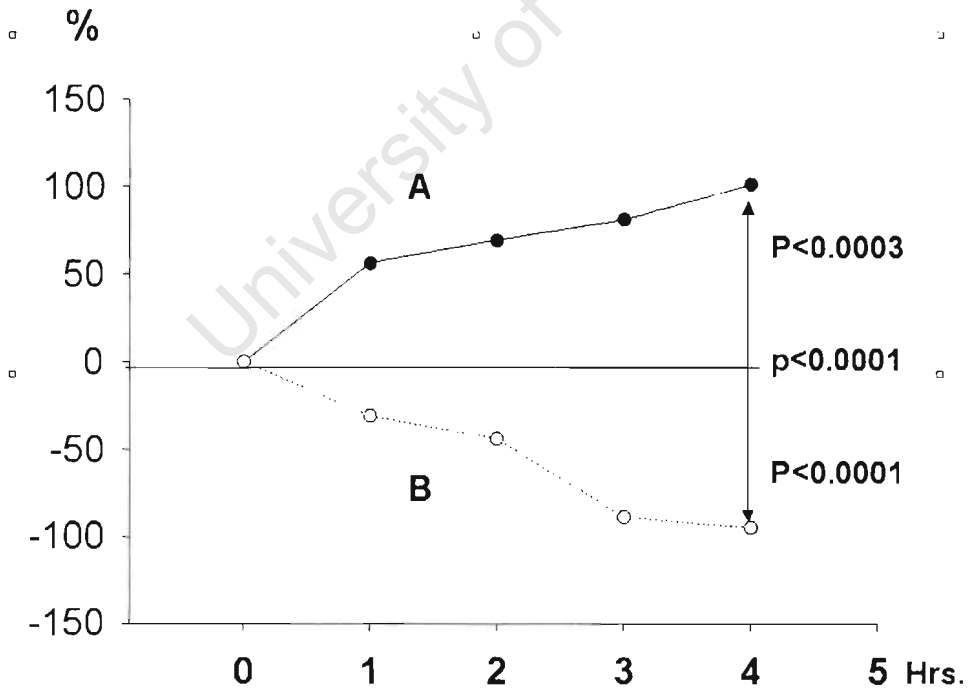


Figure 9-1

DOPAMINE REQUIREMENTS

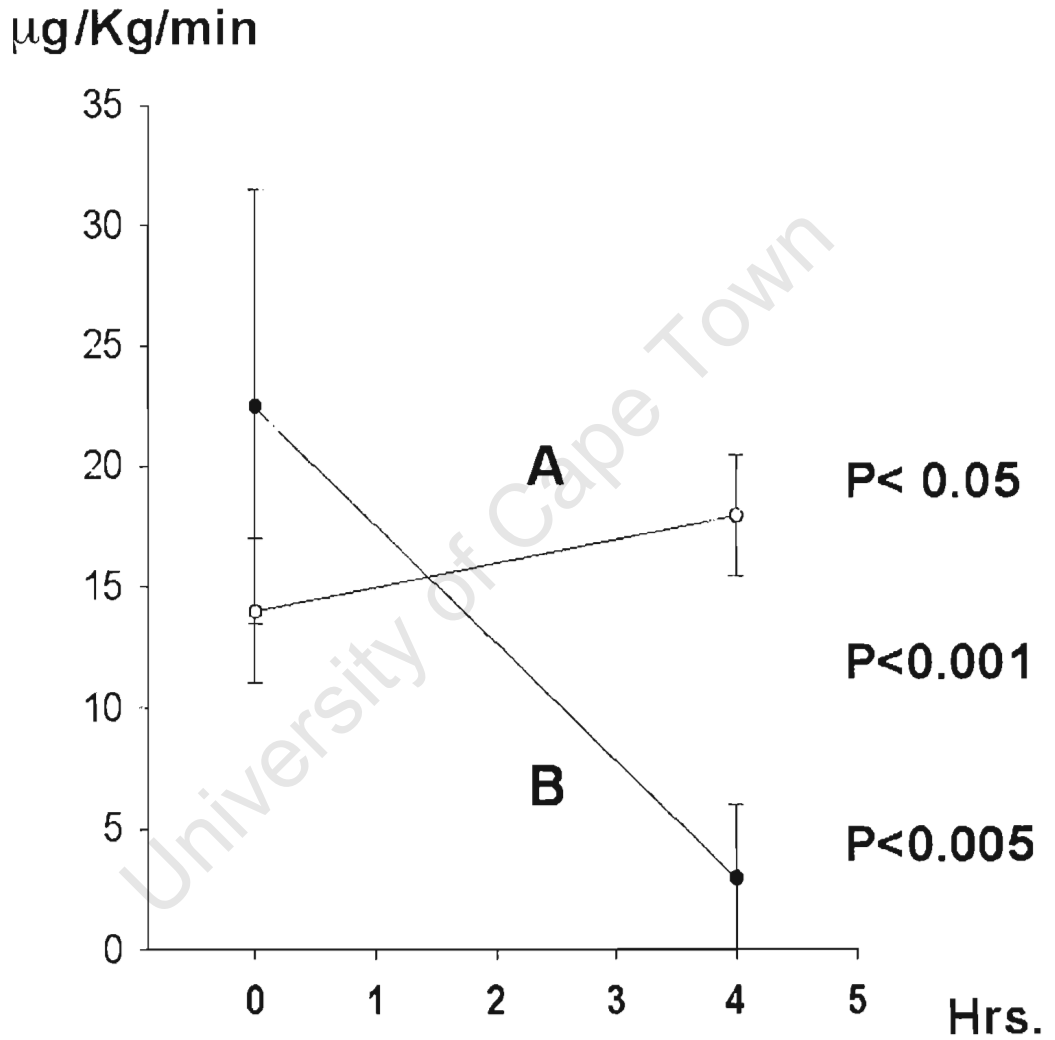


Figure 9-2

MEAN ARTERIAL PRESSURE

mmHg

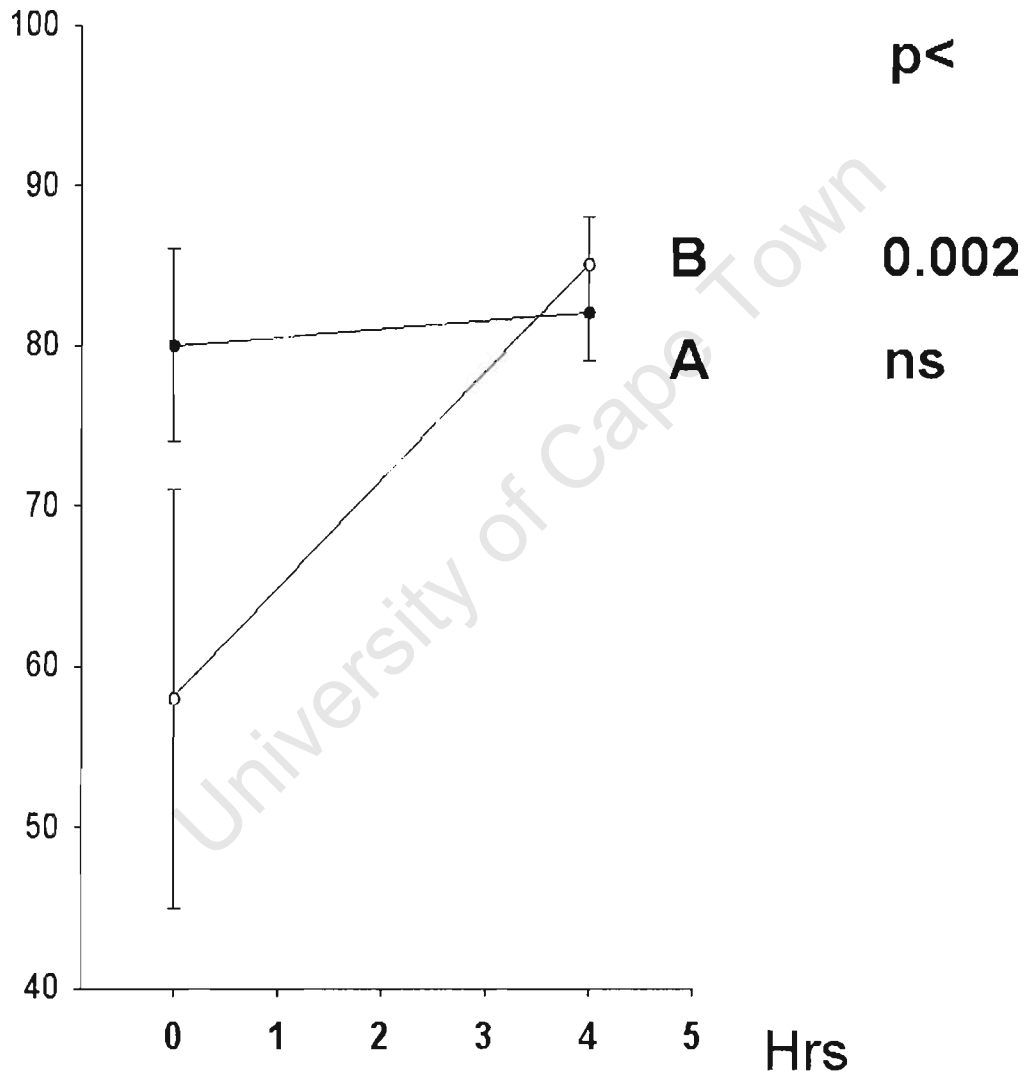


Figure 9-3

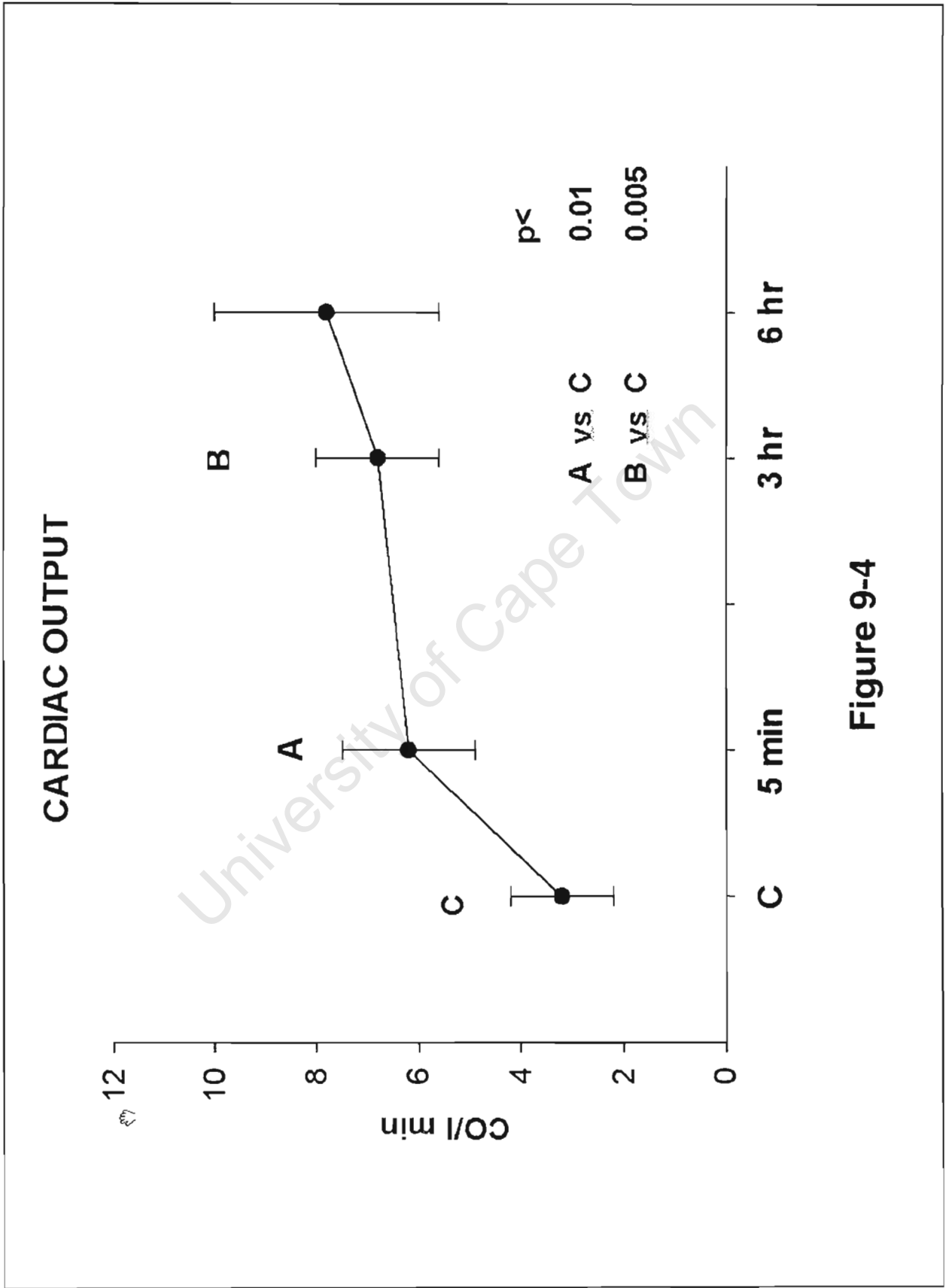


Figure 9-4

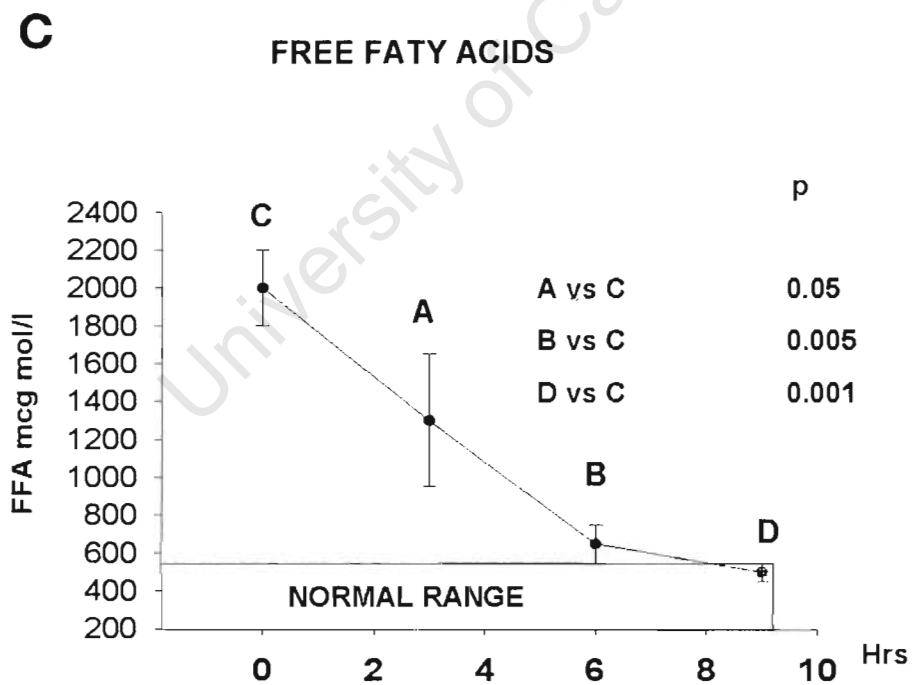
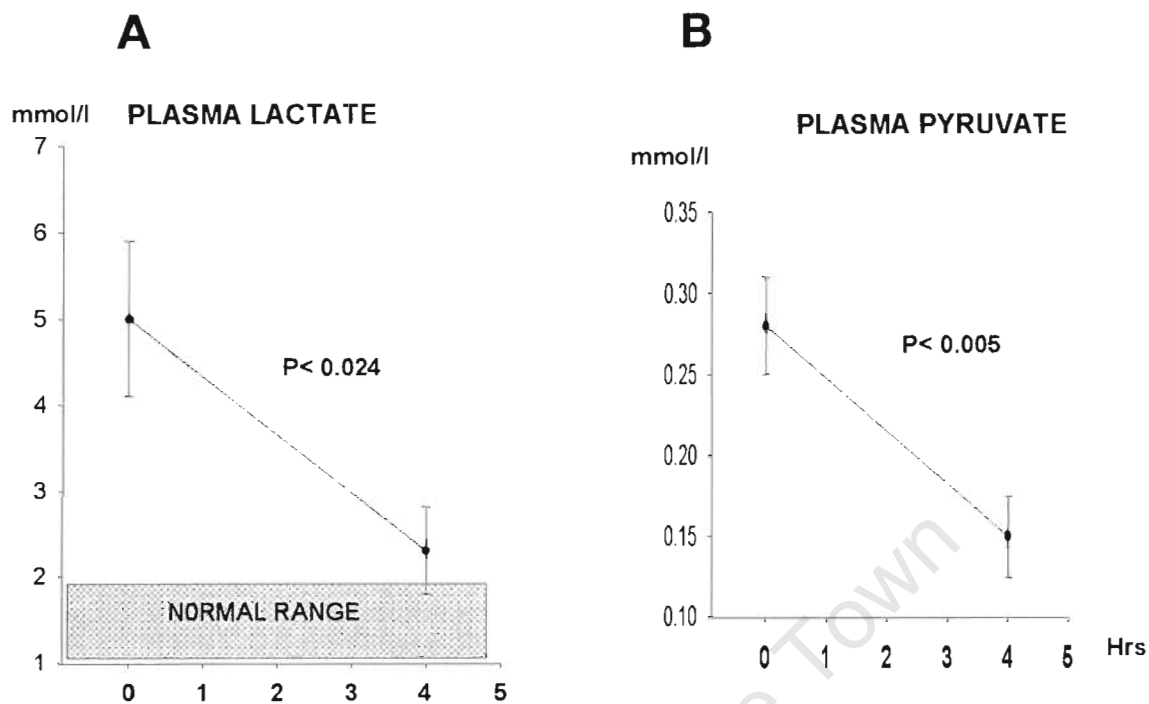


Figure 9-5

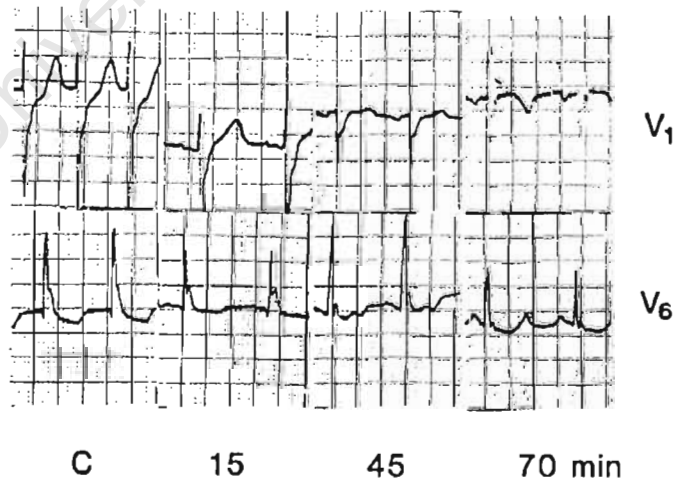
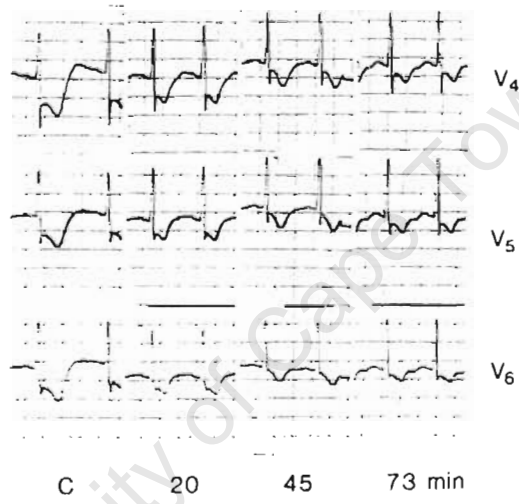
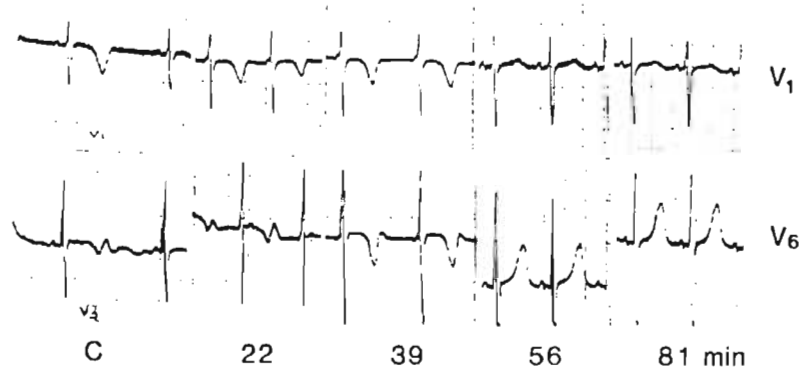
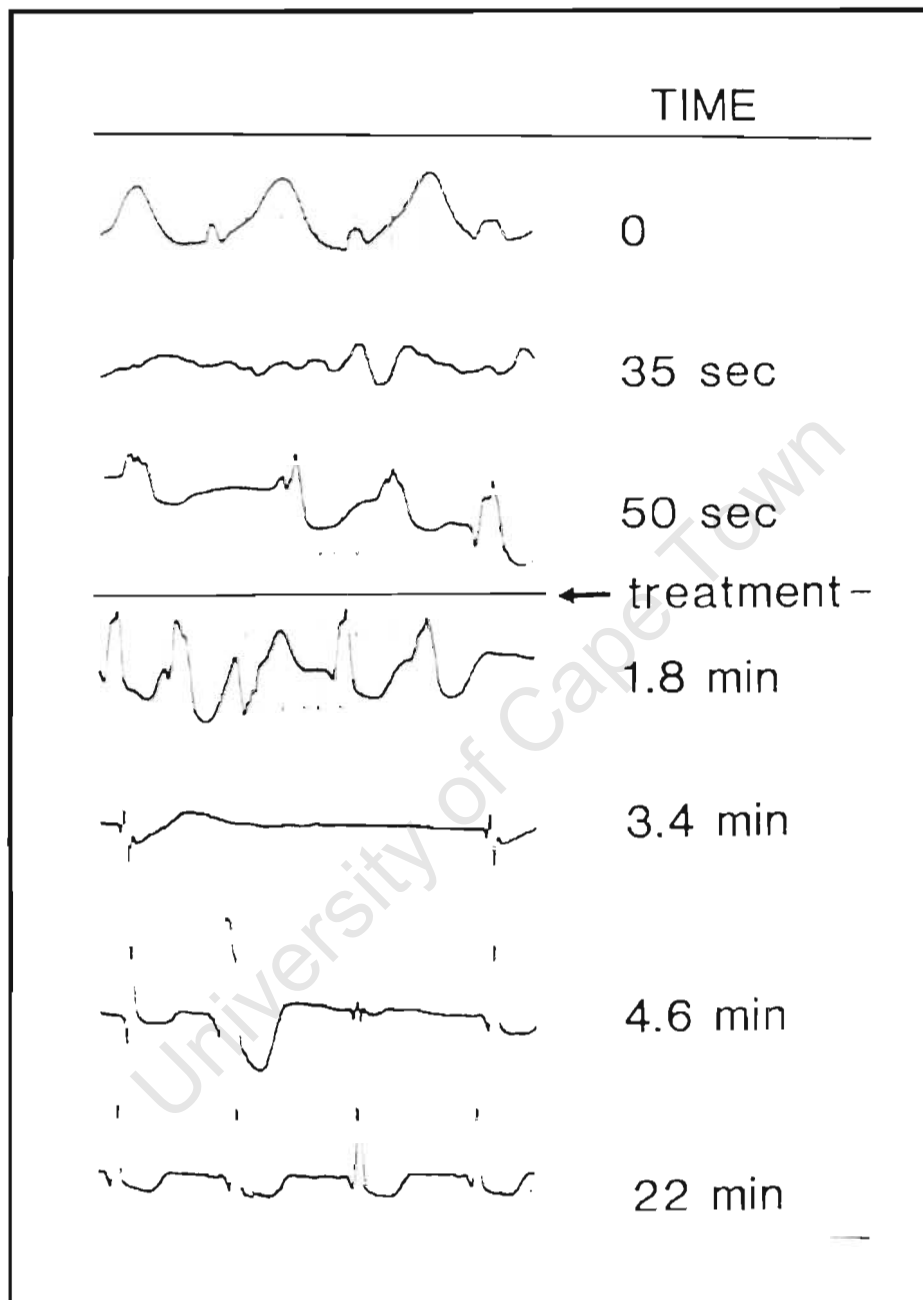


