BIOCHEMICAL INVESTIGATIONS

IN

CARDIAC DISEASE

by

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Master of Science

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ABBREVIATIONS

These are as laid down in Biochemical Journal Instructions for authors (revised, 1970) with the following additions:

BP	Blood Pressure
CHF	Congestive Heart Failure
CP	Creatine Phosphate
CPK	Creatine Phosphokinase (ATP: creatine phosphotransferase. EC 2.7.3.2.)
DC	Direct Current
ECG	Electrocardiogram
FFA	Free Fatty Acids
GOT	Glutamic-Oxaloacetic Transaminase (L-aspartate: 2 - oxoglutarate aminotransferase. EC 2.6.1.1.)
G-6-P	Glucose - 6 - Phosphate
G-6-PDH	Glucose - 6 - Phosphate Dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase. EC 1.1.1.49.)
Hb	Haemoglobin
LBBB	Left Bundle Branch Block
LDH	Lactate Dehydrogenase (L-lactate: NAD oxidoreductase. EC 1.1.1.27.)
LVF	Left Ventricular Failure
mU	Milli-Unit of Enzyme Activity

$PaCO_2$	Arterial Blood Carbon Dioxide Tension
PaO_2	Arterial Blood Oxygen Tension
SCPK	Serum Creatine Phosphokinase
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SLDH	Serum Lactate Dehydrogenase
U	Unit of Enzyme Activity

CHAPTER 1 - GENERAL INTRODUCTION

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1.1. History

The effects of cardiovascular disease were first described in 1669 by Lower in his 'Tractatus de Corde'. Subsequently Bonetus in 1679 and 1700 described several hundred cases with symptoms and signs which could be attributed to lesions of the heart and great vessels (White, 1951a). The symptoms and signs of angina pectoris were first observed by Heberden in 1768, but he did not connect them with coronary artery disease (White, 1951b). It was not until 1772 and the work of Jenner, a pupil of John Hunter, that this connection was discovered (Herrick, 1942). Indeed the dramatic story of John Hunter's illness, and the demonstration at autopsy that the coronary arteries of his heart were 'ossified', is a striking example of a case of angina pectoris culminating in a final fatal myocardial infarction (Willius and Keys, 1941).

However, more than a century was to pass before the clinical entity of acute thrombosis with myocardial infarction was described by Herrick in his classical paper of 1912. In particular, he pointed out that, while the pain of angina pectoris is connected with effort and could be readily

relieved by rest and nitroglycerine, the pain of myocardial infarction was often not associated with effort, and was generally more severe and of much longer duration. However, it was not until the publication of a paper by McNee in 1925, that Herrick's work became widely known in the United Kingdom. That myocardial infarction could be detected on the electrocardiogram (ECG), was first demonstrated by Parkinson and Bedford in 1928,

Since 1920 the incidence of ischaemic heart disease in Scotland has vastly increased, as can be seen in Table 1.1.1. the data for which was obtained from the 'Annual Report of the Registrar General for Scotland', 1968 - Part 1 - Mortality Statistics - No. 114, Table C1 - 7.

Table 1.1.1.

Death rates per 100,000 male population from heart disease and coronary thrombosis, Scotland, 1920 to 1968.

CAUSES	PERIOD	DEATHS per 100,000 males
Heart disease *	1920 - 22	136
	1930 - 32	195
	1940 - 42	366
	1950 - 52	425
	1960 - 62	451
	1968	450
Coronary thrombosis	1920 - 22	8
	1930 - 32	21
	1940 - 42	80
	1950 - 52	190
	1960 - 62	281
	1968	346

* of all types

1.2. Metabolism of the Normal Heart

The function of the heart is to perform mechanical work in the form of muscular contraction. Under normal aerobic conditions the metabolism of the myocardium is therefore geared to the production of large amounts of energy, which takes the form of the high energy compound adenosine triphosphate, (ATP). Bing and his colleagues (Bing, Siegel, Vitale, Balboni and Sparks, 1953), by means of coronary sinus catheterisation, demonstrated that the human heart utilises glucose, pyruvate and lactate, (Green and Goldberger, 1961). However, the total aerobic metabolism of these compounds accounts for only 35% of the oxygen consumption of the heart, and it has been established that the human heart also uses fatty acids, ketones and amino acids (Bing, Siegel, Ungar and Gilbert, 1954). According to these authors approximately 3/5ths of the energy expenditure of the heart, in the postabsorptive state, is sustained by fatty acids. Further to this, the work of Gordon and Cherkes (1956), and Dole (1956), proved that the principal fraction of plasma concerned with the transport of these fatty acids is the plasma nonesterified fatty acids, or free fatty acids, (FFA) fraction.

1.3. Metabolism of the Heart after Myocardial Infarction

Direct access to the myocardium *in vivo*, in man, under conditions of ischaemia and infarction is obviously, due to practical and ethical reasons, extremely difficult. However, in order to investigate the response of the myocardium to such conditions, many experiments, using both whole animals and animal tissues, have been carried out. From these it is possible to draw some inferences about the metabolism of the infarcted heart.

When an occlusion occurs in the coronary circulation that area of the myocardium which the blood vessel supplies becomes ischaemic and infarction develops. Bing, Castellanos, Gradel, Lupton and Siegel (1956), reported a significant decrease in myocardial oxygen extraction after occlusion of the coronary arteries of dogs. Due to this lack of oxygen the metabolism of this area of the myocardium becomes anaerobic.

The energy which can be derived from anaerobic glycolysis is approximately 6% of that derived from oxidative metabolism (Green and Goldberger, 1961), and Reeves (1959) showed, using the turtle heart, that this could only maintain cardiac function for a short time. In addition, the muscle's

stores of glycogen rapidly become exhausted, and from this point onwards there are no means by which the muscle cell can obtain ATP (Michal, Naegle, Danforth, Ballard and Bing, 1959; Danforth, Naegle and Bing, 1960). During ischaemia the uptake of glucose is increased (Brachfeld and Scheuer, 1967), and uptake of FFA continues and these are then transformed into neutral lipids (Scheuer and Brachfeld, 1966).

Under normal aerobic conditions the heart extracts 20 - 40% of the lactic acid content of arterial blood, but lactic acid is produced, either from endogenous glycogen or glucose, in the ischaemic myocardium. This has been demonstrated on numerous occasions in animal experiments (Clark, Gaddie and Stewart, 1932; Shea, Watson, Piotrowski, Dermksian and Case, 1962) and, more recently, in man during angina (Krasnow and Gorlin, 1963; Parker, Chiong, West and Case, 1969) and cardiogenic shock (Mueller et al., 1968). The lactic acid so produced accumulates in the myocardium and depresses its function (Clark et al., 1932; Wildenthal, Mierzwiak, Myers and Mitchell, 1968). In experiments on the dog heart in situ, Goodyer, Eckhardt, Ostberg and Goodkind (1961), noted that

the increased concentration of lactic acid causes a local fall in pH, and a corresponding disturbance of the acid-base balance.

It has been observed in dog and man that, after myocardial infarction, a large number of enzymes is present in the coronary vein and peripheral venous blood (Siegel and Bing, 1956; Konttinen and Halonen, 1963). Siegel and Bing (1956), showed that, in the dog, changes in plasma enzyme activities were associated with pathological events which take place many hours after coronary artery embolisation, and are correlated with the height of coagulation necrosis.

1.4. Outline of Present Investigation

The mechanisms which cause ischaemic heart disease and hence myocardial infarction are very much a pathological mystery. In the present investigation we are not concerned with what these causes are, be they a defect in the blood clotting mechanism or in lipid metabolism. The purpose of the present study has been the simple one of trying to obtain information, by means of chemical analysis of the blood of patients with suspected myocardial infarction, which would aid the clinicians to confirm the diagnosis, assess the severity of the lesion, and suggest the likely prognosis.

Two particular questions have been investigated:

1. The usefulness of serum enzyme estimations, and whether serial estimations provide more information on which to base a diagnosis and assess the condition, and possible prognosis, of the patient.
2. Whether haemodynamic measurements, blood gas analysis and estimations of blood lactate and pyruvate are good indices of the degree of severity of the patient's condition, and whether these are affected by oxygen therapy.

Each of these questions will be discussed in turn.

CHAPTER 2 – SERUM ENZYMES

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2.1. Introduction

Diagnosis of myocardial infarction presents some difficulty. Postmortem studies have shown that 25 to 40% of acute myocardial infarctions were not so diagnosed (Johnson, Achor, Burchell and Edwards, 1959). Until recent years diagnosis has been based on the clinical history and the ECG results. The ECG however, is fallible (Woods, Laurie and Smith, 1963), hence the need for additional diagnostic aids such as serum enzyme analysis.

2.1.1. Development of Clinical Enzymology

The use of enzyme assays as an aid to diagnosis dates back to 1908 with the introduction by Wohlgemuth of the determination of amylase (α- 1, 4- glucan 4-glucanohydrolase EC 3.2.1.1.) activity in the serum of patients with suspected pancreatic disease. Until 1930 this and one other enzyme alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1.), which is of value in the diagnosis of bone disease (Robison, 1923; Kay, 1929), were the only enzymes assayed routinely in clinical laboratories.

The period from 1935 to 1950 however, saw an immense increase in the number of enzymes known to biochemists, and

a corresponding improvement in the methods of enzyme assay. This was largely due to the development of spectrophotometers following the use of spectrophotometry by Warburg in 1935 (Warburg, Christian and Griese, 1935), in the study of enzymes responsible for oxidation/reductions involving the nicotinamide nucleotide coenzymes. These spectrophotometric methods were far easier than the existing manometric ones, and for the first time made possible the routine assay of a wide range of dehydrogenases, and of the enzymes which could be linked to them. This discovery gave a decisive stimulus to clinical enzymology, and many enzymes are now routinely used as diagnostic aids (Gowenlock, 1965). A consequence of this has been a number of textbooks entirely devoted to clinical enzymology (Wilkinson, 1962; King, 1965; Coodley, 1970a).

2.1.2. Release of Enzymes from Cells

Although we are not, in this work, primarily concerned with the mechanisms responsible for the abnormal serum enzyme levels found in myocardial infarction, it would seem pertinent at this point to discuss these briefly.

The generally held view is that in myocardial infarction hypoxic lesion of the muscle causes necrosis, and as a result of this there is an increased rate of release of enzymes from the muscle cells. This is supported by the work of Ruegsegger Nydick, Freiman and LaDue (1959), who failed to detect any

increase in the activity of the enzyme glutamic-oxaloacetic transaminase. (L aspartate: 2 - oxoglutarate aminotransferase EC 2.6.1.1.), (GOT), in the serum of dogs after induction of reversible myocardial ischaemia for forty-five minutes although in 1955 Agress et al., also using dogs, had detected a significant rise in serum GOT, (SGOT), when only 5% of the myocardium was necrotic.

Contrary to this Schmidt andSchmidt (1967a), observed a remarkable increase in enzyme activities in the extracellular space within four hours of hypoxic lesion of the isolated perfused rat liver although there was little histological or electron microscope evidence of damage (Schmid Schmidt, Herfarth, Opitz and Vogell, 1966). This is in agreement with the earlier observation of Zierler (1958), that anoxia caused increased loss of aldolase (ketose - 1 - phospha aldehyde-lyase EC 4.1.2.7.) from the isolated perfused peroneus longus muscle of rat and the suggestion by Schmidt an his colleagues, (Schmidt, Schmidt, Horn and Gerlach, 1963) tha changes in cell permeability without cell death may permit esc ape of significant amounts of intracellular enzymes. If this i so it is possible that in experimental and clinical myocardial infarction other factors, in addition to necrosis, contribute to the increased serum enzyme activities. Such a view would

explain Wróblewski's calculation that the lactate dehydrogenase (L - lactate: NAD oxidoreductase. EC 1.1.1.27) content of infarcted heart muscle could not maintain, for any length of time, the elevated serum LDH, (SLDH), levels commonly found after myocardial infarction (Wróblewski, 1963).

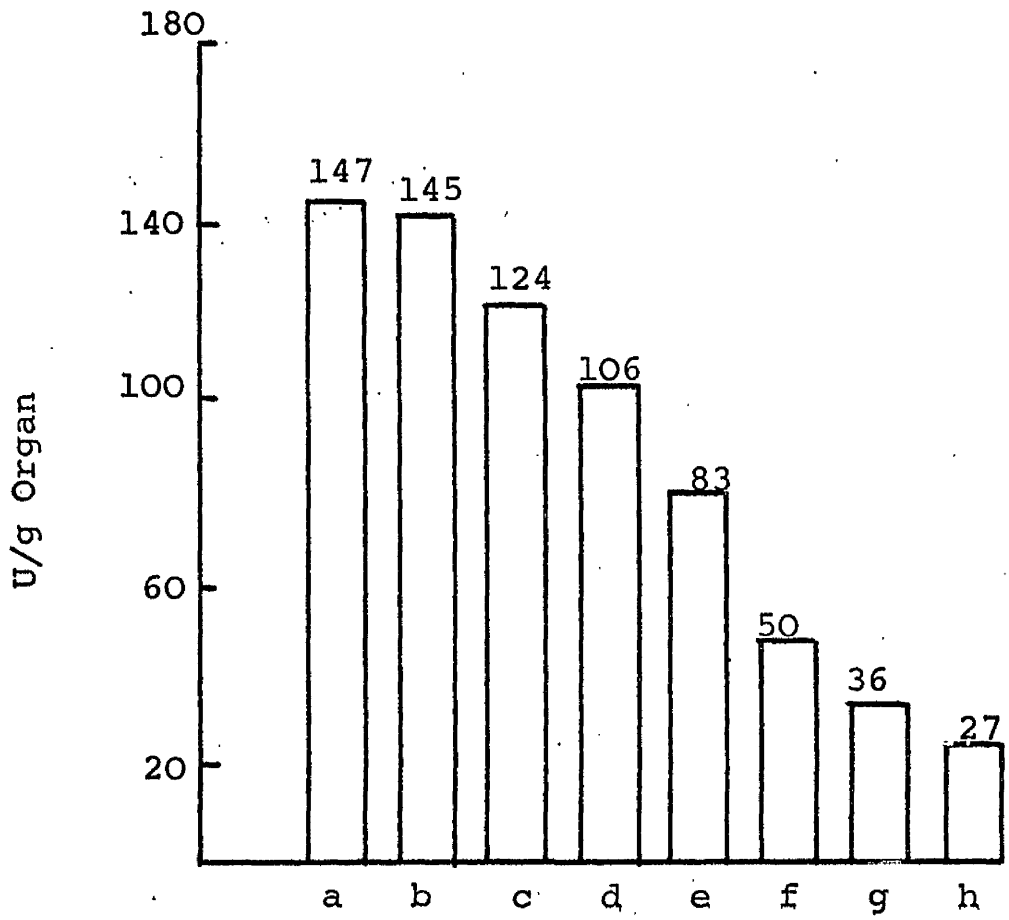
1.3. Serum Enzymes after Myocardial Infarction

The discovery that serum enzyme assays were of value in the diagnosis of myocardial infarction was made by LaDue, Wróblewski and Karmen (1954). They had observed that the serum enzyme activity of GOT was generally elevated after myocardial infarction, and therefore suggested that assays of SGOT might be useful in the diagnosis of this condition (LaDue et al., 1954; Karmen, Wróblewski and LaDue, 1955). These workers then proceeded to look at other enzymes in the myocardium.

Amongst those they investigated was LDH which, while by no means peculiar to heart muscle is nonetheless abundant there (Fig. 2.1.1.), and they did indeed find that, after infarction, there was an increase in the activity of SLDH (Wróblewski and LaDue, 1955; Wróblewski, Ruegsegger and LaDue 1956). LDH is widely distributed amongst tissues, (Fig. 2.1.1.) and therefore shows elevated activities in serum when any of

Fig. 2.1.1.

LDH Activity in Human Organs



a - skeletal muscle; b - liver; c - heart; d - kidney; e - lymph nodes; f - pancreas; g - erythrocytes; h - lung

from Schmidt and Schmidt (1967b).

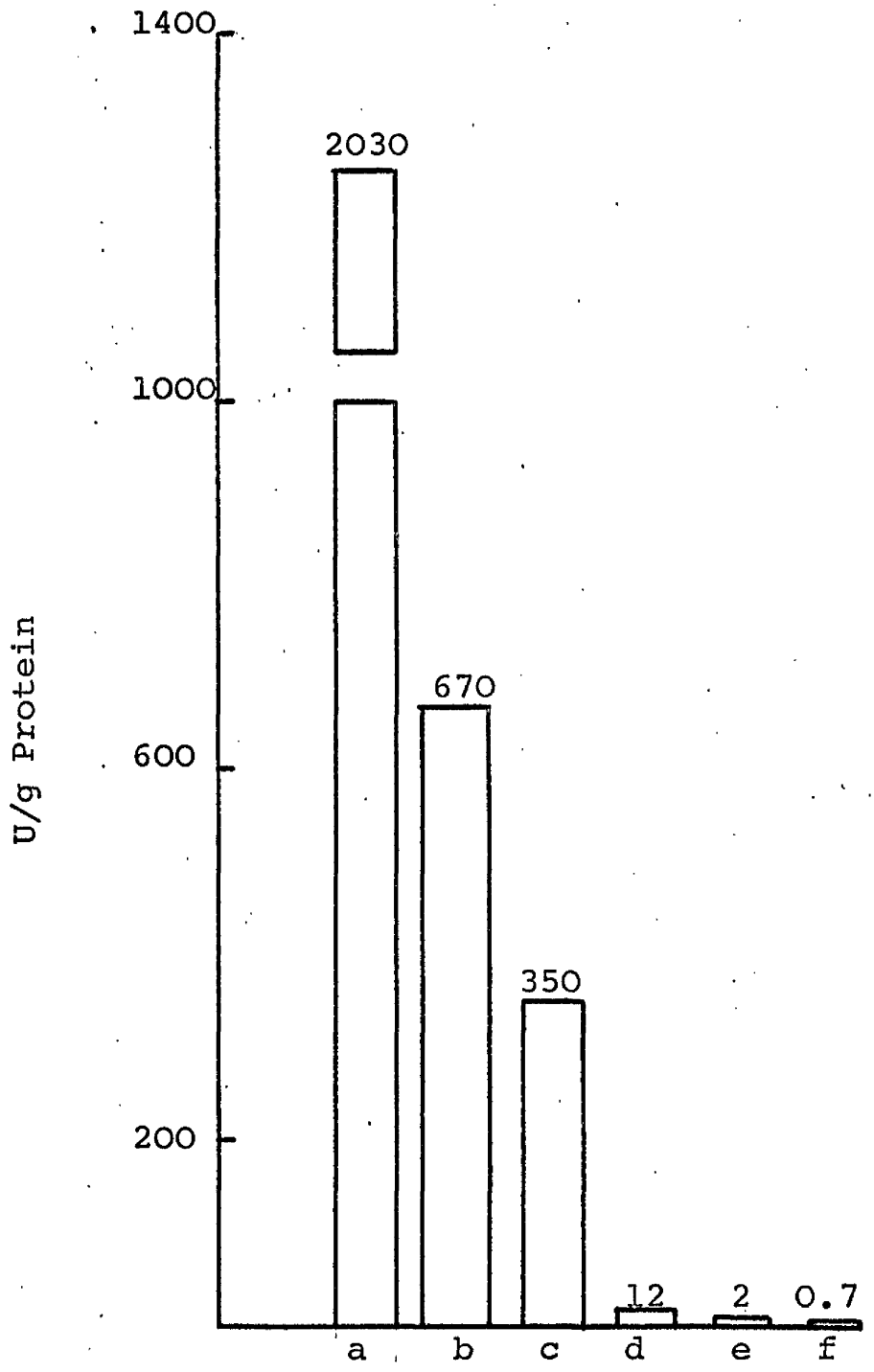
these tissues is damaged (Hill and Levi, 1954; Hsieh and Blumenthal, 1956), and this limits its usefulness in diagnosis.

Many workers have since confirmed these findings for GOT (Ostrow, Steinberg, Ticktin, Polis and Evans, 1956; Chinsky, Shmagranoff and Sherry, 1956), and for LDH (Wacker, Ulmer and Vallee, 1956; White, 1956; MacDonald, Simpson and Nossal, 1957).

However, in 1960 Dreyfus, Schapira, Resnais and Scebat noticed that after myocardial infarction the serum creatine phosphokinase (ATP: creatine phosphotransferase. EC 2.7.3.2.), (SCPK), showed a transient increase in activity. This enzyme was first seen to be of diagnostic significance by Ebashi, Toyokura, Momoi and Sugata (1959), who found high SCPK activities in patients suffering from muscular dystrophy. Unlike GOT and LDH however, this enzyme is not so widely distributed amongst the tissues (Fig. 2.1.2.). Elevated activities of SCPK are therefore only found after pathological damage to myocardial muscle, skeletal muscle and brain tissue, and in hypothyroidism (Graig and Ross, 1963). Since it is usually possible to exclude the last three of these on the grounds of other clinical evidence, SCPK is therefore the most specific enzyme for indicating myocardial necrosis (Sorensen, 1963; Hess et al., Coodley, 1966).

Fig. 2.1.2.

CPK Activity in Human Organs



a - skeletal muscle; b - brain; c - heart;

d - smooth muscle; e - kidney; f - liver.

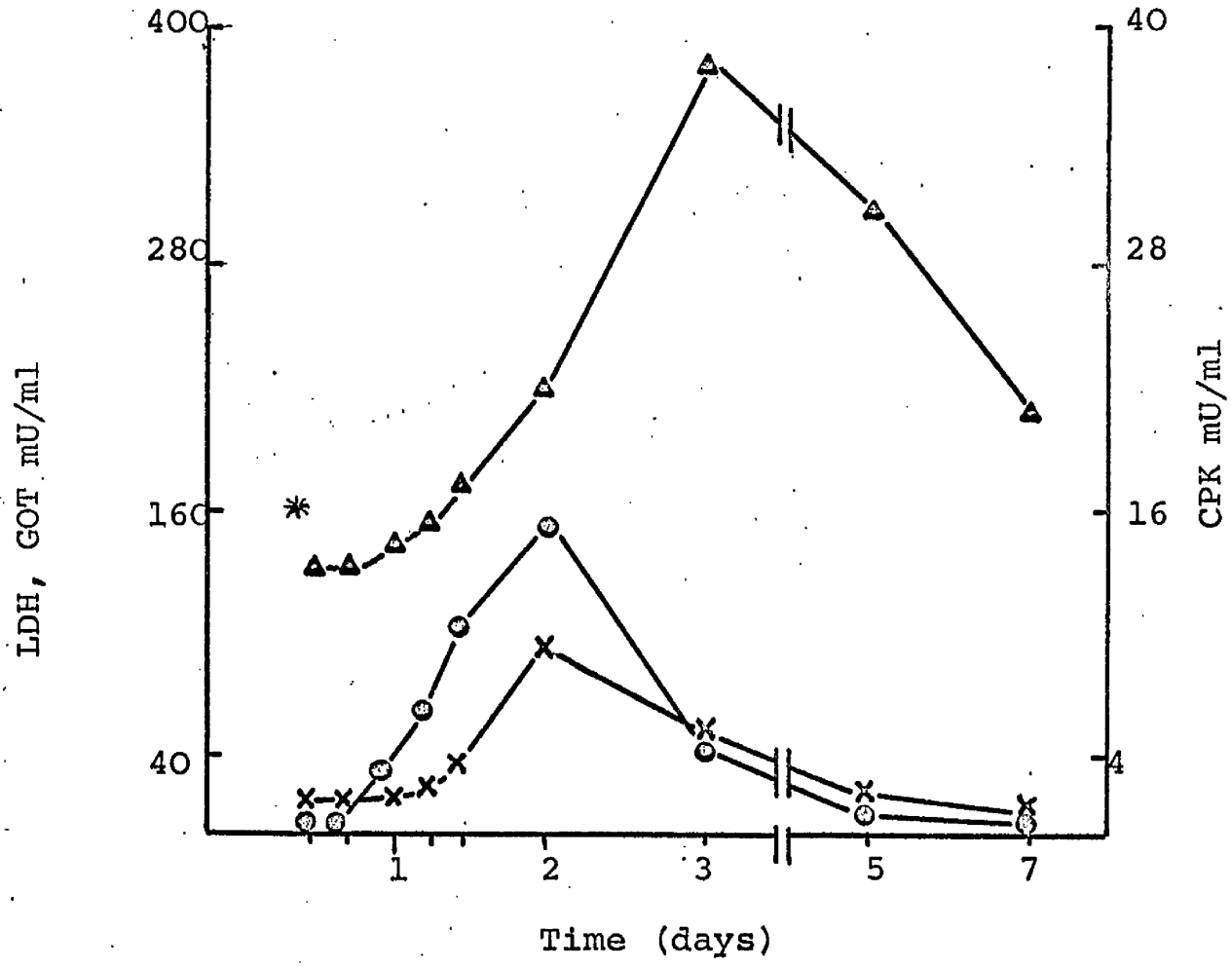
from Colombo, Richterich and Rossi (1962).

Fig. 2.1.3. shows the typical enzyme pattern exhibited in serum after a myocardial infarction.

The present investigation was based on the idea that if frequent serial enzyme estimations were carried out information of the sort shown in Fig. 2.1.3. could routinely be obtained and could be used to estimate the extent of the infarction and the time at which it occurred.

Fig. 2.1.3.

Serum Enzyme Activities in Myocardial Infarction



△ LDH

⊙ CPK

× GOT

✳ Time of onset of infarction process.

Note: The SCPK assay used here gives a normal range of up to 1 mU/ml serum.

from Schmidt and Schmidt (1967c).

2.2. Results

Thirty three patients were studied. Twenty eight of these, all males, were suspected on clinical grounds to have had a myocardial infarction. The remaining five, two females and three males, were suffering from cardiac disease other than myocardial infarction and were either in a state of cardiac fibrillation or in tachycardia. These five patients were all treated by direct current (DC) countershock.

Although the ideal is to take blood samples at very frequent time intervals i.e. two hourly intervals for at least the first forty eight hours after infarction, this is not in practice feasible. Therefore the procedure followed in these cases was to take blood samples off:

1. As soon as possible after admission.
2. At as near as possible to four or eight hourly intervals thereafter, for at least the first forty eight hours.

In some cases the patient died soon after admission, in others the patient's condition did not permit withdrawal of frequent blood samples; and occasionally samples were found to be haemolysed and therefore useless for LDH determinations since 1 ml. of completely haemolysed blood has an activity approx. one hundred times that of 1 ml. of serum (Wroblewski and LaDue, 1955).

The enzymes determined were CPK and LDH: CPK was chosen because of its rapid increase in activity in serum within hours of a myocardial infarction, and for its specificity; LDH because its activity in serum after myocardial infarction rises and falls more slowly than that of CPK. It was hoped that the pattern provided by frequent estimations of the serum activities of these two enzymes together would be a more reliable guide to the time of onset, extent of the infarction and the likely prognosis.

Determinations of SGOT were not carried out since the pattern of its activity in serum after myocardial infarction falls between that of CPK and LDH, (Fig. 2.1.3.), and it did not therefore seem likely to give much additional information.

The results for patients with suspected myocardial infarction are shown in Figs. 2.2.1. - 2.2.28, and Figs. 2.2.34. - 2.2.49. inclusive. The results for patients with cardiac disease other than myocardial infarction are shown in Figs. 2.2.29. - 2.2.33. inclusive.

The order of cases is not chronological.

Units

The unit of enzyme activity was as recommended by the International Union of Biochemistry (1964).

i.e. 'One unit (U) of any enzyme is that amount which will catalyse the transformation of 1 micromole (μ mole) of the substrate per minute under standard conditions'.

Conditions of Measurement

Temperature: CPK was assayed at 30°C

LDH was assayed at 25°C.

Figs. 2.2.1. - 2.2.28.

Changes in serum enzyme activities in patients suffering from suspected myocardial infarction.

● Activity of creatine phosphokinase, (CPK), in serum

▲ Activity of lactate dehydrogenase, (LDH), in serum.

* Denotes the time of onset of the infarction process, which we took to be onset of severe, crushing chest pain.

† Denotes the time of occurrence of a cardiac arrest.

Time zero on each figure represents 00.01 hours on the day of admission.

Note: Previous history refers to cardiac disease.

Fig. 2.2.1.

Case 1

Age: 52 years

Previous history: None

History: Severe central chest pain at 10.00 am on day of admission which persisted till one hour after admission. Cardiac arrest at 12.30 pm on day 2.

ECG interpretation: Acute antero-lateral myocardial infarction.

Fig. 2.2.1.

Case 1

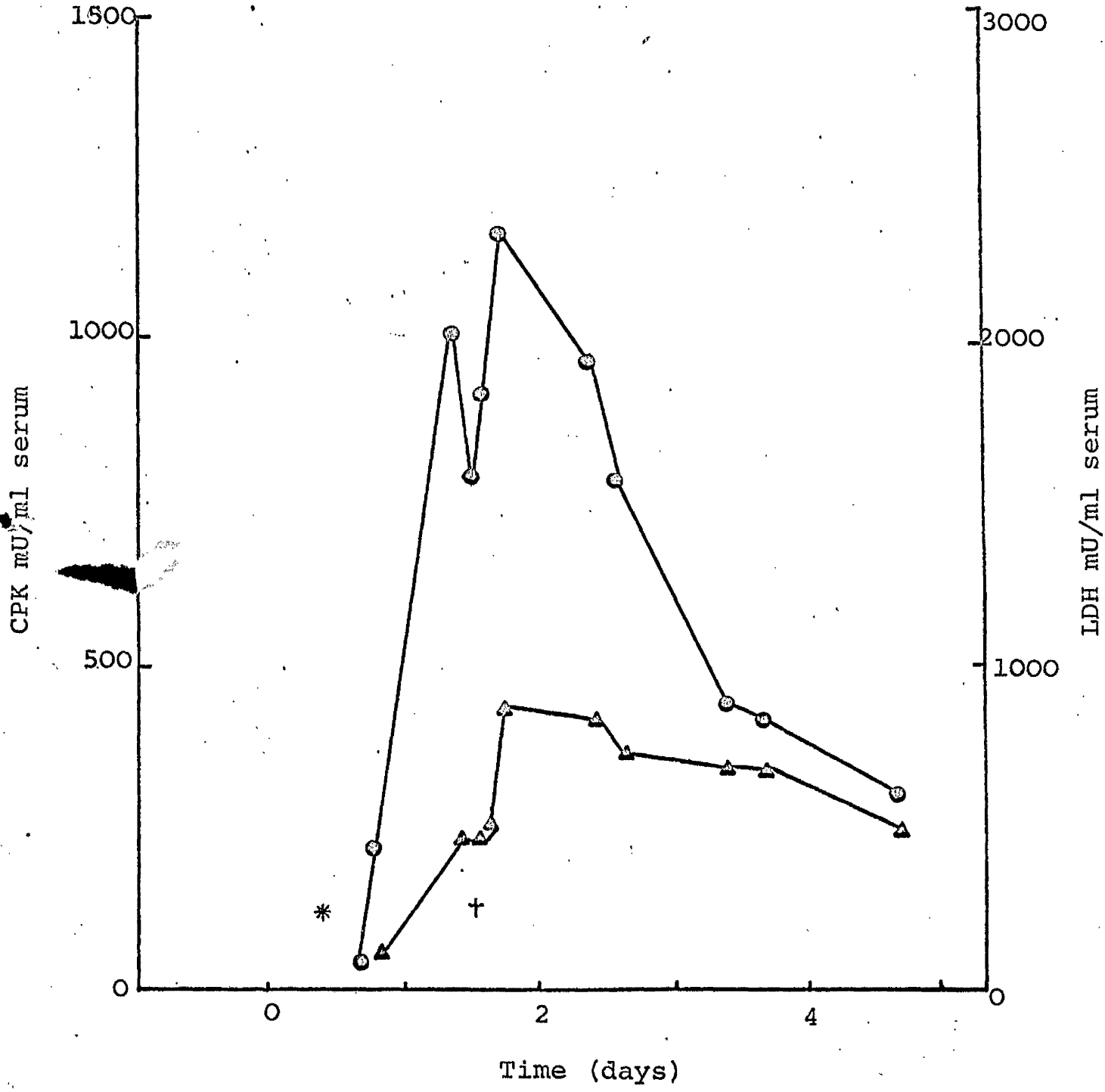


Fig. 2.2.2.

Case 2

Age: 59 years

Previous history: None

History: Intermittent attacks of severe central chest pain, nausea and sweating two days prior to admission.

ECG interpretation: Antero-septal myocardial infarction.

Fig. 2.2.2.

