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An Investigation Into The Mechanisms Responsible For The Exercise-induced Alterations In Plasma Enzyme Activity

Christopher Paul Bolter

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AN INVESTIGATION INTO THE MECHANISMS RESPONSIBLE
FOR THE EXERCISE-INDUCED ALTERATIONS IN PLASMA
ENZYME ACTIVITY

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Department of Physiology

Submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Canada
April, 1973

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ABSTRACT

The experiments to be reported were performed to elucidate the mechanism(s) responsible for the exercise-evoked increases in activity of several enzymes in blood plasma. Mongrel dogs were the experimental animal.

The behaviour of three enzymes, glutamic-oxalacetic transaminase (GOT), creatine phosphokinase (CPK) and lactic dehydrogenase (LDH) in the plasma was followed during running exercise. At the end of a run, plasma LDH (PLDH) was elevated more frequently than plasma GOT (PGOT) or plasma CPK (PCPK). Duration of exercise did not appear to be an important determinant of the enzyme response. In general, the greater the intensity of the exercise, the larger the increases in PLDH and PGOT. PCPK was elevated only after the most intense exercise.

The possible involvement of the large cardiovascular adjustments to exercise in the exercise-evoked plasma enzyme increase was examined using two different techniques. In the first, right atrial pacing enabled the heart to be driven at a rate normally achieved in hard exercise (240 beats/minute). The second method utilised electrical stimulation of selected diencephalic sites, which resulted in large increases in heart rate (to 225 beats/minute) and arterial blood pressure. In the pentobarbital-dog, there was a small increase in
PGOT, but no change in PCPK following 30 minutes of cardiac pacing. Thirty minutes of diencephalic stimulation in the chloralose-dog resulted in increased PGOT and PCPK. These changes in plasma enzyme activity were approximately one-third as large as those observed during a 15-minute run (heart rate = 229 beats/minute). These observations indicated that the cardiovascular adjustments to exercise could only account for part of the plasma enzyme response to exercise.

The transport of enzyme by the lymphatic system was also examined. The results obtained indicated that the normal levels of PCPK and PGOT were maintained, in the pentobarbital-dog, by the continual entry of lymph having high CPK and GOT activity, into the circulating blood. In contrast, the low activity of LDH in thoracic duct lymph could not have contributed to the maintenance of normal levels of PLDH.

The changes of LDH, GOT and CPK in plasma, right duct and thoracic duct lymph were followed during stimulation of hind-limb skeletal muscle. During 30 or 60 minutes of muscle stimulation, both PGOT and PLDH increased over the first 10 to 15 minutes, and then stayed at a relatively steady level for the remainder of stimulation. High levels of CPK, and a moderate increase in GOT activity in thoracic duct lymph were achieved during stimulation. LDH activity in thoracic duct lymph was much
higher during stimulation than at rest, but was considerably less than CPK or GOT activity. During stimulation, the thoracic duct lymph transport of CPK, but not of GOT or LDH, would have contributed to a significant increase in the plasma enzyme activity in the intact dog. The activities of all three enzymes in right duct lymph decreased during muscle stimulation. It was concluded that the lymphatic route was important in the transport of CPK from contracting muscle to the circulating blood, but that LDH and GOT must enter the blood by some route other than the major lymphatics.

The mechanisms involved in the plasma enzyme responses to muscle stimulation and exercise are discussed. It is suggested that both an increase in the rate of release of enzyme from the formed elements of the blood, and a decrease in clearance of enzyme from the blood may be involved. In addition, it is clear that at least one enzyme, CPK, is released from contracting muscle cells, and reaches the circulating blood via the lymphatics.
Acknowledgements

The author would like to thank Dr. J.B. Critz for his able supervision throughout this study, and Drs. W.F. Ziegler and E.K. O'Hea for their active interest during its experimental stages. I would also like to thank Drs. E.K. O'Hea, P.G. Dellow, and D.A. Cunningham for their advice during the preparation of this thesis.

This thesis was typed by Mrs. Elizabeth Rennie whose friendly help is very much appreciated.

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<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees Centigrade</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>&gt;</td>
<td>Greater than</td>
</tr>
<tr>
<td>hr</td>
<td>Hour</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>IU</td>
<td>International unit</td>
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<tr>
<td>IU/L</td>
<td>International units per litre</td>
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<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>&lt;</td>
<td>Less than</td>
</tr>
<tr>
<td>MBP</td>
<td>Mean blood pressure</td>
</tr>
<tr>
<td>mg</td>
<td>Milligrams</td>
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<tr>
<td>mg/kg</td>
<td>Milligrams/kilogram</td>
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<tr>
<td>min</td>
<td>Minute</td>
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<tr>
<td>ml</td>
<td>Millilitre</td>
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<tr>
<td>mm</td>
<td>Millimetre</td>
</tr>
<tr>
<td>mmHg</td>
<td>Millimetres of mercury</td>
</tr>
<tr>
<td>mph</td>
<td>Miles per hour</td>
</tr>
<tr>
<td>n</td>
<td>Number</td>
</tr>
<tr>
<td>N</td>
<td>Normal</td>
</tr>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>%elev</td>
<td>Percent elevation</td>
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xix
† Plus or minus
RE Reticulo-endothelial
RES Reticulo-endothelial system
RR Respiratory rate
SBP Systolic blood pressure
sec Second
SEM Standard error of the mean

ENZYMES

AcidP Acid phosphatase
Ald Aldolase
AlkP Alkaline phosphatase
CPK Creatine phosphokinase
GOT Glutamic-oxalacetic transaminase
GPT Glutamic-pyruvic transaminase
ICD Isocitric dehydrogenase
LDH Lactic dehydrogenase
MDH Malic dehydrogenase
OCT Ornithine carbamyl transferase
PHI Phosphohexoisomerase

When the abbreviation for an enzyme is preceded by either P or S, it refers to the activity of that enzyme in the blood plasma or serum, respectively.
INTRODUCTION

A large variety of enzymes can be found in the extracellular fluid of mammals. It is tacitly assumed that, in the healthy animal, intracellular enzymes enter the extracellular compartment during the regular turnover of cells. Within the extracellular fluid, these enzymes are maintained at a remarkably constant, though minute, level of activity. While this precise regulation depends upon an equilibration between the entry and removal of enzyme within the extracellular compartment, the kinetics and physiological mechanisms involved in this regulation have not been clearly elucidated.

Changes in the plasma levels of many enzymes occur in both pathological and exercise situations. While, in the former case, frank necrosis and cellular destruction undoubtedly result in a loss of intracellular enzymes to the extracellular fluid, exercise is not often accompanied by any such histologically demonstrable cellular derangement.

Some authors consider that the exercise-induced increase in plasma enzyme is entirely derived from the working muscles (Fowler et al., 1968; Dieter, 1970). However, several studies suggest that the liver, as well as other organs, may also be involved. In general the
nature and magnitude of the exercise-evoked increase in plasma enzymes depend upon the duration and intensity of the exercise, and upon the physical conditioning of the subject. Previous physical conditioning tends to reduce the magnitude of the increase in plasma enzyme activity evoked by a standardised bout of exercise.

Examination of previous work suggests that the various enzymes in the plasma may respond in two different ways to exercise. The first type of response appears to involve an increase in the plasma enzyme activity during exercise, with maximum activity reached at, or near, the termination of exercise. During recovery, the plasma enzyme activity falls sharply toward control values. In the second type of response there is a slow delayed rise in plasma enzyme activity; the maximum level may be achieved anywhere between six hours and five days following the exercise.

This division of the exercise-evoked plasma enzyme responses into two clearly separable categories has not previously been made. It is considered, by the author, that this division is valuable in that the two basically different responses may be promoted by dissimilar mechanisms, and that further investigation should be performed in this light.
A reversible increase in the permeability of the cell membrane to macromolecules is considered to be the most likely cause of a loss of enzyme from cells during exercise. Studies on the intact, in vitro, skeletal muscle preparation lend support to this concept. Several mechanisms have been proposed to account for these postulated permeability changes. The effects of high concentrations of circulating catecholamines, and of various degrees of arterial hypoxia on plasma enzyme levels, have been investigated (Highman, Maling, and Thompson, 1959; Loegering and Critz, 1968; Loegering and Critz, 1971). None of these studies provided clear evidence concerning the mechanisms promoting changes in cellular permeability during exercise.

In a recent study using anaesthetised dogs, Loegering and Critz (1971) concluded that relative hypoxia within the exercising muscles was not the principle cause of the plasma enzyme response to simulated exercise. They commented, however, that the changes in plasma enzyme levels occurring in simulated exercise were considerably different from those elicited by spontaneous exercise. Consequently, it was felt that there were additional factors, beyond rhythmic muscle contractions, that promoted the plasma enzyme response to exercise.
In the current study, with the mongrel dog as the experimental animal, an attempt was made to define as clearly as possible, the physiological responses to exercise which are involved in the exercise-evoked plasma enzyme changes.

Cardiovascular adjustments of considerable magnitude occur during spontaneous exercise. In particular, during exercise, the heart may work at a rate several-fold above that at rest, with respect to both its metabolic activity, and to its production of useful work. The effect on plasma enzyme activity of increases in cardiac work and metabolism were investigated using two fundamentally different techniques. In the first, right atrial pacing enabled the heart to be driven at a rate normally achieved in hard exercise. Using this technique, the oxygen consumption of the heart could be increased without involving the autonomic nervous system, or significantly affecting any other cardiovascular parameter. The second method chosen utilised the electrical stimulation of select diencephalic structures. Stimulation within these regions results in increases in heart rate, arterial blood pressure, and cardiac output, and in changes of the geometry of the left ventricle: an integrated cardiovascular response very similar to
that occurring in spontaneous exercise. These responses were mediated by both the parasympathetic and sympathetic nervous systems.

In the second part of the study, the effect of spontaneous exercise on plasma enzyme activity was investigated. Throughout the remainder of this text, this part of the study is presented first although, chronologically, it was performed following the above experiments. The results obtained were at variance with the little data that had previously been published (Bedrak, 1965; Wagner and Critz, 1968). One conclusion reached, however, was that simulated exercise in the dog, does in fact result in plasma enzyme changes quite similar to those evoked by spontaneous exercise. Consequently, simulated exercise was adopted for the next part of the study.

The lymphatic system has been shown to act as an important pathway in the transport of enzymes from the gastrointestinal system, and from necrotic tissues, to the circulating blood. In the present study, after cannulation of thoracic and right lymph ducts, the transport of enzymes in the lymph was monitored during periods of rest and of simulated exercise. Data obtained in these experiments suggested that the plasma enzyme
elevation evoked by simulated exercise may be primarily of intravascular or splenic origin. Simulated exercise experiments were repeated in splenectomised animals.

From the data obtained in the latter part of this study, an understanding of the transport of cellular enzymes by the lymphatic system at rest and in simulated exercise was achieved. Further analysis suggested an intravascular mechanism for the exercise-induced elevation of plasma enzyme activity.
HISTORICAL REVIEW

1. Distribution and function of the enzymes studied

This review covers material concerning a variety of enzymes while, in the current investigation, three particular enzymes were selected for study. A description of these three enzymes is provided at this point, in order that their status in previous research can be assessed.

(i) Creatine phosphokinase

The reaction involving the reversible transfer of a high energy phosphate radical from creatine phosphate (CP) to adenosine diphosphate (ADP) is catalysed by creatine phosphokinase (CPK).

\[
\text{CPK} \quad \text{CP} + \text{ADP} \leftrightarrow \text{C} + \text{ATP}
\]

Thus ATP can be obtained anaerobically from creatine phosphate stores during periods of high metabolic demand or hypoxia. Creatine phosphate stores are replenished when a net production of ATP is established. CPK is predominant in skeletal muscle, with some activity in heart and neural tissue (Hess et al. 1964). Skeletal
muscle can perform well in anaerobic conditions; myocardial and neural tissue cannot, but CPK may ameliorate the sensitivity of these latter tissues to mild hypoxia. CPK activity has also been demonstrated in human seminal fluid, but not in liver, kidney, or erythrocytes (Hess et al. 1964; Lehmann and Griffiths, 1963).