Figure 9-6

**Figure 9-7**

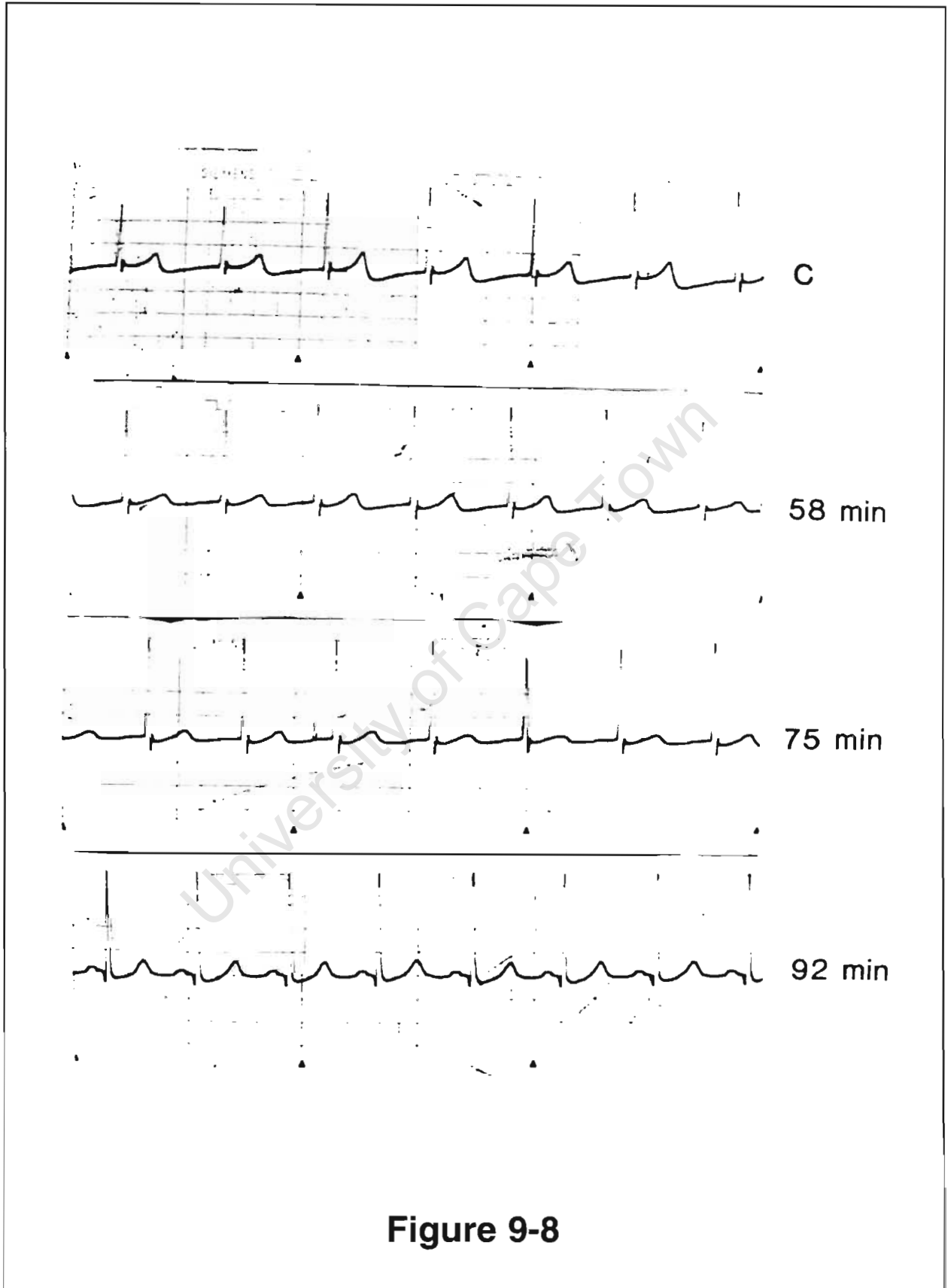
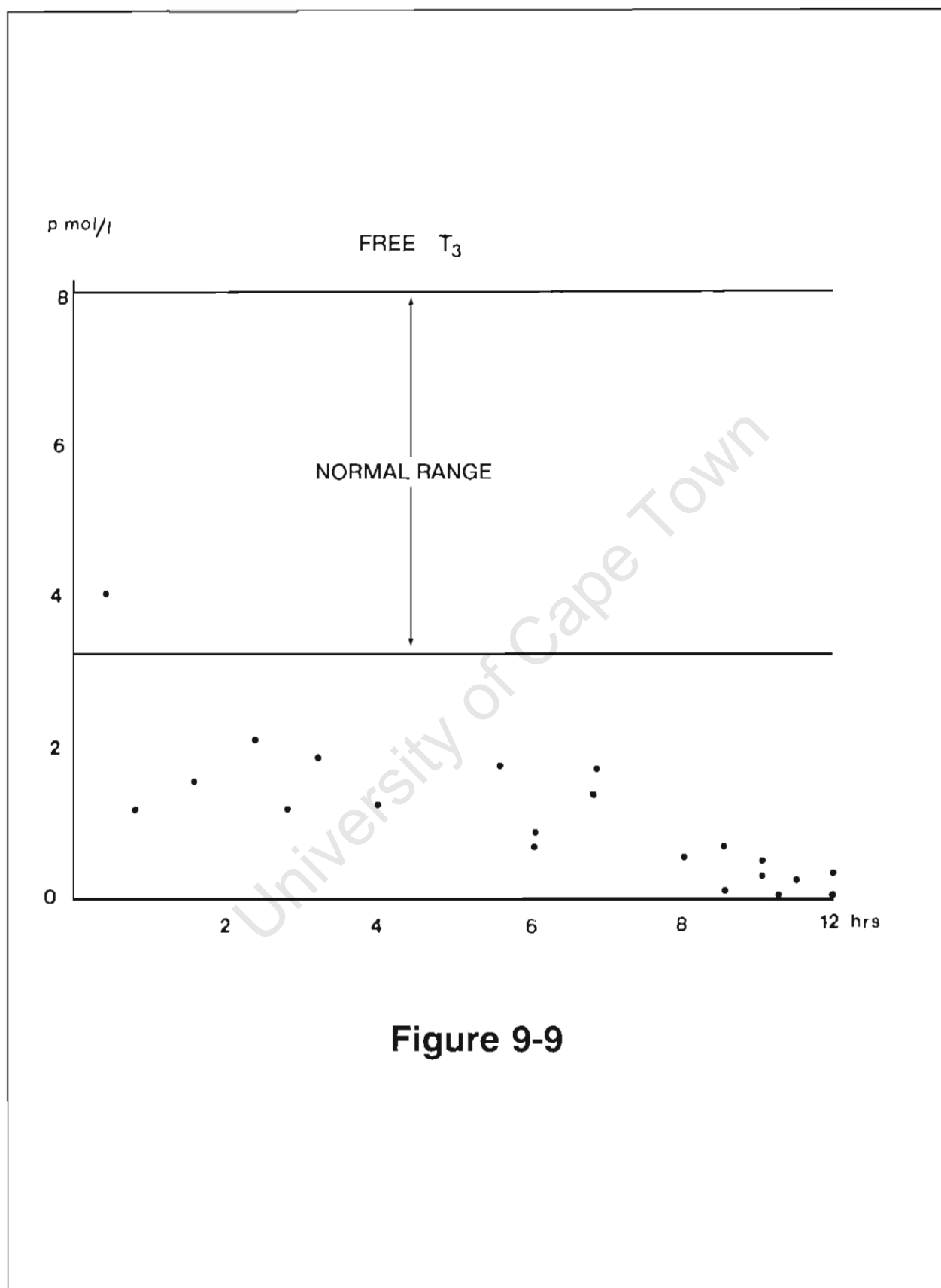


Figure 9-8

**Figure 9-9**

CHAPTER 10

OVERVIEW - STATE OF THE ART IN THE MANAGEMENT OF THE BRAIN-DEAD ORGAN DONOR

INTRODUCTION

The presented dissertation confirms that brain-dead organ donors (BDOD) are exposed during the agonal period to a massive catecholamine release (catecholamine storm) (Novitzky et al. 1984, Shilvalkar et al. 1993), resulting in systemic hypertension, elevated systemic vascular resistance, transient acute left ventricular failure, and multiple cardiac arrhythmias (Novitzky et al. 1987). This is followed by a significant reduction of free triiodothyronine (FT₃), cortisol, insulin, and antidiuretic hormone (Novitzky et al. 1984). A change from aerobic to anaerobic metabolism takes place (Novitzky et al. 1988, Sztark et al. 2000, Bruisma et al. 2001). The low plasma FT₃ state is one of the components of the euthyroid sick syndrome (ESS) (Novitzky et al. 1992, Stathatos et al. 2003, Wyne 2005, Groot 1999). As a consequence of the catecholamine storm, the endothelium of the entire body is affected (Stoica 2002, Stoica et al. 2002), triggering the initial stages of a systemic inflammatory response that continues progressing as time elapses (Szabo et al. 2002).

Release of endogenous catecholamines in the heart induces various degrees of myocyte injury and necrosis (Novitzky et al. 1989), including contraction bands in the smooth muscle of the coronary arteries and calcium deposits in the coronary media (Rose et al. 1988, Yeh et al. 2002). Progressive inhibition of mitochondrial function leads to cellular depletion of high energy phosphates (ATP, CP) and accumulation of tissue lactate (Novitzky et al. 1988, Sztark et al. 2000). The reduction in cytosolic ATP results

in the inhibition of ATP-dependant ATPases in the sarcolemma and cytosol. Calcium-induced injury may be reduced if ATP is available, mobilizing Ca^{++} into the sarcoplasmic reticulum (Davis et al. 1993, Tillmann et al. 2002). Reduced ATP will further induce cellular calcium overload, contraction band necrosis, and further mitochondrial injury (Hiroi et al. 2006).

Hormonal therapy (HT) with T_3 , cortisol, and insulin beneficially impacts the rapid recovery of mitochondrial function. Thus, depleted high energy phosphates are restored, allowing hemodynamic recovery. Therefore, following HT, initially unacceptable hearts recover cardiovascular function, and allow reduction in the initial high dependence on catecholamines (Novitzky et al. 1987, David et al. 2005). These hearts can be procured and used for transplantation, with good function in the recipient (Novitzky 1996).

Further studies in Cape Town documented for the first time the occurrence of the ESS during cardiopulmonary bypass (CPB). Following cardiac surgery on CPB, a group of patients became CPB-dependent despite the administration of inotropic support, and placement of an intra-aortic balloon pump. Administration of T_3 alone allowed rapid cardiac recovery, and hemodynamic stabilization; CPB was successfully discontinued in all patients (Novitzky et al. 1989).

This observation suggested the concept of administering T_3 to both the donor and the recipient of a cardiac allograft. At the time of reperfusion of the donor heart in the recipient, the heart is exposed to a low plasma FT_3 milieu. Potentially, the heart that has recovered from the low FT_3 state in the donor could relapse in the recipient to a pre-HT state (Novitzky et al. 1988, Jeevanandam 1997, Zaroff et al. 2002). Thus, the administration of T_3 to both donor and recipient has become a therapeutic modality in some centers.

MAGNITUDE OF THE PROBLEM

Despite major improvements in heart failure management, the demand for donor organs has outpaced donor organ availability. In the USA, the Organ Procurement and Transplantation Network and the United Network for Organ Sharing (UNOS) maintain statistics on organ donor availability and the demand for organs for such patients. In 2006, 90,600 patients were in the UNOS database (Figure 1a-1b). In the year 2005, over 7,000 patients died on the waiting list or were not transplanted for medical reasons (Novitzky et al. 2006, Rosendale et al. 2003). In that year, 25,953 living and deceased organ transplants were performed, but 41,057 potential organ recipients were added to the waiting list. As the demand grows, the number of organ donors will be increasingly insufficient to meet that demand. Furthermore, using conventional resuscitation methods, a loss of at least 35-40% and up to 64% of brain-dead organ donors hearts has been observed (Ullah et al. 2005). In the case of cardiac transplantation, one alternative is the use of left ventricular assist device support as a bridge to transplantation or as destination therapy, but this form of therapy has not resolved the problem of donor organ shortage.

An alternative is to implement the use of HT in all brain-dead organ donors. Following the initial presentation of the UCT findings in 1984 at the International Society for Heart Transplantation, the response was only modest. The UCT researchers continued to provide positive results and data on the management of brain-dead organ donors. Other research centers confirmed the UCT findings. Thus, at a slow pace, HT has gained increasing acceptance. Currently, after some years, HT in the management of potential organ donors is gaining rapidly in popularity. (Rosendale et al. 2003, UNOS 2002, Rosengard et al. 2002, Salim et al. 2005).

FURTHER BASIC RESEARCH

To understand better the body response to brain death, further studies were performed in animals and in human donors. It was found that the catecholamine storm has a significant impact on the endothelium of all organs used for transplantation Stoica et al. (2002); there is upregulation of major histocompatibility complex molecules Koo et al. (2003), expression of E-P-selectins Jassem et al. (2003) and integrins Pratscke et al. (2000), expression of Class II antigens is upregulated Lefer et al. (1991), ICAM-1 Mulligan et al. (1993), VCAM-1 Walzog et al. (2000), and platelets are activated Hirai (2003), and elevated levels of inflammatory cytokines are released Skarabal et al. (2004), Avlonitis, 2005 e.g., tumor necrosis factor (TNF) Takada et al. (1998), and IL-6 Plentz et al. (1998), Plentz et al. 2000, Stango, (2001). Levels of TNF and IL-6 are elevated 1.6 and 2.4 times, respectively, in donor hearts deemed unsuitable for transplantation compared with hearts deemed acceptable. This inflammatory response has also been observed in situations, such as during CPB Hirao (2003), sepsis (van der Meer et al. 2007), acute myocardial infarction del Fresno et al. (2007), trauma GOU et al. (2006) heart failure Villacorta et al. (2007), and in the majority of patients who are treated in an ICU setting Schweickert et al. (2007). It is associated with the ESS Powner et al. (2005, Papanicolaou (2000).

Further research on BDOD has confirmed their inability to generate high energy phosphates, not only in the heart Novitzky et al. (1987), but also in the kidneys Wicomb et al. (1986), liver Olinga et al. (2005), pancreas and gastrointestinal tract Koudstaal et al. (2005) and somatic muscle Sztark et al. (2000).

Despite a better understanding of the events occurring during and following brain death, the standard therapy of the BDOD remains the same as, or very similar to, that initially described at UCT in the 1980s.

CLINICAL STUDIES

Despite multiple clinical studies, the basic therapy remains the same as it was prior to the introduction of HT. Therapy includes ventilatory support, fluid replacement, acid-base correction, electrolyte replacement, inotropic support, control of temperature, treatment and prophylaxis for infection, and blood product replacement Ullah et al. (2006).

HT has not changed much since the research performed in Cape Town, although arginine vasopressin, which was used only intermittently at UCT, has been added to the regimen in some centers. This consists of the administration of triiodothyronine (2 μ g), corticosteroids (100mg cortisol or 125mg methylprednisolone), vasopressin (1-3U), and insulin (10U) hourly Novitzky et al (2006), Rosendale et al. (2003). Once HT has been initiated, plasma K⁺ monitoring is essential. Initially, normal K⁺ levels may rapidly be reduced to values as low as 2meq/l. This is the result of rapid activation of Na⁺/K⁺ ATPase. ATP-depleted cells lose ionic homeostasis. By restoring ATP substrate, T₃ allows intracellular K⁺ normalization. On occasion, up to 200-300meq of K⁺ are required to normalize plasma K⁺ levels.

Following the initial studies by the UCT group, other transplant groups presented results confirming the UCT findings. Although at least four groups demonstrated a beneficial clinical effect of HT on the unstable donor Wheeldon et al. (1995), Jeevanandam et al. (1996), Salim et al. (2001), Zuppa et al. (2004) not all studies showed a benefit. Subsequently, a large number of experimental and clinical studies on the effect of brain death and HT have been reported Timeket et al. 1998, Salim et al. (2001).

At Papworth Hospital in the UK Darracott-Cankovic et al. (1987, 1988), after conventional

donor management consisting of ventilation, fluid replacement, and optimizing serum electrolytes, 50 donors failed to meet minimal acceptance criteria for heart donation due to low mean arterial pressure (MAP) (<55mmHg), elevated central venous pressure (CVP) (>15mmHg), elevated pulmonary capillary wedge pressure (PCWP) (>15mmHg), low left ventricular stroke work index <15gm*m/m²/beat, or high inotrope requirement (>20µg/kg/min). After HT therapy, consisting of methylprednisolone, T₃, arginine vasopressin, and insulin, 44 of the 50 donors had improved enough to be used as heart donors. Thirty-day recipient survival was 89%, and none of the five early deaths was due to cardiac failure.

At Temple University, 22 potential donors with impaired left ventricular function, elevated left atrial pressures, and high inotrope requirement received T₃, and all demonstrated an improvement in cardiac and circulatory function concomitant with a significant reduction in inotrope requirement Jeevanandam et al. (1994). In 17 of the donors, the heart recovered and was transplanted successfully. In Los Angeles, HT with methylprednisolone, T₄, and insulin enabled similar improvement in the function of 19 inadequate donor hearts Salim et al. (2001). At the University of Pennsylvania, 91 pediatric brain-dead donors receiving T₄ had a significant reduction in their vasopressor requirements. No data were given on organ yield or quality Zuppa et al. (2004).

WIDER ADOPTION OF HORMONAL THERAPY IN THE MANAGEMENT OF HUMAN BRAIN-DEAD POTENTIAL ORGAN DONORS

For several years, few transplant groups incorporated HT into their protocols for the management of the brain-dead potential organ donor. However, in 2001, a conference was held to discuss this form of therapy. Guidelines were developed for maximizing the number of organs recovered and transplanted from deceased donors. In the cohort of

10,292 BDOD, 701 received three-drug HT Rosendale et al. (2003) (methylprednisolone, T₃/T₄, arginine vasopressin). The mean number of transplanted organs from HT donors (3.8) was 22.5% more than from non-HT donors (3.1) ($p < 0.001$). Multivariate studies on HT revealed significant increases in the number of organs from each donor that could be transplanted, and an improvement in the one-year survival of kidneys and hearts after transplantation. Kidney graft survival increased from 80% in non-HT BDOD to 90% when the kidney was procured from a HT donor ($p < 0.01$).

In a second UNOS study Rosendale et al. (2003) of 4,543 heart recipients, one-month post-transplant survival of three-drug HT donor hearts was 89.9%, compared to 83.9% of non-HT donor hearts ($p < 0.01$). In recipients of heart grafts, a 46% reduced risk of death within 30 days and a 48% reduced risk of early graft dysfunction was documented if the donor had received 3-drug HT.

