INFARCT SIZE AND FREE FATTY ACIDS IN THE EARLY PHASE OF ACUTE MYOCARDIAL INFARCTION

A Thesis submitted by

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To my mother and father,

Zoe and Henry Tansey.

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Abbreviations used in the text

ADP	= Adenosine diphosphate
ATP	= Adenosine triphosphate
AMI	= Acute myocardial infarction
CK	= Creatine kinase
DC	= Direct current
EDTA	= Ethylene diamine tetra-acetic acid
EIS	= Estimated infarct size
FFA	= Free fatty acid
ISI	= Infarct size index
LDH	= Lactic dehydrogenase
MVO_2	= Myocardial oxygen consumption
NAD^+	= Nicotinamide adenine dinucleotide
NS	= Not significant
SD	= Standard deviation
SEM	= Standard error of the mean
SER	= Serum entry ratio
SGOT	= Serum glutamic oxalo-acetic transaminase
TRIS	= Trihydroxymethylaminomethane
VF	= Ventricular fibrillation
VPB	= Ventricular premature beat
VT	= Ventricular tachycardia

INTRODUCTION

The management of acute myocardial infarction (AMI) has been improved by the realisation that the size of infarction can influence mortality (Sobel et al, 1972) and that the infarct size can be altered by subsequent therapy (Maroko et al, 1972). The identification of any factor which may have adverse effects on the ischaemic myocardium and which is amenable to treatment would therefore have important prognostic implications.

Elevation of circulating free fatty acid (FFA) concentrations is a consistent feature (Kurien & Oliver, 1966; Oliver et al, 1968) of the profound, non-specific metabolic reaction associated with the onset of AMI (Opie, 1975). The FFA rise has been correlated with the development of arrhythmias (Oliver et al, 1968) after AMI, and with the severity of ischaemic damage (Oliver et al, 1968; Gupta et al, 1969; Russell & Oliver, 1978) on clinical grounds. The method of quantifying infarct size developed by Shell et al (1972) has provided a means of correlating the degree of metabolic disturbance with extent of myocardial damage, and of assessing the benefits of metabolic interventions.

The purpose of the studies reported in this thesis was to examine in detail the FFA rise in the early phase of AMI and to correlate this rise with the development of arrhythmias and other complications of AMI and with enzymatically estimated infarct size, thus leading to a more rational approach to therapeutic interventions.

CHAPTER I

PATIENT SELECTION AND CLINICAL METHODS

PATIENT SELECTION

All patients studied were initially admitted to the coronary care unit at Groote Schuur Hospital for a period of at least 3 days. Consent was obtained in each case, though all the procedures, other than the frequency of blood sampling, were routinely carried out in all patients. Criteria for inclusion varied from study to study but in all cases patients had to satisfy the following conditions:-

1. Proven acute myocardial infarction. Acute myocardial infarction was defined by the presence of at least 2 of the following features: (a) a history of prolonged chest pain suggestive of acute myocardial infarction; (b) new Q-waves or typical evolutionary ischaemic ST-T wave changes on the electrocardiogram; (c) a characteristic rise and fall in the levels of the serum enzymes creatine kinase, amino aspartate transaminase and lactic dehydrogenase.

2. A time lapse of less than 12 hours after the onset of symptoms of acute myocardial infarction. This time limit was set because peak plasma free fatty acid (FFA) levels after acute myocardial infarction were being estimated. The major FFA rise occurs within 12 hours and a very high FFA is rarely found beyond this time (Kurien & Oliver, 1966).

3. No clinical evidence of shock, because this may cause a release of CK from skeletal muscle (Sobel & Shell, 1972) and plasma CK activity was being measured in order to calculate infarct size. Shock was defined by the presence of at least 2 of the following findings: (a) a systolic blood pressure of 90 mmHg or less for 30 minutes or more; (b) urine flow of less than 30 ml per hour despite a fluid intake of over 100 ml/hour; (c) evidence of reduced organ perfusion, as shown by cold, cyanosed peripheries or mental confusion.

4. No intramuscular injections in the week prior to admission, because

intramuscular injections result in release of CK from skeletal muscle (Meltzer et al, 1970; Klein et al, 1973).

5. No oral intake of any kind from the time of onset of symptoms of acute myocardial infarction. Carbohydrates lower FFA levels in both normal subjects (Gordon & Cherkes, 1956) and in patients with elevated FFA levels after acute myocardial infarctions (Oliver et al, 1972; Opie et al, 1975). For the same reason the only intravenous infusion permitted was either normal or half-normal saline.

6. No prior administration of heparin, clofibrate, catecholamines or steroids, which could influence blood FFA levels (Oliver et al, 1968).

7. No history or evidence of diabetes mellitus, as FFA levels may be elevated in this condition (Shafrir & Gutman, 1965).

BLOOD SAMPLING AND STORAGE

Blood was withdrawn via an indwelling venous catheter, the tip of which was placed either in the superior vena cava or in the right atrium. The position of the catheter was checked by X-ray immediately after insertion. Sampling via a catheter is better than sampling by repetitive venipuncture when CK activity is to be estimated because even minor trauma near the sampling site may result in release of skeletal muscle CK and distortion of results (Roberts et al, 1975a). The proximal end of the catheter was attached to a 3-way tap so that the catheter could serve for both the intravenous saline infusion and for sampling of venous blood. Prior to sampling the 3-way tap was turned so as to prevent saline entering the catheter, and 5 mls of fluid were then withdrawn and discarded to ensure that only venous blood, uncontaminated by the infusion, was ultimately withdrawn and analysed.

20 ml samples of blood were withdrawn and 10 mls placed into each of 2 heparinised plastic tubes, which were then sealed and centrifuged at

4000 rpm for 10 minutes. Samples which appeared haemolysed or turbid to the naked eye were discarded. The plasma from 10 mls of blood suffice for all the analyses carried out in these studies, and the second sample was taken as a precaution, in case of loss of the first sample. The determination of insulin levels could not be carried out on heparinised samples because the laboratory which carried out the insulin estimations reported spuriously high results from samples containing heparin. Where insulin levels were required 10 mls of the blood were placed in a plastic tube and spun immediately for 10 minutes at 4000 rpm, after which the clot was removed from the supernatant using an orange stick.

After spinning, the plasma or serum was poured into plain plastic tubes which were sealed and immediately frozen at -10°C. Immediately prior to analysis, specimens were thawed rapidly under tap water (Sobel et al, 1972) and then spun for 5 minutes at 4000 rpm to ensure that only clear plasma or serum was used for analysis.

Where possible, blood samples were collected hourly up to 12 hours after the onset of symptoms, 2 hourly for the next 12 hours, 4 hourly for the next 24 hours and then 6 hourly for 24 hours, or until the CK activity had returned to near normal levels.

ROUTINE CLINICAL INVESTIGATIONS

The following investigations were carried out routinely for each patient by the staff of the coronary care unit, and the results were used to follow progress in all patients studied.

1. X-rays. A portable X-ray of the chest was taken when each patient was admitted, and daily thereafter for the duration of stay in the coronary care unit. Additional X-rays were taken when this was warranted by a change in clinical status.

2. ECG. A 12-lead ECG with a 12 second rhythm was taken on admission and

daily thereafter. Further ECGs were taken after any further episode of chest pain and whenever a change of rhythm or a conduction defect was observed.

3. Arrhythmia monitoring. Arrhythmias were continuously monitored by an Electrodyne computer for the duration of the patient's stay in the coronary care unit. All arrhythmias in the preceding eight hours could be displayed by the computer, and hard copies of the display were available. The onset of any arrhythmia caused an alarm to be activated and a rhythm strip to be printed out, so that a record of all arrhythmias experienced in the coronary care unit was available for all patients.

ROUTINE BIOCHEMICAL ESTIMATIONS

The potassium level was carried out as a matter of urgency, by the hospital laboratory, whenever a patient with suspected acute myocardial infarction (AMI) was admitted to the casualty department, so that patients with low serum potassium levels could receive potassium supplements at the earliest opportunity. The full blood count, urea, creatinine, electrolytes and cardiac enzymes (Creatine kinase, Lactic Dehydrogenase and Glutamic Oxalo-acetic Transaminase) were monitored daily by the hospital laboratory. On the day of admission, serum protein, calcium, phosphate, cholesterol, urate, bilirubin and alkaline phosphatase levels were also estimated.

ROUTINE OBSERVATIONS AND RECORDS

Blood pressure, heart rate, respiratory rate and oral temperature were recorded every 4 hours by the nursing staff; observations were recorded more frequently if indicated by the clinical situation. Fluid intake and urine output were carefully monitored.

A record was kept of all drugs given orally and intravenously. No intramuscular injections were given during the stay in the coronary care unit to any patient included in these studies.

ROUTINE DRUG ADMINISTRATION

The following drugs were prescribed routinely for all patients in the coronary care unit:-

Morphine 5-10 mg intravenously, whenever required for chest pain;

Prochlorperazine 12.5 mg, or Metoclopromide 10 mg intravenously as required to counteract nausea and vomiting.

Diazepam 5 mg orally three times daily. All other drugs were prescribed in accordance with the clinical needs of the patients.

CLINICAL STATUS OF THE PATIENTS

On admission to the coronary care unit, all patients had full histories taken and were given a complete physical examination. A complete clinical examination was carried out daily. Details relevant to each study are included in the appropriate chapter.

CLASSIFICATION OF THE EXTENT OF MYOCARDIAL DECOMPENSATION BY THE METHOD OF KILLIP AND KIMBALL (1967)

Killip classes provided a clinical estimate of the severity of myocardial decompensation after acute myocardial infarction. The 4 classes are defined as follows:-

I. No signs of pulmonary or venous congestion.

II. Mild to moderate heart failure - crepitations over an area of 50% or less of the lung fields, development or intensification of a third heart sound, tachypnoea or dyspnoea, or signs of right heart failure, such as venous or hepatic distension.

III. Severe heart failure - clinical frank pulmonary oedema.

IV. Shock - as defined previously.

SURFACE AREA

The surface area was determined from the patient's height and weight, using a nomogram (Berlyne, 1974).

STATISTICAL METHODS

Full details of the statistical methods used are given in Appendix C, and are appropriately identified in the text. All statistical tests were two-tailed. Mean values are expressed $\pm$ standard error of the mean ($\pm$ SEM) unless otherwise stated.

CHAPTER II

BIOCHEMICAL METHODS

1. ANALYSIS OF PLASMA TOTAL CREATINE KINASE ACTIVITY

Serial measurements of plasma creatine kinase (CK) activity were used to calculate estimated infarct size in patients with acute myocardial infarction, as described below. The accuracy of this method is largely dependent on the accuracy of the estimations of CK activity, and the method used is therefore analysed in detail.

A. METHOD EMPLOYED IN THE ANALYSIS OF PLASMA CK ACTIVITY

Biochemistry of the reaction mixture

Plasma creatine kinase activity was estimated using an IL/Harleco model 368 CLINICARD analyzer. All reactions and photometric measurements were performed in individual disposable cuvettes.

CK catalyses the following reaction:-

ADENOSINE DIPHOSPHATE (ADP) + CREATINE PHOSPHATE $\xrightleftharpoons{CK}$ ADENOSINE TRIPHOSPHATE (ATP) + CREATINE.

The formation of ATP and creatine is reversible, but the direction of the reaction in which ATP and creatine are produced is preferable because it proceeds more rapidly (Rosalki, 1967). The Clinicard Creatine Phosphokinase Chemistry is based on a spectrophotometric method, first described by Oliver (1955), in which the above reaction is coupled with two additional enzyme reactions which link the CK reaction to reduction of oxidized nicotinamide adenine dinucleotide (NAD^+) (Tanzer et al, 1959) as in the following equations:

ATP + GLUCOSE $\xrightleftharpoons{\text{HEXOKINASE}}$ GLUCOSE-6-PHOSPHATE (G-6-P) + ADP.

G-6-P + NAD^+ $\xrightleftharpoons{\text{G-6-P Dehydrogenase}}$ 6-Phosphogluconic A + NADH.

NAD^+ is colourless but NADH absorbs Ultra Violet light, and the reaction was measured by following the increase in absorbance at 340 nm of NADH over a 48 second period and solving the equation:

$$Xu = \frac{dA}{dt} K$$

Where Xu = Activity of CK in International Units per litre (i.u./l)

dA = Change in absorbance

dt = Change in time

K = Scaling factor which produced a proper numeric readout.

The accuracy of the electronic circuits in computing this ratio was 2% or better, as stated by the manufacturer.

The pre-packed disposable cuvettes contain the complete substrate in lyophilized form. The substrate consists of:

Creatine Phosphate

ADP

Glucose

Hexokinase

NAD^+

G-6-P Dehydrogenase

Magnesium and Manganese ions

A myokinase inhibitor

A mercaptan

The myokinase inhibitor and mercaptan were included for reasons detailed below.

Procedure

3 ml of distilled water heated to 42 $\pm$ 1°C was used to reconstitute the substrate. The accuracy and the repeatability of the dispenser used was to <1%. The cuvette was then sealed and incubated for 8 minutes at 37°C. The serum sample was aspirated by a precision dispenser located on the 368 Clinicard Analyzer and 100 µl was dispensed into the cuvette, which was then incubated for a further 8 minutes at 37°C. The 100 µl dispenser was

accurate to 0.5% (95% confidence limits) and the temperature limits were ± 0.25°C for 37°C. After the second 8 minute incubation period, the change in absorbance at 340 nm was measured for 48 seconds. The light path was 1.00 cm at a point on the centre lines of the cells which was 8 mm above the base. The relative path length variation was equivalent to less than 0.2 mA from cell to cell.

Temperature and conversion factor

Using the Clinicard Analyzer the chemical reactions were all carried out at 37°C. Values were then corrected to 30°C, the standard temperature recommended by the International Union of Biochemistry (1965), and the temperature at which the constants for evaluation of infarct size have been calculated (Shell et al, 1971; Sobel et al, 1972). The factor used to convert the results from 37°C to 30°C was 0.58 (Rosalki, 1967; Jarmakani et al, 1976).

Normal range

The level of CK activity was determined in 49 normal volunteers, none of whom were known to have myocardial or skeletal muscle disease of any nature, and who had not indulged in violent physical exercise or had intramuscular injections in the preceding week. The individual levels of CK activity are given in Table 1. The range of CK activity was 2-133 i.u./l at 37°C.

The mean CK activity ± Standard Deviation (SD) was 39 ± 30 i.u./l at 37°C. The upper limit of normal for CK activity was taken to be 99.0 i.u./l (Mean ± 2 SD) at 37°C or 58 i.u./l at 30°C.

Reproducibility of results

A within day reproducibility of 8 measurements in a sample from a normal volunteer, from a patient with AMI and from a standard are shown below (i.u./l at 37°C).

Observations	Normal human serum	Human serum after myocardial infarction	Standard Mean 149 i.u./l* (119-179)
1	59	744	155
2	55	743	157
3	49	770	155
4	53	737	157
5	51	761	151
6	55	739	165
7	52	755	160
8	52	750	153
Range	49-59	743-770	151-165
Mean	53.3	749.9	156.6
SD	3.1	11.5	4.3
RSD	5.8%	1.5%	2.7%

*These results were obtained by the manufacturers using a different method.

B. FACTORS INFLUENCING THE ACCURACY OF THE ESTIMATION OF CK ACTIVITY

Enzyme stability

Storage of plasma prior to assay may result in loss of CK activity. A 30% fall in activity was reported by Kar and Pearson (1965a) in serum stored at -20°C for 4 weeks, whilst Hughes (1962) and Duma and Siegel (1965) reported no loss of activity in serum stored for 2 weeks at -20°C. The inclusion of thiol compounds in the reaction mixture ensure maximal enzyme reactivation (Miyada et al, 1975) and, provided these are present, there is no loss of CK activity in serum stored at 4°C for 7-10 days (Rosalki, 1967; Hess et al, 1968; Schwartz, 1973) or at -18°C for 1 month (Rosalki, 1967). The thiol activator used in this reaction mixture, Mercaptoethane, has been

shown to activate CK maximally (Hess et al, 1968; Miyada et al, 1975).

The samples used in the following studies were all frozen at -10°C and all assays were carried out within 48 hours. Plasma was used for CK determinations with heparin as the anticoagulant, though CK activity is similar in corresponding serum and plasma samples (Roberts & Sobel, 1978). The choice of anticoagulant was important. Heparin in conventional amounts does not affect the CK determination (Rosalki, 1967; Roberts & Sobel, 1978), though citrate and large quantities of heparin may inhibit CK activity (Sobel, Personal Communication), amd EDTA may sequester magnesium required for enzyme activity (Roberts & Sobel, 1978).

Myokinase

Myokinase is released from the myocardium after acute myocardial infarction (Sobel, 1976, Personal Communication). In the above reaction mixture it acts on ADP to produce ATP and adenosine monophosphate (AMP) according to the equation:-

$$ADP + ADP \underset{}{\overset{MYOKINASE}{\rightleftharpoons}} ATP + AMP$$

Thus myokinase could activate the auxilliary enzyme systems resulting in spurious changes in absorbance, which are not the result of CK activity. Oliver (1955) reported that myokinase activity could be inhibited by the addition of AMP to the reaction mixture in a proportion of 10:1 with ADP. Hess et al (1968) found that AMP, whilst inhibiting myokinase activity also inhibited CK activity by up to 35%. These investigators only allowed 3 minutes to elapse between substrate addition and initial absorbance readings, whereas Rosalki (1967) showed that the presence of AMP, in the above ratio, causes a delay for linear optical density readings to occur of up to 6 minutes. After this time no difference in optical density changes was observed in specimens with and without AMP.

AMP is included as a myokinase inhibitor in the Clinicard reaction

mixture, and since the absorbance is read after an 8 minute incubation period, no inhibitory effect of AMP on CK activity should be observed.

Haemolysis

Haemolysed plasma contains increased concentrations of ATP and G-6-P released from the red blood cells when there is more than minimal haemolysis. These may influence auxilliary enzyme systems by increasing the absorbance and thus the apparent CK activity (Rosalki, 1967). Samples in which naked-eye haemolysis had occurred were therefore not used in these studies.

Other substances causing interference

Zinc, copper, silver and mercuric ions are inhibitory, as are L-Thyroxine and citrate to lesser extents. Lipaemic plasma may introduce inaccuracies since the turbidity may interfere with the optical density readings (Rosalki, 1967), and high concentrations of uric acid can interfere with the assay (Sobel, Personal Communication). No patient with high plasma uric acid levels or with lipaemic plasma was included in these studies.

Dilution

The Clinicard CK chemistry has been found by the manufacturers to be linear up to activities of 1000 i.u./l and will read out values up to 1100 i.u./l. In several instances CK activity in patients with acute myocardial infarction reached levels greater than 1000 i.u./l, and where the activity in any sample from a given patient was found to be 1000 i.u./l or greater, all the samples from that patient were diluted.

Simple dilution of plasma prior to assay for CK activity is not possible however, because dilution results in a spurious increase in CK activity (Graig et al, 1967; Hess et al, 1968; Bray & Ferrendelli, 1968; Thomson, 1969; Dobosz, 1974). Graig et al (1967) found that the increase in CK activity after dilution was evident even in normal sera, but this finding was not confirmed by Bray and Ferrendelli (1968). The mechanism of the dilution

effect remains unknown (Graig et al, 1967), though the presence of inhibitors in plasma or serum has been proposed to explain the dilution effect (Menaché et al, 1966; Wiesmann et al, 1966; Graig et al, 1968; Thomson, 1969; Spikesman & Brock, 1969). On the basis of the dilution effect, Thomson (1969) found that inhibitors were only present when abnormal levels of CK activity were the result of Duchenne muscular dystrophy, but Spikesman and Brock (1969) found the same dilution effect on CK activity after myocardial infarction, and concluded that the inhibitors were not specific to Duchenne or any other neuromuscular disease. Graig et al (1967) were unable to elucidate the mechanism of the dilution effect and suggested that the progressive nature of the increase in CK activity with dilution militated against the presence of an inhibition in the serum as an explanation for this effect.

Attempts have been made to find a diluent which does not cause an increase in CK activity. Graig et al (1967) tested the dilution effect of water, Tris buffer, deproteinised serum, serum free of low molecular weight components and heat-inactivated serum. Only heat-inactivated serum showed no dilution effect, confirming the results of Hess et al (1964). Thomson (1969) found no dilution effect using heat-inactivated serum and saline to dilute serum from patients with acute myocardial infarction, but a marked effect using saline to dilute the serum of patients with muscular dystrophy.

In summary, the problem in measuring CK activity greater than 1000 i.u./l lies in the fact that dilution of the sample results in increased CK activity. The mechanism for this dilution effect remains unknown, though the presence of inhibitors to CK activity which are differentially diluted has been put forward as an explanation. Several diluents have been studied to try and overcome the dilutional effect, but so far none have proved effective.

C. STUDIES TO DETERMINE A SUITABLE METHOD OF DILUTING PLASMA WITH HIGH CK ACTIVITY

In order to assess the effects of dilution on CK activity in plasma assayed with Clinicard kits, samples from patients with acute myocardial infarction were diluted with distilled water, Tris buffer and heat-inactivated serum and the results compared with those from undiluted plasma samples within the linear range of the 368 Clinicard analyzer (0-1000 i.u./l).

Effect of dilution on plasma samples using Tris buffer

0.5 ml of plasma from 8 samples were diluted with 2 mls of 0.01M Tris buffer pH 7.4 with 0.2% Bovine albumin (Sobel et al, 1972).

The results were as follows:-

PLASMA CK ACTIVITY i.u./l AT 37°C

	Undiluted	Diluted in Tris buffer
1	653	865
2	703	825
3	714	860
4	802	940
5	937	1195
6	946	1420
7	995	1585
8	1091	1595

The results are shown in Fig.1. There was a significant difference between the regression line and the line of identity ($P<0.05$). Statistics relevant to this and the following figures are given on page 25.

The effect of dilution on plasma samples using distilled water (Fig.2)

0.5 mls of plasma from 10 samples were diluted with 1.0 ml distilled water. The results were as follows:-

PLASMA CK ACTIVITY i.u./l AT 37°C

	Undiluted	Diluted x 3 with distilled water
1	268	396
2	366	448
3	470	554
4	531	894
5	554	926
6	648	956
7	669	1084
8	700	1108
9	766	1084
10	886	1176

The effect of dilution on plasma samples using heat-inactivated serum (Fig.3)

0.5 mls of plasma from 5 samples were diluted with 2 mls of heat-inactivated serum. The serum was inactivated by heating at 56°C for 30 minutes (Graig et al, 1967). The results were as follows:-

PLASMA CK ACTIVITY i.u./l AT 37°C

	Undiluted	Diluted x 5 in heat-inactivated serum
1	711	799
2	718	854
3	766	1018
4	822	1010
5	857	952

The correlation on the few results obtained ($r = 0.71$) was so poor that this approach was abandoned.

In view of these results, no further attempts were made at diluting plasma samples prior to assay, and an alternative approach was sought.

Assay dilution

Introduction

Instead of diluting the plasma prior to assay, the concentration of plasma in the assay mixture was reduced.

A possible source of error which may arise with any assay dilution, even with the sample volumes recommended by the manufacturers, is that the concentration of plasma in the assay mixture relative to the concentrations of enzymes and substrate may affect comparative results (Menaché, 1967). The reason for this is that the protein concentration in the plasma is very high compared with enzyme concentration. Menaché (1967) found that the CK activity in a given sample diminished as the plasma concentration in the reaction mixture was increased, and that with high levels of enzyme activity, the inhibitory effect of high plasma concentrations increased. Hess et al (1968) found the same effect when serum comprised 10% or more of the reaction volume, but not when less than 10% of the reaction volume. They reasoned that at serum concentrations of 10% or above, the activity of any inhibitors present became significant. With the Clinicard system the plasma concentration in the reaction volume was never greater than 3.3%, so that no inhibitory effect was likely to be apparent.

Method

Using Socorex micropipettes, 20 µl and 10 µl samples of plasma were added to the assay mixture and the results compared with each other and with the results when the recommended 100 µl of plasma were added. For CK activities greater than 1000 i.u./l only the results from 20 µl and 10 µl samples could be compared. The normal volume of the reaction mixture is 3.0 mls and of the sample 100 µl, a total of 3.1 mls. By reducing the

volume of the sample, the total volume was therefore reduced. To compensate for this the dilution factor was modified according to the equation for calculating the results. The simplified equation for calculating the results is:-

$$Xu = \frac{dA}{dt} \times K.$$ Where Xu = CK activity in i.u./l

dA = Change in absorbance

dt = Change in time

K = Scaling factor

1 Unit of CK activity is defined as that amount of activity which will lead to the formation of 1 µM of NADH per minute (Hess et al, 1968). One litre is used as the standard unit volume for plasma assay.

More specifically the equation is as follows:-

$$\mu M/Min/L.\ NADH = A/Min \times \frac{1}{6.99} \times \text{dilution factor} \times 1000$$

Where $\frac{1}{6.22} \times$ dilution factor $\times 1000 = K$

6.22 = Extinction coefficient for NADH

$$\text{Dilution factor} = \frac{\text{Total Reaction Volume}}{\text{Plasma Volume}}$$

Dilution factors

(a) Using 100 µl of plasma

Substrate volume = 3.0 ml

Plasma volume = 0.1 ml

Total reaction volume = 3.1 ml

Dilution factor $= \frac{3.1}{0.1} = 31.$

(b) Using 20 µl of plasma

Substrate volume = 3.0 ml

Plasma volume = 0.02 ml

Total reaction volume = 3.02 ml

$$\text{Dilution factor} = \frac{3.02}{0.02} = 151$$

$$\text{Multiplication factor} = \frac{151}{31} = 4.87 \times \text{normal dilution factor.}$$

When 20 µl of plasma were added to the assay, the result was therefore multiplied by a factor of 4.87.

(c) Using 10 µl of plasma

Volume = 3.0 ml

Plasma volume = 0.01 ml

Total reaction volume = 3.0 ml

$$\text{Dilution factor} = \frac{3.01}{0.01} = 301$$

$$\text{Multiplication factor} = \frac{301}{31} = 9.7 \times \text{normal dilution factor.}$$

When 10 µl of plasma were added to the assay, the result was therefore multiplied by a factor of 9.7.

Results

(a) Comparison of CK activity in undiluted plasma and in plasma diluted x 4.87 (Fig.4)

26 samples were compared "undiluted" (100 µl of plasma added to the assay) and diluted x 4.87 (20 µl of plasma added to the assay). The results were as follows:-

PLASMA CK ACTIVITY i.u./l AT 37°C

	Undiluted	Diluted x 4.87
1	57	50
2	64	56
3	77	76
4	78	70
5	84	78
6	103	93
7	126	115
8	127	129
9	153	155
10	155	126
11	174	169
12	223	217
13	232	239
14	254	259
15	261	262
16	262	262
17	183	285
18	293	304
19	300	290
20	318	316
21	320	321
22	340	352
23	383	375
24	414	412
25	579	536
26	692	716

(b) Comparison of CK activity in undiluted plasma, in plasma diluted x 4.87, and in plasma diluted x 9.7 (Fig.5)

8 undiluted samples were compared with x 4.87 dilution and x 9.7 dilution (10 µl of plasma added to the assay). The results are as follows:-

PLASMA CK ACTIVITY i.u./l AT 37°C

	Undiluted	Diluted x 4.87	Diluted x 9.7
1	64	56	64
2	84	78	81
3	155	126	154
4	320	321	294
5	383	375	369
6	414	412	384
7	579	536	534
8	692	716	708

(c) Comparison of CK activity in undiluted plasma and in plasma diluted x 9.7 (Fig.6)

At levels of CK activity greater than 1000 i.u./l it was not possible to compare undiluted with diluted samples. A comparison was therefore made of 23 samples diluted x 4.87 and x 9.7 to include samples with CK activity greater than 1000 i.u./l.

PLASMA CK ACTIVITY i.u./l AT 37°C

	Diluted x 4.87	Diluted x 9.7
1	56	64
2	78	81
3	126	154
4	321	294
5	375	369
6	412	384
7	536	534
8	655	638
9	686	728
10	716	708
11	886	834
12	934	926
13	1039	1029
14	1071	1048
15	1188	1217
16	1225	1285
17	1578	1649
18	1948	1998
19	2221	2212
20	2325	2415
21	2347	2492
22	2474	2503
23	2630	2677

When the very high levels of CK activity were included, the two dilutions differed significantly, but no difference was observed for values of 1500 i.u./l and less.

Statistics of the regression line and the line of identity (Figs.1-5)

1. Dilution in Tris buffer (Figure 1)

a = -436.3 Sy.x = 85.4

b = 1.84 S_b = 0.2

r = 0.96 S_{Db} = 0.283

t = 2.97 P<0.05

2. Dilution in distilled water (Figure 2)

a = 6.51 Sy.x = 104

b = 1.46 S_b = 0.19

r = 0.94 S_{Db} = 0.27

t = 1.7 Not significant (NS)

3. Assay dilution - undiluted and diluted x 4.87 (Figure 4)

a = -6.08 Sy.x = 12.76

b = 1.01 S_b = 0.02

r = 0.995 S_{Db} = 0.028

t = 0.35 (NS)

4. Assay dilution - undiluted and diluted x 9.7 (Figure 5)

a = -6.56 Sy.x = 20.85

b = 0.98 S_b = 0.03

r = 0.995 S_{Db} = 0.042

t = 0.47 (NS)

5. Assay dilution - diluted x 4.87 and diluted x 9.7 (Figure 6)

a = -18.1 Sy.x = 37.34

b = 1.03 S_b = 0.01

r = 1 S_{Db} = 0.014

t = 2.1 P<0.05

Discussion of dilution methods

Plasma dilution in Tris buffer, heat-inactivated serum or distilled water gave results which were clearly unacceptable, while assay dilutions using 20 µl of plasma (dilution x 4.87) and 10 µl of plasma (dilution x 9.7) gave results which compared well with undiluted plasma. Within the limits of comparison diluted and undiluted plasma (0 - 1000 i.u./l) the regression line did not differ significantly from the line of reference whether a dilution of 4.87 or 9.7 is used. A dilution of 9.7 compared with a dilution of 4.87 resulted in a regression line different from the line of reference ($P<0.05$); it is possible that pipetting errors could account for some of this difference (Hess et al, 1967), and since the percentage error is greater with smaller volumes and plasma CK activity greater than 2500 i.u./l was never encountered, a dilution of 9.7 was not needed and not used.

Within the comparable range 0 - 1000 i.u./l, a dilution of 4.87 varied by a maximum of 5% at the very lowest levels of CK activity to a minimum of 0.07% at 500 and 600 i.u./l when compared with undiluted CK activity. Projecting the regression line to the level 2000 i.u./l, though not acceptable for statistical purposes, predicts an error of 0.8% when comparing undiluted with diluted plasma (x 4.87); since these errors are well within the standard error of the assay method, assay dilution using 20 µl of plasma added to the assay mixture was adopted as the method to be used when plasma CK activity exceeded 1000 i.u./l.

2. ANALYSIS OF PLASMA MB-CK ISOENZYME ACTIVITY

Method employed

MB-CK analysis was carried out using MERCK-1-TEST CK-MB kits. These have been found to be specific and their results reproducible (Würzberg

et al, 1977). The reaction temperature was 25°C.

The collection of specimens for analysis of MB-CK activity was carried out as for total CK, and the precautions regarding storage, haemolysis and the presence of interfering substances was the same. Dilution is required for samples in which total CK activity exceeds 800 i.u./l at 25°C, otherwise the antibody is exhausted whilst M subunits are still active.

Plasma was added to the reaction mixture and incubated at 25°C for 7 minutes in a water bath, as recommended by the manufacturers. The rate of the reaction was then measured using a Gilford 300 N spectrophotometer (light wavelength 340 nm) with a Gilford 4008 Datalister over a period of 5 minutes. Values for CK-MB equal to or exceeding 4% of the total CK activity were considered to be elevated (Varat & Mercer, 1975).

Reproducibility of the method

In order to test the accuracy of the method and the within-day reproducibility, a control serum (Boehringer, mean level of CK-MB activity 51 i.u./l, range 40.8 - 61.2 i.u./l) was assayed 5 times by the MERCK-1-TEST CK-MB test.

No.	ΔA/Min	Conversion factor	MB-CK
1	0.0450	1333 U/l	60
2	0.0450	1333 U/l	60
3	0.0425	1333 U/l	57
4	0.0425	1333 U/l	57
5	0.0450	1333 U/l	60
Range			57-60 i.u./l
Mean			59 i.u./l
SD			1.6
RSD			2.7%

Boehringer assay the control serum using a chromatographic method so that the results are not directly comparable.

3. BIOCHEMICAL ANALYSIS OF PLASMA GLUCOSE

Methods used

Plasma glucose was estimated using an IL/Harleco model 368 Clinicard analyzer and the 32100 Clinicard glucose chemistry. The chemistry uses the reaction of O-toluidine, a primary aromatic amine, in hot glacial acetic acid, with the terminal aldehyde group of glucose. One hundred micro litres of plasma are added to the chemistry and the reaction mixture is incubated at 90°C for 16 minutes, at the end of which time a blue-green condensation product of O-toluidine and glucose is produced. The absorbance of the reaction mixture is then measured at 630 nm against a 200 mg/100 ml standard run concurrently. The normal fasting range for the O-toluidine method is reported as being 70-122 mg/100 ml (Cooper & McDaniel, 1970). Plasma glucose levels were only used for comparative purposes and a normal range for the local population was therefore not established.

Requirements for accurate determinations

The manufacturers recommend that the plasma be separated from the cells immediately after collection, and that only non-turbid, non-haemolysed plasma should be used. The method is not specific for glucose and therefore xylose, mannose, fructose and galactose will react with O-toluidine and give spuriously high results, whilst haemoglobin, hypochlorites, high levels of bilirubin and the filler in oxazepam preparations also interfere with the reaction (Young et al, 1972). The manufacturers state that bilirubin levels below 25 mg/100 ml do not affect the reaction, and no patient in the study had abnormally high bilirubin levels. Samples were well spun so that no cells were present in the plasma, and haemolysed samples were not used.

Performance specifications (as determined by the manufacturers)

The coefficient of variation was 0.7 to 2.6% for within-day reproducibility, and 2.6% to 4.8% for day-to-day reproducibility, using samples with normal and samples with elevated glucose levels. Comparison of a manual O-toluidine method with the Clinicard chemistry in 63 samples, with levels ranging from normal to grossly elevated, gave a correlation coefficient of 0.993. Recovery of glucose added to human plasma varied from 100% at low levels to 86% when 300 mg/100 ml of glucose was added. The results are linear for plasma glucose values ranging from 0 mg/100 ml to 400 mg/100 ml, and values higher than 400 mg/100 ml were not found in the studies which were carried out.

All determinations of plasma glucose were carried out within 48 hours of sampling, and results expressed as mg per 100 mls of plasma.

4. BIOCHEMICAL ANALYSIS OF PLASMA FREE FATTY ACIDS

Method used

The method of Dole and Meinertz (1960) was used for the extraction and titration of long chain or free fatty acids (FFA) from plasma. Plasma is added to a mixture of water, heptane and isopropyl alcohol and shaken, after which the mixture is allowed to stand. There is rapid separation of the mixture into 2 phases: an upper "heptane" phase and a lower acqueous phase. The long chain free fatty acids are then found distributed predominantly in the upper, non-polar phase, whereas the other acids, which are more polar, remain in the lower phase. Enough acid must be added to the lower, acqueous phase to bring the pH to 2.5, otherwise the distribution of long chain free fatty acids to the upper phase is impaired. Quantitative analysis is carried out by titrating an aliquot of the upper phase against a base.

Solutions used

(a) Doles mixture

This is a mixture of 40 parts Iso-propyl alcohol, 10 parts heptane and 1 part of 1N sulphuric acid.

(b) Nile blue indicator

100 mg of Nile blue dye were dissolved in 100 ml of Carbon Dioxide-free water, filtered, and diluted to 500 mls with distilled water. This was washed with iso-octane until the washings were colourless (Stock 1). Each 10 mls of stock 1 were then further diluted to 100 mls with CO_2-free water as required (Stock 2). For titration purposes 10 mls of stock 2 were diluted to 100 mls with absolute alcohol, and the pH was adjusted to 8.0 using 0.1N sodium hydroxide. If the indicator is acid the blank titration will be excessively high; if it is neutral it will take up carbon dioxide, in which case the titration values for the FFA extracts will be too low, since part of the acidity of the extract will be neutralized by the bicarbonate buffer in the indicator solution.

(c) 0.01N Sodium hydroxide

A stock of 5N sodium hydroxide (200 gms dissolved in 1 litre) was prepared, and the 0.01N sodium hydroxide solution used in the titration was prepared fresh daily by dissolving 1 ml of the stock solution in 500 mls of carbon dioxide-free water. The solution was standardised using 0.1 mls of normal hydrochloride acid with phenolphthaline as an indicator.

(d) Palmitic acid standard

A palmitic acid standard was distributed in the extraction system with every set of analyses. Palmitic acid (molecular weight 256) was made up into a solution containing 6.0 mM/l by dissolving 76.8 grams in 500 mls of heptane. A one in six silution of this stock mixture gave a working standard containing 1.0 mM/l.

Procedure

(a) Plasma was defrosted immediately prior to the separation of free fatty acids and kept on ice.

(b) 0.6 mls of plasma were pipetted into a 25 ml quick-fit test tube.

(c) 3 mls of Doles mixture were added, and the stoppered test tube was then shaken for 5 minutes.

(d) 1.2 mls of distilled water and 1.8 mls of heptane were then added and the mixture shaken for a further 5 minutes.

(e) The mixture was allowed to stand for at least 5 minutes after shaking to allow the phases to separate.

(f) 1 ml of the upper phase was then titrated with 1 ml of the Nile blue indicator against 0.01N sodium hydroxide, whilst being continuously agitated by a stream of nitrogen. Each plasma sample was extracted twice and the mean of the two results taken.

The palmitic acid standard was prepared by adding 0.6 mls of the solution in heptane to Doles mixture, but later only 1.2 mls of heptane were added so that the total volume of heptane would not exceed 1.8 mls; a further 0.6 mls of water were then added to maintain the total volume at 3.6 mls. A blank, which was used with each set of analyses, was prepared by adding 0.6 mls of distilled water instead of 0.6 mls of plasma to the extraction system.

0.01N sodium hydroxide was titrated against the extraction mixture using a syringe attached to a micrometer screw gauge. Each unit on the syringe micrometer corresponded to 0.0002 ml of sodium hydroxide; the number of units on the screw gauge rather than the volume of sodium hydroxide required to neutralise the FFA extract was recorded on each occasion. Acceptable values for the blank and the palmitic acid standard were 5 (range 5 to 15) micrometer units and 124 (range 118 to 130) micrometer units

respectively.

Before each set of analyses 0.01N sodium hydroxide was titrated directly against the working standard (0.2 mls of palmitic acid standard + 0.6 mls heptane + 0.8 mls Nile blue dye), so that all extracted values could be corrected against this 100% standard. Analysis was carried out within 48 hours of plasma samples being withdrawn.

Calculation of FFA concentrations in millimoles per litre (mM/l)

The total volume of heptane in the extraction mixture = 0.6 + 1.8 mls = 2.4 mls

2.4 mls of heptane extracts FFA from 0.6 mls of plasma.

$\therefore$ 1 ml of heptane contains $0.6 \div 2.4 = 0.25$ mls of FFA solution.

Volume of FFA solution = 0.25 mls

Strength of FFA solution (unknown) = α

Volume of sodium hydroxide solution = β units dispensed by micrometer syringe x 0.0002 mls

Strength of sodium hydroxide solution = 0.01N.

Thus: $0.25\alpha = 0.01 \times 0.0002\ \beta$

$$\therefore \quad \alpha = \frac{0.000002}{0.25}\ \beta = 0.000008\ \beta \text{mM/ml}.$$

$$\therefore \quad \alpha = 0.000008 \times 1000 = 0.008\ \beta\ \text{mM/l}.$$

The value for the blank is subtracted from both the value obtained for the unknown FFA concentration and the value obtained for the standard. Since the standard is 1.0 mM/l, the unknown value is divided by the standard value to give the FFA concentration in mM/l:

$$\text{Plasma FFA} = \frac{\text{Unknown value - blank value}}{\text{Standard value - blank value}}\ \text{mM/l}$$

Interfering substances

Exceptional quantities of lactic, acetic, aceto-acetic and beta-hydroxybutyric acids interfere with the procedure, giving spurious high values. The presence of lactic acid is most important in the clinical setting, but it was not possible to determine lactic acid levels in the patients studied.

Storage

Separated plasma was stored for up to 48 hours at -10°C. Storage in this way did not alter the results when compared with plasma estimated immediately (Rifkind, 1966; Kurien & Oliver, 1966).

Normal values

The upper limit of normal for plasma FFA as determined in 100 healthy adults, was 0.8 mM/l (Oliver et al, 1968).

5. BIOCHEMICAL ANALYSIS OF INSULIN

Insulin was analysed by the Department of Endocrinology of the University of Cape Town. Serum insulin was measured by radio-immunoassay by a modification of the method of Hales and Randle (1963), using kits supplied by the Radiochemical Centre, Amersham. The upper limit of normal in healthy, fasting subjects, as determined by the Department of Endocrinology, University of Cape Town was 9 micro units/ml.

6. ANALYSIS OF PLASMA POTASSIUM

Plasma potassium was estimated in the Biochemistry Laboratory of Groote Schuur Hospital. The normal range is 3.5 - 5.0 mM/l.

7. THE FFA/ALBUMIN MOLAR RATIO

The albumin concentration was determined by the Hospital Laboratory and the result expressed as grams per 100 ml of serum. This is converted to mM/l as follows:

ygm albumin/100 ml = 10 ygm/litre

$$= \frac{10\ y}{68000} \text{ mol/litre (68000 = molecular weight of albumin)}$$

$$= \frac{10y \times 1000}{68000} \text{ mM/litre}$$

$$= \frac{10y}{68} \text{ mM/litre}$$

The FFA concentration in mM/l was then divided by the albumin concentration in mM/l to give the FFA/albumin molar ratio. Serum drawn at the same time as that of the peak FFA was sent to the laboratory to determine the albumin concentration.

8. BIOCHEMICAL RESULTS

FFA, glucose and insulin results in all patients are listed in Appendix A.

Table 1 CK ACTIVITY (i.u/l) MEASURED AT 37°C IN 49 NORMAL VOLUNTEERS

No.	CK activity	No.	CK activity	No.	CK activity	No.	CK activity
1	25	13	21	25	25	37	46
2	3	14	18	26	29	38	51
3	2	15	13	27	51	39	129
4	48	16	27	28	7	40	39
5	7	17	34	29	79	41	15
6	47	18	30	30	54	42	69
7	38	19	86	31	8	43	65
8	27	20	32	32	45	44	8
9	58	21	55	33	133	45	18
10	24	22	32	34	77	46	45
11	7	23	26	35	11	47	24
12	93	24	16	36	32	48	31
						49	12

Mean 39 i.u./l

S.D. ±30

Range 2 - 133 i.u./l

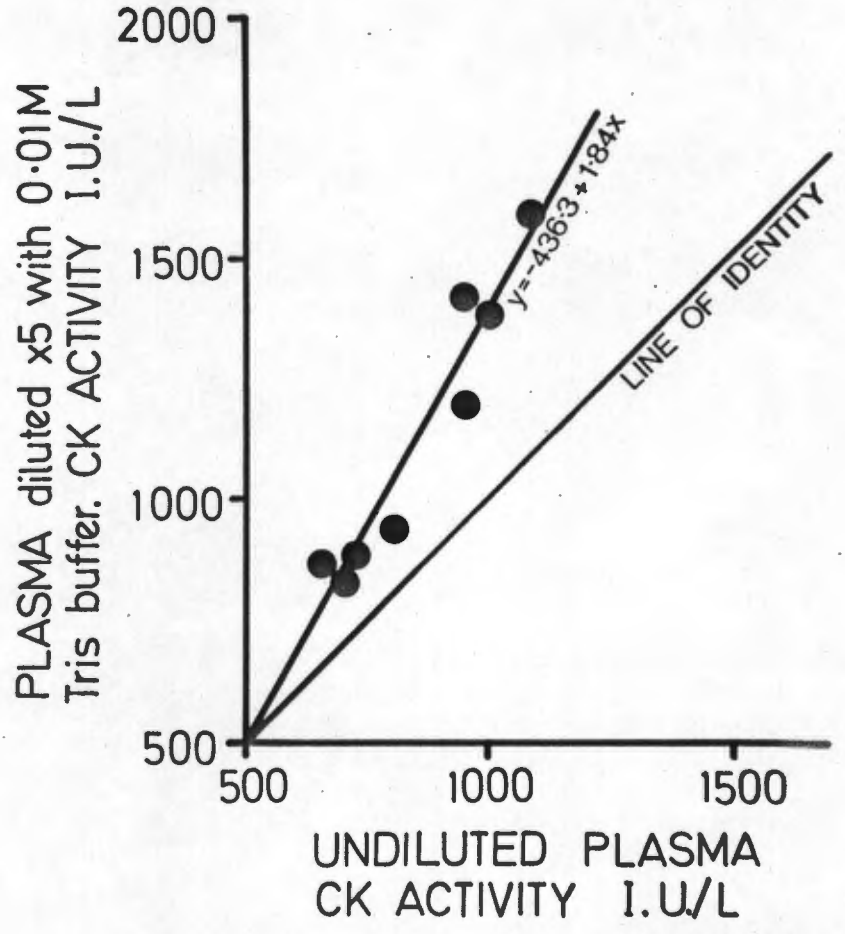


Figure 1

Plasma CK activity in undiluted plasma and in plasma diluted x 5 in 0.01M Tris buffer. The regression line differs significantly from the line of identity.

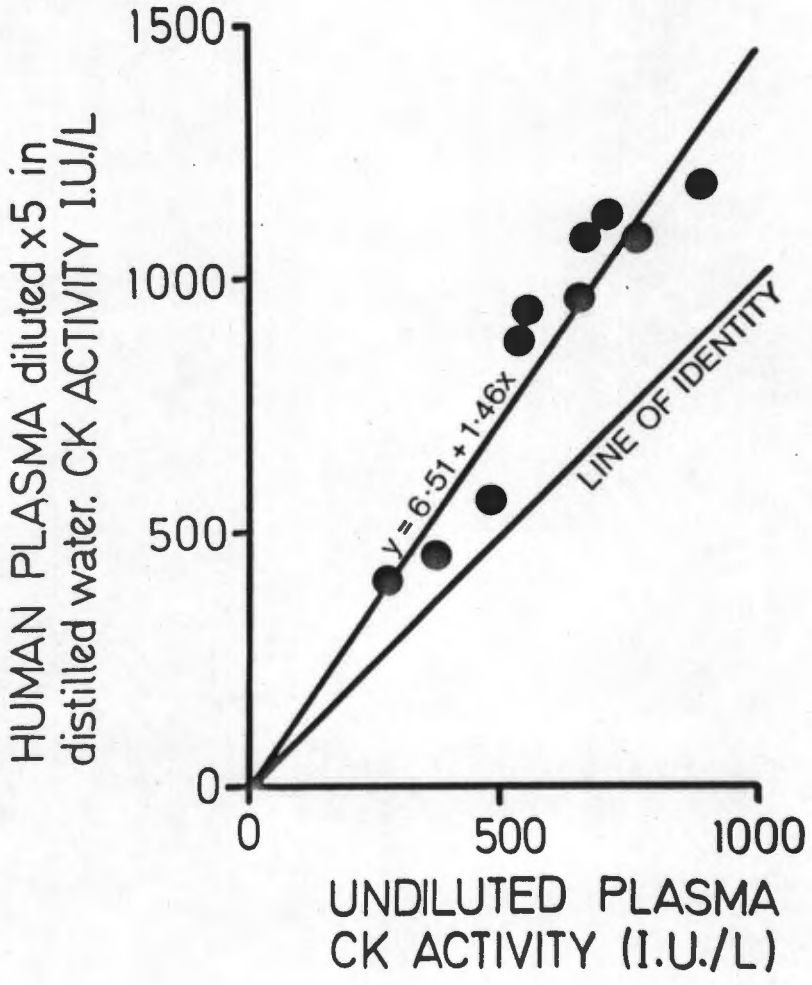


Figure 2

Plasma CK activity in undiluted plasma and in plasma diluted x 5 in distilled water.

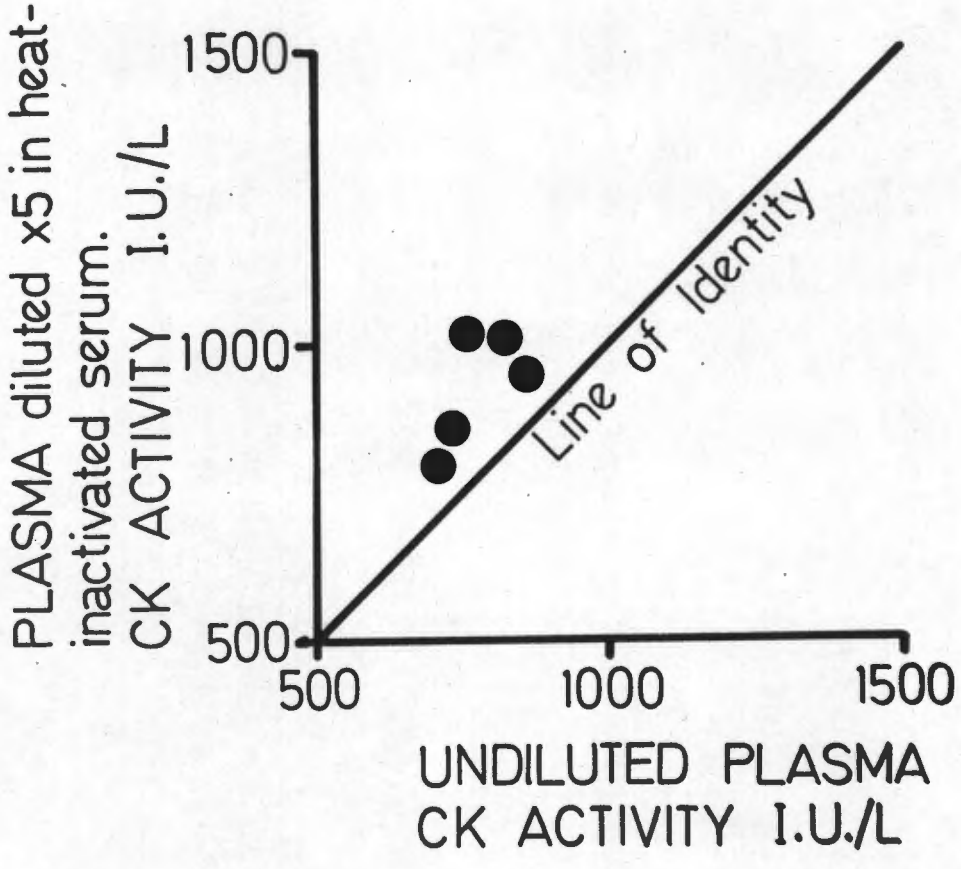


Figure 3

Plasma CK activity in undiluted plasma and in plasma diluted x 5 in heat-inactivated serum.

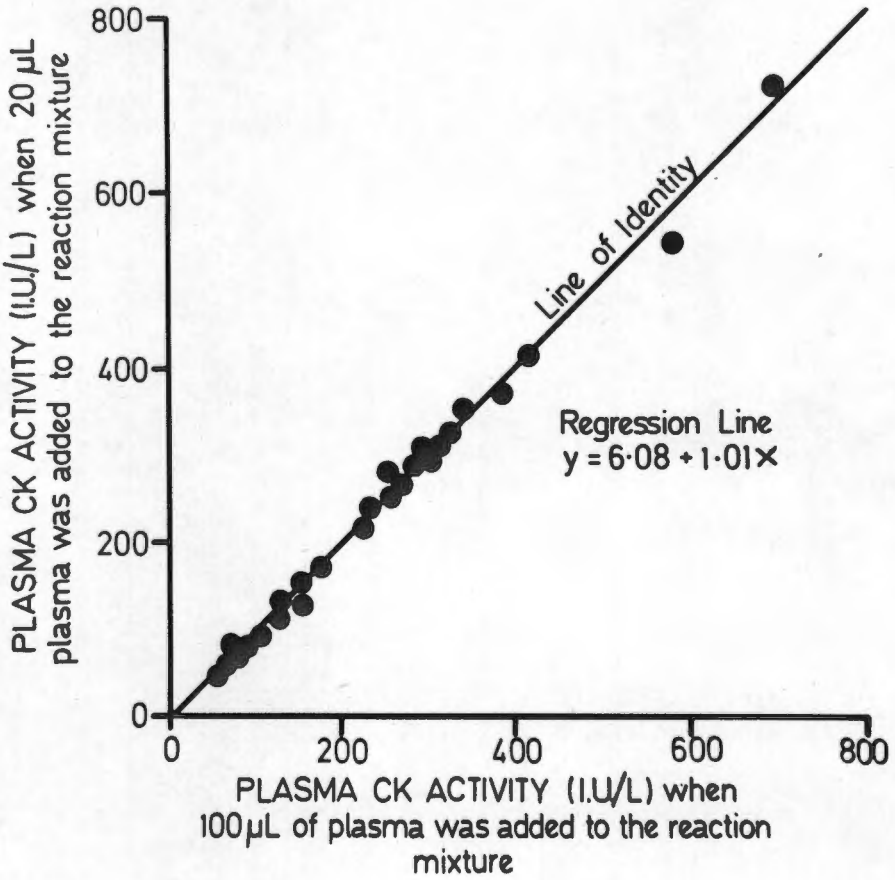


Figure 4

Plasma CK activity when 100 µl of plasma and when 20 µl of plasma (x 4.87 'dilution') were added to the Clinicard reaction mixture.

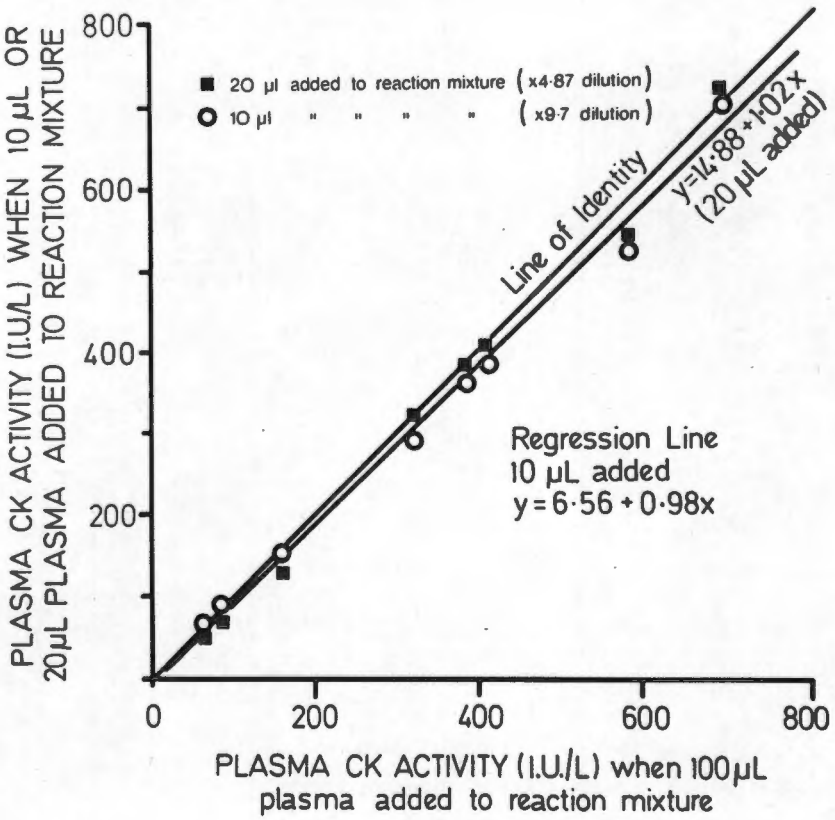


Figure 5

Plasma CK activity when 100 µl of plasma and 20 µl (x 4.87 'dilution') or 10 µl (x 9.7 'dilution') were added to the Clinicard reaction mixture.