Three isoenzymes of CPK have been distinguished electrophoretically (Eppenberger, Dawson and Kaplan, 1967). Active CPK is a dimer with two subunits: muscle type (M) and brain type (B) (Dawson, Eppenberger, and Kaplan, 1965). These subunits combine to give active dimers of types BB, MB, and MM. Brain contains the type BB enzyme, while skeletal muscle contains the MM dimer. The myocardium possessed all three isoenzymes, with the MM type predominant.

(ii) **Glutamic-oxalacetic transaminase**

Glutamic-oxalacetic transaminase (GOT) catalyses the reversible reaction involving the interchange of the alpha-amino group of L-glutamate for the alpha-keto group of oxalacetate to produce alpha-keto glutarate and aspartate.

\[
\text{GOT} \quad \text{L-glutamate} + \text{oxalacetate} \overset{\rightleftharpoons}{\rightarrow} \text{alpha-keto glutarate} + \text{aspartate}
\]

Transaminases are important in the interconversion of amino and keto acids, and possibly in the formation of
metabolic substrate during periods of high energy demand, for example during exercise (Cohen, 1940). These metabolic substrates, the keto acids, would be utilised in aerobic metabolism via the Krebs cycle.

There is a wide distribution of GOT both in plant and in animal tissues. In dogs the highest activity is found in the myocardium, with high activity also demonstrated in skeletal muscle, liver, and kidney (Nagode, Frajola, and Loeb, 1966). Both rat and dog neural tissue have been shown to possess some GOT activity (Wroblewski, 1958). A considerable amount of GOT is present in erythrocytes, leucocytes, and platelets (Koj, Zgliczynski, and Frendo, 1960; Friedel and Mattenheimer, 1971).

Four or more isoenzymes of GOT have been demonstrated in rat tissues (Decker and Rau, 1963). At the present time however, there are considered to be two principal isoenzymes of GOT; a supernatant (apodal) type, and a mitochondrial (cathodal) type (Fleisher, Potter, and Wakim, 1960). In most tissues containing GOT, both isoenzymes are present in approximately equal activity.
(iii) **Lactic dehydrogenase**

The reversible, NAD-coupled, dehydrogenation of lactate to pyruvate is catalysed by lactic dehydrogenase (LDH).

\[
\text{pyruvate} + \text{NADH} + \text{H} \underset{+ \text{LDH}}{\longrightarrow} \text{lactate} + \text{NAD}
\]

The net direction of this reaction depends upon the tissue involved and its metabolic state. In general, during hypoxia, lactic acid and NAD\(^+\) are the end-products, allowing glycolysis to proceed. This reaction is predominant in erythrocytes and skeletal muscle. Pyruvate is usually the net end-product in the heart, liver, and kidney. In these tissues, lactate may be channelled into the gluconeogenic pathway or metabolised oxidatively.

The LDH molecule is a tetramer composed of two different subunits, types H and M (Heart and Muscle). Every permutation exists, giving rise to five different isoenzymes which can be distinguished electrophoretically \((H_4 = LD_1, H_3M = LD_2, \ldots, M_4 = LD_5)\) (Jordan and White, 1967). During electrophoresis, LD\(_1\) migrates the fastest, with a decrease in migration rate through to LD\(_5\).

There are several species variations in the tissue distribution of the LDH isoenzymes (Wilkinson, 1965). In mammals, LD\(_1\) and LD\(_2\) are found in the myocardial, neural
and kidney tissues, LD₃ is found in the spleen and pancreas, while LD₄ and LD₅ predominate in skeletal muscle and liver. LD₁ and LD₂ are found in human and dog erythrocytes; LD₃, LD₄, and LD₅ in rat erythrocytes (Vessell and Bearn, 1961). There is a similar distribution of LDH isoenzymes in leucocytes. Hule (1965, 1971) found a tremendous variation in mammalian blood platelet LDH isoenzyme complement.

It was believed, until quite recently, that the tissue distribution of LDH isoenzymes could be explained purely on the direction of the lactate-pyruvate interconversion normally occurring within a given tissue. However, contrary to this concept, it now appears that the normal metabolism of lactate and pyruvate in a given tissue is not necessarily related to the species of LDH isoenzyme present in that tissue (Hule, 1965, 1971; Wunitch, Chen, and Vessell, 1970).

2. Intracellular enzymes in extracellular fluid

   (i) Introduction

There is low, but measurable, activity of many cellular enzymes in extracellular fluid. Most of the studies on enzymes in extracellular fluid have been performed on plasma or serum prepared from whole blood. Enzymes have also been demonstrated in urine (Raab, 1968), cerebrospinal
fluid (Liebermann et al. 1957), lymph (Carlson and Luckhardt, 1908), and saliva (Wolman et al. 1947). It is generally considered that these enzymes, which normally function in an intracellular environment, do not fulfil a meaningful metabolic role in the extracellular fluid. Carlson and Luckhardt (1908) made this conclusion after studying the activity of amylase in blood and lymph.

(ii) Plasma enzymes

The activities of many different enzymes in the blood have been measured in a variety of physiological and clinical situations. The ease with which blood samples can be obtained, and the development of precise assay procedures, resulted in a rapid expansion of the field of clinical enzymology during the late 1950's.

Blood enzymes are presently determined on either plasma or serum preparations. Either medium must be totally free of formed elements and, in the course of preparation, these blood components must remain undamaged. The various blood cells and platelets possess a high activity of most of the enzymes measured clinically and experimentally (Friedel and Mattenheimer, 1970).

Plasma is the preferred medium for the assay of blood enzymes, since it has been shown that during the process of clotting, many enzymes are released in considerable
quantity from platelets (Friedel and Mattenheimer, 1970). The release of enzymes from platelets during clotting can cause marked elevations in serum enzyme levels in the rat, dog, and rabbit. In man, clinically insignificant, but experimentally significant, release of enzymes from platelets occurs during clotting. Therefore, in reviewing the literature, it is important to note the medium in which the enzymes were assayed.

To date, the precise origin of the cellular enzymes in the plasma is not known. One theory suggests that there is a constant breakdown and replacement of individual cells within tissues, with a concomitant release of enzymes (White, 1963). Thus the plasma activity of an enzyme would depend upon the rate of breakdown of the tissues in which it was present, the level of activity of the enzyme in those tissues, and the rate of removal of that enzyme from the plasma (Posen, 1970).

In a study of eight different enzymes in twenty vertebrate species, Zimmerman et al. (1965) concluded that normal intact cells were probably the source of enzymes in the blood plasma. Factors such as the molecular dimensions of the enzyme, its cellular location, and various characteristics of the cell membrane may determine the loss of enzyme from a normal cell. Zimmerman, Dujovne,
and Levy (1968) demonstrated a strong correlation between the tissue and serum levels of glutamic-oxalacetic and glutamic-pyruvic transaminases in six vertebrate species.

More recently, Friedel and Mattenheimer (1971) studied the activities of the five isoenzymes of LDH in erythrocytes, leucocytes, and platelets, and concluded that a continual slow release of enzyme from these formed elements could produce the normal plasma profile of LDH isoenzymes.

Whether the cellular enzymes, after release into the interstitial fluid enter the blood directly, or enter through the lymphatic system, has not been determined. The activity of several enzymes in the lymph from the hind limb of the cat was found to be the same as, or greater than, their activity in the plasma (Lewis and Winsey, 1970). Sacks et al. (1970) found greater CPK and acid phosphatase levels in the interstitial fluid and thoracic duct lymph, than in the blood plasma of dogs.

(iii) **Turnover of plasma enzymes**

There is a fairly constant level of activity of most enzymes in the plasma of healthy man, suggesting the existence of an equilibrium between the entry of enzyme into the vascular compartment, and the subsequent removal or degradation of that enzyme. The mechanisms of enzyme
clearance from the intravascular compartment are not well understood, but the rates of clearance of some of the plasma enzymes are known (Posen, 1970).

The only mechanisms found to date which appear to assist in the removal of enzyme from the vascular compartment are the reticulo-endothelial system (RES), and renal excretion. Mahy, Rowson, and Parr (1967) found that blocking the RES of mice with the Riley virus led to an increase in the activity of some plasma enzymes. Other plasma enzymes were unaffected. Amylase is of a sufficiently low molecular weight to be filtered by the kidney (Mattenheimer, 1971). Most enzymes are larger than the threshold for filtration by the kidney.

Glutamic-oxalacetic transaminase (GOT) exists as two isoenzymes; a cytoplasmic and a mitochondrial type, with plasma half-lives of 4.0 hours and 54 minutes, respectively, in the dog (Wakim and Fleisher, 1963; Fleisher and Wakim, 1963). The plasma level of each isoenzyme therefore, does not directly reflect the rate of its cellular release. As a result, plasma GOT (PGOT) is normally represented by the cytoplasmic isoenzyme of GOT (Gabrielli and Orfanos, 1968).

No experiments have been performed to determine the rate of clearance of the isoenzymes of creatine phosphokinase (CPK) from the plasma. However, the fast
removal of plasma CPK (PCPK) from the plasma following a myocardial infarction suggests a plasma half-life of about 12 hours (Griffiths, 1966).

The LDH isoenzymes from rabbit, dog, sheep, and mice exhibit plasma half-lives of eight hours for LD5 to 48 hours for LD1 (Boyd, 1967; Mahy, Rowson, and Parr, 1967; Posen, 1970). The intermediate isoenzymes show plasma half-lives between these two values. After blocking the RES in mice, LD5 is removed at the same rate as LD1, suggesting that under normal circumstances the RES removes only LD5. The reason for this selective isoenzyme clearance is not understood (Mahy, Rowson, and Parr, 1967). The large variation in the rates of LDH isoenzyme clearance from the plasma means that the plasma level of each isoenzyme does not reflect its rate of release from tissues.

3. Plasma enzymes in pathology

 (i) Introduction

At least three basic mechanisms have been shown to play some role in the elevation of plasma enzyme levels in disease. There may be an increase in the rate of enzyme release from tissues, a decrease in the rate of enzyme clearance from the plasma, an increase in the rate of enzyme synthesis, or a combination of
two or more of these factors. If a disease state is accompanied by a decrease in the plasma level of a particular enzyme, it is usually the result of a decrease in tissue synthesis of that enzyme (Zimmerman and Seeff, 1970).

The most common cause of elevated plasma enzyme levels during disease is tissue necrosis. In acute pancreatitis, the plasma levels of several digestive enzymes is extremely high (Korn et al., 1962). Similarly, in acute hepatitis and myocardial infarction, enzymes of intermediary metabolism exhibit high plasma activities (White, 1960).

In neoplastic diseases the elevated levels of certain plasma enzymes may be related to the high activity of these enzymes in the abnormal tissue (Bodansky, 1959). Bodansky found that in some cases the degree of elevation of an enzyme in the plasma reflected the extent of the tumour.

In obstructive jaundice, the biliary excretion of leucine aminopeptidase and alkaline phosphatase is reduced, which results in elevated plasma levels of these enzymes (Gutman, 1959). Amylase, which is normally excreted by the kidney, is elevated in the plasma in renal failure (Janowitz and Dreiling, 1959).
Plasma levels of cholinesterase are low in patients with hepatic disease in which cholinesterase synthesis is reduced (Zimmerman, 1964). Decreased levels of plasma amylase are found in cases of malnutrition, or of prolonged hepatic or pancreatic diseases (Janowitz and Dreiling, 1959). Gastric mucosal atrophy usually results in a decrease in the plasma levels of pepsinogen (Bock et al., 1963).