A heart donor management algorithm (Table I) was suggested that included 4-drug HT for donors with a left ventricular ejection fraction $< 45\%$ and/or with unstable hemodynamics. Recommended hemodynamic management included a pulmonary artery catheter to assess the effect of HT in meeting six target criteria: (i) mean arterial pressure $> 60\text{mmHg}$, (ii) central venous pressure $4\text{-}12\text{mmHg}$, (iii) pulmonary capillary wedge pressure $8\text{-}12\text{mmHg}$, (iv) systemic vascular resistance $800\text{-}1200\text{ dynes/sec-cm}^5$, (v) cardiac index $> 2.4\text{ l/min-m}^2$, (vi) dopamine or dobutamine $< 10\text{ }\mu\text{g/kg-min}$ Zaroff et al. (2002).

UNOS implemented these recommendations Rosendale et al. (2003), and carried out a retrospective analysis of all brain-dead potential donors from January, 2000, to September, 2001, inclusive. HT is now becoming more widely accepted with an increase in 3-drug therapy from 8.8% of BDOD reported in the US in the year 2000 to 19.9% of

donors in 2004 (UNOS database 2004).

Similar guidelines have been developed by the Canadian Council for Donation and Transplantation Forum, 'Medical Management to Optimize Donor Organ Potential' in 2004.

These studies therefore clearly indicated that T_3 and additional HT significantly (i) increased the number of donor hearts considered acceptable for transplantation, (figure 4) (ii) reduced the incidence of inadequate heart function early after transplantation, and (iii) increased graft survival (figures 2, and 3). HT of the brain-dead donor is therefore associated with encouraging outcomes (Ulla et al. 2006).

STUDIES ON THE EFFECTS OF T_3 FOLLOWING MYOCARDIAL ISCHEMIA AND CARDIOPULMONARY BYPASS IN EXPERIMENTAL ANIMALS

A significant reduction in plasma FT_3 , which was known to have an inotropic effect, had been documented in patients undergoing open-heart procedures Novitzky et al. (1989). Our observations of the effect of T_3 administration to brain-dead organ donors confirmed that T_3 therapy restored hemodynamic stability. The reduction in circulating FT_3 during and following CPB restored FT_3 levels, improved the hemodynamics following cardiac reperfusion and prevented further deterioration of cardiac function in patients following open heart surgery. To investigate the effect of this observation, 22 pigs underwent 2 or 3 hours of myocardial ischemia during CPB at 26°C Novitzky et al. (1988). The myocardium was protected by a standard cardioplegic solution and cold saline solution at 30-minute intervals. After the pig was rewarmed to 37°C, CPB was discontinued, and measurements of hemodynamic function were made 10 and 70 minutes later. Half of the pigs received 6 μ g of T_3 i.v. immediately after removal of the aortic cross-clamp; the remainder received no T_3 .

After 2 hours of ischemia (cross-clamping of the ascending aorta), untreated pigs showed significantly reduced myocardial function 10 minutes after discontinuation of CPB. By 70 minutes, 2 of 5 untreated pigs had died of low cardiac output, but all 5 T₃-treated pigs survived. After 3 hours of ischemia, both groups showed some reduced function at 10 minutes, though the reduction was more marked in untreated animals. By 70 minutes, 4 of 6 untreated pigs had died of myocardial failure and all T₃-treated pigs remained alive. Surviving pigs in both groups still demonstrated some reduced function compared with values obtained before CPB.

When all pigs were considered together, overall survival of those that did not receive T₃ was significantly less than those that did. T₃ clearly had a significant beneficial cardioprotective effect when administered after a period of myocardial ischemia and CPB. These data suggested that its administration might be indicated in patients undergoing open-heart operations.

To clarify the effect of T₃ on myocardial high energy phosphate stores and lactate, a series of experiments was carried out in baboons undergoing 3 hours of myocardial ischemia while supported by CPB. Seven baboons received no T₃ and six received 6μg of T₃ at the end of the ischemic period. Seventy minutes after CPB, the myocardial adenosine triphosphate level was significantly higher in the treated animals. In untreated animals, a steady increase in myocardial lactate levels occurred after CPB; by 120 minutes after ischemia (70 minutes after CPB) there was a significant difference in lactate levels between the two groups Novitzky et al. (1988).

From these data, it was postulated that a combination of global ischemia and depletion of T₃ resulted in reduced mitochondrial function, inhibition of the tricarboxylic acid cycle, inability to utilize oxygen aerobically with resulting increased anaerobic metabolism, and

depletion of myocardial phosphates. T_3 replacement therapy was presumed to improve mitochondrial function and increase aerobic metabolism that led to a measured increase in myocardial phosphates. These observations strengthened the indication for the administration of T_3 to patients undergoing cardiac operations under CPB with a prolonged myocardial ischemic period, or in whom there was any evidence of low cardiac output after discontinuation of CPB.

STUDIES ON THE EFFECTS OF T_3 FOLLOWING MYOCARDIAL ISCHEMIA AND CARDIOPULMONARY BYPASS IN PATIENTS UNDERGOING OPEN HEART SURGERY

Initially, we administered T_3 (4-10 μ g i.v.) to 10 patients, either when difficulty was being experienced in weaning from CPB support (n = 5), or when myocardial function remained extremely poor (n = 5), despite inotropic and intra-aortic balloon pump support Novitzky et al. (1989). Mean preoperative NYHA functional class of the 10 patients was 3.2, left ventricular end-diastolic pressure 20mmHg, and ejection fraction 40%. The mean myocardial ischemia time was 72 (range 40-120) minutes.

Within 1 hour of T_3 administration, the mean plasma free T_3 level had risen from 1.03 to 3.56 pmol/l, and CPB was able to be discontinued in all cases. Balloon pump support (n = 2) was no longer essential within 3 hours. At 1 hour, the mean arterial pressure had risen from 42 to 78 mmHg, and heart rate from 90 to 104 beats/min. The left atrial pressure had fallen from 30 to 14 mmHg, and the central venous pressure from 20 to 11 cm H₂O. All changes were statistically significant. Inotropic support had been significantly reduced or discontinued.

To our knowledge, T_3 had not been administered previously as an inotropic agent

to patients who had undergone cardiac surgery. Although this small trial was not randomized, the observations suggested that T_3 could play an important role in the rescue of failing hearts following a period of myocardial ischemia in patients who had undergone open heart surgery.

In two small randomized trials in patients undergoing myocardial revascularization on CPB, postoperative T_3 therapy was associated with a reduced need for inotropic support and diuretic therapy in the first study and improved cardiac output in the second study Novitzky et al. (1989). A later study added support to this conclusion Novitzky et al. (1996).

Others continued to study the effects of CPB on thyroid hormones and the effect of thyroid hormone on myocardial function Clark (1993, Dyke et al. (1991), Holland et al. (1991), the mechanisms of action have also been investigated Novitzky et al. (1988), Novitzky et al. (1989), Novitzky et al. (1990), Haas et al. (2006), Haas et al. (2006), Reichert et al. (2001).

THE POTENTIAL ROLE OF T_3 IN THE TREATMENT OF BOTH DONOR AND RECIPIENT IN CARDIAC TRANSPLANTATION

In view of the findings following brain death and following CPB, it became a UCT policy to hormonally treat both the brain-dead potential organ donor and the recipient who had undergone heart transplantation Novitzky et al. (1988). Donors were treated with a combination of T_3 , cortisol, insulin (and sometimes vasopressin), whereas recipients received T_3 before being weaned from CPB. One hundred and sixteen consecutive potential donors were treated, as well as 70 of the recipients. Immediate post-transplant cardiac function was good in all but 3, and these hearts recovered to normal within a maximum of 24 hours of mechanical support Novitzky et al. (1990).

We therefore advocate the use of HT to the donor and T₃ therapy to the recipient (figure 5) Novitzky et al. (1989), Jeevanandam (1997). By correcting the metabolic derangements that take place in the donor, the heart will be excised with close to normal levels of energy stores that can be utilized during the period of myocardial ischemia while the heart is transported to the recipient center and transplanted into the recipient. T₃ replacement therapy to the recipient, administered before removal of the aortic cross-clamp (if this has been applied), will lead to rapid restoration of energy stores that may have decreased during CPB, with associated improvement in myocardial function.

In my opinion, all BDOD should receive HT, even those with stable hemodynamics. In my experience, I have observed primary graft failure of hearts that were explanted by experienced surgeons at the same institution where the cardiac transplant was performed.

Institution of new therapies is implemented in medicine based on evidence from prospective randomized studies. However, the case for HT in the resuscitation of the donor is made so compelling by the UNOS studies that undertaking a controlled clinical trial may be unethical. What is required is to carry out a propensity analysis of outcomes relating to both donor and recipient. This undertaking is close to a prospective randomized study and would be welcomed by the transplant community.

LEGENDS

TABLES

Table 1: Number of recipients for whom the donors received 3 HT, as well as each of the possible combinations of the three drugs, and the percentage of those patients who died in the first month after transplantation or experienced early graft dysfunction. Rosendale et al. (2003).

FIGURES

Figure 1 a: UNOS data showing the number of patients awaiting heart transplantation and the number of donor hearts recovered from brain-dead organ donors from 1988 to 2001. A significant shortage of hearts available for transplantation is evident Rosendale et al. (2003).

Figure 1 b: UNOS data as for July 22nd 2007 (from UNOS webpage). On this date, in the US there were 97,008 patients awaiting organ transplantation. In 2007 (January to July 22nd), 9,217 transplants had been performed using organs from 4,662 brain-dead donors. These data indicate a significant shortage of donors and of organs procured from BDOD.

Figure 2: Kaplan-Meir survival curves for two groups of recipients showing a difference in outcome following heart transplantation. The solid line represents hearts procured from brain-dead organ donors that received hormonal therapy. The dashed line shows the outcome of hearts procured from brain-dead donors that received only conventional therapy, ($P < 0.01$), From Rosendale et al. (2003).

Figure 3: Kaplan-Meir kidney graft survival of all recipients receiving three-drug hormonal therapy (3HR, $n = 1,805$) compared with those receiving no hormonal resuscitation (no-3HR,

n=3,570). The graphs also show the survival of recipients with expanded criteria donors (ECD) with three-drug hormonal resuscitation (ECD-3HR, n=212) vs those receiving no hormonal resuscitation (ECD-NoHR, n=1,051). The difference in survival for 3 HR vs ECD No-HR recipients was significant ($P<0.01$) (From Novitzky et al. (2006).

Figure 4: Dopamine requirements before (gray bar) and after (black bar) T_3 administration alone in 154 brain-dead organ donors. Dopamine requirements are shown on the left y axis. The T_3 administered to the donors is shown on the right y axis. = Circles indicate the mean T_3 dose required to reduce the dopamine needs, from the time when the donor was first seen until the time of heart procurement. The numbers indicate the patients in each group. The three left sets of bars (Groups 1, 2 and 3) indicate BDOD that were receiving $<15 \mu\text{g}/\text{min}$ (n = 111). The two right sets of bars (Groups 4 and 5) indicate BDOD that were receiving dopamine $>15 \mu\text{g}/\text{min}$ (n=43). The hearts from Groups 4 and 5 were initially considered unacceptable for transplantation. There was a significant dopamine reduction shown for each donor group. The cardiac donor pool was expanded by an extra 43 hearts (28%) being rendered acceptable. These 'salvaged' hearts performed well in the recipients From Novitzky (1990).

Figure 5: Mean plasma free T_3 levels (in pmol/l) in 22 human donors (A) at the time of referral, and 22 human transplanted recipients (C) at the time of release of the aortic cross-clamp during cardiopulmonary bypass (CPB). After hormonal therapy to donors (D+ T_3) and recipients (R+ T_3), values have become normalized. Four different therapeutic modalities are possible. Implantation of the donor heart may be from: (1) an untreated donor to an untreated recipient, (2) an untreated donor to a treated recipient, (3) a treated donor to an untreated recipient, or (4) a treated donor to a treated recipient. Option 4 is clearly the most physiologically sound and is to be preferred.

Table 1 DRUG COMBINATIONS

Drugs	n	Failed by 1 Month (%)	Early Graft Dysfunction (%)
3 HR (T_3/T_4 + steroids+ vasopressin)	394	3.8	5.6
Steroids+ T_3/T_4	577	5.2	7.8
Steroids + vasopressin	537	8.9	12.3
Steroids only	1,057	6.1	8.0
T_3/T_4 + vasopressin	104	8.7	10.6
T_3/T_4 only	240	6.7	12.9
Vasopressin only	484	6.2	9.7
None	1,155	7.9	11.6

3HR: three-drug hormonal resuscitation, T_3 : triiodothyronine, T_4 levothyroxine.

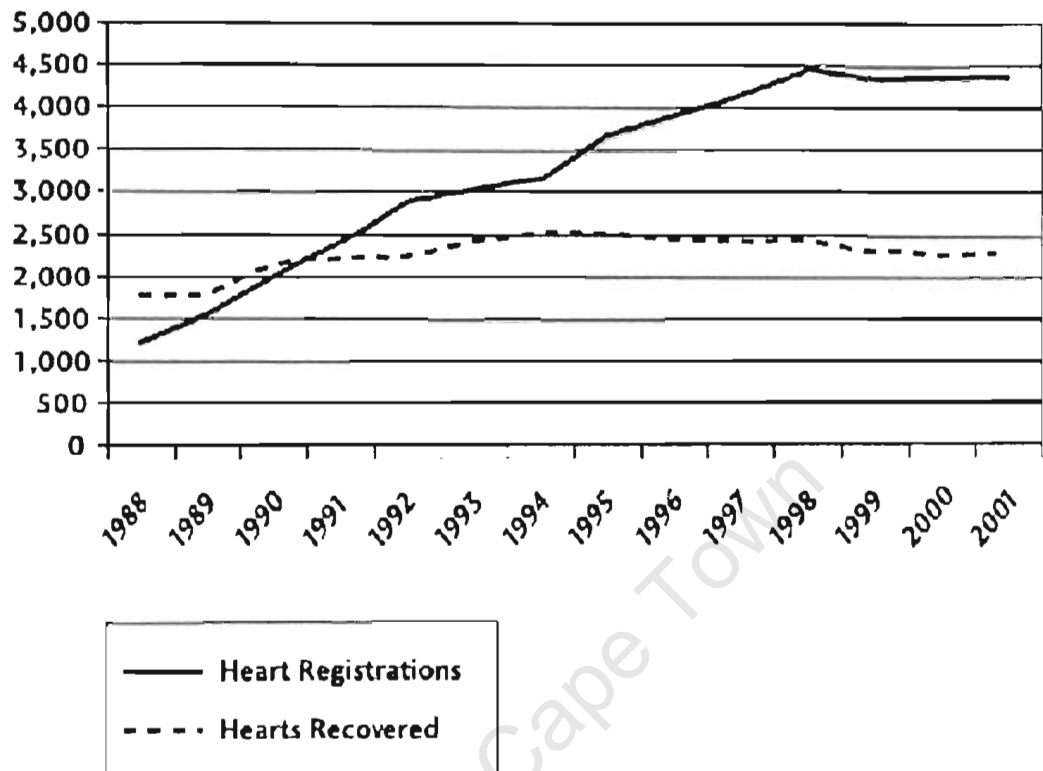


Figure 10-1A



Waiting list candidates	97,008	as of today 2:36pm
Transplants January - April 2007	9,217	as of 07/13/2007
Donors January - April 2007	4,662	as of 07/13/2007