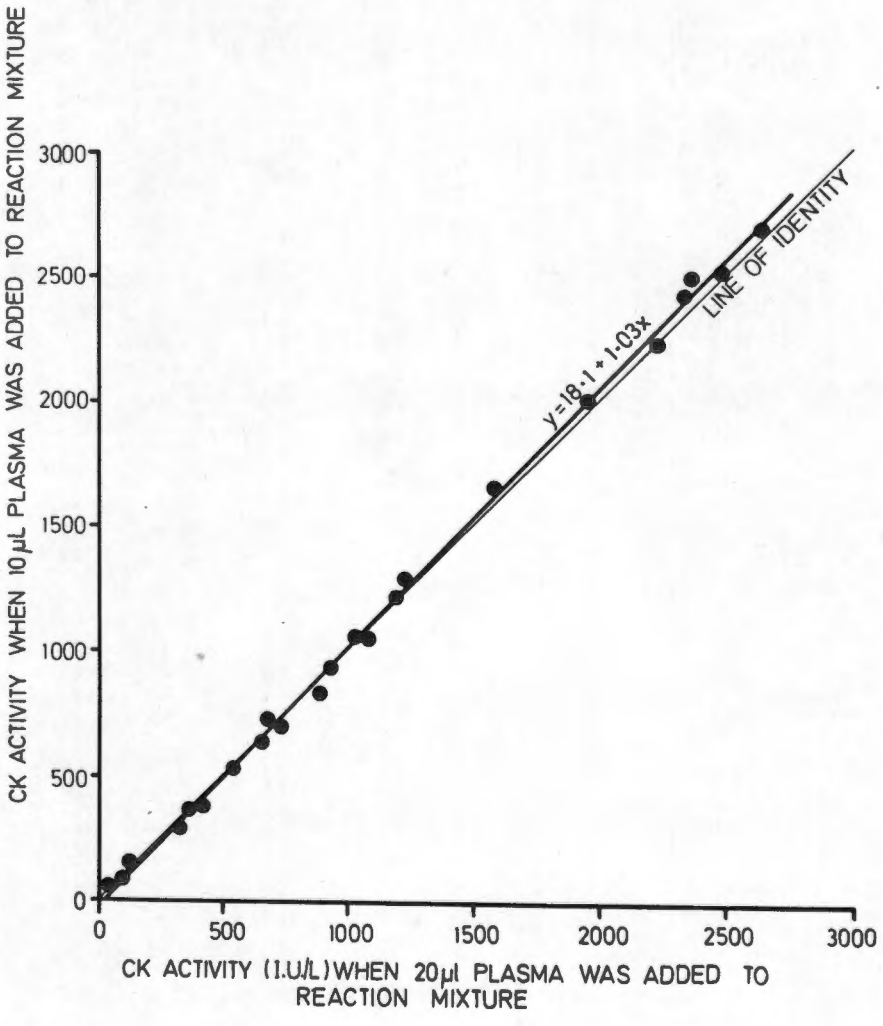


Figure 6

Plasma CK activity when 20 µl of plasma (x 4.87 'dilution') and when 10 µl of plasma (x 9.7 'dilution') were added to the Clinicard reaction mixture.

CHAPTER III

THE ESTIMATION OF MYOCARDIAL INFARCT SIZE BY SERIAL MEASUREMENTS OF BLOOD CREATINE KINASE ACTIVITY

Review of the literature and practical application of the method

1. BACKGROUND TO THE USE OF CREATINE KINASE AS A MARKER OF MYOCARDIAL ISCHAEMIC INJURY

The principle that serum enzymes may be elevated in response to tissue injury (Wohlgemuth, 1908; Warburg & Christian, 1943) was first applied to cardiology by Karmen et al (1954), who demonstrated an increase in serum glutamic oxalo-acetic transaminase (SGOT) and lactic dehydrogenase (LDH) activity in patients with recent myocardial infarction. Dreyfus et al (1960a) first reported raised levels of creatine kinase (CK) activity after myocardial infarction, and their findings were rapidly confirmed (Dreyfus et al, 1960b; Forster & Escher, 1961; Stick & Tsirimbas, 1961; Stick & Tsirimbas, 1962; Hess & MacDonald, 1963).

Investigation of the association between the magnitude of peak SGOT and LDH changes following coronary artery occlusion and the extent of myocardial infarct size suggested a definite relationship (Lemley-Stone et al, 1955; Nydick et al, 1955; Strandjord et al, 1959; Nachlas et al, 1964) but the correlation between the two was not close. Bang and LaDue (1962) showed that the maximal enzyme rise in patients who survived their infarction was half that of patients who died. Neither SGOT nor LDH is specific for the myocardium however (Sobel & Shell, 1972), so that raised enzyme activity after myocardial infarction may reflect release not only from the myocardium, but from other components in the heart involved in the inflammatory response, such as fibroblasts, marginating white cells and other blood elements (Kjekshus & Sobel, 1970), and from non-cardiac sources (Wilkinson, 1970; Agress & Kim, 1960). Furthermore, the magnitude of the enzyme peak in serum is dependent on a number of variable, namely the rate at which the enzyme is released into the circulation, the space in which it is distributed and the rate at which it is cleared from the circulation (Wilkinson, 1970; Posen, 1970). Thus direct comparison of peak enzyme values and anatomical

infarct size cannot be expected to have a high degree of correlation.

Serum CK activity is subject to many of the limitations described for SGOT and LDH, but of these three enzymes, it is the most sensitive and the most specific marker of myocardial ischaemic injury. The assay of CK by the back reaction with thiol activation, as described, is highly sensitive (Sobel & Shell, 1972). Serum CK activity is elevated in virtually all patients with acute myocardial infarction (Goldberg & Winfield, 1972; Roberts et al, 1975a), and is detectable from 4-6 hours after the onset of myocardial damage (Ahamuda et al, 1976). In the heart CK is not present in any tissue other than myocardial cells, and is therefore not released by other components involved in the inflammatory process after myocardial infarction (Kjekshus & Sobel, 1970). In contrast, LDH levels in the blood after AMI are frequently higher than the total amount of LDH present in the normal heart, possibly due to release from the blood elements involved in the inflammatory response (Sobel, 1975). CK levels in plasma are not dependent on renal function or renal blood flow because CK in plasma is not cleared via the kidney (Roberts et al, 1975b), but by the reticulo-endothelial system (Painter et al, 1977).

CK is present in tissue other than the myocardium; highest values are found in skeletal muscle, heart, brain and the gastrointestinal tract, whilst minimal amounts are found in the lung, kidney, liver and spleen and none at all in red blood cells (Roberts et al, 1975b). Significant amounts are also found in the thyroid (Dawson & Fine, 1967). The most important extracardiac source of CK is skeletal muscle. Thus serum CK activity is increased in patients with muscular dystrophy and inflammatory muscle disease (Danowski et al, 1968), with alcohol intoxication (Lafair & Myerson, 1968), with hypothermia (Maclean et al, 1968; Carlson et al, 1978a), with diabetes mellitus (with or without ketoacidosis) and with convulsions

(Savignano et al, 1969). CK activity also rises after intramuscular injections (Meltzer et al, 1970; Klein et al, 1973), after surgery (Klein et al, 1973) and after vigorous exercise (Griffiths, 1966; Vejjajiva & Teasdale, 1965). Serum CK activity may also be elevated in patients with pulmonary thromboembolism, cerebral vascular disease, pancreatitis, shock (Sobel & Shell, 1972), hypothyroidism (Roberts & Sobel, 1978), and after electrical cardioversion (Konttinen et al, 1969; Mandecki et al, 1970; Forssell et al, 1975; Ehsani et al, 1976; Reiffell et al, 1978). CK may be released from the myocardium in the absence of myocardial infarction by conditions such as pericarditis and myocarditis, but not in uncomplicated heart failure,(Hess et al, 1964), and also by investigative and therapeutic measures such as cardiac catheterization (Michie et al, 1970; Roberts et al, 1976), and radiotherapy (Muggia et al, 1970).

Whilst the applicability of total serum CK as a marker of myocardial ischaemic injury is restricted by these factors, it remains more specific than SGOT and LDH as a marker of ischaemic myocardial injury, since the extracardiac sources of CK are less numerous and easier to exclude (Sobel & Shell, 1972).

The isoenzymes of creatine kinase

The inaccuracies introduced by the use of total serum CK activity can be avoided by the use of the cardiospecific isoenzyme of CK, MB-CK. Creatine kinase exists in the form of dimers of the subunits M and B respectively. In 1964 three isoenzymes were distinguished (Burger et al, 1964; Deul & Van Bremen, 1964; Sjovall & Voight, 1964), the dimer MM, found predominantly in skeletal muscle, the dimer BB, found predominantly in the brain and the hybrid dimer MB, found in the myocardium. The distribution of CK and its isoenzymes in human tissue is as follows (Roberts et al, 1975b; Jockers-Wretou et al, 1975):-

Tissue	Total CK (i.u./l)	CK isoenzymes (%) MM	MB	BB
Skeletal muscle	3200	100%	0	0
Heart	680	86%	14%	0
Brain	180	0	0	100%
GI tract	140	0	0	100%
Lung	13	85%	0	15%
Kidney	9	89%	0	11%
Liver	2	0	0	100%
Spleen	2	0	0	100%
Red cell lysates	0	0	0	0
Uterus	10	20%	20%	60%
Aorta	5	80%	20%	0
Pancreas	2	50%	0	50%

Ogunro et al (1977) found no MB isoenzyme in uterus however and Varat and Mercer (1975) have found up to 3% MB-CK activity in skeletal muscle.

CK-MB is confined mainly to the myocardium and is not raised by intramuscular injections or other skeletal muscle trauma, uncomplicated congestive cardiac failure (Roberts et al, 1975a), pericarditis (Fleg et al, 1977) or in neck, thoracic, abdominal, genito-urinary or orthopaedic surgery (Roberts & Sobel, 1976). It is not elevated after cardiac catheterization (Roberts et al, 1976), or in patients with pneumonia, chronic lung disease and pulmonary emboli (Smith et al, 1976) even though total CK may be increased.

Apart from myocardial infarction, conditions in which MB-CK activity may occasionally be enhanced are hypothermia (Carlson et al, 1978a), cardiac massage, electrical injuries, severe drug intoxication, polymyositis,

dermatomyositis and Reye's syndrome (Merck Brochure). MB-CK is also consistently elevated in patients with muscular dystrophy (Kar & Pearson, 1965b), and though some may be released by the dystrophic heart, the main cause may be that the normal differentiation of skeletal muscle with increasing foetal maturity fails to occur, and as a result the normal progression of isoenzyme profiles within skeletal muscle, from BB in the early foetal phase to MM and MB at or before term and MM alone by birth also fails to occur (Eppenberger et al, 1964). The MB-CK isoenzyme activity is raised after acute myocardial infarction (Van der Veen & Willebrands, 1966; Wagner et al, 1973), and is both sensitive (Wagner et al, 1973; Smith et al, 1976), and is relatively specific (Roe et al, 1972; Konttinen & Sommer, 1972; Konttinen & Sommer, 1973; Wagner et al, 1973; Roberts et al, 1975a; Smith et al, 1976) for this condition. An accurate and practical quantitative method for measuring MB-CK only became available in South Africa in late 1977.

Previously published methods for the differentiation of the CK isoenzymes were based broadly on two techniques: firstly, electrophoretic separation with subsequent measurement via NADPG fluorescence, or via reduction of tetrazolium salts to formazanes (Somer & Konttinen, 1972; Smith, 1972; Roe et al, 1972; Klein et al, 1973; Elevitch, 1973), and secondly, ion exchange chromatography (Mercer, 1974; Nealon & Henderson, 1975; Henry et al, 1975; Wong & Smith, 1975). A method in which the MB isoenzyme is specifically activated with dithiothreithol has also been reported. The electrophoretic methods all suffer from lack of sensitivity and are laboriously time consuming (Ogunro et al, 1975) whilst quantitation of isoenzymes by fluoroscanning is inaccurate and irreproducible due to uneven substrate diffusion (Roberts et al, 1974). Ion-exchange chromatography and dithiothreitol activation yield false low values for the

MB-isoenzyme (Kudirka et al, 1975; Wong & Smith, 1975; Ogunro et al, 1977) and there may be insufficient separation of the isoenzymes. The selective activation method has been shown to lack specificity (Vacca, 1977).

Recently a highly specific immunological method allowing rapid, quantitative estimation of MB-CK activity has been reported (Wurtsburg et al, 1977), and has been shown to be highly sensitive (Vacca, 1977). This method only became available towards the completion of these studies and was therefore not used extensively.

2. THE CALCULATION OF INFARCT SIZE FROM SERIAL MEASUREMENTS OF CK ACTIVITY IN THE BLOOD

Using CK as the biochemical index of myocardial infarction, Shell et al (1971) developed a model which provided a quantitative assessment of infarct size in conscious dogs by analysis of serial changes in serum CK activity. The basis for this model was the following evidence:-

(a) CK depletion from the myocardium was directly proportional to histologically estimated infarct size in the rabbit (Kjekshus & Sobel, 1970) and in the dog (Maroko et al, 1971).

(b) The fraction of CK released from the centre of the infarcts in the above two studies was relatively constant.

An assumption was then made that the change in serum CK activity at any given time was the result of the combined effect of an appearance function (i.e. release of CK from the heart into the circulation) and a disappearance function (i.e. the removal of CK from the circulation, Shell et al, 1971).

A. Calculation of infarct size based on direct measurement of myocardial CK (Shell et al, 1971)

By direct measurement of myocardial CK, an equation was derived whereby infarct size could be calculated:

$$\text{Infarct size} = \frac{CK_D}{[CK_N] - [CK_I]}$$

Where:

$[CK_N]$ = myocardial CK concentration (i.u./gm) in normal left ventricular muscle determined by biopsy of non-ischaemic myocardium.

$[CK_I]$ = myocardial CK concentration (i.u./gm) in the centre of infarcts 24 hours after coronary artery occlusion.

CK_D = total amount of CK (i.u.) depleted from a given heart subjected to coronary artery occlusion.

$CK_D = CK_E - CK_M$

Where:

CK_E = total left ventricular CK (i.u.) expected in the homogenate from a normal ventricle of known weight.

$= [CK_N] \times$ left ventricular weight in grams.

CK_M = total left ventricular CK (i.u.) measured in the homogenate from a heart subjected to coronary artery occlusion 24 hours earlier.

In the dog the value for myocardial CK concentration in the centre of infarcts 24 hours after coronary occlusion, $[CK_I]$, was consistently 25% of that in normal ventricle $[CK_N]$ and the value for $[CK_N] - [CK_I]$ was 800 i.u./gm (Sobel et al, 1977a). Since this value was constant only the CK depletion (CK_D) needed to be calculated in order to determine infarct size, without resorting to necropsy studies.

B. Calculation of CK depletion (CK_D) from serial changes in serum CK activity (Shell et al, 1971)

The calculation is based on the following model:

CK release from the heart = serial changes in plasma CK activity + CK disappearance from the circulation.

The following information is required:

i. The actual volume in which CK is distributed after entering the circulation (the distribution volume, DV). This is calculated as a proportion of body weight.

ii. The proportion of CK depleted from the myocardium which appears in the CK distribution (P_{CK}).

iii. The fractional disappearance rate of CK from the circulation (Kd).

iv. Serial, observed values of CK activity in the blood after AMI.

With the above information CK_D can then be calculated as follows:

$$CK_D = CK_R/P_{CK}.$$

CK_R is derived as follows:

t = Time after onset of myocardial infarction (minutes).

$f(t)$ = CK appearance function. This is the rate at which CK appears in the blood (i.u./mg/min).

$\int_0^t f(t)dt$ = The integrated appearance function (i.u./ml). This represents the CK concentration released from the heart which would have been observed in the blood if no clearance of CK occurred. It is dependent on t.

CK_r = Cumulative CK serum entry/ml = $\int_0^t f(t)dt$ at any given time.

CK_R = Total cumulative CK serum entry.

C. Calculation of estimated infarct size (EIS)

$$EIS = \frac{CK_D}{[CK_N] - [CK_I]}$$

$$= CK_R \times \frac{1}{P_{CK}} \times \frac{1}{[CK_N] - [CK_I]}$$

$$= CK_r \times DV \times \frac{1}{P_{CK}} \times \frac{1}{[CK_N] - [CK_I]} \times \text{body weight}$$

$$DV \times \frac{1}{P_{CK}} \times \frac{1}{[CK_N] - [CK_I]} = K \quad \frac{(ml)\ (CK\text{-}g\text{-}eq)}{(Kg)\ (i.u.)}$$

K = The proportionality constant.

$$\therefore EIS = CK_r \times K \times \text{body weight (CK-gram-equivalents)}$$

A CK-gram-equivalent (CK-g-eq) is that quantity of tissue from which CK depletion has occurred equal in magnitude to CK depletion in one gram of myocardium exhibiting homogeneous necrosis (Sobel et al, 1972).

D. Values used with this model

Values used initially

Initially the following values were used with this model to estimate infarct size in man (Sobel et al, 1972):

$[CK_N]$ = 680 i.u./gram

$[CK_I]$ = 180 i.u./gram

These values were obtained from myocardial samples obtained 4-24 hours post-mortem.

DV = 11.4% body weight

P_{CK} = 0.3

These values had been observed in the dog.

Kd = 0.001 $\pm$ 0.0001 (mean $\pm$ SEM).

This value was estimated from the fractional disappearance rates observed in 24 patients with a single, discrete peak of CK elevation after acute myocardial infarction. In later studies all these values were modified.

Modification of the values for DV, P_{CK}, $[CK_N]$ and $[CK_I]$

Roberts et al (1975b) calculated the values for DV and P_{CK} in man, with the following results:

DV = 44 mls/kg (the approximate plasma volume).

P_{CK} = 0.15

Using myocardial samples analysed 90 minutes after death, the following values of $[CK_N]$ and $[CK_I]$ were obtained (Roberts & Sobel, as quoted by Bleifeld et al, 1977):

$[CK_N]$ = 1300 i.u./gm

$[CK_I]$ = 195 i.u./gm

Modification of the fractional decay rate (Kd)

Sobel et al (1972) used a mean Kd of 0.001 $\pm$ 0.0001 (mean $\pm$ SEM, n=24) when calculating infarct size in man. Norris et al (1975) pointed out that the standard deviation for this values, 0.0005, was so great that over-and under-estimations of 50% and 100% were likely to occur. They therefore proposed a method for individualizing Kd for each patient.

Values for serum CK activity were plotted against time on the y axis on semi-log paper. The values where log CK activity declined rapidly and uniformly were used for the calculation. It was inferred that when the decline was linear enzyme release from the myocardium had ceased, and Kd was therefore independent of CK release. Five or more values had to be used, and a line of best fit was calculated together with the 95% confidence limits for the slope.

Since error in calculating the integrated appearance function of CK is identical to the calculating error in the determination of Kd, cases where the 95% confidence limits were greater than $\pm$ 15% were arbitrarily excluded.

E. The proportionality constant, K, for calculating EIS using MB-CK activity-identification of misleading information in the literature

Whilst reviewing the literature concerning the calculation of EIS, it became apparent that the proportionality constant, K, for MB-CK was incorrectly reported in three key publications (Roberts et al, 1975b; Shell & Sobel, 1976; Ahamuda et al, 1976).

K was reported as being 4.1×10^{-1} for MB-CK, which is similar to K for total CK (5.9×10^{-1}). By the method of enzymatic estimation, infarct size is the product of K, body weight and CK release (Roberts et al, 1975b; Shell & Sobel, 1976; Ahamuda et al, 1976; Sobel et al, 1976) and, in the absence of extracardiac CK, total CK release is approximately ten times greater than MB-CK release (Ahamuda et al, 1976). If the value for K were similar in both instances, the calculated infarct size based on MB-CK would be approximately ten times smaller than that based on total CK. The correct K value for MB-CK was calculated using the relevant data from these same papers (Roberts et al, 1975b; Shell & Sobel, 1976; Ahamuda et al, 1976):

$$K = DV \times \frac{1}{P_{MB\text{-}CK}} \times \frac{1}{\left[MB\text{-}CK_N\right] - \left[MB\text{-}CK_I\right]}$$

$DV = 44$ ml/kg

$P_{MB\text{-}CK} = 0.15$

$\left[MB\text{-}CK_N\right] = 96$ i.u./g

$\left[MB\text{-}CK_I\right] = 25$ i.u./g

$$\therefore K = \frac{44}{0.15 \quad (96-25)} = 4.1 \frac{(ml)\ (CK\text{-}g\text{-}eq)}{(Kg)\ \ (i.u.)}$$

This value corresponds to the one quoted by Sobel et al, 1976.

In reply to a letter pointing out this error (Tansey, 1977), the relevant authors (Ahamuda, Roberts & Sobel) acknowledged that their reported values for K might have caused confusion and explained that in their own laboratory a further step in the calculation was used, one not mentioned in the literature. They used constants of similar magnitude and multiplied the result for MB-CK by the ratio of total myocardial CK to MB-CK, thus achieving the same result. They agreed, however, that the notation, as described above, was "clear, correct and desirable since it obviated the need for an implicit additional computational step".

3. LIMITATIONS OF THE METHOD OF CALCULATING ESTIMATED MYOCARDIAL INFARCT SIZE FROM SERIAL PLASMA CK MEASUREMENTS

The practical application of the model described above is dependent on certain premises with respect to the specificity of CK as an index of myocardial necrosis, the fractional disappearance rate of CK, the distribution volume of CK and the proportion of CK appearing in the circulation after AMI. The basis for these premises requires further analysis as they are fundamental to the accuracy of the calculation.

Plasma CK and MB-CK as indices of irreversible myocardial necrosis

The calculation of infarct size on the basis of serial measurements of plasma CK activity was based on the observation that the appearance of CK in the peripheral circulation correlated well with CK depletion from infarcted myocardium in the rabbit (Kjekshus & Sobel, 1970) and in the dog (Maroko et al, 1971). Increased plasma CK activity should therefore

only reflect release from irreversibly injured myocardial cells, because if CK were released from damaged cells which subsequently recovered, there would be a variable overestimation of EIS in each case. Whilst the evidence that CK release only occurs after irreversible cellular damage is strong, there have been conflicting reports in the literature.

Myocardial necrosis can only be detected histologically after 4 to 8 hours (Sommers & Jennings, 1964) though biochemical criteria may provide earlier evidence (Rose et al, 1976), but enzyme release from isolated rat hearts has been found soon after coronary artery ligation (Hearse & Humphrey, 1975), before the biochemical or histological diagnosis of necrosis can be made. Opie (1979) has postulated that metabolic changes and enzyme release precede necrosis, and that any relationship between the metabolic events underlying enzyme release and those underlying necrosis would only be indirect.

The degree of functional recovery of the arrested rat myocardium has been shown to depend in part on the levels of the high energy phosphate adenosine triphosphate (ATP), and creatine phosphate (CP) which can be maintained (Hearse & Stewart, 1974). Spieckermann et al (1973) found enzyme protein release proportional to ATP depletion in myocardial cells which were not irreversibly damaged, and Gebhardt et al (1977) argued that enzyme release may be related to intracellular energy levels and cellular membrane integrity rather than cell death. Sakai et al (1975) concluded that CK release after 20 minutes total ischaemia in the guinea pig heart was not necessarily due to irreversible damage because of the rapid recovery of mechanical activity observed following reperfusion. Despite some conflicting evidence from studies on anoxic isolated rat hearts, Hearse and Humphrey (1975) concluded that the onset of major myocardial enzyme release reflected irreversible cellular damage.

Total CK and MB-CK measured in patients after cardiac surgery were both invariably raised even in the absence of ECG or radionucleotide scan evidence of myocardial necrosis (Klein et al, 1976). During surgery, however, the myocardium was subjected to a number of gross insults, namely hypothermia, frequent defibrillation, cardiac bypass and direct trauma. Hypothermic coronary perfusion was associated with good functional recovery and high intracellular energy levels in the rat heart (Hearse & Stewart, 1974) and was thus an unlikely cause of the elevated enzyme levels after cardiac surgery. The effects of defibrillation, of cardiac bypass and of direct trauma, especially the latter, may well account for the raised enzyme levels observed after cardiac surgery.

There are other reports of patients in whom MB-CK levels were elevated in the probable absence of myocardial necrosis. MB-CK was elevated in patients after prolonged atrial tachyarrhythmias (Varat & Mercer, 1975), and in 2 out of 60 patients after coronary bypass grafting (Delva et al, 1978). Total CK was not raised in any of these reported cases, and the absence of minimal necrosis could not be excluded by the conventional methods used. Using technecium-99m pyrophosphate scans to exclude myocardial necrosis, Righetti et al (1977) demonstrated a significant rise in MB-CK in 6 patients after coronary bypass grafts. In the remaining 31 of their 41 patients with raised MB-CK post-operatively, there was objective evidence of irreversible myocardial damage. Marmer et al (1979) excluded myocardial necrosis using haptoglobin levels in 5 patients who had raised MB-CK levels during documented myocardial ischaemia. Total CK, with normal values up to 12 i.u./l,was not elevated in these patients, and the maximum rise in MB-CK found was 17% of the total CK level. The time at which these small rises of MB-CK peaked after ischaemia were up to 12 hours earlier than the times of peaking after acute myocardial infarction. In

contrast, several studies have reported the absence of MB-CK in experimental myocardial ischaemia and in patients with angina, but no evidence of infarction (Konttinen & Sommer, 1972; Roe et al, 1972; Roberts et al, 1974; Roberts et al , 1975a; Roberts et al, 1976).

The evidence in favour of CK release reflecting myocardial necrosis is strongly supported by the studies of Conrad et al (1979). Cellular potassium loss was used as a marker of irreversible injury as it has been shown that cells which are reversibly damaged can recover potassium whilst irreversibly damaged cells cannot (Jennings et al, 1964). Potassium is also an excellent marker of myocardial cellular integrity because 98% is intracellular (Blesa et al, 1970). Potassium loss after various periods of anoxia in rabbit myocardium correlated well with loss of creatine kinase and also with loss of functional recovery of the myocardium. Despite these good correlations a small amount of enzyme, amounting to approximately 0.1% of total tissue enzyme content, was lost without any evidence of irreversible cellular damage after 20 minutes anoxia. Hearse et al (1973) found an early peak of CK release after 10 to 60 minutes anoxia in the isolated perfused rat heart which amounted to 2 - 5% of total enzyme released after 8 hours anoxia. They proposed that this early rise was due to changes in cellular membrane permeability, whilst the bulk of the enzyme rise reflected massive ultrastructural damage.

Available evidence, therefore, indicates that CK and MB-CK release from the myocardium accurately reflect myocardial necrosis, though a very small but variable proportion of total CK may be released by cells which are not irreversibly damaged. Alternatively available methods may not be sensitive enough to detect all evidence of irreversible cellular damage. Quantitation of infarct size using total CK release may however be liable to slight overestimation.

The fractional disappearance rate of CK (Kd)

In the calculation of Kd from the terminal portion of the plasma CK activity curve (Norris et al, 1975) the assumption was made that there was a monoexponential decline in CK activity after acute myocardial infarction. This in turn was based on the following assumptions:

(i) CK is released from the heart into a non-physiological, 1 compartment model;

(ii) The isoenzymes of CK have identical fractional disappearance rates;

(iii) When the decay of plasma CK is monoexponential, there is no more CK being released into the circulation.

These assumptions were based on evidence that, after an initial mixing stage, there was a monoexponential decay rate for CK in the conscious dog following intravenous injections of CK (Shell et al, 1971; Rapaport, 1975; Roberts & Sobel, 1977). The assumption was also made that the decay rate of CK was exponential in man after acute myocardial infarction (Sobel et al, 1972). In common with other enzymes (Dunn et al, 1958) and proteins (Schultze & Heremans, 1966) however, CK may be distributed in an extra-vascular as well as a vascular compartment, in which case a bi-exponential curve fit to plasma CK activity would be expected to conform more clearly to observed values than a mono-exponential fit (Witteveen et al, 1975; Sobel et al, 1977b). Norris et al (1975) discounted the presence of extravascular distribution on the basis that it was if this were present CK decay would initially be rapid and would then decline slowly, a pattern they saw in only two out of more than 60 patients. Studies by Sobel et al (1977b) however, showed that after an intravenous injection the CK activity curves were fitted more accurately by a bi- than a mono-exponential curve. It was only the very early portion of the CK time-activity curve after peak values which did not behave mono-exponentially however (Sobel et al, 1977a).

Using bi-exponential curves, they calculated a faster rate of elimination of CK activity from the circulation and a higher recovery of CK released from the heart. Resupply of the vascular pool from extravascular sources would be one explanation for the slower rate of elimination when a mono-exponential fit was used.

Carlson et al (1978b) proposed a bi-exponential elimination curve on the basis that if CK clearance through the reticulo-endothelial system (in which CK elimination may occur) was slower than reticulo-endothelial blood flow, then the distribution phase may add to the elimination. Their studies showed CK clearance to be slower than reticulo-endothelial blood flow and the elimination half life of CK to be significantly longer using a bi-exponential than a mono-exponential model. Cairns and Klassen (1977) compared a bi-exponential with a mono-exponential fit in 25 cases where dogs had been injected with partially purified myocardial CK. There was better correlation in 7 of the 25 cases using the bi-exponential curve fit, but in only one case was the improvement significant, so the authors concluded that the mono-exponential expression for CK decay in plasma was only minimally improved using the bi-exponential expression. This is important from a practical viewpoint, because the parameters for a two compartment model can only be derived after intravenous injection of a bolus of CK and not from CK curves after acute myocardial infarction (Sobel et al, 1977b) because of the possibility of continued CK release from the heart and because the CK activity present in both compartments prior to infarction is unknown.

Initially is was thought that when the decay of plasma CK activity was mono-exponential there could no longer be any continuing release of CK from the heart into the circulation (Sobel et al, 1972; Norris et al, 1975) but recent experiments have shown that this is not necessarily true.

Sobel et al (1977b) found that CK activity in plasma decayed more slowly after myocardial infarction in the dog than after an intravenous bolus of purified or crude CK extracted from the myocardium. Similar results were found by Cairns et al (1978). CK extracted from the myocardium had different physical properties from CK released into plasma after AMI; CK harvested from plasma in dogs after AMI and injected into other dogs had a decay rate different from that found after injection of myocardium-extracted CK (Sobel et al, 1977b), but significantly faster than the decay rate found after AMI. Since the contribution from any extravascular compartment will be the same in all cases it seems likely that continued release from the myocardium contributes to the slower decay rate after AMI. Slutsky (1977) has provied that it is possible for CK release from the myocardium to continue throughout the period of CK decay with kinetics which still allow a mono-exponential expression for the decay to be used. Since liberation of CK from as little as 1 gm of myocardium after peak CK could reduce the elimination rate by as much as 50% (Roberts et al, 1977), estimates of the fractional disappearance rate (Kd) from the terminal portions of CK time-activity curves will be much lower than the true fractional disappearance rate.

The use of total CK introduces a further source of error in the estimation of Kd in that the individual CK isoenzymes have different fractional disappearance rates (Rapaport, 1975). There is therefore, a further reason why the total CK activity should not decay mono-exponentially. The CK-MB isoenzyme has a higher rate of decay than the MM isoenzyme, so that decline in total CK activity should approximate to exponential with an early rapid phase and a later slow phase of decline. Norris et al (1975) found this pattern in only 2 out of 50 cases in man and concluded that the different decay rates of the 2 isoenzymes were not an important factor in

the calculation of Kd for total CK activity.

Kd is dependent ultimately on the mechanism responsible for the disappearance of CK activity, and the evidence suggests that this mechanism is not dependent on any specific organ and is therefore not dependent on renal blood flow (Roberts et al, 1975b). There is evidence to suggest that CK disappearance may depend on overall reticulo-endothelial system activity (Roberts et al, 1975b; Sobel et al, 1976a; Ahamuda et al, 1976; Sobel et al, 1976b). Inhibition of the reticulo-endothelial system by zymosan brought CK disappearance virtually to a standstill (Roberts et al, 1975b), but it is possible that an action of zymosan outside the reticulo-endothelial system caused this effect (Sobel et al, 1976b) and this aspect of the problem still requires investigation. Painter et al (1977) have shown that in dogs CK is cleared by removal of protein, rather than by independent denaturation or inhibition of CK enzyme activity.

Since one of the objects of calculating infarct size is to determine the effectiveness of interventions in limiting infarct size, it is important to know whether Kd is constant in any given patient when no treatment is given, when routine non-cardiac drugs are given, and when the patient experiences haemodynamic changes. Kd did not change significantly from day to day in individual cases as determined by repeated injections of CK in dogs and in man, and from extentions of myocardial infarction in man (Roberts et al, 1975b; Ahamuda et al, 1976; Roberts & Sobel, 1977; Cairns & Klassen, 1977). The following table shows the effects of some haemodynamic and pharmaceutical interventions on Kd in the dog (Roberts et al, 1975; Roberts & Sobel, 1977; Cairns & Klassen, 1977):

Type of intervention	Effect on Kd
Control	< 10% variation
Tachycardia (induced by pacing or isoproterenol)	10% reduction
50% increase in cardiac output	None
Bilateral renal artery occlusion	None
Nephrectomy and renal artery occlusion contra-laterally	None
Hepatic artery occlusion	7 - 15% increase
Cardiac artery occlusion	None
Thiamol anaesthesia (Barbiturate)	70-90% reduction
Reticulo-endothelial blockade with Zymozan	80% reduction
Lignocaine infusion	None
Methyl prednisolone	None
Nembutal (30 mg/kg)	52% reduction
Valium (1 mg/kg)	46% reduction
Morphine (2 mg/kg)	48% reduction
Valium (0.1 mg/kg)	8% reduction
Morphine (0.2 mg/kg)	10% reduction
Propranolol (0.3 mg/kg or 2.0 mg/kg)	23% reduction

The administration of valium and morphine in therapeutic doses did not change Kd substantially as compared with control values.

Sobel et al (1977b) have proposed the use of a mean value for Kd derived by analysis with a two-compartment model after intravenous injection of CK in order to provide more accuracy by accounting for more CK than when individualised values for Kd using a one-compartment model are used. The enormous variability of Kd from patient to patient and dog to dog found in the studies by Norris et al (1975) and Cairns et al (1978), especially after pharmacologic intervention (Cairns & Klassen, 1977; Roberts & Sobel, 1977) appear to support the contention of Norris et al (1975)

and Roe and Starmer (1975) that individualised values for Kd minimize the errors which may occur in the calculation of infarct size.

The distribution volume (DV)

The distribution volume for CK is difficult to measure in individual patients (Norris et al, 1975), but was assumed to approximate to plasma volume (44 ml/kg body weight; Roberts et al, 1975b). In view of the possible extravascular distribution of CK (Dunn et al, 1968; Schultze & Heremans, 1966), the actual distribution volume may be greater. If it can be assumed that the extravascular distribution approximates to a fixed proportion of body weight (as in the case of the plasma volume), then relative infarct size between patients will not be affected.

The distribution volume for total CK is only a mean value, since the 3 isoenzymes have significantly different distribution volumes (Rapaport, 1975), and a further error is therefore introduced. The distribution volume of the MB fraction is greater than that of the MM fraction (Rapaport, 1975), but the distribution volume used in the calculation of infarct size for both total CK and MB-CK is nevertheless assumed to be the same (Roberts et al, 1975b).

More important is the probability that the distribution volume may vary during the time a patient is under study. Expansion of the plasma volume will lead to underestimation, and contraction of the plasma volume to overestimation of myocardial infarct size (Cairns & Klassen, 1977). Diuretics are known to cause large changes in plasma volume (Norris et al, 1975) and it is possible that propranolol may expand the plasma volume (Cairns & Klassen, 1977), though the latter possibility requires further investigation. It is therefore important to know whether any pharmacological intervention alters plasma volume during the time when plasma CK levels are being determined, but no studies concerning this problem have so

far been published.

The proportion of CK released from the myocardium which appears in the circulation (PCK)

The proportion of CK entering the circulation compared with the amount of CK released by the myocardium is called the serum entry ratio (SER). Since the initial studies of Shell et al (1971), it has been noted that the serum entry ratio in dogs is low, only a small proportion of the CK depleted from the myocardium reaching the circulation. Shell et al (1971) calculated the SER in dogs as being 0.3 ± 0.2, whilst Roberts et al (1975b) observed the ratio to average 0.15 within a range of $\pm$ 0.016 (SEM). The reason for this low ratio has not yet been fully explained explained; local inactivation in necrotic tissue may play a role (Ahamuda et al, 1976) but the proportion which is degraded locally remains unknown (Norris et al, 1975).

The role of the cardiac lymphatic system in controlling the SER has been increasingly emphasized (Szabo et al, 1974; Sobel et al, 1976b; Clarke et al, 1978). In the dog a significant proportion of CK (and other enzymes) released from the myocardium reaches the circulation via the lymphatic system (Malmberg, 1972a; Malmberg, 1972b; Gervin et al, 1974), though some enzymes released from the heart appear to enter the systemic venous circulation directly (Szabo et al, 1974; Pasyk et al, 1971). The exact proportion of CK entering the circulation via the lymphatics is unknown, but Malmberg (1972a) estimated that between 30% and 50% of transaminase activity after myocardial infarction in the dog reached the circulation via this route. Experimental studies in man and dog have shown that CK activity is lost at a more rapid rate when exposed to lymph than to plasma or whole blood, both in vitro and in vivo (Robinson et al, 1975). Clarke et al (1978) confirmed the role of the lymphatic system

in the transport of myocardial CK in the dog, and showed that CK is deactivated more rapidly in lymph than in blood in vitro (4% and 78% remaining active after 5 hours incubation respectively). Lymph in vivo did not deactivate CK as actively as lymph in vitro. They determined that the deactivation of CK in lymph in vitro was primarily due to thiol oxidation, and was not due to enzymatically mediated proteolysis.

The flow of cardiac lymph is about 300 times less than coronary blood flow (Sobel et al, 1976b), being approximately 1.5 - 4.0 ml/hr in the unanaesthetized dog (Miller et al, 1964), so that CK remains exposed to lymph for a prolonged period of time. Variations in lymphatic flow may therefore alter the SER and affect the calculation of infarct size. Physiological factors which may alter myocardial lymph flow include age-related thickening of endocardial portions of the heart (McMillar & Lev, 1959), variations in systemic venous pressure (Wegria et al, 1963), expansion of extracellular volume in association with congestive cardiac failure, amongst other causes (Uhley et al, 1969), and lymphatic obstruction related to myocardial injury (Kline et al, 1964).

Any situation in which the blood flow to the ischaemic portion of the myocardium is increased following the onset of myocardial infarction will affect the serum entry ratio by accelerating the rate at which CK is washed out of the myocardium and thus the rate of flow through the lymphatics (Walters et al, 1977). If this increased CK activity in the cardiac lymphatics is not diverted to the circulation (Gervin et al, 1974), washed-out CK may still alter the SER by entering the circulation by a route which by-passes the lymphatic system. This phenomenon of re-perfusion may occur spontaneously, because although coronary occlusion is the most likely cause of acute myocardial infarction in man, post-mortem studies have shown that the occlusion does not always persist (Baroldi,

1969; Walston et al, 1970; Roberts & Buja, 1972). Vatner et al (1978) suggest subtotal coronary occlusion, alternating coronary spasm and relaxation and a dynamic process with clot formation and lysis as mechanisms for coronary artery reperfusion in man.

A good correlation has been found between infarct size calculated from myocardial CK depletion and infarct size calculated from serum CK curves in the dog (Shell et al, 1971; Bleifeld et al, 1977; Norris et al, 1975; Vatner et al, 1978). In the latter study, reperfusion was specifically excluded. Gervin et al (1974) found that CK activity in myocardial lymph reached extremely high peaks 2 hours after reperfusion in the dog, without, however, finding any significant increase in serum CK activity. Bresnahan et al (1974) also found that reperfusion did not interfere with infarct size calculation from serial blood CK activity.

Roe and Starmer (1975) suggested that the situation may nevertheless arise where, following a successful pharmacological or haemodynamic intervention, the CK washed out may not be diverted to lymph, and the subsequent increase in circulating CK activity would then be misinterpreted as being the result of a larger infarct than is in fact present. This could be the explanation for the results of studies carried out in the dog by Jarmakani et al (1976), who found that whilst anatomical infarct size was reduced in animals which were reperfused, there was no difference in calculated infarct size between the reperfused and the permanently occluded groups of dogs. They concluded that infarct size could not be calculated using total CK measurements whether there was reperfusion or not. Using only the MB-CK isoenzyme however, there was no disparity between anatomical and calculated infarct size. Since some of the animals in the study developed heart failure, it is possible that extracardiac CK may have been responsible for the results observed when total CK was used.

The only study of the effects of reperfusion on infarct size which compares anatomical infarct size, infarct size determined by myocardial CK depletion and infarct size estimated by serial blood measurements of CK activity was carried out by Vatner et al (1978). They found a more rapid rise in blood CK in dogs which were reperfused; the earlier the reperfusion the more rapid was the rise in CK. Infarct size calculated from myocardial depletion correlated well with anatomical infarct size whether or not the dogs were reperfused, but infarct size calculated from serial blood CK measurements was larger in reperfused than nonreperfused dogs, though the correlation with anatomical infarct size remained linear. Thus more CK was released into the blood per unit myocardium in dogs who underwent reperfusion.

Reperfusion thus alters the serum entry ratio and may occur spontaneously, by the mechanisms described above (Vatner et al, 1978) or as a result of pharmacologic (Shell & Sobel, 1973a) or haemodynamic (Shell & Sobel, 1973b) manipulations. SER has also been shown to vary according to whether an infarct is homogenous or scattered (Cairns et al, 1978). There was a linear inverse ratio between SER and infarct size in homogeneous infarcts, but no such relationship in scattered infarcts, which exhibited a significantly higher SER and a significantly earlier cumulative CK plateau than homogeneous infarcts. All other studies mentioned above were carried out in homogeneous infarcts, though there is evidence that infarcts in humans need not be homogeneous (Levine & Ford, 1950), and that platelet emboli (Jorgensen et al, 1978) may produce scattered myocardial infarctions similar to those produced experimentally by Cairns et al (1978). In such a situation, the mean SER of 0.15 used in the calculation of infarct size would appear to be a large source of error.

In experiments where the serum entry ratio in dogs has been measured

directly, the ratio has been constant (Shell et al, 1971; Bresnahan et al, 1974; Shell & Sobel, 1974; Shell & Sobel, 1973b), though slight variations have been found following such interventions as the administration of propranolol (Shell & Sobel, 1973b), reperfusion of the myocardium (Bresnahan et al, 1974) and acceleration of heart rate by ventricular pacing (Shell & Sobel, 1973b). Small variations in the amount of CK washed out of the myocardium, as observed in the above studies, would not seriously affect the calculation of infarct size using a mean SER of 0.15, but clearly individualised serum entry ratios would result in greater accuracy, and would appear to be essential when non-homogeneous infarcts occur.

Other potential sources of error in the calculation of infarct size

Initially blood samples were drawn every 2 hours in order to determine CK activity for the calculation of infarct size in man (Sobel et al, 1972). Less frequent sampling was thought to result in gross inaccuracies, but Norris et al (1975) showed that the results obtained from 4 hourly sampling varied by only 0% to 3% from those obtained from 2 hourly sampling, and the 4 hour sampling interval is now the method of choice.

The calculated infarct size is sensitive to variation in the myocardial CK depletion parameter, CK_D (Roe & Starmer, 1975). The value for man initially quoted by Sobel et al (1972) had a range about the mean of $\pm$ 15.9% ($\pm$ 1 SD), representing an enormous potential variation. This variation is due primarily to 2 factors; individual variation from one heart to another (Roe & Starmer, 1975) and technical variation in the recovery of CK from the myocardium (Roe & Starmer, 1975; Sobel et al, 1977). The influence of the latter factor can to some extent be modified when each individual research group determines CK depletion from the myocardium after acute myocardial infarction in post-mortem specimens, using the same

homogenising procedure in each case and analysing the CK in the same way as the plasma CK was analysed, as was done in this study.

CK activity starts to rise approximately 4 hours after the onset of myocardial infarction (Bleifeld et al, 1976) but it is not unusual for patients to arrive in hospital much later after the onset of their symptoms, so that CK levels are already raised. Bleifeld et al (1976) maintain that if the first CK sample exceeds 10% of the final peak value, the cumulated activity of CK, and therefore infarct size, cannot be calculated accurately, and that the error increases with increasing level of the initial CK. In contrast Sobel (Personal Communication) states that sizing of infarcts is relatively uninfluenced by omission of early data points, provided that the peak value has not been missed, and he suggests that in such cases it is valid to assume a time at which the rise in CK might reasonably have been expected to start.

4. CORRELATION BETWEEN CALCULATED INFARCT SIZE AND ANATOMICAL INFARCT SIZE, HAEMODYNAMIC PARAMETERS, CLINICAL FEATURES, MORBIDITY AND MORTALITY AFTER ACUTE MYOCARDIAL INFARCTION

The sources of error inherent in the method of calculating infarct size from serial measurements of CK activity in blood must limit the accuracy of the method, so that its validity can only be verified by comparison with anatomical and clinical data.

Comparison between infarct size estimated by serial CK measurements in blood and infarct size determined by myocardial CK depletion and by direct anatomical measurement

CK depletion from the myocardium was found to correlate well with anatomical infarct size in the rabbit (Kjekshus & Sobel, 1970) and in the dog (Maroko et al, 1971). Shell et al (1971) found a good correlation

between enzymatically estimated infarct size and myocardial CK depletion in dogs with infarcts ranging from 300 mg to 54 grams, and similar results were obtained by Cairns et al (1978). The validity of using total CK in these estimations was supported by the findings of Roberts et al (1975b) that a good correlation existed between enzymatically estimated infarct size measured with total CK and MB-CK, provided that extracardiac sources of CK were carefully occluded.

Studies in patients dying shortly after AMI, but after the necessary CK rise and fall had been completed, showed a good correlation between enzymatically estimated infarct size and anatomical infarct size (Bleifeld et al, 1977). The mean difference between the two measurements of infarct size was 7 gms, and large infarcts were accurately distinguishable from small infarcts. Rogers et al (1977a) found that the percentage of abnormally contracting left ventricle, as determined angiographically in patients, correlated well with infarct size estimated by serial measurement of blood MB-CK activity.

Other reports do not confirm the accuracy of CK estimated infarct size. Jarmakami et al (1976) found a good correlation between anatomical and estimated infarct size in dogs, though the enzymatic method, using total CK, overestimated infarct size, and the correlation had a large standard error. Correlation was best when there was no reperfusion, or only a short period of reperfusion (15 minutes), of the infarcted area. The authors felt that the use of total CK was not an accurate way of determining infarct size, but most of the dogs in their study had heart failure and this could account for the presence of extracardiac CK and the resulting overestimation of infarct size. Having minimised the possibility of extracardiac CK, Vatner et al (1978) found that infarct size was only overestimated in the presence of reperfusion.

A comparison of enzymatically estimated infarct size and anatomical infarct size after experimental myocardial infarction in dogs showed a good correlation only when the infarcts were small (Roe et al, 1977). The authors compared the early method of Shell et al (1971), the modification for Kd introduced by Norris et al (1975) and the further modifications of Roberts et al (1975b). In each subsequent study there was an improvement over the previous study in the correlation between enzymatically estimated and anatomical infarct size provided that only infarcts smaller than 13 gms were considered and that the MB-CK isoenzyme, rather than total CK, was used. Furthermore, all the three methods overestimated infarct size, the Shell method resulting in the largest and the Roberts method in the least overestimation. It was not possible to distinguish large from small infarcts by enzymatic estimation.

A possible explanation for this observation is that a small, well-perfused infarct may lose the same amount of CK into the circulation as a large, poorly-perfused infarct. This theory was supported by the finding of Swain et al (1978) who found a poor overall correlation between histologically and enzymatically estimated infarct size except when small infarcts of 16 gms or less were considered. Using microspheres to estimate regional myocardial blood flow they found that large infarcts had a central zone with markedly reduced regional myocardial blood flow even 24 hours after coronary occlusion, suggesting a possible cause for the failure of all CK to be released into the circulation.

Cairns et al (1978) found a good correlation in dogs between CK release and increasing infarct size until approximately 20% of the myocardium was involved, after which the CK release fell. In man, however, there was a good correlation between estimated infarct size, anatomical infarct size and prognosis, as described below (Bleifeld et al, 1977). Cairns et

al (1978) have therefore suggested that the fall-off in CK release observed in the dog may not occur in man until a percentage of the myocardium incompatible with survival has been lost, so that a valid estimation of large and small infarct sizes can be made by serial blood CK measurements.

Estimated infarct size and prognosis

Enzymatically estimated infarct size correlates well with morbidity and mortality after acute myocardial infarction. Infarct size was significantly larger in patients who died than in patients who survived AMI, both during the period of hospitalization (Mathey et al, 1974; Bleifeld et al, 1977) and in the 6 months following infarction (Sobel et al, 1972). The 6-month mortality associated with estimated infarcts $\geq$65 CK-g-eq was 67% compared with 5% for infarcts <65 CK-g-eq (Sobel et al, 1972). Even better prediction was found using the infarct size index (ISI), which is the estimated infarct size (CK-g-eq) divided by the surface area in square metres (m^2), and is expressed as CK-g-eq/m^2 (Shell & Sobel, 1976). In the study by Shell and Sobel (1976) 67% of patients had ISI less than 40, with a 6 month mortality of 3%, whilst patients with ISI $\geq$ 40 had a mortality of 68%, a highly significant result when tested against an overall mortality of 23%. Survivors in this study had a significantly lower infarct size index than non-survivors. Infarct size index provided a better prognostic index than clinical classification as determined by Scheidt et al (1970), except in class IV patients, where cardiogenic shock might have resulted in extracardiac CK release and inaccuracy in ISI determination.

Estimated infarct size and ventricular performance

Cardiogenic shock occurs when 32% to 40% of the left ventricle becomes infarcted (Harnarayan et al, 1970; Page et al, 1971), and patients who develop shock early, within 6 hours of acute myocardial infarction, have a more extensive area of necrosis as estimated at post-mortem, then patients in whom shock develops over a longer period of time (Caulfield et al, 1976). The cumulative effect of a small infarct in a patient who has had a previous infarct may lead to shock. The incidence of, and mortality as result of, heart failure might therefore reasonably be expected to increase with increasing estimated infarct size. Several studies have shown that the degree of left ventricular dysfunction is related to estimated infarct size, using both clinical and haemodynamic parameters. Sobel et al (1972) found that survivors of acute myocardial infarction who had clinical evidence of heart failure had a significantly higher mean estimated infarct size (91 $\pm$ 8 SEM CK-g-eq) than survivors with minimal or no heart failure (31 $\pm$ 4 SEM CK-g-eq). In patients who died with heart failure, mean infarct size ($\pm$ SEM) was 109 $\pm$ 10 CK-g-eq, and one patient who died without heart failure had an estimated infarct size of 50 CK-g-eq. The presence or absence of pulmonary venous congestion or pulmonary oedema on a chest X-ray taken within 24 hours of hospitalization is considered to be the most important prognostic factor for both early and late survival after acute myocardial infarction by Norris and his co-workers (Norris et al, 1969). Separating 31 patients into two groups according to whether pulmonary congestion or oedema was present or absence on chest X-ray, Norris et al (1975) found that patients free from congestion had a mean cumulated CK release (CK_r) which was half that in patients with evidence of congestion or oedema ($P<0.001$). Thomson et al (1977) found that death due to cardiac failure occurred significantly more often in

patients with large than with small estimated infarct size, but only during the hospital phase, there being no prognostic value in estimated infarct size after recovery. Inoue et al (1977) measured the duration of the CK appearance function f(t) and found a shorter period of CK release in patients without clinical heart failure. They found that the major release of enzymes after infarction occurs during the period of chest pain, and that a longer duration of chest pain was associated with the development of heart failure.

Using haemodynamic data estimated infarct size was shown to correlate well with impaired left ventricular function (Kostuk et al, 1973; Rogers et al, 1976). Mathey et al (1974) divided their patients into 3 groups according to the admission pulmonary artery diastolic pressure. Patients in group I had a normal pulmonary artery diastolic pressure (<12 mmHg), patients in group II a slightly elevated pressure (12-20 mmHg) and patients in group III a grossly elevated pressure (>24 mmHg). Group I patients had a mean estimated infarct size of 17 CK-g-eq, and a normal cardiac index and left ventricular stroke work index. One patient in this group died of cardiac rupture, giving an overall mortality of 4%. Patients in group II had a mean estimated infarct size of 42 CK-g-eq with a 21% mortality. Cardiac index and left ventricular stroke work index were slightly reduced. Mean infarct size in group III patients was 99 CK-g-eq, the mortality was 60%, and cardiac index and left ventricular stroke work index were significantly reduced. After later studies which produced essentially the same results (Bleifeld et al, 1976), the authors concluded that "in individual patients estimated infarct size alone cannot be used to predict prognosis, but with haemodynamic data gives an estimation of the functional state and reserve of the residual myocardium".

Hori et al (1979) found an excellent inverse correlation between

enzymatically estimated infarct size and ejection fraction, which in turn correlates well with the percentage of non-contracting ventricle as estimated angiographically. Using stroke volume index (Swan et al, 1972) as their haemodynamic parameter, Shell and Sobel (1976) found that both stroke volume index and infarct size index correctly predicted survival or mortality, and that the mortality rate prediction associated with a combination of the two indices was not significantly different from the mortality rate prediction using either parameter alone. They felt that "the prognostic power of CK infarct size index suggests that the estimate of infarct size corresponds to a biologically significant determinant of outcome".

Estimated infarct size and ventricular dysrhythmias

Ventricular arrhythmias are, along with heart failure, the predominant cause of death in patients with acute myocardial infarction (Friedberg, 1968). A relationship has been demonstrated between enzymatically estimated infarct size and the development of ventricular arrhythmias. Bloor et al (1975) found a lowering of the ventricular fibrillation threshold with increasing infarct size in the experimental animal. Roberts et al (1975c) found a significant increase in ventricular premature beats, coupled beats, ventricular tachycardia and the peak frequency of ventricular premature beats with increasing infarct size index in patients during the first 10 hours after hospitalization. An increased incidence of ventricular tachycardia and ventricular fibrillation was also found by Bleifeld et al (1976) in patients with EIS greater than 65 CK-g-eq compared with patients with EIS less than 30 CK-g-eq, but in this study the possible role of the poorer haemodynamic status of patients with larger infarcts was emphasized. In 38 patients with AMI admitted within 6 hours of onset of symptoms the persistence and severity of ventricular dysrhythmia in the first 20 hours

hospitalization correlated well with ISI especially when patients with previous infarcts were excluded (Cox et al, 1976). In these studies the correlation between infarct size and ventricular arrhythmias were only demonstrated in the short term, but a preliminary report from a recent study (Ehsani et al, 1978) has shown that enzymatically estimated infarct size predicts ventricular electrical instability for as long as one year after the initial infarct in patients up to 60 years of age.

Conclusion

The method of calculating infarct size using serial measurements of blood CK activity after acute myocardial infarction is valid in that relative infarct sizes in man can be estimated with accuracy, though the estimated infarct size may not correspond absolutely with anatomical infarct size. Sources of error inherent in the model can be minimised by careful patient selection and by individualising as many of the parameters involved as possible.

Good correlations exist between enzymatically estimated infarct size and prognosis, arrhythmias, cardiac performance and morbidity. Sobel (1976) has shown that there is strong evidence for a causal basis to these correlations.

The CK method for estimating infarct size appears to be a valid and valuable index with which to assess metabolic, haemodynamic and pharmacological events occurring shortly after acute myocardial infarction.

5. CALCULATION OF ESTIMATED INFARCT SIZE AND INFARCT SIZE INDEX - PRACTICAL DETAILS

The derivation of the equation for the calculation of estimated infarct size has been discussed in detail.