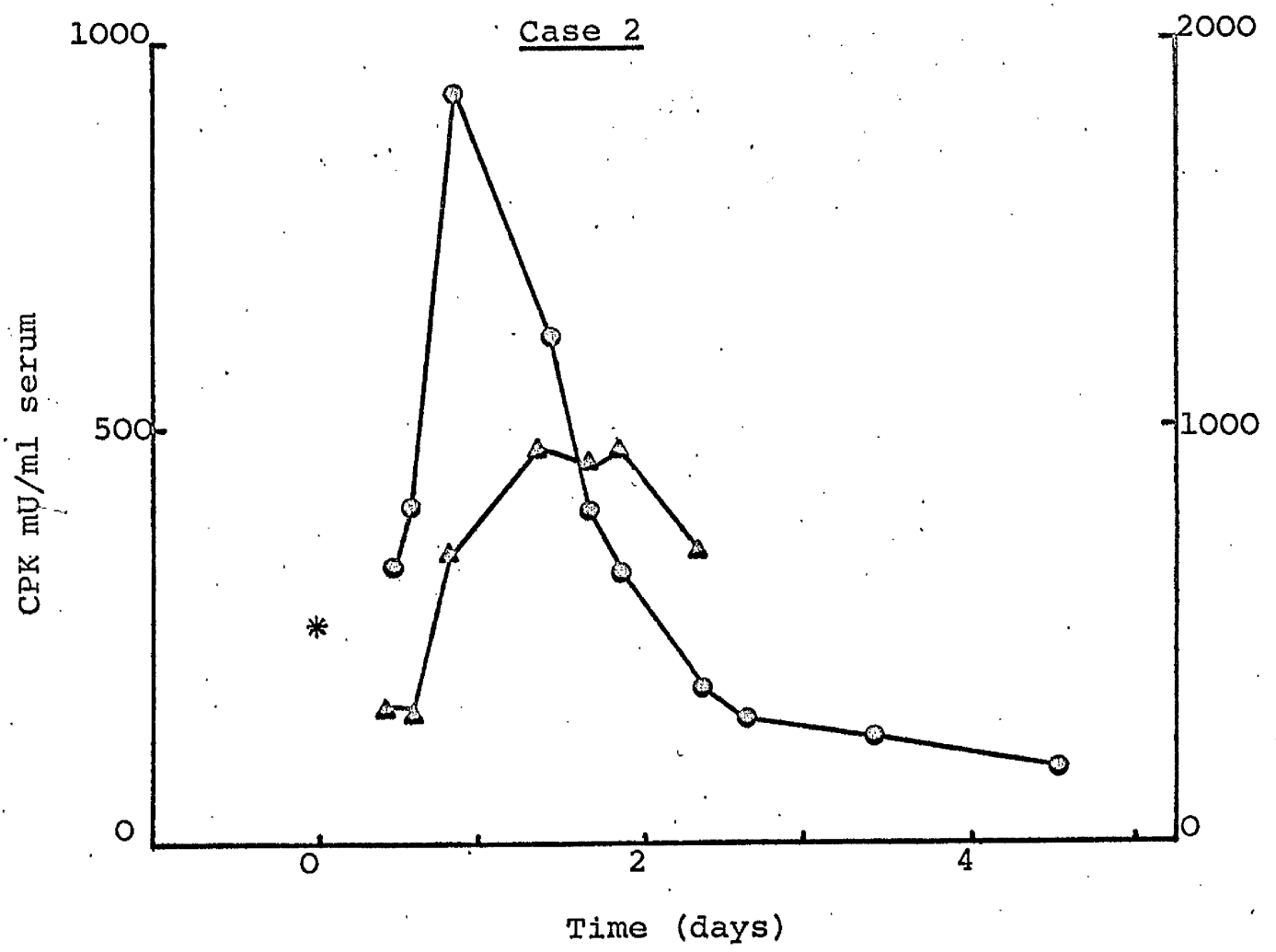

Case 2
1000
500
0
2000
1000
0
0
2
4
CPK mU/ml serum
LDH mU/ml serum
Time (days)
*

Fig. 2.2.3.

Case 3

Age: 55 years

Previous history: Myocardial infarction five months previously.

History: Increasingly severe chest pain on day before admission which recurred at 10.00 pm on same day.

ECG interpretation: Posterior myocardial infarction.

Fig. 2.2.3.

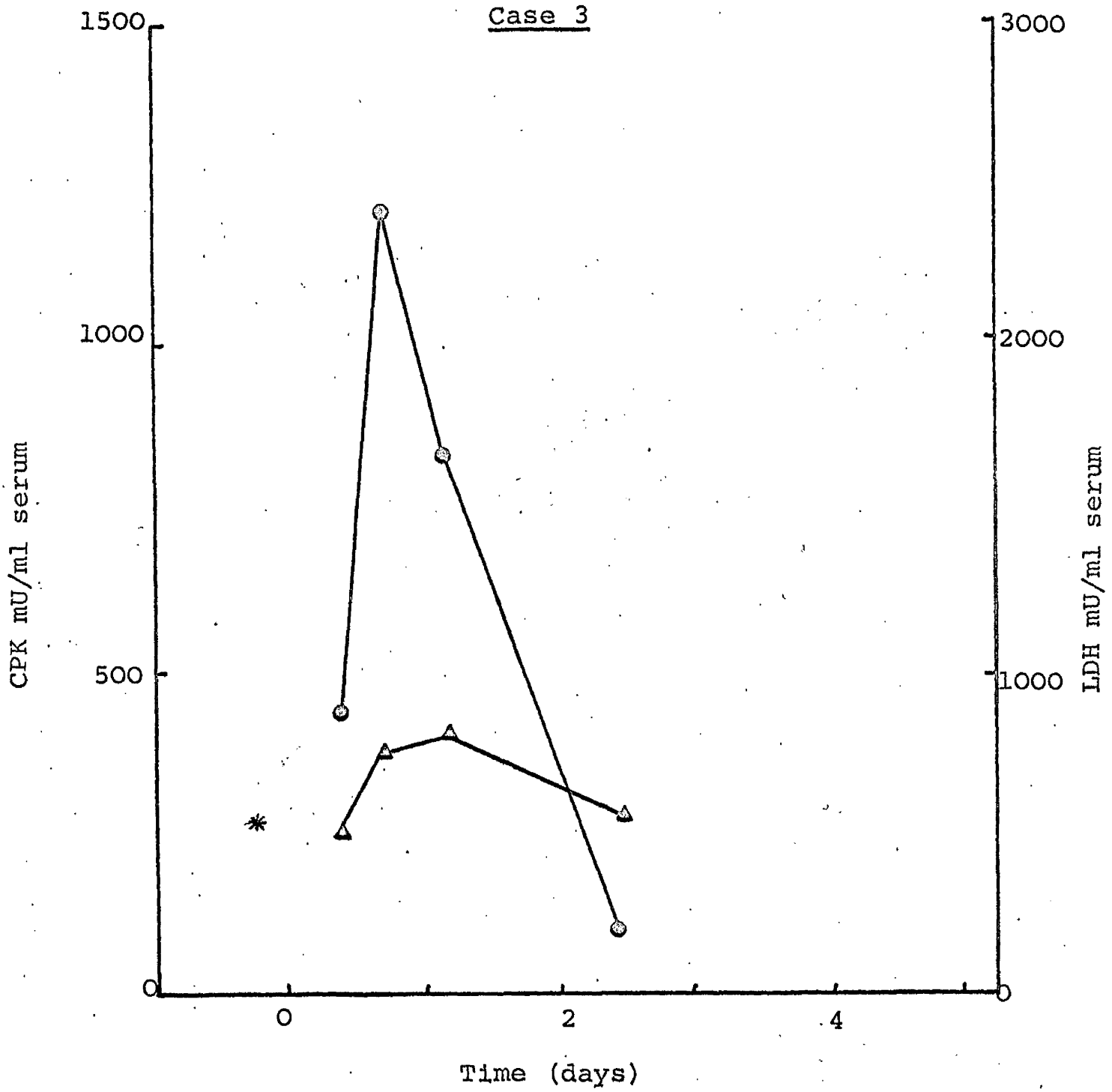

Case 3
1500
1000
500
0
3000
2000
1000
0
CPK mU/ml serum
LDH mU/ml serum
0
2
4
Time (days)

Fig. 2.2.4.

Case 4

Age: 34 years

Previous history: None

History: Constricting chest pain at 4.00 am on morning of admission and subsequently developed ventricular fibrillation and at 4.45 pm on same day had a cardiac arrest.

ECG interpretation: Antero-lateral myocardial ischaemia, but no evidence of myocardial infarction.

Fig. 2.2.4.

Case 4

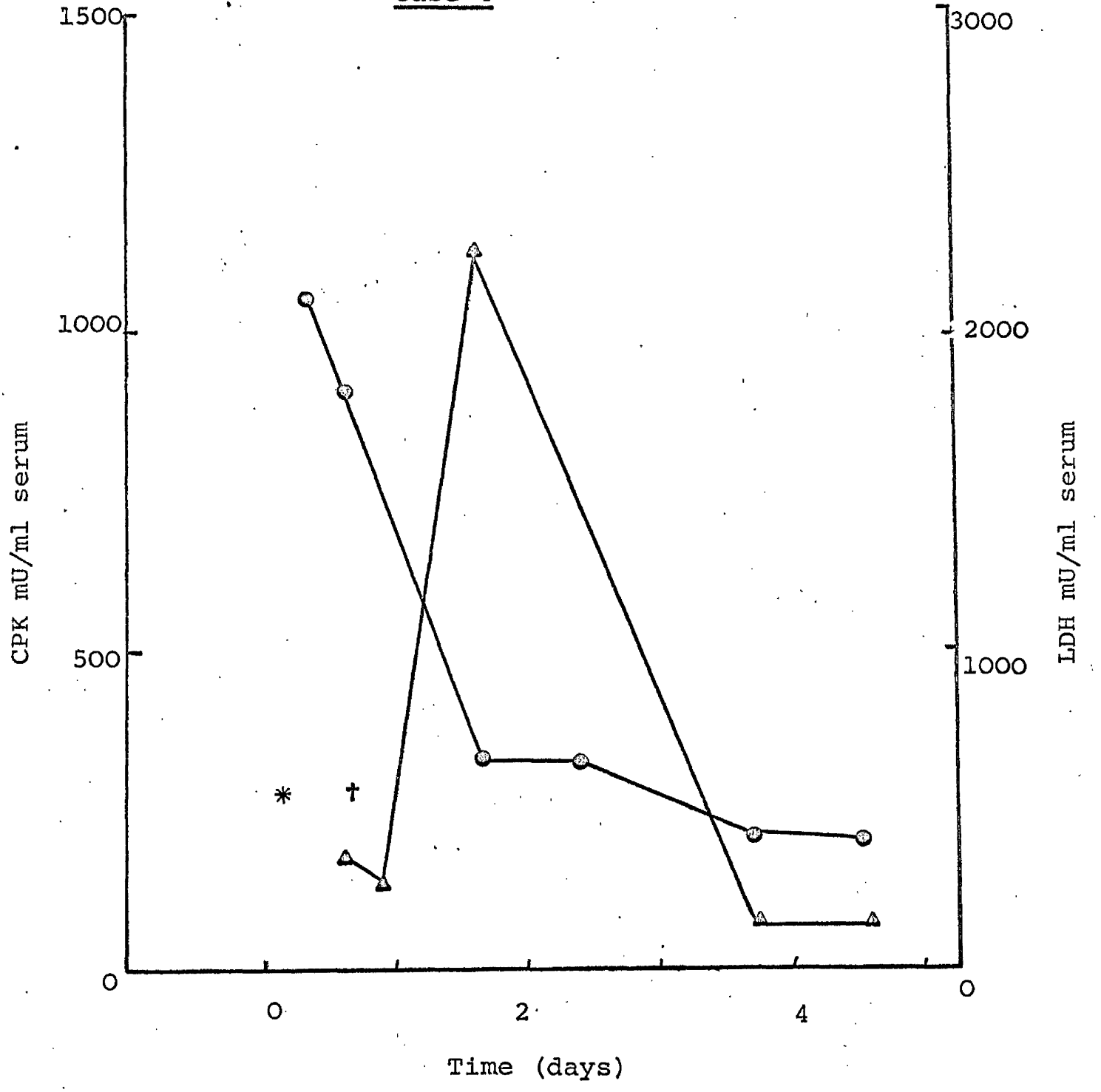


Fig. 2.2.5.

Case 5

Age: 47 years

Previous history: None

History: Angina of effort for two weeks. Severe central chest pain for more than four hours on morning of admission.

ECG interpretation: Acute posterior myocardial infarction.

Fig. 2.2.5.

Case 5

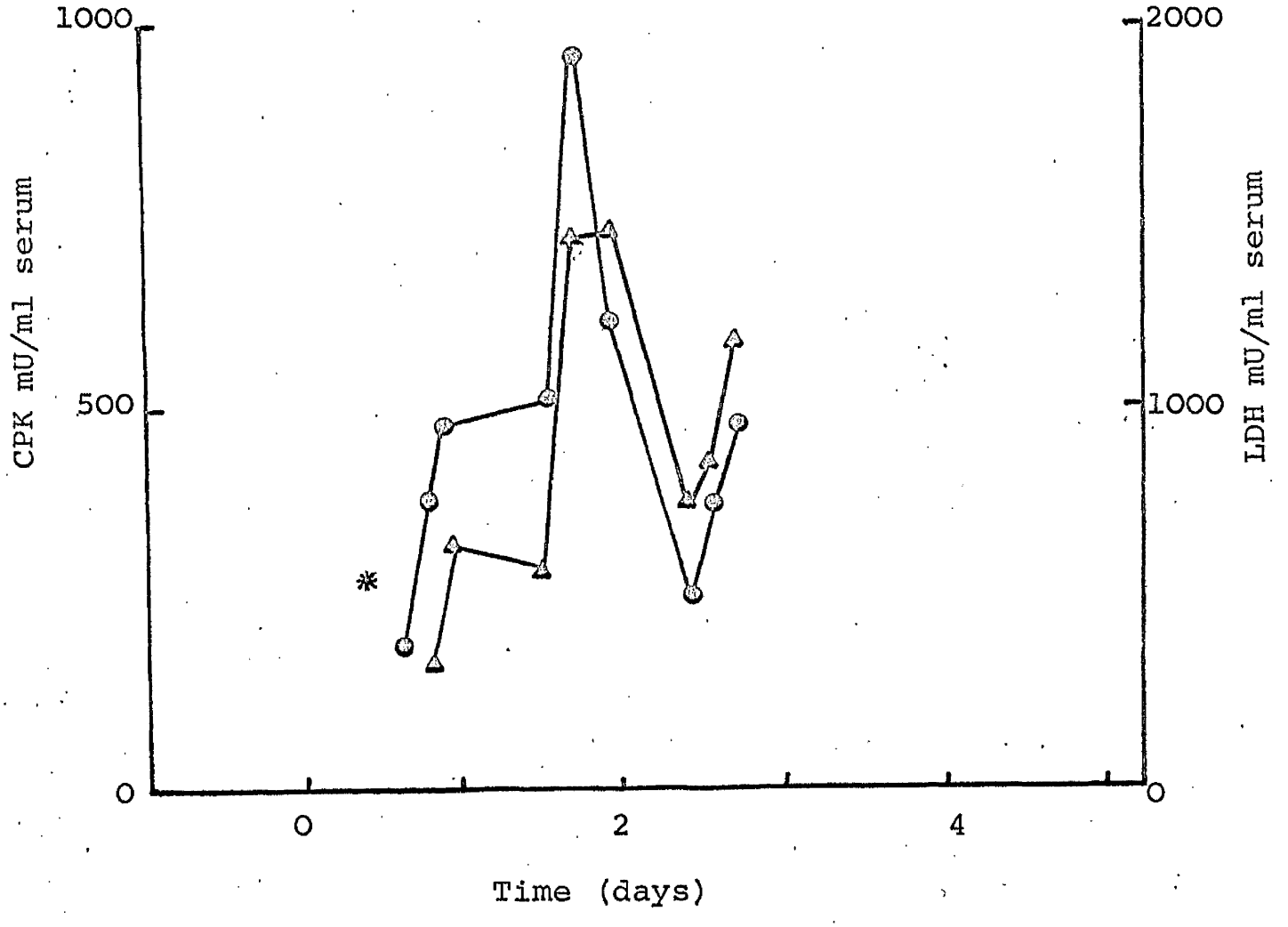


Fig. 2.2.6.

Case 6

Age: 60 years

Previous history: None

History: Severe chest pain and breathlessness for five days before admission. On morning of admission chest pain became continuous. Died six days after admission.

ECG interpretation: Antero-lateral myocardial infarction.

Post-mortem findings: Extensive areas of infarction involving left ventricle and almost the whole of the septum.

Fig. 2.2.6.

Case 6

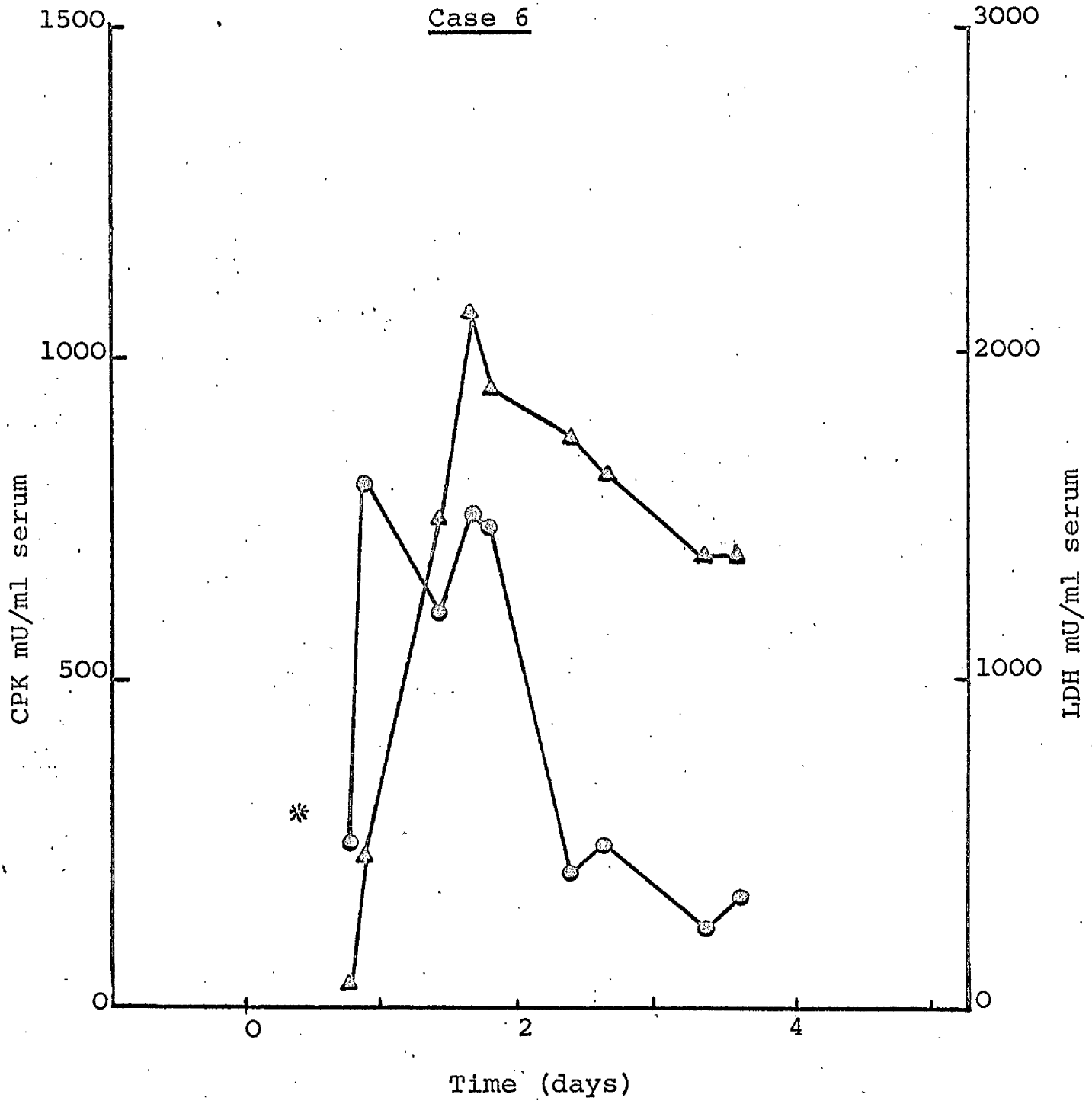


Fig. 2.2.7.

Case 7

Age: 63 years

Previous history: None

History: Angina of effort for seven days before admission. At midnight on day of admission had sudden onset of severe stabbing, then crushing, retrosternal chest pain.

ECG interpretation: Acute postero-lateral myocardial infarction.

Fig. 2.2.7.

Case 7

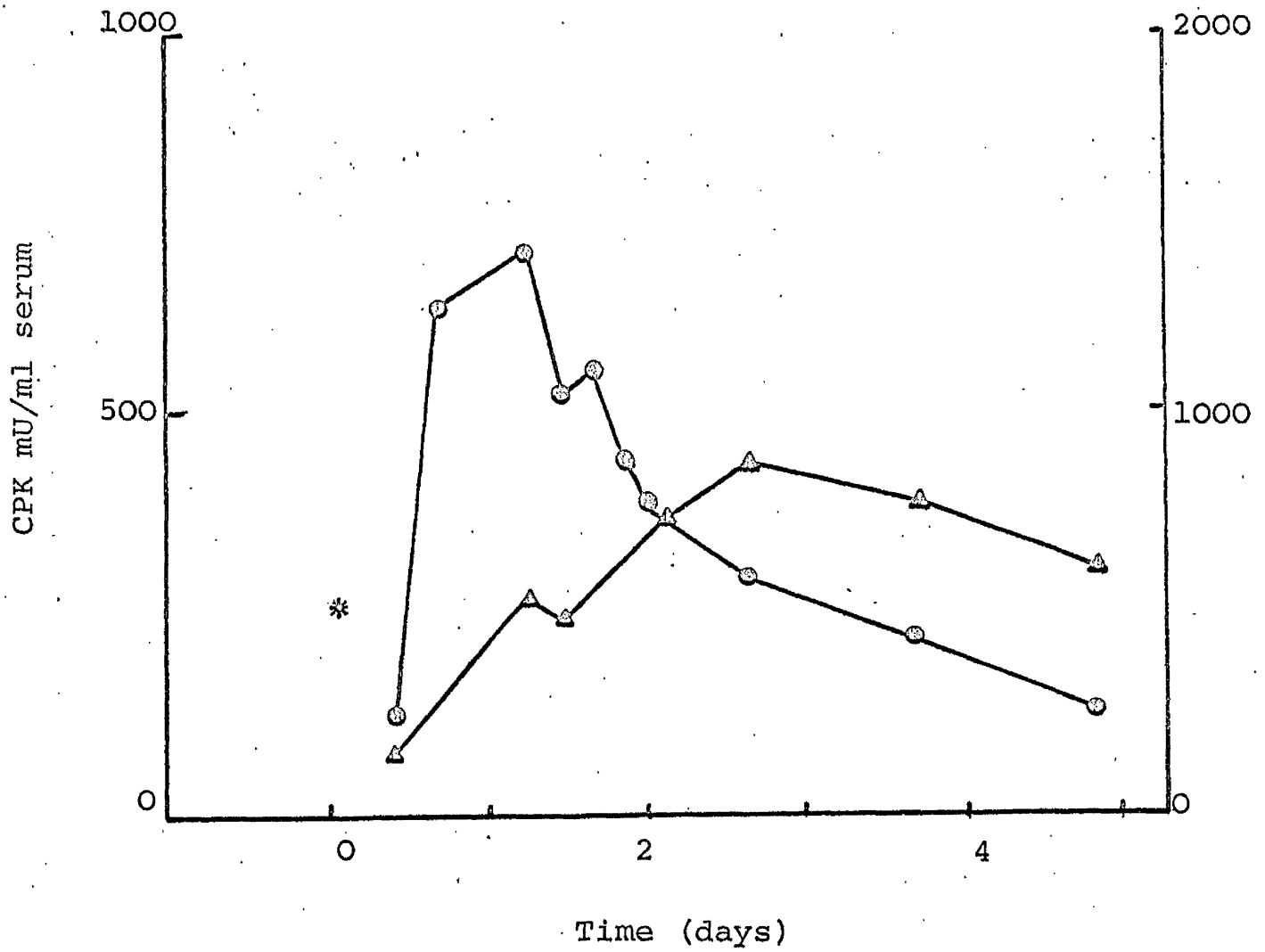


Fig. 2.2.8.

Case 8

Age: 65 years

Previous history: Slight myocardial infarction four years previously.

History: Choking sensation three days before admission. Severe chest pain on day before admission and at 4.00 am on day of admission breathless, sweating, vomiting and further pain.

ECG interpretation: Posterior myocardial ischaemia.

Fig. 2.2.9.

Case 9

Age: 56 years

Previous history: None

History: At 7.00 pm on day before admission severe retrosternal chest pain which persisted till admission.

ECG interpretation: Posterior myocardial infarction.

Fig. 2.2.8

Case 8

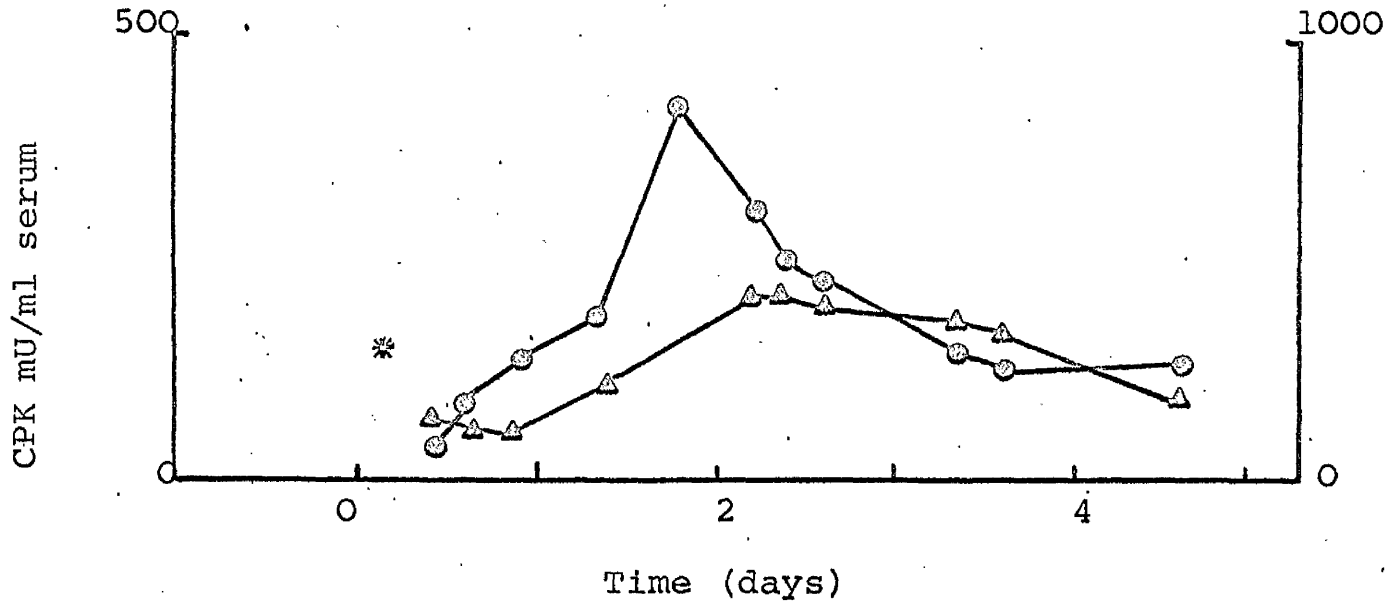


Fig. 2.2.9

Case 9

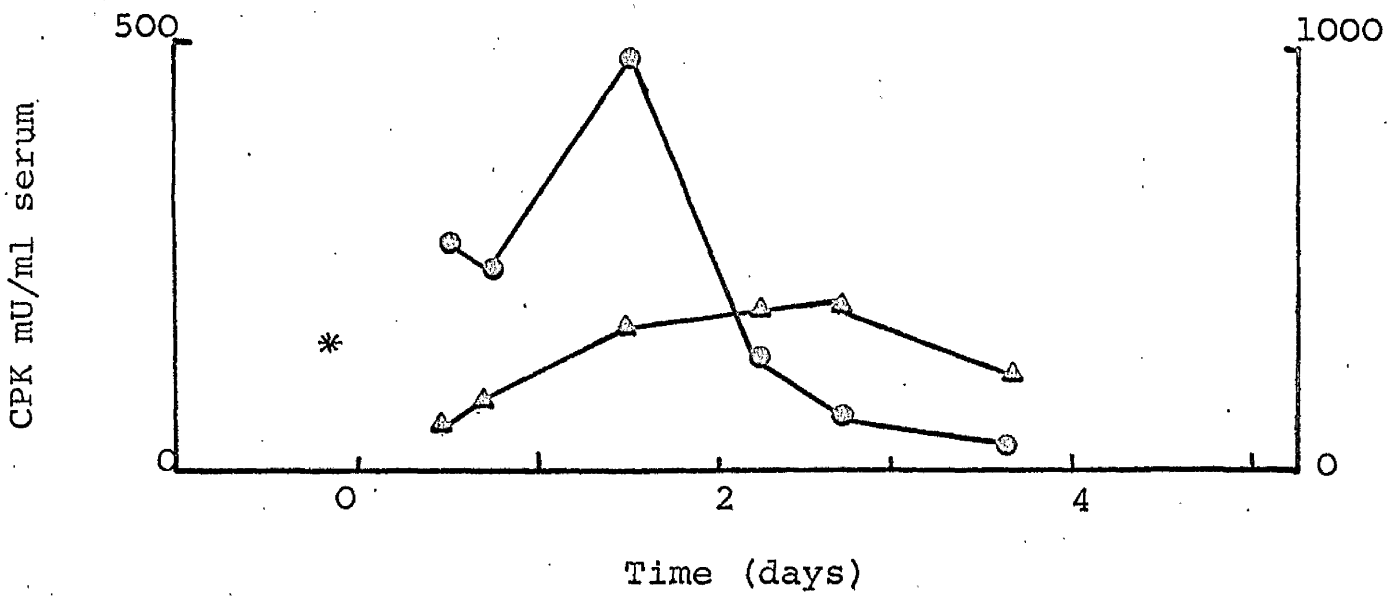


Fig. 2.2.10.

Case 10

Age: 62 years

Previous history: Myocardial infarction three years previously.

History: Chest pain for three days prior to admission. On day of admission severe chest pain at 10.30 am while working and also at rest.

ECG interpretation: Anterior myocardial infarction.

Fig. 2.2.10

Case 10

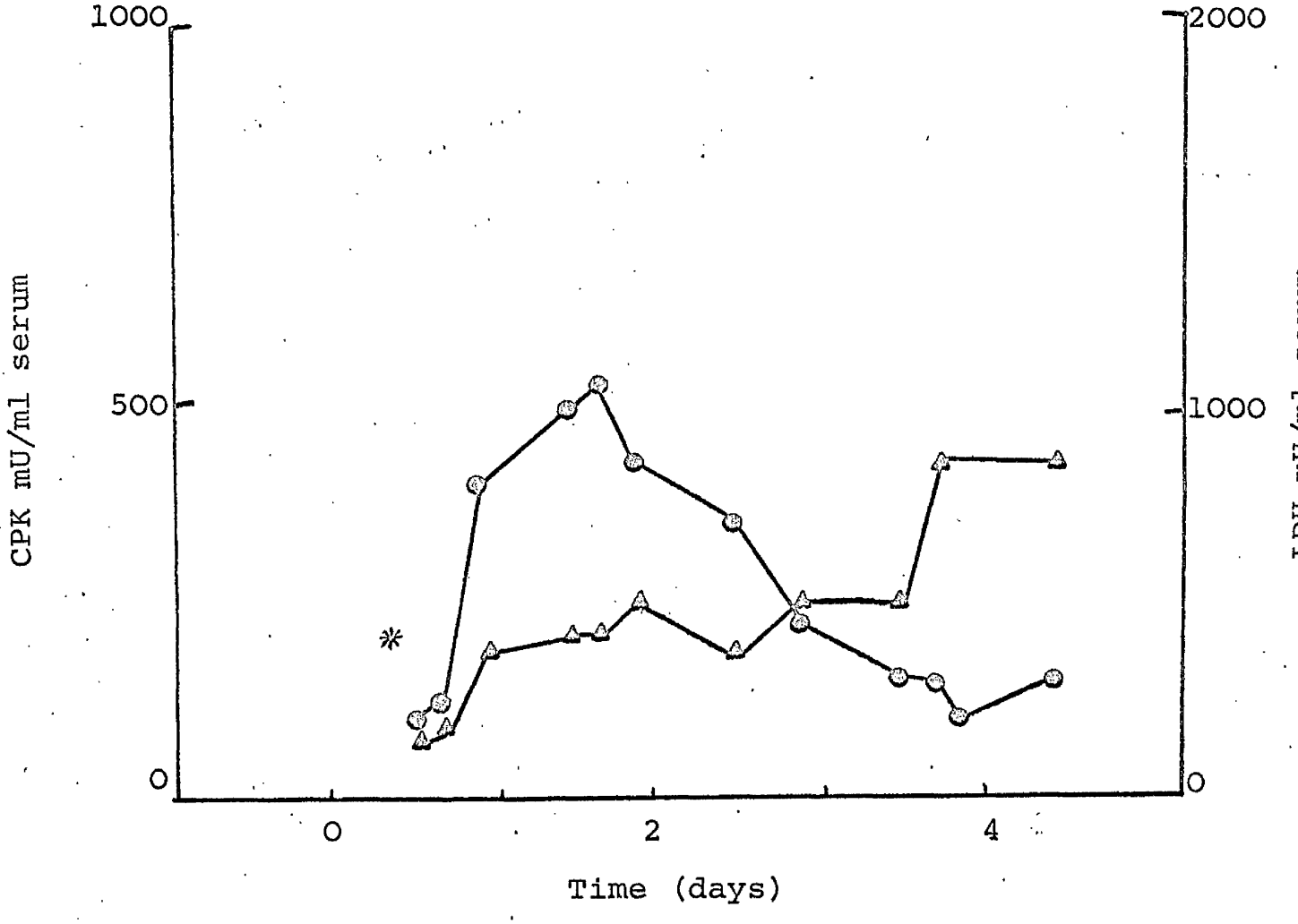


Fig. 2.2.11.