Figure 10-1B

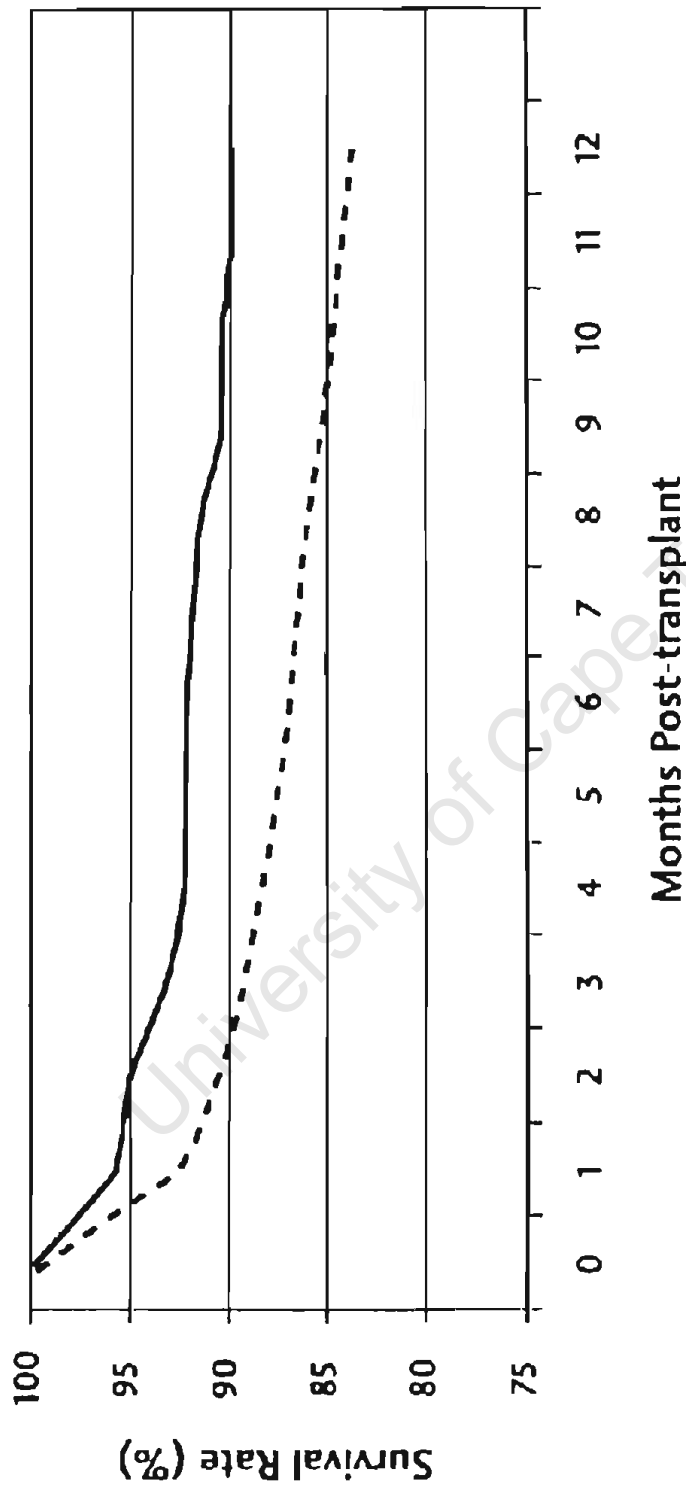


Figure 10-2

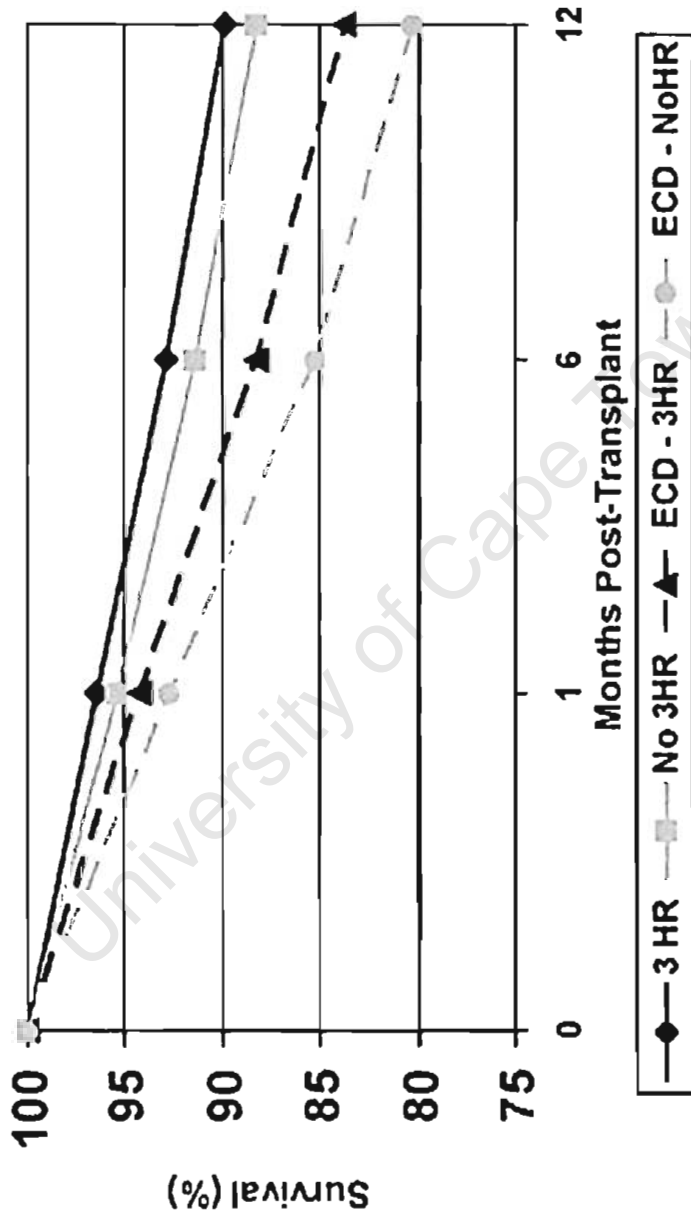


Figure 10-3

TOTAL DONORS: 154

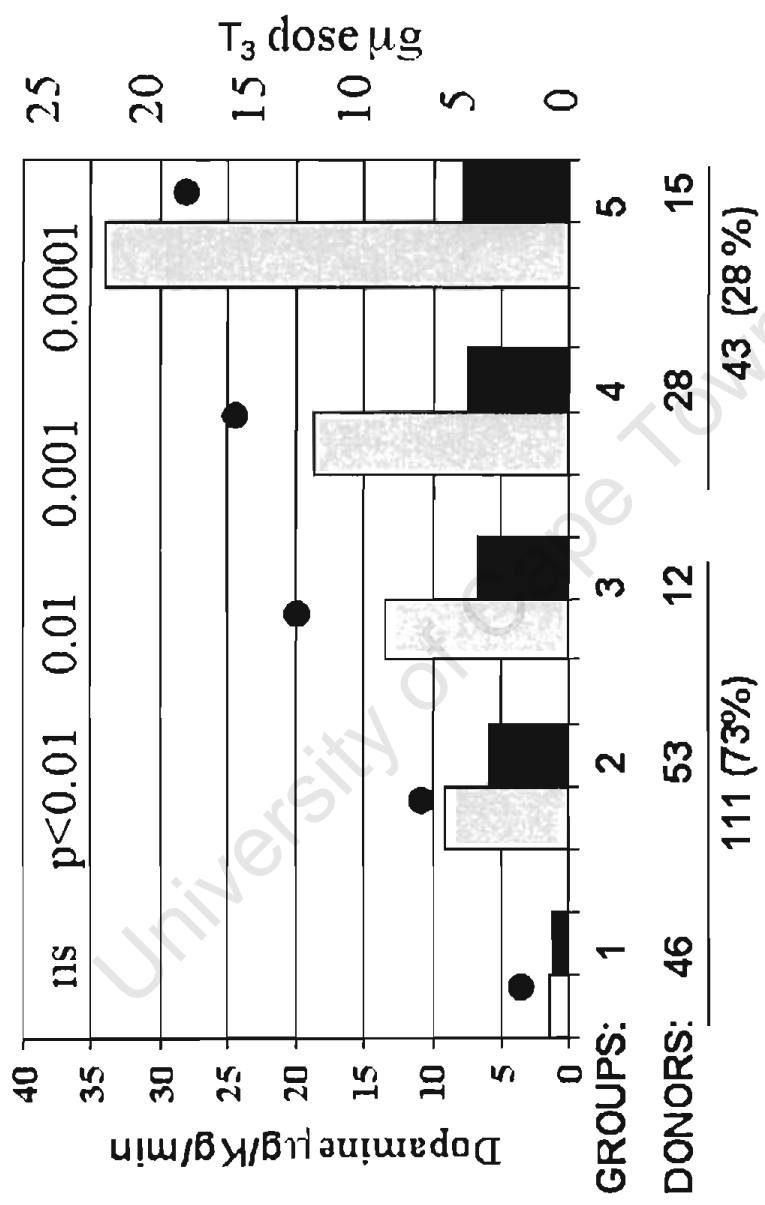


Figure 10-4

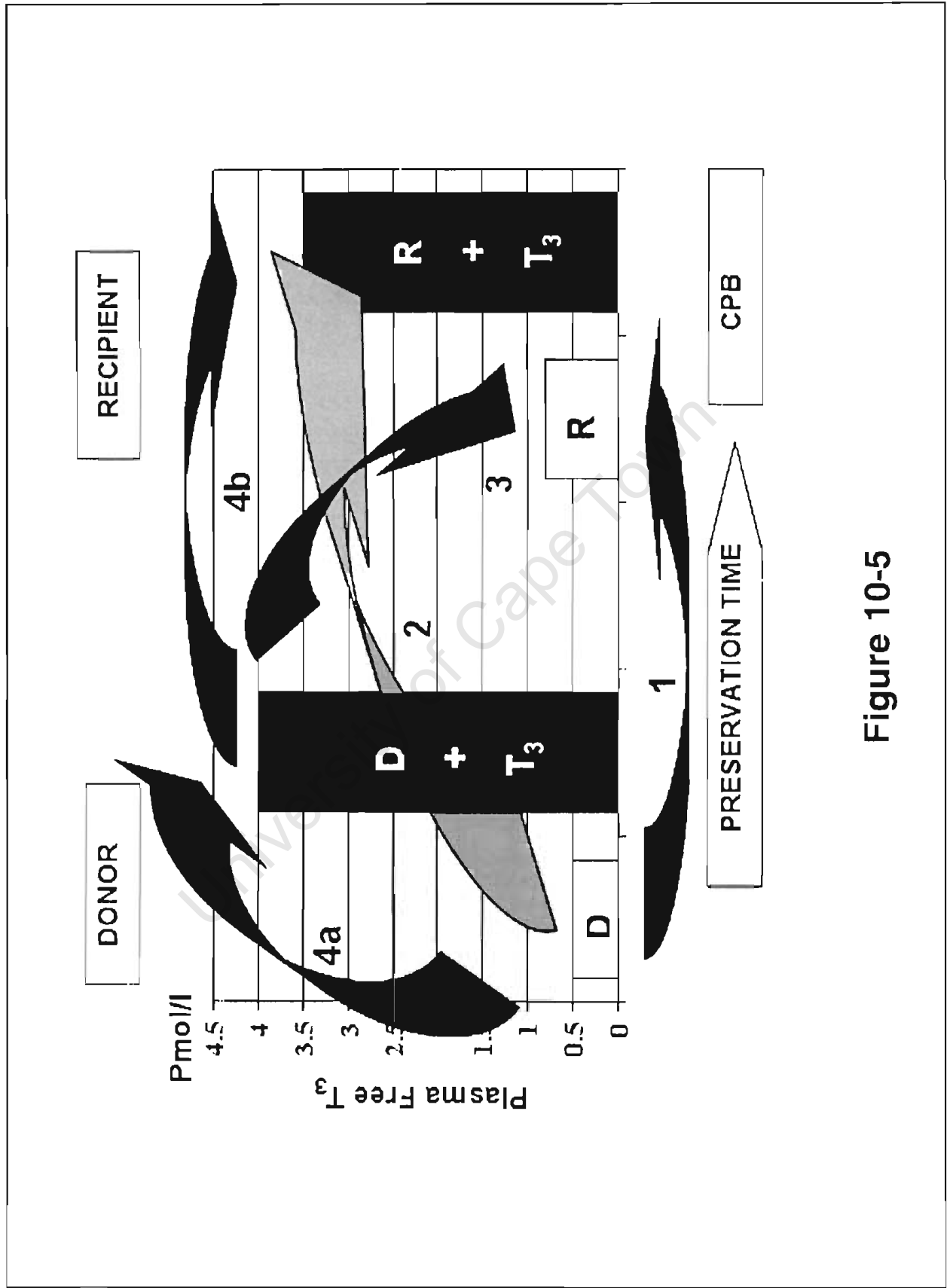


Figure 10-5

CHAPTER 11

ETHICAL ASPECTS RELATING TO THIS DISSERTATION

There are two key topics that require comment in relation to the ethics of the work carried out in animals and reported in this dissertation. The first relates to whether experiments carried out in the 1980's followed the same or similar guidelines to those required today, and the second relates to the use of nonhuman primates in these studies. In addition, a comment is included on the initial clinical studies relating to hormonal therapy in brain-dead potential organ donors and to T₃ therapy in patients who could not be weaned from cardiopulmonary bypass during open heart surgery.

Ethical Aspects of the Management of Experimental Animals: A Comparison Between the Early 1980s and Today

Today, any research worker embarking on a similar experimental program to that described in this dissertation would be required to take the following steps.

1. Describe the planned experiments in some detail and submit the protocol to the Institutional Animal Care and Use Committee (IACUC) at his or her center (Festing 2005, Aguilar-Nascimento 2005, Schapiro et al. 2006, Anderson 2007). This committee reviews the proposals, particularly with regard to humane care of the animals to be utilized, and, if necessary, asks further questions for clarification. A literature search is required (Johnson, et al. 2002), and is particularly directed to (i) minimizing the number of animals used in the study (reduction), (ii) ensuring that alternative scientific approaches that do not include experimental animals are not possible and that potential pain- and distress-inducing procedures have been refined as much as possible (refinement), and (iii)

replacing potential pain- and distress-inducing methods with other techniques where possible (replacement) (Rusche 2003, Randell et al. 2005). The investigator is also expected to have enlisted the help of a statistician to ensure the number of animals used will be sufficient but not excessive (Fitzpatrick 2003). When the IACUC is satisfied that the proposal is not only scientifically sound, but that all steps have been taken to ensure humane care of the experimental animals (Code of Federal Regulation, 1997), the protocol is approved.

In the USA, the institution is also responsible for providing training of personnel to fulfill the requirements of the Animal Welfare Act of 1991 (Rollin 2006). Furthermore, the Food Security Act of 1985, also known as the Improved Standards for Laboratory Animals Act, requires that the US Department of Agriculture (USDA 2006) registers the research facility and uses the IACUC to provide training of scientists (Anderson 2007).

The responsibilities of the IACUC continue throughout the period in which studies are performed (Gonder et al. 2001, Walker 2006, Gonder 2007).

2. The animals are purchased and, after a necessary period in quarantine, if required, are made available to the research team. With regard to nonhuman primates, they are generally today colony-bred and relatively few are captured in the wild, although these are acceptable for experimental purposes after a suitable period of quarantine. Animals are only allowed to be purchased from recognized dealers and breeders.

3. The animal is transferred to the research center where it is housed in a dedicated room with other members of the same species. It is given a period of time to acclimate to the environment and the staff. This period will vary depending on the species of animal.

4. The animal is provided with adequate food and water. The water, in particular, should be unlimited and always available to the animal. In addition, its diet should be varied with certain food 'treats' so that the diet is not monotonous. In the case of nonhuman primates, this will particularly include various fruits.
5. Before any surgical procedure, the animal is sedated with premedication and then any surgical procedure is undertaken under full inhalational anesthesia, unless there is a specific reason why this should not be so. This will have been discussed at the IACUC level before the research is begun. In particular, the use of agents that induce muscular paralysis are avoided unless a strong case can be made for their use. The reason for this is that, under deep anesthesia, the animal will be assured of feeling no pain, whereas when muscular paralysis is used, heavy sedation - anesthesia will be provided to avoid complete feeling of pain.
6. All surgical procedures are undertaken under aseptic, sterile conditions.
7. After any surgical procedure, adequate analgesia is administered to ensure a minimum of pain and discomfort during recovery from the operative procedure. This is usually for a period of three days.
8. Prophylactic antibiotics are administered, if indicated.
9. Throughout the period that the animal is in the experimental facility, it is housed in a cage of adequate size (defined by regulations) to ensure its movements are not unduly restricted. Furthermore, it is housed in a socially-acceptable environment, i.e., with other animals of the same species, and with frequent visits by human handlers who should take steps to interact with the animals. Stimulation should be provided in the form of music,

videos, radio, and various toys aimed at preventing boredom. Mirrors are valuable in the case of nonhuman primates.

10. If euthanasia is required at the end of the experiment, this is carried out under strict guidelines using accepted pharmacologic agents.

The planning of experiments and the management of experimental animals were very similar in 1980 as they are today with the exception that no IACUC approval was required. However, during the planning phase of the experiments, advice and information were sought from a number of experts (who could be considered to have formed an informal 'committee') both with regard to the scientific methods to be employed and to how these should be carried out in an ethical manner.

For example, in the brain-death studies described in this dissertation, the mode of inducing brain death was fully discussed with Professor J.C. Devilliers, the Professor of Neurosurgery at the time at UCT. Anesthesia was discussed with Dr.D. Morel of the Department of Anesthesia. Indeed, when studies on cardiopulmonary bypass were being performed, Dr. D. Morel or Dr A. Swanepoel or one of their colleagues would come to provide anesthesia for the animal. The Chairman of the Department of Cardiac Surgery at the time, initially Professor C.N. Barnard and subsequently Professor B. Reichart, were closely involved in the planning and performance of these studies, and advice was sought from several other experts at UCT at the time, including Professor L. Opie. Dr. C. Isaacs collaborated as statistician on several projects. All of these advisors and collaborators were aware of the studies being planned and undertaken, and so acted as both scientific advisors and ethical peer reviewers.

The Use of Nonhuman Primates in this Work

Several of the studies detailed in this dissertation were carried out in pigs, which were at times more readily available and less expensive to maintain than nonhuman primates. However, several of the key experiments were carried out in baboons, which are ideal for several reasons (Dormehl 1992, Dormehl, 1992, Kaup 2002, Altman 1995, Carlsson et al. 2004, National Research Council 2003, Vitale et al. 2004, Randall et al. 2005, Radzikowski 2006).

Their phylogenetic closeness to humans, particularly relating to anatomy, physiology and immunology, make them ideal experimental animals for transplantation and allied studies. The results obtained in studies using baboons can readily be extrapolated for human application.

Indeed, even today, work carried out in rodents, or even dogs or pigs, is usually not acceptable as a basis for clinical trials, and, increasingly, studies are required in nonhuman primates before they can be transferred to humans. Our ability at UCT to use baboons for these studies therefore enabled immediate transfer to the clinical transplantation program at UCT. Our findings in baboons proved highly clinically relevant and, within a very short period of time, enabled advances to be made in the clinical management of brain-dead organ donors and of patients undergoing heart transplantation and other cardiac surgical procedures on cardiopulmonary bypass. This had immense benefits for patients undergoing cardiac surgical procedures at UCT.

The baboon was being used as the standard experimental animal in the Department of Cardiac Surgery at UCT at that time. The surgical technique of heterotopic heart

transplantation had been developed in baboons by Dr. J. Losman, particularly because the thoracic cavity of the baboon is very similar in shape to that of the human. Having once been developed in the baboon, the procedure was immediately translatable to the human patient. Furthermore, Dr. W. Wicomb, working under the direction of Professor Barnard, was using pigs and baboons in his studies on organ storage. This work was again immediately transferred to the clinical transplantation program once it had been proved successful in nonhuman primates. Professor D.K.C. Cooper was also using baboons regularly in his studies of immunosuppression of heterotopic heart allografts and subsequently of pig-to-baboon heterotopic heart xenografts.

Baboons were readily available to the experimental laboratories at UCT because they were being captured by farmers in the surrounding regions as they had become pests, particularly in crop-growing areas. Rather than being shot, as was common, they were captured and transferred to a farm where they were placed in quarantine for a period of time until it was assured they were not carrying significant diseases. They were then made available to research groups, of which we were one. Nonhuman primates were therefore much more readily available in South Africa at that time than in most other countries where advanced experimental research was being undertaken.

Baboons are relatively large animals, allowing sequential blood samples to be drawn. In the brain-dead studies carried out at UCT, multiple samples were drawn over periods of perhaps 24 hours or longer. In some experiments, sequential myocardial biopsies were taken for biochemical analysis after brain death.

The baboon tolerates cardiac surgery using the heart-lung machine particularly well. The baboon's tissues, such as the aorta, are strong and easy to suture, and hemostasis is

easy to obtain. These features allow operative procedures to be carried out without a high risk of losing the animal.

Clinical Application of T₃ Therapy

The clinical use of T₃ for non-thyroid diseases was initially studied in two different conditions: (1) The unstable brain-dead potential organ donor, and (2) the cardiopulmonary bypass (CPB)-dependent patient during open heart surgery. When these studies were carried out, there was no IRB in existence at UCT, and so the studies were discussed and planned with advice from colleagues in the Departments of Cardiac Surgery and Anesthesia at UCT. In particular, with regard to the studies involving patients who could not be weaned from CPB while undergoing open heart surgical procedures, it was members of the Department of Anesthesia who urged use of T₃ in an attempt to keep the patients alive.

At the time hormonal therapy was initially introduced clinically, the management of brain-dead organ donors consisted of (i) ventilatory support, (ii) acid-base management, (iii) fluid replacement, and (iv) inotropic support. Animal studies had demonstrated a highly beneficial effect of hormonal therapy in improving the hemodynamic status of the potential donor. It was on the basis of these studies that hormonal therapy was first used clinically. The initial brain-dead organ donors to whom it was administered were those that were hemodynamically unstable and, because of this, were deemed unsuitable for organ donation. It was reasoned that it would be ethical to test this new form of therapy in such deceased individuals. Following hormonal therapy, hemodynamic recovery was so evident that we used this therapy in a series of donors. We then conducted a retrospective comparison of outcomes of historical donors with donors treated with hormonal therapy. Following hormonal therapy, the data were so compelling with regard to

favorable hemodynamic and metabolic recovery that this modality was adopted at UCT as the standard heart transplant donor management.

Our experience with human brain-dead organ donors, together with a review of the literature that indicated that T_3 levels were markedly reduced during CPB, led to studies in animals undergoing CPB. The results indicated that T_3 therapy could be valuable in improving myocardial function in patients undergoing open heart surgery. Triiodothyronine was therefore administered to a small number of patients who were undergoing open heart procedures and who had become CPB-dependent. These patients could not be weaned from CPB support despite receiving maximal inotropic support; in addition, some had had an intra-aortic balloon placed, but remained dependent on CPB. All of these patients were considered unrecoverable. We treated 10 consecutive patients with T_3 (Novitzky et al. 1987). Myocardial function improved in all cases, allowing discontinuation of CPB and successful recovery of all 10 patients.

In view of this experience, after my relocation to the USA, the first prospective, randomized clinical studies on T_3 therapy in patients undergoing coronary bypass surgery on CPB were carried out following full IRB approval; all patients gave informed consent (Novitzky et al. 1989).

SUMMARY

With the exception of the requirements to submit the proposals to a formal IACUC, care of laboratory animals at UCT was almost identical in the 1980's as it is today in leading experimental surgical centers. However, as much care was taken in planning these experimental studies in the early 1980's by discussion with expert colleagues as is achieved

today by submission to an IACUC (Greene 2007).

The advantages of using baboons for some of the studies described in this dissertation were that the results obtained were immediately applicable to the management of brain-dead organ donors and patients undergoing cardiac surgical procedures at UCT, and thus enabled patients to be helped at an earlier stage than if the studies had been carried out in lower animal species.

At the time of the initial clinical use of T_3 , there was no IRB at UCT. Donor management was largely empirical, with only limited scientific knowledge of the pathophysiology of brain death. Following successful animal studies, hormonal therapy was given initially to brain-dead potential donors that were hemodynamically unstable and considered unsuitable for purposes of transplantation. As hormonal therapy led to recovery of myocardial function in these cases, its use became routine.

The initial study on 10 high-risk patients who were CPB-dependant was of necessity also carried out in the absence of an IRB at UCT, but the use of T_3 was considered ethically justified as the patients were considered likely to die if this treatment was withheld. All further studies, carried out in the USA, were IRB approved.

APPENDIX 1

METHODS USED FOR BIOCHEMICAL ESTIMATIONS IN BLOOD AND TISSUE

Serial blood samples were taken for measurement of hormones; (catecholamines, adrenaline, noradrenaline, dopamine, T_3 , T_4 , TSH, cortisol, glucagon, insulin, ADH, and electrolytes (sodium, potassium, and ionized calcium)). Also measured were lactate, pyruvate, and free fatty acids in plasma.

Tissue biopsies of the heart were performed for measurements of ATP, CP, glycogen, and lactate.

1. Catecholamines

Measured using a radioenzymatic assay kit: CAT-A-KIT (Upjohn Diagnostics).

2. Thyroxine (T_4)

Measured using the Amerlex-M. Free T_4 RIA kit (for the immunoassay of free T_4 thyroxine in human serum). Amersham, England.

3. Triiodothyronine (T_3)

Measured using a radio immunoassay method, Amerlex-M. Free T_3 RIA kit (triiodothyronine) in human serum. Amersham, England.

4. TSH

Measured with the TSH Amerwell assay (monoclonal antibodies), Amersham, England.

5. Cortisol

Measured with a radioimmunoassay method using the kit Amerlex-M for cortisol measurement RIA kit. Amersham, England.

6. Glucagon

Plasma glucagon was measured using a radioimmunoassay technique described by Unger (1973).

7. Insulin

Measured by a radioimmunoassay method. RIA-Sedonal-MAIA (magnetic separation for antibodies), Switzerland.

8. Antidiuretic Hormone (ADH)

Measured in plasma using a bio-assay method in ethanol- anaesthetized rats, according to the method of Esturmer (1968).

9. Electrolytes (sodium, potassium)

Measured using flame photometer analysis with the FLM 3 flame photometer (Radiometer, Copenhagen).

10. Ionized Calcium

Measured in plasma by a UCT designed machine in which a Radiometer ICA-1 calcium ion exchange electrode was incorporated.

11. Lactate Dehydrogenase (LDH)

Measured in serum by the method described by Wroblewski (1955), based on spectral photometric measurements using the Boehringer Mannheim Biochemical test-combination kit N° 124893.

12. Pyruvate and Lactate

Arterial blood was injected into cold 6% perchloric acid in a pre-weighed centrifuge tube. The tube was shaken, re-weighed and centrifuged. The supernatant fluid was removed for enzymatic analysis estimations for pyruvate and lactate, using the method of Hohorst (1963). Boehringer Mannheim test-combination kit N° 12482 was used.

13. Plasma Free Fatty Acids

Plasma samples were centrifuged and then separated immediately. A flame photometer was used for free fatty acids, described by Chlouveralis (1963) and Eggstein (1966).

14. Left Ventricular Tissue Biopsy

In baboons and pigs a method of drill biopsy as described by Poole et al. (1968), was used; the rotating drill penetrates the myocardium, small cores of tissue are obtained under suction and, with a sudden burst of positive pressure, ejected into liquid nitrogen. Depending on the size of the drill, head cores of tissue (wet weight) weighing 50-200 mg were obtained. The area of the biopsy was 1-2 cm parallel to the left of the left anterior descending coronary artery. If biopsies were done following cardioplegic arrest, these were taken from the base of the heart and on the lateral wall of the left ventricle.