Estimated infarct size = CK_r x K x body weight (CK-g-eq)

A. Calculation of CK_r

i. Equation used and definition of terms

The cumulative CK serum entry per ml (CK_r) is calculated by integrating the appearance function f(t) at any given time. CK activity was measured in i.u./l so the final calculated value of CK_r was divided by 1000.

$$CK_r = \sum_{o}^{t} f(t)\,dt \text{ (the integrated appearance function)} = \sum_{o}^{t} (DE/DT + Kd\,\bar{E})\,DT$$

Where:

DE = change in CK activity over the preceding time interval DT

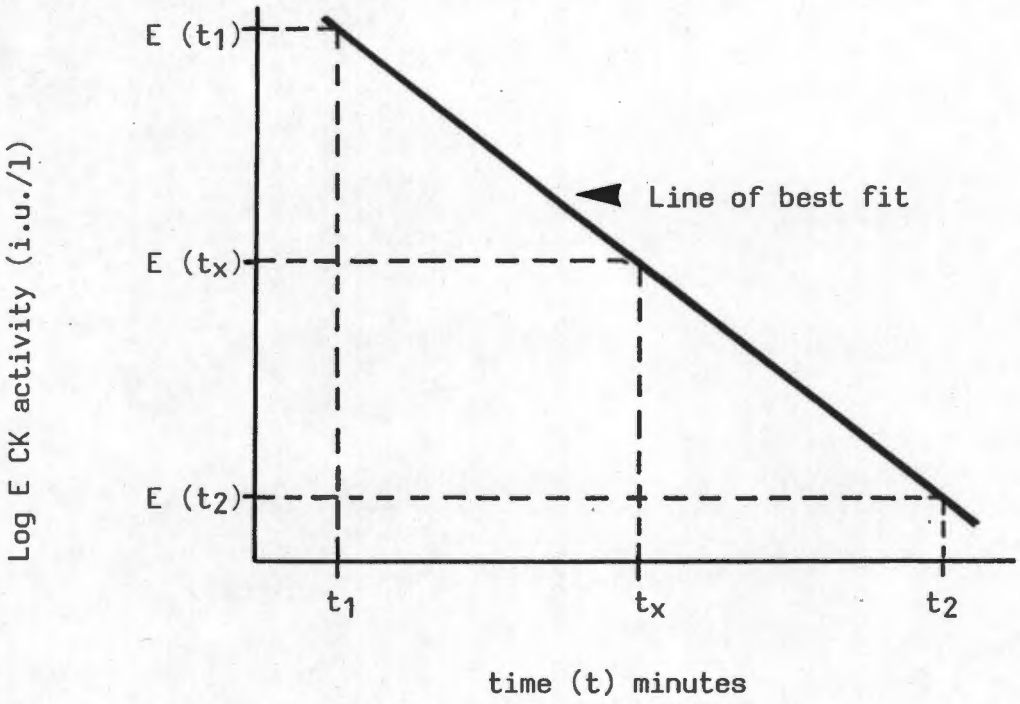
DT = the exact time interval in minutes

$\bar{E}$ = the mean CK activity during the time interval DT

Kd = the fractional disappearance rate of CK from blood

ii. Calculation of Kd (Norris et al, 1975)

The terminal portion of the CK time-activity curve was expressed as a semi-logarithmic plot, the highest value being that which was nearest to 80% of peak activity (Sobel et al, 1977b). At least 5 points had to be used, and values approaching zero were excluded because it is impossible to determine whether CK activity will return to below normal levels or not (Norris et al, 1975). The line of best fit was calculated so that the 95% confidence limits for the slope ($\pm$ 2 deviations were <15% of the value for the slope (Norris et al, 1975). The references used for these calculations are given in Appendix C.



$$t_x = t_2 - t_1/2$$

$$Kd = \frac{E(t_1) - E(t_2)}{E(t_x)\,(t_2 - t_1)} \quad mins^{-1}$$

B. The proportionality constant, K

i. The equation

$$K = \frac{DV}{P_{CK}\left(\left[CK_N\right] - \left[CK_I\right]\right)} \qquad \frac{(ml)\ \ (CK\text{-}g\text{-}eq)}{(Kg)\ \ (iu.)}$$

Where:

DV = the distribution volume of CK/unit body weight (ml/kg)
= 44 ml/kg

P_{CK} = proportion of CK released into the blood compared with CK depleted from myocardium (i.u./CK-g-eq)/(i.u./g)
= 0.15 (i.u./CK-g-eq)/(i.u./g)

$\left[CK_N\right]$ = CK activity in a homogeneous section of normal myocardium (i.u./g)

$\left[CK_I\right]$ = CK activity in a homogeneous section of infarcted myocardium (i.u./g)

Values for $[CK_N]$ and $[CK_I]$ as determined by Sobel et al (1972) and Roberts & Sobel (1977) have beeen quoted above. Since the CK-g-eq used to express infarct size are not absolute units, but are dependent on the assay conditions (Bleifeld et al, 1977), $[CK_N]$ and $[CK_I]$ were determined by the same method as that used to measure plasma CK activity.

ii. Method used in the analysis of myocardial CK activity

The CK activity in normal myocardium was determined from samples of left ventricle grossly normal to the naked eye, from 5 hearts with no clinical or post-mortem evidence of myocardial disease of any description.

The CK activity in infarcted myocardium was determined from samples taken from the centre of an area of homogeneous infarction, as determined macroscopically, from 5 hearts taken from patients who had died 24 hours to 7 days after acute myocardial infarction. Samples were taken and analysed within 12 to 24 hours post-mortem.

The tissue was minced with scissors and 200 mg was added to 5 mls of homogenizing medium consisting of 0.25M sucrose, 0.001M neutralised sodium ethylenediaminetetra-acetic acid, and 1×10^{-3}M mercaptoethanol, pH 7.0 (Shell et al, 1971). The tissue sample was homogenised with 4 x 15 second bursts of an Ultra Turrax homogeniser, speed 6. The homogenate was then centrifuged at 16000 g for 10 minutes at 0°C to -3°C, after which the supernatant was decanted and immediately analysed as described.

iii. Results

MYOCARDIAL CK CONCENTRATION (i.u./g) at 30°C

	Normal ventricle $[CK_N]$	Infarcted ventricle $[CK_I]$
1	717	42
2	804	166
3	710	137
4	579	237
5	569	156
Mean ± SEM	676±45 i.u./g	148±31 i.u./g

iv. The calculation of K

The proportionality constant K, was calculated using the above values for $[CK_N]$ and $[CK_I]$ and the values for P_{CK} and DV as determined by Roberts et al (1975).

$$K = \frac{44}{0.15\ (676 - 148)} = 0.56\ \frac{(ml)\ (CK\text{-}g\text{-}eq)}{(Kg)\ \ (i.u.)}$$

This value for K was used in all calculations of estimated infarct size.

C. Calculation of infarct size index (Shell & Sobel, 1976)

$$\text{Infarct size index (ISI)} = \frac{\text{Estimated infarct size}}{\text{surface area}}\ (CK\text{-}g\text{-}eq/m^2)$$

Surface area was determined from a nomogram using the height and the weight of the patient (Berlyne, 1974).

6. CALCULATION OF ESTIMATED INFARCT SIZE AND INFARCT SIZE INDEX - AN EXAMPLE

Table 1 gives the details of the serial CK activities and the calculation of $\Sigma\ f(t)Dt$ for patient No.28.

a. CK activity - baseline values (upper limit of normal = 60 i.u./l) have been subtracted in all cases. The initial CK value recorded had already risen above normal, but omission of early data points has relatively little effect on the calculation of infarct size by this method (Sobel, Personal Communication). CK activity after 52 hours was within normal limits.

b. CK_r

$$CK_r = \int_0^t f(t)dt$$

$$f(t) = DE/DT + Kd \times \text{mean CK activity } (\bar{E})$$

CK activity and $\Sigma\ f(t)DT$ are shown plotted against time in Figure 1.

c. Calculation of Kd

i. Semi-log plot of the relevant points

Samples number 11 to 22 inclusive were used for the calculation of Kd. CK activity 16 hours post-infarction (770 i.u./l, sample No.11) was 84% of the peak (917 i.u./l).

Log E of the CK activity was plotted against time (Figure 1) and the line of best fit was determined.

ii. Statistics of the line of best fit

Details of the statistical methods are given in Appendix C.

a = 3.48	Sa = 0.04
b = -0.04	Sb = 0.00095
r = 0.99	$S_{y.x}$ = 0.04

95% confidence limits for the slope = -0.04 ± 0.0019 (5%)

CK activity (E) was then determined from the line of best fit.

Equation for the line of best fit: Y = 3.48 + (-0.04) X

t_1 = 16 hours	E (t_1) = 794 i.u./l
t_2 = 52 hours	E (t_2) = 38 i.u./l
t_x = 36 hours	E (t_x) = 174 i.u./l

iii. Calculation of Kd

$$Kd = \frac{E_{(t_1)} - E_{(t_2)}}{E\ (t_x)\quad (t_2-t_1)\times 60} = \frac{794 - 38}{174 \times 36 \times 60} = \underline{0.002\ \text{mins}^{-1}}$$

d. Duration of CK release

This was the time from the onset of symptoms until the CK release curves reached a plateau (see example Figure 1).

e. Calculation of estimated infarct size

$EIS = CK_r \times K \times Wt$ (CK-g-eq)

$CK_r = 1924.62/1000 = 1.925$

$K = 0.56$

Weight = 87 kg

$EIS = 1.925 \times 0.56 \times 87 =$ 94 CK-g-eq

f. Calculation of infarct size index

$$ISI = \frac{EIS}{\text{surface area}}$$

EIS = 94 CK-g-eq

Weight = 87 kg

Height = 180 cm

Surface area = 2.05 m^2

$$ISI = \frac{94}{2.05} = 46 \text{ CK-g-eq/m}^2$$

7. SPECIAL CONDITIONS CONCERNING INFARCT SIZE ESTIMATION IN PATIENTS 5, 6, 11 and 33

Patient No.5 died after only 15 hours in the coronary care unit, so that insufficient time elapsed for the calculation of EIS by the CK method. Infarct size was therefore measured at post-mortem, and the infarcted portion of the myocardium, as carefully determined by a pathologist, weighed 110 gms. Whilst this estimate was not necessarily comparable, there is evidence that enzymatically calculated infarct size would have been of the same magnitude, since CK activity at 14 hours post-infarction was already 875 i.u./l above normal in this patient, and all patients with CK activities of this order 14 hours post-infarction had EIS much greater than 65 CK-g-eq, and thus ISI greater than 40 CK-g-eq/m^2, (Appendix B).

The inclusion of this patient was therefore felt to be justified.

Patients 6 and 33 had definite myocardial-infarctions, as determined by the appropriate ECH and enzyme changes. CK activity in both cases did not rise sufficiently to follow the standard pattern of rise and fall, and the decay constant, Kd, could not be calculated from the terminal portion of the curve. Accurate determination of EIS was therefore impossible; instead EIS was calculated using Kd = 0.002, this being twice the mean Kd proposed by Sobel et al (1972) for calculating infarct size in man and similar to the highest values noted in other patients in this study. Using a value for Kd larger than might be expected, EIS for both patients was less than 65 CK-g-eq, (Appendix B) and both have therefore been included as patients with small infarcts.

Patient No.11 had an extension of his infarct approximately 24 hours after the initial episode. Two figures are therefore given for this patient, the first figure relating to the initial episode and the second figure to the combined initial episode plus the extension.

Table 1 SERIAL CK ACTIVITY AND THE DERIVATION OF THE TOTAL CUMULATIVE CK RELEASE IN PATIENT NO. 28

Sample number	Hours post-infarct	DT (minutes)	CK activity (i.u./l)	Mean CK activity	DE/Dt	F(t)	F(t)Dt	ΣF(t)DT
1	4		129					
2	5	60	143	136	0.2333	0.505	30.3	30.3
3	6	60	231	187	1.467	1.841	110.46	140.76
4	7	60	412	321.5	3.017	3.66	219.6	360.36
5	8	60	592	502	3.0	4.004	240.24	600.6
6	9	60	782	687	3.167	4.541	272.46	873.06
7	10	60	833	807.5	0.85	2.465	147.9	1020.96
8	11	60	917	875	1.4	3.15	189	1209.96
9	12	60	855	886	1.033	0.739	44.34	1254.3
10	14	120	872	863.5	0.142	1.869	224.28	1478.58
11	16	120	770	821	-0.85	0.792	95.04	1573.62
12	18	120	629	699.5	-1.175	0.224	26.88	1600.5
13	20	120	542	585.5	-0.725	0.446	53.52	1654.02
14	22	120	465	503.5	-0.642	0.365	43.8	1697.82
15	24	120	412	438.5	-0.442	0.435	52.2	1750.02
16	28	240	273	342.5	-0.579	0.106	25.44	1775.46
17	32	240	220	246.5	-0.221	0.272	65.28	1840.74
18	36	240	174	197	-0.192	0.202	48.48	1889.22
19	40	240	121	147.5	-0.221	0.074	17.76	1906.98
20	44	240	67	94	-0.225	-0.037	-8.88	1898.1
21	48	240	54	60.5	-0.054	0.067	16.08	1914.18
22	52	360	33	43.5	-0.058	0.029	10.44	1924.62

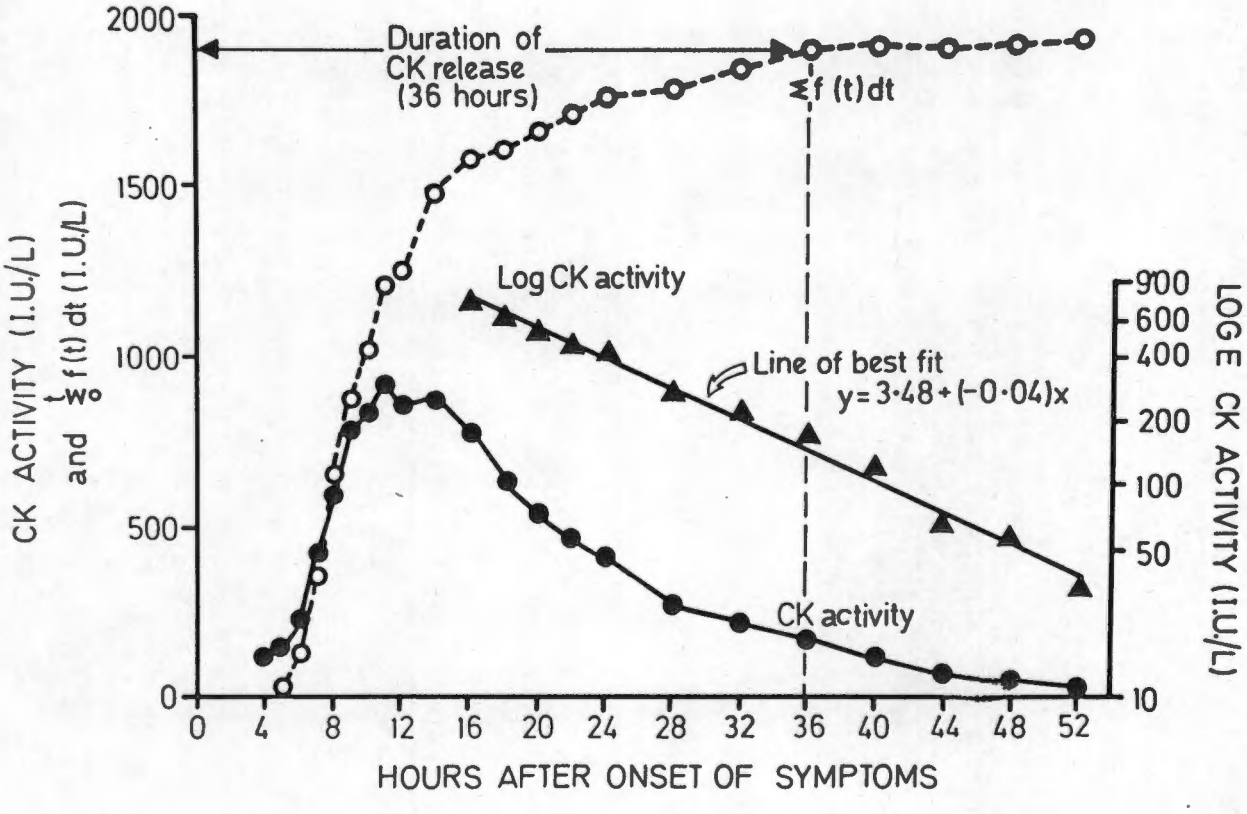


Figure 1

CK activity, total cumulative CK release ($\Sigma f(t)dt$) and Log E CK activity plotted against time after the onset of acute myocardial infarction. (Patient No. 28).

CHAPTER IV

DIRECT CURRENT EXTERNAL CARDIOVERSION, AND ITS EFFECT ON PLASMA TOTAL CREATINE KINASE AND MB-CK ISOENZYME ACTIVITIES

INTRODUCTION

Since external direct current (DC) cardioversion was shown to be a safe and effective method for the treatment of cardiac arrhythmias in man (Lown et al, 1962) a number of studies have reported a variable rise in total CK activity following cardioversion (Hunt & Bailie, 1968; Konttinen et al, 1969; Mandecki et al, 1970; Forssell et al, 1975; Eschani et al, 1976; Reiffel et al, 1978). The importance of the questions raised by these observations to clinical practice are two-fold: is the extent of the rise in total CK activity sufficient to obscure the diagnosis of myocardial infarction and the calculation of estimated infarct size by means of plasma CK activity, and does the rise in CK activity indicate that damage has occurred to the myocardium?

The extent of the rise was significant enough to interfere with the diagnosis of myocardial infarction and the calculation of infarct size in the studies of Hunt and Bailie (1968), Konttinen et al (1969), Mandecki et al (1970), Ehsani et al (1976), whilst only a few insignificant rises were observed by Forssell et al (1975) and Reiffel et al (1978).

Whether or not the rise in CK represents myocardial damage is more controversial. Histological examination of hearts from experimental animals which have undergone repeated direct current cardioversion has shown evidence of myocardial necrosis (Dahl et al, 1974; Warner et al, 1975) but the cumulative electrical energy used in these studies was up to 4000 watt-seconds, far in excess of the maximum 750 watt-seconds reported in clinical studies (Hunt & Bailie, 1968; Ehsani et al, 1976). Elevation of the relatively cardiospecific MB-CK isoenzyme (Roberts et al, 1975), which was associated only with irreversible ischaemic myocardial injury in the dog (Ahmed et al, 1976), has been shown to be nil, or minimal, in the clinical setting (Ehsani et al, 1976; Reiffel et al, 1978).

Thus total CK was elevated after cardioversion in most clinical studies, but did not appear to reflect myocardial ischaemic injury in the clinical setting, as it did in experimental studies where the cumulative electrical energy was much greater than that used in clinical practice.

Scope of the present study

One of the patients whose infarct size was estimated by means of serial plasma CK activities developed ventricular fibrillation and required a single direct current shock of 400 watt-seconds to convert him to sinus rhythm. The primary aim to this study was to determine whether this level of electrical energy was sufficient to elevate plasma CK to a degree which would make calculation of estimated infarct size inaccurate.

During the course of the study, it was noted that most patients received cumulative electrical energies in excess of the 750 watt-seconds reported in the literature, so that the relationship between total electrical energy, plasma total CK, and plasma MB-CK activities was also investigated.

Patients and Methods

The study was carried out on 9 patients admitted to the coronary care unit for elective cardioversion of supraventricular arrhythmias. There were 8 males and one female, aged from 30 to 66 years. None of the patients had clinical or electrocardiographic evidence of acute myocardial ischaemia, using the criteria defined in Chapter I. There was no history of intramuscular injections or of skeletal muscle trauma in the week prior to cardioversion and no intramuscular injections were given throughout the study period. Digoxin and all antiarrhythmic therapy had been stopped for at least 48 hours prior to cardioversion. Intravenous diazepam in doses ranging from 20 mg to 80 mg were used as sedation, and informed, written consent was obtained in each case.

Cardioversion was carried out using a defibrillator with paddles applied to the upper sternum and cardiac apex. The total number of shocks given to a patient varied from two to six, and the cumulative energy from 80 to 1400 watt-seconds, as required to effect cardioversion, which was successful in 5 patients.

Venous blood samples were collected immediately prior to cardioversion and then 4-hourly for a period of 24 hours, unless the patient was discharged sooner. Blood was sampled, stored and analysed within 24 hours as described above.

RESULTS

Clinical details and results are shown in Table 1. Two patients (numbers 2 and 8) had pre-cardioversion CK activities above the upper limit of normal (60 i.u./l). A rise in total CK activity was seen in all patients, but in 2 patients (numbers 4 and 6) the rise in CK activity did not exceed the normal limits. Total CK activity in the 24 hour period after cardioversion is shown for each patient in Figure 1. Where normal values were exceeded, a rise in CK activity was found by 4 hours, and peak levels of activity were found from 8 to 20 hours after cardioversion. Patient No.1 was discharged 16 hours after cardioversion whilst plasma CK activity still appeared to be rising and it is not known whether the maximum value recorded would have been the peak value. None of the elevated levels of CK activity had returned to normal by 24 hours, or whenever the last estimation was made.

There was no correlation between the number of shocks given or between the cumulative electrical energy administered and the peak level of CK activity ($r = 0.136$ and $r = 0.50$ respectively). There appeared to be an association between the extent of the enzyme rise and the number of 400 watt-second shocks administered (Figure 2), but the numbers were too

small to permit statistical analysis. Four patients received no 400 watt-second shocks; in two cases CK activity did not rise above the upper limit of normal and in no case did peak CK activity exceed 155 i.u./l irrespective of the number of shocks or the cumulative electrical energy. Patient No.9 received 5 shocks totalling 800 watt-seconds and peak CK activity was 132 i.u./l, just over twice the normal limit. All three patients receiving one 400 watt-second shock had peak CK activities above 200 i.u./l but not exceeding 400 i.u./l, whilst the 2 patients receiving one or more 400 watt-second shocks had very high CK peaks.

Initial MB-CK was less than 3 i.u./l in all cases and there was no rise in MB-CK activity subsequent to cardioversion in any of the 9 patients.

DISCUSSION

In five of the 9 patients the cumulative electrical energy was greater than the maximun of 750 watt-seconds reported in previous studies concerned with enzyme elevation after direct current cardioversion (Hunt & Bailie, 1968; Konttinen et al, 1969; Mandecki et al, 1970; Forssell et al, 1975; Ehsani et al, 1976; Reiffel et al, 1978). A significant rise in total CK activity was found in 7 of the 9 patients, but there was no relationship between the peak level of CK activity and the total cumulative electrical energy or the number of shocks delivered. Instead, highest levels of CK activity were observed in patients receiving one or more 400 watt-second shocks, whilst lower energy shocks had little or no effect on total CK activity, irrespective of their frequency or the resulting cumulative electrical energy.

The lowest reported percentage of patients exhibiting a rise in total CK activity was 6% in the study of Reiffel et al (1978) where only one of 18 patients exhibited a small significant rise in CK activity above pre-cardioversion levels. Maximum cumulative electrical energy was 600 watt-

seconds; more than 200 watt-seconds were only required in 4 patients, 2 of whom received 225 and 250 watt-second shocks respectively and 2 of whom received 600 watt-second shocks. Although the information was not given, it seems likely that only one of these latter 2 patients could have received a 400 watt-second shock, and it was this patient who exhibited the significant rise in CK activity. Forssell et al (1975) found a rise above normal in two of 12 patients receiving up to 650 watt-second shocks; whether or not 400 watt-second shocks were given is not recorded, but no CK rise exceeded twice the upper limit of normal. Mandecki et al (1970) found no significant CK rise in all of 43 patients given one electrical shock of 150 watt-seconds, whilst 9 of 16 patients receiving two or more DC shocks had elevated CK activities, in three cases to between 5 and 15 times the upper limit of normal. Cumulative electrical energy was not stated, but it was implied that 400 watt-second shocks were given to at least one patient. Blood samples were only take 90 minutes and 24 hours after cardioversion, so that peak CK activities may have been missed.

Konttinen et al (1969) reported a rise in CK activity in 24% of 34 patients, 7 of whom received a total energy of 600 watt-seconds and none of whom received a 400 watt-second shock. Four patients had a rise which was less than twice the normal CK level, in 2 cases the rise was three times and four times the normal level, and 2 patients, both of whom received a total of 600 watt-seconds, had 20-fold rises or more. Hunt and Bailie (1968) and Eshani et al (1976) found CK rises in 75% and 30% of cases respectively. Maximum cumulative energy was 750 watt-seconds in both studies and no tOO watt-second shocks were given.

Konttinen et al (1969) and Mandecki et al (1970) felt that the amount of enzyme leakage might be related to the total electrical energy given,

but other studies do not support this idea (Hunt & Bailie, 1968; Eshani et al, 1976; Reiffel et al, 1978). In the present study there was no correlation between peak CK activity and the number of DC shocks or the total electrical energy given.

No rise in MB-CK was found in any of the 9 patients. This suggests that patients receiving up to five shocks with cumulative electrical energy of up to 1400 watt-seconds do not suffer irreversible myocardial injury, assuming that MB-CK is a specific marker for irreversible injury in patients as it was shown to be in the dog by Ahmed et al (1976). Though the same may be true in man, the issue is not without controversy, as described in Chapter III. Although studies in dogs (Dahl et al, 1974; Warner et al, 1975) have shown myocardial necrosis after cardioversion, ten consecutive 400 watt-second shocks were given, a level never reported in clinical practice, so that no comparison can be made between these experimental and clinical studies. The results of the present study are in accordance with those of Reiffel et al (1978) who found no rise in MB-CK activity after cardioversion, but not with those of Ehsani et al (1976) who found a small but significant rise in 2 of 30 patients.

The conclusion drawn from this study is that, following DC cardioversion, infarct size cannot be measured accurately using total plasma CK activity, especially when shocks of 400 watt-seconds have been given. The lack of an association between the cumulated electrical energy or the number of shocks and the subsequent peak CK activity means that no correction factor can be formulated to take account of the additional CK rise following cardioversion in patients with AMI. As a result, no patients who had required cardioversion was included in the following studies. A total electrical energy of up to 1400 watt-seconds did not cause a rise in MB-CK, which can therefore be used to estimate infarct size in patients who have undergone cardioversion.

Table 1 DETAILS OF 9 PATIENTS UNDERGOING DIRECT CURRENT CARDIOVERSION

Patient No.	Sex	Age	Clinical details	Dose of IV valium	No. of shocks	Energy of individual shocks watt-secs	No. of 400 watt-second shocks	Cumulative watt-seconds	Total CK (iu/l) Before cardio-version	Total CK (iu/l) Peak level after cardio-version
1	M	56	Atrial fibrillation	80 mg	4	200 400 400 400	3	1400	42	696*
2	M	60	Atrial fibrillation Hypertension	40 mg	4	50 200 300 400	1	950	89	327
3	M	56	Atrial fibrillation Mild congestive heart failure	20 mg	6	50 100 150 200 300 400	1	1200	41	219
4	M	66	Atrial fibrillation Diabetes Pagets disease Hepatic cirrhosis	65 mg	3	50 100 300	0	450	41	52
5	M	42	Atrial fibrillation	20 mg	4	50 200 400 400	2	1050	15	1717
6	M	66	Atrial flutter	20 mg	2	20 60	0	80	27	40
7	F	30	Atrial fibrillation Mitral prosthesis	40 mg	4	50 100 200 400	1	750	39	224
8	M	54	Atrial fibrillation Hypertension Mild congestive heart failure	30 mg	3	50 100 200	0	350	82	155
9	M	48	Atrial fibrillation Mitral and aortic prosthesis	50 mg	5	50 100 150 200 300	0	800	6	132

*Maximum CK activity recorded. Peak may be higher.

MB-CK elevation zero throughout.

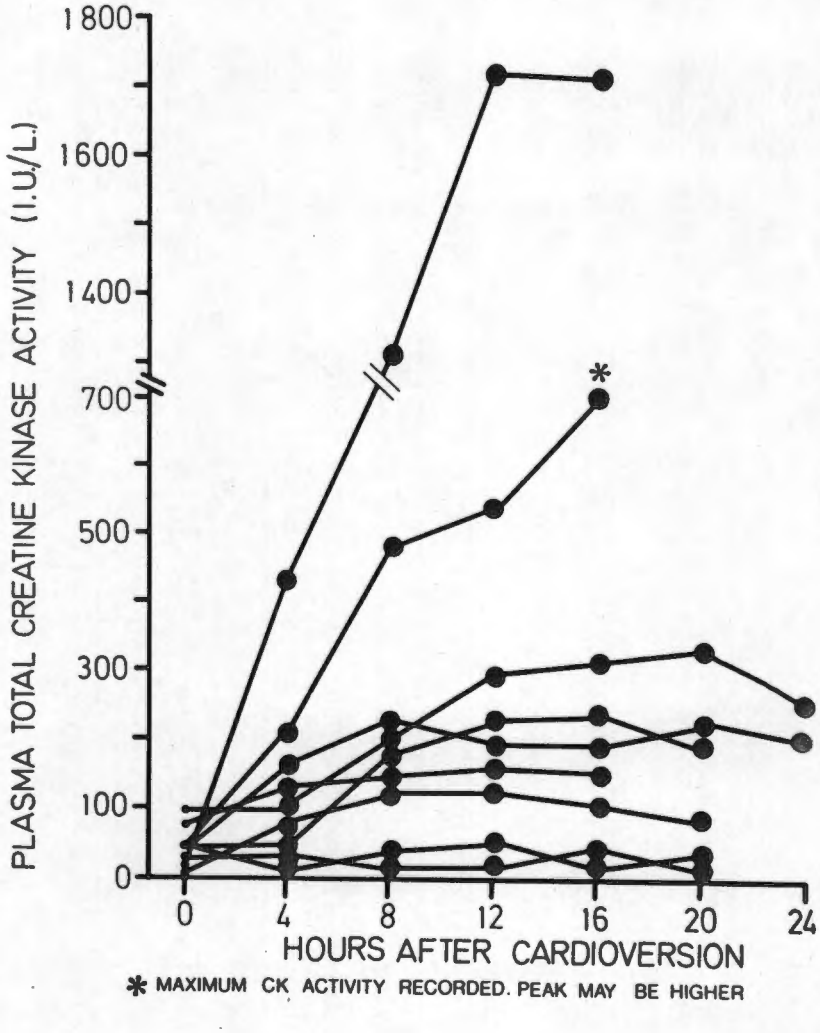


Figure 1

Plasma total creatine kinase levels in the 24 hours after DC cardioversion in 9 patients.

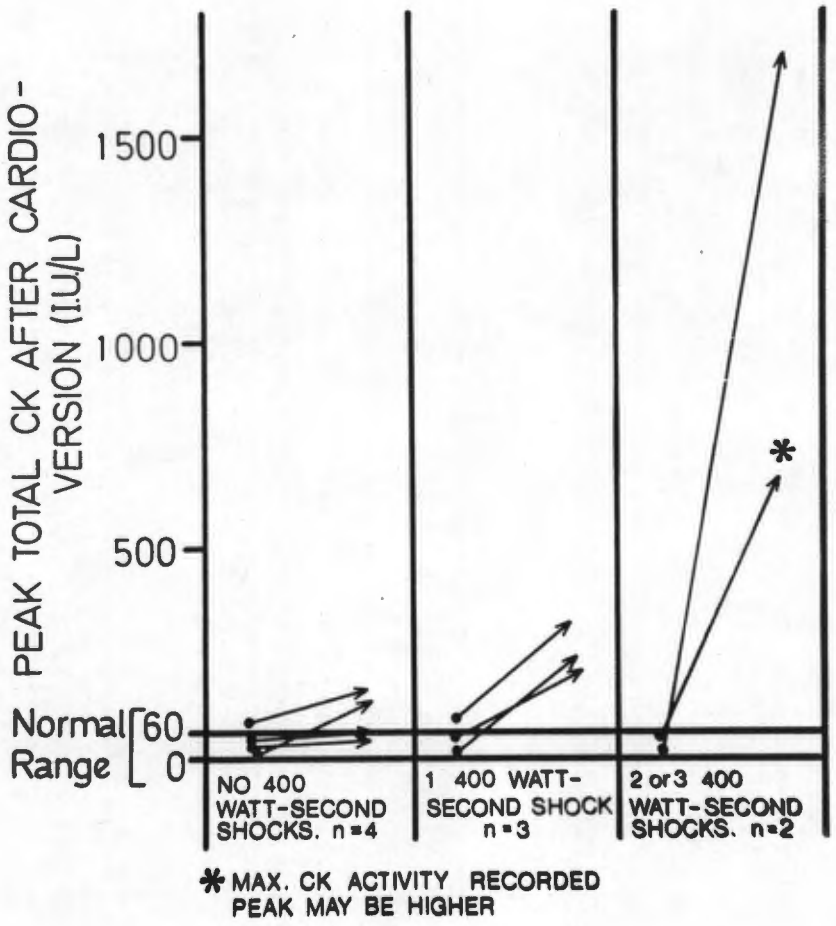


Figure 2

Rise in total CK activity after DC cardioversion according to the number of 400 watt-second shocks given.

CHAPTER V

FREE FATTY ACIDS AND ACUTE MYOCARDIAL ISCHAEMIA AND INFARCTION

A REVIEW OF THE LITERATURE

INTRODUCTION

The recognition that acute myocardial infarction (AMI) causes severe, generalised stress with resulting profound metabolic disturbances has only occurred within the past 20 years (Opie, 1971). During this time, the effects of AMI on the autonomic nervous system and on the endocrine system, as reflected by alterations in the metabolism of free fatty acids, glucose, insulin and catecholamines, have been extensively investigated and reviewed (Opie, 1968, 1971, 1972a; Oliver, 1972; Opie 1972b; Vetter et al, 1974; Opie, 1975; Mueller & Ayres, 1978).

The observation that free fatty acids (FFA) were elevated as part of the generalised metabolic response to acute myocardial infarction in man was first made by Kurien and Oliver (1966), since when much research has been carried out to determine whether or not this response is harmful to the ischaemic myocardium. The results of this research are reviewed here.

NORMAL FREE FATTY ACID METABOLISM IN MAN

Free fatty acids are the major source of energy for the heart, and other organs, in normal man (Ganong, 1977; Cahill Jnr. 1974). In the fasting state, FFA supply 60% of the fuel for oxidative metabolism (Opie et al, 1971a), glucose, lactate, pyruvate, ketone bodies and amino acids supplying the remainder (Schlant, 1974). In the fed state the FFA contribution diminishes and that of glucose rises (Opie, 1972a).

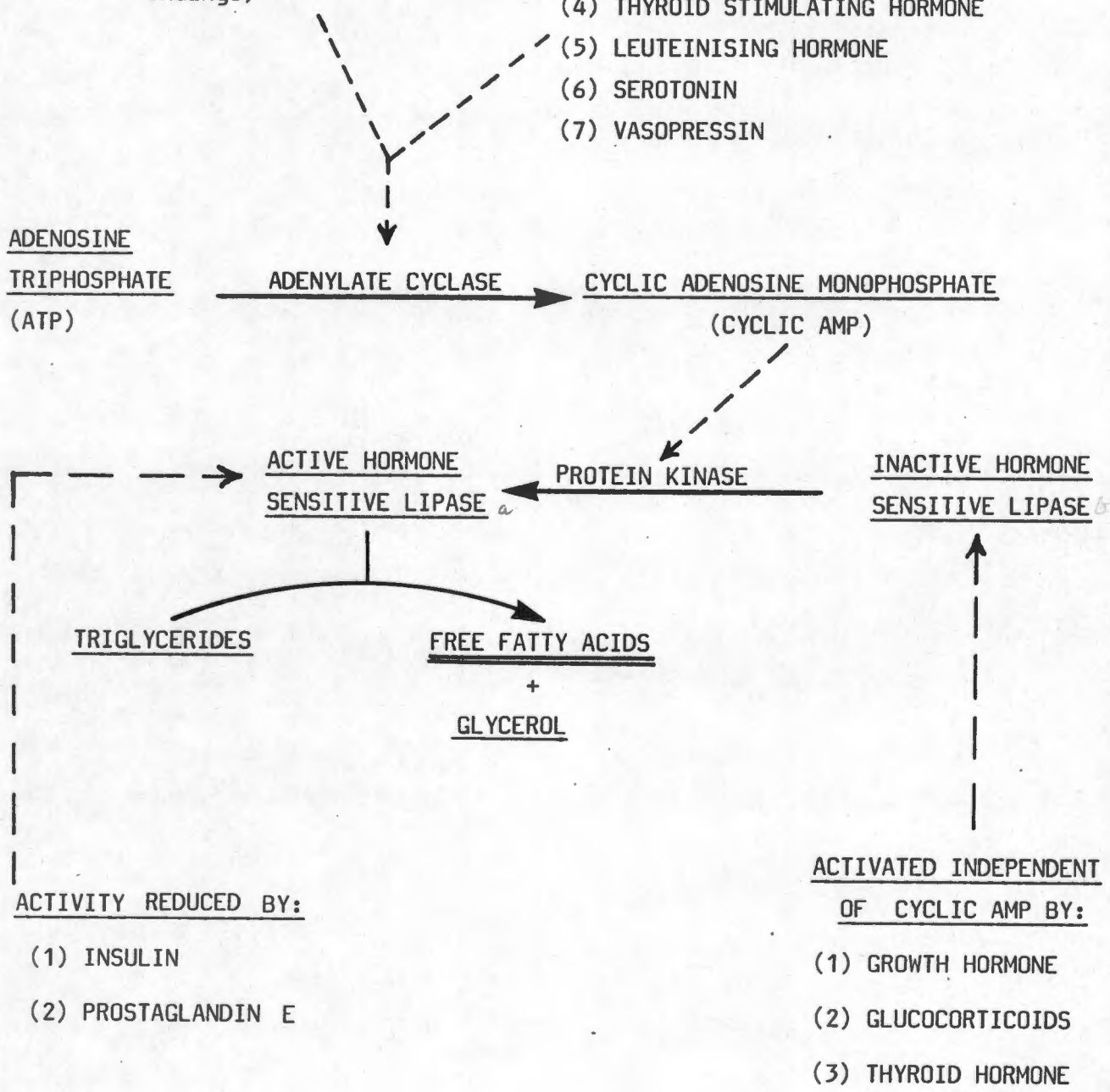
FFA are released into the circulation from stored triglycerides by the following pathway (adapted from Ganong, 1977 and Prakash et al, 1972):-

ADENYLATE CYCLASE ACTIVATED BY:

(1) CATECHOLAMINES (Gordon & Cherckes, 1956; Carlson et al, 1965; Pinter et al, 1967)

(Activated by the adrenal medulla and sympathetic post-ganglionic nerve endings)

(2) GLUCAGON
(3) ADRENOCORTICOTROPHIC HORMONE
(4) THYROID STIMULATING HORMONE
(5) LEUTEINISING HORMONE
(6) SEROTONIN
(7) VASOPRESSIN



ACTIVITY REDUCED BY:

(1) INSULIN

(2) PROSTAGLANDIN E

ACTIVATED INDEPENDENT OF CYCLIC AMP BY:

(1) GROWTH HORMONE

(2) GLUCOCORTICOIDS

(3) THYROID HORMONE

In the circulation FFA are bound to albumin (Goodman, 1958), and uptake of FFA by the myocardium is proportional to the concentration in the circulation (Dole, 1956; Evans et al, 1963; Mjøs, 1971; Nimmo et al, 1976). In the cell FFA are metabolised to acetyl coenzyme A, which enters the citric acid cycle in the mitochondria (Schlant, 1974).

Plasma FFA concentrations may be raised in obesity (Opie & Walfish, 1963), in diabetes (Shafrir & Gutman, 1965), in the fasted state (Opie, 1972a), and by stimulation of the sympathetic nervous system and catecholamine release secondary to nicotine, caffeine (Cahill Jnr. 1974) and stress (Pinter et al, 1967). Reduction of plasma FFA concentrations may occur after ingestion of food, carbohydrate having the major influence (Dole, 1956).

CIRCULATING FREE FATTY ACID CONCENTRATIONS AFTER ACUTE MYOCARDIAL INFARCTION

Since Kurien and Oliver (1966) first described a rise in FFA concentrations in patients with AMI their observations have been confirmed by other studies, including those of Oliver et al (1968), Janusjewicz et al (1971), Christensen and Videbaek (1974), Vetter et al (1974) and Opie et al (1975).

The initial account described a rise in FFA concentrations occurring within the first 12-24 hours of AMI, with concentrations returning to normal levels over the ensuing 48 hours (Kurien & Oliver, 1966), so that in this study abnormal levels could be found for up to 72 hours post-infarction. In a later study, where three or more serial measurements of serum FFA were made in 50 patients after AMI (Oliver et al, 1968), very high FFA concentrations were noted at 6 hours post-infarction, mean levels were within normal limits after 12 hours, and no individual value was above the normal limit after 36 hours post-infarction. Insufficient samples were taken to assess FFA concentrations within 6 hours. Vetter et al (1974) were able

to measure FFA concentrations in 16 patients within one hour of AMI; in four patients samples were obtained within 15 minutes. All values were elevated above the normal range, and at 2 hours post-infarction mean FFA concentrations reached a peak of about twice the upper normal limit of 0.8 ml/l (Oliver et al, 1968). Here measurements were only made during the first 5 hours. Opie et al (1975) made serial measurements in 9 patients with AMI for up to 66 hours after admission. They confirmed the rise in FFA concentrations after AMI, but their measurements were timed from the moment of admission, rather than from the onset of symptoms, so that an accurate assessment of FFA concentrations relative to the time of infarction was not possible. Individual values within 20 hours of admission showed enormous variability however.

Thus elevated FFA concentrations have been found within one hour of myocardial infarction, with peak values occurring from 2 to 12 hours and mean values returning to normal from 6 to 72 hours post-infarction, there being considerable fluctuations in FFA concentrations in individual patients.

FACTORS WHICH DETERMINE FFA CONCENTRATIONS AFTER ACUTE MYOCARDIAL INFARCTION

Several factors may be involved in causing the raised FFA concentrations reported in the acute phase of myocardial infarction. Opie (1971, 1975) has emphasized the role of stress in such patients, in whom pain and anxiety may lead to adrenal cortical and medullary stimulation (Levi, 1969) with consequent catecholamine-mediated lipolysis and elevation of circulating FFA concentrations. These patients might also have poor appetites initially, so that the effect of fasting on FFA concentrations (Opie, 1972a) may contribute to the overall effect.

The evidence that catecholamine-mediated lipolysis is responsible for

the major rise in FFA after AMI is very strong. Several studies have shown that catecholamine concentrations are elevated after AMI in the blood (Gazes et al, 1959; McDonald et al, 1969; Siggers et al, 1971; Griffiths & Leung, 1971; Januszewicz et al, 1971; Lukomsky & Uganov, 1972; Christensen & Videbaek, 1974; Vetter et al, 1974) and in the urine (Valori et al, 1967; Januszewicz et al, 1968; Jewitt et al, 1969; Gupta et al, 1969; Lukomsky & Uganov, 1972; Prakash et al, 1972).

When Kurien and Oliver (1966) first described the rise in FFA concentrations after AMI, they stated that myocardial necrosis per se was unlikely to be the cause of the rise in view of the return of the FFA concentrations to normal whilst myocardial necrosis was still occurring. They postulated that catecholamines released locally from myocardial sympathetic nerve endings as well as from the adrenals might be the cause. This theory is supported by the finding that plasma noradrenaline levels are elevated within one hour of myocardial infarction and remain so for at least 12 hours (Griffiths & Leung, 1971), and by the observations of Vetter et al (1974), that catecholamines were increased within 30 minutes of the onset of symptoms. Januszewicz et al (1971) found significantly higher plasma noradrenaline levels on the first than on the tenth day of acute myocardial infarction, with a highly significant positive correlation between plasma noradrenaline and plasma FFA concentrations, whilst Christensen and Videbaek (1974) could only predict serum FFA levels if both the plasma noradrenaline and basal insulin concentrations were known. Both studies revealed no correlation between FFA and adrenaline concentrations. Vetter et al (1974) showed a correlation between plasma-total-catecholamines and plasma FFA ($r = 0.66$); they noted a delay between the maximum rise in catecholamine concentration and FFA and suggested that this may be due to reduced cardiac output resulting in poor perfusion of adipose tissue with

slow release of FFA. They concluded that in their study "noradrenaline activation of adenyl cyclase in adipose tissue, and thence of lipolysis with resulting release of FFA, probably made the main contribution to the early rise in FFA".

Urinary excretion of adrenaline and noradrenaline was increased by up to 350% and 800% of the normal mean levels respectively in 4 patients studied by Valori et al (1967), and levels could remain elevated for up to 20 days. Similar observations were made in a later, extended study of 55 patients (Jewitt et al, 1969). Gupta et al (1969) could find no relationship between 24-hour urinary noradrenaline excretion and plasma FFA levels, or between individual values of urinary adrenaline and plasma FFA, and though there was a relationship between the rate at which mean values of urinary adrenaline and plasma FFA concentration fell from day to day, this was not however applicable to individual patients. Once daily fasting samples were taken for estimation of FFA levels and, as the authors point out, these need not correlate with 24-hour urinary catecholamine excretion, especially in view of the known fluctuations in FFA concentrations after AMI.

Plasma FFA concentrations appear to be related to plasma-catecholamine, and specifically plasma-noradrenaline, concentrations after AMI, lending support to the theory that catecholamine-mediated lipolysis is the major determinant of plasma FFA concentrations in AMI. The negative findings of Gupta et al (1969) are not necessarily contradictory in view of the observations in other studies (Klein et al, 1968; Januszewicz et al, 1968; Prakash et al, 1972) that urinary catecholamine levels need not reflect plasma-catecholamine levels, especially in patients with complications of AMI who have a decreased renal blood flow.

ELEVATED CIRCULATING FREE FATTY ACID CONCENTRATIONS AND TOXICITY TO THE ISCHAEMIC MYOCARDIUM

The relevance of the elevated FFA concentrations found in patients with AMI lies in their possible toxicity to the ischaemic myocardium, as observed in both the experimental and the clinical setting. FFA metabolism in the ischaemic and the anoxic myocardium are described first.

Free fatty acids and metabolism in the ischaemic and anoxic myocardium

Lack of oxygen impairs the utilization of FFA as a myocardial fuel (Scheuer, 1967), and in the anoxic rat myocardium the only pathway known to provide energy is anaerobic glycolysis, for which glucose is the sole exogenous substrate. In anoxic conditions, FFA uptake by the myocardium is reduced because it cannot be utilized but there is intracellular accumulation of FFA (Evans, 1964). In dogs there is an increased uptake of glucose by the ischaemic myocardium relative to that of FFA after coronary artery ligation, which is associated with decreased oxygen uptake (Opie et al, 1973). Coronary artery ligation in dogs did not lead to a complete switch to anaerobic glycolysis and away from FFA utilization (Owen et al, 1969); glucose uptake by the ischaemic zone was increased fourfold and, by inference, FFA uptake reduced by the same amount. These findings were felt to be in keeping with the concept that in myocardial infarction a central zone of total ischaemia may be surrounded by zones with lessening degrees of ischaemia and thus varying proportions of aerobic and anaerobic metabolism (Owen et al, 1969; Opie, 1975).

Glucose is therefore important to the ischaemic myocardium and essential to the anoxic myocardium for the production of energy, but studies have shown that elevated circulating FFA concentrations may increase uptake of FFA by the myocardium (Ballard et al, 1960; Carlsen et al, 1961; Scott et al, 1962) and decrease glucose uptake (Shipp et al, 1961) so that

the relative contribution of glucose to energy production decreases (Havel et al, 1964).

FFA/albumin molar ratio and the toxic effects of FFA

Spector (1971) has stressed the role of the FFA/albumin molar ratio in regulating FFA utilization by the tissues. Serum albumin in animals was found to have six high energy, or strong, binding sites for FFA and a number of weaker ones (Spector et al, 1969); saturation of these binding sites resulted in an increased concentration of unbound circulating FFA. There was an exponential relationship between increases in the FFA/albumin ratio and FFA entry into the cell (Spector, 1968).

Experimentally it has been shown that the FFA/albumin molar ratio rather than the absolute circulating FFA concentration is the determining factor in the genesis of FFA toxicity in the ischaemic myocardium. Willebrands et al (1973) found an increased incidence of arrhythmias and greater impairment of myocardial function with increasing FFA/albumin molar ratios in both ischaemic and non-ischaemic isolated rat hearts, whilst de Leiris et al (1975) found that increasing the ratio increased enzyme release from ischaemic, isolated rat hearts. Mjøs (1975) demonstrated that a decrease in the FFA/albumin molar ratio led to diminished myocardial extraction of FFA by infusing FFA-free albumin in dogs, but Kostis (1973) was unable to show an increase in the ventricular fibrillation threshold despite very high FFA/albumin molar ratios. Opie (1975) has reviewed the significance of the FFA/albumin molar ratios in several experimental studies in great detail.

The results of animal experiments may not be relevant to man because the albumin levels differ and the relative binding capacity may vary (Kurien et al, 1971). In man, assuming constant serum albumin level of 4 mg/100 ml, any increase in FFA concentrations above 1.2 mM/l (or an

FFA/albumin molar ratio of greater than 2) will result in a rise in the concentration of circulating unbound FFA (Kurien & Oliver, 1970).

Free fatty acids and myocardial oxygen consumption

High free fatty acid concentrations were found to increase myocardial oxygen consumption (MVO_2) by 40% in isolated perfused rat hearts in comparison with low FFA concentrations (Challoner & Steinberg, 1966a). Comparison of these results with subsequent in vivo studies is difficult because FFA was the only substrate supplied and because myocardial mechanical function, an important determinant of MVO_2, was not studied. Myocardial oxygen consumption was also shown to rise after elevation of serum-FFA to high concentrations in both non-ischaemic and ischaemic globally-perfused working pig hearts (Liedtke et al, 1978). In contrast, Opie (1973) found that a high FFA/albumin molar ratio of linoleic acid had no effect on MVO_2 in the perfused rat heart in non-working, working and ischaemic conditions.

Mjøs (1971a) found that increased FFA extraction by the myocardium of intact dogs resulted in increased oxygen extraction and increased MVO_2 without any increase in coronary blood flow. This is contrary to the results of previous animal (Feinberg et al, 1962) and human (Lombardo et al, 1953; Messer et al, 1962) studies in which increased myocardial oxygen demand was met by increased coronary blood flow rather than increased myocardial oxygen extraction. Subsequently Mjøs (1971b) studied the effect of isoproterenol in the intact dog. Both MVO_2 and FFA concentrations rose significantly after isoproterenol infusion; abolishing the isoproterenol-mediated FFA rise with either a nicotinic-acid analogue or high concentrations of intravenous glucose reduced MVO_2 by 30%. Whilst the nicotinic-acid analogue totally abolished the FFA rise, intravenous glucose only reduced it by 44%, but both agents were equally effective

in decreasing MVO_2. The explanation for this was that intravenous glucose might also decrease the myocardial uptake of FFA. In all the above studies, plasma FFA/albumin molar ratios were elevated well above the physiological range. Most et al (1973) carried out a study in intact anaesthetized dogs in which plasma-FFA concentrations were elevated to between 1.6 mM/l and 3.6 mM/l, which were considered to be within the range seen clinically. No change in the haemodynamic situation or in MVO_2 occurred; comparing these results with those of Mjøs (1971a), the authors suggest that a matter of degree rather than one of disagreement might be involved.

Controlled myocardial ischaemia in the open-chest dog caused ventricular dilatation and an increase in MVO_2 (Kjekshus & Mjøs, 1972). Subsequent elevation of plasma FFA concentrations increased the degree of dilatation and possibly the degree of anaerobic metabolism with no other change in mechanical function or in MVO_2. The authors interpreted their results as suggesting that the mechanism whereby high plasma FFA concentrations increased ventricular dilatation was identical to that whereby MVO_2 was increased in the normally perfused heart (Mjøs, 1971a) and not due to a primary depressive effect on myocardial contractility, and that in the hypoxic heart high FFA concentrations increased the oxygen requirements in competition with the oxygen requirements for mechanical activity. This conclusion is contrary to that of Henderson et al (1970a) who found a depression of both mechanical performance and MVO_2 in oxygenated isolated perfused rat hearts, and could not exclude a primary depressive effect on the myocardium. The possibility that these hearts were somehow oxygen-limited could not be excluded by the authors themselves, so that their conclusions may be invalid.

Mjøs et al (1974) showed that in closed-chest dogs with coronary

artery occlusion, inhibition of lipolysis reduced the norepinephrine-mediated rise in MVO_2 by 48%. The rise in arterial FFA concentrations and increased myocardial FFA extraction were abolished and there were no haemodynamic changes. The fraction of MVO_2 attributable to lipolysis in this study was larger than that observed when a similar increase in myocardial FFA extraction was induced by infusion of triglycerides and heparin in the absence of norepinephrine (Mjøs, 1971a). The difference was felt to be due to the added effect on MVO_2 of increased endogenous lipolysis by norepinephrine, as previously suggested by Challoner and Steinberg (1966a, 1966b), in addition to the effect of circulating FFA. Mjøs et al (1974) also observed that after inhibition of lipolysis there was a substantial decrease in myocardial FFA extraction which was not accounted for by the decreased MVO_2, and they concluded that only an increase in FFA concentrations above the requirement for mechanical activity would increase MVO_2.

Rogers et al (1977b) were unable to detect a rise in MVO_2 after heparin-induced elevation of FFA concentrations up to 2.75 mM/l in patients with a history suggestive of ischaemic heart disease. They postulated that there was no increase in MVO_2 in these patients because the FFA were stored as triglycerides in the myocardium and not oxidized. Dagenais and Jalbert (1977) also increased FFA concentrations by heparin infusion in patients with well-documented ischaemic heart disease and found no resulting increase in oxygen extraction by the myocardium or in MVO_2, either at rest or during atrial pacing. Patients who developed angina during pacing prior to FFA elevation had no change in the severity of their symptoms subsequently. Mean FFA concentrations rose to 1.83 mM/l after heparin infusion and peaks between 2.6 and 3.1 mM/l occurred in three of the patients. The authors could not explain the difference in the results

between this study and previous animal studies. The method whereby FFA concentrations were elevated by heparin infusion alone might explain some of the differences, in that FFA concentrations in dogs cannot be increased in this way.

Simonsen and Kjekshus (1978) elevated FFA concentrations by infusing isoproterenol in one group and by infusing Intralipid and heparin in a second group of patients with coronary artery disease who underwent atrial pacing. Pacing alone increased MVO_2 by 66% over control levels, whilst increasing heart rate to the same level by isoproterenol infusion alone increased MVO_2 to 142% above control values. MVO_2 increase was 23% and 81% respectively when the same procedures were carried out during the infusion of an antilipolytic agent, which all but abolished the FFA rise. After isoproterenol stimulation, FFA contributed 50% to the increase in MVO_2. Elevating FFA concentrations with Intralipid and heparin did not increase MVO_2 in the resting state, in keeping with the findings of Rogers et al (1977b), but during pacing MVO_2 rose by 81% as compared with 38% when no lipids were administered. When isoproterenol was infused at the same time as the lipids, MVO_2 rose by 91% of the control value during pacing compared with 67% during isoproterenol infusion alone. FFA concentrations in this study were within the limits observed during AMI. Several patients experienced angina at rest during simultaneous isoproterenol and lipid administration but not when these were administered separately, suggesting a synergistic effect. The conclusions drawn were that MVO_2 was closely related to the myocardial uptake of FFA and that catecholamines can sensitize the heart to FFA without increasing FFA uptake. The major part of the increased MVO_2 during catecholamine stimulation, as might be experienced during the early phase of AMI, was accounted for by the increased FFA uptake by the myocardium.

Sensitization of the myocardium to free fatty acid by catecholamines

Opie et al (1971b) noted that elevation of circulating FFA concentrations to up to 10.0 mM/l in open-chest dogs with coronary artery ligation did not increase the frequency of ventricular arrhythmias, but that a combined adrenaline infusion significantly increased the incidence of ventricular ectopic beats. They therefore proposed the theory that catecholamines may sensitize the myocardium to the effects of elevated FFA concentrations. This theory is supported by evidence from studies in which catecholamines exacerbated the toxic effects of high FFA concentrations in dogs with experimental myocardial ischaemia (Kjekshus & Mjøs , 1972; Kjekshus & Mjøs, 1973) and Opie (1975) concluded that catecholamine-mediated stimulation of endogenous lipolysis or increased myocardial extraction of circulating FFA might account for such sensitization. Simonsen & Kjekshus (1978) found evidence for catecholamine sensitization in patients with ischaemic heart disease but without any associated FFA uptake by the myocardium.

Intracellular free fatty acids and mechanisms of toxicity

Kurien and Oliver (1970) put forward the hypothesis that FFA toxicity occurred because of intramyocardial breakdown of stored triglycerides to FFA by local catecholamine activation of a lipase. This hypothesis is supported by the observations that in the under-perfused left ventricle, there was greater incorporation of labelled FFA into myocardial triglyceride and decreased incorporation into tissue (Scheuer & Brachfeld, 1966), and that there was a greater efflux than influx of FFA from the ischaemic zone in coronary artery ligated dogs (Opie et al, 1973). Experimental evidence that endogenous lipolysis promotes the effects of ischaemia is indirect and inconclusive however (Opie, 1975).

Kurien et al (1971) further proposed that in patients with AMI

previous ischaemic episodes might have already resulted in high intra-myocardial triglyceride concentrations, quoting the study of Wartman et al (1956) as support. During AMI the high local release of noradrenaline could stimulate lipolysis (Kruger et al, 1967) resulting in very high intracellular FFA concentrations requiring relatively little supplementation from circulating FFA levels to lead to toxicity. In this way, they could explain the discrepancy between the high plasma-FFA concentrations required to produce arrhythmias and impair myocardial function in animal experiments, as described below, compared with the much lower plasma-FFA concentrations observed in patients with AMI.