Case 11

Age: 60 years

Previous history: None

History: Collapsed with severe chest pain while at work at 10.00 am on morning of admission.

ECG interpretation: Antero-septal and posterior myocardial infarction.

Fig. 2.2.12.

Case 12

Age: 67 years

Previous history: Angina of effort for several years and myocardial infarction eleven years previously.

History: Severe chest tightness at 6.00 pm on evening before admission, still persisting when admitted. In left ventricular failure on days 2 and 3.

ECG interpretation: Postero-lateral myocardial ischaemia.

Fig. 2.2.11

Case 11

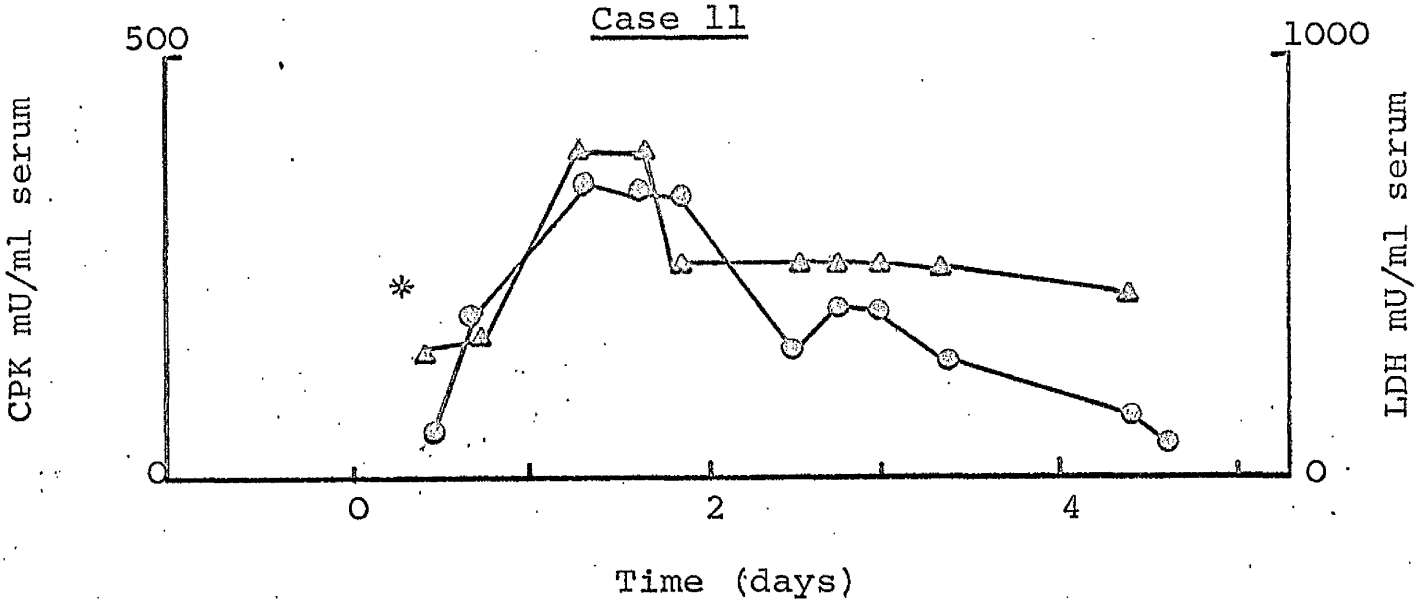


Fig. 2.2.12

Case 12

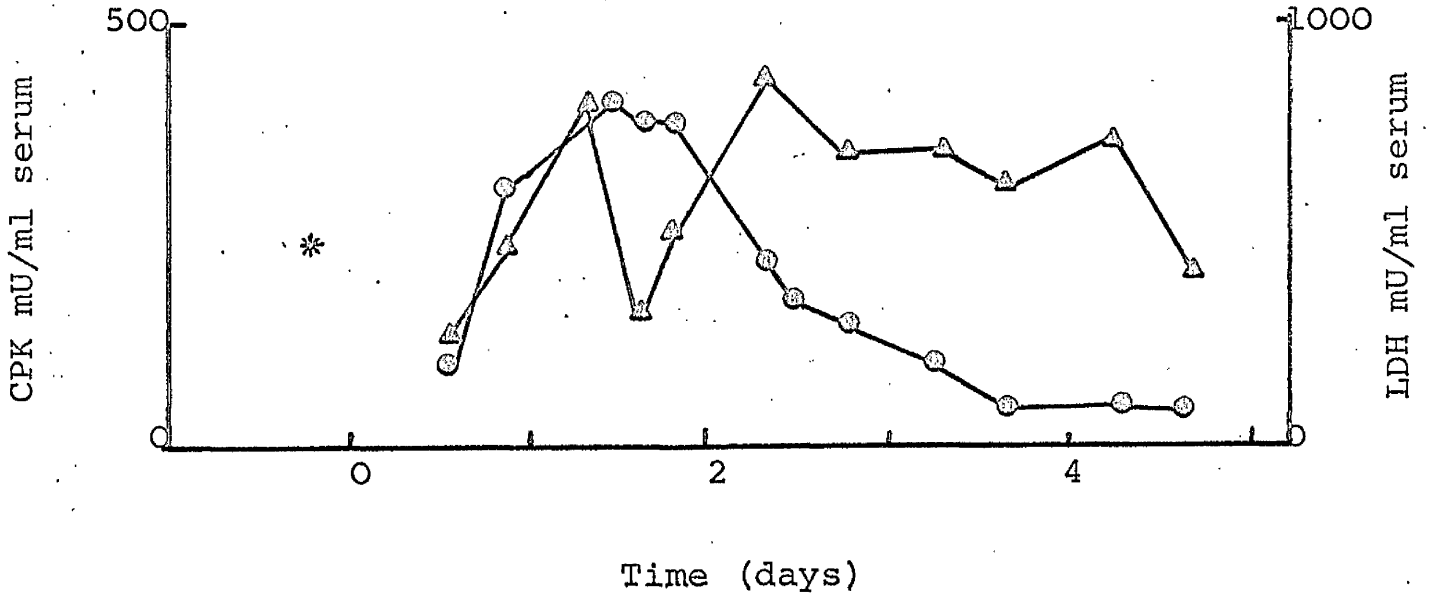


Fig. 2.2.13.

Case 13

Age: 60 years.

Previous history: None

History: Episode of central chest pain two days before admission which recurred at 3.00 pm and lasted till 10.00 pm on day before admission. At 1.30 am on day of admission wakened by severe chest pain lasting two hours.

ECG interpretation: Posterior myocardial infarction.

Fig. 2.2.14.

Case 14

Age: 47 years

Previous history: None

History: Two days before admission experienced sudden onset of severe crushing retrosternal chest pain which lasted for about three hours. At 2.00 am on morning of admission pain recurred and lasted for two hours. Further attacks of severe chest pain on day 3.

ECG interpretation: Day 1 - probable antero-septal myocardial ischaemia.

Day 2 - widespread anterior myocardial ischaemia.

Day 3 - antero-septal myocardial infarction.

Fig. 2.2.13

Case 13

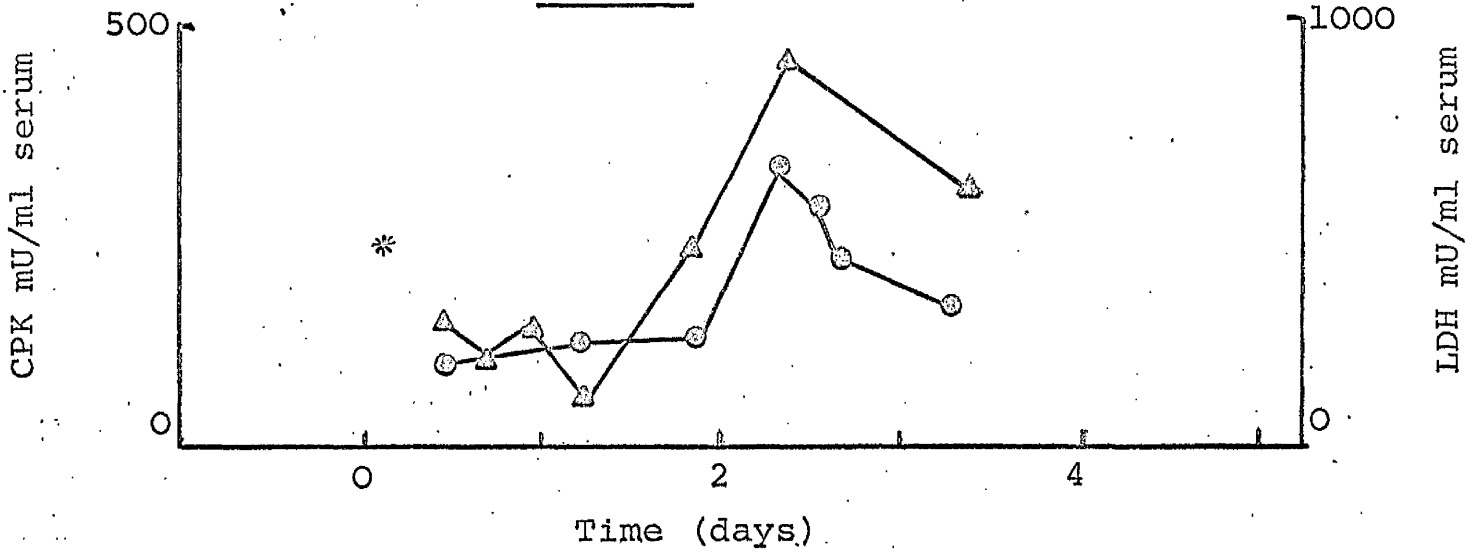


Fig. 2.2.14

Case 14

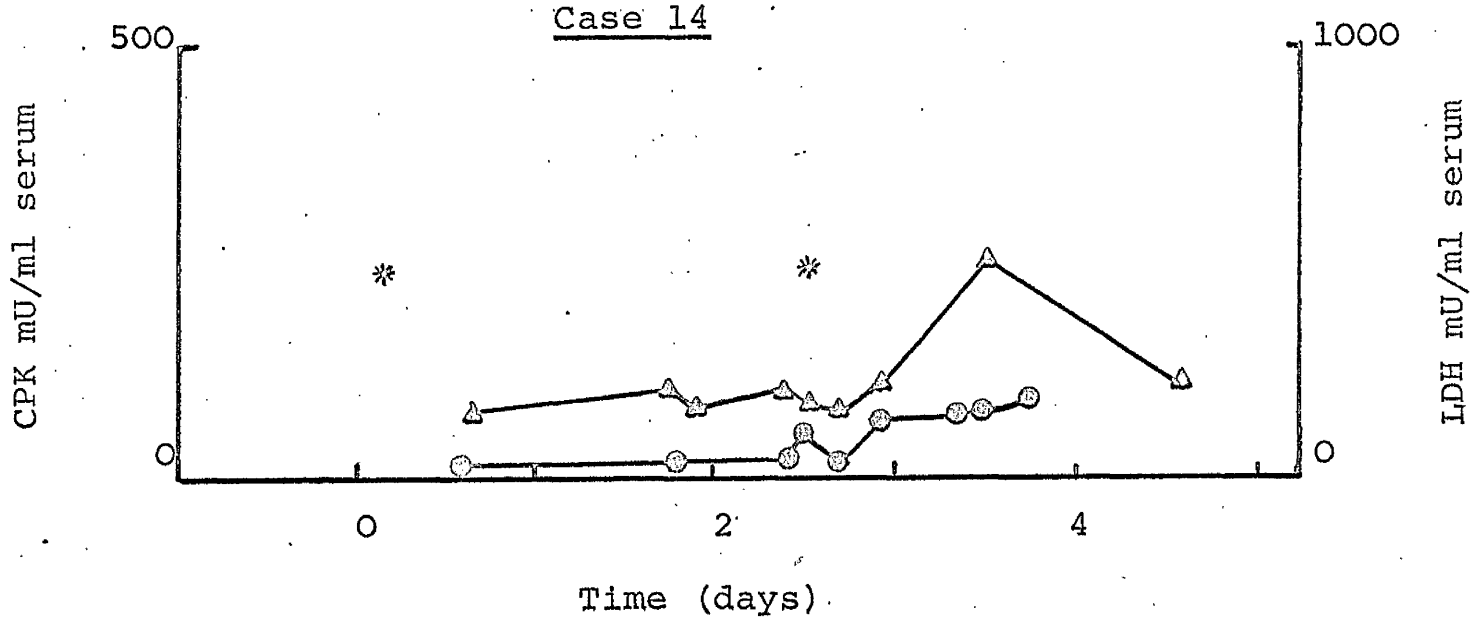


Fig. 2.2.15.

Case 15

Age: 58 years

Previous history: None

History: At 6.00 am on day previous to admission experienced tightness across front of chest which became progressively worse and by 6.00 pm was severe.

ECG interpretation: Antero-lateral myocardial infarction.

Fig. 2.2.16.

Case 16

Age: 72 years

Previous history: None

History: Three days before admission had fairly severe chest pain continuing on and off for two days. No further chest pain on day of admission.

ECG interpretation: Day 1 - evidence of recent posterior myocardial infarction.

Day 3 - evolving pattern of posterior myocardial infarction.

Day 5 - recent posterior myocardial infarction.

Fig. 2.2.15

Case 15

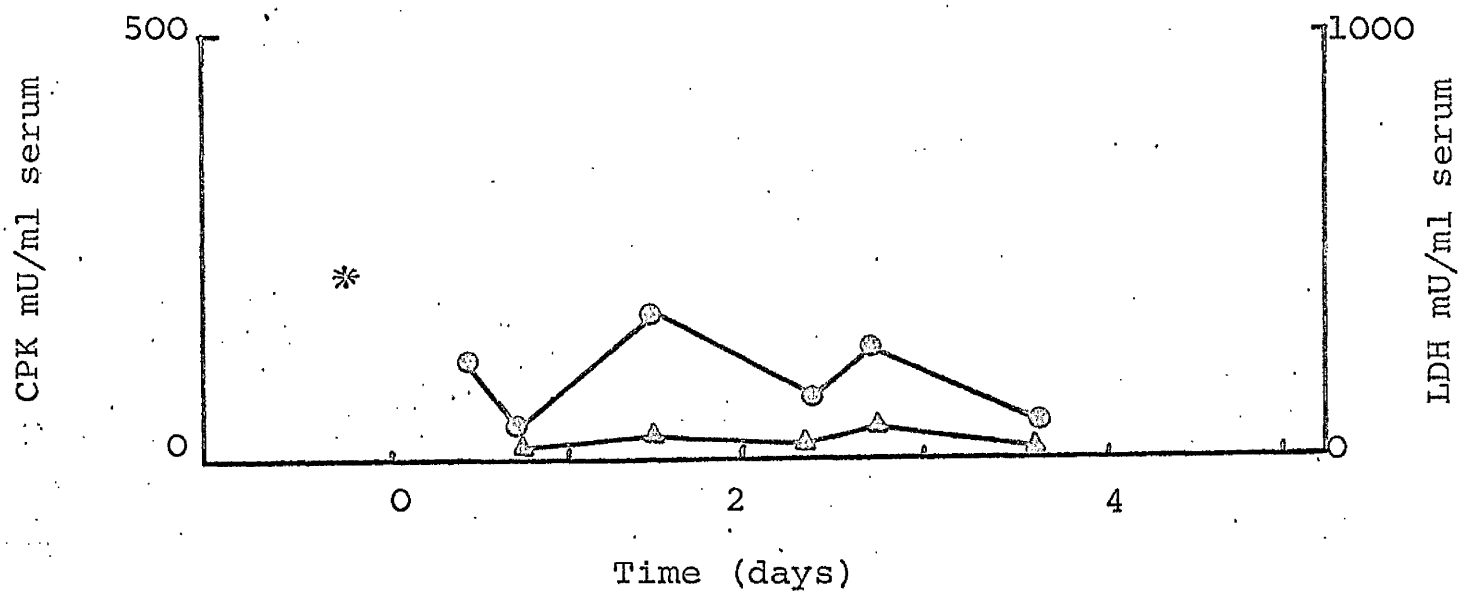


Fig. 2.2.16

Case 16

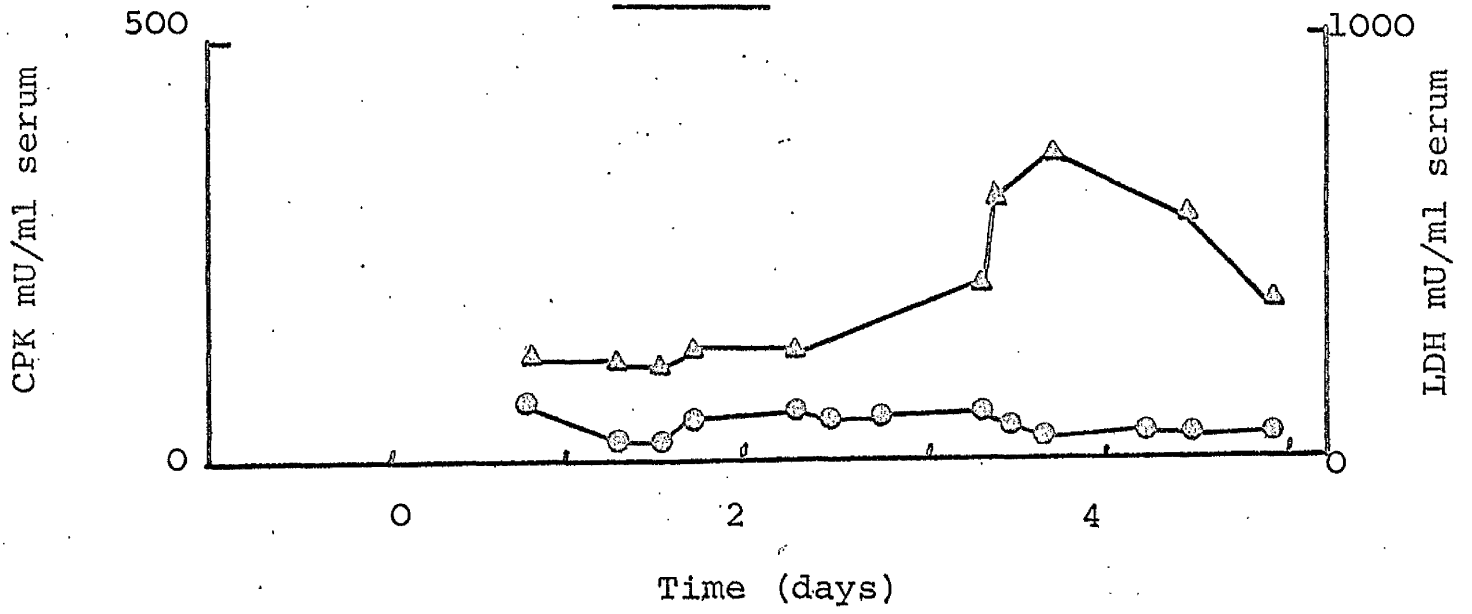


Fig. 2.2.17.

Case 17

Age: 64 years

Previous history: One year previously had a posterior myocardial infarction.

History: Sudden onset of acute breathlessness, sweating, vomiting and chest pain at 1.00 am on day of admission. On admission unconscious and in left ventricular failure. On day 5 was in atrial and ventricular fibrillation and had a cardiac arrest.

ECG interpretation: Day 1 - lateral myocardial ischaemia.

Day 5 - atrial and ventricular fibrillation and lateral myocardial ischaemia.

Fig. 2.2.18.

Case 18

Age: 54 years

Previous history: None

History: At 10.00 am on morning of admission severe tight pain across chest wall which was easing at time of admission.

ECG interpretation: Posterior myocardial infarction.

Fig. 2.2.17

Case 17

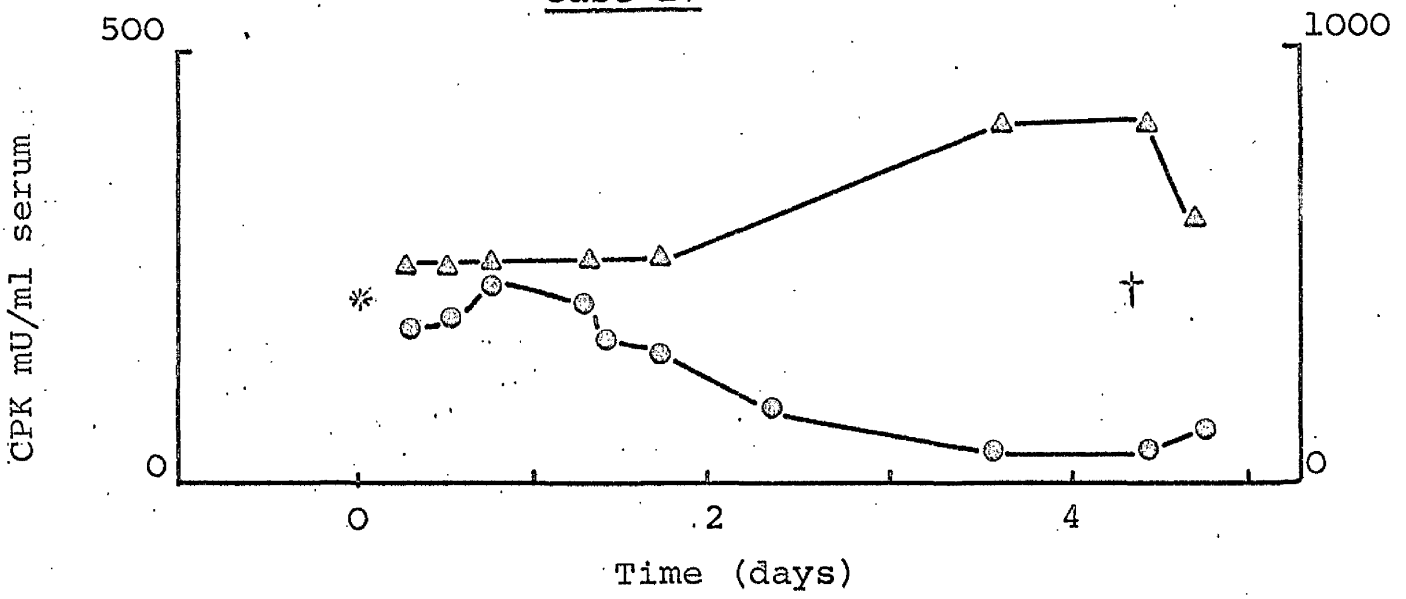


Fig. 2.2.18

Case 18

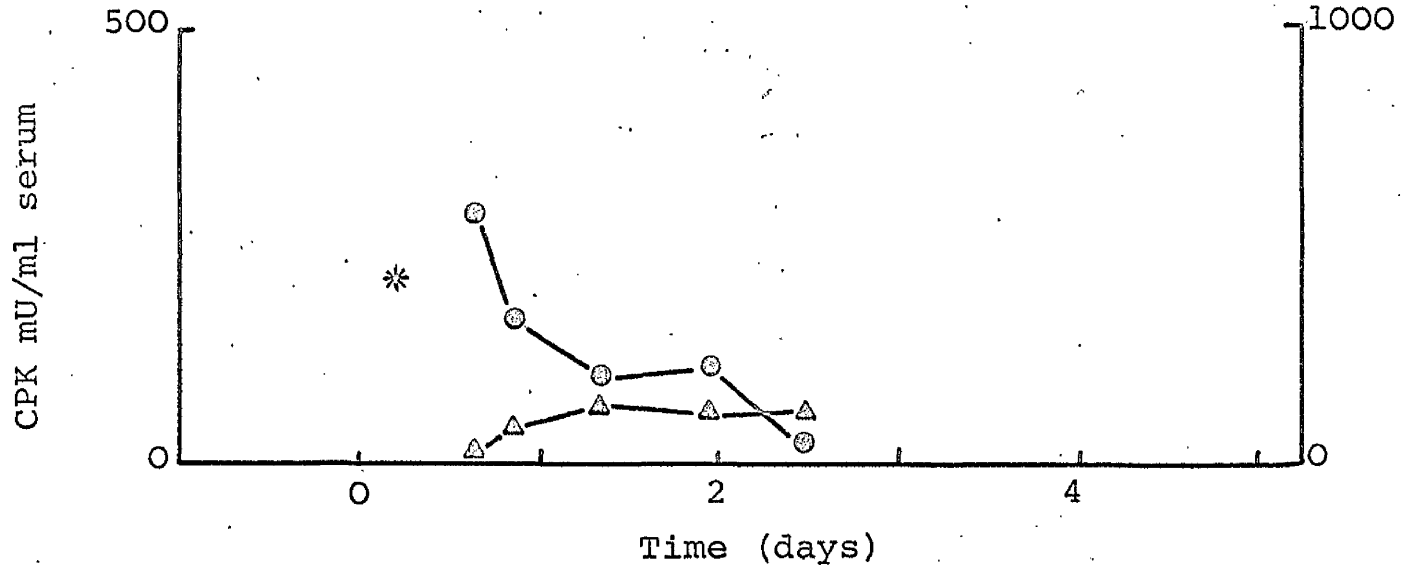


Fig. 2.2.19.

Case 19

Age: 34 years

Previous history: None

History: Sudden onset of central retrosternal chest pain at 11.00 pm on evening before admission.

ECG interpretation: Antero-lateral myocardial infarction.

Fig. 2.2.20.

Case 20

Age: 45 years

Previous history: None

History: Angina of effort for several days. Severe chest pain for three days commencing at 10.00 pm on evening before admission. No subsequent pain between then and admission.

ECG interpretation: Antero-lateral myocardial infarction.

Fig. 2.2.19

Case 19

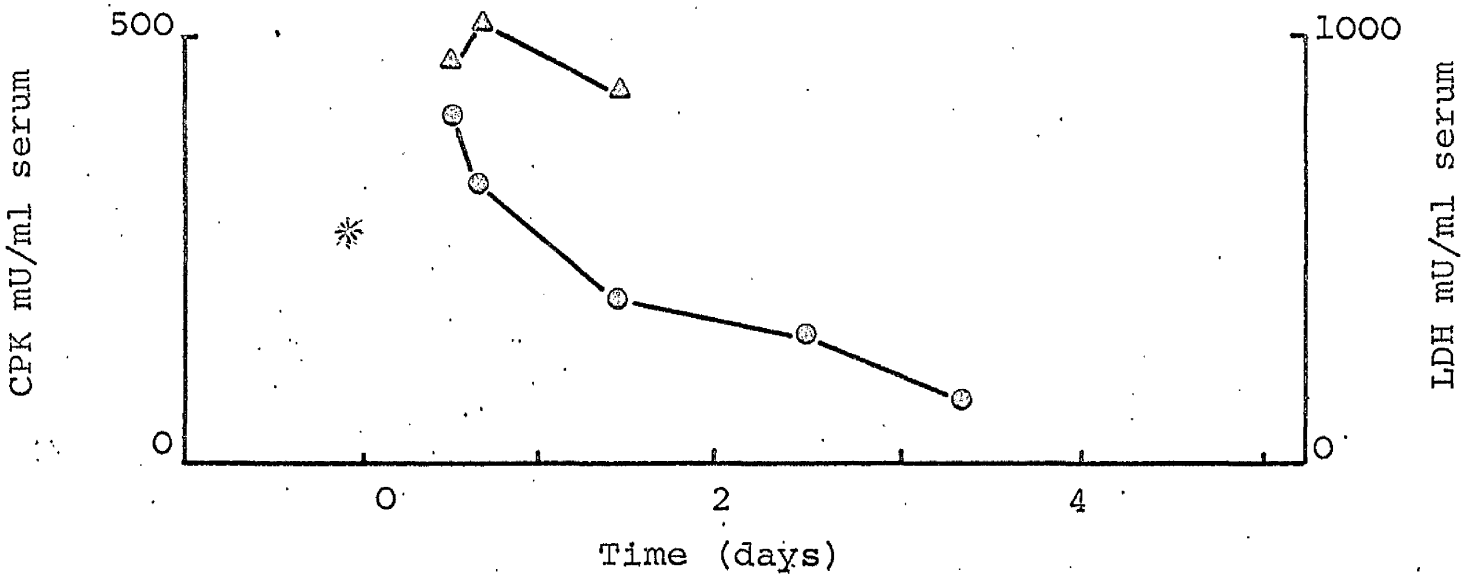


Fig. 2.2.20

Case 20

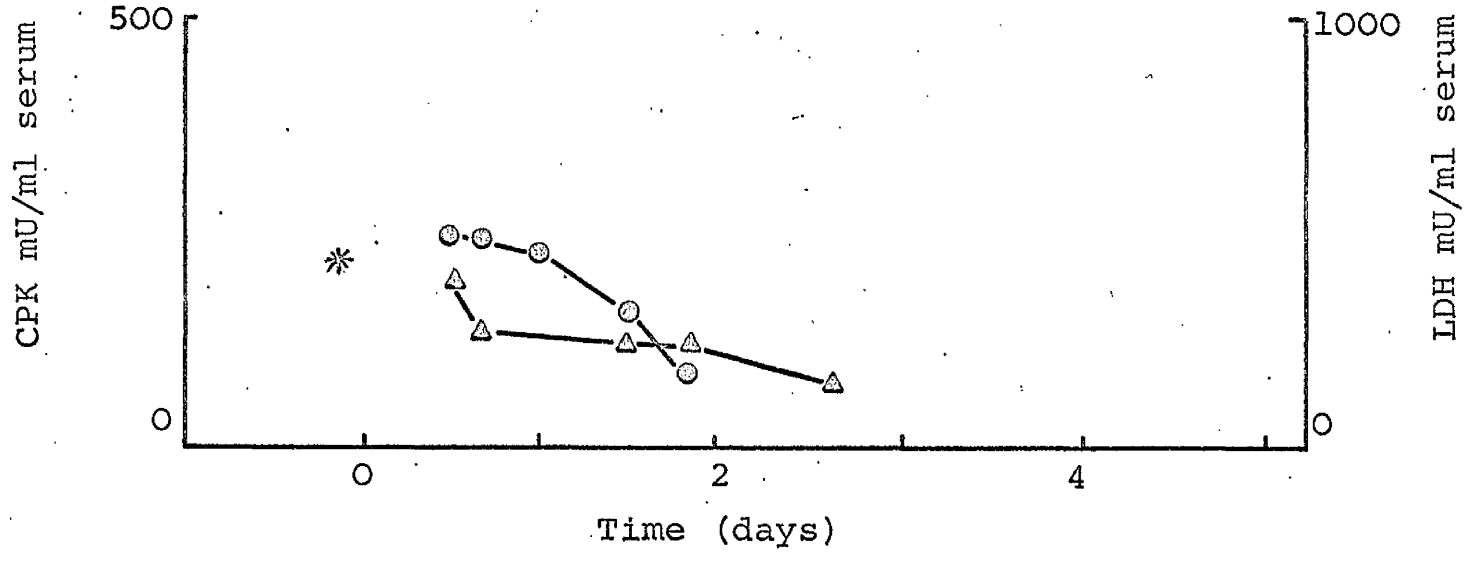


Fig. 2.2.21.

Case 21

Age: 68 years.

Previous history: Angina of effort for nine months

History: At 7.30 pm on day before admission sudden onset of central, crushing chest pain.

ECG interpretation: Day 1 - widespread myocardial ischaemia.
Day 3 - recent posterior myocardial infarction.

Fig. 2.2.22.

Case 22

Age: 67 years

Previous history: Five years previously had a posterior myocardial infarction.

History: Dizziness and incipient black-outs.

ECG interpretation: No definite evidence of myocardial infarction.