15. Glycogen

This was measured using a modification of the Guud, Kramer and Somogyi method (1933).

16. Adenosine Triphosphate and Creatine Phosphate

Estimation of ATP and CP were measured according to the method described by Lamprecht Traudschold (1963).

APPENDIX 2

ANATOMY AND PHYSIOLOGY OF MYOCARDIAL CELL CONTRACTION

The macroscopic and histological appearances of the heart have been described by many authors. In this chapter emphasis will be directed towards the different components of the myocyte and its regulation during excitation-contraction coupling.

Structure and Function of Cardiac Muscle

The myocardium represents a highly specialized structure capable of transforming chemical energy into mechanical work. Utilization of energy and production of work depends on a complex process known as excitation-contraction coupling in which the muscle is activated by an electrical depolarization to contract. Calcium ions are a vital link between excitation and contraction.

In the myocardium, contraction occurs in an all-or-none fashion; the myocardium is a "functional syncytium". The predominant feature of the myofibres is the myofibrils contained within the sarcolemma. The myofibrils appear as bundles of thin, longitudinal elements with a characteristic repetitive pattern giving the striated appearance. The myocytes include numerous mitochondria and a centrally located nucleus, an extensive internal membrane system (sarcoplasmic reticulum), and tubular invaginations of the sarcolemma (transverse tubular system) which surrounds the myofibrils.

Sarcolemma

On the exterior of the sarcolemma is a homogenous layer of material known as the glycocalyx. Chemically, the glycocalyx consists of: anionic mucopolysaccharides, gly-

Appendix 2-2

coproteins and sialic acid residues which contribute to a negative surface charge with a high capacity for cation binding. The glycocalyx may be a site for calcium binding and calcium exchange across the cell membrane (Figure 2-1 OPIE 1984).

The portion of the sarcolemma lying immediately deep to the glycocalyx is a bi-layered structure composed of phospholipids with the phosphate heads extending outwards while the hydrophobic fatty acid tails are directed inwards. Indentations or invaginations of the sarcolemma into the cell interior form what is known as the transverse tubular system (T-tube system). The invaginations occur predominantly at the Z-line of the sarcomere, penetrating to the centre of the myofibre. The T-tube membrane is heavily vesiculated, containing many membrane bound particles including the glycocalyx of the surface sarcolemma.

Embedded within the sarcolemma are numerous globular macromolecules - proteins and glycoproteins which cross the membrane playing an important role in the regulation of muscle contraction, e.g., hormone receptors, neurotransmitters, membrane-bound enzymes such as sodium NaK/ATPase and adenylate cyclase. Other properties of the sarcolemma include a selective voltage-dependent permeability to ions traversing the membrane via ion selective "gates" or channels.

Another characteristic of the myocardial cell is the presence of intercalated discs between the myocytes. These are thought to serve as a low resistance pathway between adjacent cells, facilitating electrical propagation during excitation.

Mitochondria

The mitochondria in the myocardial cells are located beneath the sarcolemma as well

as around and between the myofibrils, and represent close to 30% of the ventricular heart cell volume. They usually contain lipid droplets and glycogen granules, both constituting the energy requirements of the muscle, which is primarily dependent upon aerobic metabolism. They also play a role in calcium storage and its movement across the mitochondrial membrane into the cytosol.

The Sarcoplasmic Reticulum (SR)

The sarcoplasmic reticulum (SR) is not in continuity with the sarcolemma, but is in continuity with other internal membranes such as that of the nucleus. The SR forms a plexiform arrangement of tubular elements surrounding each myofibril. The myocardial sarcoplasmic reticulum is mainly smooth, although small amounts of ribosomes are present. Pairs of parallel terminal cisternae run transversely across the myofibril in close relation to the T-tubular system of the sarcolemma. In cardiac muscle, the sarcoplasmic reticulum terminates in dilatations of sarcotubules in close apposition to the T-tubules of the sarcolemma (sub sarcolemma cisternae). This structure plays an important regulatory role in maintaining the cytosol calcium concentration during the contraction-relaxation cycle. It is thought that the cardiac SR acts as a primary locus of calcium storage and release, thus modulating the excitation-contraction coupling. Calcium flux is regulated by an ATPase enzyme and a phosphoprotein catalyzed by the cyclic AMP-phosphokinase called phospholamban. Tada and Inui (1983) reviewed this subject showing that phospholamban is present in 4-6% of the contents of the cardiac sarcoplasmic reticulum with a ratio of one to one phospholamban and ATPase. Active calcium transport from the cytosol to the sarcoplasmic reticulum is dependent on these two ATP consuming pumps.

The Contractile Apparatus

The basic contractile structure, called the sarcomere, is delineated by two successive narrow lines known as the Z-lines (Figure 1). During relaxation, the distance between two Z-lines varies between 2-3 μm . The A-band, a dark centrally located region is situated between the Z-lines. At either end of the A-band are more lightly stained sections, known as the I-bands, which are bisected by the Z-lines. At rest, the central region of the A-band has a slightly lower density than the rest of the myofibril and is called the H-zone. In its centre, a narrow dark line, the M-line is located precisely in the middle of the A-band. Electromicroscopic studies show that the thicker filaments (myosin) form a parallel arrangement extending throughout the length of the A-band. The thinner actin filaments interdigitate with the myosin filaments. This interdigitation of the actin and myosin filaments gives rise to the dense zone of the A-band. The peripheral ends of the myosin filaments are covered with numerous projections. In the overlap region of the A-band, these projections may join to the actin filaments forming "cross bridges" between the thick and thin filaments. The I-band is composed only of actin filaments. The H-zone within the A-band contains only myosin filaments. The widths of the H-zone and I-band are determined by the degree of actin and myosin overlap. During diastole, the H-zone and the I-band become wider and when the myofibre contracts, both regions become narrower. In either case, the width of the A-band remains the same; neither the myosin filaments nor the actin change length during muscle contraction or relaxation.

The central region of the myosin filament is smooth in contrast to the peripheral ends which are covered with numerous short projections. These reveal individual myosin molecules, rod-shaped with globular projections at one end having ATPase properties. The thin actin myofilaments consist of a double stranded helix of globular sub-units.

Associated with the actin molecule are the tropomyosin and troponin. Troponin is a primary modulator of contraction. This molecule exists as a globular complex of three proteins: troponin I appears to act as an inhibitor of actinomyosin interaction. Troponin T serves primarily to bind the troponin complex to tropomyosin, and troponin C acts as a reversible binding site for calcium ions. Havers (1984) reviewed the microanatomy of contraction showing that after an increment of Ca^{++} ions in the cytosol, the Ca^{++} binds to specific sites on troponin C so that the thin filaments revert to the conformation that permits interaction between the myosin heads and adjacent actin units, inducing changes in the molecular configuration, hinges flexing the heads of myosin. The progressive recruitment of actinomyosin complexes leads to an increment of tension and mechanical work.

The second messenger theory of Sutherland et al. (1965) proposes that a hormone is the first messenger, acting as the initial extracellular signal; the second messenger is formed inside the cell and is thought to include the adenylate cyclase, calcium ions, as well as cyclic nucleotides. Tomlinson et al. (1984), in an extensive review, shows that there is now evidence that in many cellular systems the calcium ions and cyclic nucleotides act as dual interrelated messengers, and therefore some intracellular processes are regulated by calcium as well as by cyclic nucleotides. There is increasing evidence that hydrolysis of phosphoinositol is connected with sarcolemmal activity in the calcium dependent channels; Mitchell (1979) and Rasmussen (1984) have reviewed this subject.

Opie (1984) reviewed the nature of Ca^{++} fluxes. The extracellular concentration is approximately 1 mmol (10^{-3}M), and the intracellular concentration fluctuates between 10^{-7}M during diastole and 10^{-5}M during systole; the major intracellular calcium stores are the sarcoplasmic reticulum and the mitochondria.

Appendix 2-6

Activation of slow calcium channels occurs during depolarization of the cell. Beta receptor stimulation results in an increased number of Ca^{++} channels. Fabiato (1983) showed that "calcium induced-release of calcium" from the sarcoplasmic reticulum leads to calcium accumulation, initiating the activation of the contractile mechanism. Walsh (1980) showed that in cardiac muscle, calmodulin is involved in the calcium dependent activation of cyclic nucleotide phosphodiesterase which may inhibit adenylate cyclase. Calmodulin is implicated in a number of calcium modulated cellular processes including cyclic AMP metabolism, calcium fluxes, glycogen metabolism, muscle contraction, microtubular assembly-disassembly, and stimulus-secretion coupling. Calmodulin was first found in cardiac muscle by Cheung (1980).

An increase in cytosolic calcium concentration results in formation of the Ca-magnesium - calmodulin that undergoes a conformational change enabling it to interact with phosphodiesterase. This lowers the cyclic-AMP concentration within the cardiac tissue. Current evidence suggests that under normal conditions, calmodulin activity is not altered by changes in its concentration within the cell, but is altered mainly by changes in the concentration in the free intracellular calcium. In resting conditions the concentration of intracellular free calcium is too low to allow any significant binding of calcium to calmodulin. Following electrical cell stimulation a significant increment of calcium concentration occurs through the plasma membranes or from its release from intracellular structures or organelles. Calcium then binds to calmodulin which undergoes a conformational change allowing interaction with inactive enzymes to form an active complex. A reduction in intracellular free calcium by extrusion from the cell by calmodulin dependent calcium ATPase dissociates the active calmodulin enzyme complex, decreasing its activity.

The two principal mechanical effects of endogenous catecholamines on the myocardium

are increased contractility and the abbreviation of systole and accelerated rates of contraction and relaxation. The cyclic AMP and ATPase-phospholamban system in the sarcoplasmic reticulum can alter the rate of calcium uptake and subsequently change the rate of calcium release. Calcium influx across the sarcolemmal membrane was also found to increase during beta adrenergic stimulation of the myocardial cells. In the presence of cyclic AMP, the acceleration of calcium uptake by the ATPase phospholamban system may explain the acceleration of relaxation because the increased rate of calcium uptake by the sarcoplasmic reticulum would increase the rate at which calcium is removed from troponin, accelerating relaxation.

In summary, the arrival of the action potential at a myocyte will open the calcium gates, allowing entry of cytosolic calcium, permitting a "calcium-induced release of calcium" from the cardiac sarcoplasmic reticulum; this triggers the interaction between myosin-troponin C and induces contraction. As the calcium level rises within the cytosol, calcium is mobilized back into the sarcoplasmic reticulum by several mechanisms - phosphorylation of phospholamban, calmodulin-induced elimination of calcium through the sarcolemma, or through the calcium ATPase pumps and sodium- calcium channels.

LEGENDS

FIGURE

Appendix 2-1

A schematic representation of the contractile apparatus of the myocyte, the role of ionic calcium, the different fluxes to the mitochondria, sarcoplasmic reticulum, and to the sarcolemma during the arrival of the action potential initiating the depolarization-repolarization and sarcomere contraction, is shown. (Frontispiece from Opie L.H., (1984), *The Heart - Physiology, Metabolism, Pharmacology and Therapy*, Grune and Stratton, London).

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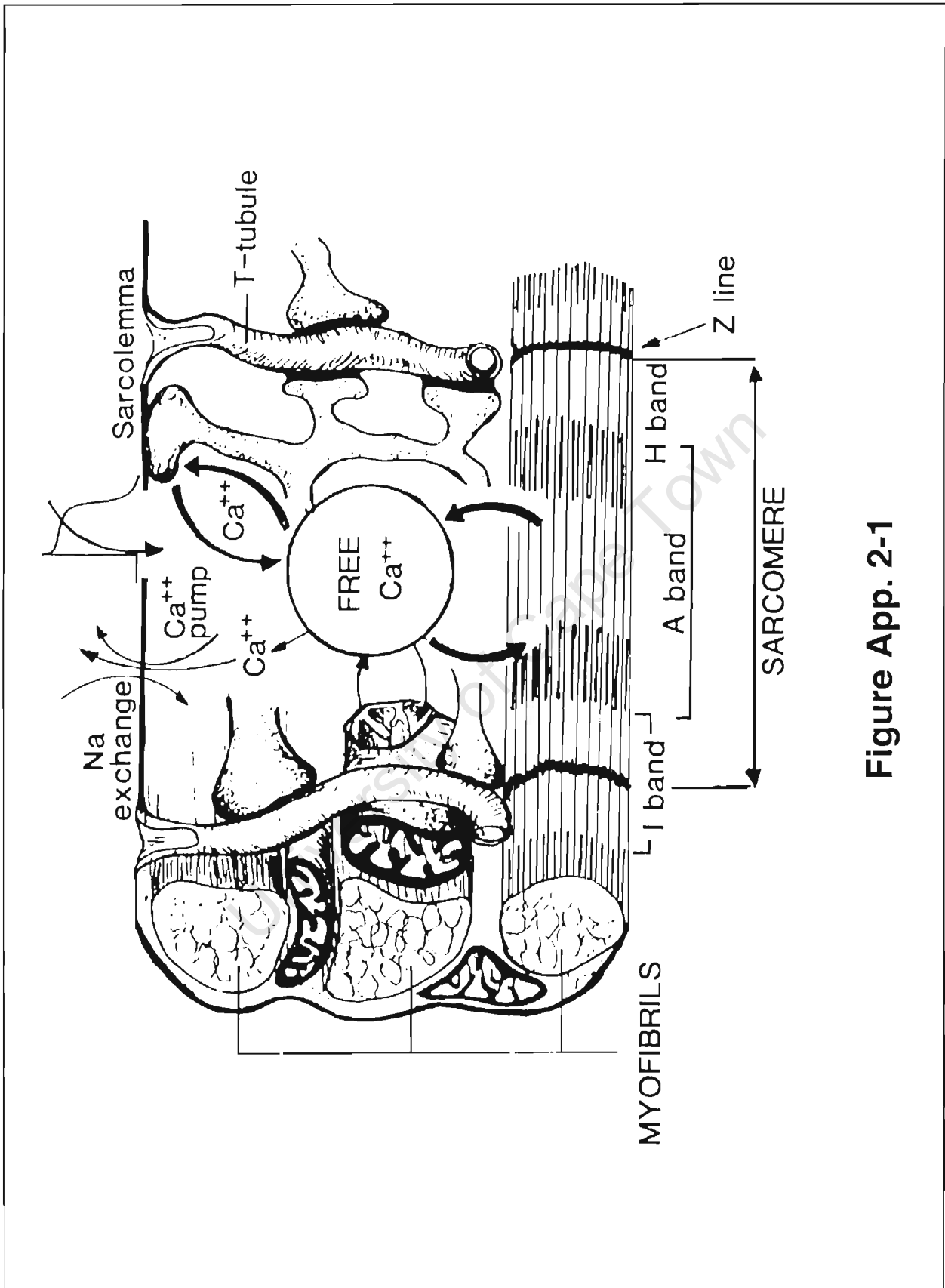


Figure App. 2-1

**DATA COLLECTED FROM THE METABOLIC STUDIES (^{14}C -R
PERFORMED IN BABOON AND PRESENTED IN CHAPTER 7**

University of Cape Town

**ANIMAL, WEIGHT, DOSE AND BEST FIT OF CURVE FOLLOWING
I.V. BOLUS OF ¹⁴C-U-GLUCOSE**

STUDY I STUDY 2 STUDY 3
C BD BD + T₃

ANIMAL	WEIGHT/KG	DOSE μCi	BF	R ²	DOSE μCi	BF	R ²	DOSE μCi	BF	R ²
1	25	20	3	.995	20	3	.995	22.1	2	.98
2	20	20	2	.971	20	2	.962	22.7	2	.836
3	29	20	2	.982	20	2	.970	23.1	1	.973
4	27	20	3	.998	20	2	.967	25.14	3	.995
5	29	20	2	.997	20	3	.998	21.8	1	.964
6	17	20	2	.99	20	3	.998	22.46	2	.986

BF = Best fit to the data points as determined by least squares regression analysis.
The numbers indicate exponential functions to describe curve.

R² = Correlation "coefficient" squared.

Dose in Study 3 = 20 μCi + remaining glucose from Study 2

Table 1

BEST FIT OF EXHALED $^{14}\text{CO}_2$ CURVE FOLLOWING ^{14}C -U-GLUCOSE I.V. INJECTION

ANIMAL	STUDY 1 C		STUDY 2 BD		STUDY 3 BD + T3	
	BF	R ²	BF	R ²	BF	R ²
1	2	.861	2	.910	2	.980
2	2	.608	2	.862	Linear	.916
3	2	.982	2	.956	4	.977
4	2	.869	2	.933	3	.846
5	2	.878	1	.928	2	.868
6	2	.891	2	.949	4	.916

BF = Best fit

Abbreviations as per Table 1

Table 2

**PERIOD OF TIME OF EACH STUDY IN HOURS (¹⁴C-U-GLUCOSE) AND PERIOD OF BRAIN DEATH
BEFORE COMMENCING STUDY 2**

ANIMAL	HOURS	STUDY I	STUDY 2	STUDY 3
		C BD TIME INTERVAL (HOURS)	BD HOURS	BD + T ₃ HOURS
1	6	15	6	6
2	6	12	4	4
3	6	14	5	4
4	5	13	5	5
5	5	16	5	5
6	6	14	4	4

Abbreviations as per Table 1

Table 3

¹⁴C-U-GLUCOSE PLASMA HALF LIFE (HOURS)

	STUDY I	STUDY 2	STUDY 3
ANIMAL	C	BD	BD + T ₃
1	6.52	10.04	2.52
2	2.62	2.85	2.14
3	2.05	2.65	1.04
4	1.93	2.16	1.33
5	1.56	2.74	1.24
6	2.40	4.88	1.72
MEAN	2.84 (.75)	4.22 (1.11)	1.66 (.23)

Statistical comparison shown in Table 7-1
 Figures in brackets = standard error of the mean

Table 4

¹⁴C-U-GLUCOSE PLASMA CLEARANCE FOLLOWING SIMPLE BOLUS I.V. INJECTION (MILS/MIN)

ANIMAL	STUDY 1		STUDY 2		STUDY 3	
	C		BD		BD = T _j	
1	.86		54		1.40	
2	1.67		.88		1.77	
3	1.62		1.16		1.61	
4	.37		.33		.72	
5	.52		.39		1.32	
6	1.63		.61		2.00	
MEAN	1.11 (0.22)		.65 (0.26)		1.62 (0.10)	

Statistical comparisons shown in Table 7-1

SEM = Standard error of the mean

Table 5

ACCUMULATED $^{14}\text{CO}_2$ COUNTS DURING FIRST TWO HOURS FOLLOWING $^{14}\text{C-U-GLUCOSE}$ I.V. ADMINISTRATION

ANIMAL	STUDY 1	STUDY 2	STUDY 3
	C	BD	BD + T ₃
1	8561	2417	12300
2	4881	636	4772
3	20047	3787	20763
4	16833	6283	20168
5	30540	1387	12078
6	18500	3318	5614
MEAN	16560.33 (3382)	2921 (746)	12616 (2551)

Statistical comparison shown in Table 7-1

Table 6

ANIMAL WEIGHT, DOSE AND BEST FIT OF CURVE FOLLOWING SIMPLE BOLUS OF ¹⁴C-1- PYRUVATE

ANIMAL NO	WEIGHT/KG	STUDY 1 C			STUDY 2 BD			STUDY 3 BD + T ₃		
		DOSE	BD	R ²	DOSE	BD	R ²	DOSE	BD	R ²
1	24	12	2	.991	12	2	.986	13.52	2	.930
2	24	12	2	.991	12	3	.991	14.12	1	.862
3	15	12	2	.986	12	3	.988	13.71	2	.988
4	15	12	3	.990	12	3	.997	12.78	2	.987

Abbreviations as per Figure 1

Table 7

BEST FIT OF EXHALED $^{14}\text{CO}_2$ CURVE FOLLOWING ^{14}C -1-PYRUVATE INJECTION

ANIMAL	STUDY 1 C		STUDY 2 BD		STUDY 3 BD + T ₃	
	BF	R ²	BF	R ²	BF	R ²
1	3	.858	2	.977	3	.986
2	3	.998	3	.971	2	.759
3	2	.998	3	.995	2	.972
4	2	.955	3	.998	3	.997

Table 8

THE PERIOD OF EACH STUDY (HOURS) ¹⁴C-1-PYRUVATE AND PERIOD OF BRAIN DEATH BEFORE COMMENCING STUDY 2

ANIMAL	HOURS	STUDY 1	STUDY 2	STUDY 3
		C	BD	BD + T ₃
		BD TIME INTERVAL (HOURS)	HOURS	HOURS
1	4	13	4	4
2	4	15	4	3.5
3	4	14	4	4
4	4	16	4	4

Abbreviations as per Table 3

Table 9

¹⁴C-1-PYRUVATE PLASMA HALF LIFE (HOURS) FOLLOWING SINGLE BOLUS I.V. INJECTION

	STUDY 1	STUDY 2	STUDY 3
ANIMAL	A	BD	BD + T ₃
1	2.79	3.23	1.46
2	2.43	6.22	0.62
3	1.79	3.21	1.33
4	1.42	2.41	1.28
MEAN	2.107 (0.16)	3.767 (0.72)	1.172 (6.16)

Statistical comparison shown in Table 7-1

Table 10

¹⁴C-1-PYRUVATE PLASMA CLEARANCE FOLLOWING SINGLE BOLUS I.V. INJECTION (MLS/MIN)

ANIMAL	STUDY 1	STUDY 2	STUDY 3
	C	BD	BD + T ₃
1	2.18	1.89	4.22
2	2.903	1.67	7.86
3	4.72	3.43	6.79
4	6.71	5.35	8.58
MEAN	4.127 (0.87)	3.08 (0.74)	6.862 (0.82)

Statistical comparison shown in Table 7-1
 Figures in brackets = standard error of the mean

Table 11

**ACCUMULATED $^{14}\text{CO}_2$ -COUNTS DURING FIRST TWENTY MINUTES FOLLOWING ^{14}C -1-PYRUVATE I.V.
ADMINISTRATION**

	STUDY 1	STUDY 2	STUDY 3
ANIMAL	C	BD	BD + T₃
1	26721	10524	13539
2	26218	9916	12165
3	20398	10393	08544
4	31665	20843	29199
MEAN	26250.50 (1996)	12919 (2290)	18341.75 (3329)

Statistical comparison shown in Table 7-1
 Figures in brackets = standard error of the mean

Table 12

ANIMAL, WEIGHT, DOSE AND BEST FIT OF CURVE FOLLOWING I.V. BOLUS OF ¹⁴C-U-GLUCOSE

ANIMAL	WEIGHT/KG	STUDY 1 C			STUDY 2 BD			STUDY 3 BD + T ₃		
		DOSE	BF	R ²	DOSE	BF	R ²	DOSE	BF	R ²
1	26	5	2	.990	5	2	.972	6.11	2	.986
2	26	5	2	.971	5	2	.997	5.71	2	.986

Abbreviations as per Table 1

Table 13

BEST FIT OF EXHALED ^{14}C CO_2 CURVE FOLLOWING ^{14}C -1-PALMITATE I.V. ADMINISTRATION

ANIMAL	A		BD		BD + T ₃	
	BF	R ²	BF	R ²	BF	R ²
1	2	.977	2	.984	2	.961
2	2	.938	2	.902	2	.968

Table 14

PERIOD OF TIME OF EACH STUDY IN HOURS (¹⁴C-U-GLUCOSE) AND PERIOD OF BRAIN DEATH BEFORE COMMENCING STUDY 2

ANIMAL	A	BD INTERVAL	BD	BD + T ₃
1	4	12	4	4
2	4	16	4	4

Table 15

¹⁴C-1-PALMITATE PLASMA HALF LIFE (HOURS)

ANIMAL	A	BD	BD + T₃
1	1.15	4.11	1.3
2	2.34	3.06	2.54
MEAN	1.74 (0.59)	3.58 (0.52)	1.92 (0.06)

Table 16

¹⁴C-1-PALMITATE PLASMA CLEARANCE (ML PER HOUR)

	STUDY 1	STUDY 2	STUDY 3
ANIMAL	C	BD	BD + T ₃
1	6.83	8.03	18.28
2	12.15	17.85	15.55
MEAN	9.49 (2.66)	12.94 (4.91)	16.92 (1.36)

Table 17

ACCUMULATED ^{14}C - COUNTS DURING THE FIRST HOUR FOLLOWING ^{14}C -1-PYRUVATE I.V.
ADMINISTRATION

ANIMAL	STUDY I C	STUDY 2 BD	STUDY 3 BD + T ₃
1	1162.26	587.16	823.329
2	374.458	78.01	572.005
MEAN	786.35 (393.90)	332.58 (254.57)	697.66 (125.669)

Table 18

APPENDIX 4

METABOLIC IMPLICATIONS

Carbohydrates and fatty acid oxidation are the main sources of cellular fuels yielding high energy bonds (ATP, CP) required for cellular life. (Neeley 1974, Newsholme and Leech 1983, Opie 1984).