Mechanisms of FFA toxicity in animals include the following. In vitro studies have shown that FFA anions may combine with intracellular cations to produce soaps which may have a detergent effect on cellular enzymes and membranes which could result in loss of potassium from the cell (Pande & Mead, 1968; Connor et al, 1963). In the perfused rat heart FFA have been observed to uncouple oxidative phosphorylation, thus raising tissue oxygen requirements (Challoner & Steinberg, 1966b). Intermediates of FFA metabolism which accumulate in the heart subsequent to impaired oxidation may have a deleterious effect. Long-chain acetyl-coenzyme A esters increased in concentration (Shug et al, 1975; Liedtke et al, 1978) in the ischaemic myocardium and affected energy metabolism by inhibiting the transport of ADP and ATP across the mitochondrial membrane (Shug et al, 1975). Long-chain carnitine esters were also increased in the ischaemic myocardium (Liedtke et al, 1978) and have been shown to effect transport mechanisms in cardiac membranes (Wood et al, 1977). Opie (1975) quoted studies which suggested that the duration of the plateau of the action potential of the myocardium was dependent on cytoplasmic ATP produced by glycolysis, which would be reduced if FFA were the predominant exogenous

fuel. On this basis he suggested that raised FFA concentrations might shorten the duration of the plateau of the action potential in the ischaemic myocardium thus increasing the likelihood of ectopic activity, but he stressed that sufficient data was not yet available to support this possible mechanism.

PLASMA FREE FATTY ACIDS AND ARRHYTHMIAS AFTER ACUTE MYOCARDIAL INFARCTION - EXPERIMENTAL AND CLINICAL STUDIES

Free fatty acids and arrhythmias after acute myocardial infarction - clinical studies

Describing the elevation of FFA concentrations in 20 patients with AMI, Kurien and Oliver (1966) noted that of three patients dying on the day of admission one had the highest FFA concentrations recorded in the study (1.75 mM/l) and a fourth patient who died on the fourth day of admission had the second highest FFA concentration recorded (1.52 mM/l). The authors also gained the impression that arrhythmias developed more frequently in patients with very high serum-FFA levels, so a study was established to determine the relationship between serum-FFA concentrations and arrhythmias in patients with AMI (Oliver et al, 1968). Significantly more patients with serum-FFA concentrations of 1.2 mM/l or greater within the first 24 hours of AMI had arrhythmias of all kinds, including atrial arrhythmias, ventricular premature beats, second or third degree heart block, ventricular tachycardia, ventricular fibrillation and complete heart block, when compared with patients having lower FFA concentrations. The number of patients with the above arrhythmias increased significantly with increasing FFA concentrations. Hospital mortality was higher in patients with serum-FFA concentrations of 1.2 mM/l or greater and also increased significantly with increasing FFA concentrations.

Although Gupta et al (1969) found an increased incidence of serious cardiac arrhythmias and poorest prognosis in patients with AMI who had

initial plasma-FFA values greater than 1.0 mM/l, they could not establish a direct role for FFA in the production of arrhythmias because of the direct relationship found between plasma-FFA and urinary adrenaline excretion. There was no evidence to show that FFA concentrations did not merely reflect the acute metabolic disturbances in AMI, and the authors suggested that studies in which either FFA or catecholamine concentrations were reduced after AMI might have important clinical implications. In this same study plasma-FFA concentrations were artificially elevated to between 1.3 to 1.8 mM/l for up to 20 minutes by intravenous injection of heparin in 4 patients without the development of serious arrhythmias. A further clinical study which may support the findings of Oliver et al (1968) was that of Prakash et al (1972) who found that 6 of 9 patients with left ventricular failure, arrhythmias, shock or death after AMI had FFA values of 1.1 mM/l or higher, whilst all of the patients who had none of the above complications had plasma-FFA levels of less than 1.1 mM/l. All patients with complications were grouped together, so that the actual number of patients developing arrhythmias is unknown.

A number of clinical studies do not support the independent role of increased plasma-FFA concentrations in the production of arrhythmias after AMI. Rutenberg et al (1969) investigated 84 patients with AMI, most of them admitted within 8 hours of the onset of symptoms, and could find no relationship between the serum-FFA concentration on admission and subsequent arrhythmias. They found that patients with a persistently normal serum-FFA level were more likely to have an uncomplicated course, and that when complications occurred serum-FFA were likely to be elevated, but that these levels bore no relationship to the admission FFA concentration. They too suggested that increased FFA concentrations at the time of complications might merely be a reflection of the catecholamine response.

Likewise Hagenfeldt & Wester (1973) could find no association between plasma-FFA levels and arrhythmias.

In view of the suggestion by Kurien et al (1969) that administration of heparin to patients with AMI might not be safe because of its effect in enhancing lipolysis and elevating FFA concentrations, the effect of heparin administration on arrhythmias after AMI was investigated by Gupta et al (1969), Russo et al (1970) and Nelson (1970). In all cases standard therapeutic doses of heparin were used and in most cases peak plasma-FFA of well over 1.2 mM/l (and in some cases over 2.0 mM/l) were achieved with no increase in ventricular arrhythmias.

An association between the initial FFA concentration, taken within 24 hours of the onset of AMI, and serious ventricular arrhythmias was therefore found in three clinical studies (Oliver et al, 1968; Gupta et al, 1969; Prakash et al, 1972), but was not confirmed in two other studies (Rutenberg et al, 1969; Hagenfeldt et al, 1973). The limitations of all these studies are similar however. The admission FFA level may not reflect the peak FFA level after AMI, which might have occurred early (Vetter et al, 1974), before the patients were admitted to hospital. Alternatively, in view of the fluctuation of FFA levels after AMI (Oliver et al, 1968), the infrequent sampling may have resulted in subsequent peaks being missed. All patients in these studies were allowed to eat normally and were given medications as required, both being factors which may influence FFA levels. The absence of an association between the artificial elevation of plasma-FFA concentrations with heparin and arrhythmias, in the absence of a concurrent generalised metabolic response, as demonstrated by Gupta et al (1969), Russo et al (1970) and Nelson (1970) may also be explained. It is possible that catecholamines sensitize the myocardium to the effects of FFA - if so, elevation of plasma-FFA in isolation after AMI may not induce arrhythmias

at the level achieved. Alternatively, elevated FFA concentrations may merely reflect the generalised metabolic response to AMI and other factors, such as catecholamines, may be responsible for arrhythmias (Gupta et al, 1969; Oliver et al, 1968; Rutenberg et al, 1969).

Free fatty acids and arrhythmias after acute myocardial infarction - experimental studies

The results of the clinical trials are contradictory with respect to FFA and arrhythmias, but the clinical circumstances and possible errors due to the time of sampling as outlined above, may have contributed to these results. Much of the variability found in clinical studies may be eliminated in experimental studies, but nevertheless the relationship between plasma-FFA levels and arrhythmias after experimental myocardial infarction is equally confused.

Kurien et al (1969) produced serum-FFA concentrations of between 4.0 and 6.0 mM/l in dogs with experimental myocardial infarction by simultaneous infusions of Intralipid and heparin and found an increased frequency of cardiac arrhythmias. Neither Intralipid nor heparin infusion alone was associated with arrhythmias in the doses used. When the expected rise in FFA concentrations was abolished or reduced by pretreatment with protamine sulphate, there was a corresponding decrease in the frequency of ventricular arrhythmias. Protamine sulphate had no intrinsic anti-arrhythmic properties. The authors concluded that the association of raised serum-FFA concentrations and ventricular arrhythmias in dogs with experimentally produced myocardial infarction without an associated rise in catecholamines (though these were not measured) suggested a direct toxic effect of FFA on the myocardium.

In their carefully controlled definitive study in dogs (Kurien et al, 1971), the same authors made the following observations. Arrhythmias only occurred after elevation of FFA concentrations in the presence of myocardial

ischaemia or infarction, and FFA concentrations of 3.0 mM/l or more were generally required. The maximal frequency of ventricular ectopic beats occurred 10-30 minutes after the peak FFA concentrations were achieved, in keeping with an earlier suggestion (Kurien et al, 1969) that an intracellular effect of FFA rather than absolute plasma levels might be responsible for inducing arrhythmias. FFA concentrations had a cumulative effect, with a greater quantity or more severe arrhythmias being associated with prolonged elevation of FFA. Arrhythmias could be abolished by suppressing lipolysis. In the same study, noradrenaline was infused into dogs with raised FFA concentrations and the effect on arrhythmias was observed. The authors noted that after noradrenaline there were early arrhythmias which appeared to be due to the haemodynamic changes. Later arrhythmias occurred when the blood pressure and heart rate had stabilised, and could be abolished by inhibiting lipolysis and thus reducing FFA concentrations without reducing the noradrenaline levels. There was therefore considerable evidence for the independent effect of FFA in producing arrhythmias in dogs with myocardial infarction.

The clinical relevance of this study may be questioned in view of the much higher plasma-FFA concentrations required to produce arrhythmias in dogs than found in patients after AMI (Oliver et al, 1968). Furthermore, FFA are more loosely bound to albumin in dogs than in man, so that a much higher unbound proportion of FFA with consequent increased uptake by the myocardium will occur in the dog. Kurien and his co-authors argued that no analogy could be made between the toxic effects of high FFA concentrations in dog and man because the infarcting human myocardium was likely to have higher concentrations of triglyceride than the dog. Local release of noradrenaline in the myocardium of patients with AMI, which is higher than in dogs, has been shown to activate a lipase (Kruger et al, 1967), which could lead to lipolysis of stored intracellular triglycerides resulting in

a high intracellular FFA concentration which might only require a relatively small increment from circulating FFA to produce toxic effects. Experiments in which dog hearts were infiltrated with triglyceride prior to vascular occlusion made them more prone to early arrhythmias and more sensitive to the effect of catecholamines.

In order to counter the argument that Intralipid-heparin elevation of FFA concentrations does not reflect the mechanism of FFA elevation in AMI, Oliver and Yates (1971/72) used a technique whereby appropriate proportions of the individual fatty acids in a stable complex with albumin were infused into dogs with myocardial infarction. Arterial levels of FFA between 1.2 and 2.1 mM/l, similar to those in patients with AMI, were achieved and 6 of 9 dogs developed serious ventricular arrhythmias within the hour after infusion. This was only a preliminary report, however.

Three experimental studies have been reported which do not support the above findings. The first was that of Opie et al (1971b) where increased FFA concentrations of up to 6.8 mM/l in greyhounds with experimentally produced myocardial infarction were not associated with ventricular arrhythmias, except in 3 of 4 dogs given simultaneous infusions of adrenaline. Increased FFA uptake by the hearts in these experiments were shown by measuring arterio-venous differences and by histology. Differences in experimental technique, as detailed by the authors and also by Oliver and Yates (1971/72) and Oliver (1972), may explain the discrepancies in the findings of this study and those of Kurien et al (1969) and Kurien et al (1971). Elevation of FFA concentrations was induced in the same way and comparable levels (2.0 to 6.0 mM/l) were achieved. Whilst Kurien et al used mongrel dogs, which in many cases were undernourished, Opie et al used greyhounds, with their different life history and habits, physiological hypertrophy of the heart and possible different

feeding. Oliver and Yates (1971/72) suggested that Opie's experiments were carried out in circumstances of reduced sensitivity to induction of arrhythmias, the dogs having higher heart rates and higher serum potassium and albumin levels than those of Kurien et al (1971). Furthermore, the latter used the more physiological closed-chest preparation in which the main circumflex artery was occluded by a balloon catheter with stimulation of the arterial sympathetic nerves, whilst Opie et al (1971b) used an open-chest model requiring pericardiectomy in which a branch of the anterior descending artery was ligated with interruption of the arterial sympathetic nerves. Local catecholamine concentrations might have been increased in the studies of Kurien et al and decreased in those of Opie et al (Oliver 1972).

In a subsequent study by Opie and Lubbe (1975) in 27 closed-chest dogs with electrolytically induced thrombosis of a coronary artery and elevation of FFA with Intralipid and heparin, there was little difference in the incidence of ectopic beats when these dogs were compared with controls within one hour of vascular occlusion (50% and 41% respectively). It was not possible to precipitate serious arrhythmias by Intralipid-heparin elevation of FFA concentration 2 to 3 days post-thrombosis in 6 dogs.

Most et al (1976) found no significant increase in ventricular ectopic beats or more serious ventricular arrhythmias after Intralipid-heparin-induced FFA elevation in close-chest pigs with balloon-catheter occlusion of the left anterior descending artery. They presented evidence to support their argument that the coronary circulation of the pig resembles that of man more closely than the coronary circulation of dogs, and they attempted to induce arrhythmias in animals both with and without ventricular excitability prior to elevation of FFA concentrations. Ventricular fibrillation occurred in one of 17 pigs after elevation of FFA levels, but

otherwise FFA did not influence the appearance of arrhythmias. FFA concentrations of between 1.86 and 5.0 mM/l were achieved, but serum-albumin levels were not measured.

The experimental results of Kurien et al (1971) could not be reproduced by Opie et al (1971b), where marked differences in experimental design were apparent, nor by Opie and Lubbe (1975) in dogs or Most et al (1976) in pigs in studies where similar experimental methods were used. The FFA/albumin ratio was not monitored in any of these studies however, and their relevance to the clinical situation is questionable in view of the very high plasma-FFA concentrations (up to 6.0 mM/l) achieved, levels far higher than those previously demonstrated in man after AMI (Oliver et al, 1968). As in the clinical studies described above, experimental studies have produced conflicting evidence for the role of plasma-FFA concentrations in producing ventricular arrhythmias after acute myocardial infarction.

The effect of reducing circulating FFA concentrations by inhibition of lipolysis on ventricular arrhythmias after acute myocardial infarction

In view of the possible arrhythmogenic effects of FFA after AMI, Oliver et al (1972) suggested that lowering excess FFA levels might produce a less toxic metabolic environment, and showed that a nicotinic-acid analogue reduced plasma-FFA concentations both in healthy men and in patients with AMI. They proposed that clinical trials with nicotinic-acid analogues in patients with AMI might help to determine the relationship between high FFA concentrations and ventricular arrhythmias.

Rowe et al (1975) conducted a controlled double-blind trial of a nicotinic-acid analogue in 70 patients with AMI, having established its efficacy in lowering FFA concentrations in a previous study (Rowe et al, 1973). The results were not conclusive. Significantly fewer patients treated early (within 5 hours of onset of symptoms) whose FFA concentration

fell rapidly and then remained within normal limits for 20 hours had ventricular tachycardia when compared with patients whose FFA levels were not as rapidly or consistently reduced. In patients treated later (5 to 12 hours post-infarct), there was no difference in the incidence of ventricular arrhythmias between treated and control groups. In assessing their results, the authors recognised several limitations. The incidence of serious ventricular arrhythmias diminishes rapidly after the onset of AMI, so that in their group of patients admitted and treated within 5 hours there might have been an increase in early serious arrhythmias in the placebo group with bias in favour of the treated group. The nicotinic-acid analogue was not entirely satisfactory in that only 50% of patients treated early had adequate control, and the time taken to absorb the analogue was not known. There was also some evidence that the nicotinic-acid analogue acted by inhibition of lipolysis at the myocardial level, independent of plasma-FFA concentrations . The possibility that the analogue might have primary antiarrhythmic properties was considered, and further stressed by Opie and Lubbe (1975). An important observation in the study of Rowe et al (1975) was that plasma-catecholamine levels were not reduced by the nicotinic-acid analogue, so that its effect, if any, on the relationship between plasma-FFA concentrations and ventricular arrhythmias was independent of the effects of catecholamines.

Whilst otherwise inconclusive, this important pilot study showed a reduction in the number of patients with ventricular tachycardia when treatment with an agent which rapidly lowered FFA concentrations to within normal limits was initiated within 5 hours and continued for 24 hours. A subsequent experimental study (Smith & Duce, 1975) showed reduction in phase III arrhythmias after administration of a nicotinic-acid analogue and reduction of FFA concentrations in dogs. Whilst the clinical and

experimental evidence for the antiarrhythmogenic effect of elevated plasma-FFA after AMI is not conclusive, the available results of studies with nicotinic-acid analogues tend to swing the balance in favour of such an effect.

FREE FATTY ACIDS, EXTENSION OF MYOCARDIAL ISCHAEMIC INJURY AND OTHER COMPLICATIONS OF ACUTE MYOCARDIAL INFARCTION - EXPERIMENTAL AND CLINICAL STUDIES

Experimental studies

In vitro studies on isolated perfused rabbit hearts (Severeid et al, 1969) have shown a depressant effect of free fatty acids. Depression of contractility of rat papillary muscle by FFA was demonstrated by Henderson et al (1970b) in the presence of hypoxia and anoxia. Under these circumstances, glucose partially counteracted the depressant effect of FFA. FFA had no depressant effect in normally oxygenated papillary muscle in this study though such an effect was observed by Henderson et al (1970a) in experiments using beating rat hearts, but the possibility that these hearts were oxygen-limited could not be excluded however. Most et al (1972) were able to demonstrate a depressive effect of increased FFA levels on the isolated hypoxic rat papillary muscle.

Ventricular dilatation produced by controlled myocardial ischaemia in open-chest dog preparations was significantly increased by raising blood-FFA concentrations from a mean of 0.36 mmM/l to a mean of 3.69 mM/l (Kjekshus & Mjøs, 1972). Reduction of FFA concentrations to initial levels reversed the dilatation completely, suggesting that elevated FFA concentration impair myocardial function in the dog in vitro.

The same authors (Kjekshus & Mjøs, 1973) investigated the effect of plasma-FFA on the magnitude and extent of myocardial ischaemic injury, which was monitored by means of epicardial ST segment elevation. This

technique has been shown to provide rapid and reproducible determination of ischaemic injury in the same animal (Maroko et al, 1972). Using β-pyridyl-carbinol, an antilipolytic agent which inhibits catecholamine-induced lipolysis, in doses which did not alter haemodynamic status, it was found that both the extent and magnitude of ST elevation which occurred after coronary artery occlusion in open-chest dogs were reduced. β-pyridyl-carbinol abolished the additional elevation and extension of ST segments which occurred with FFA elevation or isoproterenol infusion after coronary artery occlusion. Pre-treatment with this antilipolytic agent prior to coronary artery ligation decreased the creatine kinase depletion of the resulting infarcted myocardium, which is a measure of the extent of myocardial necrosis (Kjekshus & Sobel, 1970; Maroko et al, 1971). Thus inhibition of catecholamine-mediated lipolysis decreased the extent of myocardial ischaemic injury in this study, but the effect on circulating FFA was not detailed so it is not known whether the beneficial actions of inhibition of lipolysis were peripheral or intracellular.

Most et al (1974) could not confirm an FFA-mediated change in the rate of diminution of the ST segments after myocardial infarction in closed-chest pigs. Elevation of FFA concentrations to nearly 3.0 mM/l at one hour or at 2 hours post-infarction did not alter the rate of fall of the elevated ST segments, whilst isoproterenol infusion at these times caused a significant rise in the ST segments. FFA concentrations rose significantly when isoproterenol was infused at one hour post-infarction, but fell when the infusion was carried out at 2 hours, so that it was difficult to argue that the isoproterenol effect was even partially mediated by raised FFA concentrations. FFA levels rose to peak values and back to control levels within one hour in all cases, so that the short duration of the FFA rise might have been responsible for the negative results.

Kjekshus (1974) carried out experiments to determine whether any antilipolytic-induced reduction in the extent and magnitude of ST segment elevation and diminution in myocardial CK depletion represented a sufficient salvage of myocardial tissue to improve ventricular function after experimental coronary occlusion. There was significantly less impairment of cardiac output in dogs pre-treated with the antilipolytic agent prior to coronary artery occlusion than in untreated dogs, but again FFA concentrations were not monitored.

Free fatty acid levels were assayed in a study to determine the effect of the antilipolytic p-Chlorophenoyisobutyrate (CPIB) on epicardial ST segment elevation in dogs after coronary artery occlusion and isoproterenol infusion (Mjøs et al, 1976). Pre-treatment with CPIB significantly reduced the rise in ST segments and FFA concentrations occurring after coronary artery occlusion alone and the much higher rise occurring after coronary artery occlusion with concurrent isoproterenol infusion. Reduction in circulating FFA concentrations was associated with a proportionate decrease in myocardial blood flow, so that the beneficial effect reflected in the diminished rise in arterial FFA concentrations, whose toxic effect was additional to that of the infused isoproterenol. The clinical significance of this study may be limited because there are several references, as quoted by the authors, to indicate that the antilipolytic effect of CPIB is considerably less in man than in animals. This may not be a contraindication to the use of CPIB in man if the main purpose of treatment is merely to reduce excess circulating fatty acids (Kjekshus, 1975), rather than to abolish lipolysis altogether.

Further evidence of FFA toxicity to the myocardium came from the study of Liedtke et al (1978) in which excess FFA concentrations caused significant impairment in myocardial function (specifically, in mean

aortic pressure, left ventricular systolic pressure, left ventricular work and epicardial motion) in both normal control and ischaemic pig hearts. Impairment in ischaemic hearts when FFA concentrations were elevated was significantly greater than that produced by the ischaemia alone.

Most of the experimental evidence indicates that raised FFA concentrations impair myocardial function after coronary artery occlusion, whether in isolated rabbit or rat hearts, or dog and pig hearts in vivo. Some of the evidence is presumptive, in that inhibition of lipolysis proved beneficial in studies where FFA concentrations were not measured. The evidence of FFA toxicity in non-ischaemic hearts is conflicting, as is the evidence that FFA elevation increases myocardial ischaemic injury as reflected in elevation of epicardial or precordial ST segment elevation.

Clinical studies

Two clinical studies have been carried out specifically to determine the effect of lowering plasma-FFA on precordial ST segment elevation in man with ischaemic heart disease (Luxton et al, 1976) and with acute myocardial infarction (Russell & Oliver, 1978). Precordial ST segment changes correlate well with changes in underlying myocardial ischaemic injury (Reid et al, 1971; Madias et al, 1975).

Luxton et al (1976) lowered plasma-FFA with a nicotinic-acid analogue in a double-blind cross-over study in 12 men with stable angina pectoris who were exercised to the point of pain. FFA concentrations were significantly lower in treated than in control patients from 30 minutes prior to exercise until 20 minutes after exercise was discontinued because of pain. ST segment elevation was similar in the treated and control groups prior to exercise, but rose significnatly higher in the control group during the 8 minutes after starting exercise. The duration of

exercise prior to onset of chest pain was not altered. The authors interpreted the diminution in ST segment elevation in treated patients as reflecting an improved myocardial metabolic milieu as a result of decreased FFA uptake by the myocardium. They could offer no explanation for the lack of correlation between ST segment elevation and pain, but noted that Borer et al (1975) had reported similar findings.

Patients treated with a nicotinic-acid analogue shortly after AMI (Russell & Oliver, 1978) showed an increased rate of decline of ST segment elevation in the absence of any change in heart rate or Q-wave evolution. FFA concentrations were rapidly and effectively reduced to within normal limits in association with the ST segment changes when optimal doses of the antilipolytic were given, though these resulted in an unacceptably high incidence of side-effects. Lowering FFA concentrations appears to modify myocardial ischaemia as monitored by ST segment elevation in man.

Correlation between FFA concentrations and the extent of myocardial necrosis, as reflected by raised plasma enzyme activity, has produced inconsistent results. No correlation was found between FFA levels and maximum SGOT activity (Kurien & Oliver, 1966) or creatine kinase activity (Oliver et al, 1968) after AMI. Subsequently Vetter et al (1974) found a correlation between mean FFA values within the first 5 hours of AMI and the subsequent peak creatine kinase ($r = 0.9$, $P<0.001$) and peak SGOT ($r = 0.62$, $P<0.01$) levels. The value of such correlations is questionable in view of the non-specificity of SGOT as a marker of myocardial necrosis, and the factors which influence CK activity in the blood after AMI, as described in detail in the section on estimation of myocardial infarct size by serial measurements of creatine kinase activity.

When first describing the rise in FFA concentrations after AMI, Kurien and Oliver (1966) noted no difference in the pattern of FFA levels

in patients with and without heart failure. Rutenberg et al (1969) attempted to predict the development of shock, congestive cardiac failure, and late deaths with the admission fasting FFA concentration in patients with AMI, but found no relationship. Though plasma-FFA concentrations were usually elevated at the time of these complications, it was felt that this was effect (possibly catecholamine-mediated) rather than cause. This study therefore failed to support the findings of Oliver et al (1968) that there was a correlation between FFA concentrations in excess of 1.2 mM/l and the incidence of shock and late deaths (though not with heart failure) after AMI. The time of admission after onset of symptoms were apparently similar in the two studies, but sampling for FFA level estimation was less frequent in the former study so that peak FFA concentrations might have been missed. Infrequent sampling was a feature of both these studies however, as it was in that of Gupta et al (1969) who showed daily fasting FFA concentrations in patients with left ventricular failure, cardiac failure or shock were significantly higher on the third, fourth and fifth days post-infarction than in patients without these complications. Again, it was impossible to deduce from the results whether the higher FFA concentrations were the cause or the effect of the complications.

As in the experimental studies, the results of clinical observations are conflicting and no clear association between elevated FFA concentrations, myocardial ischaemic injury and complications of AMI emerge. The effect of FFA in elevating and of antilipolytic agents in reducing the post-infarction rise in precordial ST segments in man is relatively conclusive. The interpretation of these results is not straight-forward however. If the degree of ST elevation correlates with the degree of underlying ischaemia (Reid et al, 1971; Madias et al, 1975) and raised FFA concentrations increase the degree and extent of ischaemia, an association with the incidence of heart failure and shock might be expected, since

these are clinical manifestations of the extent of impaired myocardial function (Braunwald, 1976). There is no evidence that in man elevated FFA concentrations increase the extent of myocardial ischaemia; thus increasing the degree of ischaemia in tissue which is already ischaemic might not appreciably impair the function of that tissue further. The lack of an association between FFA levels, heart failure and shock after AMI in most reports would therefore be understandable. Studies to date correlating FFA concentrations with the magnitude of myocardial necrosis, as reflected by plasma SGOT and CK activity, have not been extensive enough to draw any conclusions. Clinical evidence that elevated FFA concentrations impair myocardial function after AMI is not as strong as the experimental evidence.

CHAPTER VI

THE BEHAVIOUR OF PLASMA FREE FATTY ACIDS IN INDIVIDUAL PATIENTS IN THE FIRST 24 HOURS OF ACUTE MYOCARDIAL INFARCTION

INTRODUCTION AND SCOPE OF THE STUDY

A large number of clinical investigations have been carried out to determine whether there is a relationship between plasma free fatty acid concentrations and the occurrence of complications after acute myocardial infarction (Oliver et al, 1968; Gupta et al, 1969; Rutenberg, 1969; Prakash et al, 1972; Hagenfeldt & Wester, 1973; Mueller & Ayres, 1978). Despite the important clinical implications of some of the results, no study has yet been reported in which the behaviour of FFA concentrations in the early phase of AMI in individual patients has been analysed from the results of frequent serial measurements. Vetter et al (1974) carried out an intensive investigation of the metabolic disturbances in the first 5 hours of AMI, but all the studies referred to above, as well as the original study describing the rise in FFA after AMI (Kurien & Oliver, 1966) reported that FFA concentrations were elevated for much longer than 5 hours after the onset of symptoms.

The scope of this study was to carry out frequent FFA estimations in the first 24 hours of AMI in patients who, within practical limits, were not subjected to any treatment which might influence FFA levels. In this way the observed pattern of FFA rise and fall might lead to a better understanding of the relationship between FFA concentrations and complications after AMI, and might help in the evaluation of therapy to reduce FFA levels.

Patients and Methods

Fifteen patients admitted to the coronary care unit of Groote Schuur Hospital were studied. The criteria for selection were as outlined in Chapter I. All patients were starved for at least 12 hours after the onset of symptoms, and for longer where possible. Blood sampling, storage and assay of free fatty acids were carried out as described in

Chapters I and II. Statistical analysis was carried out using the coefficient of correlation and the Mann-Whitney U test (Appendix C).

RESULTS

Fifteen patients, 13 males and 2 females with a mean age of 53 years (range 37 - 69 years) were studied. Relevant biochemical details and drug therapy during the study period are shown in Table 1, where patients are listed in ascending order of peak FFA.

The FFA concentrations in the 24 hours after the onset of acute myocardial infarction are set out for each of the 15 patients in Table 2, and are shown graphically in Figs 1a,b,c. All patients showed a rise in FFA concentration within 12 hours of myocardial infarction above the upper limit of normal of 0.8 mM/l (Oliver et al, 1968). The subsequent fall was followed by a transient rebound in FFA concentrations in all but 5 patients (Numbers 14, 14, 15, 23 and 24) and the second peak was higher than the initial peak in patients numbers 18, 19 and 23. Most values remained below 1.0 mM/l after the first 12 hours and the majority of these were within the normal range. Mean FFA concentrations for all the patients are given in Table 2 and Figure 2.

FFA peaks of 1.5 mM/l or greater within the first 12 hours of acute myocardial infarction occurred earlier than FFA peaks of less than 1.5 mM/l (Table 1). The mean time of the peaks were 6 $\pm$ 1 hour (mean $\pm$ SEM) and 10 $\pm$ 0.4 hours after the onset of symptoms in the two groups respectively ($P = 0.002$, Mann-Whitney U test). The mean time lapse from onset of symptoms to arrival in hospital was 4 $\pm$ 0.7 hours in patients with peak FFA $\geq$1.5 mM/l and 5 $\pm$ 0.6 hours in those with peak FFA <1.5 mM/l.

The mean fall from peak FFA values in the 6 hours following the FFA peak in absolute figures and as a percentage are shown for all patients together and for the two subgroups with high and low FFA peaks in Table 3

and Figure 3. The major fall from peak FFA values occurred within the first 2 hours after the peak, irrespective of the height of the peak.

There was a correlation between the fall in FFA concentrations within the first 2 hours after the FFA peak and the peak FFA concentration itself (Figure 4, $r = 0.74$, $P<0.01$). The rate of fall of FFA levels in the 2 hours after the FFA peak was greater in patients with high than in patients with low FFA peaks (Figure 5, $P = 0.003$, $U = 7$, Mann-Whitney U test).

DISCUSSION

This is the first time that frequent, serial estimations of plasma-FFA concentrations in the first 24 hours after AMI have been reported in patients who were starved for at least the first 12 hours. In their original paper describing elevation of FFA concentrations after AMI, Kurien and Oliver (1966) analysed 2-hourly blood samples, starting as soon as possible after admission, but they only reported the results of between one and three samples taken on the first day in each patient, and only a brief description of the behaviour of mean FFA concentrations was given. Opie et al (1975) measured FFA levels 2-hourly for up to 66 hours from the time of admission in 9 patients with AMI, but the interval between onset of symptoms and admission in individual patients was not stated. No accurate assessment of the behaviour of FFA concentrations relative to the time of onset of symptoms in individual patients could therefore be made. Furthermore, their patients were immediately allowed free access to food, which was associated with a significant fall in FFA values in many cases. Vetter et al (1974) measured FFA concentrations hourly after AMI but only for the first 5 hours.

The importance of the first 24 hours of AMI lies in the fact that the major FFA rise occurs during this time. Elevation of FFA concentrations

may start as soon as 30 minutes after the onset of symptoms (Kurien & Oliver, 1966; Vetter et al, 1974), and elevated levels in individual patients have been noted at 36 hours post-infarction (Oliver et al, 1968) and possibly even later (Kurien & Oliver, 1966; Opie et al, 1975). In the present study FFA concentrations were above the upper limit of normal (0.8 mM/l, as described by Oliver et al, 1968) within 12 hours of AMI in all patients studied, and admission values were elevated in all but 4 patients. Admission values did not reflect subsequent values however. The major FFA rise occurred within 12 hours of the onset of symptoms in all but 2 patients, during which time a rapid fluctuation in FFA concentrations was noted. Highest peaks occurred early, thus emphasizing the importance of including only patients admitted very early after the onset of symptoms to any clinical trials involving FFA measurements. By 24 hours, FFA levels had returned to within normal limits in 9 of 14 patients who survived that period. Mean FFA concentrations (Figure 2) did not reflect the large individual variations which occurred.

These observations may partly explain some of the conflicting results arising from the studies in which FFA concentrations after AMI have been related to ventricular arrhythmias and other complications of AMI. Oliver et al (1968) estimated FFA concentrations twice in the first 24 hours of AMI, whilst Gupta et al (1969) and Prakash et al (1972) only analysed one sample during this time. Their results conflicted with those of Rutenberg et al (1969), who based their conclusions on FFA values taken on admission and again whilst the patients were fasting, and those of Hagenfeldt and Wester (1973) who took samples a maximum of four times in the first 24 hours, immediately prior to meals. Such infrequent sampling could not result in a true reflection of the FFA concentrations in the first 24 hours of AMI, as described in this study, and, in particular, FFA peaks may well have been

missed altogether.

Oral intake and administration of drugs may have an effect on FFA concentrations. Patients in this study were carefully selected to exclude patients with prior administration of heparin, catecholamines, steroids, clofibrate or intravenous glucose, all of which might influence plasma-levels of FFA (Oliver et al, 1968; Gupta et al, 1969). None of the patients had eaten from the time of onset of their symptoms, and all were starved for at least 12 hours post-infarction. Opie et al (1975) have shown a significant fall in FFA levels after every intake of calories exceeding 90 Kcals (378 KJ) on the first day after AMI. In the present study, 10 of the patients received food between 12 and 24 hours after the onset of symptoms, and in 7 of these patients (Numbers 14, 18, 21, 22, 24, 25 and 26) a subsequent fall, sometimes dramatic (Numbers 14, 21 and 22) was observed. The significance of these falls in relation to the intake of food must be considered in the light of the rapid fluctuations in FFA levels which were noted to occur spontaneously.

Of the drugs administered to the patients during the study period (Table 1) the β-blockers given to patients 14 and 19 and the diazepam administered to almost half the patients require further comment. β-adrenergic blockade has been shown to cause inhibition of peripheral lipolysis (Himms-Hagen, 1967; Whittington-Coleman et al, 1973) and can prevent increases in plasma free fatty acid concentrations (Pinker & Pattee, 1967; Imura et al, 1971; Taggart et al, 1973). In healthy, fasting, non-stressed subjects, β-blockers decreased free fatty acid serum levels by about 35% compared with placebo (Deacon, 1978) and antagonized isoprenaline induced increases in free fatty acid concentrations (Harms et al, 1978). The effect on FFA concentrations after AMI is less certain. Mueller and Ayres (1977) administered propranolol intravenously to 20 patients with AMI

and found an insignificant decrease in plasma FFA concentrations 20 minutes later, whilst in 11 selected patients, a small but significant decrease occurred (Mueller & Ayres, 1978). In the absence of definite data, an effect by β-adrenergic blockade on plasma FFA concentrations after AMI should probably be assumed.

Melsom et al (1976) found that patients sedated with diazepam after AMI had lower circulating catecholamines than a control group. Any catecholamine-mediated FFA rise due to the stress and anxiety of AMI, over and above that rise due to the generalised metabolic response (Opie, 1968; Oliver, 1972; Vetter et al, 1974) might therefore be reduced. In the same study, Melsom et al (1976) noted that FFA concentrations fell more rapidly in the first 4 hours in diazepam-treated patients than in the control group, and ascribed this more rapid fall to the effects of diazepam. This conclusion might be false however; diazepam-treated patients had higher initial FFA concentrations than control patients; in view of the results of the present study, it is possible that a naturally-occurring phenomenon, uninfluenced by diazepam, was observed by the authors. In view of the observations that catecholamines were lower in diazepam-treated patients after AMI, it could theoretically follow that catecholamine-mediated lipolysis, and thus circulating FFA concentrations, would be reduced, but there is no conclusive evidence that this in fact occurred.

In the present study, the major fall in FFA concentrations from peak levels within 12 hours of AMI had occurred by 2 hours after the peak, and was completed by 3 hours after the peak, irrespective of the height of the peak (Figure 3). There was a correlation between peak values and the fall in the subsequent 2 hours (Figure 4) and the fall in FFA concentrations was more rapid after a high peak than after a low peak (Figure 5). A possible interpretation of these results is that FFA concentrations might

rapidly reach similar levels after they have peaked, irrespective of the height of the peak. Thus in order to compare the effect of an agent designed to reduce FFA concentrations in one group of patients with a control group, it would be essential that initial FFA concentrations in the two groups were similar.

Table 1 CLINICAL AND BIOCHEMICAL DETAILS OF 15 PATIENTS FASTED FOR AT LEAST 12 HOURS AFTER THE ONSET OF AMI

Patient Number	Age	Sex	Time after onset of pain to admission (Hours)	Hours after onset of pain to peak FFA	Peak FFA	Fall in FFA in the 2 hours after the peak	Time after onset of pain to first meal (Hours)	Site of infarct	Drugs given in the first 24 hours
A. Patients with peak FFA <1.5 mmol/l in the first 12 hours after acute myocardial infarction									
13	39	M	4	10	0.87	0.18	20	Inferior	Frusemide 40 mg
25	55	M	6	10	0.98	0.07	18	Inferior	Morphine 15 mg Lignocaine 100 mg
14	69	M	7	10	0.99	0.02	18	Inferior	Morphine 5 mg Oxprenolol 20 mg Diazepam 15 mg Lignocaine 2.6 gm
18	37	M	4	6	1.05	0.12	18	Inferior	Nil
19	61	M	6	10	1.23	0.44	23	Inferior	Propranolol 80 mg Diazepam 10 mg Morphine 5 mg Lignocaine 2 gm
23	58	M	6	12	1.31	0.17	32	Inferior	Morphine 10 mg Lignocaine 2 gm Nitroprusside Methyldopa 750 mg Diazepam 10 mg Digoxin 0.25 mg Lasix 80 mg Allopurinol 300 mg Colchicine 1.5 mg
24	39	M	6	12	1.38	0.26	18	Anterior	Morphine 5 mg Moduretic x 2 Lignocaine 100 mg Disopyramide 150 mg Procainamide 500 mg
Mean (±SEM)	51 (±5) (37-69)		5(±0.6)	10(±0.4)	1.12(±0.07)	0.18(±0.05)	21 (±2)		
B. Patients with peak FFA ≥ 1.5 mmol/l in the first 12 hours after acute myocardial infarction									
15	66	M	4	8	1.57	0.06	24	Inferior	Lignocaine 1 gram Procainamide 100 mg Methyldopa 750 mg Frusemide 80 mg Nitrazepam 10 mg Diazepam 10 mg Digoxin 0.25 mg
22	54	M	7	7	1.58	0.23	12	Inferior	Warfarin 7.5 mg Morphine 5 mg
20	55	M	6	12	1.63	0.62	25	Inferior	Nil
17	64	M	2	4	1.68	0.99	24	Inferior	Atropine 0.6 mg Diazepam 15 mg Lignocaine 2 gms
21	48	F	6	7	1.79	1.08	12	Anterior	Diazepam 10 mg Frusemide 20 mg Morphine 10 mg
12	48	M	3	3	1.87	1.15	15	Inferior	Lignocaine 200 mg
16	41	M	2	6	2.05	1.01	23	Anterior	Morphine 20 mg Diazepam 10 mg Frusemide 40 mg Lignocaine 2 gms
26	58	F	3	4	2.92	0.95	16	Anterior	Morphine 5 mg Frusemide 20 mg K^+ supplements
Mean (±SEM)	54(±3) (41-66)		4(±0.7)	6(±1)	1.89 ±0.16	0.76 ±0.15	19±2		
Overall Mean (±SEM)	53(±3) (37-69)		5(±0.4)	8(±0.8)	1.53 ±0.14	0.49 ±0.11	20±1		

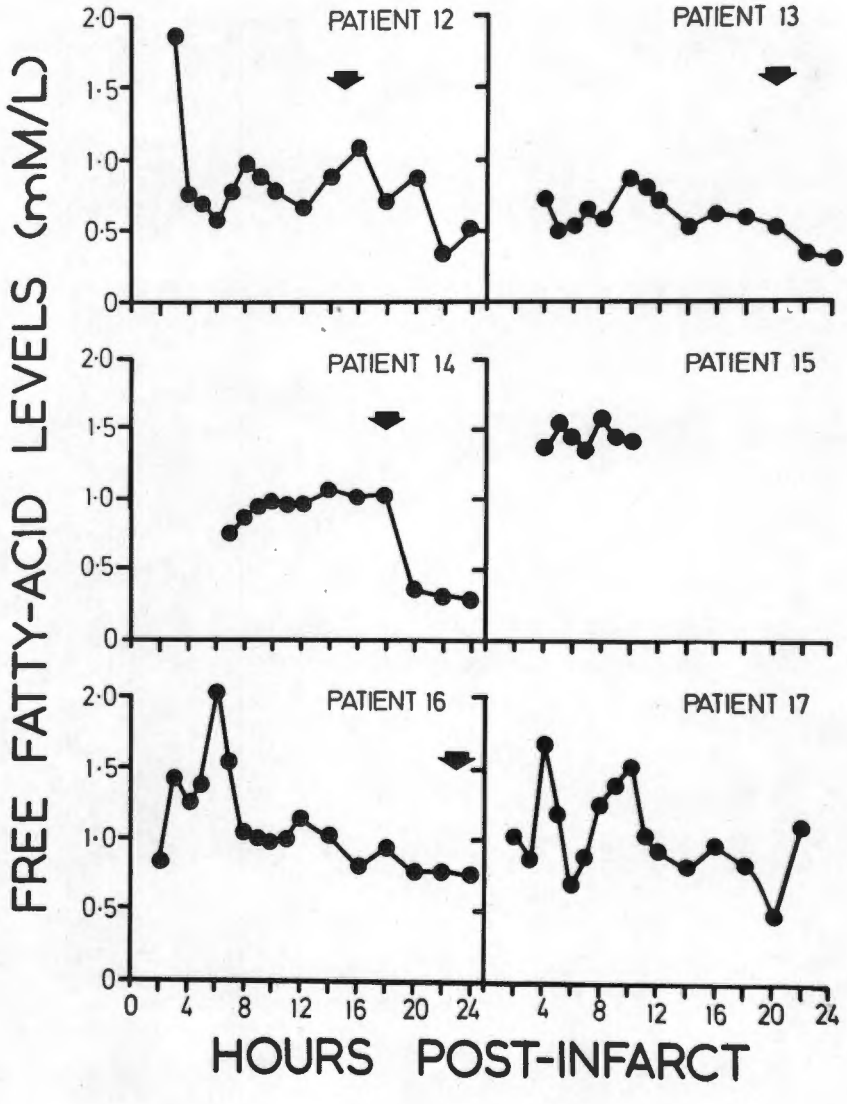


Figure 1a

FFA levels in the first 24 hours of AMI in patients fasted for at least the first 12 hours.

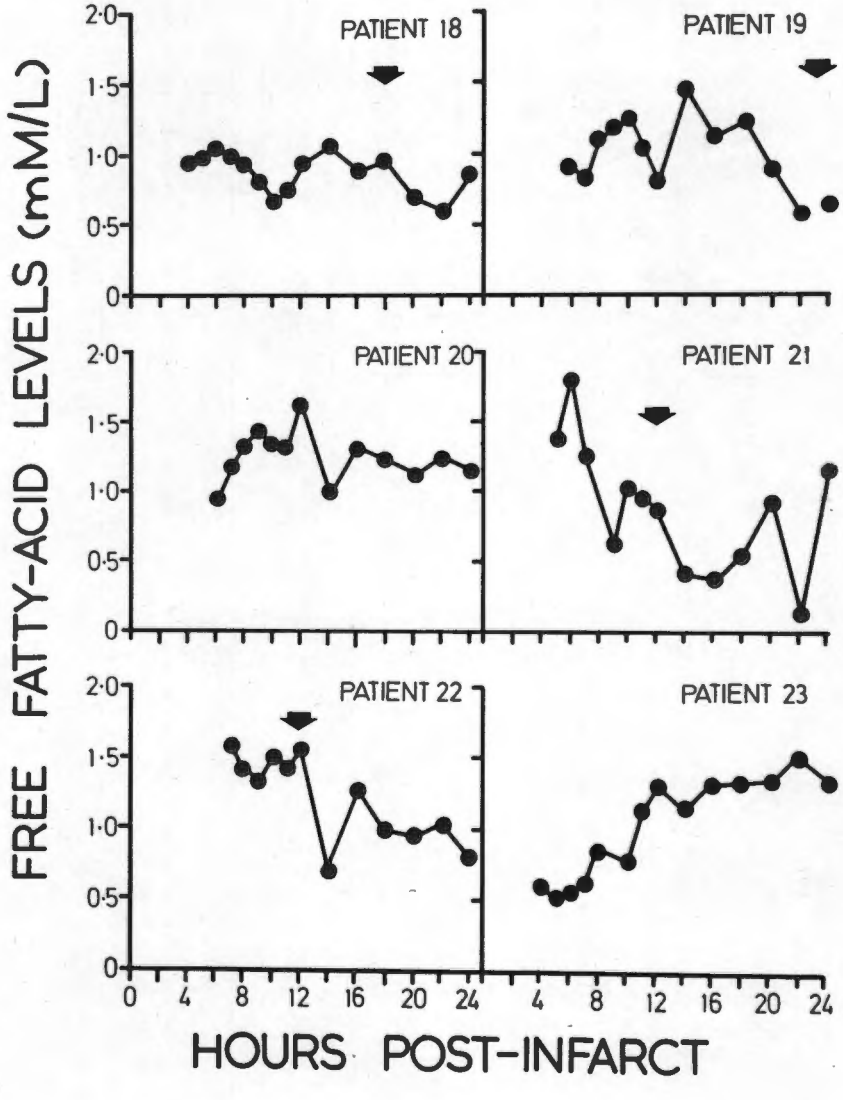


Figure 1b

FFA levels in the first 24 hours of AMI in patients fasted for at least the first 12 hours.

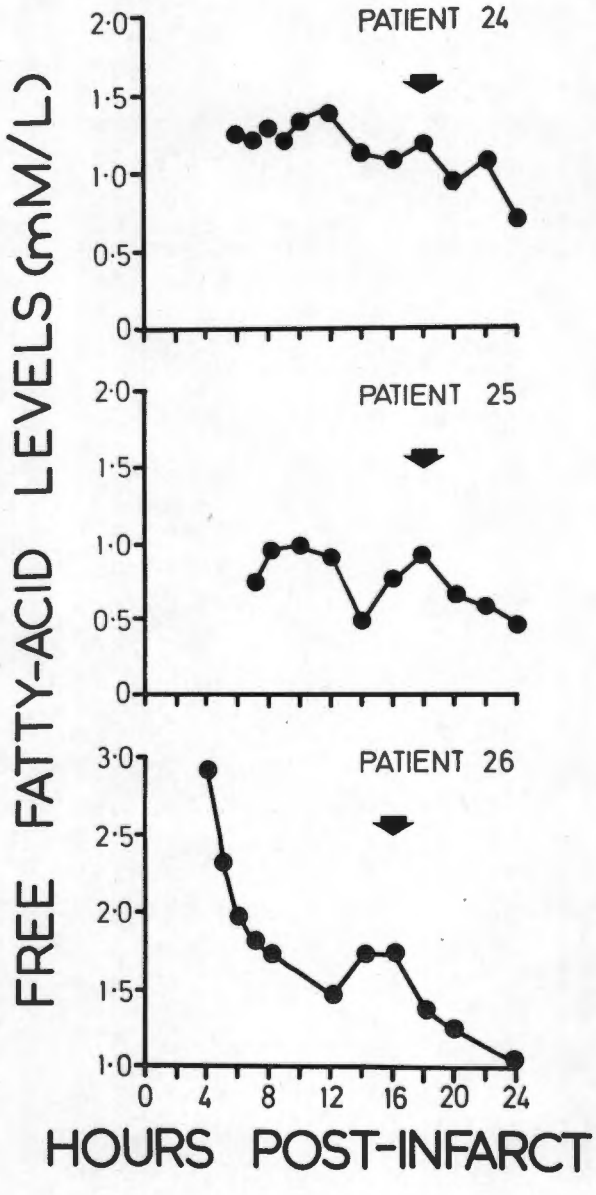


Figure 1c

FFA levels in the first 24 hours of AMI in patients fasted for at least the first 12 hours.

Table 2

PLASMA FFA CONCENTRATIONS IN 15 FASTED PATIENTS IN THE FIRST 24 HOURS OF AMI

Patient Number	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Mean
Hours post-infarct																
2	-	-	-	-	0.83	1.04	-	-	-	-	-	-	-	-	-	0.94±0.11
3	1.87	-	-	-	1.41	0.87	-	-	-	-	-	-	-	-	-	1.38±0.68
4	0.76	0.72	-	1.36	1.26	1.68	0.94	-	-	-	-	0.60	-	-	2.92	1.28±0.27
5	0.71	0.50	-	1.53	1.37	1.19	0.97	-	-	1.39	-	0.54	-	-	2.34	1.17±0.19
6	0.58	0.53	-	1.45	2.05	0.69	1.05	0.89	0.95	1.79	-	0.56	1.24	-	1.97	1.15±0.16
7	0.79	0.62	0.75	1.36	1.54	0.89	0.99	0.82	1.19	1.24	1.58	0.63	1.21	0.73	1.81	1.08±0.10
8	0.99	0.57	0.88	1.57	1.04	1.26	0.93	1.09	1.33	-	1.41	0.87	1.28	0.96	1.73	1.14±0.08
9	0.89	-	0.96	1.53	1.01	1.39	0.81	1.17	1.43	0.70	1.35	-	1.19	-	-	1.13±0.08
10	0.79	0.87	0.99	1.51	0.98	1.52	0.67	1.23	1.34	1.03	1.51	0.79	1.32	0.98	-	1.11±0.08
11	-	0.79	0.96	-	1.00	1.03	1.74	1.01	1.31	0.95	1.43	1.14	-	-	-	1.02±0.07
12	0.67	0.71	0.97	-	1.15	0.93	0.93	0.79	1.63	0.87	1.57	1.31	1.38	0.91	1.46	1.08±0.08
14	0.89	0.52	1.07	-	1.02	0.81	1.06	1.44	1.01	0.41	0.69	1.14	1.12	0.49	1.84	0.97±0.10
16	1.10	0,61	1.02	-	0.81	0.98	0.89	1.11	1.32	0.37	1.28	1.33	1.08	0.78	1.83	1.04±0.09
18	0.71	0.59	1.03	-	0.95	0.85	0.95	1.20	1.24	0.54	0.99	1.33	1.19	0.92	1.38	0.99±0.07
20	0.88	0.51	0.35	-	0.78	0.47	0.68	0.87	1.12	0.93	0.96	1.34	0.93	0.66	1.25	0.84±0.08
22	0.34	0.33	0.31	-	0.78	1.11	0.59	0.56	1.25	0.11	1.04	1.52	1.07	0.57	0.68	0.73±0.11
24	0.52	0.31	0.29	-	0.76	-	0.87	0.61	1.17	1.16	0.81	1.34	0.71	0.46	0.97	0.77±0.09

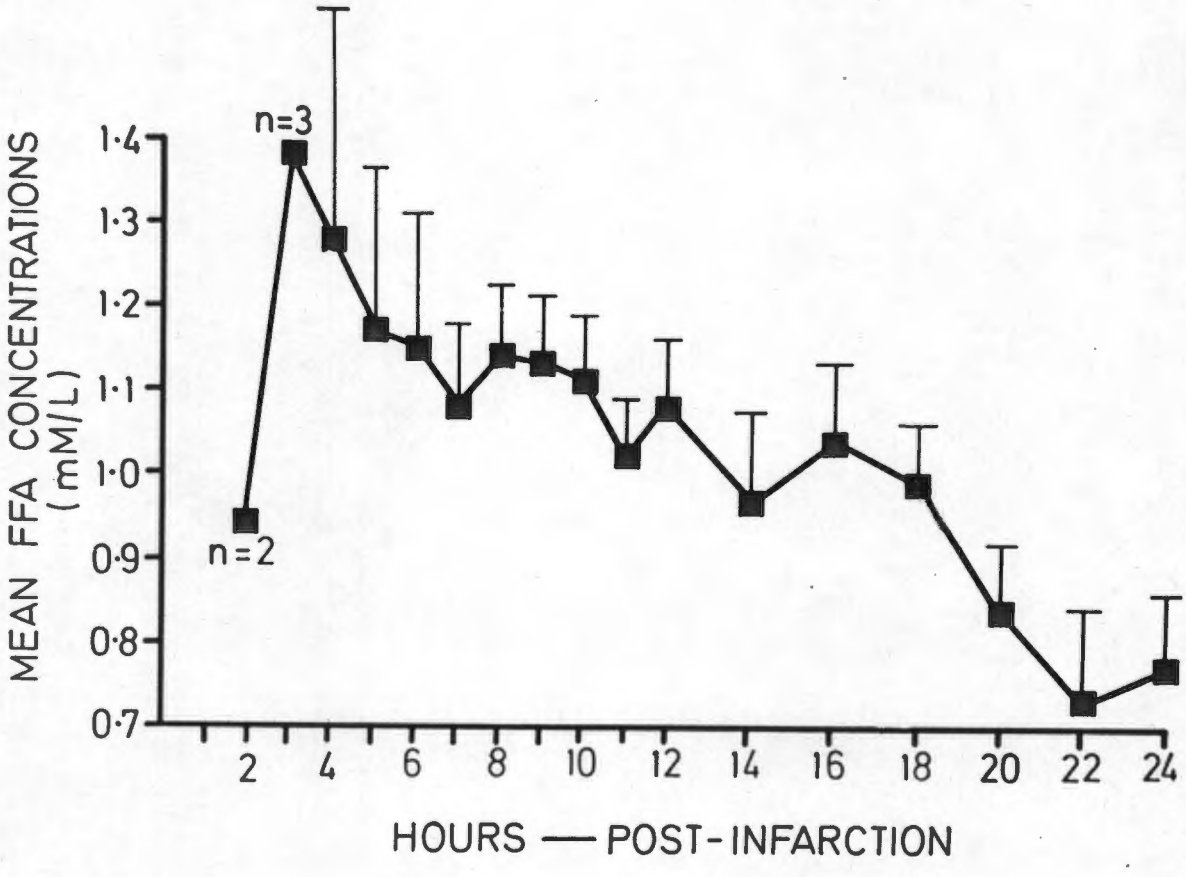


Figure 2

Mean FFA concentrations in the first 24 hours of AMI in 15 patients fasted for at least the first 12 hours.

Table 3 MEAN FALL AND PERCENTAGE FALL FROM PEAK FFA VALUES IN 15 FASTED PATIENTS AND THE TWO SUB-GROUPS WITH HIGH OR WITH LOW PEAK FFA VALUES

Hours after peak FFA	All patients				Peak FFA ≥ 1.5				Peak FFA < 1.5			
	n	Mean	SEM	% of peak	n	Mean	SEM	% of peak	n	Mean	SEM	% of peak
Peak	15	1.53	0.14	-	8	1.89	0.16	-	7	1.12	0.07	-
1	12	0.34	0.09	22	8	0.47	0.11	25	4	0.09	0.04	8
2	15	0.49	0.11	32	8	0.76	0.15	40	7	0.18	0.05	16
3	11	0.55	0.14	36	7	0.78	0.16	41	4	0.15	0.07	13
4	14	0.41	0.12	27	7	0.71	0.17	38	7	0.16	0.10	14
5	10	0.38	0.13	25	6	0.57	0.16	30	4	0.08	0.08	7
6	13	0.37	0.11	24	6	0.67	0.16	35	7	0.11	0.04	10

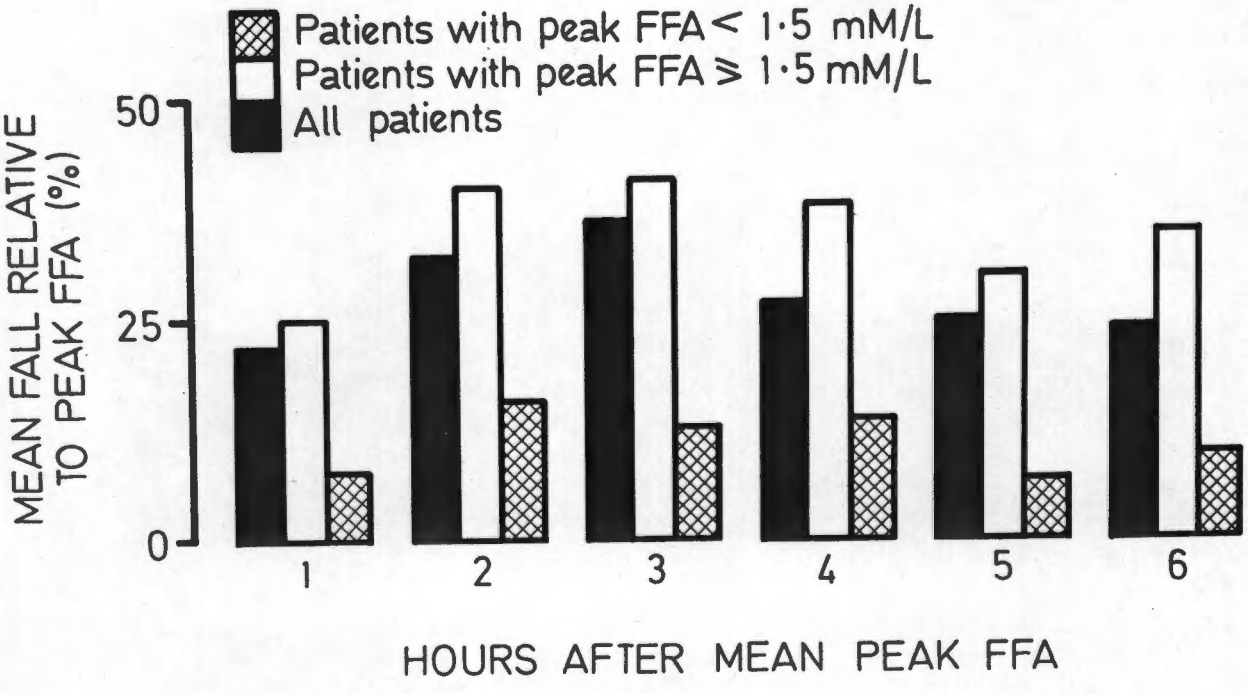


Figure 3

Percentage fall from mean peak FFA values in 15 patients fasted for the first 12 hours of AMI, according to whether peak FFA was greater or less than 1.5 mM/l.