Elevated plasma levels of several intracellular enzymes occur in several forms of muscular dystrophy. In the Duchenne form of muscular dystrophy, loss of cellular enzyme occurs as a result of an increase in permeability of the muscle cell membrane, rather than through necrotic damage (Siegel and Dowben, 1970).

The development of laboratory models of disease has been aided by the advances of clinical enzymology. A similarity between the plasma enzyme changes in a particular disease and in an experimental model indicates the probability that in both situations the same mechanisms are causing the cellular derangement. As a corollary, differences between the plasma enzyme responses to a clinical disease and to an experimental model indicate the existence of differences in the mechanisms promoting the enzyme release.
Plasma enzyme changes are often used as tools in the development of new clinical procedures, or in novel experimental situations, since changes in their composition can suggest the presence of subcellular alterations which could not necessarily be detected by conventional techniques.

(ii) **Cardiac disease**

A myocardial infarction usually occurs in a heart in which arteriosclerotic and fatty changes are already present. To this extent, myocardial infarction is a later stage of a progressive degenerative disease. It is in itself, however, an acute process, resulting from a period of myocardial ischemia, during which the area of heart muscle involved switches to anaerobic metabolism to meet the regular demand for energy. Within minutes this supply of energy becomes exhausted, and the individual myocardial cells lose their electrical and contractile functions. Depending upon the extent of the infarction, the electrical and contractile properties of the remaining tissue may be sufficiently normal for the maintenance of a reasonable cardiac output. If recovery does occur, the infarcted tissue rapidly undergoes necrosis and, over a period of weeks, is
replaced by scar tissue.

The behaviour of various plasma enzymes following myocardial infarction has been studied extensively. There is an almost immediate release of cytoplasmic enzymes from the ischemic myocardium. Depending upon its rate of clearance from the plasma, the peak plasma activity of a particular enzyme is reached from several hours to several days after the infarct (Vincent and Rapport, 1965; Stranzoid and Clayton, 1961; Sorenson, 1963).

Experimentally two techniques have been used in the dog to promote myocardial ischemia and infarction. In the first method, loose ties are placed about a major coronary artery and exteriorised. Several days later, in the closed chest animal, tension is placed on the ties thus occluding the artery. In this manner, Ruegsegger et al. (1956) demonstrated that myocardial ischemia, resulting in marked electrocardiographic abnormalities, did not produce any changes in serum GOT (SGOT) unless there was some accompanying myocardial necrosis. In these same experiments, they showed that the extent of the infarction could be estimated from the magnitude of the SGOT response.
Agress et al. (1955) observed a 20 to 30-fold increase in SGOT following bead embolisation of the coronary arterioles in the closed chest dog. There was a close correlation between the increase in SGOT and the extent of infarction.

(iii) Trauma

In the earlier experiments on myocardial infarction, it was noticed that small, but significant, increases in certain plasma enzymes were associated with the preparatory thoracic surgery (Agress et al., 1955). While these plasma enzyme changes were usually smaller than those following myocardial infarction, they were of the same magnitude as those observed after some forms of exercise. Not surprisingly, the primary source of the enzyme was considered to be the skeletal muscle transected during surgery.

In 1957, Albaum and Milch examined the effects of X-irradiation, tourniquet ischemia and crush injury of rabbit hind limb skeletal muscle on SGOT. They had previously shown that all three treatments produced a similar degree of breakdown of muscle tissue elements as determined histologically. Application of a tourniquet to a hind limb for 3 hours caused the largest increase in SGOT (150%), which peaked at 6 hours following
removal of the tourniquet. SGOT returned to control values 48 hours later. X-irradiation and crush injury resulted in small changes in SGOT (50% and 28% respectively), with peak activity reached at 2 to 3 hours after the intervention. These findings suggest that it is inadvisable to make conclusions concerning changes in plasma enzyme activity, based solely on the presence and extent of histological changes.

In a recent study, the changes in SCPK in patients undergoing major surgery was examined (Dixon, Fuchs and Ebert, 1971). In 58 patients undergoing thoracic, cardiac, or abdominal operations, SCPK was elevated 8-fold, 2 to 4 days after surgery. Normal levels were attained 6 days later.

Lawson et al. (1971) investigated the effect of muscle trauma, caused by high velocity gunshot wounds in thigh muscle, on the activity of several plasma enzymes. SCPK, SGOT, and SAld all demonstrated significant increases, reaching a maximum 24 hours after the injury. These enzymes returned to normal values 4 to 5 days later. SLDH and SAlkP increased only slightly in response to the trauma.

In general, it is clear that traumatic tissue damage of any magnitude could result in changes in the
activity of some plasma enzymes. Therefore, in experiments on plasma enzymes, adequate controls should be run since the surgical preparation alone may promote tissue enzyme release.

4. **Plasma enzyme changes in exercise**

   (i) **Introduction**

   Since 1960, several studies have been conducted on the plasma enzyme changes associated with exercise. As a consequence of the diverse forms of exercise; the inconsistency of sampling times, and the variety of assay procedures in use, a clear picture of the basic plasma enzyme response to exercise has not yet been achieved.

   Two assays for the estimation of plasma transaminases are in common use. The earlier spectrophotometric assay was developed by Karmen (1955). Reitman and Frankel (1957) adapted this assay for colorimetric determination. In this latter method, the rate of formation of oxaloacetate from glutamate or alanine, as catalysed by the appropriate transaminase, is determined. 2,4 dinitrophenylhydrazine is added to the reaction medium, and the subsequent formation of the hydrazone of oxaloacetate is measured colorimetrically.
The hydrazones of any ketone bodies present in the plasma sample are also formed, and consequently falsely high estimates of transaminase activity can be obtained. The considerable increase in the plasma concentration of ketone bodies which is known to occur following exercise, precludes discussion of work in which the Reitman and Frankel assay has been used (Jennett, Johnson, and Rennie, 1972). (When the transaminases have been assayed using the colorimetric technique, the terms GOT* and GPT* will be used. Where determined spectrophotometrically, the transaminases will be referred to as GOT and GPT.)

During exercise, the arterial haematocrit usually increases in both man and other mammals. It is important, therefore, to establish whether the observed changes in plasma enzyme activity are purely a consequence of haemoconcentration.

In man, haemoconcentration during exercise is due to loss of fluid from the circulating blood. Many authors have assumed that the percentage changes in haematocrit and plasma enzyme activity should be equal if the haemoconcentration is brought about solely by a decrease in plasma volume (Block et al., 1968; Schwartz, Carroll, and Douglas, 1971). This reasoning is clearly
incorrect since haematocrit is calculated in terms of the proportion of red blood cells in whole blood, while proteins are measured in terms of their concentration in the plasma fraction alone. Thus, when haemo-concentration occurs as a result of a decrease in plasma volume, there is a greater increase in plasma protein concentration than in haematocrit (van Beaumont, Greenleaf, and Juhos, 1972).

In most non-primate mammals the situation is more difficult to examine. During many forms of stress, splenic contraction will occur (Barcroft et al., 1925). Thus there may be a rise in haematocrit without any accompanying change in blood plasma volume or plasma protein concentration.

(ii) Man

The first detailed investigation of changes in serum enzyme activity during exercise was carried out by Cantone and Cerretelli (1960). Following a 30-minute period of slow running (5km/h), SAl was elevated 100% above control levels. SAl levels had returned to control values within 75 minutes. After 5 days of training, the resting levels of SAl were increased considerably above control. Two days rest was required for the SAl levels to fall back to normal. Similarly,
a prolonged 50 day training period resulted in elevated resting levels of SAlid. During this conditioning, the magnitude of the SAlid response to exercise was markedly reduced.

Schlang (1961) studied the changes in SGOT* and SGPT* during two minutes of swimming, five minutes of running, and ten minutes of rowing. After all three types of exercise, SGOT* and SGPT* were raised 100% and 50% above control, respectively. In samples obtained 12 hours later, SGOT* and SGPT* had returned to control values.

One year later, Fowler et al. (1962) found that the serum levels of GOT*, GPT*, LDH and MDH were elevated by running at 5mph for 15 minutes. All serum enzymes demonstrated control activity within 15 to 30 minutes after exercise. The increases in SGOT*, SGPT*, and SAlid were 100%, 50%, and 50% of their control values, respectively. In control samples SLDH and SMDH showed a wide range of activity. The size of the exercise induced changes in SLDH and SMDH, when expressed in absolute values, fell within a narrow range.

In a later study (Fowler et al. 1968), it was demonstrated that the changes in SGOT*, SGPT, and SLDH were related to the intensity of the work load, for an
exertion of constant duration. Furthermore, SLDH was only elevated after exercise at the heaviest work-load. The proportional increase in each isoenzyme was similar. Neither SAlD nor SSDH showed any real change following exercise. Fowler et al. (1968) suggested, on the basis of these results, that the plasma enzyme increase during exercise originated from skeletal muscle rather than from liver.

The effect of a prolonged bout of exercise was investigated by Halonen and Kontinen (1962). Following a 2 hour, 16 km walk, SLDH increased to over 150% of the control value. By the end of a 2-hour recovery period, SLDH had returned to control values. SGOT, SSDH, SMDH, and SAlD showed slight increases at the end of the walk, but since these enzymes demonstrated similar small increases in serum activity during a 2-hour control period, the exercise induced changes were considered negligible.

Studying the effects of even more prolonged exercise, Remmers and Kaljot (1963) found that SGOT increased 10-fold on the first day of a 15-day army training period consisting of long marches and obstacle course running. This high level of SGOT was maintained during the next 4 days. Following a partial reduction
of the daily work load, SGOT levels gradually fell towards normal over the next 10 days. The authors noted a correlation between the level of SGOT in an individual and his subjective estimation of muscle soreness.

Using the bicycle ergometer, Venerando et al. (1964) investigated the effect of work at maximum effort for periods of from 14 to 45 minutes. SGPT and SLDH showed only small inconsistent elevations following the exercise. In contrast, SAld, SCPK, and SGOT increased gradually following exercise, with peak values 5-fold, 10-fold, and 2-fold of control respectively, achieved 90 minutes after the cessation of exercise.

Nerdrum and Berg (1964) measured SGOT (using both spectrophotometric and colorimetric methods) and SLDH before and after a short, 1.5-minute maximal exertion (Swimming, bicycle ergometer, or sprinting), and before and after a long submaximal effort (15-km march, 1.5-hour run). Following the brief hard exercise, SGOT* levels were raised 50% above control values, while SLDH was unaffected. SGOT* levels returned to control within 50 minutes. The authors found that the 'increase' in SGOT* was false - almost entirely due to an increase in ketone bodies in the serum. Immediately after the prolonged exertion, SLDH levels were 20% greater than
control, reaching a value 25% greater than control, 45 minutes later. The changes in SGOT and SGOT* were small and variable.

Vejjajiva and Teasdale (1965) and Griffiths (1966) investigated the effects of hard prolonged exercise on SCPK levels. Eighty minutes of exercise (rowing or Rugby football) resulted in a 2- to 3-fold elevation in SCPK, 30 minutes after the exercise was terminated (Vejjajiva and Teasdale, 1965). Griffiths (1966) observed a 7-fold and a 24-fold elevation in SCPK after 25 and 53 miles of continuous walking, respectively.