Fig. 2.2.21

Case 21

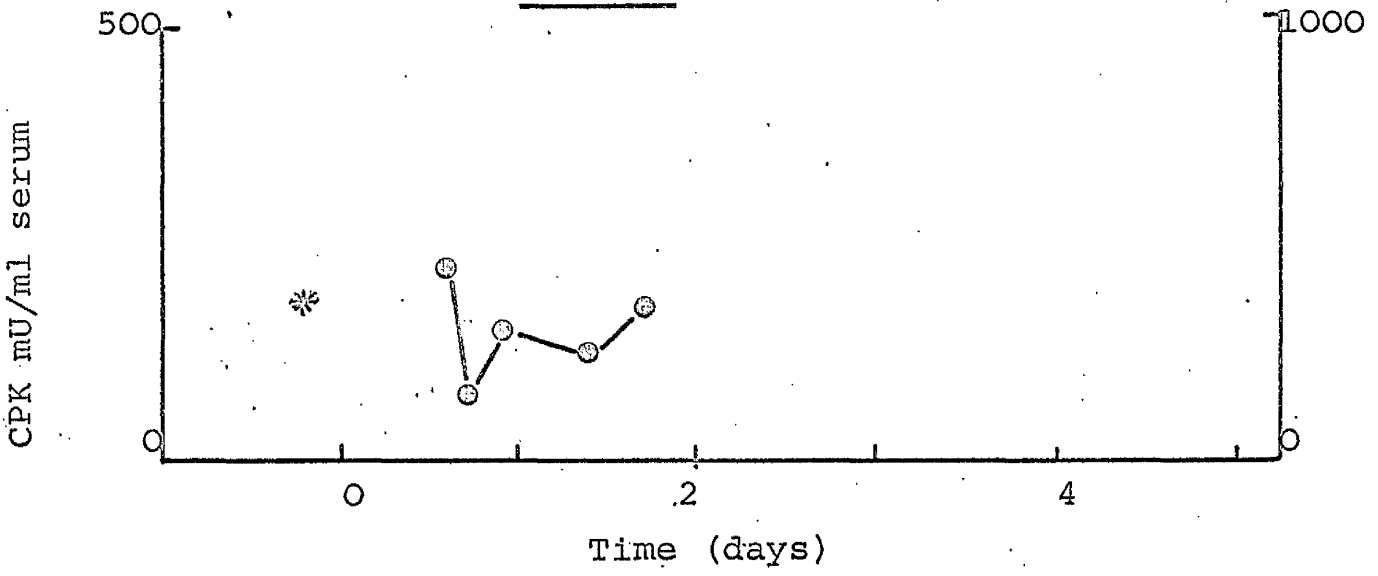


Fig. 2.2.22

Case 22

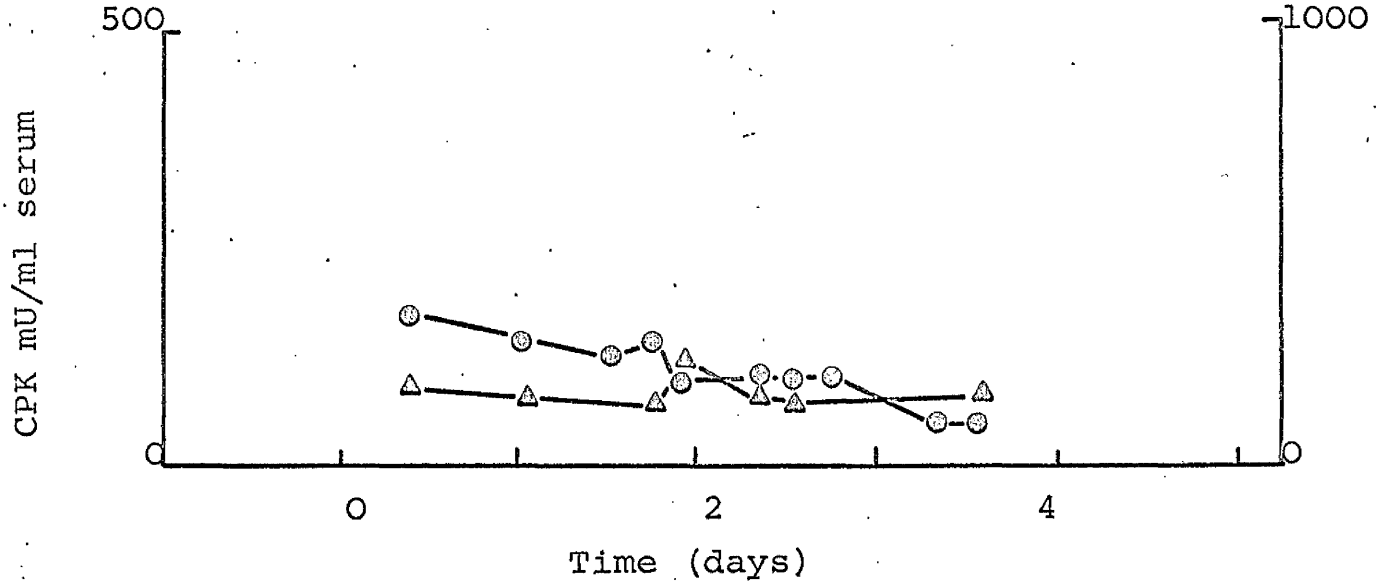


Fig. 2.2.23.

Case 23

Age: 67 years

Previous history: Angina of effort for two to three years.

History: Severe chest pain at 4.00 am on morning of admission and again on day 2. Died on day 3.

ECG interpretation: Extensive recent left ventricular myocardial infarction.

Fig. 2.2.23

Case 23

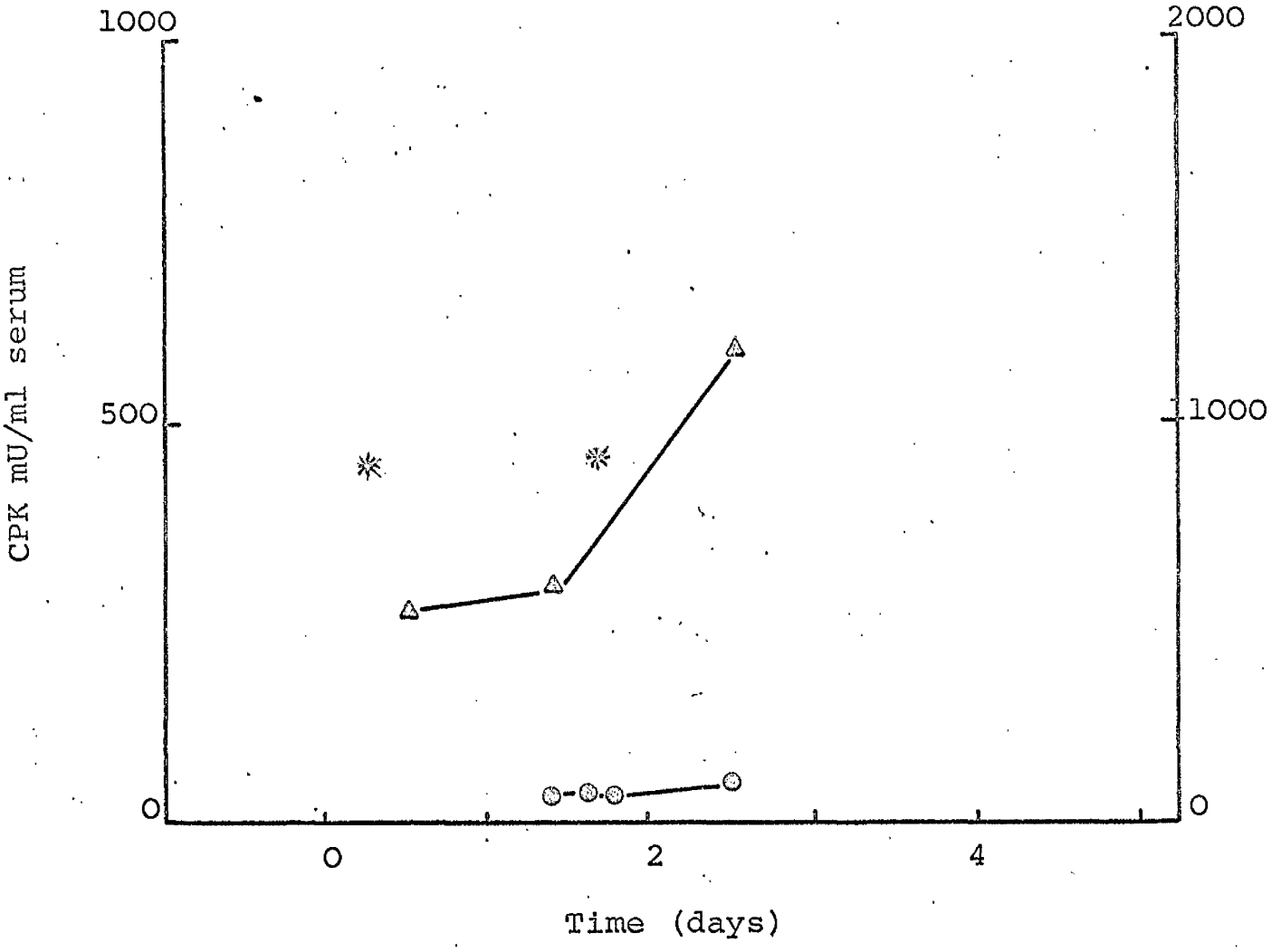


Fig. 2.2.24.

Case 24

Age: 58 years

Previous history: Angina of effort for six months.

History: Severe chest discomfort at 10.00 am on day of admission. Died on day 2.

ECG interpretation: Incomplete tracing.

Fig. 2.2.24

Case 24

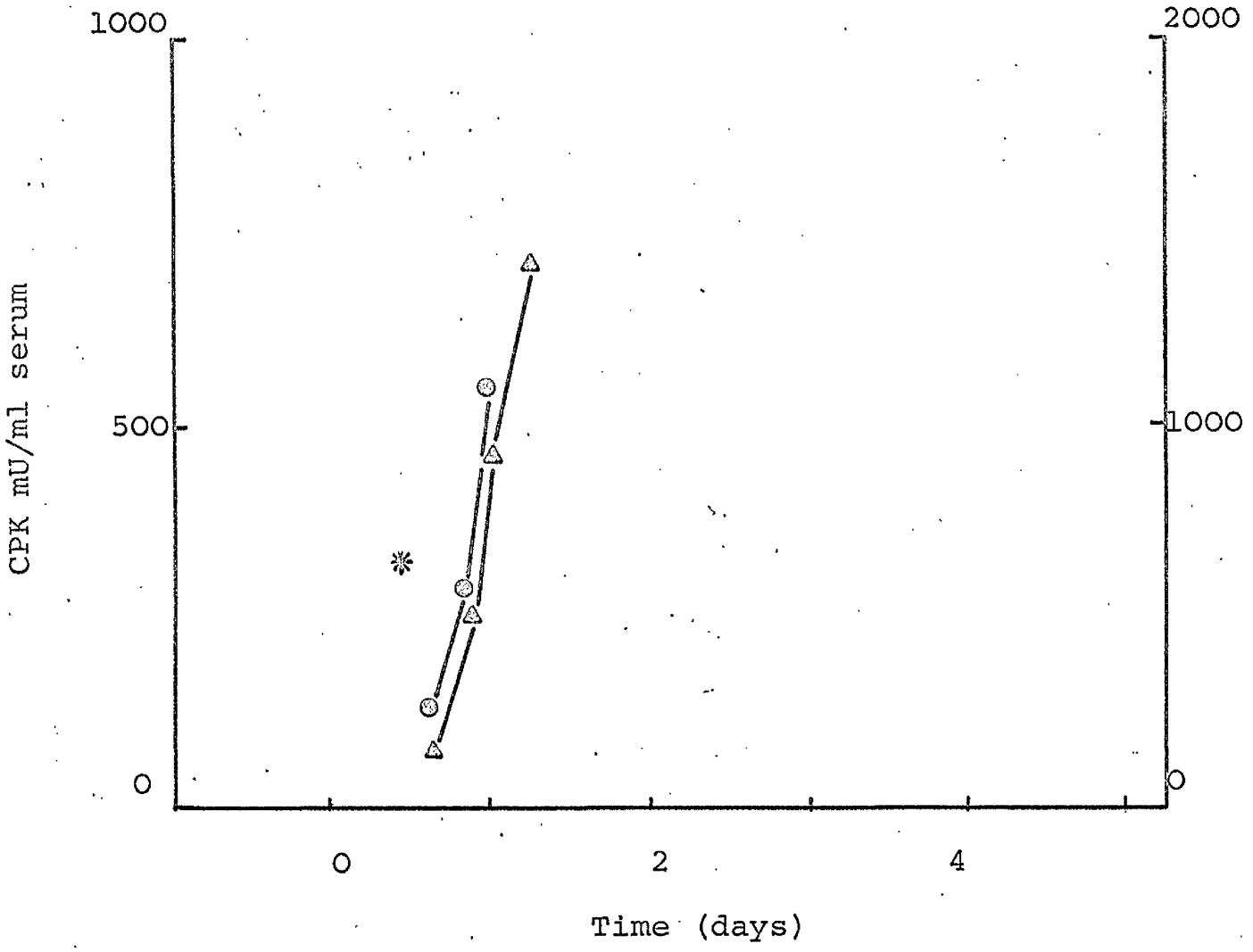


Fig. 2.2.25.

Case 25

Age: 40 years

Previous history: Angina of effort for two years.

History: Severe retrosternal pain at 1.00 pm on day of admission. Died on day 2.

ECG interpretation: Widespread anterior myocardial infarction.

Fig. 2.2.25

Case 25

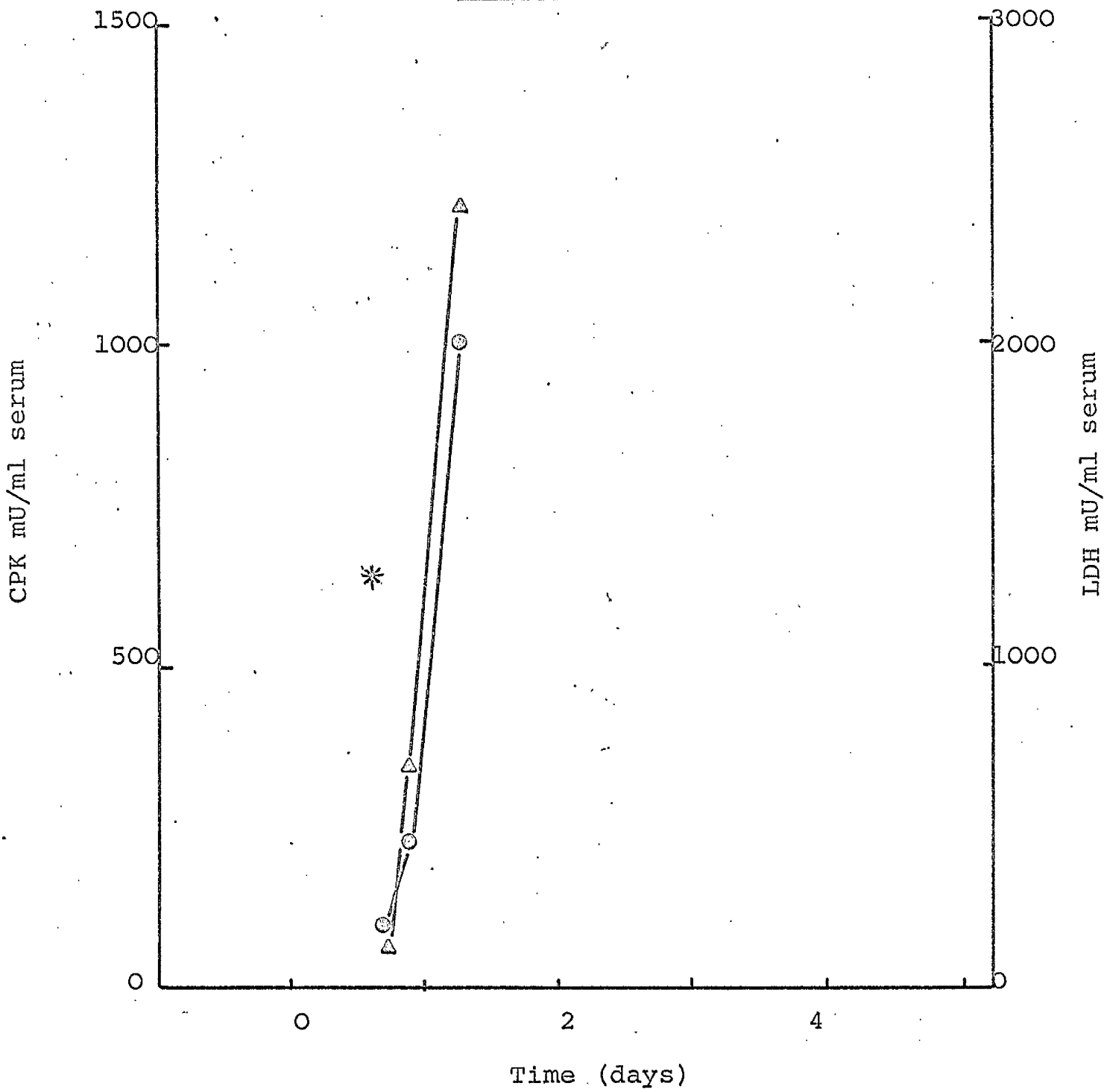


Fig. 2.2.26.

Case 26

Age: 42 years

Previous history: Six years previously had myocardial infarction and has had angina of effort since then.

History: Severe chest pain at 6.45 am on day of admission. Died a few hours after admission.

ECG interpretation: No ECG taken

Post-mortem findings: Septum and adjacent posterior wall infarcted.

Fig. 2.2.26

Case 26

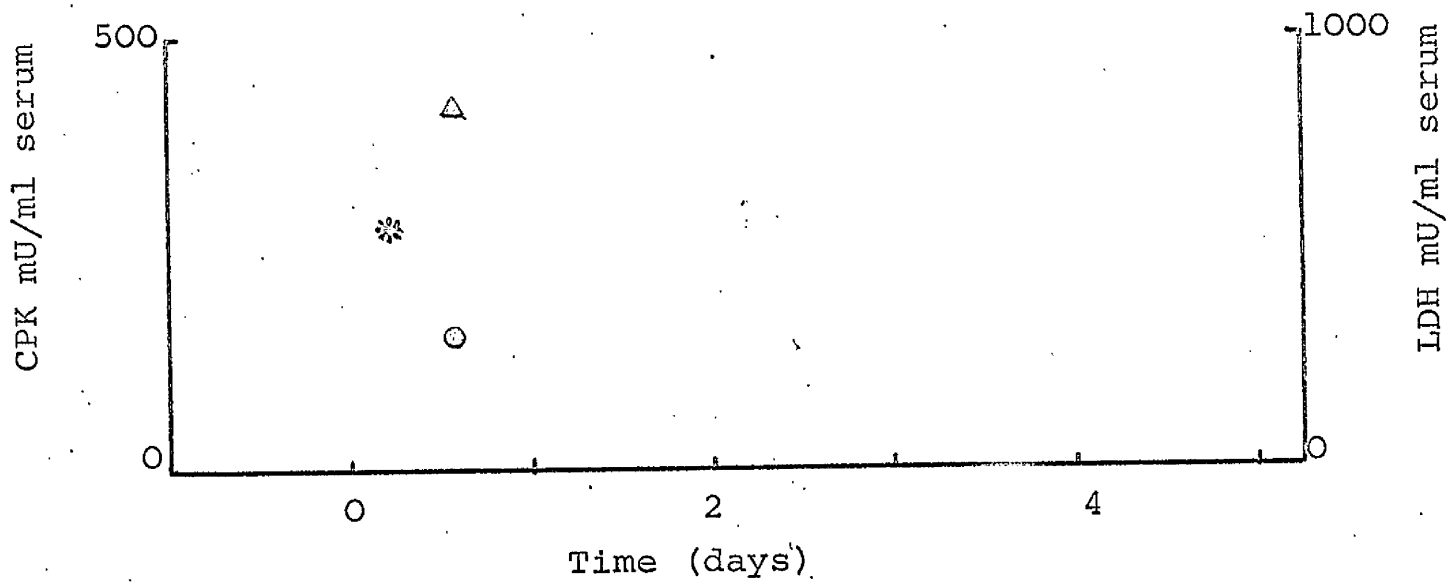


Fig. 2.2.27.

Case 27

Age: 70 years

Previous history: Three years previously had a myocardial infarction.

History: Five episodes of dizziness in past week, but did not lose consciousness. Complained of breathlessness on mild exertion.

ECG interpretation: Day 1 - left bundle branch block which dates from infarction three years previously.

Day 2 - atrial fibrillation.

Fig. 2.2.28.

Case 28

Age: 40 years

Previous history: Myocardial infarction two months previously.

History: Tachycardia and left pectoral pain.

ECG interpretation: Supraventricular tachycardia and left bundle branch block.

Fig. 2.2.27

Case 27

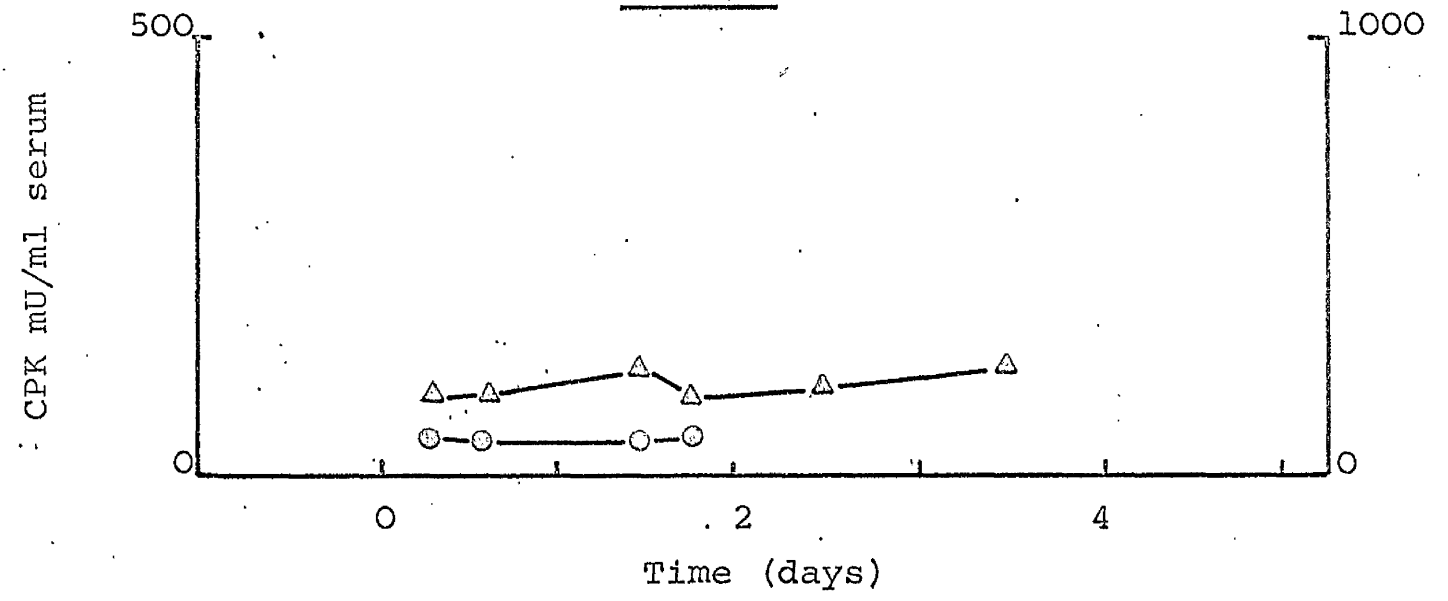
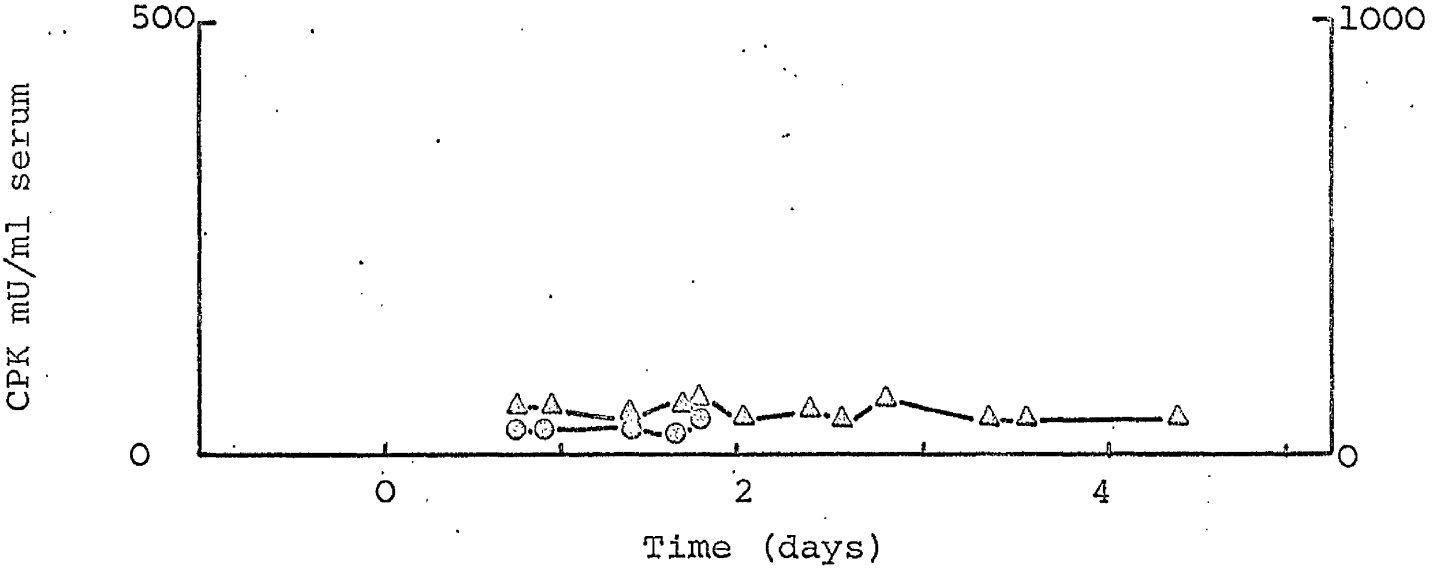


Fig. 2.2.28

Case 28



Figs. 2.2.29 - 2.2.33

Changes in serum enzyme activities in patients with cardiac disease other than myocardial infarction and who were treated by DC countershock.

⊙ Activity of creatine phosphokinase, (CPK), in serum

△ Activity of lactate dehydrogenase, (LDH), in serum

| Denotes time of DC countershock.

Time zero on each figure represents 00.01 hours on day of DC countershock.

Note: Previous history refers to cardiac disease.

Fig. 2.2.29.

Case 29

Sex: Male

Age: 29 years

Previous history: Replacement of mitral valve three years previously.

History: Complained of weakness, breathlessness and palpitations.

ECG interpretation: Atrial tachycardia.

Fig. 2.2.30.

Case 30

Sex: Female

Age: 58 years

Previous history: Three years previously in congestive heart failure and had atrial flutter.

History: Breathlessness and palpitation for last few weeks.

ECG interpretation: Left ventricular failure due to atrial flutter.

Fig. 2.2.29

Case 29

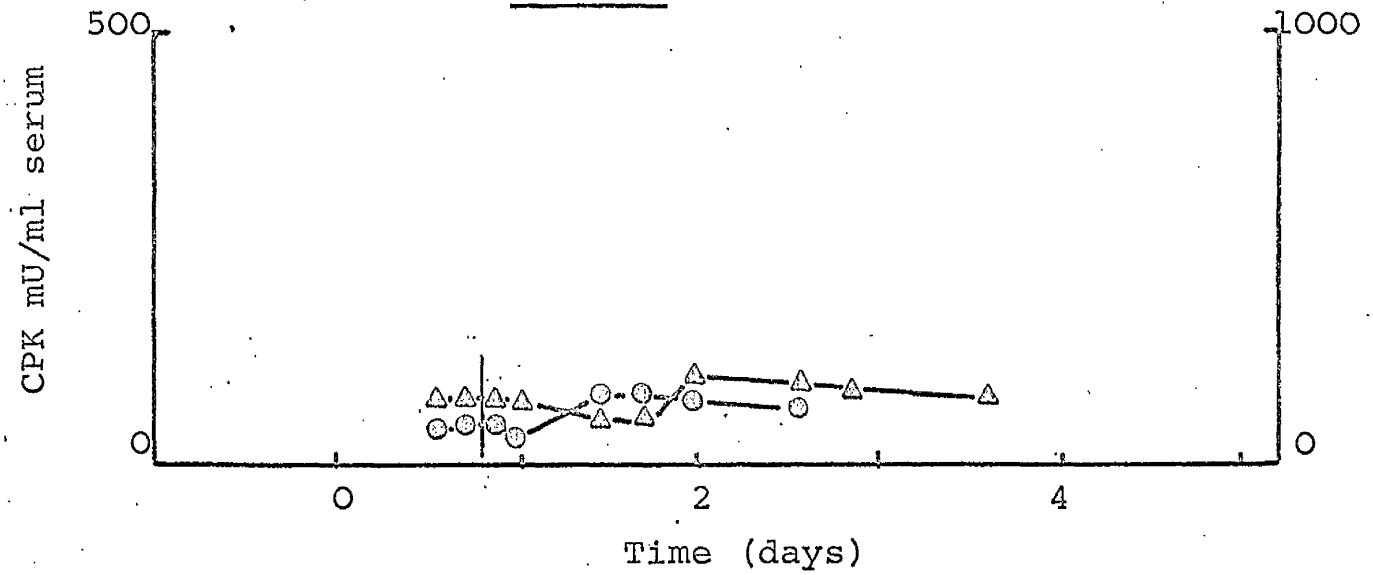


Fig. 2.2.30

Case 30

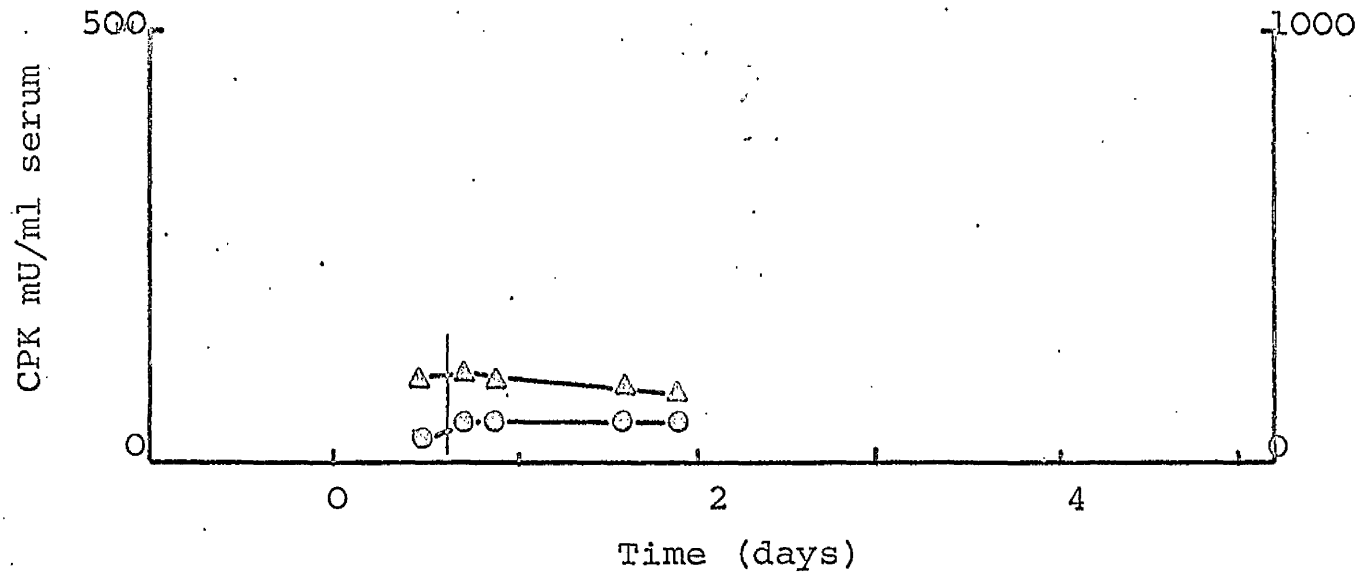


Fig. 2.2.31.

Case 31

Sex: Female

Age: 47 years

Previous history: Mitral stenosis and incompetence for three years.

History: Complained of palpitation and breathlessness.

ECG interpretation: Atrial tachycardia

Fig. 2.2.32.

Case 32

Sex: Female

Age: 66 years

Previous history: None

History: Complained of frequent episodes of palpitations.

ECG interpretation: Supraventricular tachycardia.

Fig. 2.2.33.

Case 33

Sex: Male

Age: 24 years

Previous history: Mitral and aortic valve disease for some years.

History: Mitral and aortic valve replacement six weeks previously.

ECG interpretation: Atrial fibrillation.