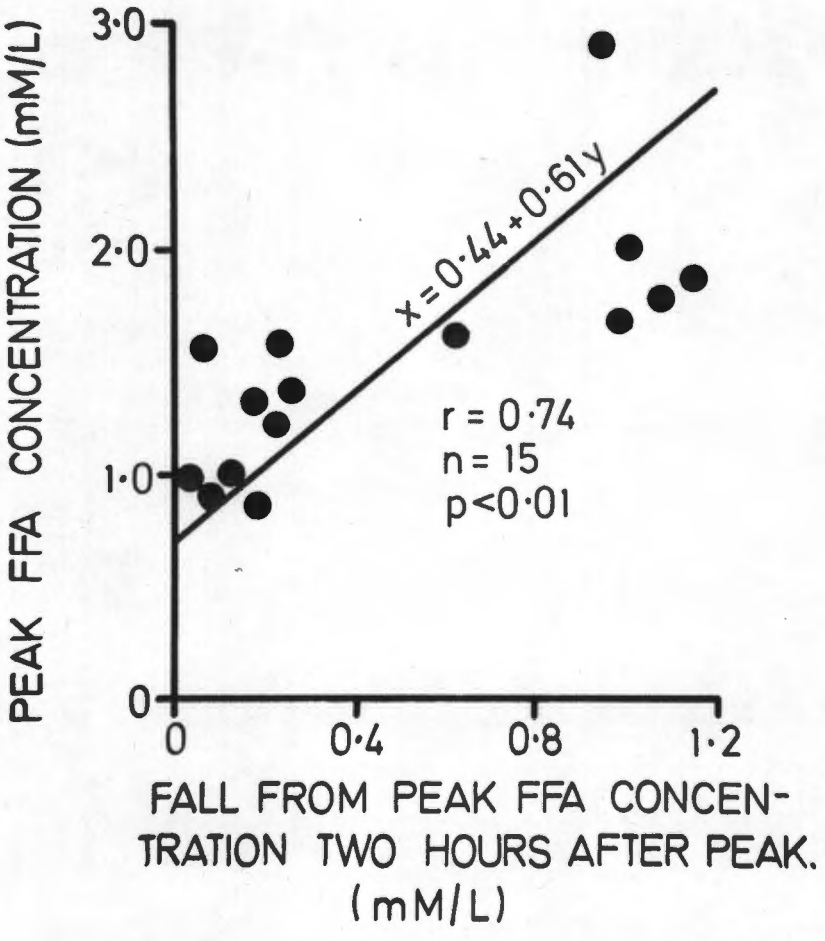


Figure 4

Correlation between the peak FFA and the fall in FFA levels in the subsequent 2 hours.

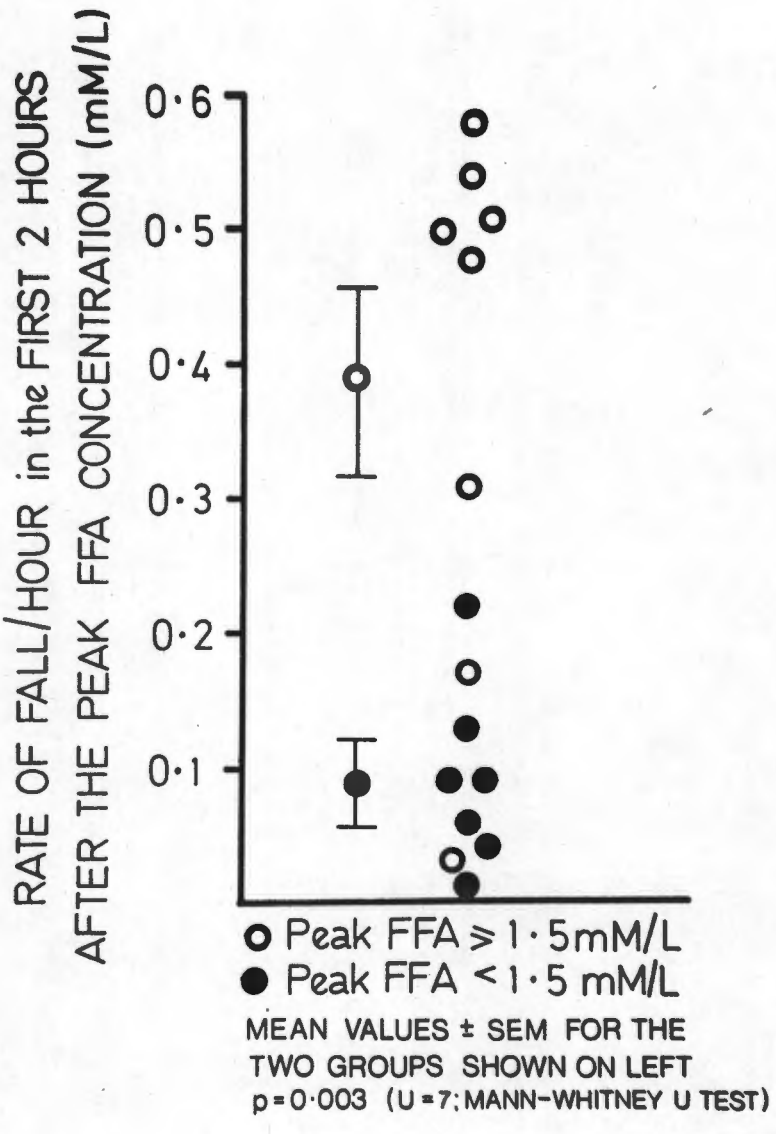


Figure 5

FFA rate of fall per hour in the 2 hours following the time of the peak in patients with peak FFA values greater or less than 1.5 mM/l. The rate of fall during this time was more rapid in patients with higher FFA peaks.

CHAPTER VII

THE RELATION OF COMPLICATIONS OF ACUTE MYOCARDIAL INFARCTION TO INFARCT SIZE AND TO FREE FATTY ACIDS

INTRODUCTION AND PURPOSE OF THE PRESENT STUDY

The method of estimating infarct size by serial measurements of plasma CK activity after AMI (Shell et al, 1971; Norris et al, 1975; Roberts et al, 1975c) provided a means of assessing the relationship between infarct size and prognosis in individual patients with AMI (Sobel et al, 1972). In this way, the efficacy of interventions designed to reduce infarct size could be tested. The validity of this method of sizing infarcts in assessing prognosis after AMI was reasonably substantiated by the strong associations found between enzymatically estimated infarct size and mortality (Sobel et al, 1972; Mathey et al, 1974; Shell & Sobel, 1976; Bleifeld et al, 1977), the degree of left ventricular dysfunction (Sobel et al, 1972; Kostuk et al, 1973; Mathey et al, 1974; Norris et al, 1975; Rogers et al, 1976; Bleifeld et al, 1976; Inoue et al, 1977; Hori et al, 1979) and the incidence of ventricular arrhythmias (Roberts et al, 1975c; Bleifeld et al, 1976; Cox et al, 1976; Ehsani et al, 1978). Similar comparisons were made in the present study as a measure of the validity of the enzymatically estimated infarct sizes as used extensively in the studies presented in this thesis.

In 1970 Kurien and Oliver put forward the hypothesis that there might be a metabolic cause for arrhythmias during acute myocardial hypoxia in which elevated FFA concentrations might play a major role. Support for this theory came from the association between elevated FFA and ventricular arrhythmias in patients with AMI found by Oliver et al (1968), Gupta et al (1969) and Prakash et al (1972), but Rutenberg et al (1969) and Hagenfeldt and Wester (1973) could not confirm these results. The correlation of high circulating FFA with shock and mortality after AMI also remains controversial (Oliver et al, 1968; Rutenberg et al, 1969). A feature of all these studies was infrequent blood sampling for FFA estimation in the

early phase of AMI, when FFA concentrations were likely to be high (Kurien & Oliver, 1966; Oliver et al, 1968). The purpose of this study was to correlate ventricular arrhythmias and other complications of AMI with FFA concentrations estimated frequently during the first 12 hours of AMI, so that no peak values would be missed and the mean FFA concentrations during this period could also be considered.

Patients and Methods

Thirty-six of the patients, 35 males and one female, were included in this study. Patient selection, blood sampling and storage, and biochemical analysis of plasma FFA and potassium concentrations and CK activity were carried out as described in Chapters I, II and III. Statistical analysis (Appendix C) was by the exact four-fold table test or Student's t test, as indicated in the text. Observations were made in all patients regarding the duration of chest pain, the occurrence of ventricular arrhythmias, the development of heart failure and the 6-month mortality after AMI; for the purpose of this study, these were collectively called the complications of AMI. The number of patients with these complications were compared according to infarct size index or free fatty acid concentrations in the first 12 hours post-infarction.

The FFA concentration of 1.2 mM/l was used as a dividing point because up to this level, with normal serum albumin levels, most of the FFA will be albumin-bound (Kurien & Oliver, 1970), whilst above this level, when the FFA/albumin molar ratio exceeds 2, the proportion of unbound FFA increases. Experimental studies (Spector, 1968; Willebrands et al, 1973) have shown that myocardial uptake of FFA increases when the FFA/albumin molar ratio is elevated so that unbound circulating FFA increase. In order to determine any association between very high FFA concentrations and the complications of AMI, a level of 1.5 mM/l was arbitrarily used as a

dividing point.

Treatment

All patients received intravenous morphine, metoclopromide or prochlorperazine as required, and oral diazepam was given three times daily to most patients. Arrhythmias were treated as stated below. Other essential treatment was initiated or continued as before, and included furosemide, β-adrenergic blockers, sodium nitroprusside, methyldopa, digoxin, allopurinol, colchicine, disopyramide, lignocaine, procainamide and warfarin.

None of this therapy has been shown to alter FFA concentrations with the exception of β-blockers, which were considered essential to the management of 4 patients (Numbers 9, 12, 19 and 35). The possible effects of β-blockade on FFA concentrations have been discussed in Chapter VI. Plasma potassium levels were measured at the first opportunity in all patients, and patients whose plasma potassium was less than 4.0 mM/l were given effervescent potassium supplements until a level of 4.0 mM/l was reached, unless the initial level was less than 3.5 mM/l, in which case intravenous potassium was given. No patients had a plasma potassium of less than 4.0 mM/l within, at the most, one hour of the recorded arrhythmias.

Calculation of infarct size index

ISI was calculated according to the method described in Chapter III, where details concerning infarct size measurements in patients 5, 6, 11 and 33 are also given. Patients were divided according to ISI <40 or ≥40 CK-g-eq/m^2 because of the significant differences in mortality and heart function found by Shell and Sobel (1976) in patients divided in this way.

Patient Number 11 had an extension of his infarction approximately 28 hours after the initial episode. Duration of chest pain on admission and ventricular arrhythmias in the first 24 hours could only be related to

the initial episode, whilst congestive heart failure and 6-month mortality were related to the total extent of infarction. Two figures are therefore given for ISI in Tables 1 and 2, the first relating to the initial infarct and the second to the combined initial infarct plus the extension (21 and 55 CK-g-eq/m^2, respectively).

Ventricular arrhythmias

Only ventricular arrhythmias occurring within 24 hours of the onset of symptoms were considered. Patients with ventricular fibrillation were not studied because of the effect of DC cardioversion on CK activity. The first episode of ventricular tachycardia (defined as a run of three or more consecutive ventricular premature beats), of second or third degree heart block or of a ventricular premature beat (VPB) rate of greater than 30 per minute lasting for at least one minute was recorded in each patient. Appropriate treatment was given immediately for ventricular tachycardia; a VPB rate of greater than 30 per minute was only treated if prolonged, if the R-on-T phenomenon was observed or if there were paired, or multifocal VPBs. The number of times each arrhythmia was observed, whilst noted, was not included in this study because of the varying efficacy of the anti-arrhythmic therapy. Arrhythmias were continuously monitored as described.

Duration of chest pain

Duration of chest pain was timed from the onset of symptoms until the last, effective dose of intravenous morphine was given in the coronary care unit. Many patients received analgesia prior to admission, and were subsequently treated as often as required to remain pain-free. In 11 cases the duration of pain was less than half an hour, having passed spontaneously or as a result of an analgesic, and in these cases the duration was recorded as zero hours.

Duration of CK release

The duration of CK release was measured from the time of onset of symptoms until the serum release curve reached a plateau (see example, Figure 1, Chapter III).

Heart Failure

Patients with heart failure were classified by the method of Killip and Kimball (1967) as described in Chapter I.

Post-mortem studies

Patients Numbers 1 and 5 underwent post-mortem examination and were reported as having well defined homogenous infarcts, both macroscopically and histologically.

RESULTS

Relevant clinical and biochemical data and the complications of AMI in the 36 patients studied are shown in Table 1. There was no correlation between the site of the infarct (anterior or inferior) and ISI, FFA concentrations, or any of the complications.

a. Infarct size index and complications

Twenty patients with ISI <40 CK-g-eq/m^2 were compared with 11 patients whose ISI was >40 CK-g-eq/m^2 (Table 2). Duration of CK release was greater in the patients with large than in the patients with small ISI (46 $\pm$ 5 and 34 $\pm$ 3 hours respectively, $t = 2.365$, $P<0.05$) but the time after onset of symptoms at which the peak CK release occurred was similar in the two groups. There was no difference in the duration of chest pain, the frequency of ventricular arrhythmias, or the 6-month mortality when the two groups were compared. Mean ISI in patients who died within 6 months was not significantly different from that in patients who survived (47 $\pm$ 12 and 30 $\pm$ 4 CK-g-eq/m^2 respectively). Elimination of patients with previous infarcts made no difference to these results. Mean ISI in 4 patients

with newly developed heart failure was larger (74 ± 16 CK-g-eq/m^2) than in 25 patients who did not develop heart failure (31 ± 5 CK-g-eq/m^2, t = 3.3, P<0.01). Whilst all patients with newly developed heart failure had ISI ≥40 CK-g-eq/m^2, and none of the patients with ISI <40 developed heart failure, these differences were not statistically significant.

b. Free fatty acid concentrations and complications

Patients were compared according to peak FFA concentrations (Table 3) and mean FFA concentrations (Table 4) within 12 hours of AMI. There was no difference in duration of chest pain, 6-month mortality, or the frequency of ventricular arrhythmias or heart failure when groups of patients divided according to the following peak FFA concentrations were compared: ≤1.2 mM/l and >1.2 mM/l, <1.5 mM/l and ≥1.5 mM/l, and ≤1.2 mM/l and ≥1.5 mM/l (Table 3).

Mean FFA concentrations correlated strongly with peak FFA concentrations in the first 12 hours of AMI (r = 0.797, P<0.001, Figure 1), but peak concentrations were significantly higher than mean concentrations and only 12 of the 20 patients with peak FFA concentrations greater than 1.2 mM/l had mean FFA concentrations greater than 1.2 mM/l during this time.

Significant differences were found when mean FFA concentrations in the first 12 hours of AMI were considered (Table 4 ,Figure 2). More patients with mean FFA concentrations greater than 1.2 mM/l had ventricular tachycardia as compared with patients whose mean FFA concentrations were 1.2 mM/l or less (83% and 35% respectively, P<0.05, Figure 2). All 12 patients with mean FFA concentrations greater than 1.2 mM/l had ventricular or heart block compared with 8 of the 23 patients with mean FFA concentrations of 1.2 mM/l or less (P<0.002). No significant differences were observed for any of the other complications.

DISCUSSION

This study comprised two distinct parts: the correlation of specified complications of AMI with infarct size, expressed as the infarct size index, and with plasma FFA concentrations in the first 12 hours after onset of symptoms. The former part of the study was not intended to explore new ground, but to compare the results with those of previously reported studies as a measure of the validity of this method of sizing infarcts. The conflicting results require explanation.

Of greater importance is the second part of the study, as this was the first time that regular, serial estimations of plasma FFA concentrations had been made within 12 hours of the onset of AMI and correlated with the incidence of ventricular arrhythmias and other complications.

Infarct size index and complications of AMI

No correlation was found between infarct size index and the duration of chest pain after the onset of symptoms of AMI, contrary to the findings of Mathey et al (1975) and Inoue et al (1977). There are good theoretical grounds for believing that the duration of chest pain might be related to the extent of infarction because, though the exact mechanism of cardiac pain remains unknown (Inoue et al, 1977), it has been observed that pain ceases once the ischaemic myocardium has died (Friedberg, 1966). Continuing pain therefore suggests continuing ischaemia, though it does not follow that all ischaemic tissue will ultimately undergo necrosis. Mathey et al (1975) observed that patients with a longer duration of CK release had larger infarcts and chest pain persisting longer than patients with a short duration of CK release. Inoue et al (1977) reported similar findings: prolonged chest pain was associated with a longer duration of CK release, total CK release being greater in these patients and the major release of CK occuured during chest pain. They noted that CK release was

shorter in patients who had suffered a previous infarction, but elimination of such patients did not alter the results of the present study. A number of factors may have been responsible for the present results. The efficacy of analgesia given prior to admission was difficult to assess, as chest pain might have subsided spontaneously. Analgesia given in this hospital might have been more effective and have lasted longer than in the other studies. Pain is a very subjective sensation, so the possibility that it was over-reported by some and under-reported by other patients, even under careful direct questioning, must be considered. The patients in the present study who had larger infarcts need not necessarily have had prolonged ischaemia, even though larger infarcts were associated with a longer duration of CK release than smaller infarcts ($P<0.05$). This observation may be explained by the experimental findings of Cairns et al (1978) that homogeneous infarcts were associated with a later cumulative CK plateau than scattered infarcts. In contrast, necropsy in patients with short duration of CK release and chest pain in the study of Mathey et al (1975) revealed homogeneous infarcts, whilst patients with prolonged CK release and pain showed a heterogeneous composition of the infarcted myocardium. If myocardial infarction in all the patients studied here was the result of rapid necrosis without prolonged ischaemia, the lack of prolonged chest pain might be explained. This possibility is supported by the fact that the time of peak CK release occurred at similar times after the onset of infarction in patients with large and patients with small ISI. In such cases the intensity rather than the duration of chest pain might be relevant. An attempt to define the intensity of chest pain, using the criteria of Waagstein and Hjalmarson (1975) was abandoned owing to the lack of objectivity which was noted in several patients during the acute phase of their infarction. Retrospective

assessment could not be made in many patients owing to partial amnesia brought on by heavy sedation.

Patients in this study who died within 6 months did not have significantly larger ISI than those who survived. Patients with shock were excluded from the study because of the large increase in extracardiac CK noted in such patients, and the only comparable reports are those of Sobel et al (1972) and Shell and Sobel (1976), where patients with shock were also excluded. In both studies patients with large infarcts (EIS $\geq$65 GK-g-eq and ISI $\geq$40 CK-g-eq/m^2 respectively) had a significantly higher 6-month mortality (67% and 68% respectively) than patients with smaller infarcts (5% and 2% respectively). No explanation for the conflicting results can be given - overall mortality in the three studies was similar (27%, 23% and 32% respectively) and the method of estimating infarct size was identical. However, Bleifeld et al (1977), who included patients with shock, concluded that infarct size alone, calculated from serial CK measurement, could not predict mortality. In an earlier study (Mathey et al, 1974) the same authors had found that the combination of haemodynamic changes and infarct size was able to predict immediate and long-term prognosis; in this study haemodynamic measurements were not made, which with the small numbers involved, may explain the lack of agreement between the present and previous reports.

Fall-off in ventricular function in relation to loss of viable myocardium is an accepted sequence (Braunwald, 1976), and has been demonstrated in several studies. Loss of 32% and 40% of the left ventricle resulted in cardiogenic shock (Harmarayan et al, 1970; Page et al, 1971) and the early development of shock in patients with AMI was associated with larger areas of necrosis at post-mortem than in patients who developed shock late (Caulfield et al, 1976). The degree of left ventricular

impairment, as assessed by haemodynamic measurements (Kostuk et al, 1973; Mathey et al, 1974; Shell & Sobel, 1976; Hori et al, 1979) and by the clinical manifestations of heart failure (Sobel & Shell, 1972; Norris et al, 1975; Bleifeld et al, 1976), correlated well with the extent of estimated infarction. In this study 6 patients had heart failure, but in 2 it was pre-existing. Mean ISI in the 4 patients with new failure was higher than in the 25 patients with no failure (74 and 31 CK-g-eq/m^2 respectively, $P<0.01$), and all the former patients had ISI greater than 40 CK-g-eq/m^2.

Ventricular arrhythmias were not significantly more common in patients with large than with small infarcts. Ventricular ectopic beats occurring at a rate of more than 30 per minute were seen with equal frequency in both groups, both patients with second or third degree heart block had small infarcts, and though ventricular tachycardia was seen more frequently in patients with large than with small infarcts, the difference was not significant, nor was mean ISI in 17 patients with VT greater than mean ISI in 14 patients without (41 ± 8 and 26 ± 5 CK-g-eq/m^2 respectively). Comparison with other studies is difficult. Both Roberts et al (1975) and Cox et al (1976) studied the frequency of premature ventricular beats and the amount of therapy required to treat them and found a positive correlation with infarct size index. Only the first episode of prolonged and frequent (>30 beats/min) ventricular ectopic activity was recorded here however; this may not reflect either overall ventricular ectopic activity or the peak rate of activity, and its value as an index of ventricular irritability may be questioned. Roberts et al (1975) found a significant correlation between the incidence of ventricular tachycardia and coupled ventricular ectopic beats, taken together, and ISI. The incidence of each individual arrhythmia was not recorded so no

comparison can be made with the present study, where the incidence of coupled VPBs was not recorded. All that can positively be stated as a result of this study is that VT and infarct size were not significantly related, although proportionately more patients with VT had larger infarcts. Early treatment of VPBs in some patients might have prevented the development of VT, and spontaneously remitting VT might have occurred prior to admission, but otherwise it is very unlikely that any episode of VT was missed. Infarct size per se might not be entirely responsible for the genesis of arrhythmias however, and concurrent metabolic and haemodynamic disturbances might also play a part.

Plasma free fatty acid concentrations and complications of AMI

The lack of any correlation between duration of chest pain, 6-month mortality, heart failure and ventricular arrhythmias with single peak FFA concentrations within 12 hours of infarction, whether above or below 1.2 mM/l or 1.5 mM/l, may have a very fundamental explanation. Transient FFA peaks greater than 1.2 mM/l, or even 1.5 mM/l, need not result in manifestations of myocardial toxicity if FFA concentrations rapidly fall again and to levels where all circulating bound FFA can once again be bound by albumin. The overall duration of elevation FFA concentrations might be more pertinent. This could explain the conflicting results of previous experimental studies. Oliver et al (1968), Gupta et al (1969) and Prakash et al (1972) found an association between raised FFA concentrations after AMI and some of the complications of AMI, particularly ventricular arrhythmias, but these results could not be confirmed by Rutenberg et al (1969) or by Hagenfeldt and Wester (1973). A feature common to all these studies was infrequent sampling for FFA estimation, as described in detail in Chapter VI.

Mean FFA concentrations in the 12 hours post-infarction were studied

because this was the duration of FFA elevation in most patients and because, as argued above, it was felt that any adverse effects of FFA would be most likely to appear when FFA concentrations were consistently high. FFA levels were estimated as soon as the patient was admitted to hospital and then hourly, where possible, or at the most 2-hourly. The number of estimations within 12 hours of infarction varied from 4 to 11 (mean $\pm$ SEM 7.0 $\pm$ 0.3). The correlation between mean and peak FFA concentrations in the first 12 hours of AMI was very strong ($r = 0.797$, $P = <0.001$, Figure 1), but peak values were significantly higher than mean values and only 12 of the patients with peak FFA greater than 1.2 mM/l had mean FFA greater than 1.2 mM/l. Patients with mean FFA concentrations greater than 1.2 mM/l (high mean FFA concentrations) in the first 12 hours post-infarction were therefore compared with patients with mean values of 1.2 mM/l or less (low mean FFA concentrations).

A possible positive association between duration of chest pain after AMI and the degree of FFA elevation was sought in view of the suggestion by Opie (1971) that pain might result in anxiety which might in turn result in further elevation of plasma FFA concentrations. No such association was found, though early and effective sedation with oral diazepam and intravenous morphine might have been the cause. Melsom et al (1976) found reduced catecholamine excretion in patients sedated with diazepam after AMI; since catecholamines can activate the release of FFA into the circulation (Gordon & Cherkes, 1956; Carlson et al, 1965; Pinter et al, 1967) any reduction in anxiety-mediated catecholamine release may prevent an associated rise in FFA concentration. Any anxiety-related rise in FFA might be dependent on the severity rather than the duration of chest pain, but the severity was not measured, for reasons stated above. Thus, although no relation between chest pain and FFA concentrations were

apparent after AMI in this study, the results were not conclusive.

Mean FFA concentrations did not correlate with heart failure or 6-month mortality. Although none of the previous clinical studies found relationship between FFA concentrations and heart failure (Kurien & Oliver, 1966; Oliver et al, 1968; Rutenberg et al, 1969; Gupta et al, 1969), FFA concentrations were higher in patients who were more seriously ill (Oliver et al, 1968; Gupta et al, 1969; Allison et al, 1969). Oliver et al (1968) were able to correlate FFA concentrations in excess of 1.2 mM/l with the incidence of shock and late deaths, but these findings were not confirmed by Rutenberg et al (1969), despite their observation that FFA concentrations were elevated at the time when patients developed shock or heart failure. The authors suggested that FFA elevation at these times was effect, possibly catecholamine-mediated, rather than cause.

Of possible clinical significance was the very strong association observed between serious ventricular arrhythmias (ventricular tachycardia and second and third degree heart block) in the first 24 hours of AMI and mean plasma FFA concentrations greater than 1.2 mM/l in the first 12 hours (Figure 2). These findings correspond with those of Oliver et al(1968), Gupta et al (1969) and Prakash et al (1972), and contradict those of Rutenberg et al (1969) and Hagenfeldt and Wester (1973), but the possible influence of infrequent sampling in those studies, as described above, may make any comparisons invalid. FFA concentrations were closely monitored, but for short periods, in three studies (Gupta et al, 1969; Russo et al, 1970; Nelson, 1970) where heparin was infused in normal therapeutic doses in patients with AMI in order to detect whether the ensuing FFA rise would provoke ventricular arrhythmias, as had been suggested might happen by Kurien et al (1969). Peak plasma FFA of well over 1.2 mM/l were achieved in most patients, with no increase in ventricular

arrhythmias. These studies were not conclusive however, and comparison with the FFA rise provoked by AMI may be invalid for two reasons. First, the duration of the study period was short (the longest period reported was two hours by Russo et al (1970), whilst after AMI abnormally high FFA concentrations may be found for up to 12 hours, if not longer (Kurien & Oliver, 1966; Oliver et al, 1968). Second, AMI provokes a generalised metabolic response (Opie, 1971) of which elevation of FFA concentrations is only one part. FFA elevation in isolation might therefore not be sufficient to provoke arrhythmias, and there is some evidence that circulating catecholamines, which also increase after AMI (Gazes et al, 1959; McDonald et al, 1969; Siggers et al, 1971; Christensen & Videbaek, 1974; Vetter et al, 1974) may sensitize the myocardium to the effects of FFA, as shown in experimental (Opie et al, 1971; Opie, 1975) and clinical (Simonsen & Kjekshus, 1978) studies.

In view of the high mortality and morbidity associated with AMI, which represents the most common serious health problem of contemporary western society (Braunwald, 1976), no available therapeutic approach should be neglected. The association between high FFA levels and arrhythmias observed in this study, lends support to the hypothesis that FFA play a role in the genesis of cardiac arrhythmias after AMI (Kurien & Oliver, 1970). In view of the reduction in serious, ventricular arrhythmias after the initiation of early, effective antilipolytic (Rowe et al, 1975) the inclusion of such therapy might be justified as an integral part of the management of all patients with AMI.

Table 1

CLINICAL AND BIOCHEMICAL DATA AND COMPLICATIONS OF ACUTE MYOCARDIAL INFARCTION

Clinical Data			Biochemical Data							Complications					
												Ventricular arrhythmias			
Patient Number	Age (years)	Site of infarct	ISI (CK-g-eq/m^2)	(Hours) Duration of CK release	Time of peak CK release rate (Hrs post-infarct)	Peak FFA (mM/l)	Peak FFA/ albumin molar ratio	Mean FFA during 1st 12 hours ± SEM (mM/l)	n	Duration of chest pain (Hours)	Congestive heart failure (Killip Class)	VPB (>30/min)	VT	2° or 3° heart block	Death within 6 months
1	43	Inferior	91	73	17	2.39	3.5	1.24±0.17	11	2	-	+	+	-	8 days
2	48	Anterior**	10	36	14	1.35	2.4	0.78±0.13	8	0	III*	+	+	-	-
3	49	Inferior	69	46	10	2.23	3.8	1.20±0.24	6	3.5	II	-	-	-	-
4	53	Anterior	-	-	-	1.07	1.9	0.70±0.38	5	5	-	-	-	-	-
5†	69	Inferior	52	-	-	2.58	4.5	1.62±0.15	10	3.5	III	-	+	-	15 hours
6†	50	Inferior**	<20	28	9	0.47	1.0	0.31±0.03	8	0	-	-	-	-	-
7	66	Inferior	20	48	10	1.96	3.3	1.34±0.11	8	0	-	-	+	-	-
8	51	Inferior	16	28	10	0.86	1.6	0.72±0.05	7	1	-	+	-	-	-
9¶	46	Anterior**	14	18	8	1.20	2.0	0.63±0.10	8	1	-	+	+	-	-
10	33	Inferior**	25	36	8	1.20	1.7	0.58±0.08	9	5	-	+	+	-	4 months
11†	43	Inferior**	21-55	20	10	1.23	2.1	1.05±0.06	6	3	II	+	-	-	3 months
12	48	Inferior**	-	-	-	1.87	3.2	0.89±0.13	9	0	-	+	-	-	-
13	39	Inferior	4	22	10	0.87	1.4	0.66±0.05	8	3	-	-	-	-	-
14¶	69	Inferior**	-	-	-	0.99	1.9	0.92±0.04	6	0	-	-	+	-	-
15	66	Inferior**	31	54	8	1.57	2.5	1.47±0.03	7	2	-	+	+	-	-
16	41	Anterior	119	40	14	2.05	3.5	1.24±0.10	11	2	II	+	+	-	5 months
17	64	Inferior	68	54	18	1.68	2.9	1.14±0.09	11	3	-	+	+	-	3 months
18	37	Inferior	30	36	10	1.05	1.7	0.89±0.04	9	0.5	-	-	-	-	-
19¶	61	Inferior	21	44	14	1.23	2.3	1.00±0.06	7	0.5	-	+	+	-	-
20	55	Inferior	34	40	18	1.63	2.9	1.31±0.08	7	8	-	-	-	+	14 days
21	48	Anterior	55	40	14	1.79	3.4	1.14±0.14	7	0	-	+	-	-	-
22	54	Inferior	18	50	10	1.58	2.5	1.48±0.04	6	0	-	-	-	+	-
23	58	Inferior**	9	24	8	1.14	2.0	0.81±0.10	8	2	-	+	-	-	3 months
24	39	Anterior	41	54	10	1.38	2.3	1.27±0.03	6	0	-	-	+	-	-
25	55	Inferior	23	56	18	0.98	1.7	0.89±0.06	4	2	-	+	-	-	-
27	54	Anterior**	66	60	9	1.59	2.4	1.50±0.04	6	5	-	+	+	-	-
28	58	Anterior	46	40	9	1.58	2.3	1.25±0.08	9	11	-	-	+	-	-
29	58	Anterior	40	16	9	1.49	2.2	1.05±0.12	5	2.5	-	-	-	-	-
30	53	Anterior**	13	32	10	1.16	1.8	1.04±0.04	5	1	-	+	-	-	-
31	37	Inferior	64	40	7	1.64	2.5	1.32±0.07	8	2	-	-	+	-	-
32	55	Inferior	25	40	9	-	-	-	-	1	-	-	-	-	-
33†	68	Inferior**	<20	18	11	1.0	1.6	0.77±0.05	8	4	-	+	+	-	-
34	61	Not known	10	30	8	1.55	2.3	1.46±0.05	4	0	II*	+	+	-	6 months
35¶	63	Inferior	10	22	12	0.77	1.8	0.55±0.08	7	3	-	+	+	-	3 months
36	37	Anterior**	-	-	-	0.81	1.4	0.51±0.15	4	0	-	-	-	-	-
37	40	Inferior	-	-	-	0.68	1.0	0.51±0.09	7	0	-	+	-	-	-

¶Patients receiving beta-blockers. ** Patients with previous infarcts. †Details regarding calculation of EIS and ISI in these patients are in the text. *Both these patients had heart failure prior to admission.

Table 2 INFARCT SIZE INDEX AND COMPLICATIONS OF ACUTE MYOCARDIAL INFARCTION

ISI	No. of patients	Mean age ± SD	Mean duration of CK release (Hours)	Mean No. hours after onset of symptoms of peak CK release	Mean duration of chest pain (Hrs± SEM)	Number with arrhythmias				Number with new heart failure	Number of deaths within 6 months
						VPBs (>30/min)	VT	2° or 3° heart block	VT or 2° or 3° heart block		
Total	31	52±10 (33-69)	38±3	11±0.6	2.3±0.44	18(58%)	17(55%)	2 (6%)	19(61%)	6 (19%)	10(32%)
< 40	20	54± 9 (33-68)	34±3	11±0.7	1.9±0.46	12(60%)	9(45%)	2(10%)	11(55%)	* 0/19	*5/19 (26%)
≥ 40	11	50±10 (37-69)	46±5	12±1	3.1±0.90	6(55%)	8(73%)	0	8(73%)	* 4/12 (33%)	*5/12 (42%)
P		NS	<0.05 (t=2.365)	NS	NS	NS	NS	NS	NS	NS	NS

*Proportion of patients with large and with small ISI changed because of patient No.11. (See patients and methods).

Table 3 PEAK FFA CONCENTRATIONS IN THE FIRST 12 HOURS POST-INFARCTION AND COMPLICATIONS OF ACUTE MYOCARDIAL INFARCTION

	Peak FFA (mM/l)	Number of patients	Mean age ±SD	Mean duration of chest pain (Hrs±SEM)	Number of arrhythmias				Number with new heart failure	Number of deaths within 6 months
					VPBs >30/min	VT	2° or 3° heart block	VT or heart block		
	Total	35	52±10	2.2±0.42	20(57%)	18(51%)	2 (6%)	20(57%)	4(11%)	10(29%)
1	≤ 1.2	15	50±11	1.8±0.46	9(60%)	5(33%)	0	5(33%)	0	3(20%)
2	> 1.2	20	53±10	2.4±0.65	11(55%)	13(65%)	2(75%)	15(75%)	4(20%)	7(35%)
3	< 1.5	20	54±10	1.7±0.38	12(60%)	8(40%)	0	8(40%)	1 (5%)	4(20%)
4	≥ 1.5	15	54±10	2.8±0.82	8(53%)	10(67%)	2(13%)	12(80%)	3(20%)	6(40%)
	P	1 vs 2	NS	NS	NS	NS	NS	NS	NS	NS
	P	3 vs 4	NS	NS	NS	NS	NS	NS	NS	NS
	P	1 vs 4	NS	NS	NS	NS	NS	NS	NS	NS

Table 4 MEAN FFA CONCENTRATIONS IN THE FIRST 12 HOURS POST-INFARCTION AND COMPLICATIONS OF ACUTE MYOCARDIAL INFARCTION

Mean FFA within 12 hours of onset of symptoms	Number of patients	Mean age ±SD	Mean duration of chest pain (Hrs±SEM)	Number with arrhythmias				Number with new heart failure	Number of deaths within 6 months
				VPBs >30/min	VT	2° or 3° heart block	VT or heart block		
Total	35	52±10	2.2±0.42	20(57%)	18(51%)	2 (6%)	20(57%)	4 (11%)	10 (29%)
≤ 1.2	23	51±10	1.7±0.35	15(65%)	8(35%)	0	8(35%)	2 (9%)	5 (22%)
> 1.2	12	54±11	2.96±1.0	5(42%)	10(83%)	2(17%)	12(100%)	2 (17%)	5 (42%)
P		NS	NS	NS	<0.05	NS	<0.002	NS	NS

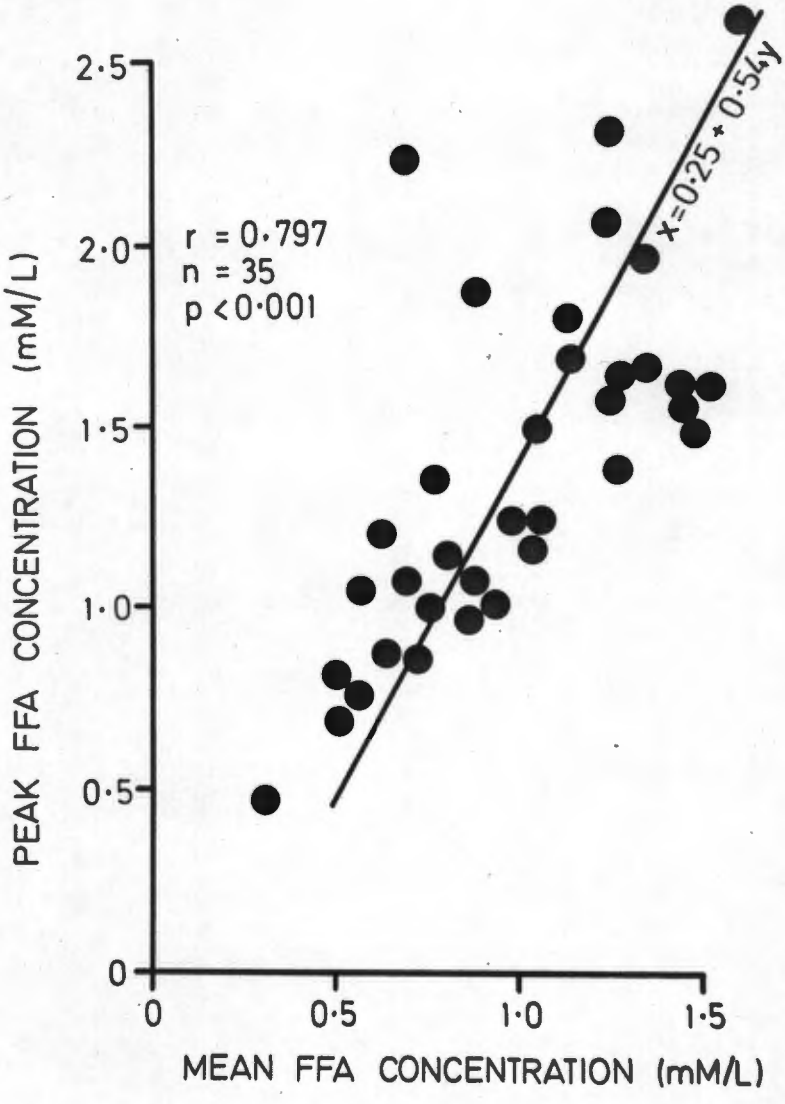


Figure 1

Correlation between peak and mean FFA concentrations in the first 12 hours of acute myocardial infarction.

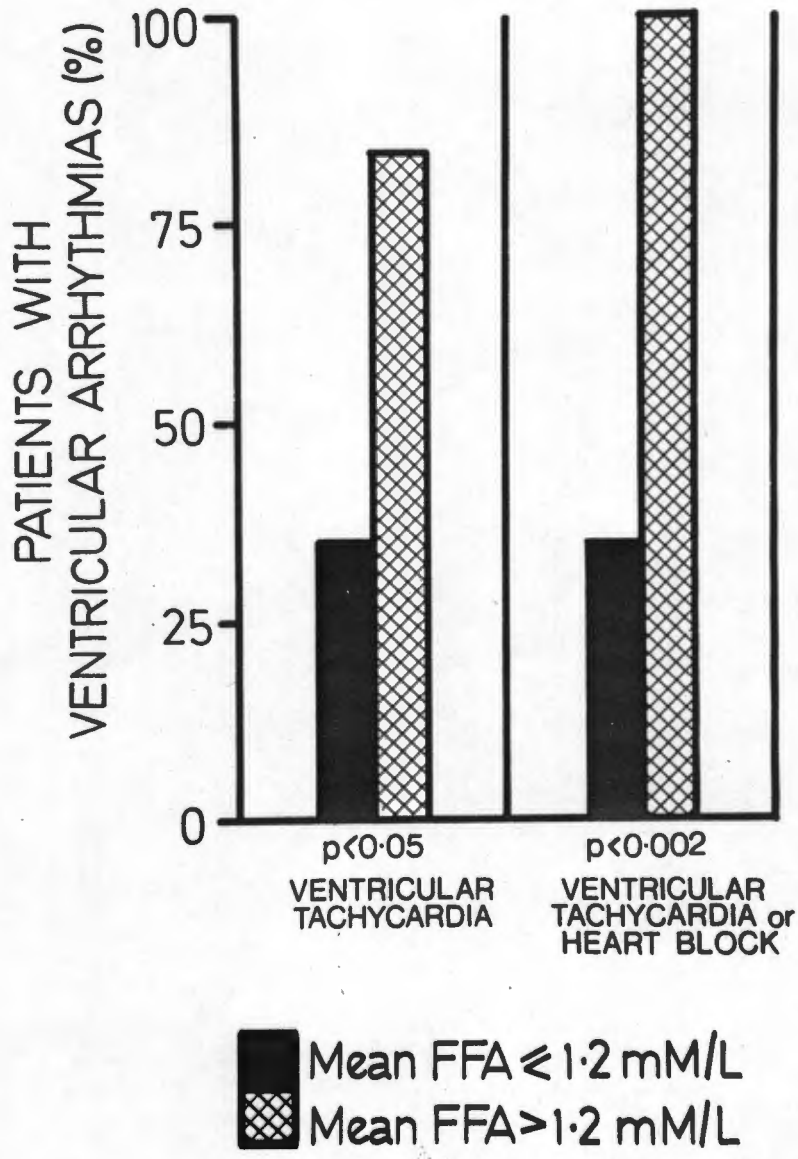


Figure 2

Percentage of patients with ventricular tachycardia or heart block (2° or 3°) according to whether mean FFA >1.2 mM/l or ≤1.2 mM/l in the first 12 hours of AMI.

CHAPTER VIII

THE RELATION BETWEEN PLASMA FREE FATTY ACIDS IN THE FIRST 12 HOURS OF ACUTE MYOCARDIAL INFARCTION AND INFARCT SIZE - A PROPOSED METABOLIC VICIOUS CIRCLE

INTRODUCTION AND PURPOSE OF THE STUDY

Myocardial infarct size, as measured by the enzymatic method of Shell et al (1971), has been reported as influencing prognosis (Sobel et al, 1972; Mathey et al, 1974; Shell & Sobel, 1976; Bleifeld et al, 1977) and morbidity (Sobel et al, 1972; Norris et al, 1975; Roberts et al, 1975); Bleifeld et al, 1976; Cox et al, 1976) after acute myocardial infarction. Other studies (Maroko et al, 1972; Pierce et al, 1973; Maroko & Braunwald, 1973; Maclean et al, 1978) have shown that myocardial infarction is a dynamic process, which can be modified by therapy initiated early enough after the onset of symptoms. Thus the identification of any factor which may have a harmful effect on the ischaemic myocardium would have important prognostic and therapeutic implications.

Plasma free fatty acids may be one such factor. Concentrations were raised in patients with AMI (Kurien & Oliver, 1966; Oliver et al, 1968; Janusjewicz et al, 1971; Christensen & Videbaek, 1974), within one hour of the onset of symptoms (Kurien & Oliver, 1966; Vetter et al, 1974). Experimental evidence suggests that elevated FFA concentrations may increase both the degree and the extent of myocardial ischaemia (Kjekshus & Mjøs, 1973) as monitored by epicardial ST-segment elevation and that treatment to reduce plasma FFA concentrations may reduce the degree of ischaemia (Mjøs et al, 1975) and the extent of necrosis (Kjekshus & Mjøs, 1973) after coronary artery ligation in the dog. Clinical studies have shown that reduction of FFA levels can reduce the degree of myocardial ischaemia, as monitored by precordial ST segment elevation, in patients with ischaemic heart disease (Luxton et al, 1976) and in patients with acute myocardial infarction (Russell & Oliver, 1978). Whilst there is evidence that FFA can influence the degree of myocardial ischaemia in patients, information concerning the

relationship between FFA concentrations and the extent of myocardial necrosis is inadequate. No correlation was found between FFA levels and maximum SGOT activity (Kurien & Oliver, 1966) or maximum CK activity (Oliver et al, 1968) after AMI, though Vetter et al (1974) subsequently found a correlation between mean FFA concentrations in the first 5 hours of AMI and the subsequent peak CK and SGOT levels.

The purpose of this study, therefore, was to investigate the relation between enzymatically estimated infarct size and the raised plasma FFA concentrations found in the early stages of AMI in man.

Patients and Methods

Twenty-eight patients, selected according to the criteria described previously and in whom both infarct size and free fatty acid estimations were available were included in this study. Biochemical analysis for CK activity and FFA concentrations, and calculations of estimated infarct size, infarct size index, total cumulative CK release and FFA/albumin molar ratio were carried out as detailed in previous chapters. Statistical analysis was by Student's t test or by the Mann-Whitney U test as appropriate (Appendix C).

Patient number 5 died before infarct size could be estimated enzymatically, and infarct size was therefore determined by weight at post-mortem, as described in Chapter III. Patient number 11 had an extension of his initial infarct approximately 24 hours later; in this study FFA concentrations within the first 12 hours were correlated with the initial infarct only.

The patients were divided into two groups according to estimated infarct size or infarct size index. Patients were considered to have had large infarcts if EIS was 65 CK-g-eq or greater and if ISI was 40 CK-g-eq/m^2 or greater, because of the significantly higher mortality in these patients

as compared with patients with EIS less than 65 CK-g-eq or ISI less than 40 CK-g-eq/m^2 (Sobel et al, 1972; Shell & Sobel, 1976). All patients with EIS $\geq$65 CK-g-eq had ISI $\geq$40 and vice versa.

Ten of the patients (numbers 1, 2, 3, 5, 7, 8, 9, 10, 11 and 35) had a mean oral intake of 256 Kcal given as sucrose, a mean of 6 hours after the onset of symptoms. Mean peak FFA in these patients was similar to mean peak FFA in the remaining 18 patients, who had been deliberately fasted for at least 12 hours after the onset of symptoms (mean 1.56 $\pm$ 0.21 and 1.44 $\pm$ 0.07 mM/l respectively) so all the patients were considered together.

RESULTS

Eleven patients had large infarcts and 17 patients small infarcts. The relevant details concerning EIS, ISI, total cumulative CK release, peak CK activity, peak and mean FFA concentrations, and FFA/albumin molar ratios are given in Table 1.

Patients with large infarcts had significantly higher mean FFA concentrations (Table 2) than patients with small infarcts (Table 3) from 3 to 7 hours after the onset of symptoms of AMI (Figure 1). Mean values were similar after 12 hours and had fallen to within the normal range (<0.8 mM/l, Oliver et al, 1968) by 24 hours.

Mean peak FFA concentrations and FFA/albumin molar ratios within 12 hours of AMI were higher in patients with large than with small infarcts ($t = 4.46$, $P<0.001$ and $t = 3.8$, $P<0.001$ respectively), as were mean FFA values within 12 hours ($t = 4.44$, $P<0.001$).

There were strong positive correlations between peak FFA in the first 12 hours and EIS ($r = 0.68$, $P<0.001$, Figure 2), ISI ($r = 0.71$, $P<0.001$; Figure 3), total cumulative CK release ($r = 0.78$, $P<0.001$, Figure 4) and

peak CK activity ($r = 0.74$, $P<0.001$). Weaker correlations were noted between the mean FFA concentrations in the first 12 hours and EIS ($r = 0.43$, $P<0.05$), ISI ($r = 0.46$, $P<0.02$), total cumulative CK release ($r = 0.46$, $P<0.02$) and peak CK activity ($r = 0.44$, $P<0.05$).

DISCUSSION

This is the first time that free fatty acid concentrations in the early phase of AMI have been studied in relation to the extent of myocardial necrosis. Apart from enzymatically estimated infarct size and infarct size index, total cumulative CK release and peak CK activity were used as less direct measurements of the extent of necrosis. Total cumulative CK release bore a direct relationship to the amount of CK depleted to the myocardium and hence to the extent of necrosis (Shell et al, 1971), and was positively correlated with EIS and ISI in this study ($r = 0.95$). Peak CK activity was included so that comparisons could be made between the results of this study and those of Oliver et al (1968) and Vetter et al (1974). Peak CK activity is theoretically less likely to reflect the extent of myocardial necrosis than the total cumulative CK release because it takes no account of the amount of CK cleared from the circulation prior to the peak value occurrence, or of the amount of CK released after the peak. There was nevertheless a positive correlation between EIS and peak CK activity in this study ($r = 0.8$).

The results of this study showed a positive correlation between infarct size and both peak and mean plasma FFA concentrations in the first 12 hours of AMI. Patients with large infarcts had significantly higher mean and peak FFA concentrations and peak FFA/albumin molar ratios than patients with small infarcts. FFA levels during the first 12 hours of AMI were used because this was noted to be the period when the major FFA rise occurred, and although FFA concentrations above the upper limit of

normal were noted up to 24 hours post-infarct, most of these were well below 1.2 mM/l, the level at which the FFA/albumin molar ratio exceeds 2 and the unbound proportion of circulating FFA may start to increase (Kurien & Oliver, 1970).

Two possible explanations for the close relations observed between FFA and infarct size are that larger infarcts increase circulating FFA concentrations or that raised FFA levels increase infarct size. There is evidence to support both sequences.

The size of a myocardial infarct has been shown to correlate well with the subsequent degree of left ventricular dysfunction, which has in turn been associated with increased circulating catecholamine levels. Increased catecholamine secretion plays a major role in stimulating lipolysis, leading to increased circulating FFA concentrations. Sobel et al (1972) found significantly higher mean estimated infarct sizes in survivors of acute myocardial infarction with clinical left ventricular failure than in those with minimal or no failure. Patients with pulmonary oedema or congestion on chest X-ray had twice the mean cumulative CK release of patients who were free from congestion (Norris et al, 1973). Inoue et al (1977) found that patients without clinical failure had a shorter period of CK release than patients with failure. Using haemodynamic criteria, infarct size was shown to correlate well with impaired left ventricular function (Kostuk et al, 1973; Mathey et al, 1974; Rogers et al, 1976; Bleifeld et al, 1976; Inoue et al, 1977; Hori et al, 1979).

Heart failure may, in turn, further increase the circulating catecholamine levels, which are initially raised as part of the general metabolic response to AMI (Gazes et al, 1959; McDonald et al, 1969; Siggers et al, 1971; Griffiths & Leung, 1971; Januszewicz et al, 1971;

Lukomsky & Uganov, 1972; Christensen & Videbaek, 1974; Vetter et al, 1974). Lukomsky and Uganov (1972) were able to link enhanced blood-catecholamine values with left ventricular failure, whilst Gupta et al (1969) found a higher urinary excretion of noradrenaline and adrenaline of the first day of AMI in patients with left ventricular failure, congestive heart failure or shock. Jewitt et al (1969) and Prakash et al (1972) reported similar findings. Catecholamines probably play a major role in stimulating lipolysis from adipose tissue (Gordon & Cherkes, 1956; Carlson et al, 1965; Pinter et al, 1967).

Fasting has also been reported as increasing plasma FFA concentrations (Opie, 1972), so that patients with larger infarcts, who might be expected to feel more ill than patients with small infarcts, could be less willing to eat and the resulting fast might contribute to the higher FFA concentrations found. In this study starved patients did not have higher peak or mean FFA concentrations than those given sucrose orally a mean of 6 hours after the onset of symptoms, so that fasting in the first 12 hours of AMI did not appear to influence FFA concentrations.

The results of these studies strongly support a catecholamine-mediated relationship between the extent of myocardial ischaemic injury and the extent of the rise in circulating FFA levels after AMI. There is also experimental and clinical evidence that raised FFA concentrations may increase myocardial ischaemic injury, independent of the effect of catecholamines. It is therefore proposed that the very high FFA concentrations observed in patients with large infarcts may not only reflect the severity of the infarct process, but may also increase the infarct size by means of a vicious circle, as illustrated in Figure 5.

The evidence for this hypothesis is as follows. Firstly, studies with experimental myocardial infarction have shown that increasing the FFA

concentrations can extend the ischaemic damage as demonstrated by increased enzyme release from the heart Kjekshus & Mjøs, 1972; de Leiris et al, 1975), impaired myocardial function (Mjøs, 1971; Liedtke et al, 1978) increased epicardial ST segment elevation (Kjekshus & Mjøs, 1972) and by increased microscopic mitochondrial abnormalities (de Leiris & Feuvray, 1977). Conversely lowering plasma FFA by antilipolytic agents such as β-pyridylcarbinol, its analogues, or glucose-insulin-potassium infusions can decrease ischaemic injury, as shown histologically or by increased epicardial ST segment elevation (Maroko et al, 1972; Mjøs et al, 1976; Opie & Owen, 1976; Mjøs et al, 1976) and by marked reduction in myocardial CK depletion after coronary artery ligation (Kjekshus & Mjøs, 1973). Furthermore, decreased FFA use by the ischaemic zone appears to be one way by which β-adrenergic blockade can beneficially influence the outcome of experimental myocardial infarction (Opie & Thomas, 1976).

Secondly, there is clinical evidence. Treatment with anti-lipolytic agents decreased precordial ST segment elevation in patients with myocardial ischaemia (Luxton et al, 1976) and infarction (Russell & Oliver, 1978). Although Oliver et al (1978) found no correlation between peak CK activity and plasma FFA levels after AMI, a subsequent study (Vetter et al, 1974), in which FFA estimations were carried out earlier and more frequently, demonstrated a positive correlation ($r = 0.9$) between mean FFA concentrations in the first 5 hours of AMI and peak CK activity. Peak CK activity may have limitations as an index of the extent of myocardial necrosis (Wilkinson, 1970; Posen, 1970) but it nevertheless correlated strongly with estimated infarct size in the present study ($r = 0.8$).

The mechanism of FFA toxicity has not yet been fully elucidated but may include increased myocardial oxygen consumption (Mjøs, 1971; Kjekshus

& Mjøs, 1972; Mjøs et al, 1974; Liedtke et al, 1978; Simonsen & Kjekshus, 1978), endogenous lipolysis (Kurien & Oliver, 1970), and decreased glucose use (Opie, 1970). Arrhythmias, which may be induced by high FFA concentrations after AMI (Oliver et al, 1968; Gupta et al, 1969; Prakash et al, 1972), could indirectly increase infarct size (Adgey et al, 1971) by causing haemodynamic imbalance (Fozzard, 1976) and by increasing myocardial oxygen consumption (Shell & Sobel, 1973b). There is also evidence that catecholamines may sensitize the myocardium to the effects of circulating FFA (Opie et al, 1971b; Kjekshus & Mjøs, 1972; Kjekshus & Mjøs, 1973; Simonsen & Kjekshus, 1978).