The effects of extended exercise on serum enzymes in trained and untrained individuals was studied by Ahlborg and Brohult (1966,1967). The exercise consisted of a 90 to 100-minute performance at maximal effort on a bicycle ergometer. Blood samples were obtained every day for 14 days following the effort. SGPT was unaffected by the exercise. Directly after the exercise, SGOT showed a 25% elevation above control level in all individuals. Control values were reached after 1 and 3 days in trained and untrained subjects, respectively. There was a 30% to 50% increase in SCPK directly following the effort. Peak levels of SCPK were reached 24 hours later, with levels 4-fold and
6-fold their control values in trained and untrained subjects, respectively. At the cessation of exercise, SLDH was elevated above control by 14% in trained, and 30% in untrained subjects. In the trained individuals SLDH rose slightly over the first day of recovery, and then returned to normal by the fourth day after exercise. SLDH continued to rise in the untrained individuals, with a maximum at 50% above control values reached on the fifth day after exercise. In these subjects, SLDH did not return to control values until the 13th day of recovery. Serum levels of OCT did not demonstrate an immediate response to the exercise. However, a gradual rise in SOCT occurred on the 3rd day following exercise, with a maximum level reached on the 7th day of recovery. The rise in SOCT was far greater in the untrained than trained subjects (238% vs 66%).

In this study, an effect of training upon the resting values of the serum enzymes was not evident, with the exception of SOCT. In the trained group, the pre-exercise levels of SOCT were 50% higher than those of the untrained group.

In 1968, Nuttall and Jones studied the effect of weight-lifting on the activities of SCPK and SGOT in men and women. Six minutes of exercise resulted in an
elevation of SCPK which peaked at a level 4-fold that of control, at 24 hours after exercise, and remained elevated for a further 48 hours. SGOT showed only a very small increase. Following a period of weight-training, SCPK was only slightly elevated after 6 minutes of weight lifting. The authors noted that muscle soreness was experienced during the post-exercise period prior to training, but that soreness did not accompany the lifting session following training. Resting levels of SCPK and SGOT were not altered by training.

The effect of a 30-minute period of ergometer exercise on serum amylase levels was studied by Drigoli et al. (1969). Seven minutes of exercise at 100 watts preceded 23 minutes of work at 50 watts. Blood samples were taken before, and at intervals during and after the exercise. A 65% increase in serum amylase occurred after the first 7 minutes of exercise. After a reduction of the work load, serum amylase was progressively lower at 20 minutes (58%), and 30 minutes (28%) of exercise. After 10 minutes of recovery, the serum amylase activity had returned to control levels.

Block et al. (1969) investigated changes in the levels of various enzymes in the serum during exhaustive exercise. The exertion consisted of a 30-minute effort
on a bicycle ergometer, with the work load increasing progressively until a terminal heart rate of 160 to 175 beats per minute was achieved. SGOT* and SGPT* had increased 50% and 27% above control values respectively at 15 minutes after the exercise. Both serum transaminases returned rapidly to control values within 1 to 2 hours. SCPK demonstrated a maximum increase of 140% between the 30th and 60th minutes of recovery. There was a slow return of SCPK levels to control by the 4th to 6th hours of recovery. SLDH was elevated following the exercise, with a peak value, 45% above control, at the 60th minute of recovery. Up to 24 hours was required for SLDH to return to normal levels. The individual LDH isoenzymes varied in their response to the exercise. LD₃, LD₂, LD₃, and LD₄ + LD₅ increased by 66%, 15%, 79%, and 285%, of their control values, respectively. However, when the changes were expressed in absolute values, LD₁ showed the largest increase. The magnitude of the increase of the four other LDH isoenzymes in the serum was similar.

The response of SLDH to strenuous long distance running was investigated by Rose et al. (1970a, 1970b). Total SLDH increased by approximately 30% in four out of five trained subjects after a 10,000-M run. All
5 isoenzymes of LDH showed similar absolute increases in their serum activity, but LD₄ and LD₅ demonstrated the largest proportional changes.

Following a marathon run, SCPK and total SLDH were elevated by 100% and 50% of control, respectively. All 5 isoenzymes of LDH in the serum increased significantly during the run. In absolute values, the increase in serum LD₅ was twice that of LD₂, LD₃, and LD₄, and four times that of LD₁. When the increases were expressed as a percentage of control values, LD₅ exhibited a 500% increase; LD₄, 100%; LD₃, 33%; LD₂, 25%; and LD₁, 11% respectively.

More recently, two studies have been conducted to test for a relationship between physical conditioning and serum enzyme levels. Hunter and Critz (1971) studied the effect, on plasma enzymes, of maximal and submaximal exertion, before and after a 10 week training period. Following a short, 10-minute ergometer ride at maximal effort, PCPK, PGOT, and PLDH levels increased 36%, 100%, and 9% above control levels, respectively. After training, the increases in PCPK, PGOT, and PLDH following the same effort were reduced to 11%, 65%, and 0% of control values. Training had a similar effect on the plasma enzyme response to submaximal exertion. PCPK showed an 18.5% elevation before
training, but did not change after training. Training reduced the PGOT increase from 55% to 17.5% of the control value. PLDH did not respond to the submaximal exercise at either time. The authors report a slight rise in the resting levels (pre-exercise values) of PLDH over the training period. However, the raw data do not support this conclusion (Hunter, 1970), and it appears that there were no significant changes, as a result of training, on the resting level of any of the serum enzymes studied.

Schwartz, Carroll, and Douglas (1971) looked for a relationship between the magnitude of the serum enzyme response to exercise and the physical condition (Max \( \dot{V}O_2 \)) of an individual. Using two different work loads on the bicycle ergometer (600 kpm/min, (20 min) and 720 kpm/min, (30 min)) they found small but significant increases in the serum levels of CPK, LDH, MDH, and GPT. However, there was no difference between the response elicited by either work load. While the magnitude of the increases in the serum levels of the different enzymes correlated well with one another, and with the time to run 1,000 M, there was no correlation between the increase in the serum enzymes and Max \( \dot{V}O_2 \). However, it is doubtful whether any of the observed increases in serum enzyme activities were significant.
There was a mean increase in haematocrit of 7.8% following exercise. This would reflect a decrease in the plasma volume of approximately 16% (van Beaumont, Greenleaf, and Juhos, 1972). Since the largest increase in any serum enzyme was 16% (SMDH), all the observed changes in serum enzyme activity may have been due to a loss of fluid from the plasma, thus concentrating enzyme activity which was previously present.

Prolonged strenuous activity, in the form of 6 to 10 hours of cross-country skiing was studied by Refsum, Stromme, and Tveit (1972). SCPK and SGOT were both elevated after exercise (300% and 150%, respectively). Twenty four hours later, the levels of SCPK and SGOT were 600% and 200% greater than control values. It required four days for the serum levels of these enzymes to return to control. In contrast, SLDH was elevated immediately after the exercise, but had returned to resting values within 24 hours. The authors state that LD₄ and LD₅ showed the greatest proportional increases. No data was provided on the absolute changes of each LDH isoenzyme.

(iii) Dog

To date, plasma enzyme levels in the spontaneously exercising dog have been studied on only two occasions.
Bedrak (1965) measured the plasma activities of 8 different enzymes before, during, and after light exercise of long duration (2-hour walk, 4 km/h, 8% grade). SGOT*, SGPT*, and SPHI increased throughout the exercise (33%, 40%, and 68%), and were further elevated at the end of a 2-hour recovery period (84%, 58%, and 94%). There was a substantial increase in SLDH after the first 30 minutes of exercise (43%), with no further change during the remainder of the exercise or recovery periods. SAcidP, SAlkP, SAld, and SLipase all increased during the exercise (79%, 18%, 18%, and 22%). These four serum enzymes, with the exception of SLipase, returned to control values during the 2-hour recovery period.

Wagner and Critz (1968) investigated the effect of running exercise (45 to 60 minutes, 10 mph, 10° grade) on SCPK. The maximum exercise heart rate was 178 beats/minute; considerably lower than the maximum heart rate that can be achieved by the dog. At the end of the exercise, the mean heart rate was 168 beats/minute. There was a 450% elevation in SCPK following exercise, and this had further increased by the 30th minute of recovery (530%). Venous haematocrit increased from a mean of 43.6% before exercise, to 47.5% at the 3rd minute of recovery; a 9% increase in haematocrit. By the 30th minute of recovery, venous haematocrit had returned to 43.7%.
(iv) **Rat**

Investigators have exercised rats in two different ways; swimming and running. In the rat, swimming describes the effort the animal performs to remain afloat. To increase the work-load, some investigators apply weight to the animal's tail. While the parameters of running exercise are easier to control, it is harder to coerce the rat to perform. However, various devices have been designed to encourage a rat to run steadily on a treadmill. These devices have had limited success.

Highman, Altland, and associates have used running exercise on numerous occasions, to study the effects of exercise on serum enzymes in the rat. Following a 16-hour walk, SGOT* and SGPT* levels were elevated to 5-fold control, and returned to normal values within 3 days (Altland and Highman, 1961). SLDH and SAld increased to 2-fold control and had normalised by the following day. SAlkP fell below control values during the exercise. Histological examination showed fatty changes in skeletal muscle, heart, liver, kidney, and adreno-cortical tissues. In some animals, necrotic lesions were observed in skeletal muscle fibres. The liver glycogen stores were almost entirely depleted.
In 1963, Highman and Altland repeated this study, investigating the effect of 6-, 12-, and 16-hour walks and of training, on serum enzyme levels and tissue changes. The 6-hour walk resulted in a 2-fold increase in SGOT*, 4-fold increase in SAld, and in some animals a small change in SLDH. Fatty changes were observed in skeletal muscle immediately after, and at 15 and 24 hours following the exercise. No fatty changes were observed in heart or liver tissue. After the 12-hour walk, SGOT* was elevated 2- to 3-fold; SLDH, 2-fold; and SAld, 3-fold. The effect of the 16-hour walk was similar to that previously described except that the SGPT* response was smaller (50% increase), and SAld increased to 4-fold control. SAlkP decreased during the 6-, 12-, and 16-hour walks. However, a similar decrease in SAlkP was observed in control fasting animals. Although there was no direct correlation between the degree of skeletal muscle fibre necrosis and the serum enzyme elevation, all animals with severe lesions demonstrated high serum enzyme levels after exercise. However, some animals with only minor necrotic changes exhibited serum enzyme levels of the same magnitude. Training for 120 days completely abolished the serum enzyme and histological changes evoked by a 16-hour walk.
Sangster and Beaton (1966) found an 85% increase in PMDH following a 2-hour swim; PGPT levels were not altered. After 4 weeks of training, 2 hours of swimming did not result in any increase in PMDH.

In 1967, Papadopoulos, Leon, and Bloor showed that the standard method of preparing serum from rat blood obtained by cardiac puncture, induced the release of LDH isoenzymes LD₁, LD₂, and LD₅ from various tissues. By preparing plasma from carefully withdrawn and heparinised blood samples, this problem was avoided.

Using this technique, the same group studied the effect of a 4-hour swim on the isoenzymes of LDH in the plasma (Papadopoulos, Leon, and Bloor, 1968). Total PLDH increased 4-fold, with the increase almost entirely due to changes in LD₁, LD₂, and LD₅. Six and 12 hours, respectively, were required for LD₅, and LD₁ and LD₂ to return to normal levels. Training for 4 hours per day gradually reduced the PLDH response to exercise. However, LD₁ and LD₂ changes were more persistent than those of LD₅.

Doty, Bloor, and Sobel (1971) repeated the previous experiment, providing additional data on the precise variations in each isoenzyme of LDH in the plasma. Total PLDH increased to almost 5-fold control.
LD₁ and LD₂ increased 4-fold (absolute changes of 45 IU/L and 70 IU/L, respectively). LD₅ increased by 75 IU/L to a level 24-fold control. During recovery, all three isoenzymes showed a rapid return to normal values with plasma half-lives of between 1 and 2 hours.