The oxidation of glucose involves three separate physiological pathways: (i) glycolysis from glucose: (ii) pyruvate conversion to acetyl CoA: (iii) oxidation through the tricarboxylic acid cycle coupled to the electron transfer chain resulting in the high energy bond production (Appendix Figures 4-1, 4-2).

The two major regulators of ATP production within the mitochondria are the cytosol-mitochondria calcium changes and the ATP/ADP concentration. (Appendix Figure 4-3).

Therefore, in broad terms, regulation of ATP synthesis depends on the ADP production and ionic calcium concentration in the cytosol and feedback regulation from changes in the concentrations of adenine nucleotides.

The integration of fatty acids and carbohydrate oxidation takes place within the mitochondrial pool of acetyl-CoA dependent on aerobic glycolysis to pyruvate and the oxidation of the fatty acids within the mitochondrial matrix.

The fundamental biochemical mitochondrial function is the oxidation of acetyl groups (i) through the tricarboxylic acid to CO_2 and concomitant reduction of the

Appendix 4-2

electron carriers (NAD⁺/FAD coupled with the oxidative phosphorylation and ATP production (Sterling 1977, Sestat 1980, Azzi 1984).

Therefore, from the previously presented studies, it appears that T₃ has a major role in the ionic calcium modulation within the cytosol and mitochondria.

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LEGENDS

FIGURES

Figure 4-1

A: The conventional metabolic pathway of glycolysis.

B: The pathway of tricarboxylic acid cycle. (Newsholme, Leech, 1983, Biochemistry for the Medical Sciences, Chapter 4, The oxidation of acetyl-coenzyme A. John Wiley & sons, Amersham, England, (page 93).

Figure 4-2

Schematic representation of carbohydrates and fatty acid catabolism and the key role played by acetyl-CoA in the pathway of the tricarboxylic acid cycle and oxidative phosphorylation through the electron transfer chain. A: After Opie LH, (1984), Chapter 9, Fuels. In the Heart, Physiology, Metabolism, Pharmacology and Therapy. Fuels. Gruen & Stratton, London, England, page 111. B: Newsholme & Leech, (1983), Chapter 7. Regulation of glucose and fatty acid oxidation in relation to energy demand in muscle. In, Biochemistry for the Medical Sciences, Chapter 4, page . The oxidation of acetyl coenzyme A. John Wiley & sons, Norwich, England, page 93.

Figure 4-3

Regulation of the major metabolic pathways. A: The regulation of non-equilibrium enzymes in glycolysis by external regulators. B: The regulation of non-equilibrium reactions in the tricarboxylic acid cycle; the role of calcium is clearly shown. C: The

Appendix 4-4

regulation of the pyruvate dehydrogenase system; the role of calcium is shown. D: A summary of ATP control synthesis in muscle by calcium ions and adenine nucleotides. The major action in the breakdown of glycogen to glucose, and the pyruvate dehydrogenase system and the tricarboxylic acid cycle, is shown. The role of calcium on the myofibrillar ATPase system, converting ADP into ATP, is clearly seen. (Same as for Figure 4-1).

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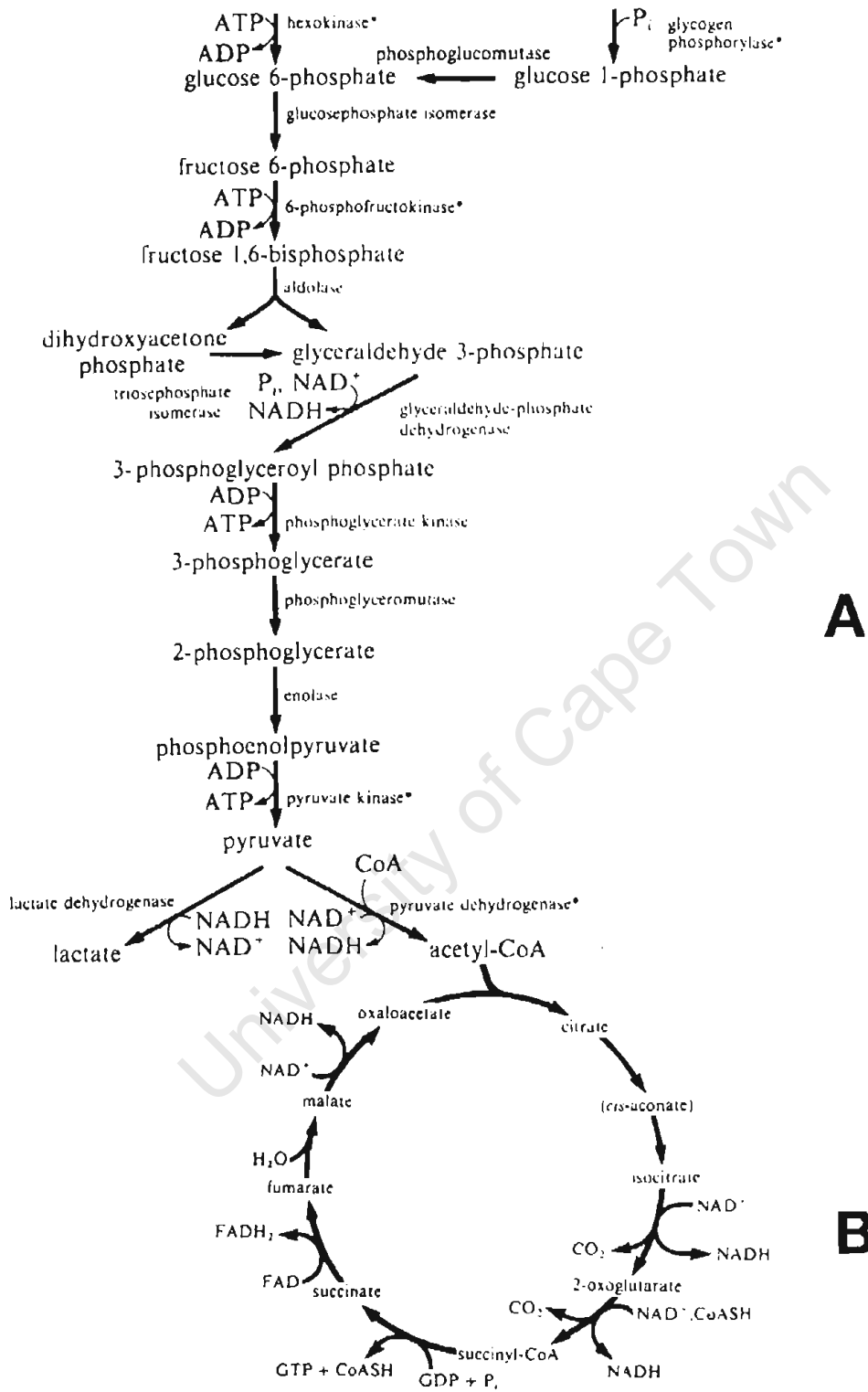


Figure Appendix 4-1

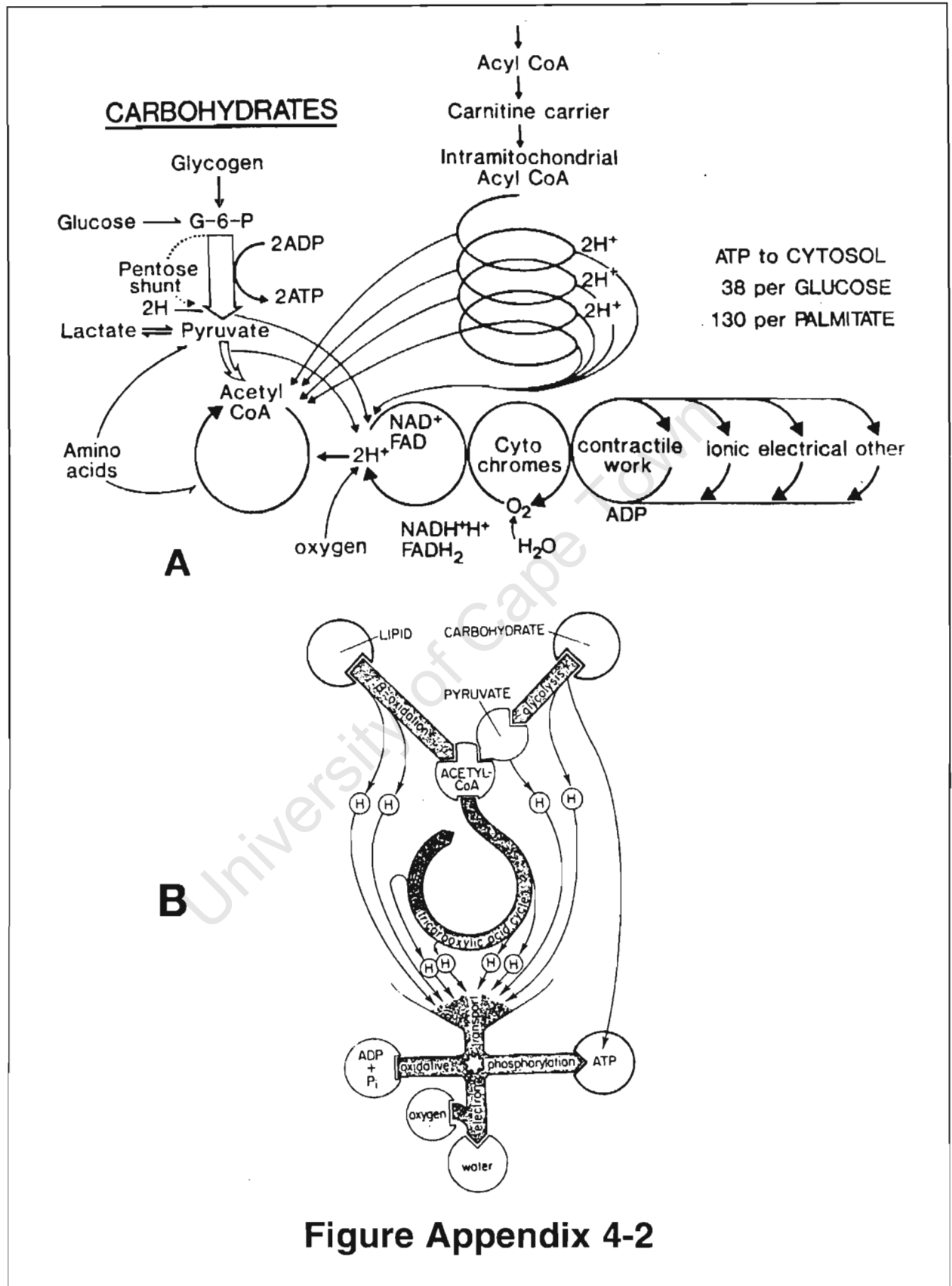


Figure Appendix 4-2

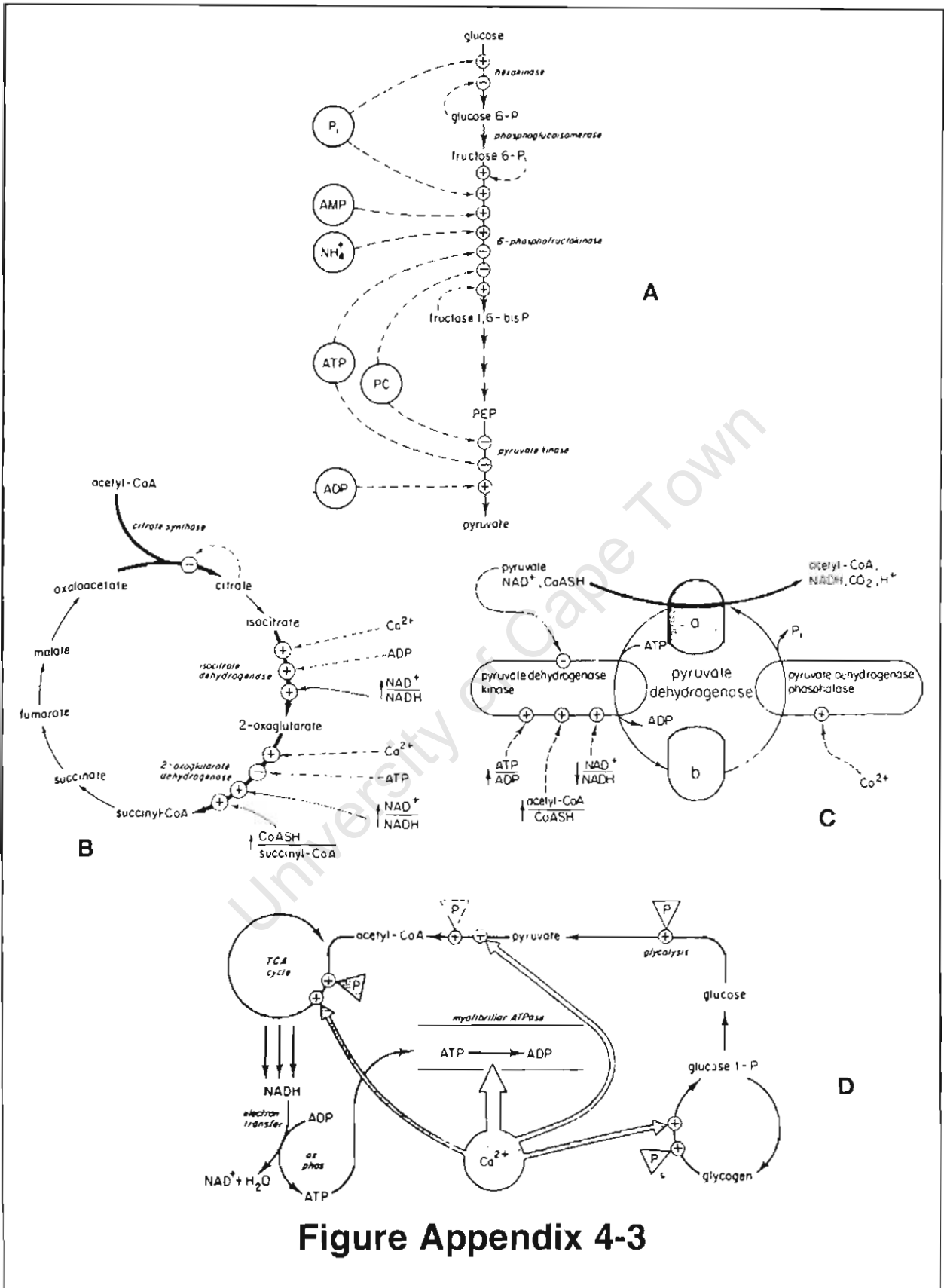


Figure Appendix 4-3

REFERENCES

ABILDSKOV JA, (1976), Adrenergic effects on the QT interval of the electrocardiogram, Am Heart J, 92, 210.

ADAMS JH, GRAHAM DI, MURRAY LS, SCOTT G. (1982), Diffuse axonal injury due to non missile head injury in humans: an analysis of 45 cases. Ann Neurol, 12, 557.

ADAMS JH, RAHA DI, MURRAY LS, and SCOTT G, (1982), Diffuse axonal injury due to non missile head injury in humans: an analysis of 45 cases, Ann Neurol, 12, 557.

AHNVE S, (1985), Correction of the QT interval for heart rate : Review of different formulae and the use of Bazett's formula in myocardial infarction, Am Heart J, 109, 568.

AKINS RP, (1979), Methods for measurement of free thyroid hormones, In, Proceedings of the International Symposium, held in Venice, December 1978. Ed. by R Akins, Faglia G, Pennisi F, and Pinchera A, Amsterdam, Excerpta Medica, 298.

ALTMAN LK, (1995), AIDS test involving baboon is approved, NY Times, July 15, 7.

AMES A, WRIGHT RL, KOWADA M, (1968), Cerebral ischaemia. The no-flow phenomenon, Am J Pathol, 52, 437.

ANDERSON LC, (2007), Institutional and IACUC responsibilities for animal care and use educational and training programs, ILAR J, 48(2), 90

AVLONTIS VS, WIGFIELD CH, KIRBY JA, DARK JH, (2005), The hemodynamic

References-2

mechanisms of lung injury and systemic inflammatory response following brain death in the transplant donor, *Am J Transplant*, 5(4 Pt1), 684.

BARNARD MS, VAN HEERDEN J, HOPE A, O'DONOHAY TG, BARNARD CN, 1969, Total body perfusion for cardiac transplantation, *S Afr Med J*, 43, 64.

BAROLDI G, (1975), Different types of myocardial necrosis in coronary heart disease: A pathophysiologic review of their functional significance, *Am Heart J*, 89, 742.

BAYLIES PH, (1983), Posterior pituitary function in health and disease, *Clinics Endocrinology & Metabolism*, 12, 747.

BAZETT HC, (1920), An analysis of the time relations of electrocardiograms, *Heart*, 7, 353.

BIRCHER J, PREISING R, (1981), Exhalation of isotopic CO₂, *Methods Enzymol*, 77, 3.

BLACK PMcL, (1978) Brain Death (first of two parts), *N Engl J Med*, 229, 338.

BLACK PMcL, (1978), Brain Death (second of two parts), *New Eng J Med*, 299, 393.

BLACK PMcL, ZERVAS NT, (1984), Declaration of brain death in neurosurgical and neurological practice *Neurosurgery*, 15, 170.

BORWMAN WC, RAND NJ, (1980) Chapter 40, Absorption, Distribution, Excretion and Metabolism of Drugs: Biopharmaceutics and Pharmacokinetics, In, *Text book of Pharmacology*, Ed Blackwell Scientific, London, pg 40-1.

BRANDON BRAVO BRUINSMA GJ, van de KOLK CWA, NEDERHOFF MGH, BREDEE JJ, RUIGROK TJC, van ECHELD CJA, (2001), Brain death-related energetic failure of the donor heart becomes apparent only during storage and reperfusion; an ex vivo phosphorus-31 magnetic resonance spectroscopy study of the feline heart, *The Journal of Heart and Lung Transplantation*, 20, 996.

BROWNE MF, ZIPES DP, HEGER JJ, and PRYSTOWSKY CN, Influence of the autonomic nervous system on the QT interval in man, *Am J Cardiol*, 50, 1099.

CARLSSON HE, SCHAPIRO SJ, FARAH I, HAU J, (2004), Use of Primates in Research: A global overview, *American Journal of Primatology*, 63, 225.

CFR (Code of Federal Regulations), 1997a, Title 21 (Food and Drugs). Subchapter A, Part 58 (Good laboratory practice for nonclinical laboratory studies), Washington DC: Office of the Federal Register.