It could be argued that, since increased circulating catecholamines can per se increase myocardial ischaemic damage (Raab et al, 1962; Jewitt et al, 1969; Herbaczynska-Cedro, 1970), the intermediate action of FFA is not necessary for the establishment of another vicious circle involving infarct size and catecholamine secretion. The independent, harmful action of FFA has, however, been demonstrated in the isolated rat heart (de Leiris et al, 1975), in dogs (Mjøs, 1971; Mjøs et al, 1974), and in man (Simonsen & Kjekshus, 1978). Thus catecholamines probably act both directly and by increasing circulating FFA concentrations to increase the severity of ischaemic injury in patients with developing infarction. The administration of therapy to lower FFA concentrations very early after AMI might therefore reduce the extent of myocardial necrosis, though this effect has yet to be studied.

Table 1 FFA AND INFARCT SIZE MEASUREMENTS IN 11 PATIENTS WITH LARGE AND 17 PATIENTS WITH SMALL INFARCTS

Patient Number	Age (years)	Estimated infarct size (CK-g-eq.)	Infarct size index (CK-g-eq/m^2)	Total cumulated CK release (i.u./l)	Peak CK activity (i.u./l)	Peak FFA (mM/l)	Mean FFA (mM/l)	Peak FFA/ albumin molar ratio
Group I - Large estimated infarct size (n=11)								
1	43	195	91	3898	1307	2.39	1.24±0.17	3.5
3	49	117	69	3629	2030	2.23	1.2 ±0.24	3.8
5	69	110	52	-	-	2.58	1.62±0.15	4.5
16	41	262	119	4672	1796	2.05	1.24±0.10	3.5
17	64	125	68	2977	1223	1.68	1.14±0.09	2.9
21	48	88	55	2616	1465	1.79	1.14±0.14	3.0
24	39	77	41	1934	991	1.38	1.27±0.03	2.3
27	54	131	66	3021	1152	1.59	1.50±0.04	2.4
28	58	94	46	1925	872	1.58	1.25±0.08	2.3
29	58	76	40	1731	1129	1.49	1.05±0.12	2.2
31	37	108	64	3043	1389	1.64	1.32±0.07	2.5
Mean (±SEM)	51 (±SD 11)	126 (±17)	65 (±7)			1.85 (±0.12)	1.29* (±0.04)	3.0 (±0.2)
Group II - Small estimated infarct size (n=17)								
2	48	19	10	482	169	1.35	0.78±0.13	2.4
7	66	36	20	956	503	1.96	1.34±0.11	3.3
8	51	32	16	744	246	0.86	0.72±0.05	1.6
9	46	26	14	698	465	1.2	0.63±0.10	2.0
10	33	46	25	1233	649	1.04	0.58±0.09	1.7
11	43	44	21	983	926	1.23	1.05±0.06	2.1
13	39	9	4	185	122	0.87	0.66±0.05	1.4
15	66	58	31	1340	552	1.57	1.47±0.03	2.5
18	37	59	30	1286	389	1.05	0.89±0.04	1.7
19	61	34	21	1121	850	1.23	1.0 ±0.06	2.3
20	55	52	34	1804	1075	1.63	1.31±0.08	2.9
22	54	31	18	591	265	1.58	1.48±0.04	2.5
23	58	17	9	393	239	1.14	0.81±0.10	2.0
25	55	54	23	767	340	0.98	0.89±0.06	1.7
30	53	26	13	636	467	1.16	1.04±0.04	1.8
34	61	19	10	404	274	1.55	1.46±0.05	2.3
35	63	19	10	434	255	0.77	0.55±0.08	1.8
Mean (±SEM)	52 (±SD 10)	34 (±4)	18 (±2)			1.25 (±0.01)	0.94* (±0.04)	2.1 (±0.1)
P						<0.001	<0.001	<0.001

*Calculated from all FFA values within 12 hours of acute myocardial infarction.

Table 2 PLASMA FFA CONCENTRATIONS IN THE FIRST 72 HOURS OF AMI IN 11 PATIENTS WITH LARGE INFARCTS

Patient number / Hours post-infarct	1	3	5	16	17	21	24	27	28	29	31	n	Mean	SEM
2	0.71	-	-	0.83	1.04	-	-	-	-	-	-	3	0.86	0.09
3	1.39	-	1.92	1.41	0.87	-	-	-	-	-	-	4	1.40	0.18
4	2.39	-	2.58	1.26	1.68				1.10	1.12	-	6	1.69	0.27
5	1.91	-	2.18	1.37	1.19	1.39	-	-	0.88	0.81	1.64	8	1.42	0.23
6	1.53	-	1.71	2.05	0.69	1.79	1.24	1.57	1.05	0.52	1.16	10	1.33	0.16
7	1.16	2.23	1.28	1.54	0.89	1.24	1.21	1.56	1.09	0.65	1.48	11	1.30	0.17
8	1.11	1.59	1.11	1.04	1.26	-	1.28	1.52	1.14	0.88	1.26	10	1.22	0.07
9	1.06	1.13	1.22	1.01	1.39	0.70	1.19	1.59	1.58	1.14	1.50	11	1.23	0.07
10	0.75	0.83	1.34	0.98	1.52	1.03	1.32	1.49	1.34	1.49	1.20	11	1.21	0.08
12	1.27	0.68	1.53	1.15	0.93	0.87	1.38	1.29	1.45	1.46	1.16	11	1.19	0.08
14	1.02	0.55	1.09	1.02	0.81	0.41	1.12	0.97	1.27	1.49	0.98	11	0.98	0.09
16	1.03	1.06	-	0.81	0.98	0.37	1.08	0.99	1.35	1.27	0.84	10	0.98	0.09
18	0.89	1.20	-	0.95	0.85	0.54	1.19	0.83	1.35	-	-	8	0.98	0.09
20	0.42	1.33	-	0.78	0.47	0.93	0.93	0.72	1.28	-	-	8	0.86	0.12
22	0.40	1.00	-	0.78	1.11	0.11	1.07	0.87	1.03	-	-	8	0.79	0.13
24	0.35	1.49	-	0.76	-	1.16	0.71	0.77	1.03	-	-	7	0.89	0.14
28	0.39	0.57	-	-	0.99	0.69	0.91	-	-	-	-	5	0.71	0.11
32	0.59	0.37	-	0.68	0.85	0.54	1.02	-	-	-	-	6	0.68	0.09
36	0.49	0.90	-	0.51	0.90	0.13	0.68	-	-	-	-	6	0.60	0.12
40	0.56	0.77	-	0.71	1.00	0.08	0.99	-	-	-	-	6	0.69	0.14
44	0.21	0.76	-	0.51	-	0.19	1.02	-	-	-	-	5	0.54	0.16
48	0.44	0.40	-	-	-	0.22	0.79	-	-	-	-	4	0.46	0.12
54	-	0.23	-	0.48	-	0.48	0.75	-	-	-	-	4	0.49	0.11
60	0.65	-	-	0.52	-	0.13	1.00	-	-	-	-	4	0.58	0.18
66	0.43	0.48	-	0.44	-	-	0.57	-	-	-	-	4	0.48	0.03
72	-	0.32	-	0.52	-	0.23	-	-	-	-	-	3	0.36	0.09

Table 3 PLASMA FFA CONCENTRATIONS IN THE FIRST 72 HOURS OF AMI IN 17 PATIENTS WITH SMALL INFARCTS

Patient Number / Hours post-infarct	2	7	8	9	10	11	13	15	18	19	20	22	23	25	30	34	35	n	Mean	SEM
2	-	-	-	0.72	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.72	-
3	-	-	0.78	0.54	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.66	0.12
4	-	-	0.79	0.51	0.65	-	0.72	1.36	0.94	-	-	-	0.60	-	0.89	-	0.77	9	0.80	0.08
5	1.35	1.06	0.78	0.30	1.04	-	0.50	1.53	0.97	-	-	-	0.54	-	-	-	0.71	10	0.88	0.12
6	1.11	1.26	0.49	0.39	0.92	-	0.53	1.45	1.05	0.89	0.95	-	0.56	-	1.16	1.54	0.74	14	0.93	0.10
7	0.34	1.1	-	-	0.5	1.23	0.62	1.36	0.99	0.82	1.19	1.58	0.63	-	-	-	0.39	12	0.90	0.12
8	0.29	1.42	0.59	0.58	0.43	1.17	0.57	1.57	0.93	1.09	1.33	1.41	0.87	0.96	1.06	1.44	0.28	17	0.94	0.10
9	0.52	1.45	-	-	0.51	0.91	-	1.53	0.81	1.17	1.43	1.35	-	0.90	-	-	-	10	1.06	0.12
10	0.74	1.96	0.73	0.83	0.37	1.12	0.87	1.51	0.67	1.23	1.34	1.51	0.79	0.98	1.01	1.55	0.30	17	1.03	0.11
12	1.06	0.99	0.86	1.2	0.41	0.84	0.71	-	0.93	0.79	1.63	1.57	1.14	0.91	1.07	1.32	0.66	16	1.01	0.08
14	1.49	1.79	0.41	1.02	0.79	1.53	0.52	-	1.06	1.44	1.01	0.69	1.31	0.49	1.04	1.6	-	15	0.98	0.12
16	1.15	1.27	0.56	1.14	0.73	1.55	0.61	-	0.89	1.11	1.32	1.28	1.14	0.78	0.60	1.07	-	15	1.01	0.08
18	1.17	1.23	0.93	0.99	0.94	1.31	0.59	-	0.95	1.20	1.24	0.99	1.33	0.92	0.84	-	-	14	1.05	0.06
20	1.08	1.22	0.88	0.92	0.77	1.12	0.51	-	0.68	0.87	1.12	0.96	1.34	0.66	-	-	-	13	0.93	0.07
22	1.48	1.17	0.87	1.05	0.74	1.28	0.33	-	0.59	0.56	1.25	1.04	1.52	0.57	-	-	-	13	0.96	0.11
24	0.93	1.14	0.53	1.24	-	1.21	0.31	-	0.87	0.61	1.17	0.81	1.34	0.46	-	-	-	12	0.89	0.10
28	1.02	0.42	0.53	0.87	0.36	1.08	0.46	-	0.69	0.52	0.80	0.59	0.79	0.59	-	-	-	13	0.67	0.06
32	0.77	1.04	0.49	0.61	0.35	1.06	0.61	-	0.83	0.74	0.43	0.65	0.95	0.75	-	-	-	13	0,71	0.06
36	1.15	0.89	0.47	0.50	0.56	0.80	0.56	-	0.62	0.52	0.41	0.58	0.68	0.65	-	-	-	13	0.65	0.06
40	0.43	0.80	0.66	0.42	0.44	0.97	0.58	-	0.75	0.73	0.75	0.70	0.82	0.84	-	-	-	13	0.68	0.05
44	0.99	0.75	0.60	0.67	0.76	1.01	0.47	-	0.51	0.42	1.15	0.91	0.77	0.55	-	-	-	13	0.74	0.06
48	1.03	0.42	0.48	0.83	0.57	0.75	0.21	-	0.50	0.59	0.93	0.80	0.74	0.84	-	-	-	13	0.67	0.06
54	0.98	0.64	0.35	0.66	0.41	0.66	0.32	-	0.85	0.64	0.46	0.63	0.80	0.59	-	-	-	13	0.61	0.05
60	0.67	0.75	0.30	0.59	0.20	-	0.56	-	0.77	0.85	0.58	0.81	-	0.62	-	-	-	11	0.61	0.06
66	0.97	0.95	0.39	0.78	0.29	-	0.54	-	-	0.63	1.02	-	0.63	0.54	-	-	-	10	0.67	0.07
72	0.47	-	-	0.63	0.41	-	0.32	-	-	0.44	0.80	0.83	-	0.51	-	-	-	8	0.55	0.07

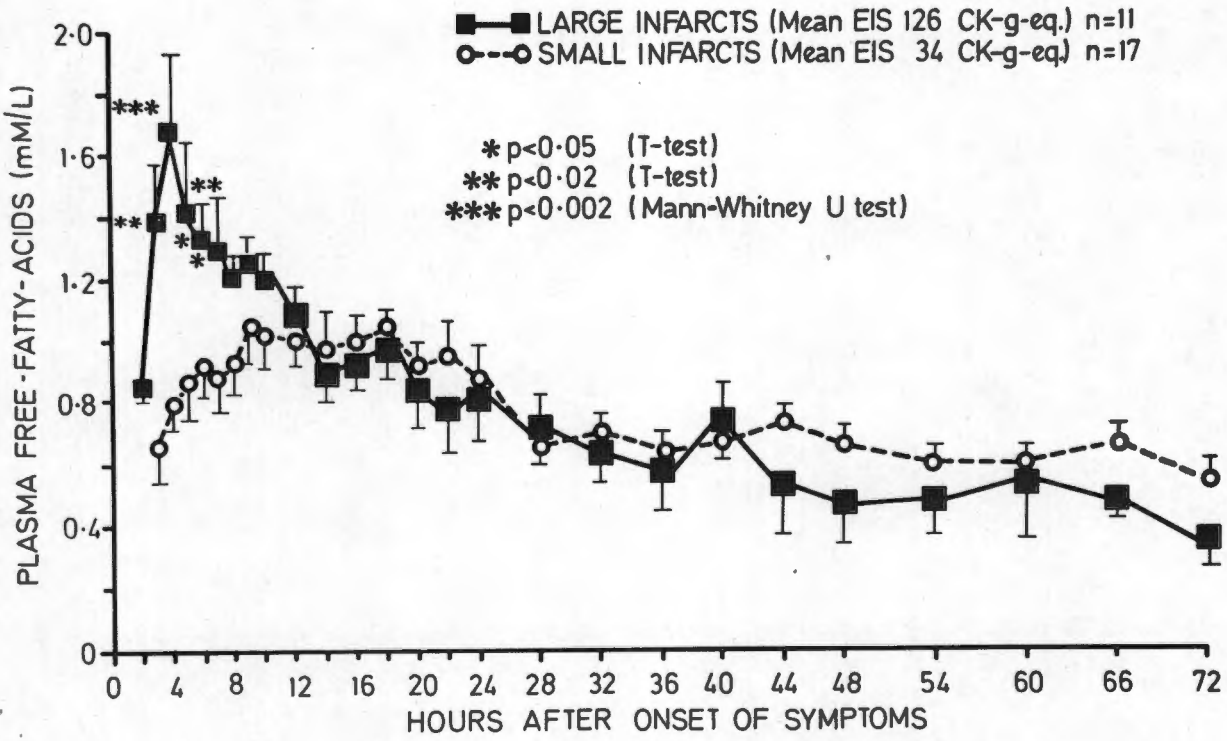


Figure 1

Mean FFA concentrations in 11 patients with large and 17 patients with small EIS in the first 72 hours of AMI. Patients with large infarcts had higher mean FFA levels at 3, 4, 5, 6 and 7 hours post-infarct.

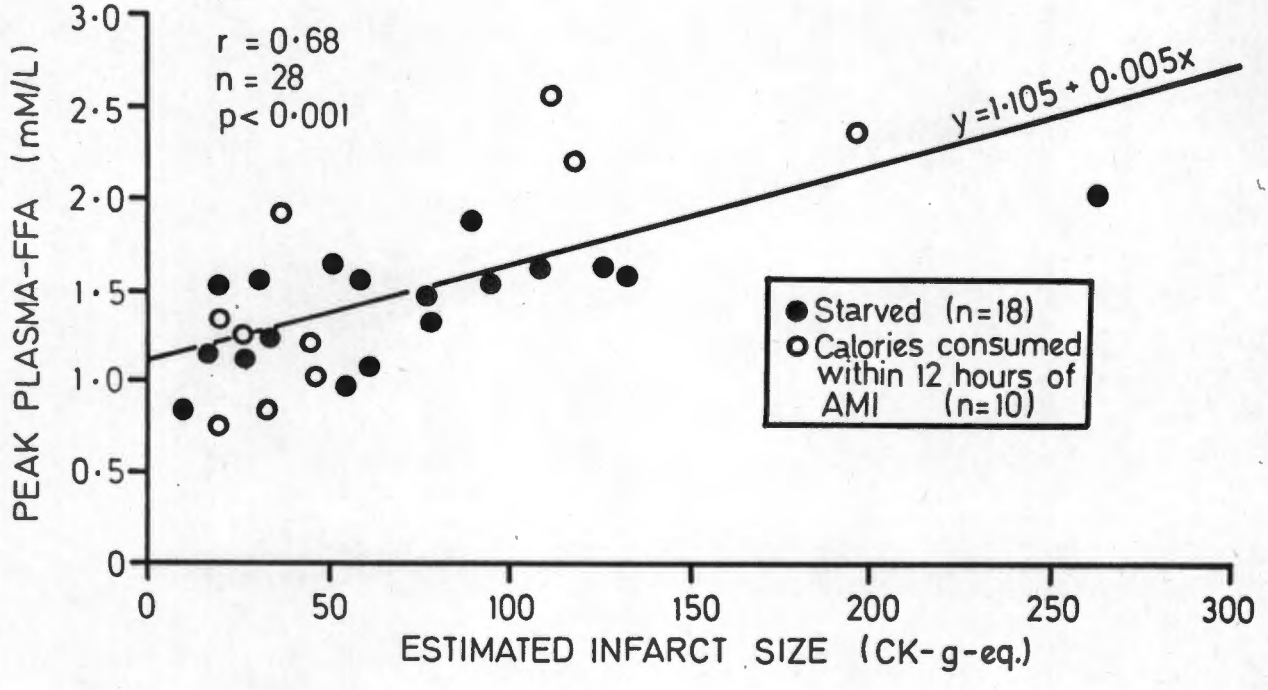


Figure 2

Correlation between peak plasma FFA (mM/l) and EIS (CK-g-eq) in the first 12 hours of AMI in 28 patients.

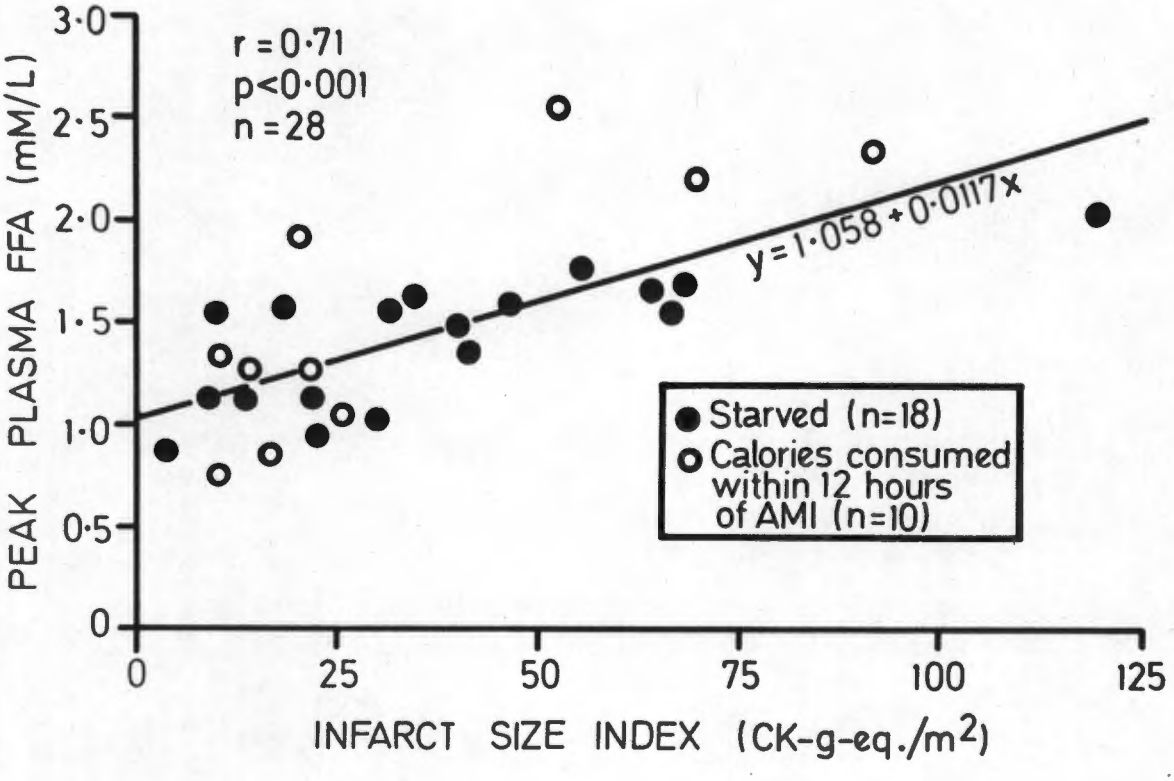


Figure 3

Correlation between peak plasma FFA (mM/l) and ISI (CK-g-eq/m²) in the first 12 hours of AMI in 28 patients.

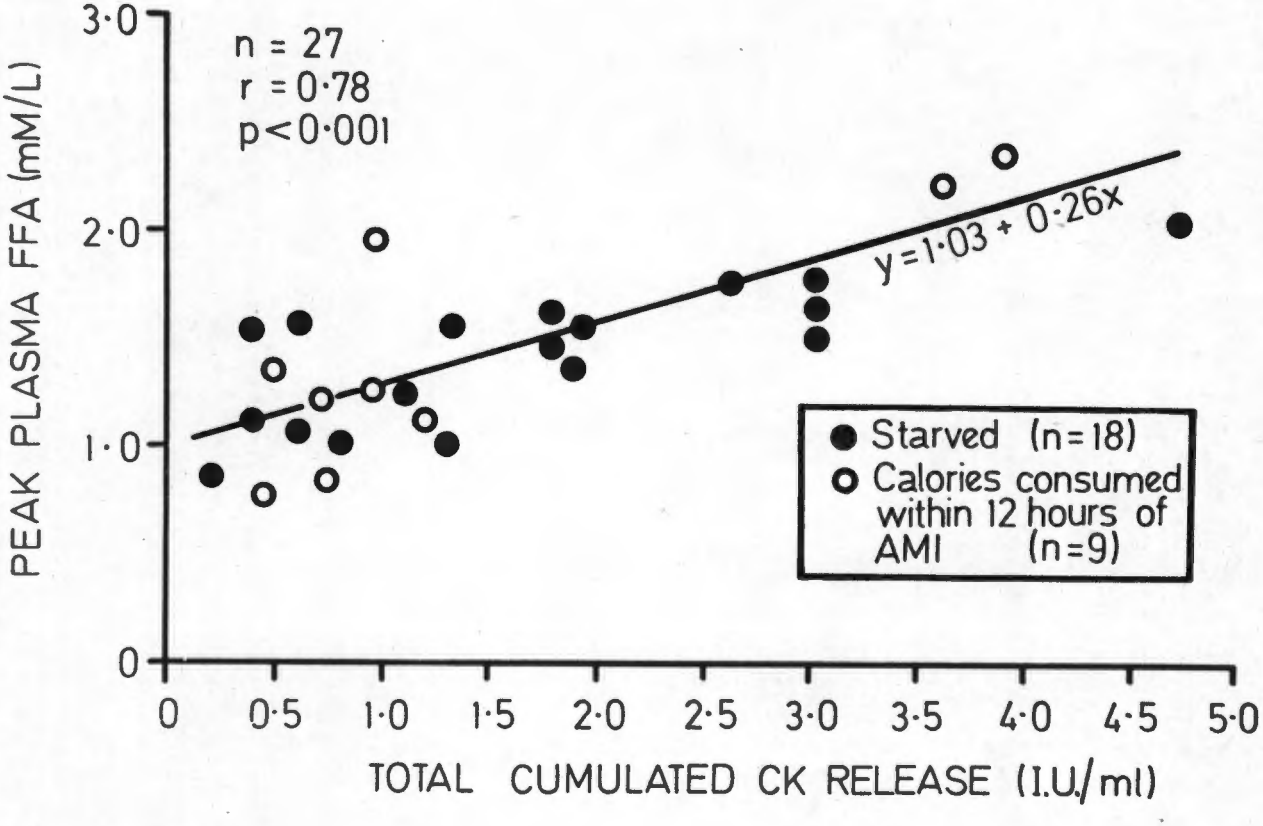


Figure 4

Correlation between peak plasma FFA (mM/l) and total cumulated CK release (i.u./l) in the first 12 hours of AMI in 27 patients.

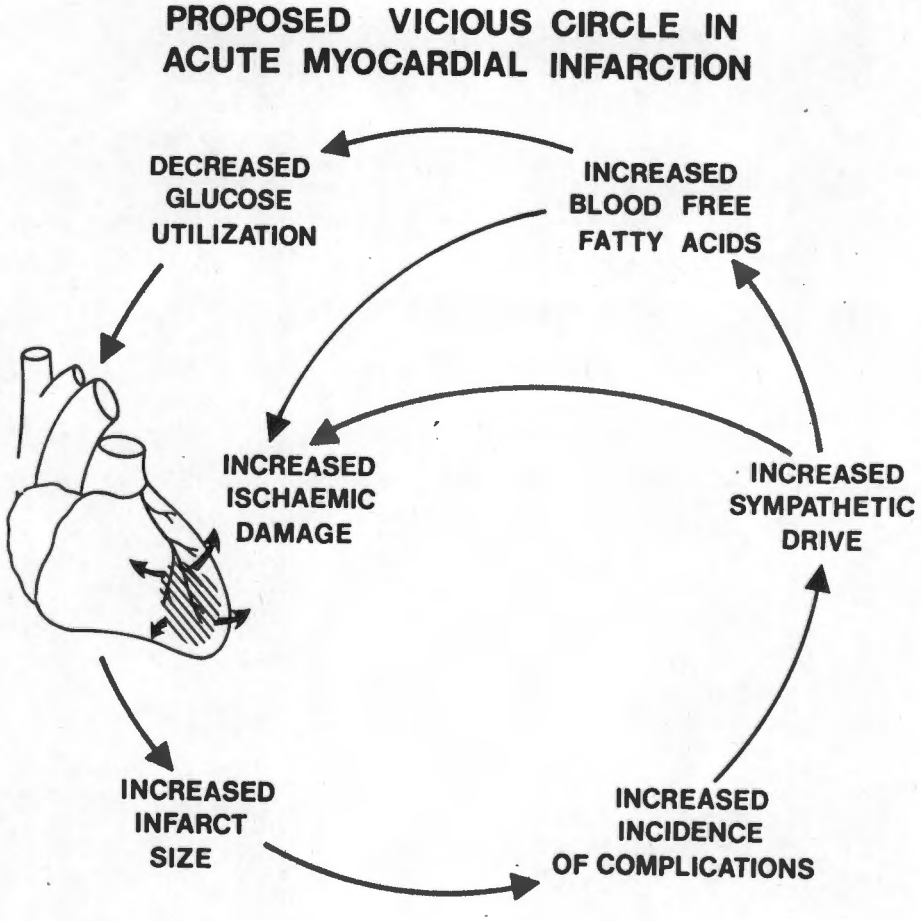


Figure 5

Proposed metabolic vicious circle in acute myocardial infarction.

CHAPTER IX

THE EFFECT OF ORAL SUCROSE ON PLASMA FREE FATTY ACIDS IN THE EARLY PHASE OF ACUTE MYOCARDIAL INFARCTION

INTRODUCTION AND PURPOSE OF THE STUDY

The increase in plasma free fatty acid concentrations which occurs in the early stages of acute myocardial infarction (Kurien & Oliver, 1966; Oliver et al, 1968; Janusjewicz et al, 1971; Christensen & Videbaek, 1974; Vetter et al, 1974) correlates with arrhythmias (Oliver et al, 1968; Gupta et al, 1969; Prakash et al, 1972; Chapter VII, present study) and with infarct size (Vetter et al, 1974; Chapter VIII, present study). It has been proposed that such increases in FFA have harmful effects in the ischaemic myocardium (Kurien & Oliver, 1970; Kurien et al, 1974; Opie, 1975), whilst lowering FFA levels by inhibition of lipolysis may help to reduce the severity of ischaemic injury (Luxton et al, 1976; Russell & Oliver, 1978). Blood FFA rise within 2 hours after the onset of symptoms of AMI (Vetter et al, 1974), but most patients seek help after this time (Armstrong et al, 1972). If lowering FFA concentrations were beneficial it would be desirable to do so as early as possible after the onset of symptoms of AMI, and a cheap and easily available substance which could be administered either by a physician or by the patient would be extremely valuable.

Oral carbohydrate can lower FFA in normal persons (Gordon & Cherkes, 1956; Swan et al, 1965) and in patients with AMI (Oliver et al, 1972; Opie et al, 1975), so the effects of oral carbohydrate, in the form of sucrose, on plasma FFA and glucose in the early stages of AMI were studied.

Patients and Methods

The 22 patients who took part in this study were selected according to the criteria outlined in Chapter I and were assigned to one of two groups by random numbers. A venous blood sample was taken on admission to hospital and the first group (sucrose-fed) received sucrose orally as soon as a second blood sample had been taken one hour later, after which they were starved until at least 12 hours had elapsed since the onset of symptoms.

The second group (starved) were starved for at least 12 hours after the onset of AMI. Both groups of patients were allowed to drink water. Sucrose was given as a 25 g/100 ml solution in water flavoured with pure lemon juice. Mean ages ($\pm$ SD) were 50 $\pm$ 10 years (range 33 - 69 years) in the sucrose-fed group and 53 $\pm$ 11 years (range 37 - 69) in the starved group. Sucrose-fed patients received a mean of 90 $\pm$ 9 grams of sucrose (range 50 - 150 grams).

Blood sampling, storage, biochemical estimations of FFA and glucose, and calculations of infarct size were carried out as described in Chapters I, II and III. The t test or the Mann-Whitney U test, as identified in the text, were used for statistical analysis (Appendix C).

RESULTS

Eleven sucrose-fed and 11 starved patients were studied. All the sucrose-fed patients were male and one of the starved patients was female. The relevant biochemical and pharmacological details are given in Tables 1 and 2. Mean time of admission was similar in the sucrose-fed and starved groups (4.5 $\pm$ 0.6 and 4.6 $\pm$ 0.6 hours after the onset of symptoms respectively) as was the mean time of the peak FFA (7.9 $\pm$ 0.9 and 7.5 $\pm$ 0.08 hours after the onset of symptoms respectively) and the mean peak FFA concentration (1.49 $\pm$ 0.21 and 1.48 $\pm$ 0.04 mM/l respectively). One sucrose-fed patient and two starved patients received β-adrenergic blockers during the study period; the possible influence on FFA levels is discussed in Chaper VI.

The mean plasma FFA and glucose concentrations in the sucrose-fed and starved patients in the first 12 hours after the onset of myocardial infarction are shown in Table 3 and Figure 1. FFA levels were consistently higher in starved than in sucrose-fed patients in the 6 hours following the administration of sucrose, a mean of 5.6 $\pm$ 0.6 hours after the onset

of symptoms.

Table 4 and Figure 2 show mean FFA and glucose concentrations in the two groups from the time of admission and from the time of sucrose administration (since sucrose was given one hour after the admission in all patients). In the sucrose-fed patients, mean glucose levels rose by 40 mg/100 ml to a peak of 161 $\pm$ 17 mg/100 ml in the 2 hours following sucrose administration ($P<0.05$, $U = 13$, Mann-Whitney U test, compared with mean glucose levels in starved patients). Mean FFA levels fell by 0.38 mM/l in the 3 hours following sucrose administration, whilst FFA levels in the starved group did not change significantly during the comparable period. Mean FFA concentrations were significantly lower in the 5-hour period following sucrose administration in the sucrose-fed than in the starved patients (0.85 $\pm$ 0.08 and 1.12 $\pm$ 0.05 mM/l respectively; $t = 2.14$, degrees of freedom 61, allowing for unequal variances, $P<0.05$).

In view of the similar mean interval between the onset of symptoms and the peak FFA in the two groups, mean FFA concentrations were adjusted so that individual peak FFA values coincided with time zero (Table 5, Figure 3). The rate of fall from mean peak levels was similar in sucrose-fed and in starved patients in the 2 hours following the peak. There was then an early rebound in FFA levels in the starved patients from 3 to 5 hours after the peak, at which time the mean FFA concentration was significantly higher than in the sucrose-fed patients ($P<0.05$, $U = 29$, Mann-Whitney U test); mean FFA levels then fell to within normal limits 11 hours after the peak. In contrast FFA levels in sucrose-fed patients remained lower initially but there was a later rebound to abnormal levels from 9 to 18 hours after the peak.

Table 6 compares the mean fall in FFA concentrations from the peak level to the nadir in subgroups of the sucrose-fed and the starved patients

according to the peak FFA level or the amount of sucrose administered. Patients with peak FFA 1.5 mM/l or greater had larger estimated infarct sizes (115 ± 32 and 103 ± 35 CK-g-eq in sucrose-fed and starved patients respectively) than patients with peak FFA less than 1.5 mM/l (33 ± 5 and 34 ± 14 CK-g-eq respectively). The larger FFA fall was always observed in the sucrose-fed patients, though the differences never reached significant levels. In patients with peak FFA less than 1.5 mM/l, a significantly greater fall in FFA concentrations was observed in sucrose-fed than in starved patients in the second hour after the FFA peak (0.33 ± 0.1 and 0.09 ± 0.04 mM/l respectively, P = 0.036, U = 4, Mann-Whitney U test) (Figure 4); no differences were observed in patients with peak FFA 1.5 mM/l or greater (Figure 5).

DISCUSSION

Oral sucrose did not modify the peak FFA within 12 hours of the onset of symptoms of AMI in the patients studied here. There are a number of possible explanations for this. It may be that too little sucrose was given, it may have been given too late after the onset of chest pain, it may have been poorly absorbed, or it may not have been an effective anti-lipolytic agent. The dose of sucrose varied between 50 and 150 grams (mean 90 grams), which would seem to be an adequate quantity; Oliver et al (1972) found that 75 grams of glucose given as a single oral dose caused a fall in serum FFA to within normal limits in 3 patients with AMI, and that the effect was identical when the same quantity of glucose was given as an intravenous infusion over 3 hours. Furthermore, patients in the present study given 100 grams of sucrose or more (mean 106 ± 4 grams) did not have a significantly greater fall in FFA compared with patients given less than 100 grams (mean 66 ± 7 grams, Table 6). Sucrose was given too late to influence peak FFA in 6 patients, and did not appear to have had any

influence in the 5 patients who received it before the peak. The earliest that any patient received sucrose was 3 hours after the onset of symptoms, so that early peak FFA levels might have already occurred (Vetter et al, 1974) and the effect of early sucrose administration still requires investigation. It is also unlikely that inadequate absorbtion occurred. The mean rise in plasma-glucose (40 mg/100 ml) was similar to the mean rise of 43 mg/100 ml observed in normal volunteers after ingestion of 100 grams of sucrose (Swan et al, 1966). The efficacy of sucrose as an antilipolytic agent, as discussed below, may be doubtful in some patients, but the main reason why it did not influence peak FFA levels is most likely to be the delay in administration after the onset of AMI.

FFA concentrations were lower in sucrose-fed than in starved patients for 5 hours after sucrose was given (Figure 2) and this effect was observed after the peak FFA levels had occurred (Figure 3). Although not always within normal limits during this time, FFA concentrations after sucrose remained well below 1.2 mM/l, the level at which unbound FFA may increase (Kurien & Oliver, 1970). Thus sucrose would appear to be an effective antilipolytic agent. In starved patients, FFA concentrations rose again immediately after the initial fall from peak levels, and did not return to normal levels until 8 hours later. This rebound was delayed for 5 hours in sucrose-fed patients, but was then more prolonged. A similar rebound effect was observed after inhibition of lipolysis with a nicotinic-acid analogue (Rowe et al, 1975), and repeated doses of sucrose for up to 24 hours post-infarction might be necessary to prevent this, as was the case for a nicotinic-acid analogue (Russell & Oliver, 1978).

The influence of sucrose on the rate and extent of fall of FFA levels after the peak was examined with respect to peak levels above and below 1.5 mM/l (Table 6, Figures 4 and 5). This was done for two reasons.

Firstly, the higher FFA peaks were associated with larger estimated infarct sizes, which may be associated with a more intensive metabolic response which might in turn have influenced the effect of sucrose. Secondly, the rate of fall after the peak may be influenced by the peak level itself, so that patients with high peak FFA might have obscured the effect of sucrose in patients with low peak FFA. The mean fall in FFA in patients with high peaks ($\geq$1.5 mM/l) was greater in sucrose-fed than in starved patients (Table 6). This could be entirely accounted for by the higher mean FFA peak in the former group, especially as FFA concentrations fell to similar absolute levels in the two groups (Figure 4). In patients with low FFA peaks (<1.5 mM/l) there was a greater overall subsequent fall in sucrose-fed then in starved patients (Table 6), although the difference was not statistically significant. The fall was significantly larger in sucrose-fed than in starved patients in the second hour after the FFA peak however (Figure 4), suggesting that sucrose was a more effective antilipolytic agent in patients with smaller than in patients with larger infarcts.

Oral sucrose may not be as effective an antilipolytic agent as nicotinic-acid analogues after AMI (Rowe et al, 1975; Russell & Oliver, 1978) but it has several advantages. It is a cheap, commonly-available substance which could be administered very early after the onset of symptoms by a medical practitioner or even by the patient himself. The nicotinic-acid analogues used in clinical trials to date (Rowe et al, 1975; Russell & Oliver, 1978) were associated with an unacceptably high incidence of gastro-intestinal side-effects when used in doses high enough and frequent enough to maintain FFA concentrations within normal levels. In the present study, as given with lemon juice, sucrose was acceptable to all patients. One patient, not included in the study, vomited after taking sucrose but morphine had been administered concurrently, so that which of

the two caused the vomiting was not known. Large, frequent doses of sucrose given to patients over many hours might induce nausea, but patients could be given dextrose intravenously once admitted to hospital with equal effect (Gupta et al, 1969; Oliver et al, 1972). A final possible advantage of oral sucrose over nicotinic-acid analogues or other antilipolytic agents might be the beneficial effect that increased blood glucose levels may have on the ischaemic myocardium (Opie, 1970).

If it is desirable to decrease circulating FFA as early as possible in patients with symptoms of acute myocardial infarction, then oral sucrose could be a simple, cheap and easily available antilipolytic agent, at least in patients with smaller infarcts.

Table 1 CLINICAL AND BIOCHEMICAL DETAILS OF ELEVEN SUCROSE-FED PATIENTS

Patient No.	Age (years)	Estimated infarct size (CK-g-eq.)	Hours from onset of pain to admission	Hours from onset of pain to peak FFA	Peak FFA (mM/l)	Grams of* sucrose given (k.Cal)	Drugs given in the first 24 hours of AMI.
1	43	195	2	4	2.39	50 (200)	Methyldopa 250 mg Nitroprusside infusion Morphine 30 mg Lignocaine 50 mg Metoclopramide 20 mg
2	48	19	6	6	1.34	100 (400)	Quinidine gluconate 1.32 g Frusemide 20 mg Digoxin 0.125 mg
3	49	117	6	7	2.23	100 (400)	Diazepam 4 mg Frusemide 40 mg
4	53	-	8	9	1.06	150 (600)	Morphine 15 mg Diazepam 10 mg
5	69	110	3	4	2.58	80 (320)	Morphine 10 mg Diazepam 15 mg Prochlorperazine 12.5 mg Lignocaine 50 mg
6	50	<20*	4	11	0.47	75 (300)	Frusemide 80 mg Digoxin 0.25 mg
7	66	36	5	10	1.96	115 (460)	Morphine 5 mg Lignocaine 1 g
8	51	32	3	12	0.86	50 (200)	Lignocaine 2.5 g Diazepam 15 mg Metoclopramide 10 mg Nitrazepam 10 mg
9	46	26	2	12	1.20	75 (300)	Morphine 10 mg Propranolol 120 mg Diazepam 15 mg Frusemide 40 mg
10	33	46	4	5	1.03	100 (400)	Diazepam 15 mg Morphine 5 mg Prochlorperazine 12.5 mg
11	43	44	7	7	1.22	100 (400)	Digoxin 1.5 mg Diazepam 15 mg Morphine 10 mg Prochlorperazine 25 mg
Mean ±SEM	50±10 (SD) (33-69)	64±19	4.5±0.6	7.9±0.9	1.49±0.21	90±9 (362±35)	

* Given 1 hour after admission (5.5±0.6 hours after onset of symptoms).

Table 2

CLINICAL AND BIOCHEMICAL DETAILS OF ELEVEN STARVED PATIENTS

Patient No.	Age (years)	Estimated infarct size (CK-g-eq.)	Hours from onset of pain to admission	Hours from onset of pain to peak FFA	Peak FFA (mM/l)	Drugs given in the first 24 hours of AMI
12	48	-	3	3	1.87	Lignocaine 200 mg
13	39	9	4	10	0.87	Frusemide 40 mg
14	69	-	7	0	0.98	Morphine 5 mg Oxprenolol 20 mg Diazepam 15 mg Lignocaine 2.5 mg
15	66	58	4	8	1.57	Lignocaine 1 g Procainamide 100 mg Methyldopa 750 mg Frusemide 80 mg Nitrazepam 10 mg Diazepam 10 mg Digoxin 0.25 mg
16	41	262	2	6	2.05	Morphine 20 mg Diazepam 10 mg Frusemide 40 mg Lignocaine 2 g
17	64	125	2	4	1.68	Atropine 0.6 mg Diazepam 15 mg Lignocaine 2 g
18	37	59	4	6	1.05	Nil
19	61	34	6	10	1.23	Propranolol 80 mg Diazepam 10 mg Morphine 5 mg Lignocaine 2 g
20	55	52	6	12	1.63	Nil
21	48	88	6	7	1.79	Diazepam 10 mg Frusemide 20 mg Morphine 10 mg
22	54	31	7	7	1.58	Warfarin 7.5 mg Morphine 5 mg
Mean ±SEM	53±11 (SD) (37-69)	80±25	4.6±0.6	7.5±0.8	1.48±0.04	

Table 3 MEAN FFA (mM/l) AND BLOOD GLUCOSE (mg/100 ml) CONCENTRATIONS IN THE FIRST 24 HOURS OF AMI.

Hours post-infarct	Mean FFA ± SEM		Mean Blood Sugar ± SEM	
	Sucrose-fed	Starved	Sucrose-fed	Starved
2	0.72±0.01	0.94±0.11	142±16	150±12
3	1.16±0.31	1.38±0.29	150±15	143±18
4	1.21±0.41	1.12±0.15	125±25	145±15
5	1.12±0.24	1.09±0.14	135±19	128± 9
6	0.96±0.19	1.11±0.18	151±24	131± 8
7	1.01±0.23	1.07±0.10	142±28	114± 6
8	0.87±0.14	1.11±0.09	136±18	121± 6
9	0.90±0.13	1.12±0.09	112±11	103± 1
10	0.95±0.14	1.13±0.09	144±22	119± 8
11	0.78±0.14	1.02±0.07	166±33	-
12	0.86±0.12	1.02±0.11	133±19	116± 8
14	0.94±0.16	0.89±0.09	145±19	111± 7
16	0.94±0.12	0.95±0.09	109± 9	113±10
18	0.99±0.08	0.91±0.07	118±10	120± 7
20	0.87±0.11	0.76±0.08	121± 9	135±16
22	0.90±0.12	0.64±0.12	123±10	122± 7
24	0.83±0.15	0.72±0.11	128± 7	124±14

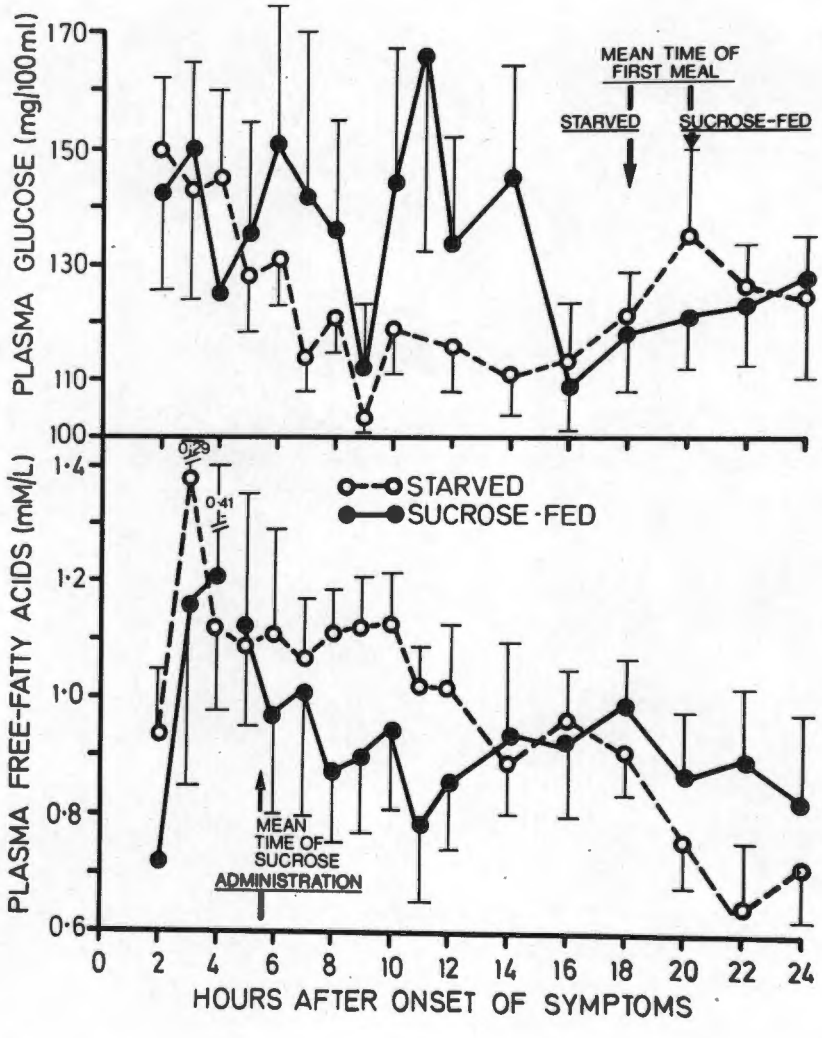


Figure 1

Mean plasma FFA and glucose concentrations in the first 24 hours of AMI in 11 sucrose-fed and 11 starved patients.

Table 4 MEAN PLASMA FFA (mM/l) AND GLUCOSE (mg/100 ml) CONCENTRATIONS FROM THE TIME OF ADMISSION

Hours post-admission	Plasma FFA		Plasma Glucose		
	Sucrose-fed	Starved	Sucrose-fed	Starved	P
0	1.09±0.17	1.12±0.11	117±11	125± 6	
1	1.17±0.18	1.10±0.12	121± 9	128± 8	
					sucrose given
2	1.04±0.20	1.15±0.10	145±13	128±11	
3	1.02±0.22	1.12±0.10	161±17	113± 7	<0.05
4	0.78±0.17	1.11±0.14	140±32	125±10	
5	0.87±0.17	1.17±0.09	135±24	114± 7	
6	0.83±0.18	1.07±0.11	140±11	121±13	
7	0.90±0.13	0.92±0.08	145±24	118±13	
8	0.66±0.16	1.10±0.13	96±16	111±10	
9	1.24±0.18	0.90±0.13	146±32	117± 9	
10	0.84±0.17	1.02±0.11	118±12	112±11	
11	1.07±0.14	0.82±0.15	119±20	121±11	

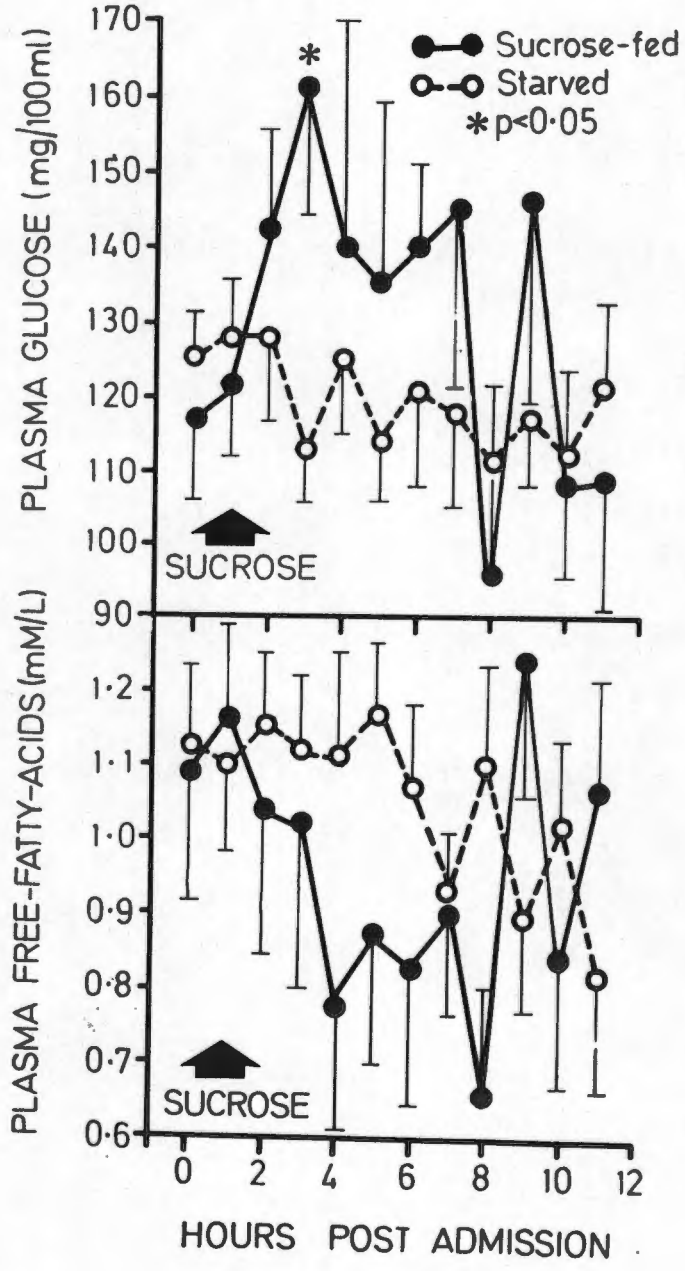


Figure 2

Mean plasma FFA and glucose concentrations in 11 sucrose-fed and 11 starved patients in the 12 hours following admission. Sucrose was given one hour after admission, and plasma glucose was higher in sucrose-fed than in starved patients 3 hours post-admission.

Table 5 MEAN FFA CONCENTRATIONS AFTER THE PEAK OCCURRING WITHIN THE FIRST 12 HOURS OF AMI IN 11 SUCROSE-FEED AND 11 STARVED PATIENTS

Hours after peak FFA	Sucrose-fed patients (Mean ± SEM)	Starved patients (Mean ± SEM)	P
-3	0.66±0.44	1.02±0.15	
-2	0.78±0.19	1.02±0.10	
-1	1.29±0.21	1.15±0.10	
Peak FFA	1.49±0.21	1.48±0.12	
1	1.22±0.16	1.16±0.08	
2	0.84±0.15	0.95±0.08	
3	0.79±0.13	0.98±0.09	
4	0.82±0.14	1.05±0.11	
5	0.81±0.12	1.08±0.10	P = 0.05
6	0.83±0.11	1.02±0.09	
7	0.85±0.13	0.92±0.09	
8	0.94±0.11	0.84±0.12	
9	1.02±0.09	0.85±0.09	
10	1.03±0.10	0.68±0.10	
11	0.97±0.11	0.78±0.09	
12	0.94±0.11	0.79±0.09	
13 - 14	0.96±0.11	0.72±0.09	
15 - 16	0.84±0.11	0.64±0.11	
17 - 18	0.86±0.19	0.82±0.09	
19 - 20	0.48±0.05	0.39±0.05	
21 - 22	0.75±0.19	0.60±0.05	
23 - 24	0.48±0.09	0.70±0.03	

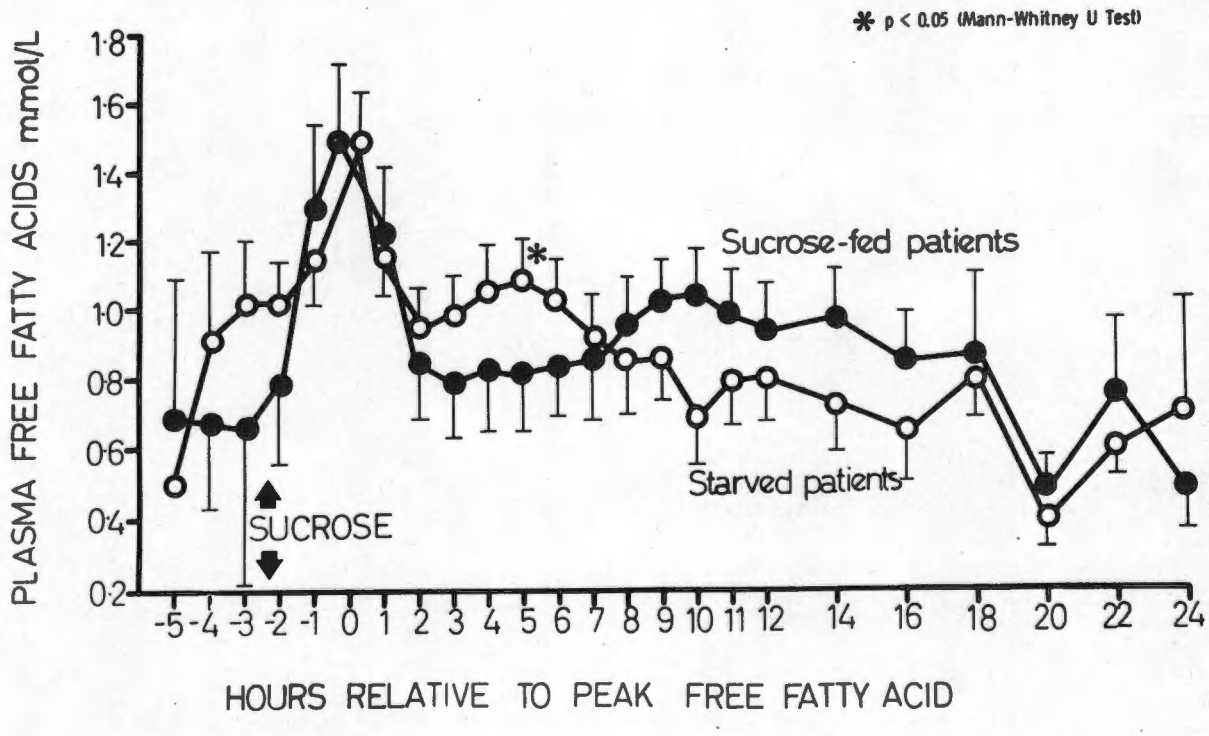


Figure 3

Mean FFA concentrations in 11 sucrose-fed and 11 starved patients adjusted so that individual peak FFA coincided with time zero.

Table 6 MEAN FALL IN PLASMA FFA CONCENTRATIONS FROM THE PEAK LEVEL TO THE NADIR

	Peak FFA ≥1.5		Peak FFA <1.5		≥ 100 g sucrose given		<100 g sucrose given	
	Mean fall	Mean Peak FFA	Mean fall	Mean Peak FFA	Mean fall	Peak Mean FFA	Mean fall	Peak Mean FFA
Sucrose-fed patients	1.52±0.21 (n=4)	2.29±0.13	0.51±0.14 (n=7)	1.03±0.11	0.92±0.19 (n=6)	1.48±0.20	0.83±0.45 (n=5)	1.50±0.42
Starved patients	0.76±0.18 (n=7)	1.74±0.07	0.29±0.09 (n=4)	1.04±0.08	0.58±0.14 (n=11)	1.48±0.04	0.58±0.14 (n=11)	1.48±0.04
P	NS	t=2.76 <0.025	NS	NS	NS	NS	NS	NS

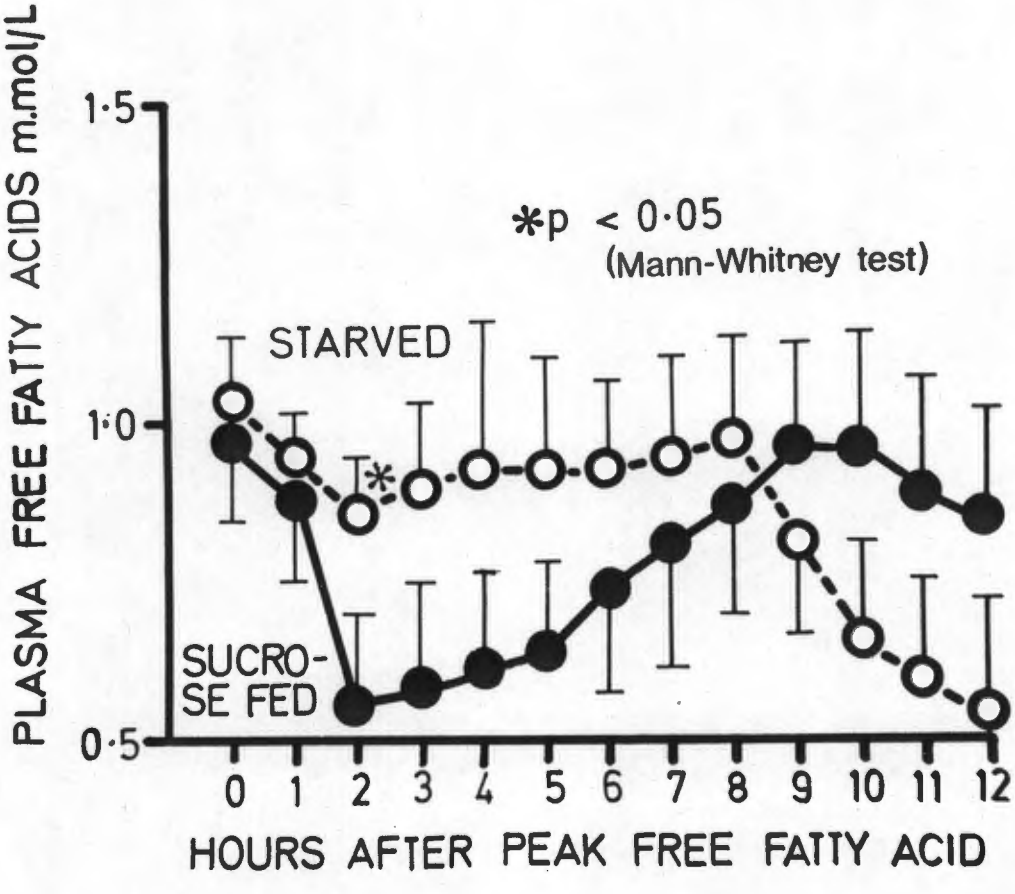


Figure 4

Mean FFA concentrations from peak levels in 7 sucrose-fed and 4 starved patients with peak FFA levels <1.5 mM/l. FFA levels in sucrose-fed patients fell significantly lower than in starved patients after 2 hours.