5. **Mechanism of plasma enzyme changes and release of tissue enzymes**

   (i) **Introduction**

   In physical effort of long duration or of high intensity, skeletal muscle might be subject to some form of trauma. However, it is unlikely that damage to skeletal muscle tissue results from the majority of exercise sessions.

   It has been postulated that an increase in cell membrane permeability to enzyme molecules, may occur during exercise. Catecholamine secretion and hypoxia have both been considered as factors coinciding with and possibly eliciting the release of enzymes from tissues during exercise through this mechanism (Loegering and Critz, 1968; Loegering and Critz, 1971).

   Blood flow to the exercising muscles may be limiting oxygen supply, thus causing a 'relative hypoxia' within the actively contracting muscle (Loegering and Critz, 1971). Recently, the concept of a cardiorespiratory
limit to the oxidative metabolism of exercising muscle has been superceded by that of a cellular limit to oxidative performance (Holloszy et al., 1971). If this is indeed the case it is unlikely that skeletal muscles, even during maximal exercise, are embarrassed by a circulatory deficiency.

In acute severe total body hypoxia, as exists at high altitude (hypobaric chamber), or during the administration of low oxygen mixtures, an increase in the plasma activity of some enzymes does occur (Highman and Altland, 1960; Altland, Highman, and Garbus, 1964; Asvall, 1960). The plasma enzyme response to acute hypoxic episodes can be prevented by the prior administration of sympathetic blocking agents, indicating that the secondary release of catecholamines during hypoxia is a principal promoter of the enzyme response (Altland, Highman and Garbus, 1964). Since there can be very little physiological similarity between total body hypoxia and the 'relative hypoxia' purported to exist in exercising skeletal muscle, this topic will not be further discussed.

In exercise there is an increase in the rate of adrenal catecholamine secretion and in the release of noradrenaline from postganglionic sympathetic fibres, giving rise to an increase in the circulating levels of catecholamines. These in turn may bring about regional
vasoconstriction and ischemic changes within tissues with a consequent release of enzymes (Loehering, 1965).

Owing to the lack of sensitivity of the assays for catecholamines then available, earlier workers had to determine catecholamine secretion values from the urinary excretion figures. A 10-fold increase in the excretion of noradrenaline following strenuous exercise was demonstrated by von Euler and Heller (1952). The excretion of adrenaline was not increased. After lighter exercise loads neither catecholamine was excreted at a greater rate than at rest.

The specific secretion of noradrenaline during exercise has been observed in several studies (Gray and Beetham, 1957; Vendsalu, 1960). Both Meehan and Jacob (1957) and Vendsalu (1960) found a correlation, during exercise, between the increase in circulating noradrenaline and the work-load. Gray and Beetham (1957) demonstrated a 3-fold rise in the plasma level of noradrenaline following an exhaustive effort. They also found that subjects who reacted emotionally during work had an increase in the plasma levels of adrenaline while those individuals who had the greatest difficulty in completing the exercise had the highest plasma noradrenaline levels. At the cessation of exercise, plasma catecholamine levels fell
to resting values within 5 minutes.

A combined stress of prolonged exercise (12 days walking) and high altitude hypoxia (4,500 M) caused a marked increase in the plasma levels and urinary excretion of noradrenaline (Cunningham et al., 1965). Adrenaline levels showed very little alteration from control.

(ii) **Catecholamines and plasma enzymes**

The acute effects of catecholamines on the level of various enzymes in the plasma has been investigated by several workers. In these studies the dosage of catecholamine used was often sufficient to cause tissue necrosis; increases in the plasma level of an enzyme could be accounted for by a loss of enzyme from necrotic tissue.

Highman, Maling, and Thompson (1959) found that in unanaesthetised dogs, infusion of large amounts of either adrenaline or noradrenaline caused an elevation in SGOT*, SGPT*, and SAlkP. Both liver and myocardial tissue exhibited areas of patchy necrosis. Larger increases in the serum enzymes were obtained following subcutaneous injection of comparable doses of adrenaline. Administration by this route, however, resulted in less evident necrotic changes in heart and liver tissue, but scattered lesions were observed in skeletal muscle. This difference in
serum enzyme and histological alteration, dependant upon the route of administration of the catecholamines, was attributed to the difference in the evoked cardiovascular response. After intravenous administration blood pressure increased; this, they suggested, reflected a strong peripheral vasoconstriction which was accompanied by ischemia, tissue necrosis and enzyme release. Subcutaneous administration, on the other hand, did not cause marked systemic cardiovascular changes, but probably caused local vasoconstriction and ischemia, with scattered necrosis and enzyme release. Their findings suggested that histologically-demonstrable necrosis may not be the only prerequisite for the escape of enzymes from tissues.

Dibenzyline, an adrenergic alpha-receptor blocking agent, prevented both the histological changes and the serum transaminase response to the subcutaneous injection of adrenaline. The SAlkP response, however, was not affected.

Other studies have implicated the sympathetic nervous system in the serum enzyme response to specific types of stress. Restraining conscious rats for 6 hours by securing them, with ties, to a board, resulted in an elevation of both SGOT* and SGPT* (Peral, Balazs, and Buyske, 1966). Subcutaneous ACTH or the administration of corticosterone had no effect on the serum transaminase
levels, while subcutaneous noradrenaline and adrenaline both brought about increases in serum transaminases comparable to those seen after restraint. The combined administration of adrenaline and corticosterone caused a larger response than the effect of adrenaline alone; thus suggesting a permissive role for corticosterone. Following adrenalectomy, there was only a minimal serum transaminase response to 6 hours of restraint. However, the administration of catecholamines or corticosterone gave responses similar to those observed in normal rats. The combined effect of restraint and corticosterone administration resulted in a considerable elevation of the serum transaminases; again a permissive role of corticosterone was evident.

Peral, Balazs, and Buyske (1966) found that the alpha blocking agent phenoxybenzamine was completely effective in preventing the SGOT response to adrenaline, but only partly effective in reducing the SGOT response to restraint. Dichloroisoproterenol, a beta-blocker, did not compromise the SGOT response to adrenaline. Thus the adrenergic alpha-receptor appears to be involved in the serum enzyme response to stress.

In 1968 Loegering and Critz studied the effect of a 90 minute intravenous infusion of noradrenaline on the levels of SGOT in the anaesthetised dog. Using
384 ug/kg of noradrenaline there was a steady rise in SGOT throughout the infusion period. SGOT continued to rise at a steady but slower rate following the infusion (10 hours). The pre-administration of the alpha-blocking agent phenoxybenzamine prevented the rise in SGOT which previously occurred during the infusion. However, in the post-infusion period SGOT increased rapidly and reached the same levels observed in the unblocked dogs. Propranolol, a beta-blocking agent, partly reduced the SGOT response during infusion, but during the post-infusion period SGOT gradually rose to attain control, unblocked, levels. The combined administration of both alpha and beta blockers entirely prevented the SGOT response to noradrenaline.

The authors attempted to explain differences in the SGOT responses, by the different effects of various combinations of the three drugs on the cardiovascular system. They considered that regional hypoxia, due to vasoconstriction promoted enzyme release, and suggested that the liver was an unlikely site for this to occur since there is a paucity of alpha receptors in the hepatic vascular bed. However, Highman, Maling, and Thompson (1959) showed definite histological changes in the liver following catecholamine administration.
Furthermore, these latter authors and Peral, Balazs, and Buyske (1966) found GPT* a predominantly liver enzyme, to be markedly elevated in the serum following catecholamine administration. Since Loegering and Critz (1968) did not check for tissue necrosis this problem remains unresolved.

(iii) **Release of intracellular enzymes from isolated tissues**

There have been several studies on the release of intracellular enzymes from isolated heart and skeletal muscle preparations. In these experiments, the tissue was either vascularity perfused, or bathed in a suitable physiological salt solution, and the change in enzyme activity of the perfusate or bathing medium determined. These techniques have two inherent problems; during preparation it is difficult to avoid microscopic damage and temporary hypoxia of the tissue. Both of these factors influence the release of enzymes from an isolated organ.

(a) **Isolated heart**

Butterworth et al. (1970) investigated the release of enzymes from the isolated perfused rat heart. The temperature of the perfusate was maintained at either 30°, 35°, or 37°C. At all three temperatures
there was a similar release of LDH. GOT was only released when the perfusate was warmed to 37°C. A one minute period of anoxia increased the rate of release of both LDH and GOT at 37°C, but not at the lower temperatures. The maximal rate of release was reached one hour after the anoxic episode.

In a more recent experiment, Butterworth et al. (1972) found that if the anoxic episode at 37°C was immediately followed by a reduction in perfusate temperature to 30°C, the post-anoxic increase in LDH release was prevented. However, if the perfusate temperature was at any subsequent time raised to 37°C, the expected release of enzyme occurred. These experiments show a marked temperature dependence of anoxia-stimulated enzyme release, possibly related to the rate of metabolism of the tissue.

(b) **Skeletal muscle**

In 1953, Zierler, Levy, and Andres found that all the enzymes in the glycolytic pathway were released into the bathing medium during incubation of the isolated rat diaphragm.

The diaphragm of a small mammal is suitable for the study of enzyme release since its thinness permits *in vitro* incubation. However, it is sufficiently thick
that the inner muscle fibres probably do not maintain a normal exchange of gases, proteins, and other metabolites (Zierler, 1957).

In a series of studies Zierler demonstrated that many variables could affect the release of aldolase from the rat diaphragm. He found that anaerobic conditions, lack of glucose in the medium, increased potassium in the medium, and transfer to a fresh medium (temporarily depressing metabolism), all resulted in a greater efflux of aldolase than in control (Zierler, 1956b). A reduction in temperature reduced the efflux (Zierler, 1956a). All these observations suggest that the muscle membrane permeability may be related to the rate of metabolism of the tissue.

The diaphragm is not a particularly satisfactory preparation since cut fibres are present. In later experiments Zierler (1957) studied the permeability of the rat peroneus longus muscle to aldolase. In addition to studying the effects of anoxia, glucose, and potassium, he determined the effect of metabolic inhibitors. Aldolase efflux was increased by dinitrophenol, cyanide, and iodoacetate. These experiments indicate that while muscle cell permeability to enzyme molecules may depend on the metabolism of the tissue, it is probably not an active process.
In the same series of experiments, Zierler studied the effect of muscle stimulation on permeability. The in situ peroneus longus muscle was repeatedly stimulated with tetanic stimuli for 10 minutes. The muscle was excised and the release of aldolase measured. The efflux of aldolase from the previously stimulated muscle was several-fold that from a paired control. Furthermore, following stimulation the aldolase content of the stimulated muscle was 10% less than in the paired control.

Dawson (1966) examined the efflux of 7 enzymes, primarily of cytoplasmic origin, from the biceps brachii muscle of the chicken. The rate of release was calculated as a percent of the total original tissue content of enzyme. All seven enzymes were released at approximately the same rate, despite a large variation in their molecular weights. Furthermore, each isoenzyme of LDH was released at a similar rate; a surprising finding in view of the different cellular location of each isoenzyme. Muscle stimulation did not alter the rate of release of any of the enzymes.
6. The lymphatic system

(i) Lymph formation

All the cells comprising a tissue are bathed by the interstitial fluid. Exchange of metabolites takes place between the intracellular and interstitial fluid, and further exchange occurs between the interstitial fluid and the circulating blood at the capillary level.

Interstitial fluid is formed as the ultrafiltrate of blood through the endothelial walls of the vascular exchange vessels. The composition of interstitial fluid is similar to blood plasma, with the exception of the protein concentration which is determined by the permeability of the vascular bed. Chemically it differs from blood plasma in that it is an intermediate in the pathway of diffusion of cellular metabolites to and from the blood.