Fig. 2.2.31

Case 31

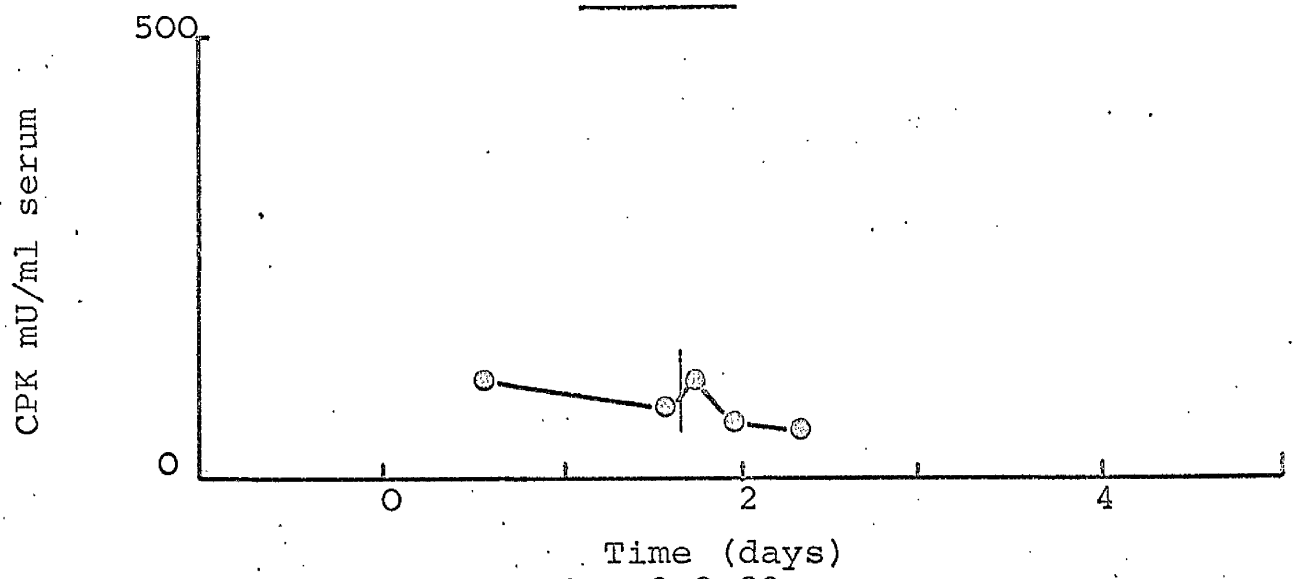


Fig. 2.2.32

Case 32

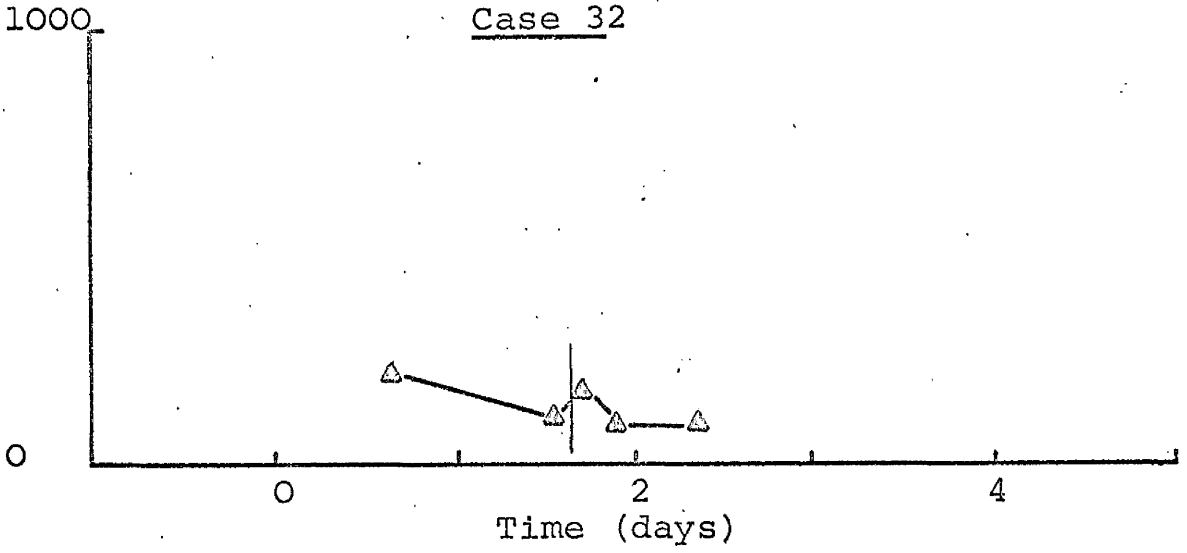
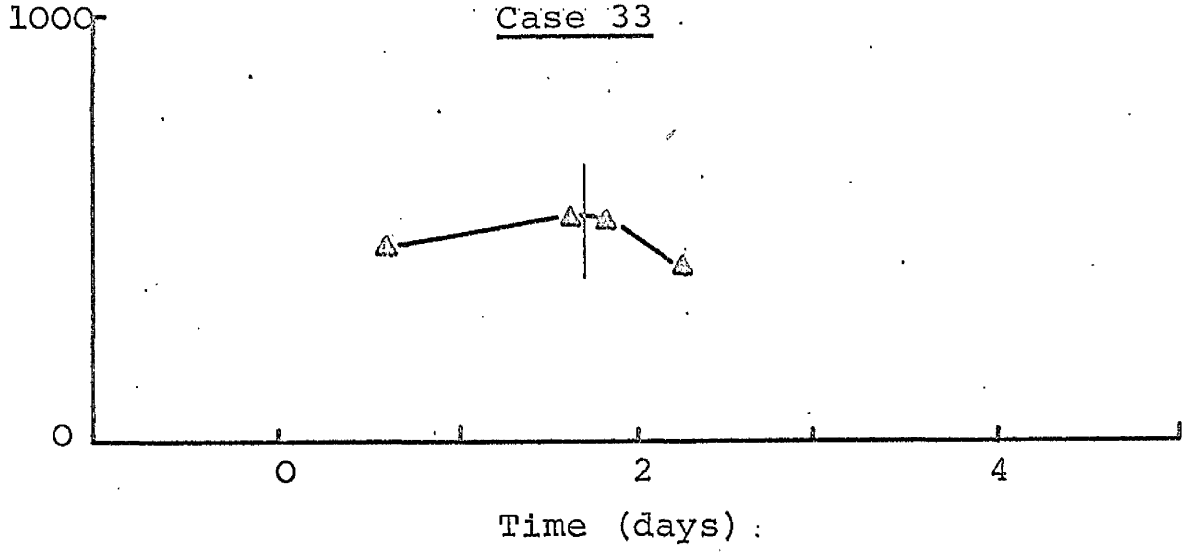


Fig. 2.2.33

Case 33



Figs. 2.2.34 - 2.2.49.

These show semilogarithmic plots of the SCPK activities of some cases. The half-life of CPK in serum was calculated in each of these cases and these are shown in Table 2.2.50.