CHEUNG JY, LEAF A, BONVENTRE JV, (1984), Mechanism of protection by verapamil and nifedipine from anoxic injury in isolated cardiac myocytes, *Am J Physiol*, 246, C323.

CHEUNG WY, (1980), Calmodulin plays a role in cellular regulation, *Science*, 207, 19.

CHIANG J, KOWADA M, AMES A, WRIGHT RL, and MAYO J, (1968), Cerebral ischaemia. Part 3: Vascular changes, *Am J Pathol*, 52, 455.

CHLOUVERAKIS C, (1963), Parasympathomimetic agents and the metabolism of rats adipose tissue, *Metabolism*, 12, 936.

References-4

CLARK RE, (1993), Cardiopulmonary bypass and thyroid hormone metabolism, *Ann Thorac Surg*, 56(1Suppl), S35.

CLIFTON GL, ZIEGLER MG, and GROSSMAN R, (1981), Circulating catecholamines and sympathetic activity after head injury, *Neurosurg*, 8, 10.

CLUSIN WD, BUCHBINDER M, HARRISON DC, (1983), Calcium overload,"injury" current, and early ischaemic cardiac arrhythmias - a direct connection, *Lancet*, 1, 272.

COHEN RD, (1976), Disorders of lactic acid metabolism. *Clin Endocrinol Metab*, 5, 613.

CONNOR RCR, (1968), Heart damage associated with intracranial lesions, *Br Med J*, 3, 29.

CONSTAM JR, (1974), Chapter 13, The Pancreas, In, Labhart A, *Clinical Endocrinology, Therapy and Practice*, Springer-Verlag, New York. 739.

COOLEY DA, REUL TJ, and WAKSACH DC, (1972), Ischaemic contracture of the heart: "stone heart", *Am J Cardiol*, 29, 575.

COOPER DKC, DE VILLIERS JC, SMITH LS, KROMBIE Y, BOYD ST, JACOBSEN JE, BARNARD CN, (1982), Medical, legal, and administrative aspects of cadaveric organ donation in the Republic of South Africa, *S Afr Med J*, 62, 933.

COOPER DKC, WICOMB WN, and BARNARD CN, (1983), Storage of the donor heart by a portable hypothermic perfusion system: experimental development and clinical experience, *Heart Transplantation*, 2, 104.

COOPER DKC, WICOMB WN, BARNARD CN. (1983), Storage of the donor heart by portable hypothermic perfusion system: experimental development and clinical experience, *Heart Transplantation*, 2, 104.

DANFORTH E, (1983), The role of thyroid hormones and insulin in the regulation of energy metabolism, *Am J Clin Nutr*, 38, 1006.

DARRACOTT-CANKOVIC S, HUTTER J, WALLWORK J, WHEELDON D, ENGLISH TA, (1988), Biopsy Assessment of myocardial preservation in 160 human donor hearts, *Transplant Proc*, 20(5 Suppl7), 44.

DARRACOTT-CANKOVIC S, WHEELDON D, CORY-PEARCE R, WALLWORK J, ENGLISH TA, (1987), Biopsy assessment of fifty hearts during transplantation, *J Thorac Cardiovasc Surg*, 93(1), 95.

DAVIES AO, and LEFKOWITZ RJ, (1984), Regulation of beta adrenergic receptors by steroid hormones, *Ann Rev Physiol*, 46, 119.

DAVIS AO, KOWITZ RJ, (1984), Regulation of beta-adrenergic receptors by steroid hormones, *Ann Rev Physiol*, 46, 119.

DAVIS PJ, DAVIS FB, (1993), Acute cellular actions of thyroid hormone and myocardial function, *Ann Thorac Surg*, 56(1), S16.

DAVIS PJ, TILLMANN HC, DAVIS FB, WEHLING M, (2002), Comparison of the mechanisms of nongenomic actions of thyroid hormone and steroid hormones, *Endocrinol Invest*, 25(4), 377.

References-6

deAGUILAR-NASCIEMENTO JE, (2005), Fundamental steps in experimental design for animal studies, *Acta Cirurgica Brasileira*, 20, 2.

DE GROOT, LJ, (1999), Dangerous dogmas in medicine: the nonthyroidal illness syndrome, *JCE & M*, 84(1), 151.

Del FRESNO C, SOLER-RANGEL L, SOARES-SCHANOSKI A, GOMEZ-PINA V, GONZALEZ-LEON L, MENDOZA-BARBERA E, RODRIGUES-ROJAS A, GARCIA F, FUENTES-PRIOR P, ARNALICH F, LOPEZ-COLLAZO E, (2007), Inflammatory responses associated with acute coronary syndrome up-regulate IRAK-M and induce endotoxin tolerance in circulating monocytes, *Endotoxin Res*, 13, 39.

DE VILLIERS JC, (1984), Chapt 23, In, *Heart Transplantation*, Cooper DKC and Lanza RP, NTP Press, Hingham USA.

DHALLA NS, PIERCE GN, PANAGIA VK, SINGAL PK, and BEAMISH RE, (1982), Calcium movement in relation to heart function, *Basic Res Cardiol*, 77, 117.

DICKERMAN RM, DUNN EL, WHITE MG. (1986), The impact of an air evacuation system on cadaveric kidney retrieval, *Transplant Proc*, 18, 413.

DILLON RS, (1980), Diagnosis and management of endocrine and metabolic disorders, Chapter 6, *Handbook of Endocrinology, Disorders of the Thyroid Gland*, Pub. Lea & Febiger, Philadelphia. 297.

DORMEHL IC, HUGO N, BEVERLEY G, (1992), The baboon: an ideal model in biomedical research, *Anesth Pain Control Dent*, 1, 109.

DORMEHL I, REDELINGHUYS F, HUGO N, OLIVER D, PILLOY W, (1992), The baboon model under anesthesia for in vivo cerebral blood flow studies using single photon emission computed tomographic (SPECT) techniques, *J Med Primatol*, 21, 270.

DRORY Y, OUAKNINE G, KOSARY IZ, and KELLERMANN JJ, (1975), Electrocardiographic findings in brain deaths; description and presumed mechanisms, *Chest*, 67, 425.

DREYER JL, (1984), Electron transfer in biological systems: an overview, *Experientia*, 40, 653.

DUCKER BT, SIMMONS RL, ANDERSON RW, (1968), Increased intracranial pressure and pulmonary oedema. Part III. The effect of increased intracranial pressure on the cardiovascular haemodynamics of chimpanzees, *J Neurosurg*, 29, 475.

DUCKER BT, SIMMONS RL, ANDERSON R, (1968), Increased intracranial pressure and pulmonary oedema. Part III: The effect of increased intracranial pressure on the cardiovascular haemodynamics of chimpanzees, *J Neurosurg*, 29, 475.

DYKE CM, YEH T Jr, LEHMAN JD, ABD-ELFATTAH A, DING M, WECHSLER AS, SALTER DR, (1991), Triiodothyronine-enhanced left ventricular function after ischemic injury, *Ann Thorac Surg*, 52, 14.

EGGSTEIN M, KREUTZ FH, (1966), Eine neue bestimmung der neutral ette im blutserum und gemebe. 1. Prinzip, durchfuhrung und Besprechung der Methode, *Klin Wscht*, 44, 267.

References-8

ELKOF B, and SIESJO BK, (1971), Cerebral blood flow and cerebral energy state, *Acta Physiol Scand*, 82, 409.

ESTURMER S, (1968), In, *Handbook of Experimental Pharmacology*, Bioessay procedures for neurohypophyseal hormones and similar neuropeptides. Ed. Bird, Pub. Springhill Verlag, 130.

FABIATO A, (1983), Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum, *Am J Physiol*, 245, C1.

FABIATO A, (1983), Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum, *Am J Physiol*, 245, C1.

FABIATO A, FABIATO F, (1975), Relaxing and inotropic effects of cyclic AMP on skinned cardiac cells, *Nature*, 253, 556.

FABIATO A, FABIATO F, (1979), Calcium and cardiac excitation-contraction coupling, *Ann Rev Physiol*, 41, 473.

FEEK CM, MARANDE DJ, EDWARDS CRW, (1983) The hypothalamic-pituitary-adrenal axis, *Clinics in Endocrinology and Metabolism*, 12, 597.

FEIBEL JH, (1981) Reduced catecholamine excretion at onset of brain death, *Lancet*, 2, 890.

FESTING MFW, (2003), Principles: the need for better experimental design, *Trends Pharmacol Sci*, 24, 341.

- FIESCHI C, BATTISSTINI N, BEDUSCHI A, BOSSELLI L, ROSSANDA M, (1974), Regional cerebral blood flow and intraventricular pressure in acute head injuries, *J Neurol Neurosurg Pyschiatr*, 37, 1378.
- FITZPATRICK A, (2003), Ethics and animal research, *J Lab Clin Med*, 41, 89.
- FRENCH JE, (1952), A histological study of the heart lesions in potassium-deficient rats, *Arch Path*, 53, 485.
- FRIEDMAN A, (1984), Sympathetic response and brain death, *Arch Neurol*, 41, 15.
- GANOTE CE, (1983), Contraction band necrosis and irreversible myocardial injury, *J Mol Cell Cardiol*, 15, 67.
- GARCIA-URIA, J, HOLL JT, MIRANDA S, NISHIMURA M, (1981), Experimental neurogenic pulmonary oedema. The role of cardiopulmonary pressure change, *J Neurosurg*, 54, 632.
- GENNARELLI TA, ADAMS JH, and GRAHAM DI, (1981), Acceleration induced head injury in the monkey. The model, its mechanical and physiological correlates, *Acta Neuropathol, Suppl*, 7, 23.
- GEVERS W, (1981), Calcium: The managing director, *S Afr Med J*, 59, 406.
- GEVERS W, (1984), Chapt 8, The mechanism of myocardial contraction, In, *The Heart, Physiology, Metabolic, Pharmacology and Therapy*, Grune and Stratton, London, 98.

References-10

GONDER JC, (2007), Recent studies, new approaches, and ethical challenges in animal research, ILAR Journal, 48, 1.

GONDER JC, SMEBY RR, WOLFLE TL, EDS, (2001), Performance standards and animal welfare: definition, application and assessment, parts I & II, Greenbelt MD: Scientists Center for Animal Welfare.

GOOD CA, KRAMER H, and SOMOGYI M, (1933), The determination of glycogen, J Biol Chem, 100, 485.

GORDON ES, GOLDBERG M, (1964), Carbon-14 studies of energy metabolism in various thyroid states. Metabolism, 13, 591.

GOU DY, SU W, SHAO YC, LU YL, (2006) Euthyroid sick syndrome in trauma patients with severe inflammatory response syndrome, Chin J Traumatol, 9(2), 115.

GRAHAM DI, ADAMS JH, DOYLE D, (1978), Ischaemic brain damage in fatal non missile head injuries, J Neurol Sci, 39, 213.

GRAHAM DI, ADAMS JH, DOYLE D, (1978), Ischaemic brain damage in fatal non missile head injuries, J Neurol Sci, 39, 213.

GREEN ME, PITTS ME, JAMES ML, (2007), Training strategies for IACUC members and the institutional official, ILAR J, 48(2), 131

GREIF RL, and SLOANE D, (1978), Mitochondrial binding sites for triiodothyronine, Endocrinology, 103, 1899.

HAAS NA, CAMPHAUSEN CK, (2006) Triiodothyronine in neonatal heart surgery: Looking for an answer, J Thorac Cardiovasc Surg, 131, 505.

HAAS NA, CAMPHAUSEN CK, KECECIOGLU D, (2006), Clinical review: thyroid hormone replacement in children after cardiac surgery – is it worth a try?, Crit Care, 10(3), 213.

HALL GM, MASHITER K, LUMLEY J, ROBSON JG, (1980), Hypothalamic-pituitary function in the brain dead patient, Lancet, 2, 1259.

HAMMON SR, BLOUIN RA, and McALISTER RG, (1984), Clinical pharmacokinetics of verapamil, Clin Pharmacokinet. 9, 26.

HETZER R, WARNECKE H, SCHULER S. (1986), The donor heart: procurement, selection, and preservation. Transplant Proc, 18, 27.

HIRAI S, (2003), Systemic inflammatory response syndrome after cardiac surgery under cardiopulmonary bypass, Ann Thorac Cardiovasc Surg, 9, 6.

HIROI Y, KIM H-H, YING H, FURUYA F, HUANG Z, SIMONCINI T, MONA K, UEKI K, NGUYEN N-H, SCANLAN TS, MOSKOWITZ MA, CHENG S-Y, LIAO JR, (2006), Rapid nongenomic actions of thyroid hormone, PNAS, 103, 38.

HOCH FL, (1962), Thyrotoxicosis as a disease of mitochondria, N Engl J Med, 266, 446.

HOCH FL, (1962), Thyrotoxicosis as a disease of mitochondria (concluded), N Engl J Med, 266, 498.

References-12

HOCH FL, (1967), Early action of injected L-thyroxin on mitochondrial oxidative phosphorylation, *Physiology*, 58, 506.

HOCH FL, (1968), Thyroid hormone action on mitochondria. Effects of inhibitors of respiration, *Archiv Biochem Biophys*, 124, 238.

HOFF JT, REIS DJ, (1970), Localization of regions mediating the Cushing response in CNS of cat, *Arch Neurol*, 23, 228.

HOLBOURN AHS, (1945), The mechanics of brain injury, *Brit Med Bull*, 3, 147.

HOLLAND PC, (1979), Biosynthesis of the Ca^{2+} and Mg^{2+} -dependent adenosine triphosphatase of sarcoplasmic reticulum in cell cultures in embryonic chick hearts, *Biol Chem*, 254, 7604.

HOLLAND FW 2ND, BROWN PS Jr, WEINTRAUB BD, CLARK RE, (1991), Cardiopulmonary bypass and thyroid function a "euthyroid sick syndrome", *Ann Thorac Surg*, 52(1), 46.

HOROWITZ S, BORCHERDING W, MOORTHY AV, CHESNEY R, and SHULTE-WISSERMANN H, (1977), Thyroid hormone action: The mitochondrial pathway, *Science*, 197, 996.

HORST HJ, (1963), L-Lactate, In, *Methods of Enzymatic Analysis*, Ed. by H. Bergmeyer, Academic Press, London, 266.

HUCKABEE, (1958), Relationship of pyruvate and lactate during anaerobic metabolism, local T shock components of total body O_2 -depth, *Am J Physiol*, 196, 253.

HUNT D, and GORE L, (1972), Myocardial lesions following experimental intracranial haemorrhage: Prevention with propranolol, *Am Heart J*, 83, 232.

HUNT D, GORE L, (1972), Myocardial lesions following experimental intracranial haemorrhage: Prevention with propranolol, *Am Heart J*, 83, 232.

INGBAR SH, WOEBER KA, (1981), Chapt 5, The Thyroid Gland, In, *The Textbook of Endocrinology*, Ed RH Williams, Pub W B Saunders Co., Philadelphia, USA, 117.

ISMAEL-BEIGI, EDELMAL IS, (1971), The mechanisms of the calorogenic action of thyroid hormones: Stimulation of Na⁺ and K⁺ activated adenosine triphosphate activity, *J Gen Physiol*, 57, 710.

JACHUCK SJ, RAMANI PS, CLARK F, KALBAG RM, (1975), Electrocardiographic abnormalities associated with raised intracranial pressure, *Brit Med J*, 1, 242.

JASSEM W, KOO DD, CERUNDOLO L, RELA M, HEATON ND, FUGGLE SV, (2003), Cadaveric versus living-donor livers: differences in inflammatory markers after transplantation, *Transplantation*, 76, 1599.

JEEVANANDAM V, (1997), Triiodothyronine: spectrum of use in heart transplantation, *Thyroid*, 7, 139.

JEEVANANDAM V, TODD B, REGILLO T, HELLMAN S, ELDRIDGE C, McCLURKEN J, (1994), Reversal of donor myocardial dysfunction by triiodothyronine replacement therapy, *J Heart Lung Transplant*, 13(4), 681.

References-14

JOHNSON PD, BESSELS DG, (2002), Practical aspects of experimental designs in animal research, *ILAR J*, 43, 202.

JOHNSTON CI, NEWMAN M, and WOODS R, (1981), Role of vasopressin in cardiovascular haemostasis and hypertension, *Clin Sci*, 61, 129.

JURGENSEN EO, (1973), Spinal man after brain death *Acta Neurochir*. 28, 259.

KAHN CR, (1985), *Ann Rev Med*, 36, 429.

KAUP FJ, (2002) Infectious diseases and animal models in primates, *Primate Rep*, 62, 3.

KIM D, and SMITH TW, (1985), Effects of thyroid hormone on calcium handling in cultured chick ventricular cells, *J Physiol*, 364, 131.

KIM D, and SMITH TW, (1984), Effect of thyroid hormone on sodium pump sites, sodium content, and contractile responses to cardiac glycosides in cultured chick ventricular cells, *J Clin Invest*, 74, 1481.

KOO SW, CASPER KA, OTTO KB, GIRA AK, SWERLICK RA, (2003), Iron chelators inhibit VCAM-1 expression in human dermal microvascular endothelial cells, *Invest Dermatol*, 120(5), 871.

KOUDSTAAL LG, 't HART NA, van den BERG A, OLINGA P, van GOOR H, PLOEG RJ, LEUVENINK HGD, (2005), Brain death causes structural and inflammatory changes in donor intestine, *Transplantation Proceedings*, 37(1), 448.

KREISBERG RA, (1984), Pathogenesis and management of lactic acidosis, *Ann Rev Med*, 35, 181.

KUSAKA M, PRATSCHKE J, WILHELM MJ, ZIAI F, ZANDI-NEJAD K, MacKENZIE HS, HANCOCK WW, TILNEY NL, (2000), Activation of inflammatory mediators in rat renal isografts by donor brain death, *Transplantation*, 69(3), 405.

LABHART A, (1974), Chapter VII, Adrenal Cortex, In, Labhart A, *Clinical Endocrinology Therapy and Practice*, Pub. Springer-Verlag, New York. 285.

LAMPRECHT W, TRAUTSCHOLD I, (1963), In, *Methods of Enzymatic Analysis*, Ed. by H. Bergmeyer, Academic Press, London, 543.

LEFER AM, LEFER DJ, (1991), Endothelial dysfunction in myocardial ischemia and reperfusion: role of oxygen-derived free radicals, *Basic Res Cardiol*, 86 Suppl2, 109.

LIDDLE GW, (1981), Chapt 5, The adrenals, In, *Textbook of Endocrinology*, Ed RH Williams, Pub W B Saunders Co, Philadelphia, USA, 249.

LIMAS CJ, (1978), Enhanced phosphorylation of myocardial sarcoplasmic reticulum, in experimental hyperthyroidism, *Am J Physiol*, 234, H426.

LINN MH, ALERA T, (1978), Increased Na^+ , K^+ ATPase concentration in various tissues of rats caused by thyroid hormone treatment, *J Biol Chem*, 253, 723.

LOWN B, and VERRIER RL, (1976) Neural activity and ventricular fibrillation, *N Engl J Med*, 294, 1165.

References-16

MACPHERSON P, and GRAHAM DI, (1978), Correlations between angiographic findings and the ischaemia of head injury, *J Neurol Neurosurg Psychiatr.* 41, 122.

McCLELLAN G, WEISBERG A, and WINEGRAD S, (1983), Energy transport from mitochondria to myofibril by a creatine phosphate shuttle in cardiac cells, *Am J Physiol*, 245, C423.

MILEI J, NUNEZ RG, and RAPPAPORT M, (1978), Pathogenesis of isoprenalol-induced myocardial lesions; its relation to human "coagulative myocytolysis". *Cardiol*, 63, 139.

MITCHELL DE, and ADAMS JH, (1973), Primary focal impact damage to the brain stem in blunt head injuries: Does it exist?, *Lancet*, 2, 216.

MONTEFUSCO CM, MOLLENKOPF FP, KAMHOLZ SL, GOLDSMITH J, VEITH FJ, (1984), Maintenance protocol for potential organ donors in multiple organ procurement, *Hospital Physician*, 20, 9.

MUHLBERG J, WAGNER W, ROHLING R, LINK J, NEUMAYER HH. (1986), Haemodynamic and metabolic problems in the preparation for organ donation, *Transplant Proc*, 18, 391.

MULLIGAN MS, VAPORCIYAN AA, MIYASAKA M, TAMATANI T, WARD PA, (1993), Tumor necrosis factor alpha regulates in vivo intrapulmonary expression of ICAM-1, *Am J Pathol*, 42(6), 1739.

NATHAN MA, and REIS DJ, (1975), Fulminating arterial hypertension with pulmonary oedema from release of adrenomedullary catecholamines after lesions of the anterior hypothalamus in the rat, *Circ Res*, 37, 226.

NATIONAL RESEARCH COUNCIL, (2003), International perspective; the future of nonhuman primate resources, Washington DC: National Academy Press

NAYLOR WG, MERRILLEES CNR, CHIPPERFIELD D, and KURTZ JB, (1971), Influence of hyperthyroidism on the uptake and binding of calcium by cardiac microsomal fractions and on mitochondrial structure. *Cardiovasc Res*, 5, 469.

NEELY JR, and MORGAN HE, (1974), Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle, *Circ Res*, 38, 122.

NELSON RJ, MANDRELL TD, (2005), Enrichment and Nonhuman Primates: "First, do no harm", *ILAR J*, 46(2), 171.

NEWSHOLME EA, LEECH AR, (1983), Chapt 7, Regulation of glucose and fatty acids oxidation in relation to energy demand in muscle. In *Biochemistry for the Medical Sciences*, John Willie & Sons, Norwich, 300.

NICHOLLS D, AKERMAN K, (1982), Mitochondrial calcium transport, *Biochemic Biophys Acta* 683, 57.