Under normal conditions there is a slight net production of interstitial fluid, which is explained on the basis of a partial imbalance between hydrostatic and osmotic forces at the blood capillary and venule exchange vessels (Starling, 1896). In regions where there is a low vascular permeability to proteins (e.g. skeletal muscle), net interstitial fluid production is
low; the converse is true in regions of high vascular permeability (e.g. the liver). The excess plasma filtrate is continuously drained away from a tissue through the lymphatic system. Consequently there is, at rest, a small production of lymph of low protein concentration by skeletal muscle, and a large production of lymph of high protein concentration by the liver.

In the healthy animal, substances leaving the cell enter the interstitial fluid and small molecules are able to diffuse into nearby blood capillaries. These exchange vessels are relatively impermeable to large molecules which are therefore more likely to follow the gradual movement of interstitial fluid into nearby lymphatic capillaries. Thus enzyme molecules escaping a cell are most likely, in the healthy animal, to enter the circulating blood via the lymphatics. Under certain physiological or pathological conditions, enzyme molecules may be able to directly enter the capillaries serving that organ.

(ii) Lymphatic drainage in mammals

On each side of the body, three lymphatic ducts drain into the veins at the base of the neck; a cervical or tracheal duct drains the head and neck, and a sub-clavian duct drains the forelimb and some of the rostral
thoracic musculature. Embryologically the third pair of ducts pass caudally through the thorax to the abdomen; each draining one side of the body. During development, the left duct takes over drainage of the abdomen and hind limbs, and the right duct drains the lungs, heart and thoracic musculature. Thus, in the adult mammal, everything posterior to the diaphragm is drained by the thoracic or left duct (Miller, 1964).

Excess fluid within the peritoneal cavity is removed through a special system of pores in the peritoneal surface of the diaphragm. In the cat, the majority of excess peritoneal fluid (80%) is drained by the right duct, with the remainder finding its way into the thoracic duct (Courtice and Steinbeck, 1950).

Since, during development, there are considerable changes in the lymphatic drainage system, it is not surprising that there are many variations in the lymphatic anatomy of the adult. Anastomoses between the thoracic and right duct systems can be demonstrated both anatomically and physiologically. It is important to assess these anastomoses in any experimental work on the lymphatic system. Even in the adult, the lymphatic system is very amenable to the formation of collaterals and anastomoses. In an adult, right duct to thoracic
duct anastomosis can be induced by ligation of one system (Blalock et al., 1937).

In the dog the principal lymphovenous junctions may be situated within a centimetre of the junction of the internal and external jugular veins. Occasionally each of the three ducts enters the venous system separately. More often there is a considerable anastomosis of these vessels before the lymphovenous junction, and from one to many lymph vessels may finally enter the vein(s) (Miller, 1964).

A further complication is that there may be lymphovenous communications at sites other than at the base of the neck. Following ligation of the thoracic duct, lymphovenous connections have been noted in the abdomen of both rat and dog (Threefoot, 1965). However, in a recent study on the dog, labelled albumin was injected centripetally into the lymphatic of a hind limb. Over 97% was recovered in the thoracic duct (Patterson et al., 1958). It is generally believed that although lymphovenous communications can be demonstrated at sites other than the base of the neck, physiological anastomoses between different lymphatic systems and extraordinary lymphovenous communications are the exception in the normal adult animal (Yoffey and Courtice, 1970).
(iii) Enzymes in lymph

Despite the fact that the probable source of enzyme in lymph is the interstitial fluid bathing the tissues, there is only one report providing data on the enzyme activity of interstitial fluid (Sacks et al., 1970). They obtained samples of interstitial fluid from anaesthetised dogs in which capsules had previously been implanted in subcutaneous tissue (Guyton, 1963). Creatine phosphokinase levels in the plasma, thoracic duct lymph, and interstitial fluid were 6.6, 18.0, and 29.8 units/ml (n = 3), respectively; acid phosphatase levels were 0.49, 0.92, and 0.77 units/ml.

That enzymes are present in lymph was known as early as 1845, when both Bernard and Magendie noticed that lymph from the thoracic duct possessed the ability to split starch. They recognised diastase (amylase) to be the substance responsible for this fermentation process. Several studies on the diastasic action of body fluids culminated in a comprehensive report by Carlson and Luckhardt, (1908). Their findings, though not often mentioned in current literature, provide a basis to all subsequent studies in this field.

They found that the following fluids demonstrated diastasic activity in a decreasing order; serum, thoracic
duct lymph, and limb or neck lymph. Following an intravenous injection of the lymphagogic agent peptone, amylase activity in thoracic duct lymph increased, in neck lymph remained unaltered, and in serum was either unchanged or slightly raised. In no case was thoracic duct lymph activity greater than serum activity. There was no change in serum amylase activity in dogs when the diet was changed from one of mainly protein, to one predominantly carbohydrate in composition. In two cats, following pancreatectomy there was no change in serum amylase. In contrast, Schlesinger (1908) had found that pancreatectomy in the dog resulted in a complete disappearance of amylase activity from the serum.

Carlson and Luckhardt summarised their findings in the following statement:... "All the facts so far at hand seem to point to the conclusion that the blood and lymph diastases are 'discards' of the tissues in general. When once in the body fluids, these substances are in all probability on the road to destruction or elimination, and serve no further essential end in body economy, at least so far as regards their starch-splitting power."

This literature review failed to reveal any further work on the enzyme activity of lymph until 1948, when Flock and Bollman studied the enzyme activity
of intestinal lymph during fasting and feeding. Earlier studies had indicated that during fasting serum alkaline phosphatase (SAlkP) levels were reduced, and that subsequent administration of a carbohydrate meal in dogs, and of a fatty meal in rats, raised the SAlkP levels back to their normal values (Bodansky, 1934; Weil and Russell, 1940). Their findings indicated that the intestinal mucosa, which has the highest AlkP activity of any tissue in the body, may be the principle source of SAlkP.

Flock and Bollman (1948) found that, in rats, during fasting, the activity of SAlkP in intestinal lymph was low. Feeding a fat-free meal caused an increase in the lymph activity of AlkP; a fatty meal brought about an even larger increase. The activity of AlkP in intestinal or thoracic duct lymph rose to much greater levels following a fatty meal than did SAlkP in intact rats. Continuous removal of intestinal lymph resulted in a considerable reduction of SAlkP. Thus it appears that in the rat, the normal level of SAlkP is maintained by delivery of AlkP rich intestinal lymph.

In a later study, Flock and Bollman (1950a) demonstrated that the presence of bile in the intestinal tract was required to obtain an increment in lymph AlkP following feeding. The bile itself, however, was not
the source of AlkP, but in some way promoted the release of AlkP from the intestinal mucosa.

These studies prompted the investigation of the behaviour of the digestive enzymes in intestinal lymph and plasma (Flock and Bollman, 1950b). In the rat, the activity of amylase in intestinal lymph was found to be less than that in plasma. Continuous drainage of lymph did not affect the plasma amylase levels. Tributyrinase activity of intestinal lymph was similarly less than that of plasma. However, a fatty meal increased its activity in intestinal lymph. External drainage of intestinal lymph did not affect the plasma tributyrinase levels.

The enzyme content of lymph during normal and abnormal pancreatic function has been the subject of more recent work. Bartos, Brzek, and Groh (1966) studied the effects of intravenously administered secretin and pancreozymin on the amylase and lipase levels in the serum and thoracic duct lymph of 40 human subjects. Basal levels of lipase and amylase were slightly lower in the lymph than in the serum. Secretin did not affect lymph flow, but the levels of amylase increased above those in the serum. Pancreozymin, and pancreozymin + secretin caused marked increases in lymph flow. Lymph amylase levels were unaffected by pancreozymin, while
pancreozymin and secretin combined resulted in a much greater increase in lymph amylase than brought about by secretin alone. A combination of secretin and morphine caused a similar response to the combination of secretin and pancreozymin.

In ten patients, a simultaneous administration of secretin and pancreozymin was given on two different occasions; (a) while thoracic duct lymph was continuously drained; (b) with the thoracic duct intact. Drainage of lymph from the thoracic duct resulted in lower levels of serum amylase. The authors suggested that during maximal pancreatic stimulation, ductal pressure is sufficiently high that there is a considerable escape of pancreatic digestive enzymes into the lymph.

A similar lymphatic drainage of pancreatic enzymes was observed by Kochner and Sharafislamov (1970). Acute pancreatitis was induced in dogs by injecting autogenous bile into the main pancreatic duct. Thoracic duct lymph and serum were analysed intermittently over the next 6 days. Lymph flow increased almost immediately and remained high from 6 to 72 hours after the initiation of pancreatitis. There were increases in both the amylase and lipase levels of lymph and serum. Amylase levels in the lymph increased to values 5-fold above
serum levels, while lymphatic lipase levels were approximately 25% higher than serum values. Although lymphatic transport of enzymes was evident, drainage of thoracic duct lymph did not modify the response of serum amylase or lipase to the experimental pancreatitis.

Guglielmi et al. (1963) found considerable increases in the serum and thoracic duct lymph activities of GOT and GPT resulting from ischemia of the liver after ligation of the hepatic artery in the anaesthetised dog. The serum and lymphatic activities of aldolase were not altered by this procedure. The activity of the transaminases in the lymph reached levels 2- to 3-fold those in the serum. However, the lymphatic transport of enzymes away from the ischemic liver was probably quantitatively unimportant.

Malek, Kolc, and Skodova (1970) examined the effects of cardiac and skeletal muscle ischemia on lymphatic enzymes. A tourniquet was applied for 4 hours to the hind limb of a rabbit. Following removal of the tourniquet, serum and thoracic duct lymph samples were obtained for several hours. Levels of GPT, GOT and LDH reached values 4-, 12-, and 70-fold that of control; concurrent serum values were 1-, 2-, and 9-fold of control, respectively. These results indicate the
importance of the lymphatic transport of enzymes in this particular experimental model.

Transient (90-minute) ischemia of the dog heart was established by a ligature on the left coronary artery. Prior to ischemia no CPK was detected in cardiac lymph. Following ischemia, fairly high levels of lymphatic CPK were observed. LDH and MDH reached levels 12- and 270-fold control values within one hour after the ischemic episode. Serum enzyme levels were not determined in this latter experiment.

Lewis and co-workers have performed a series of investigations on the flow and composition of limb lymph under a variety of conditions. Two reports are pertinent to this review. In his first study, Lewis (1967) investigated the effects of burning and freezing on serum and lymph enzymes. Under anaesthesia, flow from limb lymphatics is minimal or non-existent. To obtain a steady flow, Lewis applied a rhythmic movement to the lower limb. Under control conditions the lymphatic activity of GOT, GPT, and LDH varied. In the majority of animals, lymphatic activities of LDH and GOT were greater than their plasma activities, while GPT exhibited a lower activity in limb lymph than in plasma. One hour of circulatory ischemia had only a
minimal effect on the enzyme activities in both limb lymph and plasma. In contrast, immersing the limb at either 60°C for 1 minute or 80°C for 20 seconds caused a marked increase in the activities of all three enzymes in the lymph, but only small increases in their plasma activities. There was no increase in the activity of three lysosomal enzymes in the lymph. Freezing the limb for 3 minutes, followed by thawing, resulted in a large increase in the activities of both lysosomal and cytoplasmic enzymes in the lymph. Again the changes in the lymphatic activity of the cellular enzymes was more marked than changes in their serum values.