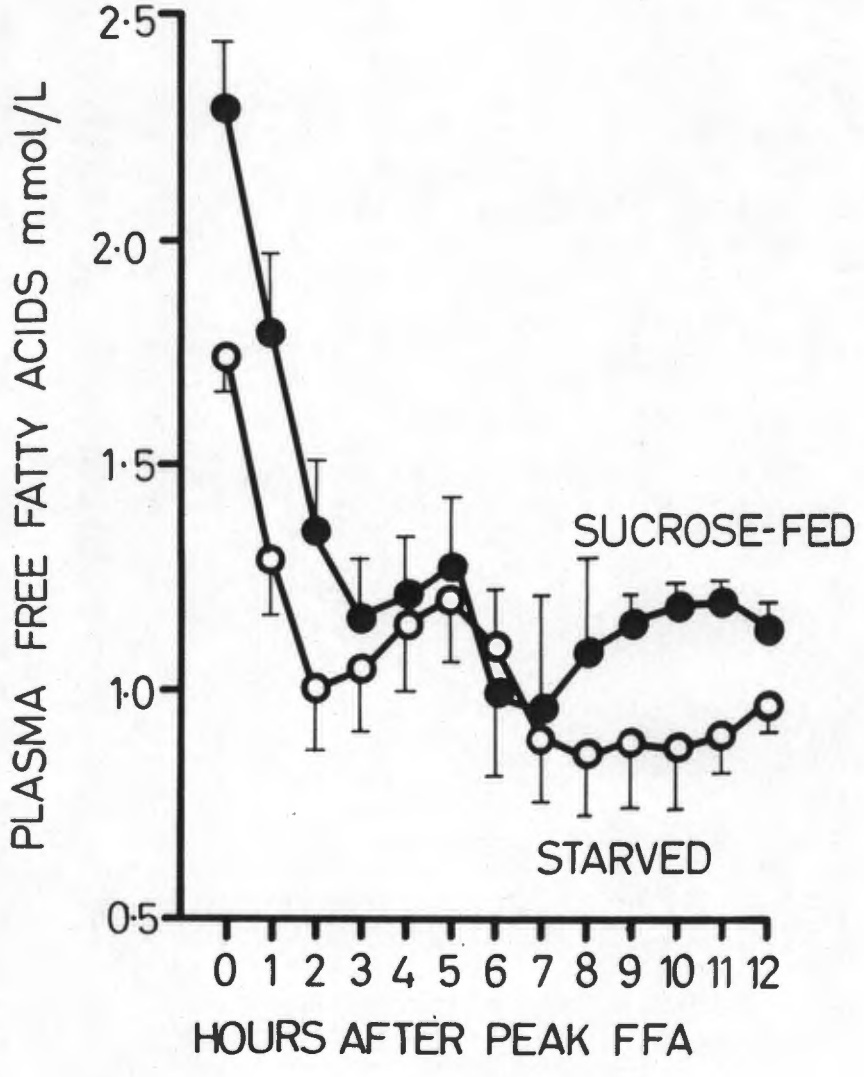


Figure 5

Mean FFA concentrations from peak levels in 4 sucrose-fed and 7 starved patients with peak FFA levels > 1.5 mM/l. There is no difference in the rate of fall from peak levels in the two groups.

CHAPTER X

THE EFFECT OF ESTIMATED INFARCT SIZE ON THE METABOLISM OF PLASMA FREE FATTY ACIDS, PLASMA GLUCOSE AND SERUM INSULIN IN THE EARLY PHASE OF ACUTE MYOCARDIAL INFARCTION

INTRODUCTION AND PURPOSE OF THE STUDY

The disturbed metabolism of free fatty acids (Kurien & Oliver, 1966; Janusjewicz et al, 1971; Christensen & Videbaek, 1974; Vetter et al, 1974), glucose (Raab & Rabinowitz, 1936; Sowton, 1962; Daley & Nanda, 1967; Allison et al, 1969; Vetter et al, 1974), and insulin (Allison et al, 1967, 1969; Lebowitz et al, 1969; Taylor et al, 1969; Vetter et al, 1974) in patients with acute myocardial infarction are well documented. So far, these biochemical disturbances, with the exception of plasma FFA levels, have been correlated with the severity of infarction only on clinical grounds (Ellenberg et al, 1952; Gupta et al, 1969; Taylor et al, 1969), although clinical severity correlates well with estimated infarct size (Sobel et al, 1972; Shell & Sobel, 1976). The purpose of this study was to determine the relation between the degree of biochemical disturbance and estimated infarct size.

Patients and Methods

Twenty-two patients in whom estimated infarct size and plasma free fatty acids, plasma glucose and serum insulin had been estimated in the first 12 hours of acute myocardial infarction were included here. Ten of the patients received sucrose one hour after admission and the remaining 12 patients were starved for at least 12 hours after the onset of symptoms. The sucrose-fed and starved groups of patients were divided according to infarct size. Large infarcts were 65 CK-g-eq or greater and small infarcts less than 65 CK-g-eq, as described previously. Patient 6 was included in the small-infarct group as described in Chapter III. Sucrose-fed patients with large infarcts received a mean of 77 ± 15 grams of sucrose and those with small infarcts 88 ± 8 grams one hour after admission, as described in Chapter IX.

Patient selection, blood sampling and storage, calculation of infarct

size and biochemical methods have been detailed in Chapters I, II and III. Statistical analysis was by the Mann-Whitney U test (Appendix C).

RESULTS

Eight patients had large and 14 had small EIS. Relevant clinical and biochemical details are given in Table 1. The mean delay in admission from the time of onset of symptoms was similar in the two groups (4.1 $\pm$ 0.7 and 4.6 $\pm$ 0.4 hours respectively. Plasma glucose on admission was higher in patients with large infarcts than in patients with small infarcts (mean 168 $\pm$ 20 and 112 $\pm$ 6 mg/100 ml respectively, $P<0.002$, $U = 11$, Mann-Whitney U test). Eight of 9 patients with large infarcts had admission blood sugars greater than 130 mg/%, whilst 10 of 13 patients with small infarcts had admission blood sugars less than 130 mg% ($P<0.05$, exact fourfold table test). Admission values for plasma FFA and serum insulin were also higher in patients with large infarcts (Table 1), but not significantly so.

Mean FFA, glucose and insulin in 5 patients with large infarcts and 7 with small infarcts were compared (Table 2, Figure 1) during the first 12 hours of AMI. Plasma FFA were significantly higher at 6 hours and plasma glucose and serum insulin at 6, 8, 10 and 12 hours after onset of symptoms in patients with large than with small infarcts.

In Table 3 and Figure 2, the mean FFA, glucose and insulin concentrations in 3 sucrose-fed patients with large infarcts and 7 sucrose-fed patients with small infarcts were compared relative to the time that sucrose was given. Plasma FFA concentrations were higher in the large infarct patients throughout. Plasma glucose concentrations reached higher and more sustained levels in patients with large than with small infarcts. Oral sucrose provoked a significant insulin response in patients with small infarcts, whereas no such response was found in patients with large infarcts. The response of FFAs, glucose and insulin to 100 gram oral sucrose in 9 normal subjects (Swan et al, 1966) have been included in

Figure 2 for comparison.

DISCUSSION

In this study a marked difference in the circulating levels of plasma FFA, plasma glucose and serum insulin in the first 12 hours of acute myocardial infarction was found according to infarct size. Admission glucose values were significantly higher in patients with large infarcts, and remained so for the first 12 hours of AMI. Patients with large infarcts also had higher FFA and insulin values during the study period than patients with small infarcts. A marked difference in the response of FFA, glucose and insulin to oral sucrose was also found according to infarct size; in patients with small infarcts, the response was similar to that found in normal volunteers (Swan et al, 1966), whilst patients with large infarcts had higher peak FFA and higher glucose concentrations with a minimal insulin response.

Increased catecholamine release after AMI (Gazes et al, 1959; McDonald et al, 1969; Siggers et al, 1971; Januszewixz et al, 1971; Lukomsky & Uganov, 1972; Christensen & Videbaek, 1974; Vetter et al, 1974) appears to be basic to the general biochemical disturbance (Opie, 1971), and higher levels are associated with a poor prognosis (Jewitt et al, 1969; Griffiths & Leung, 1971; Prakash et al, 1972). The relation between FFA and catecholamines has been described in detail in Chapter V. Raised FFA levels may also be due in part to decreased insulin release (Allison et al, 1969; Lebowitz et al, 1969; Vetter et al, 1974) which is especially reduced in shocked patients (Taylor et al, 1969) and when there are increased catecholamine levels (Porte et al, 1966; Miller & Soeldner, 1969). Suppression of insulin release was noted in this study in patients with large infarcts given oral sucrose, but not in patients with small infarcts. However, in starved patients insulin levels were higher during the first 12 hours of AMI in those with large than in those with small infarcts,

though glucose levels in the former group remained high. These observations suggest that insulin secretion, though very much reduced after AMI, is not totally abolished, and that a degree of insulin resistance may also be present, as proposed by Gupta et al (1974). Decreased insulin release and insulin resistance may also partly account for the glucose intolerance observed after AMI (Raab & Rabinowitz, 1936; Sowton, 1962; Allison et al, 1969). Ellenberg et al (1952) showed that there was a greater degree of hyperglycaemia in shocked than in non-shocked patients with AMI, and postulated that mobilization of glycogen by adrenaline and gluconeogenesis under the influence of adrenocortical hormones might be the cause.

In view of the finding that a significantly larger number of patients with large than with small infarcts had an admission blood sugar exceeding 130 mg/100 ml, this value could be used as a crude indirect index of the extent of myocardial infarction. Blood sugar levels on admission, which can be simply and rapidly estimated, might therefore be of use to indicate which patients might benefit most from early metabolic intervention.

The results of this study show that the degree of biochemical disturbance is related to infarct size, and that patients with small infarcts have a normal insulin response to a sucrose load in the first 12 hours of AMI whilst patients with large infarcts do not. This might explain the failure of oral sucrose to influence FFA concentrations in patients with large infarcts, as described in Chapter IX. Oral sucrose might, therefore, be a more effective antilipolytic agent in these patients if insulin were given at the same time.

Table 1 CLINICAL AND BIOCHEMICAL DETAILS IN 8 PATIENTS WITH LARGE AND 14 PATIENTS WITH SMALL ESTIMATED INFARCT SIZE

Patient Number	Age (years)	Whether sucrose-fed or starved	Hours post-infarct admitted	Estimated infarct-size (CK-g-eq.)	Admission plasma/FFA (mmol/l)	Admission plasma glucose (mg%)	Admission serum insulin (μU/ml)
Group I - Patients with large EIS (≥ 65 CK-g-eq)							
1	43	sucrose-fed	2	195	0.71	158	78
3	49	sucrose-fed	6	117	2.23	106	20
5	69	sucrose-fed	3	110	1.92	190	11
16	41	starved	2	262	0.83	162	41
17	64	starved	2	125	1.04	138	28
21	48	starved	6	88	1.39	149	18
24	39	starved	6	77	1.24	293	26
27	54	starved	6	131	1.57	149	20
Mean	51±11 (SD)		4.1±0.7	138±22	1.37±0.19	168±20	30±7
Group II - Patients with small EIS (<65 CK-g-eq)							
2	48	sucrose-fed	6	19	1.35	96	17
6	50	sucrose-fed	4	<20	0.31	51	23
7	66	sucrose-fed	5	36	1.06	100	22
8	51	sucrose-fed	3	32	0.78	141	36
9	46	sucrose-fed	2	26	0.72	126	22
10	33	sucrose-fed	4	46	0.65	83	17
11	43	sucrose-fed	7	44	1.23	120	25
13	39	starved	4	9	0.72	134	21
15	66	starved	4	58	1.36	137	23
18	37	starved	4	59	0.94	109	16
19	61	starved	6	34	0.89	114	9
20	55	starved	6	52	0.95	110	21
22	54	starved	7	31	1.58	114	25
23	58	starved	3	17	0.60	132	29
Mean	51±10 (SD)		4.6±0.4	34±4	0.94±0.09	112±6	22±2
P	NS		NS		NS	<0.002	NS

Table 2 MEAN PLASMA FFA, PLASMA GLUCOSE AND SERUM INSULIN IN THE FIRST 12 HOURS OF AMI IN 5 PATIENTS WITH LARGE AND 7 PATIENTS WITH SMALL ESTIMATED INFARCTS

	Plasma FFA			Plasma glucose			Serum insulin		
	Large EIS	Small EIS	P	Large EIS	Small EIS	P	Large EIS	Small EIS	P
2	0.94±0.11	-	-	150±12	-	-	35±7	-	-
3	1.14±0.27	-	-	159±12	-	-	32±9	-	-
4	1.47±0.21	0.91±0.17	NS	188±19	128± 7	NS	46	22±3	NS
5	1.32±0.06	0.89±0.24	NS	-	128± 7	-	-	23±4	-
6	1.47±0.24	0.91±0.14	0.041	185±37	122± 7	0.032	33±5	19±3	0.028
7	1.29±0.12	1.03±0.14	NS	-	114± 6	-	-	19±2	-
8	1.28±0.10	1.11±0.13	NS	172±26	112± 7	0.009	33±4	20±3	0.015
9	1.18±0.15	1.26±0.12	NS	-	-	-	-	-	-
10	1.27±0.11	1.13±0.13	NS	171±22	111±10	0.009	31±4	20±4	0.026
11	0.99±0.02	1.07±0.11	NS	-	-	-	-	-	-
12	1.12±0.10	1.16±0.16	NS	174±19	97± 5	0.002	35±3	17±3	0.002

P derived by the Mann-Whitney U Test.

NS = Not significant.

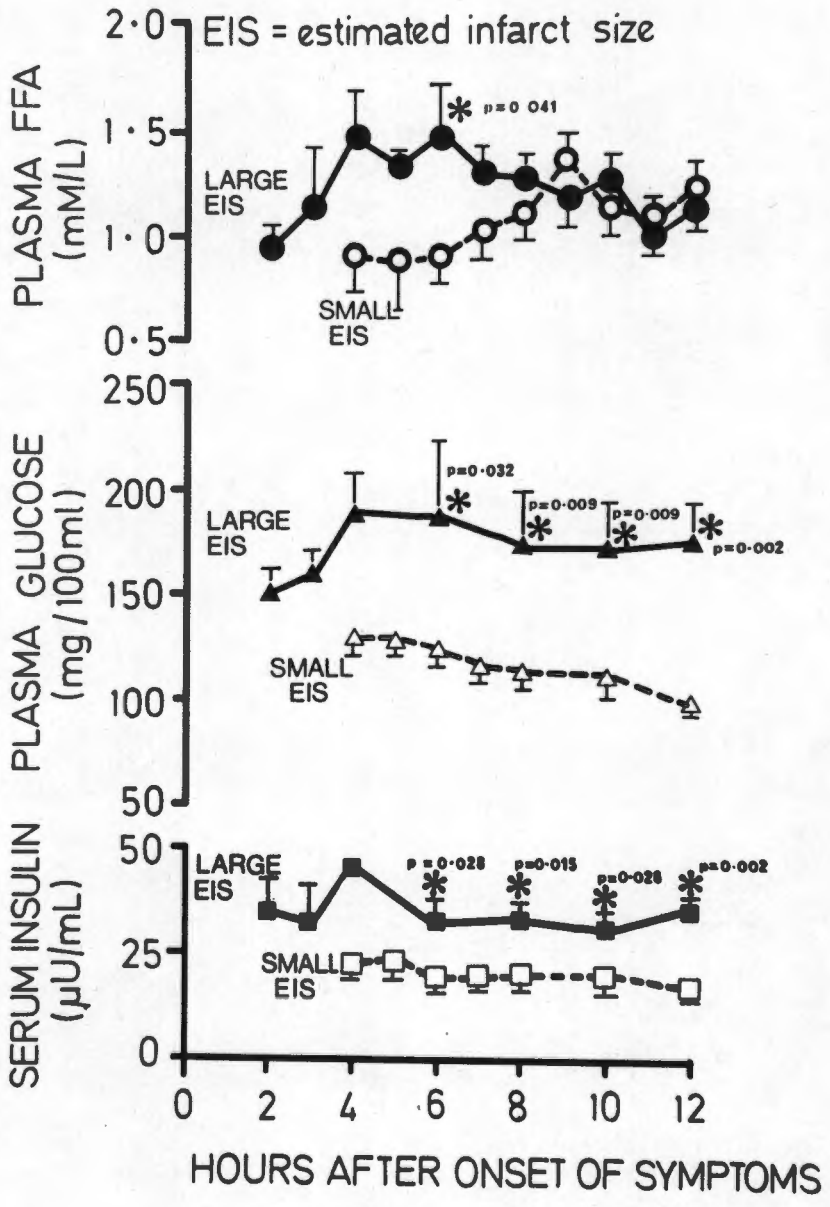


Figure 1

Mean FFA, glucose and insulin levels in 5 patients with large and 7 patients with small EIS who were starved for the first 12 hours of AMI.

Table 3 MEAN PLASMA FFA, PLASMA GLUCOSE AND SERUM INSULIN VALUES IN 7 PATIENTS WITH SMALL AND 3 PATIENTS WITH LARGE ESTIMATED INFARCT SIZES AFTER ADMINISTRATION OF ORAL SUCROSE

Hours relative to time of sucrose administration		Free fatty acids			Glucose			Insulin		
		Large EIS	Small EIS	P	Large EIS	Small EIS	P	Large EIS	Small EIS	P
	-2	0.71	0.83±0.12	NS	158	108±17	NS	-	25±6	
	-1	1.66±0.26	0.96±0.14	0.028	153±37	101±10	NS	-	24±5	
Immediately prior to sucrose	0	2.40±0.10	0.85±0.12	0.008	137±30	117± 6	NS	21±4	24±3	NS
	1	1.90±0.17	0.64±0.15	0.008	159±42	148±13	NS	21±6	93±34	0.017
	2	1.42±0.29	0.64±0.21	NS	209±77	137± 4	NS	25±0.5	62±17	NS
	3-4	1.06±0.11	0.74±0.22	NS	200±45	114±10	0.008	45±14	56±19	NS
	5-6	1.02±0.19	0.78±0.17	NS	204±51	104± 8	0.012	40± 5	29±11	NS

P derived by the Mann-Whitney U test.

NS = not significant

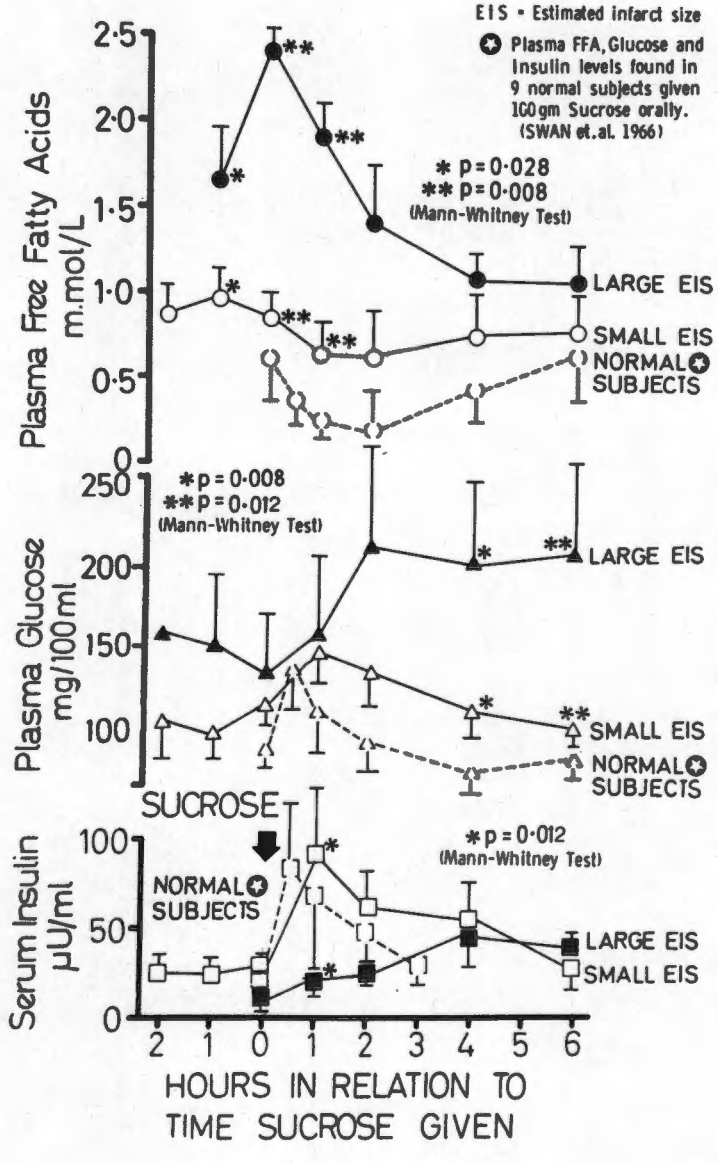


Figure 2

Mean FFA, glucose and insulin levels in 3 sucrose-fed patients with large infarcts and 7 sucrose-fed patients with small infarcts relative to the time sucrose was given. Levels in 9 normal subjects given oral sucrose (Swan et al, 1966) are included for comparison.

SUMMARY AND CONCLUSIONS

Measurement of serial levels of plasma creatine kinase activity and calculation of infarct size

Plasma creatine kinase activity was measured using commercially available kits. The main problem encountered with the method was that of diluting plasma with CK activity greater than 1000 i.u./l, which was above the limits capable of being read by the equipment used. Dilution with Tris buffer, heat-inactivated human serum or distilled water was shown to be unsuitable, but the addition of smaller amounts of sample to the reaction mixture was shown to produce no significant error up to levels of CK activity encountered.

Infarct size was calculated by serial measurement of CK activity and the errors inherent in this method were reviewed in detail. The calculation is dependent in part on knowing the concentration of CK in normal and in infarcted myocardium. These concentrations were determined using the same methods of measuring CK activity as were used for plasma CK; in this way a possible source of error in using results from previous studies arrived at by different methods was eliminated. Whilst reviewing the literature an error in the published method for enzymatic estimation of infarct size was identified, and the error was acknowledged by the authors.

In order to determine whether patients requiring direct current cardioversion could be included in the studies the effect of administering different levels of electrical energy on total CK and MB-CK were studied. Though low-energy shocks did not greatly elevate CK activity, if at all, the effect was not predictable so that accurate calculation of infarct size could not be guaranteed, and patients requiring cardioversion were therefore not included in the study. Cumulative shocks to a level

greater than previously reported did not elevate MB-CK activity, which could therefore be used to calculate infarct size after cardioversion.

Free fatty acids in the first 12 hours of AMI

This was the first time that frequent, serial measurements were used to study the pattern of FFA rise and fall in the first 12 hours of AMI. A rise in plasma FFA concentrations above normal levels was noted in all 15 patients who were studied within 7 hours of the onset of symptoms of AMI. The major rise in FFA occurred within 12 hours of AMI, during which time all patients were starved, and was characterized by rapid fluctuations with one or more peak levels within this time. The mean FFA concentrations in the 15 patients did not reflect the enormous variations observed in individual patients. Plasma FFA estimated on admission did not reflect subsequent levels. High peaks occurred significantly earlier than low FFA peaks. Following peak levels of FFA concentrations fell at a rate which was related to the peak, so that after a high peak the fall was more rapid than after a low peak. A fall in FFA concentrations was seen after oral intake in several patients, but the exact association between the two was not investigated.

Practical conclusions drawn from this study are that any investigations to correlate plasma FFA concentrations with complications of AMI should be based on frequent, early FFA estimations so as to take into account the fluctuation in FFA levels observed in the first 24, and especially the first 12 hours of AMI, and so as not to miss FFA peak concentrations.

Comparison of agents designed to influence plasma FFA concentrations after AMI should only be made between groups where the pre-treatment levels are similar.

Free fatty acids, infarct size and complications of acute myocardial infarction

The relation of heart failure, ventricular arrhythmias, 6-month

mortality and duration of chest pain to infarct size and to plasma free fatty acid concentrations was studied.

Patients who developed heart failure were found to have higher mean ISI than patients who did not, in keeping with the findings reported from other clinical studies. There was no association between infarct size duration of chest pain, 6-month mortality and ventricular arrhythmias, contrary to some previous reports, and possible reasons for the lack of agreement have been discussed.

All patients with mean FFA concentrations greater than 1.2 mM/l in the first 12 hours of AMI developed serious ventricular arrhythmias within 24 hours of AMI, as compared with 35% of patients with mean FFA of 1.2 mM/l or less ($P<0.002$). No other relations were observed. Whilst the role of FFA in the genesis of arrhythmias after AMI is controversial, this is the first study in which frequent and regular FFA estimations have been carried out within 12 hours of AMI, and it is argued that as a result a more valid assessment of the relationship between FFA and the complications of myocardial infarction has been made.

In the light of these results, the important therapeutic implications of early, effective antilipolytic therapy to reduce FFA concentrations after AMI have been emphasized.

Free fatty acids and infarct size

Peak FFA concentrations in the first 12 hours of AMI were correlated with enzymatically estimated infarct size.

A very strong relation was found between high plasma FFA concentrations and patients with large mycoardial infarcts. It was therefore proposed that the association between high plasma FFA and infarct size may give rise to a vicious circle which increases the severity of the ischaemic process in patients with high plasma FFA. This hypothesis was supported by evidence from previous experimental and clinical studies.

It was concluded that treatment to reduce plasma FFA levels after AMI, if initiated early enough, might reduce the extent of myocardial necrosis.

The effect of oral sucrose on FFA concentrations

Oral carbohydrate has been shown to lower FFA concentrations in normal volunteers and patients with AMI. If it were beneficial to lower FFA levels after AMI, sucrose would be a cheap and easily available substance to administer. The effect of oral sucrose on FFA concentrations after AMI was therefore examined. In sucrose-fed patients, mean FFA concentrations were significantly lower in the 5 hours following sucrose administration than in starved patients over the comparable period. FFA rose to a peak and then fell spontaneously by 32% of the peak level in 2 hours. Sucrose had no effect on the peak FFA level, possibly because it was administered too late, and did not accelerate the initial rate of fall in the first 2 hours, except in patients with FFA peaks less than 1.5 mM/l, who also had smaller infarcts than patients with higher peak FFA levels. A subsequent rebound of FFA values, as seen in starved patients, was delayed for about 6 hours in sucrose-fed patients.

Thus sucrose was an effective antilipolytic agent in patients with smaller infarcts. The possible advantages of oral sucrose over the nicotinic-acid analogues used to date are discussed. It is proposed that sucrose should be given much earlier after the onset of symptoms of AMI if it is to have any influence on early peak FFA concentrations, and that it should be given for a longer period, possibly 24 hours, to prevent the late rebound in FFA observed with this and other antilipolytic agents.

Infarct size, plasma glucose and serum insulin

Glucose intolerance and suppression of insulin secretion have previously been noted after AMI. The degree of these metabolic disturbances was examined in relation to infarct size. Patients with large infarcts were found to have higher glucose and insulin levels in the first 12 hours

of AMI than patients with small infarcts. Insulin secretion was therefore not totally abolished, but glucose levels remained high, suggesting the possibility of insulin resistance. These observations may explain the poor antilipolytic effect of sucrose in patients with high FFA peaks and large infarcts observed in the previous section. The results indicate that the addition of insulin to regimes where glucose is administered to improve myocardial metabolism after AMI is essential in patients with large infarcts, but not in patients with small infarcts. Most patients with large infarcts might be identifiable by the admission blood glucose level, since significantly more patients with large than with small infarcts had admission blood sugar values exceeding 130 mg/100ml. Thus patients most likely to benefit from metabolic interventions could be identified on admission by means of a simple and readily available investigation.

APPENDIX A

PLASMA FREE FATTY ACIDS, PLASMA GLUCOSE AND SERUM INSULIN CONCENTRATIONS IN THE PATIENTS STUDIED

Table 1 PLASMA FREE FATTY ACIDS (mM/l) IN THE FIRST 36 HOURS OF ACUTE MYOCARDIAL INFARCTION

Patient Number / Hours post-infarct	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	33	34	35	36	37
2	0.71	-	-	-	-	-	-	-	0.72	-	-	-	-	-	-	0.83	1.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.44
3	1.39				1.92			0.78	0.54			1.87				1.41	0.87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.68
4	2.39	-	-	-	2.58	0.31	-	0.79	0.51	0.65	-	0.76	0.72	-	1.36	1.26	1.68	0.94	-	-	-	-	0.60	-	-	2.92	-	1.10	1.12	0.89	-	-	-	0.77	-	0.55
5	1.91	1.35	-	-	2.18	0.32	1.06	0.78	0.30	1.04	-	0.71	0.50	-	1.53	1.37	1.19	0.97	-	-	1.39	-	0.44	-	-	2.34	-	0.88	0.81	-	1.64	0.64	-	0.71	-	0.31
6	1.53	1.11	-	-	1.71	0.29	1.26	0.49	0.39	0.92	-	0.58	0.53	-	1.45	2.05	0.69	1.05	0.89	0.95	1.79	-	0.56	1.24	-	1.97	1.57	1.05	0.52	1.16	1.16	0.57	1.54	0.74	-	0.18
7	1.16	0.34	2.23	-	1.28	0.24	1.10	-	-	0.50	1.23	0.79	0.62	0.75	1.36	1.54	0.89	0.99	0.82	1.19	1.24	1.58	0.63	1.21	0.73	1.81	1.56	1.09	0.65	-	1.48	0.75	-	0.39	-	-
8	1.11	0.29	1.59	1.06	1.11	0.23	1.42	0.59	0.58	0.43	1.17	0.99	0.57	0.88	1.57	1.04	1.26	0.93	1.09	1.33	-	1.41	0.87	1.28	0.96	1.73	1.52	1.14	0.88	1.06	1.26	1.00	1.44	0.28	-	0.51
9	1.06	0.52	1.13	1.07	1.22	0.21	1.45	-	-	0.51	0.91	0.89	-	0.96	1.53	1.01	1.39	0.81	1.17	1.43	0.70	1.35	-	1.19	-	-	1.59	1.58	1.14	-	1.50	0.74	-	-	0.53	-
10	0.75	0.74	0.83	0.85	1.34	-	1.96	0.73	0.83	0.37	1.12	0.79	0.87	0.99	1.51	0.98	1.52	0.67	1.23	1.34	1.03	1.51	0.79	1.32	0.98	-	1.49	1.34	1.49	1.01	1.20	0.84	1.55	0.90	0.81	0.90
11	0.43	0.85	0.75	0.29	1.34	0.47	1.48	-	-	0.39	1.01	-	0.79	0.96	-	1.00	1.03	0.74	1.01	1.31	0.95	1.43	1.14	-	-	-	-	1.58	1.42	-	1.14	0.89	-	-	0.59	-
12	1.27	1.06	0.68	0.25	1.53	0.39	0.99	0.86	1.20	0.41	0.84	0.67	0.71	0.97	-	1.15	0.39	0.93	0.79	1.63	0.87	1.57	1.31	0.38	0.91	1.46	1.29	1.45	1.46	1.07	1.16	0.75	1.32	0.66	0.12	-
14	1.02	1.49	0.55	0.17	1.09	0.45	1.79	0.41	1.02	0.79	1.53	0.89	0.52	1.07	-	1.02	0.81	1.06	1.44	1.01	0.41	0.69	1.41	1.12	0.49	-	0.97	1.27	1.49	1.04	0.98	0.86	1.16	-	0.12	0.22
16	1.03	1.15	1.06	0.25	-	0.62	1.27	0.56	1.14	0.73	1.55	1.10	0.61	1.02	-	0.81	0.98	0.89	1.11	1.32	0.37	1.28	1.33	1.08	0.78	-	0.99	1.35	1.27	0.60	0.84	0.70	1.07	-	0.15	0.19
18	0.89	1.17	1.20	0.57	-	0.62	1.23	0.93	0.99	0.94	1.31	0.71	0.59	1.03	-	0.95	0.85	0.95	1.20	1.24	0.54	0.99	1.33	1.19	0.92	-	0.83	1.35	-	0.84	-	0.72	-		0.17	0.31
20	0.42	1.08	1.33	0.23	-	0.73	1.22	0.88	0.92	0.77	1.12	0.88	0.51	0.35	-	0.78	0.47	0.68	0.87	1.12	0.93	0.96	1.34	0.93	0.76	-	0.72	1.28	-	-	-	0.95	-	-	0.39	0.77
22	0.40	1.48	1.00	0.39	-	0.59	1.17	0.87	1.05	0.74	1.28	0.34	0.33	0.31	-	0.78	1.11	0.59	0.56	1.25	0.11	1.04	1.52	1.07	0.57		0.87	1.03	-	-	-	0.35	-	-	0.60	0.64
24	0.35	0.93	1.49	0.29	-	0.32	1.14	0.53	1.24	-	1.21	0.52	0.31	0.29	-	0.76	-	0.87	0.61	1.17	1.16	0.81	1.34	0.71	0.46	-	0.77	1.03	-	-	-	0.36	-	-	0.31	-
28	0.39	1.02	0.57	0.45	-	0.21	0.42	0.53	0.87	0.36	1.08	0.39	0.46	1.20	-	-	0.99	0.69	0.52	0.80	0.69	0.59	0.79	0.91	0.59	-	-	-	-	-	-	-	-	-	0.35	0.34
32	0.59	0.77	0.37	0.28	-	0.29	1.04	0.49	0.61	0.35	1.06	0.99	0.61	0.88	-	0.68	0.85	0.83	0.74	0.43	0.54	0.65	0.95	1.02	0.75	-	-	-	-	-	-	-	-	-	0.11	0.22
36	0.49	1.15	0.90	0.55	-	0.26	0.89	0.47	0.50	0.56	0.80	0.49	0.56	0.71	-	0.51	0.90	0.62	0.52	0.41	0.13	0.58	0.68	0.68	0.65	-	-	-	-	-	-	-	-	-	0.13	0.53

Table 2

PLASMA GLUCOSE (mg/100 ml) IN THE FIRST 24 HOURS OF ACUTE MYOCARDIAL INFARCTION

Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	27
Hours post-infarct																									
2	158	-	-	-	-	-	-	-	126	-	-	-	-	-	-	162	138	-	-	-	-	-	-	-	-
3	116	-	-	-	190	-	-	141	152	-	-	110	-	-	-	171	147	-	-	-	-	-	-	-	-
4	108	-	-	-	198	51	-	112	197	83	-	115	134	-	137	169	207	109	-	-	-	-	132	-	-
5	137	96	-	-	239	117	100	112	190	91		105	125	-	147	-	-	113	-	-	149	-	126	-	-
6	-	110	-	-	286	146	111	158	148	99	-	101	109	-	146	156	127	127	114	-	164	-	112	293	-
7	158	173	106	-	295	118	114	-		149	120	-	102	102	142	-	-	-	114	110	-	114	99	285	149
8	-	124	100	118	284	113	102	116	118	167	113	111	101	104	154	147	143	117	105	104	138	102	101	276	157
9	148	-	132	113	-	68	103	-	-	-	109	-	-	103	-	-	-	-	-	105	-	101	-	259	167
10	-	82	152	162	305	-	94	118	116	146	120	108	95	104	157	137	169	108	97	112	123	95	-	247	178
11	201	-	-	239	-	99	-	-	-	-	125	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	130	102	159	131	309	129	105	95	109	66	122	102	91	108	-	144	158	113	97	107	145	94	82	245	176
14	125	149	165	155	314	124	98	157	121	77	115	-	94	95	-	129	158	111	101	106	122	85	95	219	164
16	115	146	156	71	-	113	90	96	116	76	114	106	56	99	-	145	167	103	98	105	132	115	85	250	165
18	96	169	155	149	-	99	93	77	126	84	134	120	95	101	-	158	152	118	99	97	144	116	87	215	148
20	157	156	144	133	-	103	92	91	133	81	115	108	86	159	-	176	257	120	113	106	109	112	84	295	135
22	141	163	155	152	-	97	90	84	137	87	119	135	106	90	-	165	139	122	125	104	120	110	97	291	129
24	136	154	149	130	-	139	111	83	126	-	125	108	102	94	-	192	-	119	200	79	113	106	85	312	125

Table 3

SERUM INSULIN (μU/ml) IN THE FIRST 12 HOURS OF ACUTE MYOCARDIAL INFARCTION

Patient Number	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	18	19	20	21	22	23	24	27
Hours post-infarct																								
2	78	-	-	-	-	-	-	-	22	-	-	-	-	-	41	28	-	-	-	-	-	-	-	-
3	-	-	-	-	11	-	-	36	37	-	-	16	-	-	40	23	-	-	-	-	-	-	-	-
4	28	-	-	-	16	23	-	46	130	17	-	13	21	3	46	46	16	-	-	-	-	29	-	-
5	32	-	-	-	15	26	22	-	90	16	-	11	35	18	-	-	17		-	18	-	22	-	-
6	-	17	-	-	24	68	9	278	42	23	-	12	27	17	46	44	18	9	-	30	-	25	26	20
7	72	56	20	-	30	55	13	-	-	66	25	-	19	19	-	-	-	10	21	-	25	21	26	19
8	-	33	116	18	29	55	-	153	19	130	26	39	20	33	49	29	24	7	20	31	20	17	32	25
9	46	-	25	16	-	14	18	-	-	-	33	-	-	-	-	-	-	-	15	-	21	-	25	30
10	-	14	33	42	44	-	18	78	21	100	44	13	18	36	39	31	22	6	17	22	20	-	22	40
11	105	-	-	87	-	11	-	-	-	-	44	-	-	-	-	-	-	-	-	-	-	-	-	-
12	47	14	31	33	24	39	12	31	22	16	21	16	23	-	39	41	21	5	15	35	21	15	26	32

APPENDIX B

DATA RELATING TO CALCULATION OF ESTIMATED INFARCT SIZE

DATA RELATING TO CALCULATION OF ESTIMATED INFARCT SIZE

ient ber	Peak CK activity (i.u./l)	Total cumulative CK release (i.u./ml)	Duration of CK release (hours)	Time of peak CK release (Hrs post-infarct)	Kd ($mins^{-1}$)	95% confidence limits for the slope	Surface area (m^2)	Estimated infarct size (CK-g-eq.)	Infarct size index (CK-g-eq/m^2)
1	1307	3.87	73	17	0.0013	-0.024 ±0.0033	2.1	195	93
2	169	0.48	36	14	0.0019	-0.04 ±0.003	1.9	19	10
3	2030	3.59	46	10	0.0007	-0.02 ±0.0009	1.7	117	69
6*	69	0.14	28	9	0.002	-	-	5	-
7	503	0.93	48	10	0.007	-0.02 ±0.00098	1.8	36	20
8	254	0.73	28	10	0.002	-0.05 ±0.006	1.98	33	17
9	465	0.62	18	8	0.0008	-0.02 ±0.003	1.9	26	14
0	649	1.18	36	8	0.0009	-0.02 ±0.0018	1.87	46	25
1*	926/878	0.94/2.34	20	10	0.0008	-0.02 ±0.0012	2.05	44/110	21/54
3	122	0.16	22	10	0.0013	-0.03 ±0.004	2.15	95	44
5	552	1.33	54	8	0.0009	-0.02 ±0.0012	1.9	58	31
6	1796	4.67	40	14	0.0013	-0.03 ±0.0038	2.2	262	119
7	1265	2.98	54	18	0.0011	-0.03 ±0.00015	1.85	125	68
8	389	1.29	36	10	0.002	-0.04 ±0.0048	1.95	59	30
9	850	1.07	44	14	0.0005	-0.01 ±0.001	1.6	34	21
0	1075	1.80	40	18	0.0009	-0.02 ±0.0009	1.55	52	34
1	1465	2.62	40	14	0.0012	-0.03 ±0.002	1.6	88	55
2	271	0.59	50	10	0.0013	-0.03 ±0.0016	2.1	31	15
3	239	0.38	24	8	0.0012	-0.03 ±0.003	1.98	17	9
4	991	1.93	54	10	0.0012	-0.03 ±0.0015	1.88	77	41
5	340	0.75	56	18	0.0012	-0.03 ±0.0015	2.35	54	21
7	1152	3.00	60	9	0.0016	-0.03 ±0.0016	2	131	66
8	917	1.92	40	9	0.002	-0.04 ±0.0019	2.05	94	46
9	1129	1.66	16	9	0.0014	-0.03 ±0.003	1.9	76	40
0	467	0.61	32	10	0.0016	-0.03 ±0.0033	1.95	26	13
1	1378	3.01	40	7	0.001	-0.02 ±0.0014	1.7	108	64
2	468	1.06	40	9	0.0011	-0.02 ±0.002	1.95	49	25
3*	182	0.51	18	11	0.002	-	-	7	4
4	274	0.40	30	8	0.0015	-0.03 ±0.0047	2.0	19	10
5	255	0.41	22	12	0.0017	-0.04 ±0.0016	1.95	19	10

*See Chapter III.

APPENDIX C

STATISTICAL METHODS

STATISTICAL METHODS

Statistical analysis of results was carried out using the following tests. Unless otherwise stated, the statistical tables in Geigy (Seventh Edition) were used.

1. Students t test for the differences between means of 2 samples, where the variances were similar (Swinscow, 1976).

2. The F distribution, for comparison of the variances of 2 samples (Geigy, Seventh Edition, p. 168).

3. The modification of the t test to allow for unequal variances (Geigy, Seventh Edition, p. 173).

4. The Mann-Whitney U test, for the comparison of samples with unequal variances (Siegel, International Edition). Tables for the derivation of p from U published in the same reference were used.

5. The exact fourfold table test (Geigy, Seventh Edition, p. 109-123). This was used when the number of values in a fourfold table were less than 20, or when the number of values was between 20 and 39 and the smallest expected value less than 5.

6. The line of regression and the coefficient of correlation (Colton, 1974).

Abbreviations used in the text in relation to statistical methods

n = The number of observations

r = The coefficient of correlation

a = The intercept

b = The slope

S_a = The standard error of the intercept

S_b = The standard error of the slope

$S_{y.x}$ = The standard deviation of the points above the fitted line.

REFERENCES

REFERENCES

ADGEY, A.A.J., ALLEN, J.D., GEDDES, J.S., JAMES, R.G.G., WEBB, S.W., ZAIDI, S.A. and PANTRIDGE, J.F.
Acute phase of myocardial infarction.
Lancet, 1971, 2, 501.

AGRESS, C.M. and KIM, J.H.C.
Evaluation of enzyme tests in the diagnosis of heart disease.
American Journal of Cardiology, 1960, 6, 641.

AHAMUDA, G., ROBERTS, R. and SOBEL, B.E.
Evaluation of myocardial infarction with enzymatic indices.
Progress in Cardiovascular Diseases, 1976, 18, 405.

AHMED, S.A., WILLIAMSON, J.R., ROBERTS, R., CLARK, R.E. and SOBEL, B.E.
The association of increased plasma MB CPK activity and irreversible ischaemic myocardial injury in the dog.
Circulation, 1976, 54, 187.

ALLISON, S.P., PROWSE, K. and CHAMBERLAIN, M.J.
Failure of insulin response to glucose load during operation and after myocardial infarction.
Lancet, 1967, 1, 478.

ALLISON, S.P., CHAMBERLAIN, M.J. and HINTON, P.
IV glucose tolerance, insulin, glucose and free fatty acid levels after myocardial infarction.
British Medical Journal, 1969, 4, 776.

ARMSTRONG, A., DUNCAN, B., OLIVER, M.F., JULIAN, D.G., DONALD, K.W., FULTON, M., LUTZ, W. and MORRISON, S.L.
Natural history of acute coronary heart attacks.
British Heart Journal, 1972, 34, 67.

BALLARD, F.B., DANFORTH, W.H., NAEGLE, S. and BING, R.J.
Myocardial metabolism of fatty acids.
Journal of Clinical Investigation, 1960, 39, 717.

BANG, N.U. and LaDUE, J.S.
Comparison of serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase and lactic dehydrogenase activity following acute myocardial infarction.
Diseases of the Chest, 1962, 41, 384.

BAROLDI, G.
Lack of correlation between coronary thrombosis and myocardial infarction or sudden 'coronary' heart attack.
Annals of the New York Academy of Sciences, 1969, 156, 504.

BERLYNE, G.M.
A course in renal diseases.
4th Edition. Blackwell Scientific Publications, Oxford, 1974, p. 394.

BLEIFELD, W., HANRATH, P. and MATHEY, D.
Serial CPK determinations for evaluation of size and development of acute myocardial infarction.
Circulation, 1976, 53, Suppl. I, 108.

BLEIFELD, W., MATHEY, D., HANRATH, P., BUSS, H. and EFFERT, S.
Infarct size estimated from serial serum creatine phosphokinase in relation to left ventricular haemodynamics.
Circulation, 1977, 55, 303.

BLESA, E.S., LANGER, G.A., BRADY, A.J. and SERENA, S.D.
Potassium exchange in rat ventricular myocardium : its relation to rate of stimulation.
American Journal of Physiology, 1970, 219, 747.

BLOOR, C.M., EHSANI, A., WHITE, F.C. and SOBEL, B.E.
Ventricular fibrillation threshold in acute myocardial infarction and its relation to myocardial infarct size.
Cardiovascular Research, 1975, 9, 468.

BORER, J.S., BRENSIKE, J.F., REDWOOD, D.R., ITSCOITZ, S.B., PASSAMANI, E.R., STONE, N.J., RICHARDSON, J.M., LEVINE, R.I. and EPSTEIN, S.E.
Limitations of the electrocardiographic response to exercise in predicting coronary artery disease.
New England Journal of Medicine, 1975, 293, 367.

BRAUNWALD, E.
Protection of the ischaemic myocardium - introductory remarks.
Circulation, 1976, 53, Suppl. I, 1.

BRAY, G.M. and FERRENDELLI, J.A.
Serum creatine phosphokinase in muscle disease. An evaluation of two methods of determination and comparison with serum aldolase.
Neurology, 1968, 18, 480.

BRESNAHAN, G.F., ROBERTS, R., SHELL, W.E., ROSS, J. and SOBEL, B.E.
Deleterious effects due to hemorrhage after myocardial reperfusion.
American Journal of Cardiology, 1974, 33, 82.

BURGER, A., RICHTERICH and AEGI, H.
Die heterogenität der Kreatin-Kinase.
Biochemische Zeitschrift, 1964, 339, 305.

CAHILL, G.F. Jr.
Intermediary metabolism of protein, fat and carbohydrate.
In: Harrison's Principles of Internal Medicine, 7th Edition, McGraw-Hill, 1974, Chapter 72, pp. 396-408.

CAIRNS, J.A. and KLASSEN, G.A.
The effect of propranolol on canine myocardial creatine phosphokinase distribution space and rate of disappearance.
Circulation, 1977, 86, 284.

CAIRNS, J.A., MISSIRLIS, E. and FALLEN, E.L.
Myocardial infarction size from serial CPK : variability of CPK serum ratio with size and model of infarction.
Circulation, 1978, 58, 1143.

CARLSEN, A., HALLGREN, B., JAGENBORG, R., SVANBORG, A. and WERKÖ, L.
Myocardial metabolism of glucose, lactic acid, amino-acids, and fatty acids in healthy human individuals at rest and at different work loads.
Scandinavian Journal of Clinical and Laboratory Investigation, 1961, 13, 418.

CARLSON, L.A., BOBERG, J. and HOGSTEDT, B.
Some physiological and clinical implications of lipid mobilisation from adipose tissue.
In: Handbook of Physiology, Section 5, Adipose Tissue, A.E. Renold and G.F. Cahill, Jr. eds, Washington, DC, American Physiology Society, 1965, Chapter 63, p.625.

CARLSON, C.J., EMILSON, B. and RAPAPORT, E.
Creatine phosphokinase MB isoenzyme in hypothermia : case reports and experimental studies.
American Heart Journal, 1978a, 95, 352.

CARLSON, C.J., EMILSON, B. and RAPAPORT, E.
Evidence for a biexponential perfusion model of CPK elimination.
American Journal of Cardiology, 1978b, 41, 394 (Abstract).

CAULFIELD, J.B., LEINBACH, R. and GOLD, H.
Relationship of myocardial infarct size and prognosis.
Circulation, 1976, 53, Suppl. I, 141.

CHALLONER, D.R. and STEINBERG, D.
Effect of free fatty acid on the oxygen consumption of perfused rat heart.
American Journal of Physiology, 1966a, 210, 280.

CHALLONER, D.R. and STEINBERG, D.
Oxidative metabolism of myocardium as influenced by free fatty acids and epinephrine.
American Journal of Physiology, 1966b, 211, 897.

CHRISTENSEN, N.J. and VIDEBAEK, J.
Plasma catecholamines and carbohydrate metabolism in patients with acute myocardial infarction.
Journal of Clinical Investigation, 1974, 54, 278.

CLARK, C.L., ROBISON, A.K., GNEPP, D.R., ROBERTS, R. and SOBEL, B.E.
Effects of lymphatic transport of enzyme on plasma creatine kinase time-activity curves after myocardial infarction in dogs.
Circularion Research, 1978, 43, 162.

COLTON, T.
Statistics in Medicine, 1st Edition, Little, Brown & Co., Boston, 1974.

CONNOR, W.E., HOAK, J.C. and WARNER, E.D.
Massive thrombosis produced by fatty acid infusion.
Journal of Clinical Investigation, 1963, 42, 860.

CONRAD, G.L., RAU, E.E. and SHINE, K.I.
Creatine kinase release, potassium-42 content, and mechanical performance in anoxic rabbit myocardium.
Journal of Clinical Investigation, 1979, 64, 155.

COOPER, G.R. and McDANIEL, V.
The determination of glucose by the ortho-toluidine method.
In: Standard Methods of Clinical Chemistry. R.P. MacDonald (ed). Vol. 6, Academic Press, New York, 1970, p. 167.

COX, J.R. Jr., ROBERTS, R., AMBOS, H.D., OLIVER, G.C. and SOBEL, B.E.
Relations between enzymatically estimated infarct size and early ventricular dysrhythmia.
Circulation, 1976, 53, Suppl. I, 150.

DAGENAIS, G.R. and JALBERT, B.
Effect of increased free fatty acids on myocardial oxygen extraction and angina threshold during atrial pacing.
Circulation, 1977, 56, 315.

DAHL, C.F., EWY, G.A., WARNER, E.D. and THOMAS, E.D.
Myocardial necrosis from direct current countershock. Effect of paddle electrode size and time interval between discharges.
Circulation, 1974, 50, 956.

DANOWSKI, T.S., SABEH, G., VERSTER, J.W., ALLEY, R.A., ROBBINS, T.J., TSAI, C.T., PAZITANDEH, M. and SEKATAN, K.
Serum creatine phosphokinase in muscular dystrophy and myotonia dystrophica.
Metabolism, 1968, 17, 808.

DATEY, K.K. and NANDA, N.C.
Hyperglycaemia after acute myocardial infarction. Its relation to Diabetes Mellitus.
New England Journal of Medicine, 1967, 276, 262.

DAWSON, D.M. and FINE, I.H.
Creatine kinase in human tissues.
Archives of Neurology, 1967, 16, 175.

DEACON, S.P.
The effects of atenolol and propranolol upon lipolysis.
British Journal of Clinical Pharmacology, 1978, 5, 123.

DE LEIRIS, J., OPIE, L.H. and LUBBE, W.F.
Effects of free fatty acids and glucose on enzyme release in experimental myocardial infarction.
Nature, 1975, 253, 746.

DE LEIRIS, J., and FEUVRAY, D.
Ischaemia-induced damage in the working rat heart preparation: The effect of perfusate substrate composition upon subendocardial ultrastructure of the ischaemic left ventricular wall.
Journal of Molecular and Cellular Cardiology, 1977, 9, 365.

DELVA, E., MAILLE, J.G., SOLYMOSS, B.C., CHABOT, M., GRONDIN, C.M. and BOURASSA, M.G.
Evaluation of myocardial damage during coronary artery grafting with serial determinations of serum CPK MB isoenzyme.
Journal of Thoracic and Cardiovascular Surgery, 1978, 75, 467.

DEUL, D.H. and VAN BREEMEN, J.F.L.
Electrophoresis of creatine phosphokinase from various organs.
Clinica Chimica Acta, 1964, 10, 276.

DOBOSZ, I.
The influence of the dilution effect on serum creatine phosphokinase activity in neuromuscular diseases.
Clinica Chimica Acta, 1974, 50, 301.

DOLE, V.P.
A relation between non-esterified fatty acids in plasma and the metabolism of glucose.
Journal of Clinical Investigation, 1956, 35, 150.

DOLE, V.P. and MEINERTZ, H.
Microdetermination of long-chain fatty acids in plasma and tissue.
Journal of Biological Chemistry, 1960, 235, 2595.

DREYFUS, J.-C., SCHAPIRA, G., RENAIS, J. and SCEBAL, L.
La creatine-kinase serique dans le diagnostic de l'infarctus myocardique.
Revue Francaise d'Etudes Cliniques et Biologiques, 1960a, 5, 386.

DREYFUS, J.-C., SCHAPIRA, G., SCEBAT, L., RENAIS, J. and LENEGRE, J.
Les enzymes seriques dans le diagnostic des lesions myocardiques, d'origine coronarienne. II La creatine kinase.
Archives des Maladies du Coeur et des Vaisseaux, 1960b, 2, 187.

DUMA, R.J. and SIEGEL, A.L.
Serum creatine phosphokinase in acute myocardial infarction.
Archives of Internal Medicine, 1965, 115, 443.

DUNN, M., MARTIN, J. and REISSMANN, K.R.
The disappearance rate of glutamic oxaloacetic transaminase from the circulation and its distribution in the body's fluid compartments and secretions.
Journal of Laboratory and Clinical Medicine, 1958, 51, 259.

EHSANI, A.A., EWY, G.A. and SOBEL, B.E.
Effects of electrical countershock on serum creatine phosphokinase (CPK) isoenzyme activity.
American Journal of Cardiology, 1976, 37, 12.

EHSANI, A.A., CAMPBELL, M.K., GELTMAN, E.M., ROBERTS, R. and SOBEL, B.E.
Correlations between late ventricular dysrhythmias and infarct size.
American Journal of Cardiology, 1978, 41, 424 (Abstract).

ELEVITCH, F.R.
Fluorometric techniques in Clinical Chemistry. Littlebrown, Boston, 1973.

ELLENBERG, M., OSSERMAN, K.E. and POLLACK, H.
Hyperglycaemia in coronary thrombosis.
Diabetes, 1952, 1, 16.

EPPENBERGER, H.M., EPPENBERGER, M., RICHTERICH, R. and AEBI, H.
The ontogeny of creatine kinase isoenzymes.
Developments in Biology, 1964, 10, 1.

EVANS, J.R., OPIE, L.H. and SHIPP, J.C.
Metabolism of palmitic acid in perfused rat heart.
American Journal of Physiology, 1963, 205, 766.

EVANS, J.R.
Cellular transport of long chain fatty acids.
Canadian Journal of Biochemistry, 1964, 42, 955.

FEINBERG, H., KATZ, K.N. and BOYD, E.
Determinants of coronary flow and myocardial oxygen consumption.
American Journal of Physiology, 1962, 202, 45.