Fig. 2.2.34

Case 1

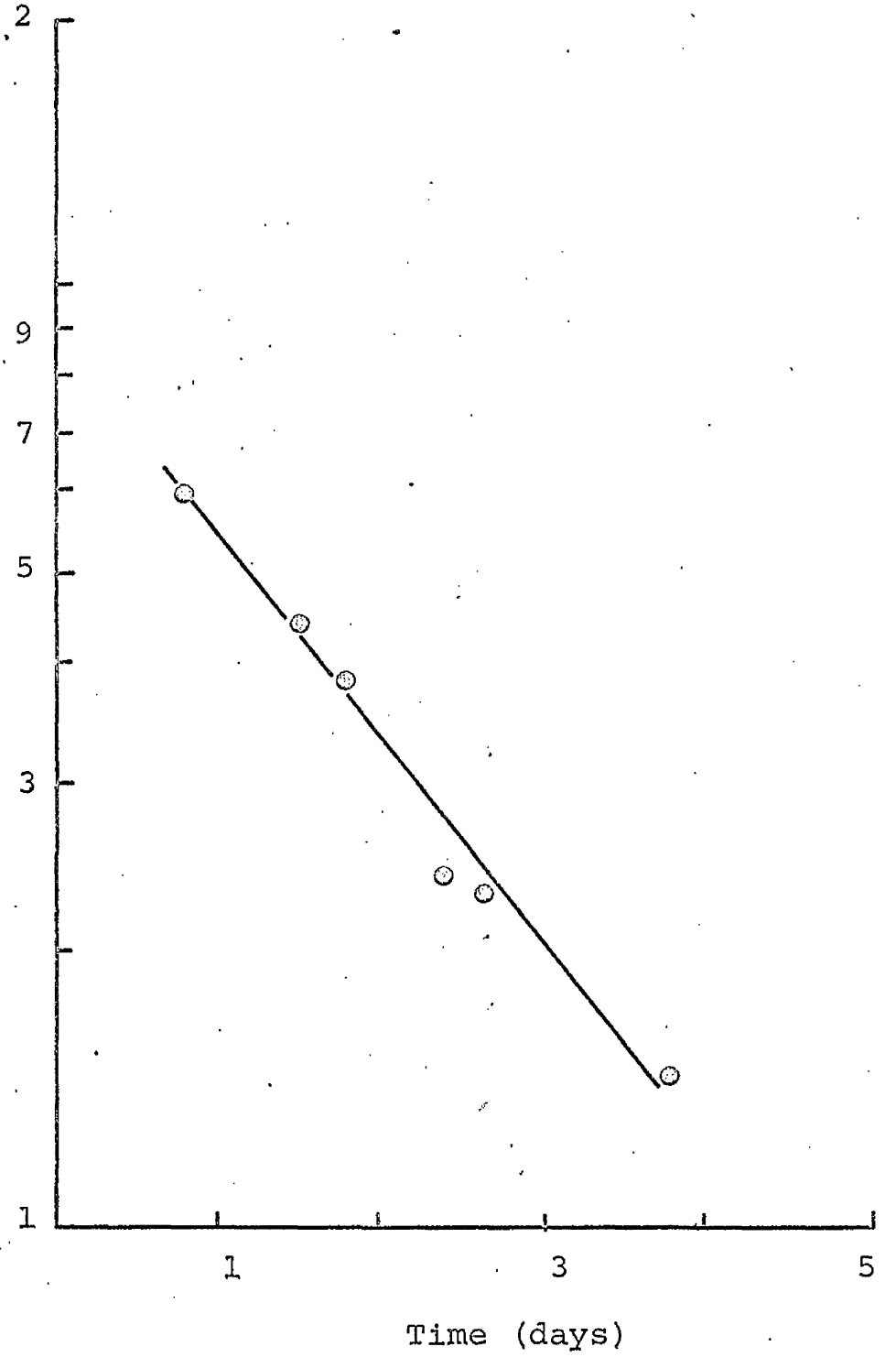


Fig. 2.2.35

Case 2

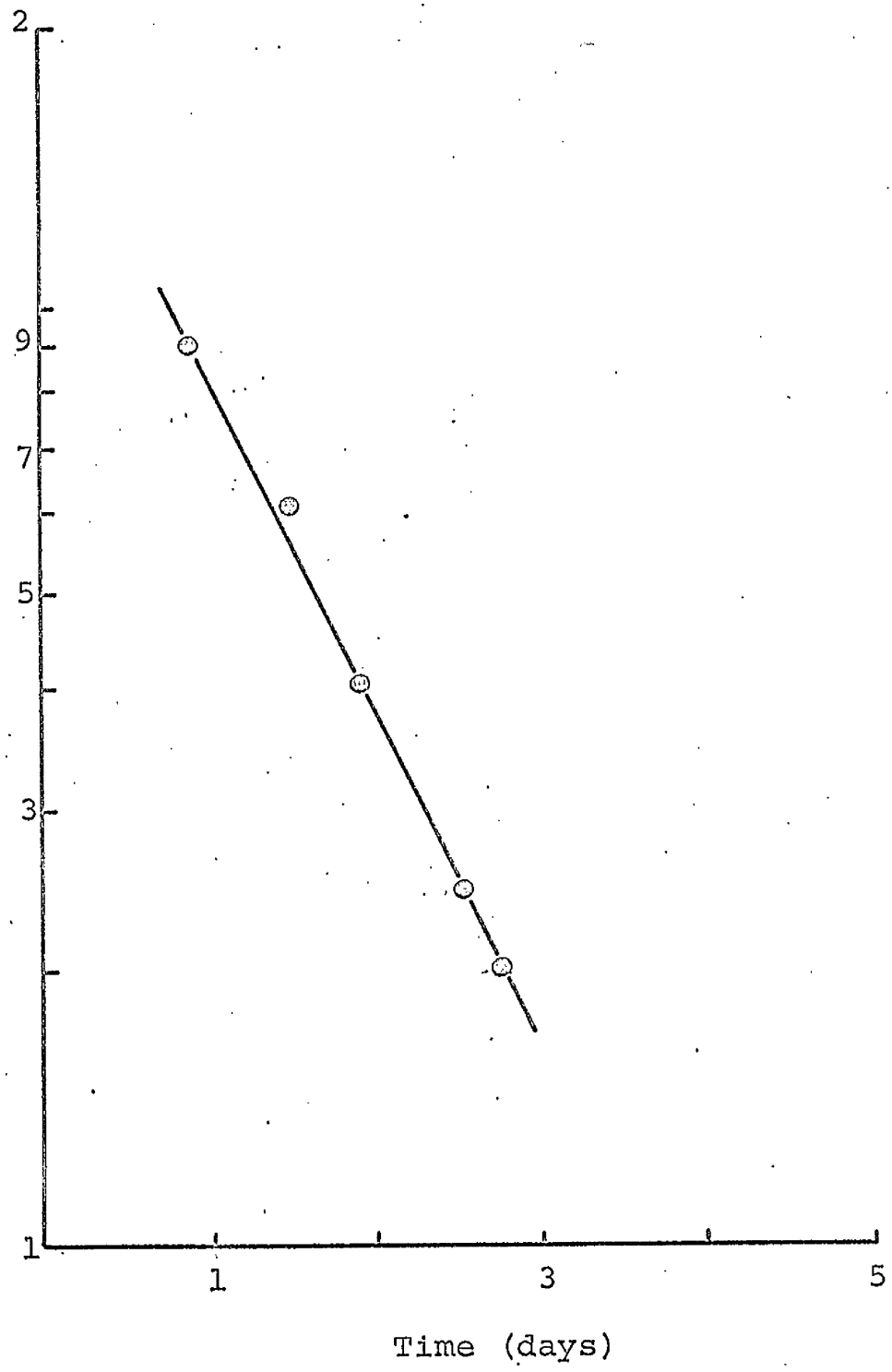


Fig. 2.2.36

Case 3

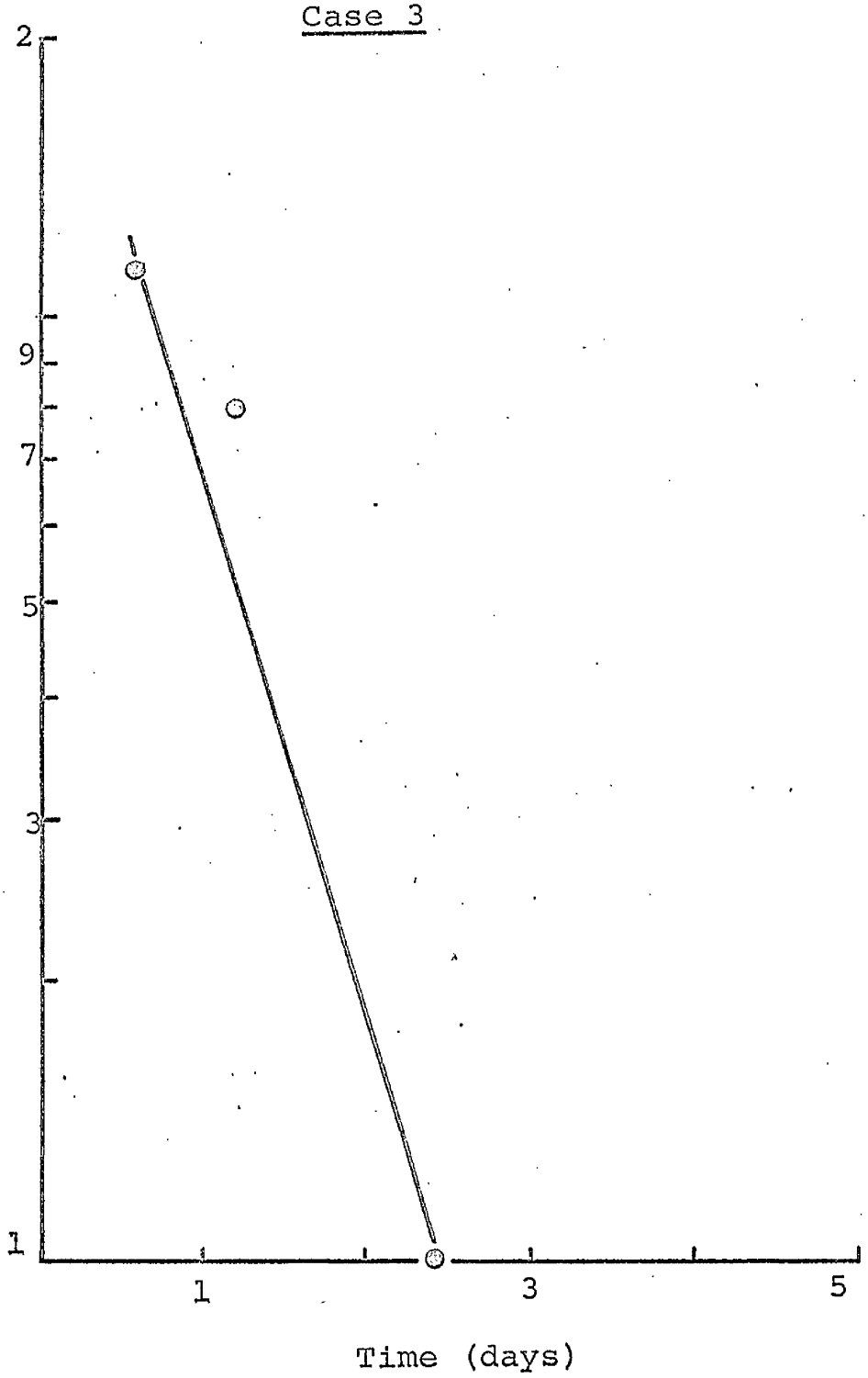


Fig. 2.2.37

Case 4

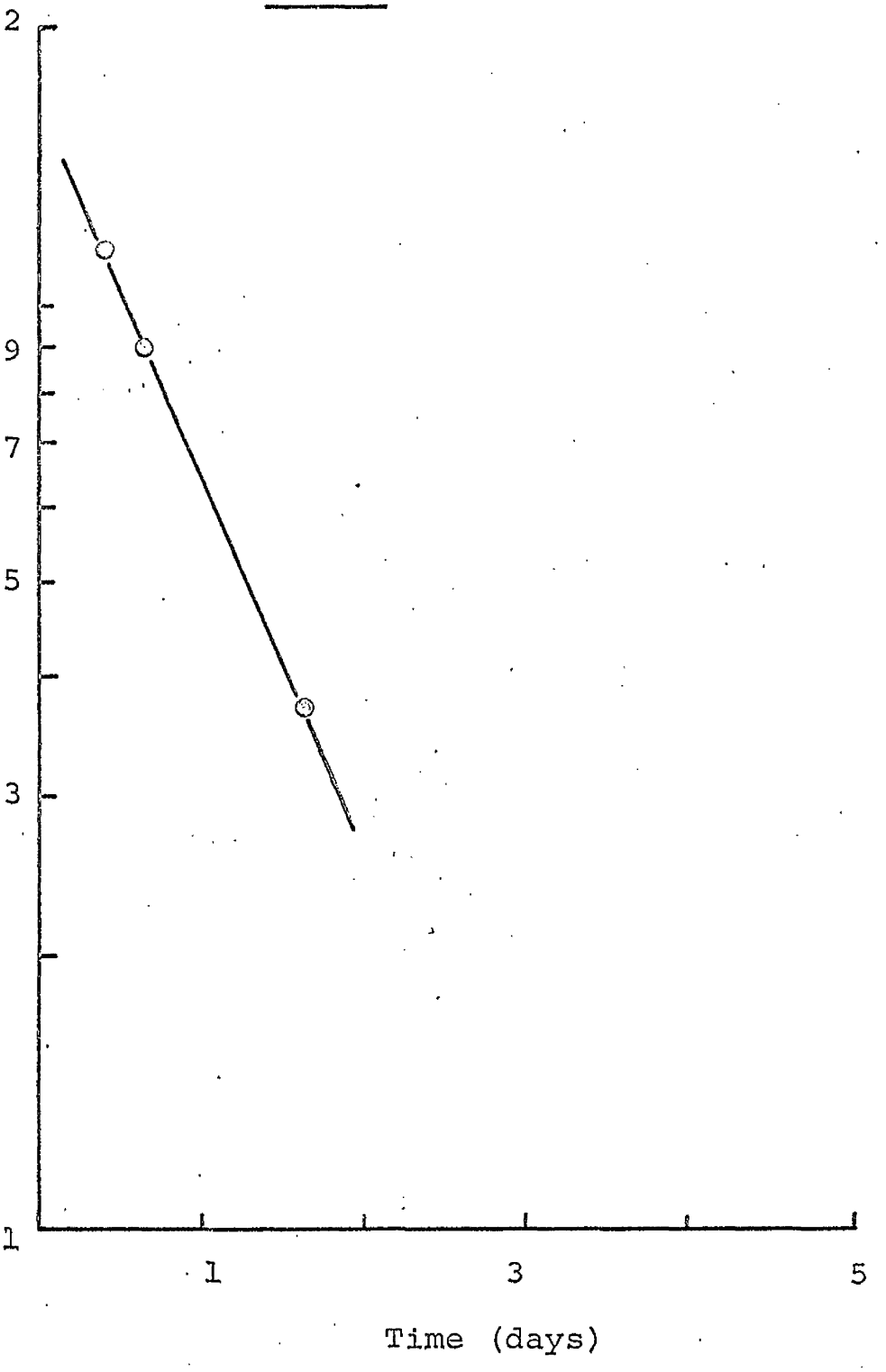


Fig. 2.2.38

Case 5

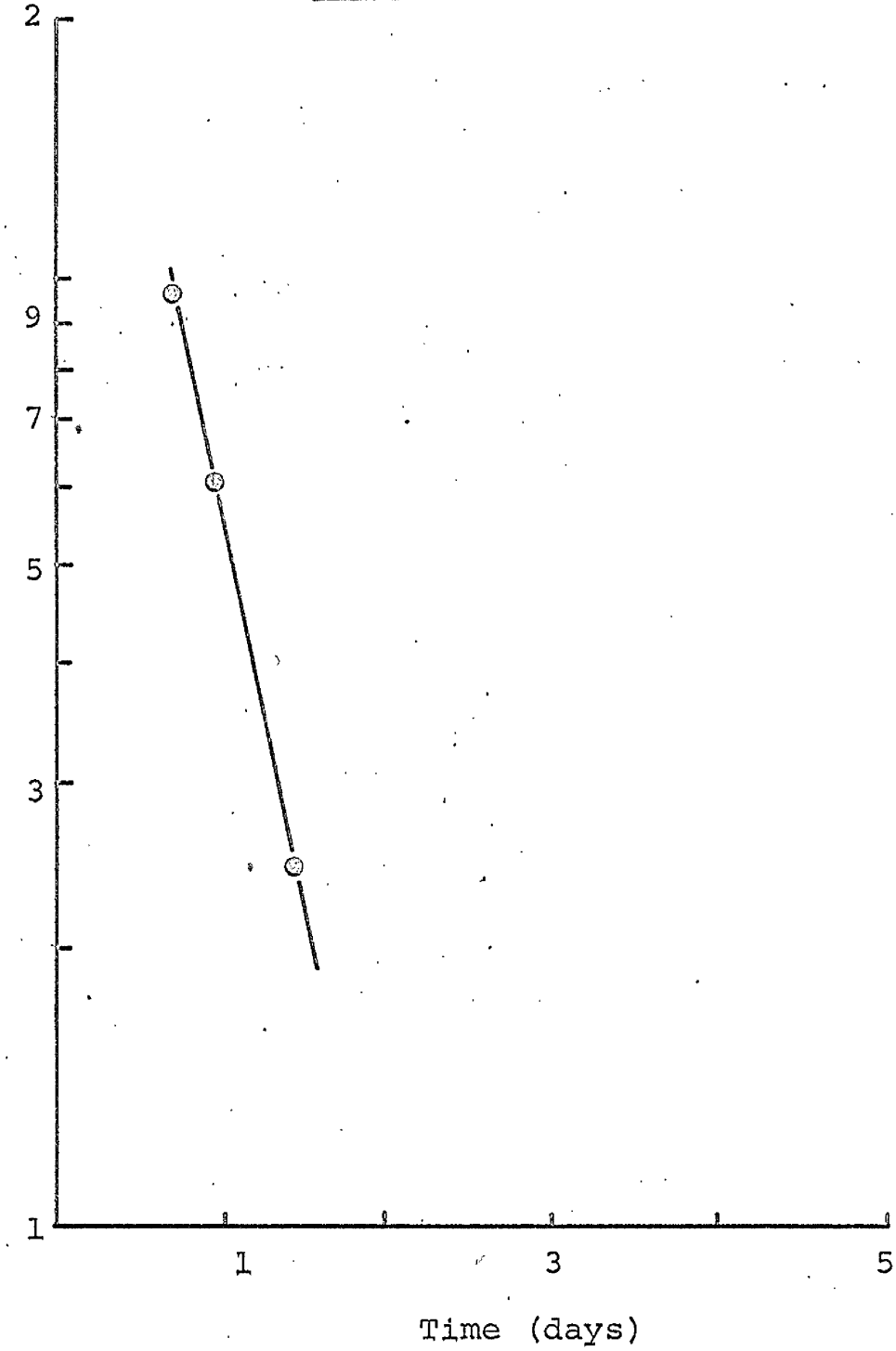


Fig. 2.2.39

Case 6

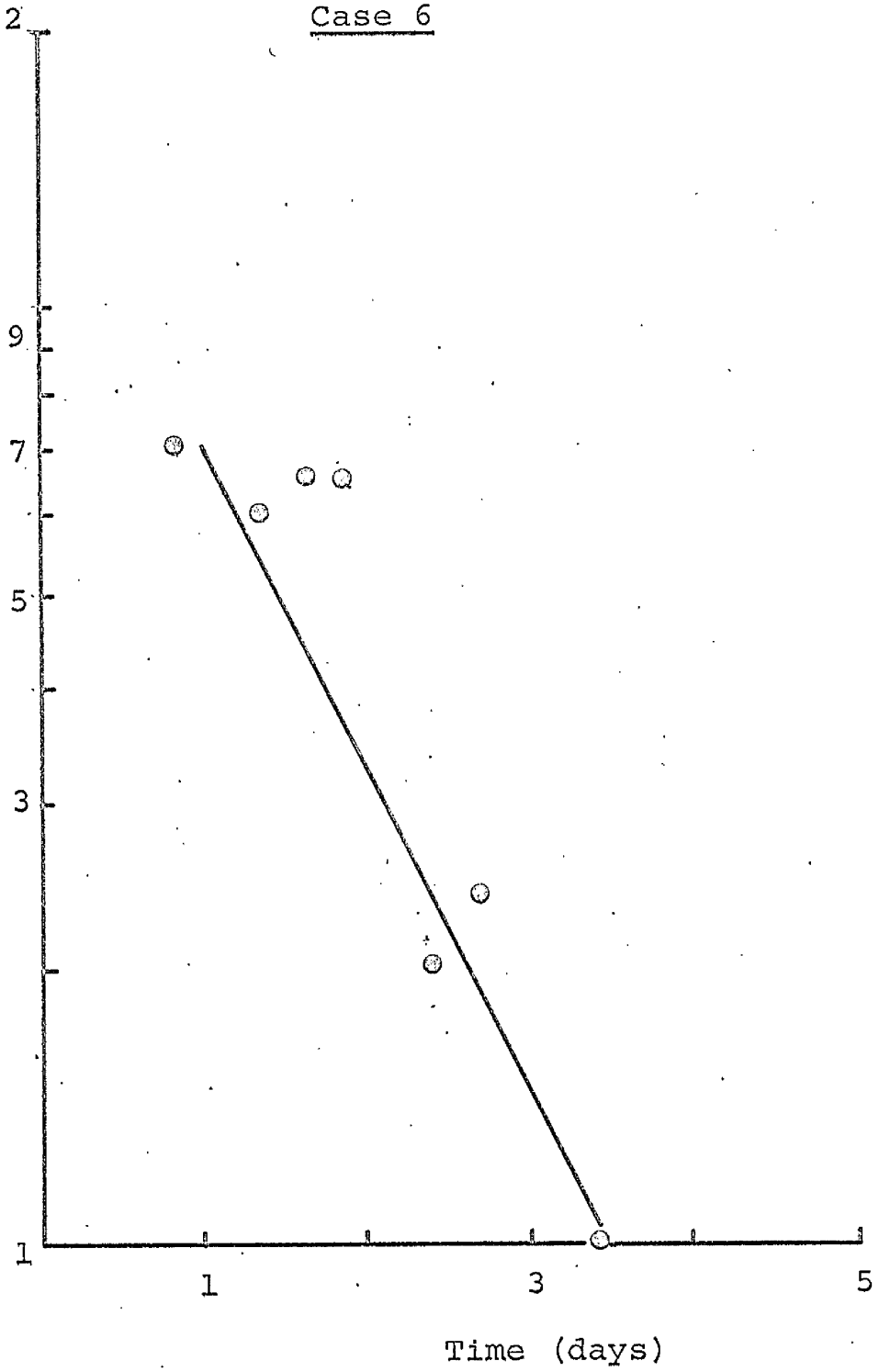


Fig. 2.2.40

Case 7

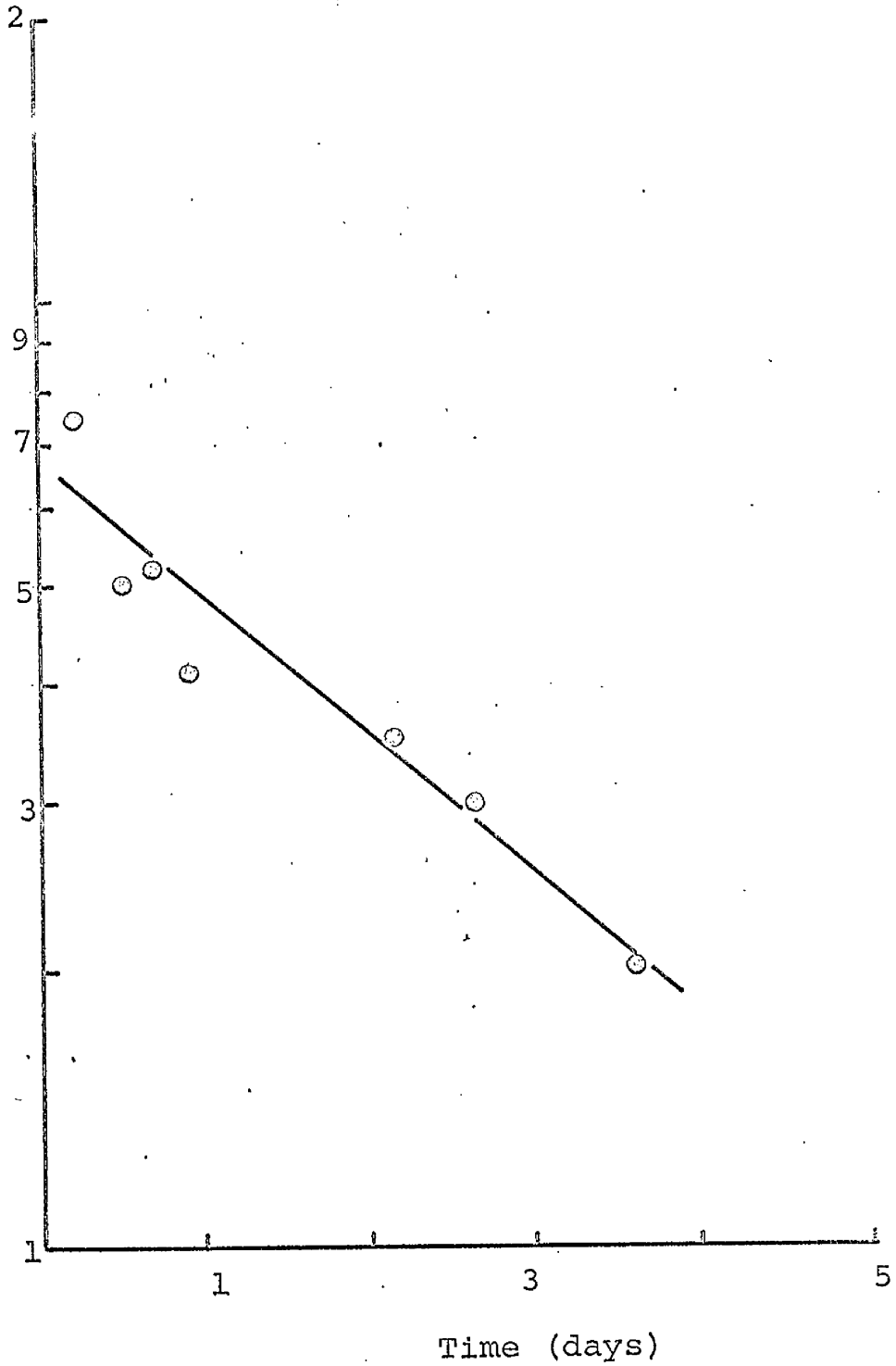


Fig. 2.2.41

Case 8

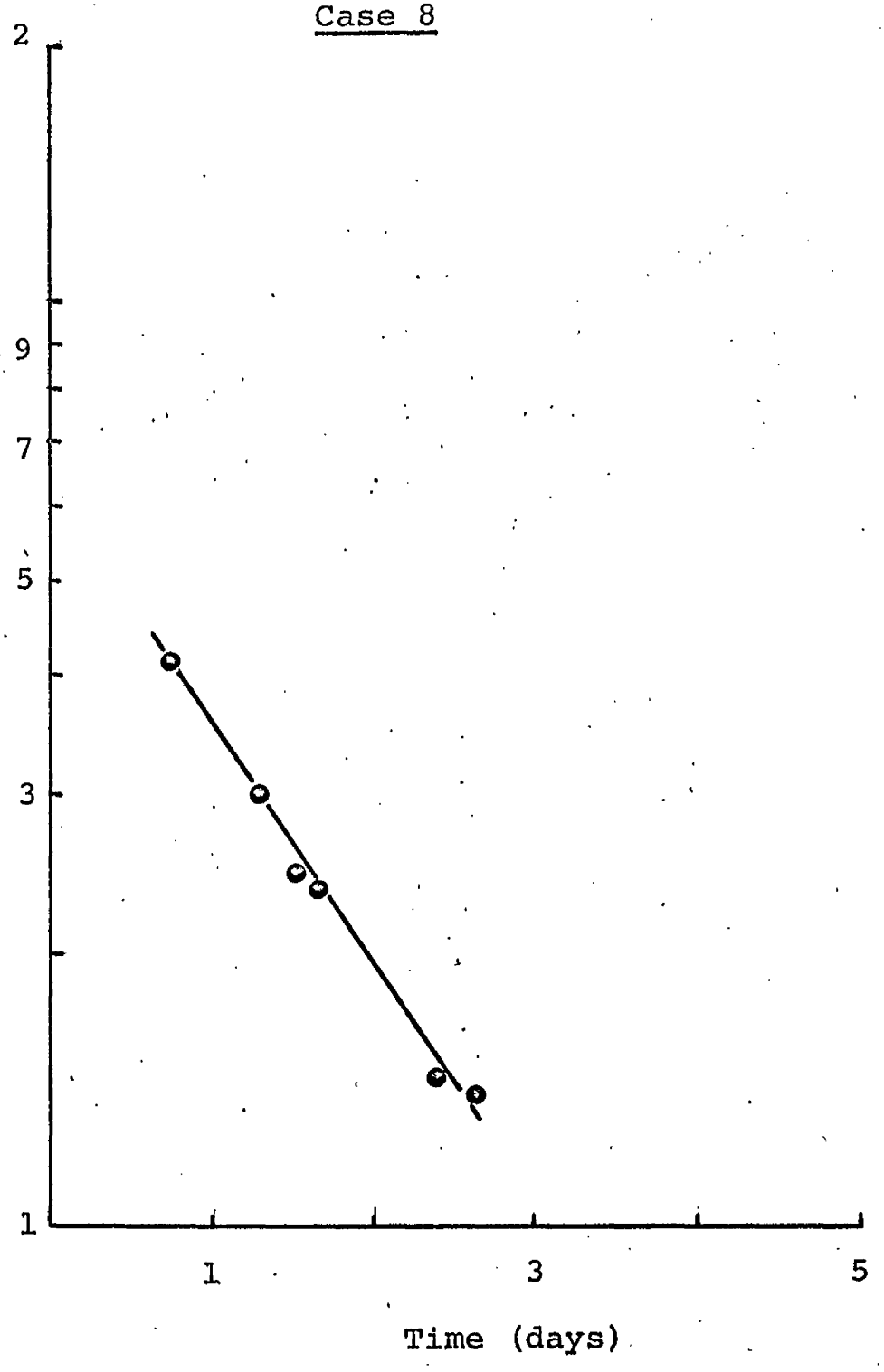


Fig. 2.2.42

Case 10

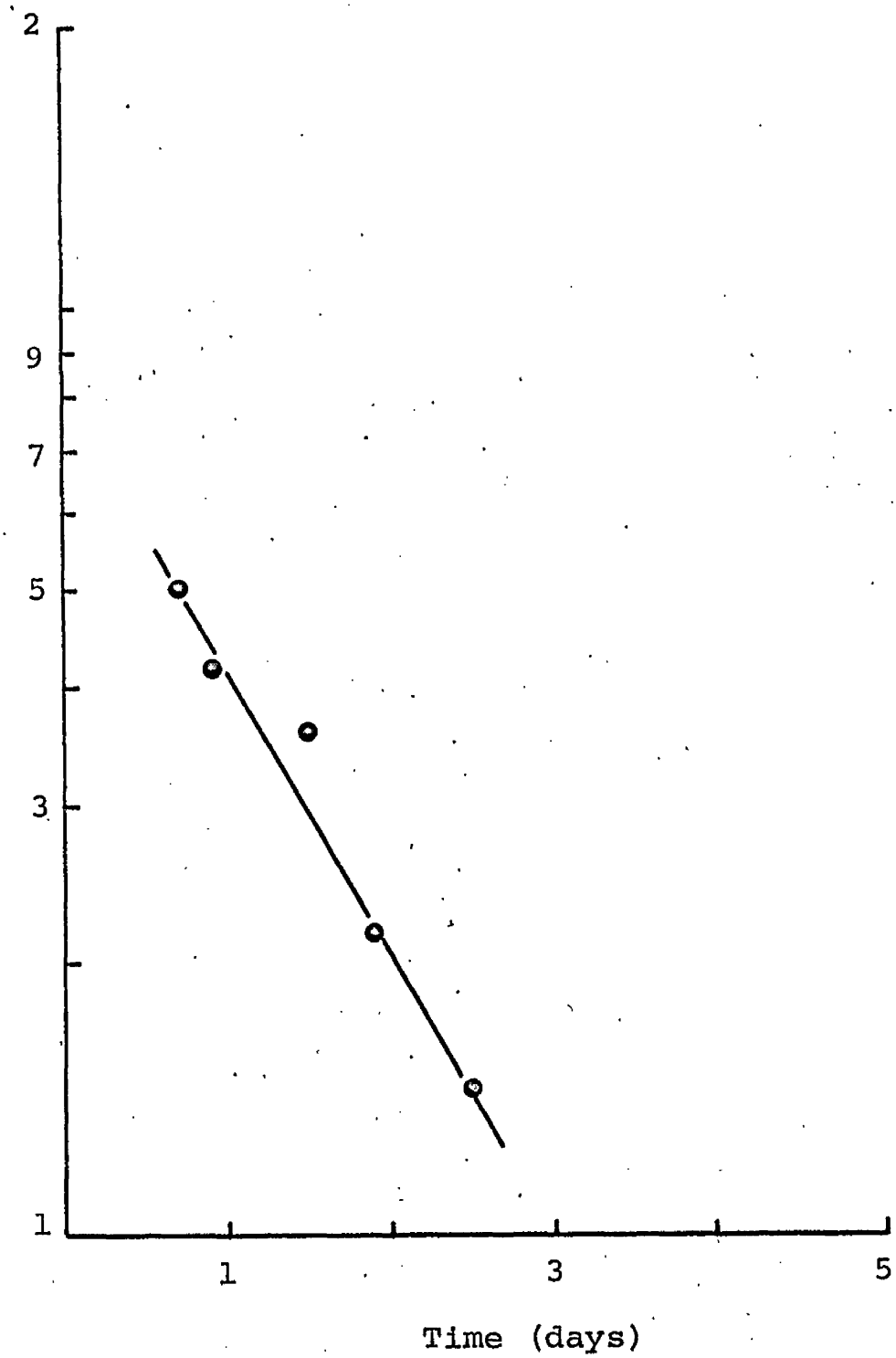


Fig. 2.2.43

Case 11

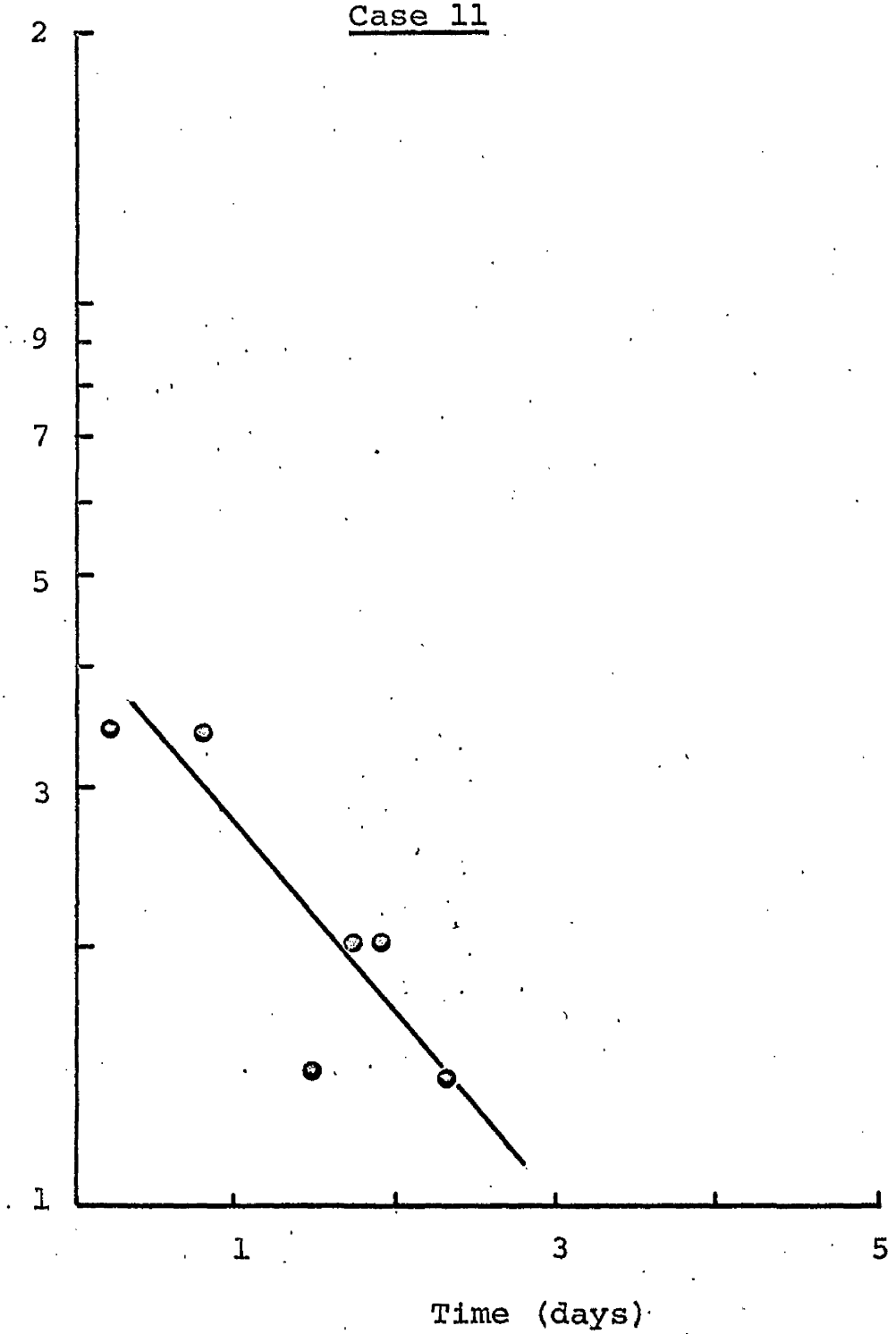


Fig. 2.2.44

Case 12

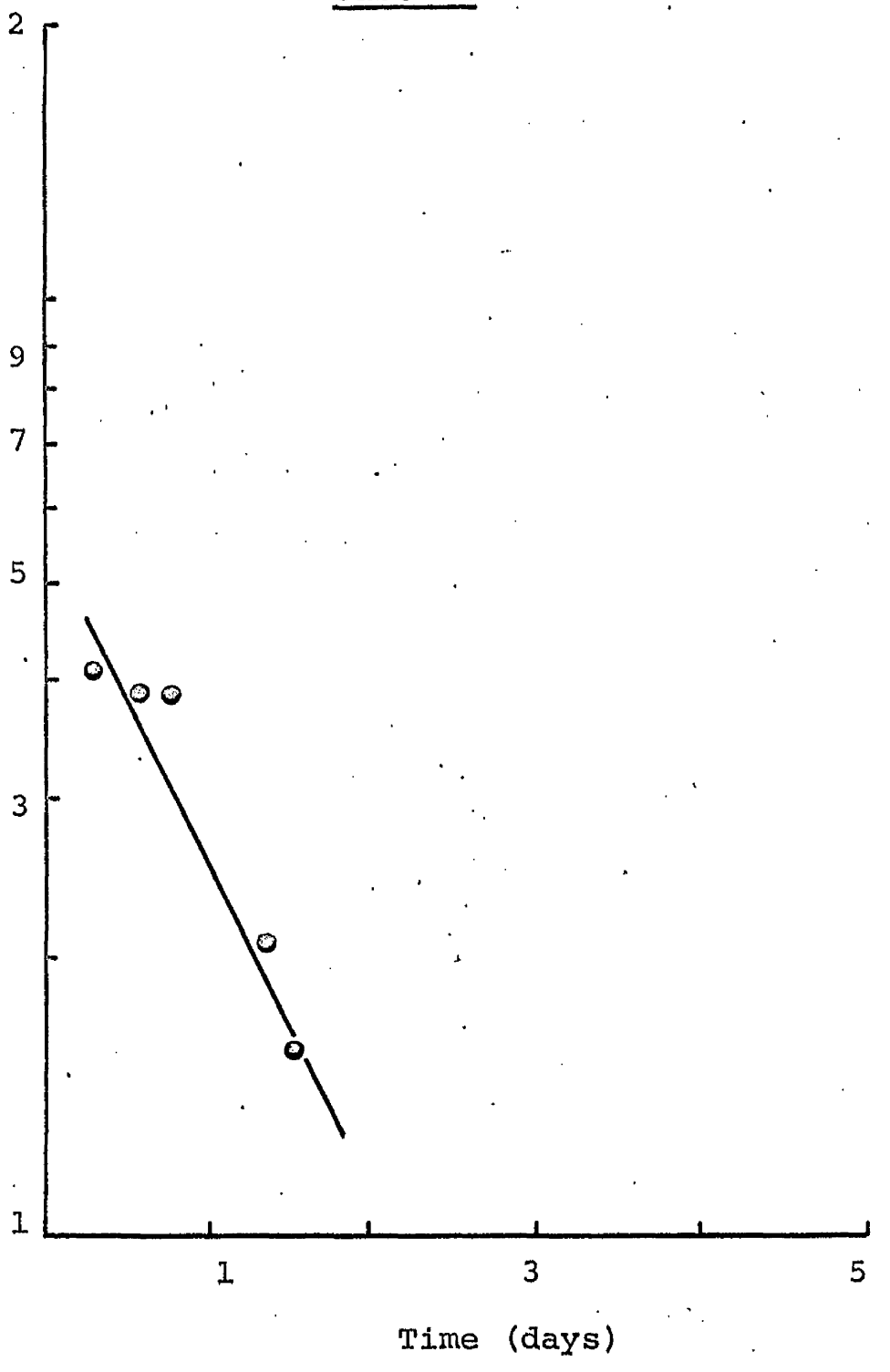


Fig. 2.2.45

Case 13

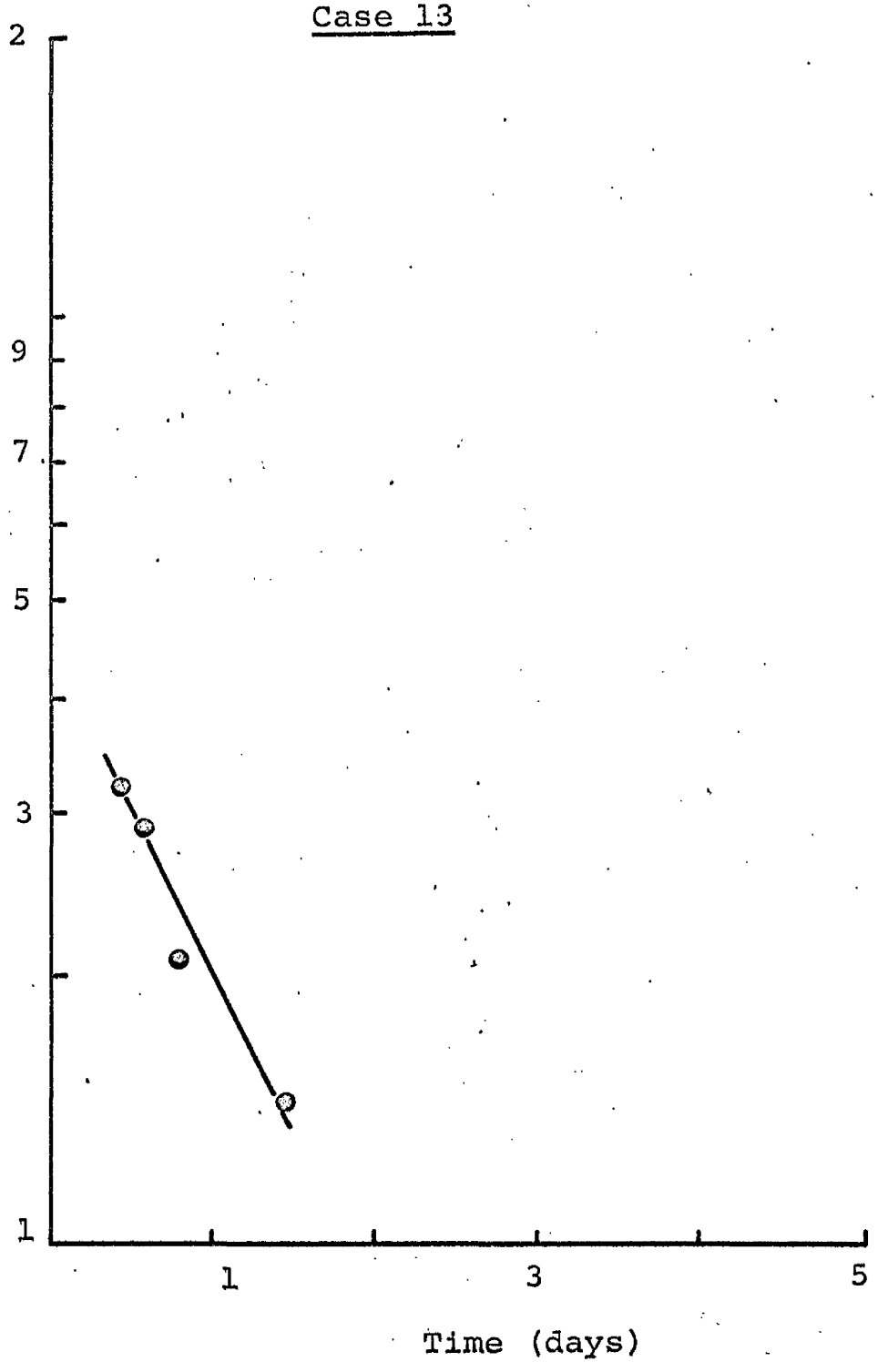


Fig. 2.2.46

Case 17

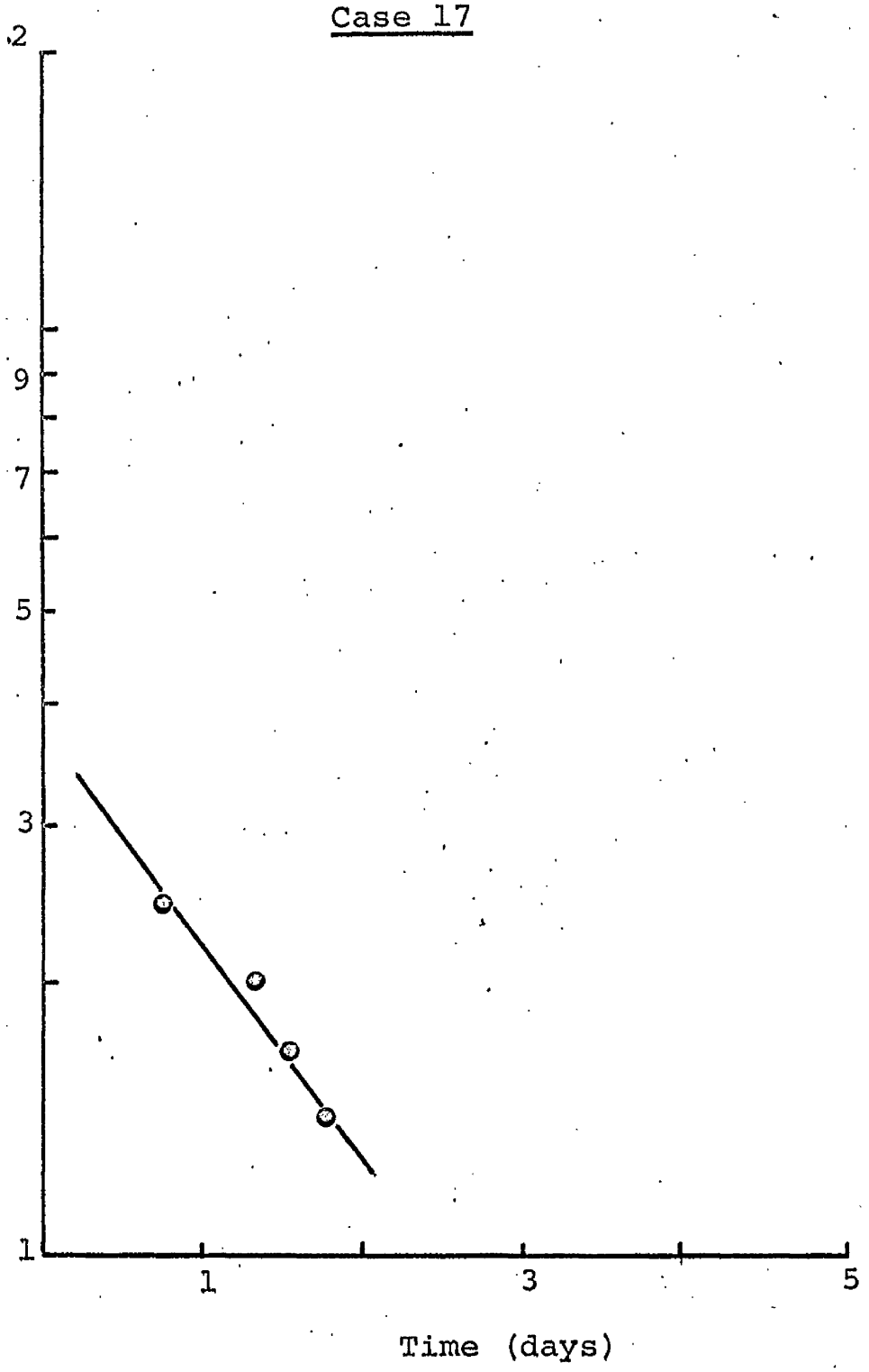


Fig. 2.2.47

Case 18

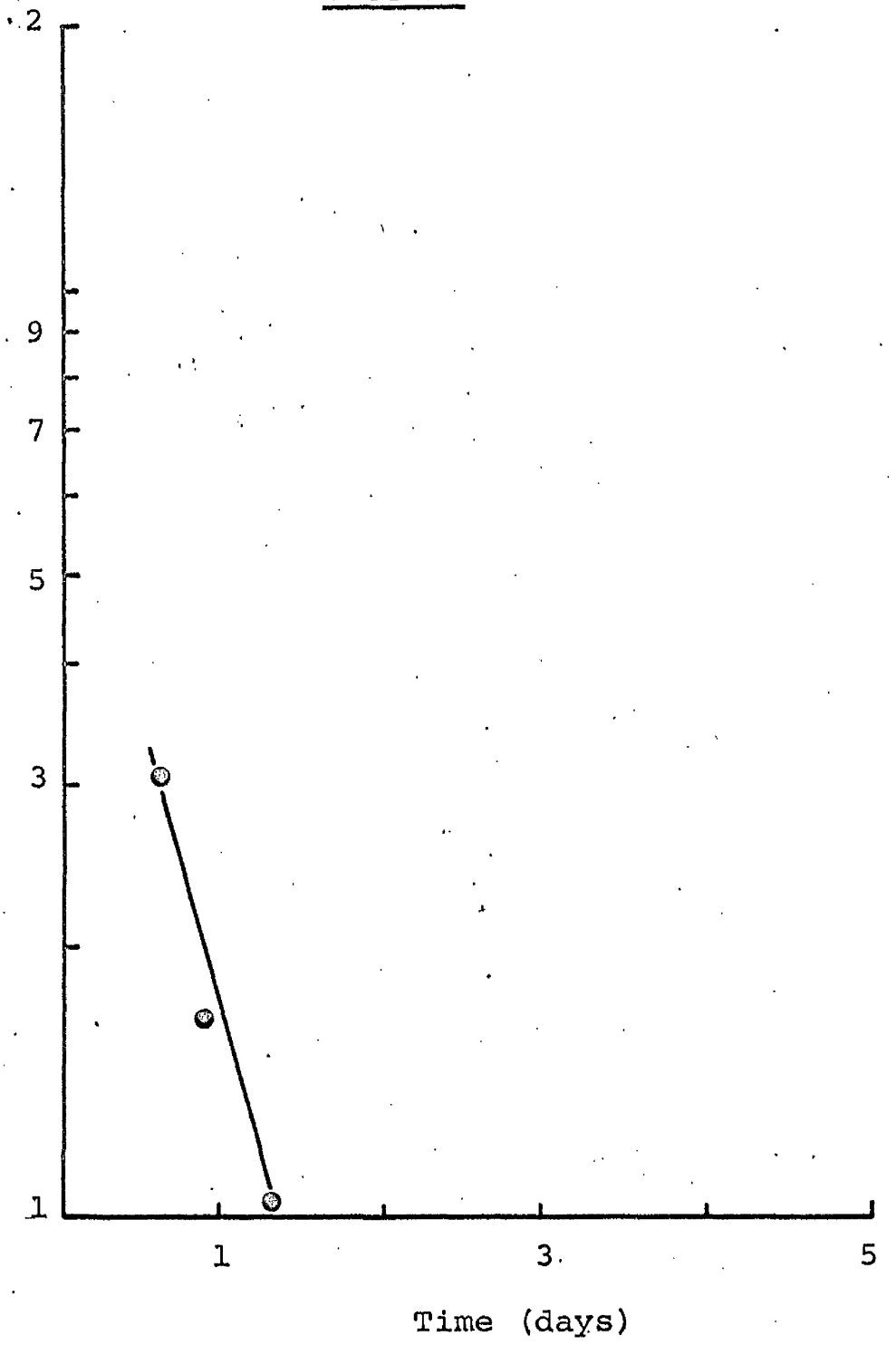


Fig. 2.2.48

Case 19

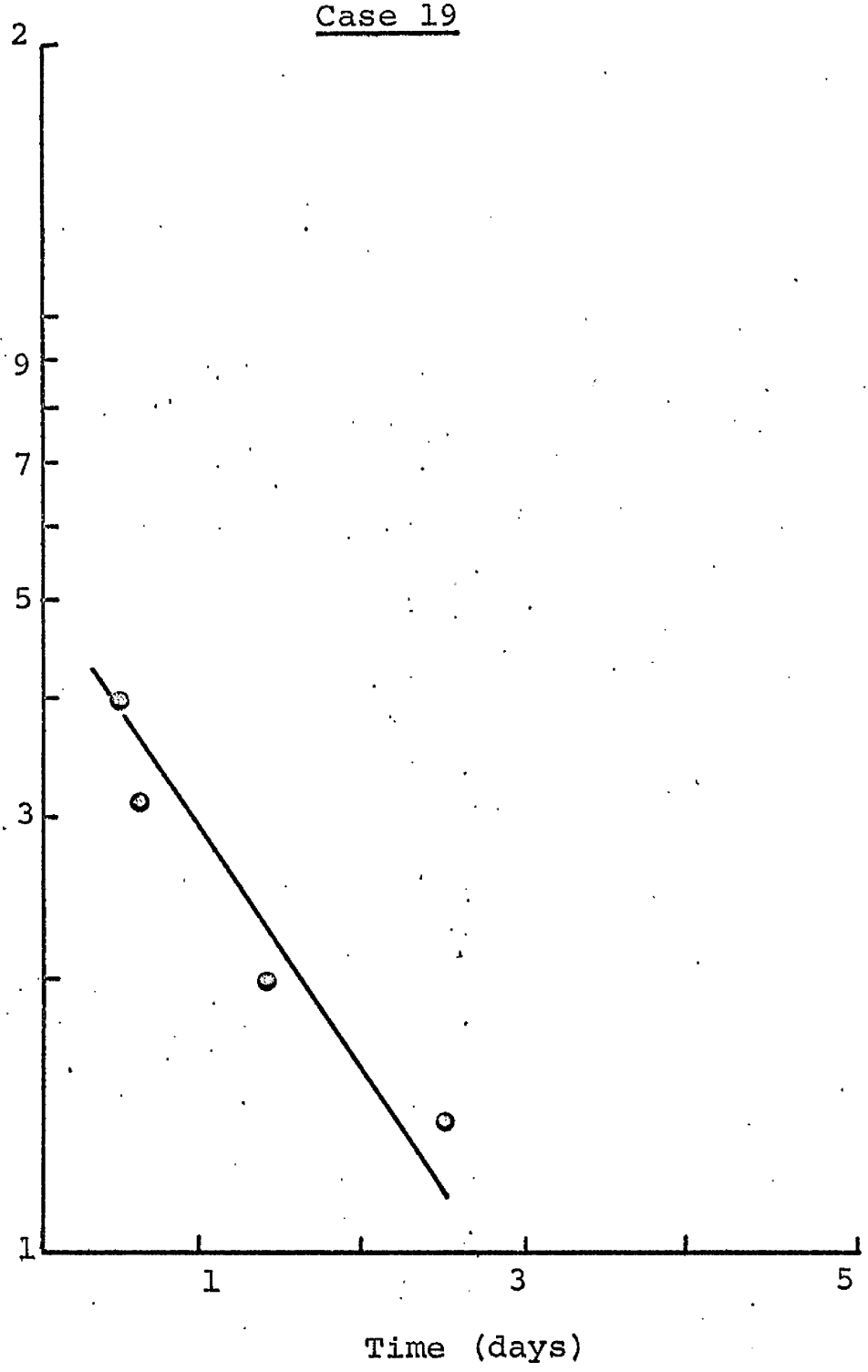


Fig. 2.2.49

Case 20

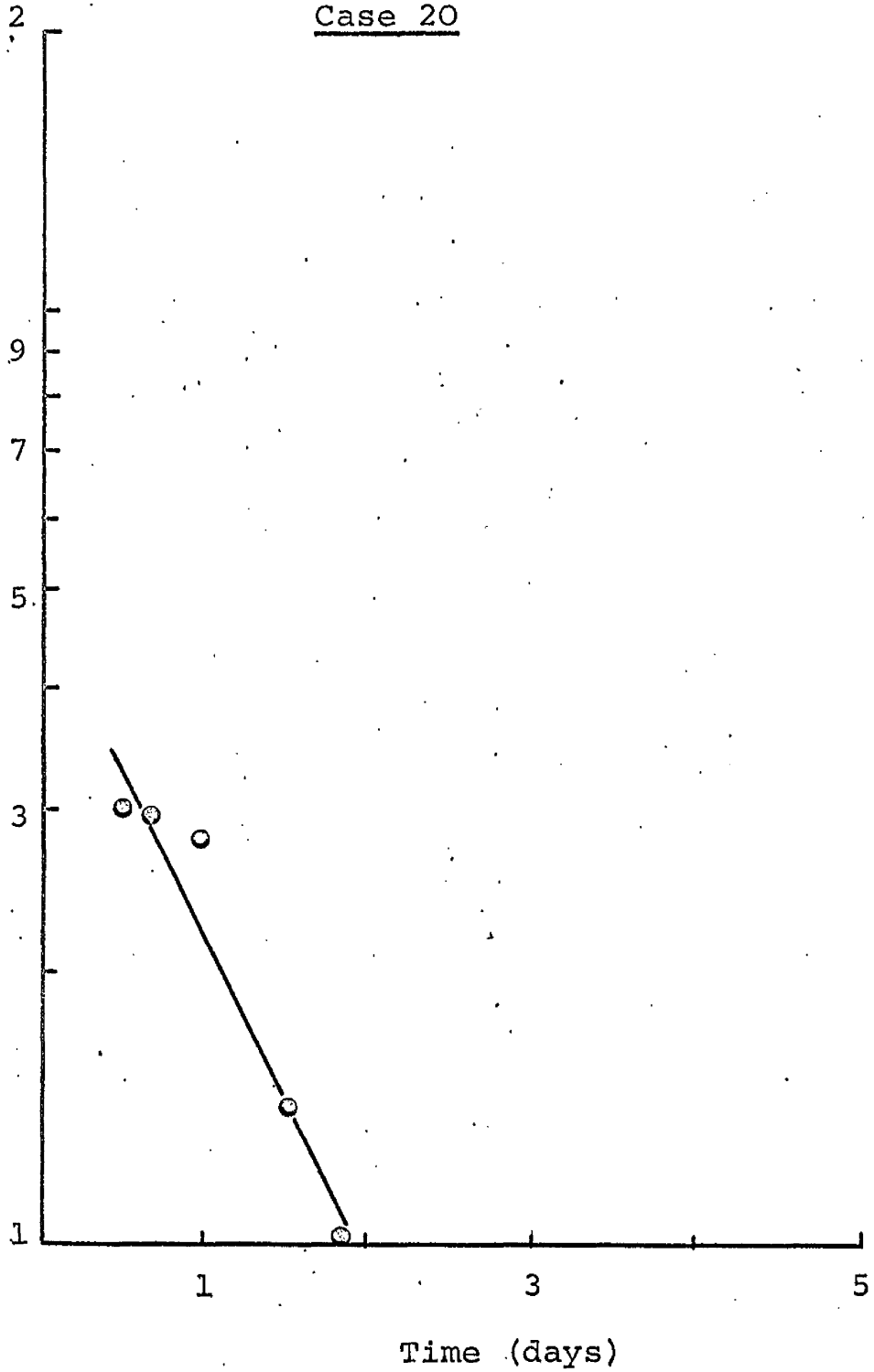


Table 2.2.50

Case	Half-lives of CPK in serum (hours)
1	33
2	18
3	12
4	18
5	9
6	21
7	48
8	25
10	24
11	33
12	18
13	22
17	30
18	12
19	30
20	20

2.3. Discussion

2.3.1. Patients with Suspected Myocardial Infarction

All of the twenty-eight patients in this group were suspected on clinical grounds to have suffered a myocardial infarction. Synopses of the case histories are given in Section 2.2.

Twenty five of these patients, (cases 1 - 21, 23 - 26), showed increased activity in serum of either CPK and/or LDH and to that extent the enzyme assays bear out the correctness of the diagnoses. In almost every case it was possible to identify in the history given to the attending physician (section 2.2.) a point at which the patient experienced chest pain of quite unprecedented severity, and this was taken as marking the occurrence of the infarction.

2.3.1.1. Patients with Increased Serum Activities of CPK and LDH

In twelve of the patients (cases 1 - 3, 5 - 13) a fairly complete pattern showing the rise and fall of the activities in serum of both of these enzymes was obtained. In almost all of these an increase in SCPK was unequivocally demonstrable within ten hours of the supposed infarct. The rise in SLDH was in most cases, somewhat less dramatic and was demonstrative within twenty hours of infarction. The SCPK activity usually reached a maximum within thirty hours and then declined rapidly

The peak of SLDH activity was usually reached not much later, and its decline was slower, than that of SCPK. These results are similar to those of Sørensen (1963); Hess et al., (1964); Duma and Siegel (1965); Smith (1967), for CPK and White (1956); Wacker et al., (1956); MacDonald, et al., (1957), for LDH.

2.3.1.2. Mathematical Analysis of Serum Enzyme Activities

It would be very helpful if it were possible, where reasonably complete data are available, to analyse mathematical the rise and fall in serum enzyme activities. It is not diffic to devise a simple mathematical model for release of the enzyme from the damaged muscle into the blood-stream and for its subsequent disappearance and, on this basis, to calculate how the serum enzyme activities will vary with time. Unfortunately this possibility is more attractive in theory than in practice.