In a later study, Lewis (1969) measured the enzymes in limb lymph and plasma of rabbits before and after thermal injury. The control levels of LDH and ICDH were similar in lymph and plasma. GOT exhibited a greater activity in the lymph than in the plasma, while the converse was true of GPT, and of the lysosomal enzymes B-glucuronidase, cathepsin, and acid phosphatase. Directly after burning there was an increase in the activity of the cytoplasmic enzymes in the lymph. PLDH was also higher. Two hours later, the activities of both cytoplasmic and lysosomal enzymes in the lymph were greater than control, with the exception of cathepsin.
At this time both LDH and GOT were elevated in the plasma. The control levels of acid phosphatase and glucuronidase were much higher in the plasma than in the lymph. Lewis concluded that an increase in capillary permeability rather than a de novo release was responsible for the increase in lymphatic lysosomal enzymes.

(iv) Lymph flow in exercise

The effect of exercise on lymph flow has been studied infrequently. White, Field, and Drinker (1933) cannulated a lymphatic in the hind paw of a dog. At rest there was no discernable lymph flow, but during walking or slow running, a steady flow of lymph developed. The lymphatic vessel that they cannulated drained the skin and connective tissue rather than muscle.

Similar increases in lymph flow can be obtained by passive movement of the limbs. Almost any type of movement will compress lymphatic vessels and propel lymph in a central direction. If there is a maintained, elevated, flow of lymph from an organ, it is unlikely that this pump-like mechanism is solely responsible. However, a temporary increase in lymph flow, especially when followed by a decrease in flow to below control values, is indicative of a passive transfer of lymph, rather than its de novo production.
During a study on liver lymph, Nix et al. (1951) found that during a slow walk (40 yards/minute), there was an increase in the flow of lymph from both the liver and the thoracic duct (83% and 270%, respectively). These authors only determined the flow of lymph during a very short period of exercise, and it was likely that much of the increase in lymph flow was due to a passive propulsion. An increase in the mean arterial pressure associated with the cardiovascular adjustments to exercise may have resulted in an increase in the lymph formation at the highly permeable intestinal and hepatic vascular beds. More precisely however, lymph production is dependent upon the mean capillary pressure, which is a function of mean arterial and venous pressures, and the pre- and post-capillary resistance.

A common finding is that lymph flow from the limbs is low or non-existent in the resting or anaesthetised animal. By cannulating the deep lymphatics of the thigh, a fairly pure sampling of muscle lymph can be made. Using this technique, Arturson and Kjellmer (1964) found that during rhythmic contraction of skeletal muscle, lymph flow initially increased to values several-fold control, but dropped to values at or below control within a short time. The increase in lymph flow under these conditions
was probably entirely due to a propulsion of lymph already formed.

Recent anatomical studies suggest that the extracapsular arrangement of skeletal muscle lymph capillaries may account for the findings in the previous study (Rodbard, 1971). During exercise there is an initial gain in water content by the muscle which would tend to raise the intracapsular pressure and compress the lymphatic capillaries preventing lymph flow, and counteracting further possible net fluid gain.

It appears that lymph flow from the skeletal muscle lymphatics is low both at rest and during exercise. While passive movements can stimulate a production of lymph, this lymph is probably cutaneous in origin and its production is associated with an active peripheral vasodilation.

7. Circulatory adaptation to exercise

Changes in many cardiovascular parameters accompany the initiation and maintenance of exercise. The primary function of these cardiovascular adaptations is to increase blood flow to the working muscles, the heart, and the heat exchange areas. At submaximal work-loads, a steady-state cardiac output is achieved which is directly related to the total body oxygen
consumption (Astrand et al., 1964).

The exercise induced changes in the basic cardiovascular parameters have been studied to any extent in only man and the dog. Most of the knowledge about the neural control of the cardiovascular response to exercise has been obtained from work on the chronically prepared dog. The increase in cardiac output during exercise is achieved through an increase in both heart rate and stroke volume in man but not in the dog (Bevegaard, Holmgren and Jonsson, 1960; Rushmer, Smith and Franklin, 1959). In man, mean arterial blood pressure increases only slightly during exercise (Bevegaard, Holmgren, and Jonsson, 1960), whereas in the dog there is a gradual rise in mean arterial blood pressure with increasing work loads (Cerretelli et al., 1964). In both man and the dog, total peripheral resistance drops during exercise as a result of skeletal muscle arterio-vasodilation, while there is an increase in the vascular resistance of the splanchnic bed (Folkow, 1960; Clausen, 1969).

Earlier workers considered the cardiovascular response to exercise to be entirely reflex in origin; initiated by a decrease in arterial blood pressure caused by the initial muscle contractions (Rushmer 1956). In the late 1950s, Rushmer and co-workers began a series
of experiments in which they demonstrated that there was central nervous control of the cardiovascular response to exercise. Using chronically-instrumented dogs, Rushmer, Smith, and Franklin (1959) noticed that the augmented stroke volume in exercise was achieved from a smaller end-diastolic volume. Furthermore, they found that by electrically stimulating selected points within the diencephalon of a chloralose-anaesthetised dog, they could elicit an overall cardiovascular response remarkably similar to that evoked by spontaneous exercise. These experiments were repeated in the conscious dog with the same result. The responsive areas of the diencephalon included the H2 fields of Forel, and the periventricular grey. Following ablation of these areas, the normal cardiovascular response to exercise was absent, the rapid cardioacceleration and positive inotropic changes at the initiation of exercise were abolished. Respiration was often increased during stimulation, and rhythmic leg movements were sometimes observed. These responses could be extinguished however by a slight change in the electrode position, without affecting the cardiovascular response. The most reasonable explanation for these findings may be that many nerve fibres involved in the control of respiratory, cardiovascular and motor systems, passed through or originated within the field of the stimulating
electrode. The 'coincidence' of this anatomical arrangement may or may not be of physiological significance.

Other workers have found considerable differences in the cardiovascular responses elicited by stimulation of the right and left side of the diencephalon. Fang and Wang (1962) found that in the dog, stimulation of cardiovascularly responsive areas in the right side of the hypothalamus resulted in a cardioacceleration with some augmentor response, whereas stimulation of the left side usually gave a marked cardioaugmentor response with some acceleration. Prior to this, in 1957, Randall et al. had demonstrated a similar asymmetrical functional division of thoracic sympathetic nerves in the dog.

The concept of a unique centre for the control of the cardiovascular system during exercise is misleading. Although Rushmer demonstrated the importance of the diencephalon in the normal cardiovascular response to exercise, many basic problems remain unsolved. The mechanisms through which cardiac output is precisely controlled are not known. Donald and Shepherd (1964) found that dogs with total cardiac autonomic denervation could tolerate hard exercise. Furthermore, at any workload their cardiac output and blood pressure were the same as prior to denervation. This suggests that while neural structures may determine the pattern of the
cardiovascular response to exercise, the precise regulation of cardiac output is determined by peripheral factors.

8. **The reticulo-endothelial system (RES) and the removal of circulating enzyme.**

Almost all of the evidence for the function of the RES in the clearance of enzyme from the plasma comes from experiments on mice infected with the Riley virus (lactic dehydrogenase elevating agent), or from experiments in which the RES, in the mouse, has been depressed or stimulated by various chemical agents.

In mice infected with the Riley virus, the plasma levels of LDH, and ICD were elevated 8- to 11-fold, and the plasma levels of PHI, MDH and GOT, were elevated 2- to 5-fold. AlkP, AcidP, Ald and GPT levels in the plasma were unaffected (Plagemann, Watanabe and Swim, 1962; Notkins et al., 1963; Mahy et al., 1964).

Bailey, Clough and Stearman (1964) studied the plasma clearance rate of LDH, GOT, and MDH in mice infected with CD/5 lymphoma. Haemolysed mouse erythrocytes were used as the source of enzyme. Clearance rates were approximately half normal in the infected mice. The authors calculated that the greater activities of these enzymes in the plasma of infected mice could not be achieved solely by an
increase in tissue enzyme release since this would require extensive tissue necrosis.

In the dog, plasma amylase appears to be cleared by the RES in the liver. Hiatt and co-workers, in a series of experiments found that intravenously injected amylase was initially bound to erythrocytes, and eventually released into the plasma and cleared by the liver. Thorotrast, a compound which blocks the RES, reduced the rate of clearance of amylase by the liver (Hiatt and Bonorris, 1966; Hiatt et al., 1966).

Wakim and Fleisher examined the effect of stimulation and blockade of the RES by zymosan, a polysaccharide of fungal origin, on the clearance of GPT, GOT I, GOT II, and LDH from the serum in the dog. Following injection of zymosan, there is an initial depression of the RES (at 2 hours), followed by a stimulation (at 24 hours). GPT clearance was not noticeably affected by the administration of zymosan. The clearance of both GOT I and GOT II paralleled the activity of the RES (Wakim and Fleisher, 1963a; Fleisher and Wakim, 1963b). When the RES was blocked by zymosan, there was a marked increase in SLDH (Wakim and Fleisher, 1963b). By using a chemical method to differentially inhibit the fast and slow isoenzymes of LDH, it was determined that a reduction in the clearance of both the fast and slow isoenzymes was responsible for the increase in SLDH, with a greater reduction in the clearance of the faster isoenzymes.
In 1957, Benacerraf and Sebestyen found that in rabbits, injection of bacterial endotoxin caused an initial decrease in RES activity (12 hours), followed by a stimulation of the RES (at 60 hours). In an independent investigation, Konttinen, Rajasalmi and Paloheimo (1964) followed the serum activities of GOT, LDH and CPK during endotoxin shock in rabbits. SGOT and SLDH increased during the first 12 hours and then returned to pre-infection values over the next 48 hours. SCPK was not affected by the endotoxin.

In summary, these findings seem to indicate that GOT, LDH and ICD are cleared from the plasma by the RES, at least in mice, rabbits and dogs; and that GPT, CPK, Ald, AlkP, and AcidP are not cleared to any extent by this system. In addition, MDH and amylase appeared to be cleared by the RES in the mouse and dog, respectively.
METHODS

1. Animals

Mongrel dogs, unselected as to sex, were used in all experiments. The source of these dogs varied during this study but, as far as could be determined, all animals were disease-free. Dogs were held in quarantine for one week before experimentation. They were housed in standard cages and allowed one meal per day and water ad libitum. Food was withheld for 24 hours prior to an experiment.

2. Experiments on the conscious exercising dog

Plasma enzyme changes were investigated in the spontaneously exercising dog (10 to 14 Kg in body weight; of either sex). The exercise run was performed on a treadmill (Quinton Model 24-72), the speed and elevation of which could be varied from 1.5 to 15 mph, and 0 to 40% grade (vertical distance/horizontal distance x 100).

All dogs used in these experiments had been previously accustomed to the laboratory procedures and to short, five minute, bouts of treadmill running.
Three dogs ran a total of four trials each, with a recovery of at least one week between trials. These test runs varied in duration, speed and elevation. A further three dogs were tested at the work load that induced the largest increases in plasma enzyme levels in the first three animals.

All trials were performed between 10.00 a.m. and 3.00 p.m. Prior to the run, each animal was allowed to rest in the experimental area for 30 minutes. During this period, the dog was placed in a harness, and a control rectal temperature was recorded (Yellow Springs Instrument Co.). To record the exercise electrocardiogram (ECG), while avoiding artifacts arising from skeletal muscle contractions, two electrodes were attached to the skin overlying the sternum. The leads were taped to the dog's chest and passed to a Grass Tachograph Pre-amplifier Model 7P4. Heart rate and ECG were recorded on a Grass Model 7 Polygraph.

Two minutes before the run, a control sample (4-5 ml) of blood was collected, in a lightly oiled syringe, from the cephalic vein. One ml of the blood was immediately added to ice-cold 3% perchloric acid and stored for blood lactate determination. The remainder was used for the determination of haematocrit.
(Adams Autocrit CT-2905 microhaematocrit centrifuge), and prepared for plasma enzyme analysis.