FLEG, J.L., SIEGEL, B.A. and ROBERTS, R.
Detection of pericarditis with ^{99m} Technetium pyrophosphate images.
American Journal of Cardiology, 1977, 39, 273. (Abstract)

FORSSELL, G., NORDLANDER, R., NYQUIST, O., ORINUS, E. and STYRELIUS, I.
Creatine phosphokinase following cardioversion.
Acta Medica Scandinavica, 1975, 198, 349.

FORSTER, G. and ESCHER, J.
Die kreatinphosphokinase in der diagnostik von Herzinfarkt und Myopattien.
Helvetica Medica Acta, 1961, 28, 513.

FOZZARD, H.
Have we solved the arrhythmia problem?
Circulation, 1976, 53, Suppl. I, 158.

FRIEDBERG, C.K.
Diseases of the heart, 3rd Edition, W.B. Saunders, Philadelphia, 1966, p. 799.

FRIEDBERG, C.K.
General treatment of acute myocardial infarction.
Circulation, 1968, 39, Suppl. IV, 252.

GANONG, W.F.
In: Review of Medical Physiology, 8th Edition, Lange, 1977, Chapter 17, pp. 199-235.

GAZES, P.C., RICHARDSON, J.A. and WOODS, E.F.
Plasma catecholamine concentrations in myocardial infarction and angina pectoris.
Circulation, 1959, 19, 657.

GEBHARD, M.M., DENHAUS, H., SAKAI, K. and SPIECKERMANN, P.G.
Energy metabolism and enzyme release.
Journal of Molecular Medicine, 1977, 2, 271.

GEIGY.
Scientific Tables, 7th Edition, K. Diem and C. Lentner (Eds)
Ciba-Geigy Ltd, Basle, Switzerland.

GERVIN, C.A., HACKEL, D.B. and ROE, C.R.
Enzyme content of canine cardiac lymph during acute myocardial infarction.
Surgical Forum, XXV, 1974, 175.

GOLDBERG, D.M. and WINFIELD, D.A.
Diagnostic accuracy of serum enzyme assays from myocardial infarction in a general hospital population.
British Heart Journal, 1972, 34, 1597.

GOODMAN, D.J.
The interaction of human serum albumin with long-chain fatty acid anions.
Journal of the American Chemical Society, 1958, 80, 3892.

GORDON, R.S., Jr. and CHERKES, A.
Unesterified fatty acid in human blood plasma.
Journal of Clinical Investigation, 1956, 35, 206.

GRAIG, F.A., SMITH, J.C. and FOLDES, F.F.
Effect of dilution on the activity of serum creatine phosphokinase.
Clinica Chimica Acta, 1967, 15, 107.

GRIFFITHS, P.D.
Serum levels of ATP: Creatine phosphotransferase (creatine kinase). The normal range and the effect of muscular activity.
Clinica Chimica Acta, 1966, 13, 413.

GRIFFITHS, J. and LEUNG, F.
The sequential estimation of plasma catecholamines and whole blood histamine in myocardial infarction.
American Heart Journal, 1971, 82, 171.

GUPTA, D.K., YOUNG, R., JEWITT, D.E., HARTOG, M. and OPIE, L.H.
Increased plasma free fatty acid concentrations and their significance in patients with acute myocardial infarction.
Lancet, 1969, II, 1209.

GUPTA, P.R., SINHA, M.K., DASH, R.J., WAHI, P.L. and RASTOGI, G.K.
Insulin, free fatty acid and glucose response to IV glucagon following myocardial infarction.
Circulation, 1974, 49, 357.

HAGENFELDT, L. and WESTER, P.O.
Plasma levels of individual free fatty acids in patients with acute myocardial infarction.
Acta Medica Scandinavica, 1973, 194, 357.

HALES, C.N. and RANDLE, P.J.
Immunoassay of insulin with insulin-antibody precipitate.
Biochemical Journal, 1963, 88, 137.

HARMS, H.H., GOOREN, L., SPOELSTRA, A.J.G. and HESSE, C.
Blockade of isoprenaline-induced changes in plasma free fatty acids, immunoreactive insulin levels and plasma renin activity in healthy human subjects by propranolol, pindolol, practolol, atenolol, metoprolol and acebutolol.
British Journal of Clinical Pharmacology, 1978, 5, 19.

HARNARAYAN, C., BENNETT, M.A., PENTECOST, B.L. and BREWER, D.B.
Quantitative study of infarcted myocardium in cardiogenic shock.
British Heart Journal, 1970, 32, 728.

HAVEL, R.J., CARLSON, L.A., EKELUND, L-G. and HOLMOREN, A.
Studies on the relation between mobilisation of free fatty acids and energy production in man : effects of norepinephrine and nicotinic acid.
Metabolism, 1964, 13, 1402.

HEARSE, D.J., HUMPHREY, S.M. and CHAIN, E.B.
Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart : A study of myocardial enzyme release.
Journal of Molecular and Cellular Cardiology, 1973, 5, 395.

HEARSE, D.J. and HUMPHREY, S.M.
Enzyme release during myocardial anoxia : a study of metabolic protection.
Journal of Molecular and Cellular Cardiology, 1975, 7, 463.

HEARSE, D.J. and STEWART, D.A.
Functional recovery of the myocardium after elective cardiac arrest in the isolated rat heart.
Lancet, 1974, I, 192.

HENDERSON, A.H., CRAIG, R.J., GORLIN, R. and SONNENBLICK, E.H.
Free fatty acids and myocardial function in perfused rat hearts.
Cardiovascular Research, 1970a, 4, 466.

HENDERSON, A.H., MOST, A.S., PARMLEY, W.W., GORLIN, R. and SONNENBLICK, E.H.
Depression of myocardial contractility in rats by free fatty acids during hypoxia.
Circulation Research, 1970b, 26, 439.

HENRY, P.D., ROBERTS, R. and SOBEL, B.E.
Rapid separation of plasma CK isoenzymes by batch adsorption on glass beads.
Clinical Chemistry, 1975, 21, 844.

HERBACZYNSKA-CEDRO, K.
The influence of adrenaline secretion on the enzymes in heart muscle after acute coronary occlusion in dogs.
Cardiovascular Research, 1970, 4, 168.

HESS, J.W. and MacDONALD, R.P.
Serum creatine phosphokinase activity : a new diagnostic aid in myocardial and skeletal muscle disease.
Journal of the Michigan Medical Society, 1963, 62, 1095.

HESS, J.W., MacDONALD, R.P., FREDERICK, R.J., JONES, R.N., NEELY, J. and GROSS, D.
Serum creatine phosphokinase (CPK) activity in disorders of heart and skeletal muscle.
Annals of Internal Medicine, 1964, 61, 1015.

HESS, J.W., MURDOCK, K.J. and NATHO, G.J.W.
Creatine phosphokinase - A spectrophotometric method with improved sensitivity.
American Journal of Clinical Pathology, 1968, 50, 89.

HIMMS-HAGEN, J.
Sympathetic regulation of metabolism.
Pharmacology Review, 1967, 19, 367.

HORI, M., INOUE, M., FUKUI, S., SHIMAZU, T., MISHIMA, M., OHGIKANI, N., MINAMINO, T. and ABE, H.
Correlation of ejection fraction and infarct size estimated from the total CK released in patients with acute myocardial infarction.
British Heart Journal, 1979, 41, 433.

HUGHES, B.P.
A method for the estimation of serum creatine kinase and its use in comparing creatine kinase and aldolase activity in normal and pathological sera.
Clinica Chimica Acta, 1962, 7, 597.

HUNT, D. and BAILIE, M.J.
Enzyme changes following direct current countershock.
American Heart Journal, 1968, 76, 340.

IMURA, H., KATO, Y., IKEDA, M., MORIMOTO, M. and YAWATA, M.
Effect of adrenergic blocking or stimulating agents on plasma growth hormone, immunoreactive insulin, and blood free fatty acid levels in man.
Journal of Clinical Investigation, 1971, 50, 1069.

INOUE, M., HORI, M., FUKUI, S., ABE, H., MINAMINO, T., KODAMA, K. and OHGIKANI, N.
Evaluation of evolution of myocardial infarction by serial determination of serum creatine kinase activity.
British Heart Journal, 1977, 39, 485.

INTERNATIONAL UNION OF BIOCHEMISTRY.
Enzyme Nomenclature, Elsevier Publishing Co., Amsterdam, 1965.

JANUSZEWICZ, W., SZNAJDERMAN, M., WOCIAL, B. and PREIBISZ, J.
Urinary excretion of free norepinephrine and free epinephrine in patients with acute myocardial infarction in relation to its clinical course.
American Heart Journal, 1968, 76, 345.

JANUSZEWICZ, W., SZNAJDERMAN, M., CISWICKA-SZNAJDERMAN, M., WOCIAL, B. and RYMASZEWSKI, Z.
Plasma free fatty acids and catecholamine levels in patients with acute myocardial infarction.
British Heart Journal, 1971, 33, 716.

JARMAKANI, J.M., LIMBIRD, L., GRAHAM, T.C. and MARKS, R.
Effect of reperfusion on myocardial infarction and the accuracy of estimating infarct size from serum creatine phosphokinase in dog.
Cardiovascular Research, 1976, 10, 245.

JENNINGS, R.B., SOMMERS, H.M., KALTENBACH, J.P. and WEST, J.J.
Electrolyte alterations in acute myocardial ischaemic injury.
Circulation Research, 1964, 14, 260.

JEWITT, D.E., MERCER, C.J., REID, D., VALORI, C., THOMAS, M. and SHILLINGFORD, J.P.
Free noradrenaline and adrenaline excretion in relation to the development of cardiac arrhythmias and heart failure in patients with acute myocardial infarction.
Lancet, 1969, I, 635.

JOCKERS-WRETOU, E. and PFLEIDERER, E.
Quantitation of creatine kinase iso-enzymes in human tissues and sera by an immunological method.
Clinica Chimica Acta, 1975, 58, 223.

JORGENSON, L., HAEREM, J.W., CHANDLER, A.B. and BORCHGREVINK, C.F.
The pathology of acute coronary death.
Acta Anaesthetica Scandinavica, 1968, 29 (Suppl.), 193.

KAR, N.C. and PEARSON, C.M.
Activation of creatine phosphokinase by sufhydryl compounds in normal and muscular dystrophy sera.
Proceedings of the Society for Experimental Biology and Medicine, 1965a, 118, 662.

KAR, N.C. and PEARSON, C.M.
Creatine phosphokinase isoenzymes in muscle in human myopathies.
American Journal of Clinical Pathology, 1965b, 43, 207.

KARMEN, A., WROBLEWSKI, F. and LaDUE, J.S.
Transaminase activity in human blood.
Journal of Clinical Investigation, 1954, 34, 126.

KILLIP, T. and KIMBALL, J.T.
Treatment of myocardial infarction in a coronary care unit. A two-year experience with 250 patients.
American Journal of Cardiology, 1967, 20, 457.

KJEKSHUS, J.K. and SOBEL, B.E.
Depressed myocardial creatine phosphokinase activity following experimental myocardial infarction in the rabbit.
Circulation Research, 1970, 27, 403.

KJEKSHUS, J.K. and MJØS, O.D.
Effect of free fatty acids on myocardial function and metabolism in the ischemic dog heart.
Journal of Clinical Investigation, 1972, 51, 1767.

KJEKHUS, J.K. and MJØS, O.D.
Effect of inhibition of lipolysis on infarct size after experimental coronary artery occlusion.
Journal of Clinical Investigation, 1973, 52, 1770.

KJEKSHUS, J.K.
Effect of inhibition of lipolysis on heart failure following acute coronary occlusion in the dog.
Cardiovascular Research, 1974, 8, 73.

KJEKSHUS, J.K.
Effects of lipolytic and inotropic stimulation on myocardial ischaemic injury.
Acta Medica Scandinavica, 1975, 587 (Suppl.), 35.

KLEIN, R.F., TROYER, W.G., THOMPSON, H.K., BOGDONOFF, M.D. and WALLACE, A.G.
Catecholamine excretion in myocardial infarction.
Archives of Internal Medicine (Chicago), 1968, 122, 476.

KLEIN, M.S., SHELL, W.E. and SOBEL, B.E.
Serum creatine phosphokinase (CPK) isoenzymes after IM injections, surgery, and myocardial infarction. Experimental and clinical studies.
Cardiovascular Research, 1973, 7, 412.

KLEIN, M.S., EDWARD COLEMAN, R., WELDON, C.S., SOBEL, B.E. and ROBERTS, R.
Concordance of electrocardiographic and scintigraphic criteria of myocardial injury after cardiac surgery.
Journal of Thoracic and Cardiovascular Surgery, 1976, 71, 934.

KLINE, I.K., MILLER, A.J., PICK, R. and KATZ, L.N.
The effects of chronic impairment of cardiac lymph flow on myocardial reactions after coronary artery ligation in dogs.
American Heart Journal, 1964, 68, 515.

KONTTINEN, A., HUPLI, V., LOUHIJA, A. and HÄRTEL, G.
Origin of elevated serum enzyme activities after direct-current countershock.
New England Journal of Medicine, 1969, 281, 231.

KONTTINEN, A. and SOMER, H.
Determination of serum creatine kinase isoenzymes in myocardial infarction.
American Journal of Cardiology, 1972, 29, 817.

KONTTINEN, A. and SOMER, H.
Specificity of serum creatine kinase isoenzymes in diagnosis of acute myocardial infarction.
British Medical Journal, 1973, 1, 386.

KOSTIS, J.B., MAVROGEORGIS, E.A., HORSTMANN, E. and GOTZOYANNIS, S.
Effect of high concentrations of free fatty acids on the ventricular fibrillation threshold of normal dogs and dogs with acute myocardial infarction.
Cardiology, 1973, 58, 89.

KOSTUK, W.J., EHSANI, A.A., KARLINER, J.S., ASHBURN, W.L., PETERSON, K.L., ROSS, J., Jr. and SOBEL, B.E.
Left ventricular performance after myocardial infarction assessed by radioiosotope angiocardiography.
Circulation, 1973, 47, 242.

KRUGER, F.A., LEIGHTY, E.G. and WEISSLER, A.M.
Catecholamine stimulation of myocardial lipolysis and fatty acid re-esterification.
Journal of Clinical Investigation, 1967, 46, 1080.

KUDIRKA, P.J., BUSBY, M.G., CAREY, R.N. and TOREN, E.C.
Separation of creatine kinase isoenzymes by high pressure liquid chromatography.
Clinical Chemistry, 1975, 21, 450.

KURIEN, V.A. and OLIVER, M.F.
Serum free fatty acids after acute myocardial infarction and cerebral vascular occlusion.
Lancet, 1966, 2, 122.

KURIEN, V.A., YATES, P.A. and OLIVER, M.F.
Free fatty acids, heparin and arrhythmias during experimental myocardial infarction.
Lancet, 1969, 2, 185.

KURIEN, V.A. and OLIVER, M.F.
A metabolic cause for arrhythmias during acute myocardial hypoxia.
Lancet, 1970, I, 813.

KURIEN, V.A., YATES, P.A. and OLIVER, M.F.
The role of free fatty acids in the production of ventricular arrhythmias after acute coronary artery occlusion.
European Journal of Clinical Investigation, 1971, 1, 225.

LAFAIR, J.S. and MYERSON, R.M.
Alcoholic myopathy with special reference to the significance of creatine phosphokinase.
Archives of Internal Medicine (Chicago) 1968, 122, 417.

LEBOVITZ, H.E., SCHULTZ, K.T., MATTHEW, M.E. and SCHEELE, R.
Acute metabolic responses to myocardial infarction. Changes in glucose utilisation and secretion of insulin and growth hormone.
Circulation, 1969, 39, 171.

LEMLEY-STONE, J., MERRILL, J.M., GRACE, J.T. and MENEELY, G.R.
Transaminase in experimental myocardial infarction.
American Journal of Physiology, 1955, 183, 555.

LEVI, L.
Neuro-endocrinology of anxiety. In: Studies of Anxiety, British Journal of Psychiatry, Special Publication.
Ed. M.H. Lader, Headley, London, No. 3, 1969, p. 40.

LEVINE, H.D. and FORD, R.V.
Subendocardial infarction : report of six cases and critical survey of the literature.
Circulation, 1950, 1, 246.

LIEDTKE, A.J., NELLIS, S. and NEELY, J.R.
Effects of excess free fatty acids on mechanical and metabolic function in normal and ischaemic myocardium in swine.
Circulation Research, 1978, 43, 652.

LOMBARDO, T.A., ROSE, L., TAESCHLER, M., TULEY, S. and BING, J.R.
The effect of exercise on coronary blood flow, myocardial oxygen consumption and cardiac efficiency in man.
Circulation, 1953, 7, 71.

LOWN, B., AMARASINGHAM, R. and NEUMAN, J.
A new method for terminating cardiac arrhythmias. Use of a synchronised capacitor discharge.
Journal of the American Medical Association, 1962, 182, 548.

LUKOMSKY, P.E. and OGANOV, R.G.
Blood plasma catecholamines and their urinary excretion in patients with acute myocardial infarction.
American Heart Journal, 1972, 83, 182.

LUXTON, M.R., MILLER, N.E. and OLIVER, M.F.
Antilipolytic therapy in angina pectoris. Reduction of exercise-induced ST segment depression.
British Heart Journal, 1976, 38, 1204.

MACLEAN, D., GRIFFITHS, P.D. and EMSLIE-SMITH, D.
Serum-enzymes in relation to electrocardiographic changes in accidental hypothermia.
Lancet, 1968, 2, 1266.

MACLEAN, D., FISHBEIN, M.C., BRAUNWALD, E. and MAROKO, P.R.
Long-term preservation of ischaemic myocardium after experimental coronary artery occlusion.
Journal of Clinical Investigation, 1978, 61, 541.

McDONALD, L., BAKER, C., BRAY, C., McDONALD, A. and RESTIEAUX, N.
Plasma catecholamines after cardiac infarction.
Lancet, 1969, 2, 1021.

McMILLAN, J.B. and LEV, M.
The aging heart. I. Endocardium.
Journal of Gerontology, 1959, 14, 268.

MADIAS, J.E., VENKATARAMAN, K. and HOOD, W.B., Jr.
Precordial ST segment mapping. (1) Clinical studies in the coronary care unit.
Circulation, 1975, 52, 799.

MALMBERG, P.
Time course of enzyme escape via heart lymph following myocardial infarction in the dog.
Scandinavian Journal of Clinical and Laboratory Investigation, 1972a, 30, 405.

MALMBERG, P.
Aspartate amino transferase activity in dog heart lymph after myocardial infarction.
Scandinavian Journal of Clinical and Laboratory Investigation, 1972b, 30, 1.

MANDECKI, T., GIEC, L. and KARGUL, W.
Serum enzyme activities after cardioversion.
British Heart Journal, 1970, 32, 600.

MARMOR, A., KAHANA, L., ALPAN, G., GRENADIER, E., KEIDAR, S. and PALANT, A.
Creatine kinase isoenzyme MB (CK-MB) in acute coronary ischaemia.
American Heart Journal, 1979, 97, 574.

MAROKO, P.R., KJEKSHUS, J.K., SOBEL, B.E., WATANABE, T., COVELL, J.W., ROSS, J., Jr. and BRAUNWALD, E.
Factors influencing infarct size following experimental coronary artery occlusions.
Circulation, 1971, 43, 67.

MAROKO, P.R., LIBBY, P. SOBEL, B.E., BLOOR, C.M., SYBERS, H.D., SHELL, W.E., COVELL, J.W. and BRAUNWALD, E.
Effect of glucose-insulin-potassium infusion on myocardial infarction following experimental coronary occlusion.
Circulation, 1972, 45, 1160.

MAROKO, P.R. and BRAUNWALD, E.
Modification of myocardial infarct size after coronary occlusion.
Annals of Internal Medicine, 1973, 79, 720.

MATHEY, D., BLEIFELD, W., HANRATH, P. and EFFERT, S.
Attempt to quantitate relation between cardiac function and infarct size in acute myocardial infarction.
British Heart Journal, 1974, 36, 271.

MATHEY, D., BLEIFELD, W., BUSS, H. and HANRATH, P.
Creatine kinase release in acute myocardial infarction : correlation with clinical, electrocardiographic, and pathological findings.
British Heart Journal, 1975, 37, 1161.

MELSOM, M., ANDREASSEN, P., MELSOM, H., HANSEN, T., GRENDAHL, H. and HILLESTAD, L.K.
Diazepam in acute myocardial infarction - clinical effects and effects on catecholamines, free fatty acids and cortisol.
British Heart Journal, 1976, 38, 804.

→ Meltzer & Klein (1970)

MELTZER, H.Y., MROZAK, S. and BOYER, M.
Effect of intramuscular injections on serum creatine phosphokinase activity.
American Journal of Medical Science, 1970, 259, 42.

MENACHÉ, R., IHIEL, D. and RUBINSTEIN, J.
Sullo stato della creatina kinasi nel siero di soggetti affetti da Infarto del myocardio e de distrofia muscolare progressiva.
Biochem. Appl. (Parma), 1966, 13, 15.

MENACHÉ, R.
Effect of serum concentration on the assay of enzyme activities of diagnostic importance.
Israeli Journal of Medical Science, 1967, 3, 907.

MERCER, D.
Separation of tissue and serum creatine kinase isoenzymes by ion-exchange column chromatography.
Clinical Chemistry, 1974, 20, 36.

MERCK, E. (Darmstadt)
Cardiac Infarction.
Brochure 16/799/10/576D.

MESSER, J.V., WAGMAN, R.J., LEVINE, H.J., NEILL, W.A., KRASNOW, N. and GORLIN, R.
Patterns of human myocardial oxygen extraction during rest and exercise.
Journal of Clinical Investigation, 1962, 41, 725.

MILLER, A.J., ELLIS, A. and KATZ, L.N.
Cardiac lymph - flow rates and composition in dogs.
American Journal of Physiology, 1964, 206, 63.

MILLER, R.E. and SOELDNER, J.S.
Suppression of portal venous insulin concentration by epinephrine in the conscious monkey.
Diabetologia, 1969, 5, 179.

MICHIE, D.D., CONLEY, M.A., CARRETTA, R.D. and BOOTH, R.W.
Serum enzyme changes following cardiac catheterisation, with and without selective angiography.
American Journal of Medical Science, 1970, 260, 11.

MIYADA, D.S., DINOVO, E.C. and NAKAMURA, R.M.
Creatine kinase reactivation by thiol compounds.
Clinica Chimica Acta, 1975, 58, 97.

MJØS, O.D.
Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs.
Journal of Clinical Investigation, 1971a, 50, 1386.

MJØS, O.D.
Effect of inhibition of lipolysis on myocardial oxygen consumption in the presence of isoproterenol.
Journal of Clinical Investigation, 1971b, 50, 1869.

MJØS, O.D., KJEKSHUS, J.K. and LEKVEN, J.
Importance of free fatty acids as a determinant of myocardial oxygen consumption and myocardial ischemic injury during norepinephrine infusion in dogs.
Journal of Clinical Investigation, 1974, 53, 1290.

MJØS, O.D.
Effect of reduction of myocardial free fatty acid metabolism relative to that of glucose on the ischaemic injury during experimental coronary artery occlusion in dogs.
Acta Medica Scandinavica, 1975, 587 (Suppl.), 29.

MJØS, O.D., MILLER, N.E., RIEMERSMA, R.A. and OLIVER, M.F.
Effects of p-Chlorophenoxyisobutyrate on myocardial free fatty acid extraction, ventricular blood flow, and epicardial ST-segment elevation during coronary occlusion in dogs.
Circulation, 1976, 53, 494.

MOST, A.S. SZYDLIK, P.A. and SOREM, K.R.
Effect of free fatty acid on myocardial function during hypoxia.
Cardiology, 1972, 57, 322.

MOST, A.S., LIPSKY, M.H., SZYDLIK, P.A. and BRUNO, C.
Failure of free fatty acids to influence myocardial oxygen consumption in the intact, anaesthetised dog.
Cardiology, 1973, 58, 220.

MOST, A.S., CAPONE, R.J., SZYDLIK, P., BRUNO, C.A. and DE VONA, T.S.
Failure of free fatty acids to influence degree of myocardial injury following acute coronary artery occlusion in pigs.
Cardiology, 1974, 59, 201.

MOST, A.S., CAPONE, R.J. and MASTROFRANCESCO, P.A.
Free fatty acids and arrhythmias following acute coronary artery occlusion in pigs.
Cardiovascular Research, 1976, 10, 198.

MUELLER, H.S. and AYRES, S.M.
The role of propranolol in the treatment of acute myocardial infarction.
Progress in Cardiovascular Disease, 1977, 19, 405.

MUELLER, H.S. and AYRES, S.M.
Metabolic responses of the heart in acute myocardial infarction in man.
American Journal of Cardiology, 1978, 42, 363.

MUGGIA, F.M., GHOSSEN, N.A. and HANOK, A.
Creatine phosphokinase and other serum enzymes during radiotherapy.
Journal of the American Medical Association, 1970, 211, 1345.

NACHLAS, M.M., FRIEDMAN, M.M. and COHEN, S.P.
A method for the quantitation of myocardial infarcts and the relation of serum enzyme levels to infarct size.
Surgery, 1964, 55, 700.

NELSON, P.G.
Effect of heparin on serum free fatty acids, plasma catecholamines, and the incidence of arrhythmias following acute myocardial infarction.
British Medical Journal, 1970, 3, 735.

NIMMO, I.A., SMITH, R.H., DOLDER, M.A. and OLIVER, M.F.
Turnover of plasma glucose and free fatty acids in patients on the first day after myocardial infarction.
Clinical Science and Molecular Medicine, 1976, 50, 401.

NORRIS, R.M., BRANDT, P.W.T., CAUGHEY, D.E., LEE, A.J. and SCOTT, P.J.
A new coronary prognostic index.
Lancet, 1969, I, 274.

NORRIS, R.M., WHITLOCK, R.M.L., BARRATT-BOYES, C. and SMALL, C.W.
Clinical measurement of myocardial infarct size. Modification of a method for the estimation of total creatine phosphokinase release after myocardial infarction.
Circulation, 1975, 51, 614.

NYDICK, I., WROBLEWSKI, F. and LaDUE, J.S.
Evidence for increased serum glutamic oxalacetic transaminase (SGOT) activity following graded myocardial infarcts in dogs.
Circulation, 1955, 12, 161.

OGUNRO, E.A., HEARSE, D.J. and SHILLINGFORD, J.P.
Creatine kinase isoenzymes : their separation, quantitative determination and use in the assessment of acute myocardial infarction.
Biochemical Society Transactions, 1975, 3, 425.

OGUNRO, E.A., HEARSE, D.J. and SHILLINGFORD, J.P.
Creatine kinase isoenzymes : their separation and quantitation.
Cardiovascular Research, 1977, 11, 94.

OLIVER, I.T.
A spectrophotometric method for the determination of creatine phosphokinase and myokinase.
Biochemical Journal, 1955, 61, 116.

OLIVER, M.F., KURIEN, V.A. and GREENWOOD, T.W.
Relation between serum free fatty acids and arrhythmias and death after acute myocardial infarction.
Lancet, 1968, I, 710.

OLIVER, M.F. and YATES, P.A.
Induction of ventricular arrhythmias by elevation of arterial free fatty acids in experimental myocardial infarction.
Cardiology, 1971/72, 56, 359.

OLIVER, M.F., ROWE, M.J. and VETTER, N.
Metabolic Intervention. In: Effect of Acute Ischaemia on Myocardial Function. Eds. M.F. Oliver, D.G. Julian and K.W. Donald, Churchill, Livingstone, 1972, pp. 354-366.

OLIVER, M.F.
Metabolic response during impending myocardial infarction. II. Clinical implications.
Circulation, 1972, 45, 491.

OPIE, L.H. and WALFISH, P.G.
Plasma free fatty acid concentrations in obesity.
New England Journal of Medicine, 1963, 268, 757.

OPIE, L.H.
Metabolism of the heart in health and disease. Part I.
American Heart Journal, 1968, 76, 685.

OPIE, L.H.
The glucose hypothesis : relation to acute myocardial infarction.
Journal of Molecular and Cellular Cardiology, 1970, 1, 107.

OPIE, L.H.
Acute metabolic response in myocardial infarction.
British Heart Journal, 1971, 33 (Suppl.), 129.

OPIE, L.H., MANSFORD, K.R.L. and OWEN, P.
Effects of increased heart work on glycolysis and adenine nucleotides in the perfused heart of normal and diabetic rats.
Biochemical Journal, 1971a, 124, 475.

OPIE, L.H., NORRIS, R.M., THOMAS, M., HOLLAND, A.J., OWEN, P. and VAN NOORDEN, S.
Failure of high concentrations of circulating free fatty acids to provoke arrhythmias in experimental myocardial infarction.
Lancet, 1971b, I, 818.

OPIE, L.H.
Metabolic response during impending myocardial infarction. I. Relevance of studies of glucose and fatty acid metabolism in animals.
Circulation, 1972a, 45, 483.

OPIE, L.H.
The general and local metabolic response to acute myocardial infarction.
Acta Biologica et Medica Germanica, 1972b, 28, 873.

OPIE, L.H.
Lipid metabolism of the heart and arteries in relation to ischaemic heart disease.
Lancet, 1973, I, 192.

OPIE, L.H., OWEN, P. and RIEMERSMA, R.A.
Relative rates of oxidation of glucose and free fatty acids by ischaemic and non-ischaemic myocardium after coronary artery ligation in the dog.
European Journal of Clinical Investigation, 1973, 3, 419.

OPIE, L.H.
Metabolism of free fatty acids, glucose and catecholamines in acute myocardial infarction. Relation to myocardial ischemia and infarct size.
American Journal of Cardiology, 1975, 36, 938.

OPIE, L.H., BRUYNEEL, J. and KENNELLY, B.M.
Effects of early oral feeding on plasma free fatty acid concentrations in patients in a coronary care unit.
British Heart Journal, 1975, 37, 400.

OPIE, L.H. and LUBBE, W.F.
Are free fatty acids arrhythmogenic?
Journal of Molecular and Cellular Cardiology, 1975, 7, 155.

OPIE, L.H. and OWEN, P.
Effects of glucose-insulin-potassium infusions on arteriovenous differences of glucose and of free fatty acids and on tissue metabolic changes in dogs with developing myocardial infarction.
American Journal of Cardiology, 1976, 38, 310.

OPIE, L.H. and THOMAS, M.
Propranolol and experimental myocardial infarction: substrate effects.
Postgraduate Medical Journal, 1976 52, (Suppl. 4), 124.

OPIE, L.H.
Metabolism, enzyme release, and cell death: possibilities for future investigation. In: Enzymes in Cardiology : diagnosis and research. Eds. D. Hearse, J. DeLeiris; John Wiley, London, 1979. Chapter 24, pp. 561-567.

OWEN, P., THOMAS, M. and OPIE, L.
Relative changes in free fatty acid and glucose utilisation by ischaemic myocardium after coronary artery occlusion.
Lancet, 1969, I, 1187.

PAGE, D.L., CAULFIELD, J.B., KASTOR, J.A., DeSANCTIS, R.W. and SANDERS, C.A.
Myocardial changes associated with cardiogenic shock.
New England Journal of Medicine, 1971, 285, 133.

PAINTER, A.A., SOBEL, B.E. and ROBERTS, R.
The dependence of clearance of creatine kinase on removal of protein.
Circulation, 1977, 56 (Suppl. III), 86.

PANDE, S.V. and MEAD, J.F.
Inhibition of enzyme activities by free fatty acids.
Journal of Biological Chemistry, 1968, 243, 6180.

PASYK, S., BLOOR, C.M., KHOURI, E.M. and GREGG, D.E.
Systemic and coronary effects of coronary artery occlusions in the unanaesthetised dog.
American Journal of Physiology, 1971, 220, 646.

PIERCE, W.S., CARTER, D.R., McGAVRAN, M.H. and WALDHAUSEN, J.A.
Modification of myocardial infarct volume. An experimental study in the dog.
Archives of Surgery, 1973, 107, 682.

PINTER, E.J. and PATTEE, C.J.
Effect of beta-adrenergic blockade on resting and stimulated fat mobilisation.
Journal of Clinical Endocrinology, 1967, 27, 1441.

PINTER, E.J., PETERFY, G., CLEGHORN, J.M. and PATTEE, C.J.
The influence of emotional stress on fat mobilisation; the role of endogenous catecholamines in the β-adrenergic receptors.
American Journal of the Medical Sciences, 1967, 254, 634.

PORTE, D. Jnr., GRABER, A.L., KUZUYA, T. and WILLIAMS, R.H.
The effect of epinephrine on immunoreactive insulin levels in man.
Journal of Clinical Investigation, 1966, 45, 228.

POSEN, S.
Turnover of circulating enzymes.
Clinical Chemistry, 1970, 16, 71.

PRAKASH, R., PARMLEY, W.W., HORVAT, M. and SWAN, H.J.C.
Serum cortisol, plasma free fatty acids, and urinary catecholamines as indicators of complications in acute myocardial infarction.
Circulation, 1972, 45, 736.

RAAB, A.P. and RABINOWITZ, M.A.
Glycosuria and hyperglycemia in coronary thrombosis.
Journal of the American Medical Association, 1936, 106, 1705.

RAAB, W., VAN LITH, P. and LEPESCHKIN, E.
Catecholamine-induced myocardial hypoxia in the presence of impaired coronary dilatability independant of external cardiac work.
American Journal of Cardiology, 1962, 9, 455.

RAO, P.S., LUKES, J.J., AYRES, S.M. and MUELLER, H.
New manual and automated method for determining activity of the creatine kinase isoenzyme MB, by use of Dithiothreitol : Clinical applications.
Clinical Chemistry, 1975, 21, 1612.

RAPAPORT, E.
The fractional disappearance rate of the separate isoenzymes of creatine phosphokinase in the dog.
Cardiovascular Research, 1975, 9, 473.

REID, D.S., PELIDES, L.H. and SHILLINGFORD, J.P.
Surface mapping of RS-T segment in acute myocardial infarction.
British Heart Journal, 1971, 33, 370.

REIFFEL, J.A., GAMBINO, S.R., McCARTHY, D.M. and LEAHEY, E.B. Jnr.
Direct current cardioversion. Effect on creatine kinase, lactic dehydrogenase and myocardial isoenzymes.
Journal of the American Medical Association, 1978, 239, 122.

RIFKIND, B.M.
Plasma free fatty acid levels and intravenous glucose tolerance after myocardial infarction.
Journal of Atherosclerosis Research, 1966, 6, 26.

RIGHETTI, A., CRAWFORD, M.C., O'ROURKE, R.A., HARDARSON, T., SCHELBERT, H., DAILY, P.O., DeLUCA, M., ASHBURN, W. and ROSS, J. Jnr.
Detection of perioperative myocardial damage after coronary artery bypass graft surgery.
Circulation, 1977, 55, 173.

ROBERTS, R., HENRY, P.D., WITTERVEEN, S.A.G.J. and SOBEL, B.E.
Quantification of serum creatine phosphokinase isoenzyme activity.
American Journal of Cardiology, 1974, 33, 650.

ROBERTS, R., GOWDA, K.S., LUDBROOK, P.A. and SOBEL, B.E.
Specificity of elevated serum MB creatine phosphokinase activity in the diagnosis of acute myocardial infarction.
American Journal of Cardiology, 1975a, 36, 433.

ROBERTS, R., HENRY, P.D. and SOBEL, B.E.
An improved basis for enzymatic estimation of infarct size.
Circulation, 1975b, 52, 743.

ROBERTS, R., HUSAIN, A., AMBOS, H.D., OLIVER, G.C., COX, J.R. Jr., and SOBEL, B.E.
Relation between infarct size and ventricular arrhythmia.
British Heart Journal, 1975c, 37, 1169.

ROBERTS, R. and SOBEL, B.E.
Elevated plasma MB creatine phosphokinase activity. A specific marker for myocardial infarction in perioperative patients.
Archives of Internal Medicine, 1976, 136, 421.

ROBERTS, R., SOBEL, B.E. and LUDBROOK, P.A.
Determination of the origin of elevated plasma creatine phosphokinase after cardiac catheterisation.
Catheterization and Cardiovascular Diagnosis, 1976, 2, 329.

ROBERTS, R., KARLSBERG, R.P. and SOBEL, B.E.
Factors retarding the decline of plasma CK activity after myocardial infarction.
American Journal of Cardiology, 1977, 39, 2 (Abstract).

ROBERTS, R. and SOBEL, B.E.
Effect of selected drugs and myocardial infarction on the disappearance of creatine kinase from the circulation in conscious dogs.
Cardiovascular Research, 1977, 11, 103.

ROBERTS, R. and SOBEL, B.E.
Creatine kinase isoenzymes in the assessment of heart disease.
American Heart Journal, 1978, 95, 521.

ROBISON, A.K., GNEPP, D.R. and SOBEL, B.E.
Inactivation of creatine phosphokinase in lymph.
Circulation, 1975, 52, (Suppl. II) 5.

ROE, C.R., LIMBIRD, L.E., WAGNER, G.S. and NERENBERG, S.T.
Combined isoenzyme-analysis in the diagnosis of myocardial injury: application of electrophoretic methods for the detection and quantitation of the creatine phosphokinase MB isoenzyme.
Journal of Laboratory and Clinical Medicine, 1972, 80, 577.

ROE, C.R. and STARMER, C.F.
A sensitivity analysis of enzymatic estimation of infarct size.
Circulation, 1975, 52, 1. (Editorial).

ROE, C.R., COBB, F.R. and STARMER, C.F.
The relationship between enzymatic and histological estimates of the extent of myocardial infarction in conscious dogs with permanent coronary occlusion.
Circulation, 1977, 55, 438.

ROGERS, W.J., McDANIEL, H.G., SMITH, L.R., MANTLE, J.A., RUSSELL, R.O.,Jr. and RACKLEY, C.E.
Correlation of CPK-MB and angiographic estimates of infarct size in man.
Circulation, 1976, 54, (Suppl. II), 28.

ROGERS, W.J., McDANIEL, H.G., SMITH, L.R., MANTLE, J.A., RUSSELL, R.O., Jr. and RACKLEY, C.E.
Correlation of angiographic estimates of myocardial infarct size and accumulated release of creatine kinase MB isoenzyme in man.
Circulation, 1977a, 56, 199.

ROGERS, W.J., McDANIEL, H.G., MORASKI, R.E., RACKLEY, C.E. and RUSSELL, R.O., Jr.
Effect of heparin-induced free fatty acid elevation on myocardial oxygen consumption in man.
American Journal of Cardiology, 1977b, 40, 365.

ROSALKI, S.B.
An improved procedure for serum creatine phosphokinase determination.
Journal of Laboratory and Clinical Methods, 1967, 69, 696.

ROSE, A.G., OPIE, L.H. and BRICKNELL, O.L.
Early experimental myocardial infarction. Evaluation of histologic criteria and comparison with biochemical and electrocardiographic measurements.
Archives of Pathologicaland Laboratory Medicine, 1976, 100, 516.

ROWE, M.J., DOLDER, M.A., KIRBY, B.J. and OLIVER, M.F.
Effect of nicotinic acid analogue on raised plasma free fatty acids after acute myocardial infarction.
Lancet, 1973, II, 814.

ROWE, M.J., NEILSON, J.M.M. and OLIVER, M.F.
Control of ventricular arrhythmias during myocardial infarction by antilipolytic treatment using a nicotinic acid analogue.
Lancet, 1975, I, 295.

RUSSELL, D.C. and OLIVER, M.F.
Effect of antilipolytic therapy on ST segment elevation during myocardial infarction.
British Heart Journal, 1978, 40, 117.

RUSSO, J.V., FRIESINGER, G.C., MARGOLIS, S. and ROSS, R.S.
Heparin and ventricular arrhythmias after myocardial infarction.
Lancet, 1970, II, 1271.

RUTENBERG, H.L., PAMINTUAN, J.C. and SOLOFF, L.A.
Serum free fatty acids and their relation to complications after acute myocardial infarction.
Lancet, 1969, II, 559.

SAKAI, K., GEBHARD, M.M., SPIECKERMANN, P.G. and BETSCHNEIDER, H.J.
Enzyme release resulting from total ischaemia and reperfusion in the isolated, perfused guinea pig heart.
Journal of Molecular and Cellular Cardiology, 1975, 7, 827.

SAVIGNANO, T., HANOK, A. and HUO, J.
Creatine phosphokinase activity: a study of normal and abnormal levels.
American Journal of Clinical Pathology, 1969, 51, 76.

SCHEIDT, S., ASCHEIM, R. and KILLIP, T. III.
Shock after acute myocardial infarction.
American Journal of Cardiology, 1970, 26, 556.

SCHEUER, J. and BRACHFELD, N.
Myocardial uptake and fractional distribution of palmitate-1-C^{14} by the ischaemic dog heart.
Metabolism, 1966, 15, 945.

SCHEUER, J.
Myocardial metabolism in cardiac hypoxia.
American Journal of Cardiology, 1967, 19, 385.

SCHLANT, R.C.
Metabolism of the heart. In: The Heart, Arteries and Veins. 3rd Edition, Ed. J.W. Hurst, McGraw-Hill, 1974, pp. 115-128.

SCHULTZE, H.E. and HEREMANS, J.F.
Nature and metabolism of extracellular proteins. In: Molecular Biology of Human Proteins, Elsevier, Amsterdam, Vol. 1, 1966.

SCHWARTZ, D.D.
Interferences in diagnostic biochemical procedures. In: Advances in Clinical Chemistry, Eds. O. Bodansky and A. Latner, 1973, 16, 155.

SCOTT, J.C., FINKELSTEIN, L.J. and SPITZER, J.T.
Myocardial removal of free fatty acids under normal and pathological conditions.
American Journal of Physiology, 1962, 203, 482.

SEVEREID, L., CONNOR, W.E. and LONG, J.P.
The depressant effect of fatty acids on the isolated rabbit heart.
Proceedings of the Society for Experimental Biology and Medicine, 1969, 131, 1139.

SHAFRIR, E. and GUTMAN, A.
Patterns of decrease of free fatty acids during glucose tolerance tests.
Diabetes, 1965, 14, 77.

SHELL, W.E., KJEKSHUS, J.K. and SOBEL, B.E.
Quantitative assessment of the extent of myocardial infarction in the conscious dog by means of analysis of serial changes in serum creatine phosphokinase activity.
Journal of Clinical Investigation, 1971, 50, 2614.

SHELL, W.E. and SOBEL, B.E.
Changes in infarct size following administration of propranolol in the conscious dog.
American Journal of Cardiology, 1973a, 31, 157 (Abstract).

SHELL, W.E. and SOBEL, B.E.
The deleterious effects of increased heart rate on infarct size in the conscious dog.
American Journal of Cardiology, 1973b, 31, 474.

SHELL, W.E. and SOBEL, B.E.
Protection of jeopardised ischaemic myocardium by reduction of ventricular afterload.
New England Journal of Medicine, 1974, 291, 481.

SHELL, W. and SOBEL, B.E.
Biochemical markers of ischaemic injury.
Circulation, 1976, 53 (Suppl. I), 98.

SHIPP, J.C., OPIE, L.H. and CHALLONER, D.
Fatty acid and glucose metabolism in the perfused heart.
Nature, 1961, 189, 1018.

SHUG, A., SHRAGO, E., BITTAR, N., FOLB, J.D. and KOKES, J.R.
Long chain fatty acyl-CoA inhibition of adenine nucleotide translocation in the ischaemic myocardium.
American Journal of Physiology, 1975, 228, 689.

SIEGEL, S.
Nonparametric statistics for the behavioural sciences.
International Student Edition, McGraw-Hill-Kogakusha Ltd.

SIGGERS, D.C., SALTER, C. and FLUCK, D.C.
Serial plasma adrenaline and noradrenaline levels in myocardial infarction using a new double isotope technique.
British Heart Journal, 1971, 33, 878.

SIMONSEN, S. and KJEKSHUS, J.K.
The effect of free fatty acids on myocardial oxygen consumption during atrial pacing and catecholamine infusion in man.
Circulation, 1978, 58, 484.

SJÖVALL, K. and VOIGT, A.
Creatine-phospho-transferase isoenzymes.
Nature, 1964, 202, 701.

SLUTSKY, A.S.
Individual values for the disappearance rate parameter (Kd) in the enzymatic estimation of infarct size. - A critique.
Circulation, 1977, 56, 545.

SMITH, A.F.
Separation of tissue and serum creatine kinase isoenzymes on polyacrylamide gel slabs.
Clinica Chimica Acta, 1972, 39, 351.

SMITH, E.R. and DUCE, B.R.
Anti-arrhythmic and serum free-fatty acid lowering effects of 5-fluoro-nicotinyl alcohol following experimentally induced myocardial infarction in the dog.
Cardiovascular Research, 1974, 8, 550.

SMITH, A.F., RADFORD, D., WONG, C.P. and OLIVER, M.F.
Creatine kinase MB iso-enzyme studies in diagnosis of myocardial infarction.
British Heart Journal, 1976, 38, 225.

SOBEL, B.E., BRESNAHAN, G.F., SHELL, W.E. and YODER, K.R.D.
Estimation of infarct size in man and its relation to prognosis.
Circulation, 1972, 46, 640.

SOBEL, B.E. and SHELL, W.E.
Serum enzyme determinations in the diagnosis and assessment of myocardial infarction.
Circulation, 1972, 45, 471.

SOBEL, B.E.
Applications and limitations of estimation of infarct size from serial changes in plasma creatine phosphokinase.
Acta Medica Scandinavica, 1975, Suppl. 587, 151.

SOBEL, B.
Infarct size, prognosis and causal contiguity.
Circulation, 1976, 53, Suppl. I, 146.

SOBEL, B., ROBERTS, R. and LARSON, K.B.
Estimation of infarct size from serum MB creatine phosphokinase activity: Applications and limitations.
American Journal of Cardiology, 1976a, 37, 474.

SOBEL, B.E.,ROBERTS, R. and LARSON, K.B.
Considerations in the use of biochemical markers of ischaemic injury.
Circulation Research, 1976b, 38, Suppl. I, 99.

SOBEL, B.E., MARKHAM, J. and ROBERTS, R.
Factors influencing enzymatic estimates of infarct size.
American Journal of Cardiology, 1977a, 39, 130.

SOBEL, B.E., MARKHAM, J., KARLSBERG, R.P. and ROBERTS, R.
The nature of disappearance of creatine kinase from the circulation and its influence on enzymatic estimation of infarct size.
Circulation Research, 1977b, 41, 836.

SOMER, H. and KONTTINEN, A.
Demonstration of serum creatine kinase isoenzymes by a fluorescence technique.
Clinica Chimica Acta, 1972, 40, 133.

SOMMERS, H.M. and JENNINGS, R.B.
Experimental acute myocardial infarction: Histologic and histochemical studies of early myocardial infarcts induced by temporary or permanent occlusion of a coronary artery.
Laboratory Investigation, 1964, 13, 1491.

SOWTON, E.
Cardiac infarction and the glucose-tolerance test.
British Medical Journal, 1962, 1, 84.

SPECTOR, A.A.
The transport and utilisation of free fatty acid.
Annals of the New York Academy of Science, 1968, 149, 768.

SPECTOR, A.A., JOHN, K., FLETCHER, J.E.
Binding of long-chain fatty acids to bovine serum albumin.
Journal of Lipid Research, 1969, 10, 56.

SPECTOR, A.A.
Metabolism of free fatty acids.
Progress in Biochemical Pharmacology, 1971, 6, 130.

SPIEKERMANN, P.G., GEBHARD, M.M., KALBOW, K., KNOLL, D., KOHL, F., NORDBECK, H., SAKAI, K. and BRETSCHNEIDER, H.J.
Freisetzung von enzymen aus der herzmuskelzelle während sauerstoffmangel.
Verhandlungen der Deutschen Gesellschaft für Kreislaufforschlung, 1973, 39, 193.

SPIKESMAN, A.M. and BROCK, D.J.H.
The significance of the dilution effect in serum creatine kinase diagnosis of muscular dystrophy.
Clinica Chimica Acta, 1969, 26, 387.

STICH, W. and TSIRIMBAS, A.
Die enzymediagnostik des herzinfarktes.
Internist (Berlin), 1961, 2, 592.

STICH, W. and TSIRIMBAS, A.
Der creatin-phosphokinase test. Eine neue enzymatische methode zur biochemischen diagnose des hertzinfarktes.
Klinische Wochenschrift, 1962, 40, 115.

STRANDJORD, P.E., THOMAS, K.E. and WHITE, L.P.
Studies on isocitric and lactic dehydrogenases in experimental myocardial infarction.
Journal of Clinical Investigation, 1959, 38, 2111.

SWAIN, J.L., COBB, F.R. and ROE, C.R.
Relationship between creatine kinase estimates and histologic extent of myocardial infarction and regional blood flow in awake dogs.
Clinical Research, 1978, 26, 46-A (Abstract).

SWAN, D.C., DAVIDSON, P. and ALBRINK, M.J.
Effect of simple and complex carbohydrates on plasma non-esterified fatty acids, plasma-sugar and plasma-insulin during oral carbohydrate tolerance tests.
Lancet, 1966, I, 60.

SWAN, H.J.C., FORRESTER, J.S., DIAMOND, G., CHATTERJEE, K. and PARMLEY, W.W.
Haemodynamic spectrum of myocardial infarction and cardiogenic shock: a conceptual model.
Circulation, 1972, 45, 1097.

SWINSCOW, T.D.V.
Statistics at square one. The t tests (continued).
British Medical Journal, 1976, 2, 358.

SZABO, G., MAGYAR, Z. and REFFY, A.
Lymphatic transport of enzymes after experimental myocardial infarction.
Lymphology, 1974, 7, 37.

TAGGART, P., CARRUTHERS, M. and SOMERVILLE, W.
ECG, plasma catecholamines and lipids, and their modification by oxprenolol when speaking before an audience.
Lancet, 1973, II, 341.

TANSEY, M.J.B.
Calculating infarct size. (Letter, with answer from G.A. Hamuda, R. Roberts and B. Sobel).
Circulation, 1977, 56, 688.

TANZER, M.L. and GILVARG, C.
Creatine and creatine kinase measurement.
Journal of Biological Chemistry, 1959, 234, 3201.

TAYLOR, S.H., CAXTON, C., MAJID, P.A., DYKES, J.R.W., GHOSH, P. and STOKER, J.B.
Insulin secretion following myocardial infarction with particular respect to the pathogenesis of cardiogenic shock.
Lancet, 1969, II, 1373.

THOMSON, W.H.S.
An investigation of physical factors influencing the behaviour in vitro of serum creatine phosphokinase and other enzymes.
Clinica Chimica Acta, 1969, 23, 105.

THOMPSON, P.L., FLETCHER, E.R. and KATARATIS, V.
Influence of myocardial infarct size on short and long-term prognosis.
Circulation, 1977, 56, Suppl. III, 147 (Abstract).

UHLEY, H.N., LEEDS, S.E., SAMPSON, J.J. and FRIEDMAN, M.
The cardiac lymphatics in experimental chronic congestive heart failure.
Proceedings of the Society for Experimental Biology and Medicine, 1969, 131, 379.

VACCA, G.
Creatine kinase MB isoenzyme: a comparison of the electrophoretic method with the selective activating method and the immunological method.
Clinica Chimica Acta, 1977, 75, 175.

VALORI, C., THOMAS, M. and SHILLINGFORD, J.P.
Urinary excretion of free noradrenaline and adrenaline following acute myocardial infarction.
Lancet, 1967, I, 127.

VAN DER VEEN, K.J. and WILLEBRANDS, A.F.
Isoenzymes of creatine phosphokinase in tissue extracts and in normal and pathological sera.
Clinica Chimica Acta, 1966, 13, 312.

VARAT, M.A. and MERCER, D.W.
Cardiac specific creatine phosphokinase isoenzyme in the diagnosis of acute myocardial infarction.
Circulation, 1975, 51, 855.

VATNER, S.T., BAIG, H., MANDERS, W.T. and MAROKO, P.
Effects of coronary artery reperfusion on myocardial infarct size calculated from creatine kinase.
Journal of Clinical Investigation, 1978, 61, 1048.

VEJJAJIVA, A. and TEASDALE, G.M.
Serum creatine kinase and physical exercise.
British Medical Journal, 1965, 1, 1653.

VETTER, N.J., STRANGE, R.C., ADAMS, W. and OLIVER, M.F.
Initial metabolic and hormonal response to acute myocardial infarction.
Lancet, 1974, I, 284.

WAAGSTEIN, F. and HJALMARSON, Å.C.
Effect of cardioselective β-blockade on heart function and chest pain in acute myocardial infarction.
Acta Medica Scandinavica, 1975, Suppl. 587, 193.

WAGNER, G.S., ROE, C.R., LIMBIRD, L.E., ROSATI, R.A. and WALLACE, A.G.
The importance of identification of the myocardial-specific iso-enzyme of creatine phosphokinase (MB form) in the diagnosis of acute myocardial infarction.
Circulation, 1973, 47, 263.

WALSTON, A., HACKEL, D.B. and ESTES, E.H.
Acute coronary occlusion and the 'power failure' syndrome.
American Heart Journal, 1970, 79, 613.

WARBURG, O. and CHRISTIAN, W.
Garungsfermente im bluteserum von tumor-ratten.
Biochemische Zeitschrift, 1943, 314, 399.

WARNER, E.D., DAHL, C. and EWY, G.A.
Myocardial injury from transthoracic defibrillator counter-shock.
Archives of Pathology, 1975, 19, 55.

WARTMAN, W.B., JENNINGS, R.B., YOKOYAMA, H.O. and CRABAUGH, G.F.
Fatty change of the myocardium in early experimental myocardial infarction.
Archives of Pathology, 1956, 62, 318.

WATERS, D., SHELL, W.E., DA LUZ, P., WYATT, H.L., TOTTEN, G., KLIGERMAN, M. and FORRESTER, J.S.
Effect of changes in residual coronary perfusion upon serum creatine phosphokinase levels.
American Journal of Cardiology, 1977, 39, 2 (Abstract).

WEGRIA, R., HORST, Z., WALTER, K.E., ENTRUP, R.W., DESHRYVER, C., KENNEDY, W. and PAIEWONSKY, D.
Effects of systemic venous pressure on drainage of lymph from the thoracic duct.
American Journal of Physiology, 1963, 204, 284.

WHITTINGTON-COLEMAN, P.J., CARRIER, D., Jr., and DOUGLAS, B.H.
The effects of propranolol on cholesterol-induced atheromatous lesions.
Atherosclerosis, 1973, 18, 337.

WIESMANN, K., COLOMBO, J.P., ADAM, A. and RICHTERICH, R.
Determination of cysteine activated creatine kinase in serum.
Enzymologia Biologica et Clinica (Basel), 1966, 7, 266.

WILKINSON, J.H.
Clinical significance of enzyme activity measurements.
Clinical Chemistry, 1970, 16, 882.

WILLEBRANDS, A.F., TER WELLE, H.F. and TASSERON, S.J.A.
The effect of a high molar FFA/albumin ratio in the perfusion medium on rhythm and contractility of the isolated rat heart.
Journal of Molecular and Cellular Cardiology, 1973, 5, 259.

WITTEVEEN, S.A.G.J., HEMKER, H.C., HOLLAAR, L. and HERMENS, W.T.R.
Quantitation of infarct size in man by means of plasma enzyme levels.
British Heart Journal, 1975, 37, 795.

WOHLGEMUTH, J.
Uber eine neue methode zur quantiativen bestimmung des diastatischen ferments.
Biochemische Zeitschrift, 1908, 9, 1.

WONG, P.C.P. and SMITH, A.F.
Comparison of three methods of analysis of the MB isoenzyme of creatine kinase in serum.
Clinica Chimica Acta, 1975, 65, 99.

WOOD, J.M., BUSH, B., PITTS, B.J.R. and SCHWARTZ, A.
Inhibition of bovine heart Na^+, K^+-ATPase by palmitylcarmitine and palmityl-CoA.
Biochemical and Biophysical Research Communications, 1977, 74, 677.

WÜRZBURG, U., HENNRICH, N., ORTH, H.-D., LANG, H., PRELLWITZ, W., NEUMEIER, D., KNEDEL, M. and RICK, W.
Quantitative determinations of creatine kinase isoenzyme catalytic concentrations in serum using immunological methods.
Journal of Clinical Chemistry and Clinical Biochemistry, 1977, 15, 131.

YATES, F.
Contingency tables involving small numbers and the X^2 test.
Journal of the Royal Statistical Society, 1934, Suppl. I, 217.

YOUNG, D.S., THOMAS, D.W., FRIEDMAN, R.B. and PESTANER, L.C.
Effects of drugs on clinical laboratory tests.
Clinical Chemistry, 1972, 18, 1041.