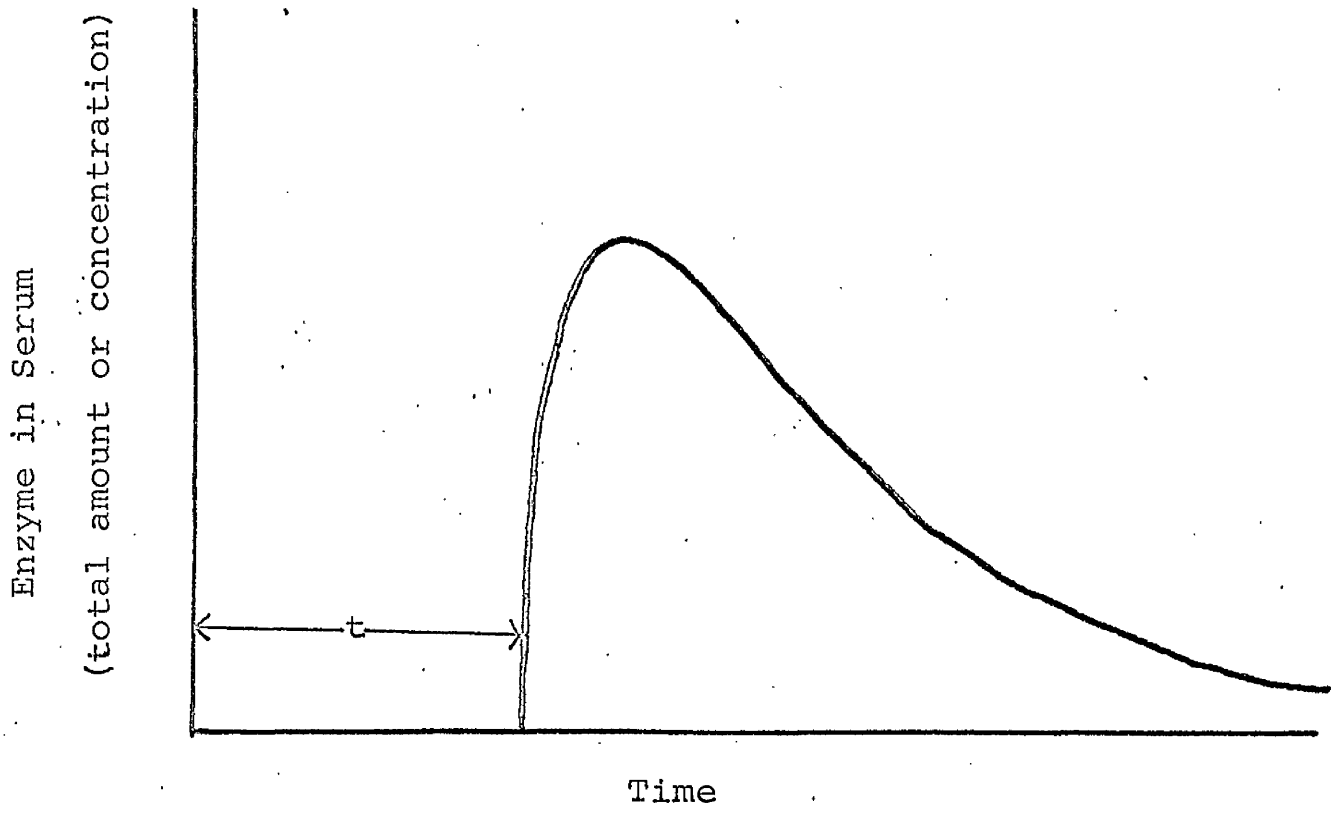
To take the simplest possible model one might assume that an infarction makes available to the circulation a finite quantity of enzyme x which will be released exponentially with a rate constant V_1 and that when it reaches the blood it will be removed by a second process, which we can assume will also be exponential, with a rate constant V_2. We know that after

infarction there is a time delay, which we will denote by t, before the release of enzyme from the damaged muscle begins A model of this type will give a relationship between the serum enzyme level and time of the form shown in Fig. 2.3.1. which is very roughly in agreement with the results obtained in practice. In theory, given the shape of the curve found experimentally, it should be possible to calculate the values of the unknowns x, V_1, V_2 and t. Unfortunately, however, the shape of the experimental curve was defined by no more than, on average, six to eight actual measurements, the number being limited by the fact that there was an obvious limit to the extent to which an acutely ill patient could be subjected to withdrawal of blood. This limited number of points was quite insufficient to establish the shape of the curve in such an unambiguous manner as to permit calculation of x, V_1, V_2 and t.

2.3.1.3. Half-lives of SCPK

It would seem, from the results of experiments on animals by other workers (Dunn, Martins and Reissmann, 1958; Wróblewski and LaDue, 1955), that the removal of enzyme from the circulation is an exponential process, and some of the data

Fig. 2.3.1.



If an enzyme is released from infarcted muscle into the serum an exponential process of rate constant V_1 and is removed from the serum by a second exponential process of rate constant V_2 it can be shown that the amount of enzyme present e in the serum at a given time a is given by the equation:

$$e = \frac{x\ V_1}{V_2 - V_1} \times (e^{-V_1 a} - e^{-V_2 a})$$

where x is the total amount of enzyme made available by the infarction. The curve above shows the sort of result which would be obtained if there is a delay (t) between the infarction and the start of the release of enzyme.

obtained for SCPK activities were accordingly replotted on a semilogarithmic grid (Figs. 2.2.34 - 2.2.49). The curves obtained tend to confirm that the fall in SCPK activity after myocardial infarction is indeed exponential and allow one to calculate the half-life of CPK in serum for at least some patients, Table 2.2.50.

It is of interest, that, according to the calculations of Buecher, Schmidt and Schmidt, the half-lives of enzymes in the serum after myocardial infarction are much longer than those obtained from animal experiments (Hess, 1963).

2.3.1.4. Patients with Increased Serum Activities of either CPK or LDH

In a second group of patients only one of the two enzymes showed increased activity in serum.

In cases 14, 16 and 17 SLDH was elevated, and it is to be noted that, in all three of these patients the increases began on the third day. The possible explanations of this are:

1. Due to a lapse in time between onset of chest pain and admission to hospital, the peak of SCPK activity was misse
2. The increased SLDH activity was not due to LDH of myocardial origin.

In cases 14 and 16 both patients complained of severe chest pa

two and three days, respectively, previous to admission, and therefore, on the basis of this and the ECG evidence the first of these explanations is the more likely. In case 17 however the situation was much more complex and we shall discuss this patient in more detail later.

In cases 15 and 21 only the SCPK showed a slight increase in activity, and it may be that both of these patients suffered very slight myocardial infarctions which were not sufficient to produce a perceptible change in SLDH.

2.3.1.5. Incomplete Enzyme Curves

In cases 18 - 20 only the descending portions of the enzy curves were evident. In cases 19 and 20 this was probably due to the fact that the times of onset of infarction in these patients, according to the clinical histories, were more than twelve hours before the first blood samples were taken off.

2.3.1.6. Cases in which ECG Evidence was not Indicative of Myocardial Infarction

In most of the cases studied the clinical history, ECG evidence and serum enzyme results were in accordance with a diagnosis of myocardial infarction. However in a number of ca this was not so, and these fell into two categories which were

1. Those patients in whom the ECG evidence was not indicative of myocardial infarction.
2. Those patients in whom the ECG evidence and serum enzyn results were not indicative of myocardial infarction.

Cases 4, 12 and 17 fall into the first of these two cate gories, and we shall discuss these in detail.

In case 4 the patient suffered what was believed to be an infarction at about 4.00 am on the morning of admission and about twelve hours later developed ventricular fibrillat subsequently arrested and was then DC countershocked and successfully resuscitated. The ECG evidence in this patient was typical of antero-lateral myocardial ischaemia. The ser enzyme results showed very high SCPK and SLDH activities. Since elevated activities of SCPK are found only in diseases myocardial and skeletal muscle, acute brain damage, and hypothyroidism (Graig and Ross 1963) this enzyme is therefor the most specific of those at present assayed for indicating myocardial necrosis (Sørensen 1963; Hess et al., 1964; Coodley, 1966). Although only the descending limb of the SCPK curve was evident in this particular case, the high values observed would seem to indicate myocardial damage.

The SLDH activity was also extremely high, but, since this enzyme is distributed over a large number of tissues, it shows increased serum activities in a considerable number of pathological states (Hill and Levi, 1954; Hsieh and Blumenthal 1956). On the basis of the elevated serum enzyme activities, particularly that of CPK, the diagnosis favoured in this case was one of myocardial infarction.

In case 12 the ECG pattern was one of myocardial ischaemia and this patient had had an infarction about eleven years previously. The patient complained of severe prolonged cardiac pain on both the second and third days and was also then in left ventricular failure (LVF). However this patient had definite increased activity of SCPK on the first and second days which eventually returned to normal on the fourth day. The SLDH activity reached a peak and started to descend within the first two days, but on the third day had risen again, and then slowly returned to normal over

a subsequent period of three days. The possible explanations of this serum enzyme pattern are:

1. This patient suffered two infarctions, one on the evening before admission and one on the second day.
2. This patient sustained an initial infarction and further to this developed congestive heart failure, (CHF), with accompanying hepatic congestion.

The two peaks of SLDH activity tend to support the first of these explanations. However the second increase in SLDH activity may be due to hepatic congestion, as a result of CHF. Only one peak of SCPK activity was observed and this returned to normal relatively slowly. This, and the fact that this enzyme is not present to any significant degree in hepatic tissue (Hughes, 1962; Sørensen, 1963), would support the second diagnosis.

In case 17 the ECG evidence was also typical of myocardial ischaemia and this patient had a myocardial infarction about one year previously. The enzyme pattern

in this case showed a small brief increase in SCPK with a long continuous elevation of SLDH. This patient improved clinically for two days post admission then, on the fourth day, he developed atrial and ventricular fibrillation, subsequently arrested and was then DC countershocked, twice, the second time being successful. The continued elevation o SLDH and not of SCPK in addition to the clinical evidence suggested a possible diagnosis of an initial myocardial infarction followed by the complication of CHF.

2.3.1.7. Negative Results

Cases 22, 27 and 28 gave negative serum enzyme results. In case 22 the ECG tracing was difficult to interpret and no definite conclusions could be drawn from it. In cases 27 an 28 the ECG showed left bundle branch block, (LBBB). Accordi to MacDonald et al., (1957), and Coodley (1970b), this syndr masks ECG evidence of myocardial infarction, therefore in these cases the serum enzyme results would appear to be a mo valuable diagnostic aid. It seems unlikely therefore that either of these patients had a myocardial infarction.

2.3.1.8. Estimation of Extent of Infarction

It has been suggested that the level of activity of SGOT is a valuable index of myocardial necrosis (Agress et al., 1955; Nydick, Wróblewski and LaDue, 1955). We

therefore studied the peak activities of SCPK and SLDH which we found in our patients in order to ascertain whether either of these was of use in estimating the extent of the infarction.

The SCPK activities varied greatly, in cases 1 - 6 they were extremely high compared to cases 7 - 13. If we take the height of SCPK to be an index of the degree of severity of the lesion, then in cases 1 - 6 the damage to the myocardium was great. More than 50% (cases 1 - 3, 7 - 10 the cases for which we had increased serum activities of both enzymes (cases 1 - 3, 5 - 13) had SCPK activities which were much greater than the corresponding SLDH activities. Coodley (1966) observed that the magnitude of elevation of SCPK was much greater than that of SLDH and he suggested that the heigl of the SCPK peak may be proportional to the extent of myocardial necrosis. Therefore it would appear that SCPK activity is a sensitive index of the degree of myocardial necrosis and this is also the view of Hess et al., (1964). Our results would tend to agree with this.

We did not find any evidence to suggest that the level of activity of SLDH was an index of myocardial damage.

2.3.1.9. Value of Serial Estimations of SCPK and SLDH in Prognosis

In cases 1 - 4 the activities of both enzymes were tending to return to normal within two to four days of onset of infarction, however in cases 5 and 6 this was not so. In case 5 the SCPK activity took four days to return to normal then started to rise again and the SLDH followed a similar course, and this patient developed a pulmonary infarction on the fifth day. In case 6 the SCPK returned to normal within four days and the SLDH remained elevated and this patient died on the sixth day. In case 10 the explanat of the secondary rise in SLDH was difficult since there was no evidence either in the clinical history or ECG to explain it, and this patient appeared to have an uneventful recovery In the other two cases, 12 and 17, the secondary and continued elevation of SLDH activity, respectively, was probably due to hepatic congestion as a result of CHF as previously discussed (section 2.3.1.6.). Therefore, with the exception of case 10, we may state that a continued elevation of SCPK activity and a secondary or continued elevation ofSLDH activity was indicative of a poor prognosis.

These results therefore agree with those of Sørensen (1963), who found a mortality of 40% in patients with increas

SCPK on the third day and hence concluded that if the SCPK was elevated for more than two days the prognosis was poor. The work of MacDonald et al., (1957), also agreed with our findings on LDH since they found that a delay in return to normal or a secondary rise in SLDH activity implied a poor prognosis.

2.3.1.10. Deaths

Of the twenty-eight patients suspected to have had a myocardial infarction five died (case 6, 23 - 26). Case 6 has been discussed in section 2.3.1.9. The cause of death in case 23 was probably a second infarction which gave rise to the rapid increase of SLDH activity observed. Those patients, cases 24 and 25, who died within twenty four hours of admission showed extremely high activities of SCPK and SLDH. Case 26 also died very shortly after admission and therefore only one sample was obtained.

In these cases there was the possibility that the increased serum enzyme activities observed may have been reflective of the general critical condition of the patient and not of damage to one particular organ.

2.3.1.11. Effects of DC Countershock on SCPK and SLDH Activities

Three patients (cases 1, 4 and 17), were treated with DC countershock. After this treatment SCPK and SLDH activities rose in case 1, but in case 4 only SLDH activity increased, and in case 17 neither enzyme was increased.

We therefore investigated the effects of DC countershock on these two enzymes in a small group of patients with cardiac disease other than myocardial infarction (cases 29 - 33). We found that in no case did shock treatment result in increased serum activities of either enzyme. Therefore, it appeared unlikely that the elevated SCPK and/or SLDH activities observed in cases 1 and 4 were caused by countershocking. However, it must be remembered that there was the possibility that the increased SCPK activity observed in case 1 may have been due to brain damage as a result of cardiac arrest (Fig. 2.1.2.).

CHAPTER 3 - EFFECTS OF OXYGEN THERAPY IN MYOCARDIAL INFARCTION

CHAPTER 3 - THE EFFECTS OF OXYGEN THERAPY IN MYOCARDIAL INFARCTION

3.1. Introduction

3.1.1. Haemodynamic Changes after Myocardial Infarction

It would seem reasonable to assume that any myocardial infarction, unless it was very small, would impair the mechanical function of the heart and hence reduce cardiac output. The extent of the impairment would presumably be roughly proportional to the size of the infarction.

The haemodynamic changes in patients with acute myocardial infarction have been investigated by a number of authors (Pritchard and Hellerstein, 1950; Gilbert, Goldber and Griffin, 1954; Lee, 1957; Broch, Humerfelt, Haarstad and Myhre, 1959; Murphy, Glick, Schreiner and Yu, 1963). In general the results obtained by these workers show that in most of the seriously ill patients investigated there was a fall in cardiac output accompanied by peripheral vasoconstriction, whereas many of the moderately ill patients had low cardiac outputs but normal peripheral resistance. Malmcrona and Varnauskas (1964), reported that patients who had a myocardial infarction and a high temperature had higher cardiac outputs than similar patients with lower temperatures and attributed this to a fall in peripheral

resistance caused by the elevated temperature. Thomas, Malmcrona and Shillingford (1965a), found a wide variation in the haemodynamics of patients after myocardial infarction, some having high cardiac outputs and low peripheral resistance and vice versa.

3.1.2. Biochemical Changes after Myocardial Infarction

If the output of the heart is diminished and the circulation depressed after myocardial infarction it might be anticipated that metabolic changes would ensue. The cyanosis sometimes observed in patients with myocardial infarction might be an example of such metabolic upset.

One of the important findings made by previous investiga (MacKenzie et al., 1964; McNicol et al., 1965; Valentine et al., 1966), who used small groups of patients, has been a reduction in the arterial blood oxygen tension. In addition they observed that when a myocardial infarction was associate with LVF and cardiogenic shock, the criteria for the latter being a low blood pressure, sweating and cyanosis, the hypoxaemia was even more marked, in the presence of considera metabolic acidosis and lacticacidaemia. Increased levels of lactate in the circulation are generally accepted as evidence of anaerobic metabolism (Best and Taylor, 1961).

3.1.3. Oxygen Therapy

The practice of administering oxygen to patients with myocardial infarction has long been established. One of the first reports on the effects of oxygen therapy in these patients was made by Barach who, in 1931, had observed that the pain associated with this condition could be alleviated by inhalation of an oxygen enriched atmosphere. However, subsequent to this Boland (1940), stated that only high concentrations of oxygen were effective in relieving the pain of these patients. It has been suggested that oxygen therapy may also increase the tissue-oxygen tension at the margin of the area of infarction (Sayen, 1951).

Thus, administration of oxygen has been recommended in the management of patients with myocardial infarction (Dunlo and Alstead, 1966; Friedberg, 1966). However the general effects of oxygen therapy were not studied at all until recently and then only in a limited number of cases (MacKenzie et al., 1964; Thomas, Malmcrona and Shillingford, 1965 b; Cameron, Hutton, Kenmure and Murdoch, 1966).

The present investigation was undertaken in order to establish, in a large series of patients, what haemodynamic and biochemical changes followed myocardial infarction and t what extent these were modified or reversed by administratio of high concentrations of oxygen.

3.2. Results

Fifty men with acute myocardial infarction were studied. Their ages ranged from 34 to 77, with a mean of 57.6 years. The diagnosis was based on a history of cardiac pain lasting more than 30 minutes, of acute left ventricular failure, or of unconsciousness, and was confirmed by ECGs which showed pathological Q waves and sequential ST-T wave changes. In all cases investigations were carried out within 24 hours of infarction.

Table 3.2.1.

Haemodynamic Findings in 50 Patients with Myocardial Infarction, Breathing Air

Case No.	Heart Rate (per min.)	Arterial B P (mmHg)	Mean Arterial B P (mm Hg)	Cardiac Output (l/min)	Systemic Vascular Resistance (dynes sec cm^{-5})	Stroke Volume (ml)
1	125	135/90	105	4,83	1,739	39
2	68	127/65	86	8.70	791	128
3	58	105/45	68	2.00	2,720	35
4	54	124/60	80	5.76	1,111	107
5	72	80/52	61	3.50	1,394	49
6	68	112/90	97	6.24	1,243	92
7	56	75/40	52	3.70	1,124	66
8	100	137/65	85	7.01	970	70
9	66	150/90	110	6.21	1,417	94
10	72	157/98	115	7.50	1,227	104
11	80	140/92	100	6.15	1,301	77
12	66	137/83	94	3.80	1,980	58
13	124	152/85	95	4.45	1,708	36
14	100	75/47	57	5.20	877	52
15	70	114/55	80	4.44	1,441	63
16	120	125/75	80	7.83	817	65
17	120	112/55	70	9.55	586	80
18	80	65/30	50	4.93	811	62
19	84	117/70	87	5.68	1,225	68

Table 3.2.1. (Continued)

Case No.	Heart Rate (per min)	Arterial B P (mmHg)	Mean Arterial B P (mmHg)	Cardiac Output (l/min)	Systemic Vascular Resistance (dynes sec cm^{-5})	Str Vol (m
20	72	110/45	62	7.19	690	100
21	60	102/55	70	5.59	1,002	93
22	68	87/45	60	3.84	1,250	57
23	64	140/82	102	3.66	2,229	57
24	74	87/57	67	4.28	1,252	58
25	64	180/80	115	5.58	1,649	87
26	100	130/100	110	6.11	1,440	61
27	57	100/52	68	3.47	1,568	61
28	56	108/74	88	4.53	1,554	81
29	83	96/58	68	4.41	1,234	53
30	83	152/74	100	5.40	1,481	65
31	61	136/54	78	3.41	1,830	56
32	75	240/137	180	3.61	3,989	48
33	83	100/64	80	5.80	1,104	70
34	115	106/74	88	2.82	2,496	25
35	102	60/40	44	1.32	2,667	13
36	107	150/96	114	5.17	1,764	48
37	82	118/84	96	3.46	2,220	42
38	88	160/104	116	6.16	1,506	70
39	96	125/53	68	4.66	1,167	48
40	48	116/56	77	3.30	1,867	69
41	90	100/48	62	5.79	857	64

Table 3.2.1. (Continued)

Case No.	Heart Rate (per min)	Arterial B P (mmHg)	Mean Arterial B P (mmHg)	Cardiac Output (l/min)	Systemic Vascular Resistance (dynes sec cm^{-5})	Stro Volu (ml
42	71	136/68	90	5.60	1,286	79
43	98	142/76	88	5.71	1,233	58
44	81	116/70	81	5.08	1,276	63
45	81	140/91	110	5.63	1,563	69
46	73	94/56	70	4.58	1,223	63
47	100	138/44	66	7.53	701	75
48	83	90/56	66	6.11	864	74
49	94	90/60	70	5.86	956	62
50	71	100/64	76	7.43	818	105
Mean Values	81	120/68	84	5.21	1,424	66

Table 3.2.2.

Arterial Blood Oxygen and Carbon Dioxide Tensions, pH, and Lactate and Pyruvate Concentrations in 50 Patients with Myocardial Infarctio Breathing Air.

Case No.	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	Lactate (mM)	Pyruvate (mM)
1	69	37	7.42	-	-
2	85	36	7.45	-	-
3	86	39	7.46	-	-
4	98	17	7.58	2.55	-
5	97	38	7.33	-	-
6	97	-	-	-	-
7	88	40	7.30	-	-
8	53	38	7.43	-	-
9	90	44	7.40	-	-
10	67	39	7.46	-	-
11	50	34	7.44	1.36	-
12	100	39	7.40	-	-
13	43	29	7.47	1.66	0.05
14	24	37	7.25	5.70	0.17
15	61	37	7.45	1.97	0.05
16	63	41	7.45	2.53	0.04
17	84	32	7.44	3.36	0.03
18	70	37	7.40	2.02	0.04
19	59	35	7.52	0.91	0.08
20	61	34	7.48	-	-

Table 3.2.2. (Continued)

Case No.	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	Lactate (mM)	Pyruvate (mM)
21	52	34	7.50	2.50	0.05
22	60	37	7.50	0.87	0.05
23	81	31	7.53	2.58	0.06
24	82	33	7.46	1.32	0.04
25	54	39	7.45	1.28	0.04
26	51	44	7.40	1.63	0.04
27	65	46	7.47	1.93	0.03
28	70	42	7.44	1.51	0.02
29	51	37	7.38	1.22	0.06
30	56	45	-	4.72	0.02
31	68	-	-	1.26	0.01
32	58	-	-	4.49	0.01
33	56	37	7.45	2.74	0.02
34	73	35	7.32	6.65	0.03
35	54	32	7.35	7.06	-
36	73	36	7.45	-	-
37	59	39	7.48	1.48	0.06
38	61	41	7.43	1.16	0.04
39	34	40	7.39	2.81	0.09
40	59	47	7.41	0.50	0.02
41	79	41	7.42	1.31	0.03
42	73	34	7.43	-	-

Table 3.2.2. (Continued)

Case No.	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	Lactate (mM)	Pyruvate (mM)
43	63	35	7.42	2.48	0.04
44	51	35	7.55	2.01	0.02
45	72	39	7.48	4.06	0.02
46	56	40	7.45	0.73	0.04
47	73	39	7.37	3.13	0.02
48	51	32	7.50	3.37	0.08
49	28	35	7.44	3.78	-
50	62	35	7.51	2.60	-
Mean Values	65	37	7.44	2.52	0.04

Table 3.2.3.

Haemodynamic Findings in 50 Patients with Myocardial Infarction, Breathing approx. 100% Oxygen

Case No.	Heart Rate (per min)	Arterial B P (mmHg)	Mean Arterial B P (mmHg)	Cardiac Output (l/min)	Systemic Vascular Resistance (dynes sec cm^{-5})	S V (
1	117	137/92	107	4.56	1,877	
2	66	136/70	92	6.51	1,131	
3	55	125/60	82	1.73	3,791	
4	58	154/64	98	4.66	1,682	
5	71	77/55	62	3.48	1,425	
6	70	120/77	90	4.97	1,448	
7	58	77/40	60	3.85	1,247	
8	96	155/80	107	5.14	1,665	
9	66	158/120	133	5.45	1,953	
10	76	180/112	142	5.70	1,993	
11	76	140/82	100	5.96	1,343	
12	70	138/90	105	3.62	2,320	
13	112	150/90	100	4.16	1,923	
14	108	107/65	77	4.66	1,322	
15	68	145/70	88	4.14	1,700	
16	100	110/67	82	6.66	985	
17	110	117/60	77	7.78	792	
18	64	87/42	54	3.44	1,256	
19	84	125/70	90	4.92	1,463	
20	74	120/60	75	6.65	902	

Table 3.2.3 (Continued)

Case No.	Heart Rate (per min)	Arterial B P (mmHg)	Mean Arterial B P (mmHg)	Cardiac Output (l/min)	Systemic Vascular Resistance (dynes sec cm^{-5})	Stro Volu (m
21	56	102/52	70	5.42	1,033	9
22	58	97/55	70	4.15	1,349	7
23	66	125/75	95	3.56	2,135	5
24	82	113/75	88	4.40	1,600	5
25	76	180/90	125	5.88	1,701	7
26	100	140/110	124	4.53	2,190	4
27	55	132/66	88	3.77	1,867	6
28	54	122/72	94	4.10	1,834	7
29	83	94/60	72	3.41	1,689	4
30	83	152/68	96	5.16	1,488	6
31	42	76/28	44	2.03	1,734	4
32	65	230/125	160	5.39	2,375	8
33	85	132/72	90	5.01	1,437	5
34	116	102/66	80	3.11	2,058	2
35	114	52/40	40	2.56	1,250	2
36	125	154/100	118	4.96	1,903	4
37	70	134/74	94	3.67	2,049	5
38	88	162/112	126	5.57	1,810	6
39	105	146/71	97	3.79	2,047	3
40	51	116/58	82	3.63	1,807	7
41	99	102/50	72	5.25	1,097	5
42	74	132/64	88	5.86	1,201	7

Table 3.2.3. (Continued)

Case No.	Heart Rate (per min)	Arterial B P (mmHg)	Mean Arterial B P (mmHg)	Cardiac Output (l/min)	Systemic Vascular Resistance (dynes sec cm^{-5})	St Vo (
43	89	144/74	100	4.73	1,691	5
44	96	126/70	96	4.64	1,655	4
45	92	145/95	110	5.72	1,538	6
46	73	106/62	72	4.28	1,346	5
47	99	150/70	100	6.41	1,248	6
48	93	112/62	78	5.63	1,108	6
49	108	92/64	73	6.27	931	5
50	69	136/70	92	6.41	1,148	9
Mean Values	81	127/72	91	4.75	1,610	6

Table 3.2.4.

Arterial Blood Oxygen and Carbon Dioxide Tension, pH and Lactate and Pyruvate Concentrations in 50 Patients with Myocardial Infarction, Breathing approx. 100% Oxygen.

Case No.	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	Lactate (mM)	Pyruvate (mM)
1	575	26	7.55	-	-
2	430	34	7.47	-	-
3	491	36	7.41	-	-
4	486	30	7.58	1.54	-
5	295	32	7.42	-	-
6	538	-	-	-	-
7	522	44	7.30	-	-
8	261	42	7.40	-	-
9	435	40	7.53	-	-
10	281	39	7.37	-	-
11	260	35	7.44	1.06	-
12	342	39	7.38	-	-
13	390	34	7.47	1.97	0.04
14	141	36	7.37	4.54	0.16
15	367	33	7.44	1.66	0.08
16	470	38	7.46	1.18	0.04
17	423	41	7.47	2.58	0.03
18	406	38	7.44	2.07	0.03
19	406	39	7.51	1.33	0.04
20	496	36	7.45	-	-

Table 3.2.4. (Continued)

Case No.	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	Lactate (mM)	Pyruvate (mM)
21	481	31	7.55	1.56	0.04
22	565	37	7.49	1.18	0.05
23	542	31	7.49	1.95	0.06
24	603	36	7.45	1.33	0.03
25	366	42	7.43	1.33	0.05
26	486	41	7.38	1.35	0.04
27	625	46	7.48	0.68	0.02
28	610	50	7.47	0.77	0.02
29	459	39	7.44	0.99	0.05
30	496	48	7.32	3.40	0.03
31	378	-	-	1.23	0.02
32	475	-	-	3.36	0.01
33	423	43	7.42	1.43	0.03
34	649	34	7.33	2.33	0.04
35	181	35	7.32	6.43	-
36	452	37	7.45	-	-
37	440	44	7.42	1.07	0.02
38	474	50	7.37	0.63	0.02
39	237	45	7.35	2.13	0.05
40	542	39	7.50	0.49	0.04
41	571	43	7.43	1.46	0.04
42	311	30	7.48	-	-

Table 3.2.4 (Continued)

Case No.	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	Lactate (mM)	Pyruvate (mM)
43	396	38	7.44	1.20	0.02
44	510	36	7.57	1.04	0.02
45	555	27	7.52	0.96	0.01
46	435	41	7.45	0.55	0.03
47	237	42	7.38	2.29	0.03
48	441	37	7.43	2.46	0.06
49	395	36	7.44	3.38	-
50	305	37	7.48	3.33	-
Mean Values	433	38	7.44	1.82	0.04

Fig. 3.2.5.

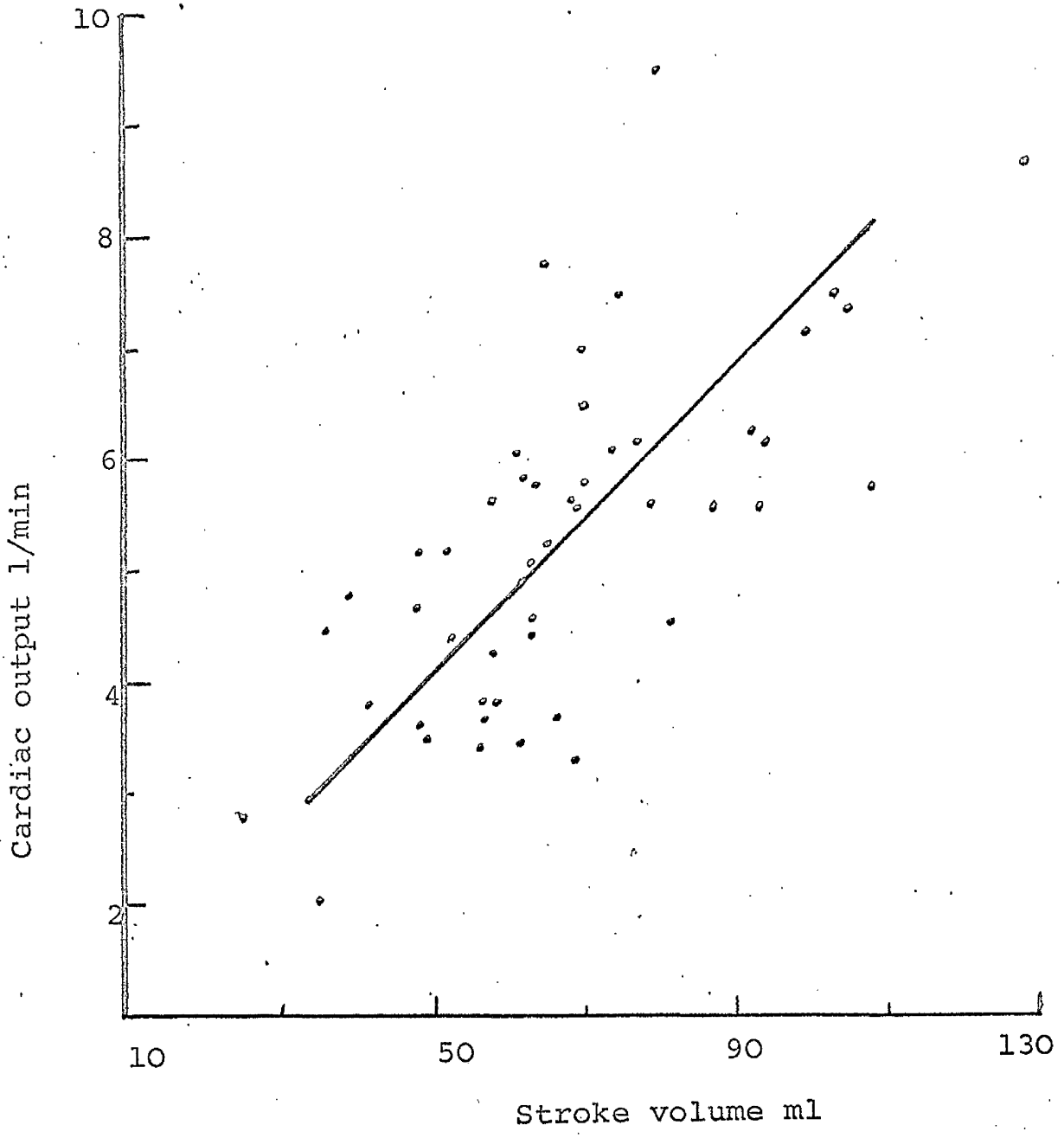


Fig. 3.2.6.