NOVITZKY D, (1992), Heart transplantation, euthyroid sick syndrome, and triiodothyronine replacement, *J Heart Lung Transplant*, 11, 196.

NOVITZKY D, (1996), Selection and management of cardiac allograft donors, *Current Opinion in Cardiology*, 11, 174.

References-18

NOVITZKY D, COOPER DK, CHAFFIN JS, GREER AE, DeBAULT LE, ZUHDI N, (1990), Improved cardiac allograft function following triiodothyronine therapy to both donor and recipient, *Transplantation*, 49(2), 311.

NOVITZKY D, COOPER DKC, HUMAN PA, REICHART B, ZUHDI N, (1988), Triiodothyronine therapy for heart donor and recipient, *J Heart Transplant*, 7, 370.

NOVITZKY D, COOPER DKC, MORRELL D, ISAACS S, (1988), Change from aerobic to anaerobic metabolism after brain death, and reversal following Triiodothyronine (T_3) therapy, *Transplantation* 45, 32.

NOVITZKY D, COOPER DKC, REICHART B, (1987), Haemodynamic and metabolic responses to hormonal therapy in brain-dead potential organ donors, *Transplantation*, 43, 852.

NOVITZKY D, COOPER DKC, ROSENDALE JD, KAUFFMAN HM, (2006), Hormonal therapy of the brain-dead organ donor: experimental and clinical studies, *Transplantation*, 82, 1396.

NOVITZKY D, COOPER DKC, SWANEPOEL A, (1989), Inotropic effect of triiodothyronine (T_3) following myocardial ischemia and cardiopulmonary bypass: Initial experience in patients undergoing open heart surgery, *Eur J Cardiothorac Surg*, 3, 140.

NOVITZKY D, FONTANET H, SNYDER M, COBLIO N, SMITH D, PARSONNET V, (1996), Impact of triiodothyronine on the survival of high-risk patients undergoing open heart surgery, *Cardiology*, 87, 509.

NOVITZKY D, HORAK A, COOPER DKC, ROSE AG, (1989), Electrocardiographic and histopathologic changes developing during experimental brain death in the baboon, *Transplant Proc*, 21, 2567.

NOVITZKY D, ROSE AG, COOPER DK, (1988), Injury of myocardial conduction tissue and coronary artery smooth muscle following brain death in the baboon, *Transplantation*, 45(5), 964.

NOVITZKY D, WICOMB WN, COOPER DKC, ROSE AG, FRASER RC, BARNARD CN, (1984), Electrocardiographic, haemodynamic, and endocrine changes occurring during experimental brain death in the Chacma baboon, *J Heart Transplant*, 4, 63.

NOVITZKY D, WICOMB WN, COOPER DKC, TJAALGARD MA, (1987), Improved cardiac function following hormonal therapy in brain dead pigs: relevance to organ donation, *Cryobiology*, 24, 1.

OLINGA P, van der HOEVEN JAB, MEREMA MT, FREUND RL, PLOEG RJ, GROOTHUIS GMM, (2005), The influence of brain death on liver function, *Liver International*, 25, 109.

OPIE LH (1984), Chapt 9, Fuels, In, *The Heart, Physiology, Metabolism, Pharmacology and Therapy*, Grune and Stratton, London, 111.

OPIE LH, (1984), Frontispiece in *The Heart, Physiology, Metabolic, Pharmacology and Therapy*, Grune and Stratton, London.

OPIE LH, 1984. Chapt 6, Adrenergic effects of cyclic AMP, In, *The Heart, Physiology, Metabolism, Pharmacology and Therapy*, Grune and Stratton, 68.

References-20

OPIE LH, MANSFORD KRL, OWEN P, (1971), Effects of increased heart work on glycolysis and adenine nucleotides in the perfused heart of normal and diabetic rats. *Biochem J*, 124, 475.

OPIE LH, NAYLOR W, and GEVERS W, (1984), Chapt 7, Calcium Fluxes, In, Opie LH, *The heart*, Gruen and Stratton, London, 88.

OPITZ E, SCHNEIDER M, (1950), *Veber die sauerstoff versorgung des gehirns und den mechanisms von mangel wir kingen*, *Ergeb Physiol*, 46, 126.

OPPENHEIMER H, (1979), Thyroid hormone action at the cellular level, *Science*, 203, 971.

OPPENHEIMER JH, (1979), Thyroid hormone action at the cellular level, *Science*, 203, 971.

OVERGAARD J, and TWEED WA, (1974), Cerebral circulation after head injury. Part 1: Cerebral blood flow and its autoregulation after closed head injury with emphasis on clinical correlations, *J Neurosurg*, 41, 531.

PALLIS C, (1982), ABC of brain stem death. Diagnosis of brain stem death - II, *Brit Med J*, 285, 1641.

PALLIS C, (1982), ABC of brain stem death. Diagnosis of brain stem death - I, *Brit Med J*, 285, 1558.

PALLIS C, (1982), ABC of brain stem death. Prognostic significance of a dead brain stem, *Brit Med J*, 286, 123.

PALLIS C, (1982), ABC of brain stem death. From brain death to brain stem death, Brit Med J, 285, 1487.

PALLIS C, (1982), Whole brain death recognized - physiological facts and philosophy, J Med Ethics, 9, 32.

PALLIS C, (1982), ABC of brain stem death, reappraising death, Brit Med J, 285, 1409.

PALLIS C, (1982), ABC of brain stem death. Pitfalls and safeguards, Brit Med J, 285, 1720.

PALLIS C, (1982), ABC of brain stem death. The declaration of death, Brit Med J, 286, 39.

PAPANICOLAOU D, (2000), Euthyroid sick syndrome and the role of cytokines, Review in Endocrine & Metabolic Disorders, 1, 43.

PEERLESS SJ, (1981), Letter in Neurosurg, 8, 13.

PETRINELLI R, TARAZI RC, (1984), Interference of calcium entry blockade in vivo with pressor responses to alpha-adrenergic stimulation: Effect of two unrelated blockers on responses to both exogenous and endogenously related norepinephrin, Circulation, 69, 1171.

PLENZ G, ESCHERT H, ERREN M, WICHTER T, BOHM M, FLESCH M, SCHELD HH, DENG MC, (2002), The interleukin-6/interleukin-6-receptor system is activated in donor hearts, J Am Coll Cardiol, 39(9), 1508.

References-22

POOL PE, NORRIS GF, LEWIS RM, and COVELL JW, (1968), A biopsy drill permitting rapid freezing, *J Appl. Physiol.* 24, 832.

PORTE D, HALTER JB, (1981), Chapt 15, In *Textbook of Endocrinology, The endocrine pancreas and diabetes mellitus*, Ed RH Williams, Pub W B Saunders Co, Philadelphia, USA, p 715.

POWNER DJ, HERNANDEZ M, (2005), A review of thyroid hormone administration during adult donor care, *Progress in Transplantation*, 15, 202.

POVLISHOCK JT, BECKER DP, SULLIVAN HG. (1978), Vascular permeability alteration to horseradish peroxidase in experimental brain injury, *Brain Res*, 153, 223.

PRATSCHKE J, WILHELM MJ, KUSAKA M, et al. (1999), Brain death and its influence on donor organ quality and outcome after transplantation, *Transplantation*, 67(3), 343.

PRATSCHKE J, WILHELM MJ, KUSAKA M, BEATO F, MILFORD EL, HANCOCK WW, TILNEY NL, (2000), Accelerated rejection of renal allografts from brain-dead donors, *Ann Surg*, 232(2), 263.

RAJS J, and JACOBSSON S, (1976), Severe trauma and subsequent cardiac lesions causing heart failure and death, *Forensic Sci*, 8, 13.

RASMUSSEN H, and BARRETT PQ, (1984), Calcium messenger system: An integrated view, *Physiological Reviews*, 64, 938.

REEVES JG, (1984) The relative haemodynamic effects of calcium entry blockers, *Anaesthesiology*, 61, 3.

REICHENBACH DD, and BENDITT EP, Catecholamines and cardiomyopathy: The pathogenesis and potential importance of myofibrillar degeneration, *Human Path*, 1, 125.

REICHENBACH DD, and MOSS NS, (1975), Myocardial cell necrosis and sudden death in humans, *Circulation*, Suppl 3, 51, 60.

REICHENBACH DD, and MOSS NS, (1975), Myocardial cell necrosis and sudden death in humans, *Circulation*, 51 (III), 60.

REICHERT MG, VERZINO KC, (2001), Triiodothyronine supplementation in patients undergoing cardiopulmonary bypass, *Pharmacotherapy*, 21(11), 1368.

REIDER CF, KEUSHKERIAN SM, CRAWFORD BG, HERMRECK AS, PIERCE GE, and THOMAS JH, (1986) Total body hypothermia for multiple organ retrieval. *Transplant Proc*, 18, 431.

REITZ B, BARTON NA, JAMESON S, BEAVER CT, PENNOCK JL, STEENSON EB, and SHUMWAY NE, (1980), Heart and lung transplantation. Autotransplantation and other transplantations in primates with extended survival, *J Thorac Cardiovasc Surg*, 80, 360.

ROGERS HJ, SPECTOR RG, TROUNCE JR, (1981), Introduction to pharmacokinetics, In, *A Textbook of Clinical Pharmacology*, Hodder & Stoughton, London, Chapt 1, 3.

ROLLIN, BE, (2006) The regulation of animal research and the emergence of animal ethics: a conceptual history, *Theoretical Medicine and Bioethics*, 27, 285.

References-24

ROSE AG, NOVITZKY D, FACOR SM, (1988), Catecholamine-associated smooth muscle contraction bands in the medial of coronary arteries of brain-dead baboons, *Am J Cardiovasc Pathol*, 2, 63.

ROSENDALE JD, KAUFFMAN HM, McBRIDE MA, CHABALEWSKI FL, ZAROFF JG, GARRITY ER, DELMONICO FL, ROSENGARD BR, (2003), Aggressive pharmacologic donor management results in more transplanted organs, *Transplantation*, 75(4), 482.

ROSENDALE JD, KAUFFMAN HM, McBRIDE MA, CHABALEWSKI FL, ZAROFF, JG, GARRITY ER, DELMONICO FL, ROSENGARD BR, (2003), Hormonal resuscitation yields more transplanted hearts, with improved early function, *Transplantation*, 75, 1336.

ROSENGARD BR, FENG S, ALFREY EJ, et al. (2002), Report of the Crystal City meeting to maximize the use of organs recovered from the cadaver donor, *Am J Transplant*, 2, 701.

RUSCHE B, (2003), The 3Rs and animal welfare – conflict or the way forward?, *Altex*, 20, 63.

SALIM A, MARTIN M, BROWN C, RHEE P, DEMETRIADES D, BELZBERG H, (2006), The effect of a protocol of aggressive donor management: implications for the national organ donor shortage, *J Trauma*, 61, 429.

SALIM A, VASSILLU P, VELMAHOS GC, et al. (2001), The role of thyroid hormone administration in potential organ donors, *Arch Surg*, 136, 1377.

SALIM A, VELMAHOS GC, BROWN C, BELZBERG H, DEMETRIADES D, (2005), Aggressive organ donor management significantly increases the number of organs available for transplantation, *The Journal of Trauma Injury, Infection, and Critical Care*, 58, 5.

SCHAPIRO SJ, EVERITT JI, (2006), Preparation of animals for use in the laboratory: Issues and challenges for the institutional animal care and use committee (IACUC), *ILAR Journal*, 47, 370.

SCHWARTZ A, (1984), Cellular action of calcium channel blocking drugs, *Ann Rev Med*, 35, 325.

SCHWEICKERT WD, HALL J, (2007), ICU-acquired weakness, *Chest*, 131(5), 1541.

SEARLE GL, (1976), The use of isotope turnover techniques in the study of carbohydrate metabolism in man, *Clin of Endocrinol Metab*, 5, 783.

SESTOF L, (1980), Metabolic aspects of the calorogenic effect of thyroid hormone in mammals, *Clin Endocrinol*, 13, 489.

SHERMAN MR, (1984), Structure of mammalian steroid receptors, evolving concepts and methodological developments, *Ann Rev Physiol*, 46, 83.

SHIVALKAR B, VAN LOON J, WIELAND W, TJANDRA-MAGA TB, BORGERS M, PLETS C, (1993), Variable effects of explosive or gradual increase of intracranial pressure on myocardial structure and function, *Circulation*, 87, 230.

SIEGELMAN S, SINHA SBP, and VEITH FJ, (1973), Pulmonary reimplantation response, *Ann Surg*, 177, 30.

SIMON RP, BAYNE LL, (1984), Pulmonary lymphatic flow alterations during intracranial hypertension in sheep, *Ann Neurol*, 15, 188.

References-26

SKRABAL CA, THOMPSON LO, POTAPOV EV, SOUTHARD RE, JOYCE DL, YOUKER KA, NOON GP, LOEBE M, (2005), Organ-specific regulation of Pro-inflammatory molecules in heart, lung, and kidney following brain death, *J Surg Res*, 123(1), 118.

SLAPAK M, (1978), The immediate care of potential donors for cadaveric organ transplantation. *Anaesthesia*, 33, 700.

STANGL M, ZERKAULEN T, THEODORAKIS J, ILLNER W, SCHNEEBERGER H, LAND W, FAIST E, (2001), Influence of brain death on cytokine release in organ donors and renal transplants, *Transplant Proc*, 33(1-2), 1284.

STATHATOS N, WARTOFSKY L, (2003), The euthyroid sick syndrome: is there a physiologic rationale for thyroid hormone treatment?, *J Endocrinol Invest*, 26, 1174.

STAUB NC, (1978) Pulmonary oedema due to increased microvascular permeability to fluid and protein, *Circ Res*, 43, 143.

STAUB NC, (1980), The pathogenesis of pulmonary oedema *Progress in Cardiovascular Diseases*, 23, 53.

STERLING K, (1979), Thyroid hormone action at the cell level (second of two parts), *N Engl J Med*, 300, 173.

STERLING K, (1979), Thyroid hormone action at the cell level, (first of two parts), *N Engl J Med*, 300, 117.

STILES GL, LEFKOWITZ RJ, (1984) Cardiac adenergetic receptors, *Ann Rev Med*, 35, 149.

STOICA SC, GODDARD M, LARGE SR, (2002), The endothelium in clinical cardiac transplantation, *Ann Thorac Surg*, 73, 1002.

STRAUB NC, (1980), The pathogenesis of pulmonary oedema, *Prog Cardiovasc Dis*, 23, 53.

STRICH SJ, (1956), Diffuse degeneration of the cerebral white matter in severe dementia following head injury, *J Neurol Neurosurg Psychiatr*. 19, 163.

SUTHERLAND EW, YE I, BUTCHER RW, (1965), The action of epinephrin and the role of the adenyl cyclase system in hormone action. *Recent Prog Horm Res*, 21, 623.

SZABO G, BUHMANN V, BAHRLER S, VAHL CF, HAGL S, (2002), Brain death impairs coronary endothelial function, *Transplantation*, 73, 11.

SZAKACS JE, and CANNON A, (1958), 1-norepinephrin myocarditis, *Am J Clin Path*, 30, 425.

SZTARK F, THICOIPE M, LASSIE P, PETITJEAN ME, DABADIE P, (2000), Mitochondrial energy metabolism in brain-dead organ donors, *Transplantation*, 5(4), 41.

TADA M, and KATZ AM, (1982), Phosphorylation of the sarcoplasmic reticulum and sarcolemma, *Ann Rev Physiol*, 44, 401.

TADA M, and KATZ AM, (1982), Phosphorylation of the sarcoplasmic reticulum and sarcolemma, *Ann Rev Physiol*, 44, 401.

TAKADA M, NADEAU KC, HANCOCK WW, et al. (1998), Effects of explosive brain death on cytokine activations of peripheral organs in the rat, *Transplantation*, 65, 1533.

References-28

TEASDALE G, and JENNET B, (1974) Assessment of coma and impaired consciousness, *Lancet*, 2, 81.

TEASDALE G, BROWN JO, TURNER J, (1977), Cerebral perfusion failure and corticoelectrical activity, *Acta Neurol*, 56, 430.

TEPPERMAN J, (1983), Chapt 14, Endocrine function of the pancreas. In, *Metabolic and Endocrine Physiology*, Pub Year Book Medical Publishers, Inc, London, England, 225.

THEODORE J, ROBIN ED, (1976), Speculations on neurogenic pulmonary oedema, *Amer Rev Resp Dis*, 113, 405.

TIMEK T, BONZ A, DILLMANN R, VAHL CF, HAGL S, (1998), The effect of triiodothyronine on myocardial contractile performance after epinephrine exposure: implications for donor heart management, *J Heart Lung Transplant*, 17(9), 931.

TOLEDO-PEREYRA LH, MacKENZIE GH, (1980), Simultaneous multiple organ perfusion and transplantation, *Am Surg*, 46, 161.

TOMLINSON S, WALKER SW, BROWN BL, (1982), Calmodulin and insulin secretion, *Diabetologia* 22, 1.

TOMLINSON S, McNEIL S, WALKER SW, OLLIS CA, MERRITT JE, BROWN B, (1984), Calmodulin and cell function, *Clinical Science*, 66, 497.

ULLAH S, ZABALA L, WATKINS B, SCHMITZ ML, (2006), Cardiac organ donor management, *Perfusion*, 21, 93.

UNGER RH, (1961), Radioimmunoassay for glucagon measurement. *J Clin Invest*, 45, 1280.

VAN DER VLIET JA, KROOTSTRA G, and KROM RAF, (1982), Cadaveric organ retrieval for transplantation, *World J Surg*, 6, 478.

van der MEER W, PICKKERS P, SCOTT CS, van der HOEVEN JG, GUNNEWIEK JK, (2007), Hematological indices, inflammatory markers and neutrophil CD64 expression: comparative trends during experimental human endotoxemia, *Endotoxin Res*, 13(2), 94.

VAUGHN TR, DE MARINO EM, STAND CN, (1976), Indicator dilution lung water and capillary blood volume in prolonged heavy exercise in normal men, *Am Med Resp Dis*, 113, 757.

VILLACORTA H, MASETTO AC, MESQUITA ET, (2007), C-reactive protein: an inflammatory maker with prognostic value in patients with decompensated heart failure, *Arq Bras Cardiol*, 88, 585.

VITALE A, MANCIOCCO A, (2004), Environmental enrichment techniques in non-human primates. The case of Callitrichids, *Ann 1st Super Sanita*, 40, 181.

WALDENSTROM AP, HJALMARSON AC, THORNELL L, (1978), A possible role of noradrenalin in the development of myocardial infarction. An experimental study in the isolated rat heart, *Am Heart J*, 95, 43.

WALSH MP, LE PEUCH CJ, VALLET B, CAVADORE JC, and DEMAILLE JG, (1980), Cardiac calmodulin and its role in the regulation of metabolism and contraction. *J Mol Cell Cardiol*, 12, 1091.

References-30

WALZOG B, GAEHTGENS P, (2000), Adhesion Molecules: the path to a new understanding of acute inflammation, *News Physiol Sci*, 15, 107.

WATANABE T, SMITH MM, ROBINSON FW, KONO T, (1984), Insulin action on glucose transport in the cardiac muscle, *J Biol Chem*, 259, 13117.

WAUCHOB TD, BROOKS RJ, HARRISON KM, (1984), Neurogenic pulmonary oedema, *Anaesthesia*, 39, 529.

WHEELDON DR, POTTER CDO, ODURO A, et al. (1995), Transforming the "unacceptable" donor: outcomes from adoption of a standardized donor management technique, *J Heart Lung Transplant*, 14, 734.

WICOMB WN, BOYD ST, COOPER DKC, ROSE AG, and BARNARD CN, (1981), Ex vivo functional evaluation of the pig heart subjected to 24 hours preservation by hypothermic perfusion, *S Afr Med J*, 60, 245.

WICOMB WN, COOPER DKC, NOVITZKY D, and BARNARD CN, (1984), Cardiac transplantation following storage of the donor heart by a portable hypothermic perfusion system, *Ann Thorac Surg*, 37, 243.

WIKMAN-COFFELT J, SIEVERS R, COFFELT RG, PARMLEY WW, (1983), Oscillation of CAMP with a cardiac cycle, *Biochem. Biophys. Res. Comm*, 111, 450.

WICOMB WN, NOVITZKY D, COOPER DK, WELLS M, HILL JD, (1989), Early extranuclear effect of triiodothyronine (T_3) on tissue slices: relevance to organ donor viability, *Transplant Proc*, 21 (1 Pt 2), 1263.

WROBLEWSKI F, and LA DUE JS, (1955), Lactate dehydrogenase measurements, Proc Soc Exp Bio Med, 90, 210.

WYNE KL, (2005), The role of thyroid hormone therapy in acutely ill cardiac patients, Critical Care, 9, 333.

YAMOUR BJ, SRIDHARAN MR, RICE JF, FLOWERS NC, (1980), Electrocardiographic changes in cerebrovascular haemorrhage, Am Heart J, 99, 294.

YEH T, WECHSLER AS, GRAHAM L, LOESSER KE, SICA DA, WOLFE L, JAKOI ER, (2002), Central sympathetic blockage ameliorates brain death-induced cardiotoxicity and associated changes in myocardial gene expression, J Thorac Cardiovasc Surg, 124, 1087.

ZAROFF JG, ROSENGARD BR, ARMSTRONG, et al. (2002), Maximizing use of organs recovered from the cadaver donor: cardiac recommendations, J Heart Lung Transplant, 21, 1153.

ZUPPA AF, NADKARNI V, DAVIS L, et al. (2004), The effect of a thyroid hormone infusion on vasopressor support in critically ill children with cessation of neurologic function, Crit Care Med, 32, 2318.