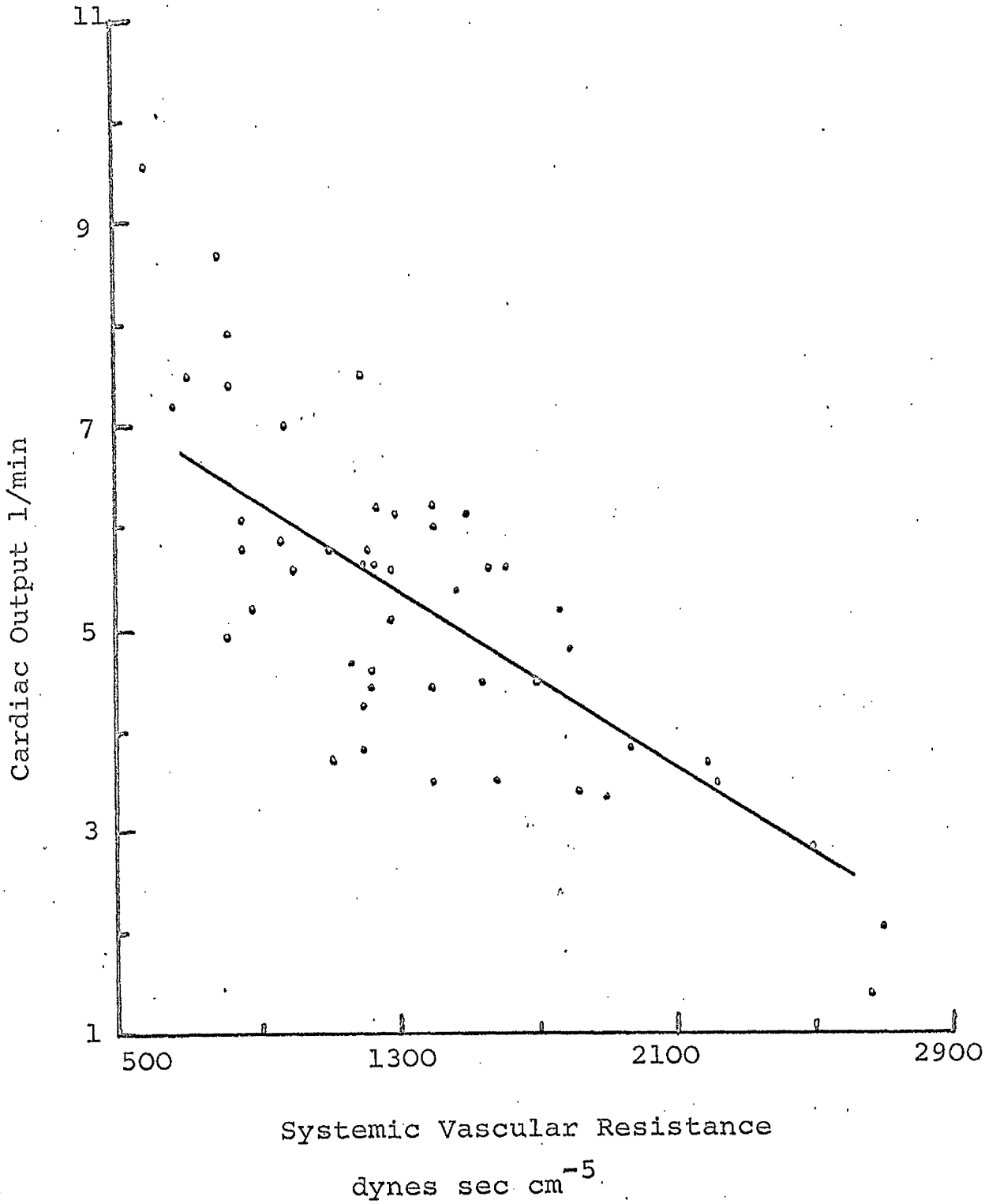
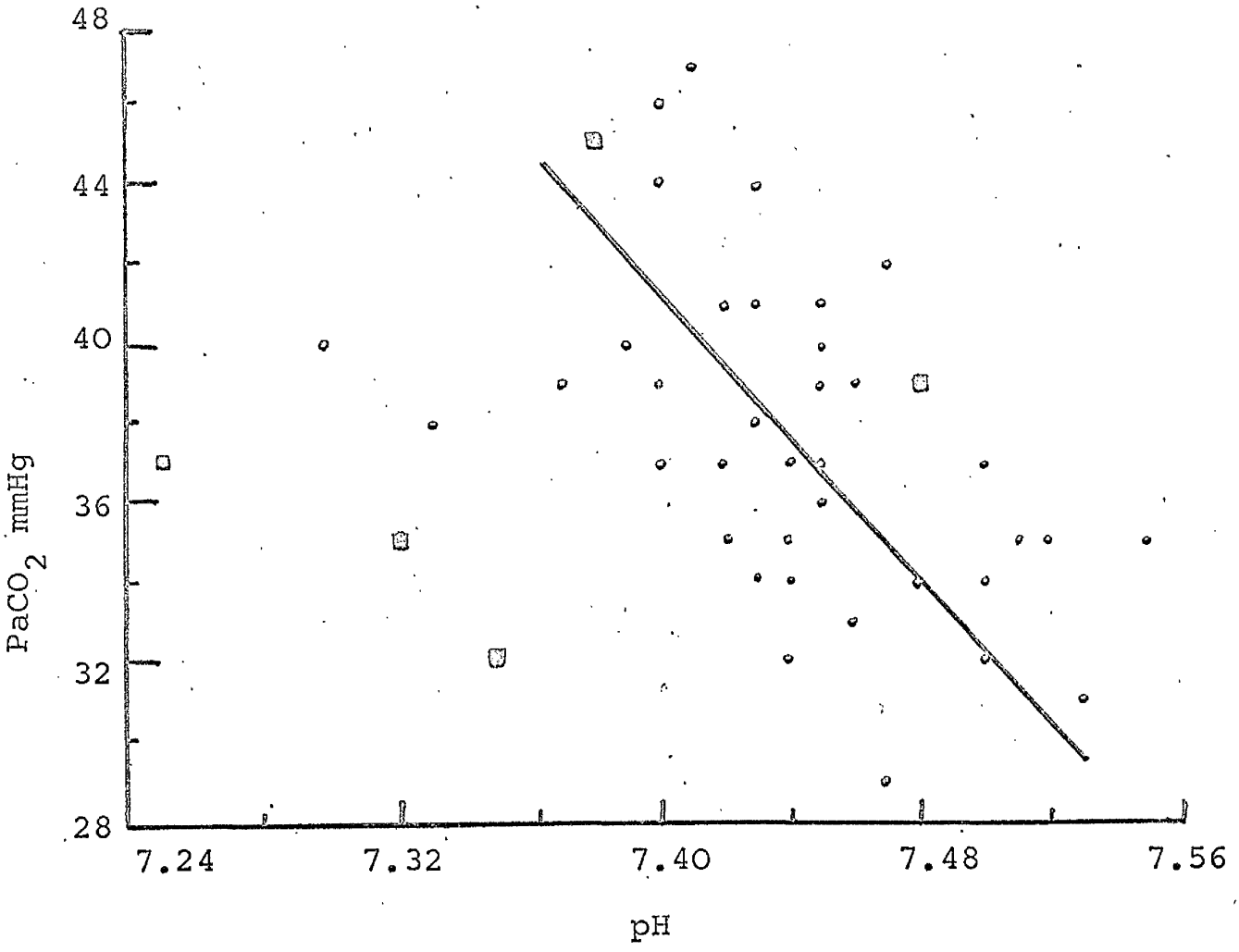

11
9
7
5
3
1
Cardiac Output l/min
500
1300
2100
2900
Systemic Vascular Resistance
dynes sec cm^{-5}

Fig. 3.2.7.



▣ High arterial blood lactate

3.3. Discussion

3.3.1. Haemodynamic Findings while Breathing Air

The haemodynamic findings before oxygen are shown in Table 3.2.1. Even on casual inspection it is apparent that there were wide variations between individuals in all the parameters measured. The heart rate varied from 48 to 125/minute, mean arterial pressure from 44 to 180 mmHg, cardiac output from 1.32 to 9.50 l/minute, stroke volume from 13 ml to 128 ml and systemic vascular resistance from 586 to 3,989 dynes sec cm^{-5}. There was no very obvious correlation between most of the parameters measured. There was however a direct relationship between cardiac output and stroke volume (Fig. 3.2.5.) and an inverse relationship between cardiac output and systemic vascular resistance (Fig. 3.2.6.).

The average value for each of the haemodynamic measureme was remarkably close to normal. One cannot say of course what the corresponding figures would have been for each individual patient immediately before infarction occurred, but it seems reasonable to conclude that infarction does not produce a consistent pattern of haemodynamic changes.

3.3.2. Biochemical Findings while Breathing Air

The corresponding biochemical findings are shown in Table 3.2.2. Again there was a striking variation between individuals, arterial blood oxygen tension (PaO_2), varied from 28 to 100 mmHg, arterial blood carbon dioxide tension, ($PaCO_2$), varied from 17 to 46 mmHg, pH from 7.25 to 7.58, arterial blood lactate from 0.50 to 7.05 mM and arterial blood pyruvate from 0.01 to 0.17 mM. As in the haemodynamic findings there was little correlation between these quantities PaO_2 and $PaCO_2$ showed no relationship, but there was a suggestion of one between pH and $PaCO_2$, particularly if cases with high lactate values were excluded (Fig. 3.2.7.).

With two exceptions the average values obtained for these biochemical parameters were normal. The exceptions were PaO_2 which gave an average value of 65 mmHg where one would normally expect a value of 100 mmHg, and arterial blood lactate which had an average value of 2.52 mM instead of one in the normal range of 0.10 to 1.78 mM.

We could not find any correlation between the haemodynamic and biochemical results.

3.3.3. Haemodynamic Effects of Oxygen Therapy

The effects of administration of high concentrations of oxygen on the haemodynamic measurements are shown in Table 3.2.3. The effects on heart rate and blood pressure

were remarkably small. On the other hand however the average cardiac output and the stroke volume were significant diminished after oxygen and the systemic vascular resistance was increased ($p < 0.001$ for all three of these parameters). Again it was notable that changes in individuals, though consistent, were seldom dramatic.

3.3.4. Biochemical Effects of Oxygen Therapy

The corresponding biochemical changes are shown in Table 3.2.4. Oxygen therapy does of course produce a tremendous increase in PaO_2. Although the oxygen was administered by an efficient method the values of PaO_2 attained were variable and showed no obvious correlation with the PaO_2 values in air. The effect of oxygen on elevated blood lactates was, in all but one case (no 18), to reduce it. The average $PaCO_2$, pH values and blood pyruvate levels were not much altered by oxygen and where changes did occur in individuals they were generally not great and did not exhibit any intelligible pattern.

3.3.5. Possible Explanations of these Findings

The most striking feature of these results is the low arterial blood PaO_2, arterial hypoxaemia, found in almost

all of the fifty patients investigated. The explanation of this is not however absolutely clear. It has been observe that pulmonary oedema is sometimes a complication of myocardial infarction (Malmcrona and Varnauskas, 1964; McNicol et al., 1965) and is frequently associated with arterial hypoxaemia, both in man (Cosby, Stowell, Hartwig an Mayo, 1957) and animals (Williams 1953; Foster 1957). Since arterial hypoxaemia is almost invariably found in myocardia- infarction it may be that a similar process to pulmonary oedema occurs, but to a lesser degree which is not clinicall detectable, in these cases. Therefore it seems most likely that this hypoxaemia is a consequence of impairment of respiratory function. This impairment could be either inadequate ventilation, veno-arterial shunting, ventilation/ blood flow inequality or incomplete diffusion of gas within the terminal airways, or a combination of these (West, 1966)

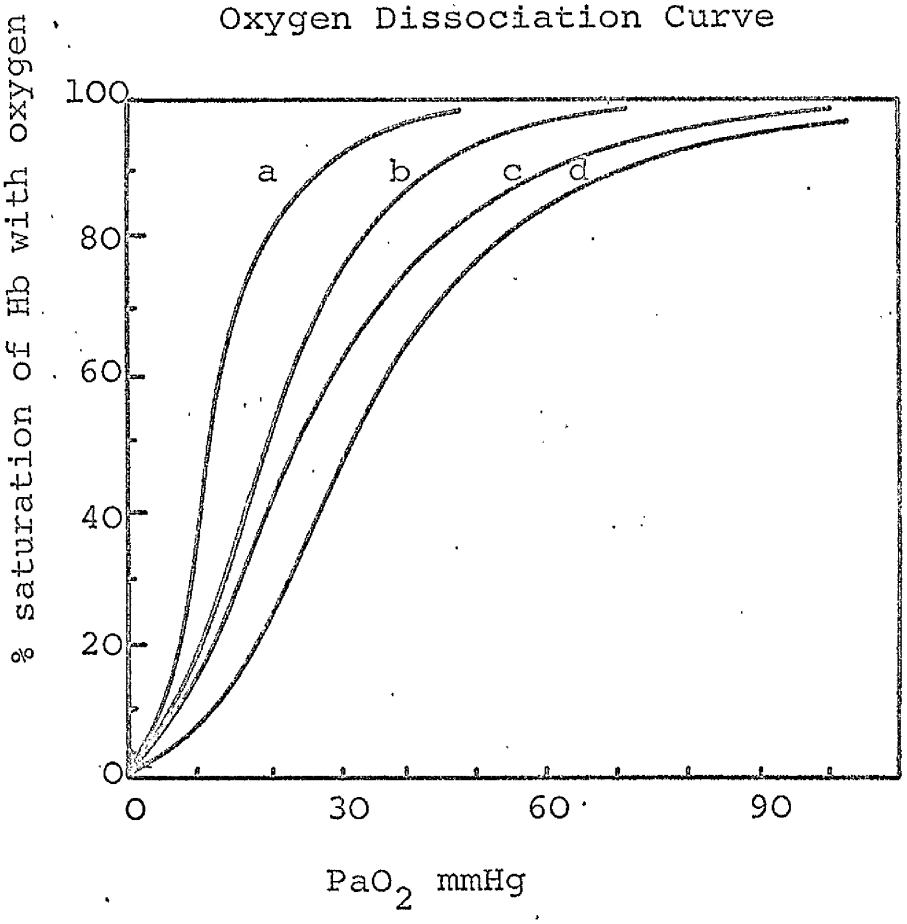
Whatever may be the cause of arterial hypoxaemia after infarction the question is whether or not it is harmful to t patient and if so does oxygen therapy have a beneficial effe

Although decreased blood-tissue oxygen tension gradient have been found in myocardial infarction and been attributed to disturbance of pulmonary function and diminished tissue perfusion (Valentine et al., 1966) arterial hypoxaemia

does not always imply tissue hypoxia. The rate at which oxygen is made available to the tissues depends on the product of the cardiac output, haemoglobin concentration and arterial oxygen saturation. In the patients we studied the average PaO_2 before oxygen was administered was 65 mmHg although this was only 2/3rds of the normal level it still corresponded to 90% saturation of the haemoglobin (Fig. 3.3 In other words in spite of the substantial fall in PaO_2 the oxygen content of the blood was nearly normal. In the norm subject it would be anticipated that the PaO_2 of mixed venc blood would be approximately 40 mmHg and this would corresp to approximately 75% saturation with oxygen (Fig. 3.3.1). If we assume the same quantity of oxygen is withdrawn from the blood of a normal healthy person, the PaO_2 in the venou blood of the patient would be around 32 mmHg. Therefore, i these patients, although the same quantity of oxygen is available to the tissues after infarction as before the oxygen would be liberated in the PaO_2 range 32 to 65 mmHg instead of the normal range of 40 to 100 mmHg. This would not be a particularly drastic change since only a small proportion of the oxygen yielded up in normal circumstance is given off at high tensions. If however the PaO_2 were as

Fig. 3.3.1.

Oxygen Dissociation Curve



a - 3 mmCO_2; b - 20 mm CO_2; c - 40 mm CO_2; d - 80 mm CO_2.

from Bock, Field and Adair (1924).

low as 28 mmHg corresponding to 60% saturation with oxygen (Fig. 3.3.1) the situation would be rather different. Assuming that the same quantity would be taken from the circulating blood, the returning venous blood would be only 35% saturated and oxygen would be made available to the tissues in the pressure range 18 to 28 mmHg. Therefore only in cases of severe arterial hypoxaemia would one expect to find tissue hypoxia and the consequent acidosis and lacticacidaemia.

3.3.6. Cardiogenic Shock

In the present series of experiments there was a number of patients (nos 3,5,7,14,18,34 and 35) who, on the grounds of either low cardiac output or blood pressure or both, could be considered to be in a state of cardiogenic shock. Cases nos 3, 5 and 7 had low cardiac outputs and PaO_2s near normal, cases nos 14 and 18 had normal cardiac outputs and reduced PaO_2s, cases 34 and 35 had reduced cardiac outputs and PaO_2s. These results could possibly be explained by veno-arterial shunting, since failure of the hearts ability to pump is the primary factor in cardiogenic shock (MacKenzie et al., 1964).

Three of these cases (nos 14,34 and 35) were acidotic an had high arterial blood lactates and two of these (nos 14 and 35) died within the first forty-eight hours while oxygen was being given continuously.

In general the effects of oxygen therapy in these cases were slight. The PaO_2s were appreciably increased in cases nos 3,7,18 and 34 but not in cases nos 5,14 and 35. The arterial blood lactate was considerably reduced after oxygen therapy in case no 34 but not in cases nos 14 and 35.

3.3.7. The Value of Oxygen Therapy

As we have seen the effect of oxygen on the haemodynamics was to increase the systemic vascular resistance and presumably in consequence to diminish the stroke volume and cardiac output. These results are similar to these obtained after administration of high concentrations of oxygen to normal man at rest (Daly and Bondurant, 1962; Eggers, Paley, Leonard and Warren, 1962).

Our results indicate that administration of high concentrations of oxygen greatly increased the PaO_2 in patient who were moderately ill but in cardiogenic shock the PaO_2s were appreciably increased in only some cases.

Therefore we may conclude that oxygen therapy is beneficial in moderate cases of myocardial infarction to the extent that it tends to increase blood pressure, systemic vascular resistance and PaO_2 and decrease elevated arterial blood lactates. However the value of oxygen in severe cases of myocardial infarction is doubtful

CHAPTER 4 - CONCLUSIONS

CHAPTER 4 - CONCLUSIONS

4.1. Serum Enzymes

On examination of this section of the work the first conclusion which was drawn was that estimations of SCPK and SLDH were valuable diagnostic aids in cases of suspected myocardial infarction. In addition we found that serial estimations of these two enzymes, as opposed to one, provided much additional information about the course of the disease and the prognosis. CPK appeared to be a more sensitive index of myocardial damage than LDH and it may be that the level of SCPK activity was proportional to the extent of the infarctio Elevated activities of SCPK on the third and fourth days seem to indicate a poor prognosis. LDH although not so specific f myocardial damage as CPK was particularly useful in those cas where admission to hospital had been delayed, since it exhibi increased activity in serum later than CPK. In those cases where the ECG evidence was difficult to interpret, for exampl where previous infarctions had occurred or where there was LB the assay of these two enzymes proved invaluable, and CPK was particularly valuable in this respect.

Since there seemed to be a definite time relationship between the onset of the infarction process and the start of the elevation of SCPK and SLDH activities we attempted to analyse our results mathematically. We concluded that

estimations of serum enzymes at four to eight hourly intervals were insufficient and in order to do this it would be necessary to take numerous blood samples at intervals of ½ to 1 hour until the peaks of the enzyme activities were reached. We also found that the activity of CPK in serum appeared to follow an exponential course and were therefore able to calculate the half-life of this enzyme in some cases

As a number of our patients with myocardial infarction were treated by DC countershock we therefore investigated th effects of this, on patients with cardiac disease other thar myocardial infarction, with respect to SCPK and SLDH. In nc case was the activity of either SCPK or SLDH increased by such treatment.

Our results illustrated a number of practical points. Firstly they showed that when working with serum enzymes th time factor was of primary importance. Therefore in order to get the maximum information in these cases it is essenti to obtain blood samples as soon as possible after onset of the infarction process and frequently thereafter. We also concluded that it was advantageous to assay for enzymes whi one would expect to have different time courses in serum e.g. CPK which showed an early, short-lived increase and

LDH which showed a somewhat later and longer-lived increase.

Finally, we concluded that in every case the serum enzyme results must be interpreted in conjunction with both the ECG evidence and the clinical history.

4.2. The Effects of Oxygen Therapy in Myocardial Infarction

The first conclusion drawn from this section of the wor was that in most of the patients investigated the haemodynam and biochemical results, before oxygen therapy, were remarka close to normal. The one general finding was that almost all of these patients exhibited some degree of arterial hypoxaemia. Only in those patients who were severely ill di we find any appreciable fall in cardiac output and/or blood pressure, and arterial blood lactate levels much above norma

The effects of administration of high concentrations of oxygen were, in general, to raise the blood pressure, syster vascular resistance and PaO_2 and reduce the cardiac output, stroke volume and arterial blood lactate. Since oxygen therapy did not, to any great extent, alter the haemodynami and metabolic findings in severely ill patients we conclude that it was of little benefit in these cases.

APPENDIX - MATERIALS AND METHODS

MATERIALS AND METHODS

Analyses were carried out in all cases with minimum delay after withdrawal of the blood specimen. When this was not possible serum was stored at $4^{\circ}C$, and plasma and deproteinised blood at $-10^{\circ}C$. Serum enzymes were always estimated within 24 hours, arterial blood lactate and pyruvate and concentration of dye in plasma within 48 hours. Each analysis was repeated until consistent results were obtained.

1. Enzyme Assay Methods

a. Estimation of Creatine Phosphokinase

Principle

This enzyme was estimated in serum by the method described by Rosalki (1967), whereby the change in extinction at 340nm (ΔE_{340}) due to the conversion of the oxidised form of nicotinamide adenine dinucleotide phosphate ($NADP^{+}$) to its reduced form (NAPDH) is measured. Adenosine triphosphate (ATP) formed by the action of the enzyme on adenosine diphosphate (ADP) and creatine phosphate (CP) is linked to the reduction of $NADP^{+}$ with glucose, hexokinase (ATP : D-hexose 6 - phosphotransferase EC 2.7.1.1.) and glucose - 6 - phosphate dehydrogenase (D-glucose - 6 - phosphate : NADP oxidoreductase EC 1.1.1.49) (G - 6 - PDH).

1. ADP + CP $\underset{\text{hexokinase}}{\overset{\text{CPK}}{\rightleftharpoons}}$ ATP + creatine

2. ATP + glucose $\longrightarrow$ glucose-6-phosphate + ADP

3. Glucose-6-phosphate + $NADP^+$ $\xrightarrow{\text{G-6-PDH}}$ 6-phosphogluconate + NAPDH + H^+

The ΔE at 340nm provides a measure of CPK activity.

Reagents:

The test capsules manufactured by Calbiochem were used. These contained:

1. Substrate: 0.001 M-ADP, 0.01M-CP, 0.02M-glucose, 0.03M-magnesium chloride, hexokinase (0.6 U/ml), G-6-PDH (0.3 U/ml), 0.008 M-$NADP^+$ in concentrations chosen to provide optimal reaction conditions.
2. 0.01M-adenosine monophosphate (AMP).
3. 0.05M-cysteine hydrochloride.

The reagents were dissolved in 0.05 M - tris buffer and the final pH adjusted to 6.8 at 25°C and then freeze dried. The capsule contents were reconstituted immediately prior to assaying by the addition of distilled water. This ' activated' substrate was kept ice-cold and was not stored.

Method

Measurements were carried out on a Unicam SP 500 series 2 spectrophotometer with an SP 505 programme controller and

a thermostatically controlled cuvette carriage. The reaction took place in a 1 ml silica cuvette with a 1 cm light path.

1 ml of 'activated' substrate was pipetted into the test cell and allowed to equilibrate to 30°C. 25 μ l of serum were blown into the test cell and gently mixed. After a pre-incubation period of 6 minutes the extinction at 340nm (E_{340}) was recorded and this was repeated every minute for the next fifteen minutes. Measurements were made against a distilled water blank.

Units

The unit of enzyme activity (U) was as recommended (1964), by the International Union of Biochemistry which was 'One unit of any enzyme is that amount which will catalyse the transformation of 1 micromole (μmole) of substrate per minute under standard conditions.

1 milli-Unit (mU) = 0.001 U.

Calculation

The Molar Extinction Coefficient (ϵ) of NAPDH at 340nm is 6.22×10^6 1 $mol^{-1} cm^{-1}$

$$\text{Activity} = \frac{\Delta E/\text{minute} \times 1000 \times V}{6.22 \times v} \text{ mU/ml}$$

where V = total assay volume in ml, and v = sample volume in ml.

Activity = ΔE/minute x 6,430mU/ml

b. Estimation of Lactate Dehydrogenase

All LDH assays were performed on unhaemolysed serum specimens.

Principle

The method used to estimate LDH in serum was an adaptation of that of Wróblewski and LaDue (1955), whereby the change in extinction at 340nm (ΔE_{340}) due to the conversion of the reduced form of nicotinamide-adenine dinucleotide (NADH) to its oxidised form (NAD^+) is measured. The reduction of pyruvate to lactate by NADH is catalysed by LDH.

$$\text{Pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{Lactate} + NAD^+$$

The ΔE at 340nm provides a measure of the LDH activity.

Reagents

The test combinations manufactured by the Boehringer Corporation (London) Ltd. were used.

1. Buffered substrate: 0.05M-phosphate buffer, pH 7.5;
   0.003M-pyruvate
2. 0.009M-NADH

Method

Measurements were carried out as for CPK using a 3 ml silica cuvette of 1 cm light path.

3 ml of reagent 1 were pipetted into the test cell and allowed to equilibrate to 25°C. 100 µl of serum were then added and gently mixed. The extinction at 340nm (E_{340}) was recorded immediately and thereafter every minute for the following ten minutes.

If the serum gave an E_{340} greater than 0.20/minute it was diluted 1 in 10 with physiological saline.

Units

As for CPK.

Calculation

As for CPK since (ϵ of NADH is 6.22×10^6 l mol^{-1} cm^{-1})

Activity = ΔE/minute x 5,064mU/ml

For diluted samples the result was multiplied by 10.

2. Lactate and Pyruvate Assay Methods

Deproteinization of Blood

Immediately after withdrawal, without stasis, blood samples were vigorously mixed with 3.5% (w/v) perchloric acid volume to volume, centrifuged at 1400 g for 20 minutes and the supernatant decanted and assayed.

a. Estimation of Arterial Blood Lactate

Principle

LDH catalyses the oxidation of lactate by NAD^+

$$\text{Lactate} + NAD^+ \rightleftharpoons \text{Pyruvate} + NADH + H^+$$

In order to obtain quantitative oxidation the reaction products were removed, protons by using an alkaline reaction medium and pyruvate by trapping with hydrazine.

$$\text{Lactate} + NAD^+ + \text{hydrazine} \xrightarrow{LDH} \text{pyruvate hydrazone} + NADH + H_3O^+$$

Since this is a stoichiometric reaction (1 mole NAD^+/ 1 mole lactate) it is possible to calculate the lactate concentration by following it.

Reagents

The test combinations manufactured by the Boehringer Corporation (London) Ltd. were used.

1. 0.5M-glycine buffer, pH 9.0; 0.4M-hydrazine
2. LDH (2 mgms/ml)
3. 0.027M-NAD^+

Method

Measurements were carried out on a Hilger Watts spectrophotometer with a thermostatically controlled cuvette carriage. 3 ml silica cuvettes with a 1 cm light path were used.

Test: 2 mU reagent 1, 0.10 ml supernatant, 0.03 ml reagent 2, 0.20 ml reagent 3.

Blank: 2 ml reagent 1, 0.10 ml 3.5% (w/v) perchloric acid, 0.03 ml reagent 2, 0.20 ml reagent 3.

After an incubation at 25°C for 1 hour the E at 340nm of test and blank were used.

Calculation

ϵ_{340} of NADH is 6.22×10^6 1 mol^{-1} cm^{-1}

Concentration of lactate in cuvette = $\frac{\Delta E \times V}{6.22 \times v}$ mM

Concentration of lactate in whole blood = $\frac{\Delta E \times V}{6.22 \times v} \times F$ mM

Where V = total assay volume in ml v = sample volume in ml.

F = dilution factor.

Concentration of lactate in whole blood = $\Delta E \times 7.5$mM.

b. Estimation of Arterial Blood Pyruvate

Principle

LDH catalyses the reduction of pyruvate with NADH

$$\text{Pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{Lactate} + NAD^+$$

Pyruvate is quantitatively converted to lactate. This is a stoichiometric reaction (1 mole NADH/1 mole pyruvate) therefore the pyruvate concentration is measured by following it.

Reagents

The test combinations of the Boehringer Corporation (London) Ltd. were used.

1. 2.2M-dipotassium hydrogen phosphate ($K_2H\ PO_4$)
2. 0.012M-NADH
3. LDH (0.75 mgm/ml)

Method

Measurements were made as for lactate.

1 ml of reagent 1 was added to 3 ml supernatant, mixed, and allowed to stand in an ice-bath for at least ten minutes. The resultant supernatant was filtered off and the filtrate allowed to equilibrate to 25°C. 2 ml of filtrate was pipetted into a cuvette and 0.05 ml of reagent 2 added, mixed and E_{340} read. 0.05 ml of reagent 3 was then added to the test cell, mixed, and E_{340} was read after 5 minutes.

Calculation

As for lactate except ΔE must be corrected for dilution caused by addition of LDH

Concentration of pyruvate in whole blood = ΔE x 0.5mM.

3. Estimation of Dye Concentration in Plasma

Cardiac output was measured by a dye-dilution technique, the procedure described by Taylor and Shillingford (1959), being followed. The calibration of the dye dilution curve requires the measurement of the concentration of dye, in this case Coomassie Blue, in plasma and to do this we used a modification of the method of Clausen and Lifson, (1956).

Method

To each plasma sample (one before and one after injection of dye), and two standards (standard 1 - 20 mgms dye/l, standard 2 - 10 mgms dye/l), the following were added.

1. 2 ml saturated urea
2. 4 ml acetone
3. 0.5 ml $ZnSO_4 . 7H_2O$ (10% w/v)
4. 0.5 ml NaOH (0.5M)

The tubes were shaken after each addition, and after centrifugation at 700 g for 15 minutes the supernatant was pipetted off and their E at 575 nm read against an acetone blank. Measurements were made in a Unicam SP 600 spectrophotometer using a 3 ml glass cuvette with a 1 cm light path.

Calculation

$$\text{Concentration of dye in plasma} = \frac{E_s - E_b}{E_{st}} \text{ mgm/l}$$

(E_s - E of sample plasma; E_b - E of blank plasma; E_{st} - E of standard). This was multiplied by the haematocrit to give the concentration of dye in whole blood.

4. Estimation of Aterial Blood Gases and pH

Oxygen was administered by means of a close-fitting face-mask connected to a humidifier and low-resistance demand valve (McDowall, Ledingham, Jacobson and Norman, 1965).

Blood gases and pH were measured using an Instrumentation Laboratory Inc. system (Model I.L.113). The arterial oxygen tension (PaO_2) was measured by a Clark electrode, carbon dioxide tension ($PaCO_2$) by a Severinghaus electrode and pH by a glass electrode. The system was calibrated using nitrogen, oxygen, carbon dioxide and nitrogen mixtures of known composition and buffers of known pH, and tonometered blood.

SUMMARY

SUMMARY

This work consists of two main sections. In the first of these we investigated the value of serum enzyme assays in cases of suspected myocardial infarction. The second part was concerned with the effects of oxygen therapy on patients who had had a myocardial infarction.

Serial assays of serum creatine phosphokinase, (SCPK), and serum lactate dehydrogenase, (SLDH), were carried out in a group of twenty-eight patients. From the results obtained we assessed the value of such estimations in confirming, or otherwise, the diagnosis, in following the course of the disease and in prognosis. We found that there was a definite relationship between onset of the infarction process and the commencement of increased activities in serum of both of these enzymes. In general SCPK activity started to rise, reached a peak, and returned to normal more rapidly than did SLDH. The assay of these two enzymes, as opposed to one, proved to be of value in following the course of the disease. SCPK appeared to be a more sensitive index of myocardial necrosis than SLDH and it seemed likely that the level of activity of this enzyme in serum may have been proportional to the extent of myocardial damage.

Since a number of these patients were treated by direct current, (DC), countershock we investigated the effects of this in a small group of patients with cardiac disease other than myocardial infarction. In no case did we find that this

treatment gave rise to increased SCPK and/or SLDH activities.

Haemodynamic and biochemical measurements were carried out, before and after administration of high concentrations of oxygen, on a group of fifty patients who had had a myocardial infarction within the previous twenty-four hours. The haemodynamic parameters measured were heart rate, blood pressure cardiac output, systemic vascular resistance and stroke volume. The biochemical measurements were arterial blood oxygen and carbon dioxide tension, pH, and lactate and pyruvate concentrati In general we found that the values of most of these parameters were close to normal and only in the severely ill patients was there a fall in cardiac output and/or blood pressure, and a rise in arterial blood lactate of any significance. The only consist finding was that almost all of these patients exhibited some degree of arterial hypoxaemia. The effects of oxygen therapy were, in general, to raise the blood pressure, systemic vascular resistance and arterial blood oxygen tension, and to lower the cardiac output, stroke volume and arterial blood lactate concentration. We found that oxygen appeared to have little therapeutic value in severely ill patients i.e. patients in cardiogenic shock. Possible explanations of these findings were suggested.

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