The animal was placed on the treadmill at an initial speed of 1.5 mph, and elevation of 0%, and the treadmill then rapidly adjusted (within 20 sec) to the desired speed and elevation. The ECG and heart rate were recorded continuously. At the end of the run, the treadmill was stopped and the dog allowed to rest. A second venous blood sample was taken within two minutes following the run, and treated in an identical manner to the control sample. Rectal temperature was recorded at the fifth minute following exercise. Blood lactate, PCPK, PGOT, PLDH, and the PLDH ratio were determined as described later.

3. Design of acute experiments on anaesthetised dogs

Except where otherwise qualified, all acute experiments were performed in accordance with a basic design. This allowed for comparative analysis of data from animals undergoing different experimental interventions. Following anaesthesia, the animal was surgically prepared, allowed to stabilise, and an initial blood sample obtained. A one hour control period elapsed and a second blood sample was collected. A 30-minute
period followed during which the appropriate experimental intervention, or control, was applied. Arterial blood samples were obtained at the end of this period, and at \( \frac{1}{4}, 1, 2, \) and 4 hours after. (See Figure 1.)

4. **Right atrial pacing**

Adult mongrel dogs of either sex weighing between 10 and 14 kg were used. After an initial sample of venous blood had been taken from the conscious dog, anaesthesia was induced with intravenous sodium pentobarbital (27 mg/kg body weight) (n=6). Supplemental doses of anaesthetic were administered as required. In a further series of experiments, atrial pacing was attempted in dogs under chloralose anaesthesia (n=3). Anaesthesia was induced with intravenous sodium pentothal and maintained with intravenous alphachloralose (80 mg/kg body weight).

Experiments were performed with the dog in supine position. The trachea was cannulated and spontaneous respiration continued. A catheter was advanced to the lower abdominal aorta through the right femoral artery and the right femoral vein was cannulated. A standard lead II ECG, heart rate, and arterial blood pressure were continuously recorded on a Grass Model 7
FIGURE 1

A diagram illustrating the general protocol which was followed in the acute experiments on anaesthetised dogs.

Several variations of this protocol were used and these are explained at the appropriate points in the text.

Blood samples were obtained directly before and during the last minute of an experimental intervention. In the text, these sampling times are referred to as 'before intervention', and 'end of intervention', respectively.
Polygraph. Rectal temperature was maintained between 38.0°C and 39.0°C using heat lamps.

Through an incision in the neck, a polyethylene catheter, filled with heparinised saline, was advanced along the right external jugular vein, until it had just entered the right atrium. To pace the heart, a flexible stainless steel electrode, insulated to within 3 mm of its tip was passed through the catheter and extended 5 mm beyond. An external ground electrode was applied to the chest.

After a 1 hour control period, the heart was paced at 240 beats per minute for 30 minutes using a direct coupled Grass Model S4 Stimulator. The stimuli were monophasic impulses of 0.5 to 1.0 volts, 5.0 milliseconds in duration, at a pulse frequency of 4.0 hertz. The stimulus artifact was clearly defined on the ECG, and was usually immediately followed by a P wave, P-R interval, and QRS complex. A rate of 240 beats per minute was chosen since it approached the maximum heart rate that can be attained during spontaneous exercise in the dog (Cerretelli et al., 1964b).

Arterial blood samples for blood gas, plasma enzyme and haematocrit determinations were obtained at one hour, and immediately before pacing. One minute before the termination of pacing, and at 30 minutes,
1, 2, and 4 hours after, further arterial blood samples were obtained. Haematocrit, PCPK, and PGOT were determined. Arterial PO$_2$ and PCO$_2$ were measured using an Instrumentation Laboratory Incorporated pH/Blood Gas System 113-S1. Measurements were made at 37°C and corrected to body temperature.

In control experiments (n=6) the complete surgical preparation was made; a 30 minute control period replaced the pacing period.

At the termination of an experiment, the animal was killed by an overdose of sodium pentobarbital followed by a bilateral thoracotomy. The exact position of the intra-atrial electrode was determined at death.

5. **Simulation of the cardiovascular responses to exercise by diencephalic stimulation**

It was necessary to select dogs within a narrow weight range for these experiments (9-11 kg) (n=9). Stereotaxic coordinates were obtained from an atlas (Lim, Liu, and Moffitt, 1960), and from preliminary experiments, on three dogs, not included in this report. The atlas was mapped from brains of hound-like mongrel dogs with a mean body weight of 10 Kg (range 8 to 12 Kg). Even using this limited size range the authors found
a considerable variation in the anatomy of and extent of structures. Anaesthesia was induced with sodium pentothal and maintained with alpha-chloralose (80 mg/kg body weight). The trachea and right femoral artery and vein were cannulated. The dog was turned into a prone position and its head adjusted in a stereotaxic unit (David Kopf Instruments #1504). After careful exposure of the brain surface, a coaxial bipolar electrode (In Vivo Metrics NE-100, tip separation- 0.5 mm) was placed within a predetermined area in the diencephalon from which the desired cardiovascular responses (large increases in heart rate and blood pressure) could be evoked.

Short trains of stimuli lasting 5 to 10 seconds were delivered to determine if stimulation of a specific point would elicit a suitable response. The stimuli were rectangular biphasic pulses of 10 volts, 2 ms in duration, at a frequency of 100 hz. Wilkus and Peiss (1963) have shown these stimulus parameters to be optimal for eliciting cardiovascular responses from hypothalamic structures, while keeping current flux and consequent damage to neural tissue at a minimum. The vertical position of the electrode was altered in small steps until a suitable response was elicited. Once such a response was obtained, the experiment was continued
following the protocol outlined in Figure 1. In control experiments (n=3), the electrode was positioned within a diencephalic region from which electrical stimulation elicited no discernable cardiovascular responses.

Arterial haematocrit, $\text{PO}_2$ and $\text{PCO}_2$ were determined, and PCPK, and PGOT were assayed. The position of the electrode was verified histologically after the experiment from frozen sections stained with cresyl violet.

6. **Drainage of thoracic duct lymph and simulated muscular exercise**

i) **Cannulation of the thoracic duct**

Dogs of either sex weighing between 13 and 26 kg were used. Animals were anaesthetised with sodium pentobarbital (27 mg/kg body weight), with supplemental doses administered as required. The whole experiment was performed with the animal in the supine position. A patent airway was maintained by cannulating the trachea. Arterial blood pressure was recorded from a cannula advanced from the right common carotid artery into the thoracic aorta. The right cephalic vein was cannulated.

An incision, about 8 cm long, was made extending from the sternum over the left external jugular vein. In the cannulation of lymphatics, it is essential to
maintain a virtually blood-free field since, owing to
their fragility and transparency, lymphatic vessels are
easily overlooked. Thus, any small arteries and veins
in the surgical area were ligated and sectioned to avoid
bleeding. The fascia overlying the external jugular
vein was carefully dissected away up to the junction of
the external jugular vein with the subclavian vein.
The external jugular vein was laterally retracted and
the fascia medial to the vein was dissected until the
flaccid transparent thoracic duct was observed.

A 1 to 2 cm length of the thoracic duct was
separated from connective tissue and tied off at the
venous end. The now slightly distended vessel was
cannulated with a 20 cm length of PE 150 polyethylene
tubing, making the initial incision with a 17 gauge
hypodermic needle. The free end of the tubing was held
at the same height as the external jugular vein. Free
lymph flow was usually established within 30 s. At
least 45 min elapsed before any lymph was collected for
analysis. This allowed for normal flow to be resumed
following cannulation.

Lymph was collected in heparinised graduated
glass centrifuge tubes. Flow was measured in ml over
a 30 min collection period. Collection over a shorter
time interval was used when flow was high, or when changes in lymph flow or enzyme content were under investigation. All lymph samples were centrifuged at 1600 X g to remove the erythrocytes, leucocytes, and platelets normally present in thoracic duct lymph.

In control experiments, after surgical preparation as described above, arterial blood and lymph samples were collected at and between the same time intervals as outlined in Figure 1. All lymph and plasma samples were analysed for PCPK, PGOT, PLDH, and protein. Heart rate, arterial blood pressure, respiratory rate, and rectal temperature were monitored throughout the experiment.

A continuous recording of respiratory rate was obtained by monitoring the fluctuations in carbon dioxide content of expired air. Tracheal air was drawn off and analysed for carbon dioxide content by a Beckman Medical Gas Analyser Model LBl. The output from the analyser was amplified by a Grass Low-level DC pre-amplifier Model 7Pl.

ii) Simulated muscular exercise

The animal was prepared and the thoracic duct cannulated as described above. Large brass ECG electrodes were attached to the hind limbs over the biceps femoris,
semitendinosus, gracilis, and sartorius muscles. Repeated muscular contractions were obtained by electrically stimulating the hind limbs at a pulse frequency of 10 Hz, with a pulse amplitude and width of 70 volts and 10 ms, respectively. This technique has previously been used and described by Loegering and Critz (1971).

Following stabilisation and a 1 hour control period, the hind limbs were stimulated for 30 min. To enable precise changes in lymph flow and composition to be examined, three 10 min samples were collected during stimulation. In addition to the measurements taken in control experiments, oxygen consumption (\( \dot{V}O_2 \)) was determined in 5 preparations using a closed circuit technique (Collins Respirometer P-900). \( \dot{V}O_2 \) was measured over 3 min periods during which the animal breathed 100% oxygen. At all other times the animal breathed room air. In 3 experiments, arterial blood pH was monitored during stimulation.

In three dogs, a second period of stimulation was initiated 2 hours following termination of the first.

Variations of the above experiment were later performed in an attempt to define the time course of the plasma and lymph enzyme changes during muscular stimulation; (a) Lymph and plasma samples were collected at 10 min
intervals during 60 min of muscle stimulation (n=1); (b) Samples were obtained every 5 min during 30 min of muscle stimulation (n=2).

7. **Drainage of thoracic and right duct lymph and simulated exercise**

The animal was initially prepared as in Section 6 of Methods. The thoracic duct was isolated and a loose ligature passed about it.

After careful exposure, the right external jugular vein was retracted outwards, and the cervical lymph duct traced along the trachea towards its entry into the venous system. In several preparations, prior to its venous entry, the cervical duct was joined by the right duct. In these animals, ligatures were placed about the right internal jugular vein, and about the right external jugular vein, both rostral and caudal to the entry of the right duct (Figure 2). The isolated venous segment was then incised, carefully flushed out with heparinised saline, and cannulated with a 20 cm length of PE 100 tubing following the procedure described for the thoracic duct. Within about 5 min, the venous segment had filled with lymph, and a steady flow began. The thoracic duct cannulation was then completed.
FIGURE 2

A schematic diagram illustrating the method of collecting lymph from the thoracic and right lymph ducts

1. Left external jugular vein.
2. Right external jugular vein.
3. Left axillary vein.
4. Right axillary vein.
5. Left internal jugular vein.
6. Right internal jugular vein.
7. V. thyroidea ima.
8. Left caudal thyroid vein.
10. The segment of the right external jugular vein which receives the right lymph duct has been isolated, by ligatures, from the circulating blood. This permits collection of lymph draining into that segment.
   a. Right lymph duct as it comes from the cranial mediastinal lymph nodes.
   b. Right cervical lymph duct.
   c. Cervical portion of the thoracic duct.
A. A length of PE tubing draining the thoracic duct.
B. A length of PE tubing draining lymph from the isolated segment of the right external jugular vein.

ligated vessel ...
Three tests were applied to detect anastomoses between the right and thoracic duct systems.

(a) The cannulae were raised. Mean pressure in the thoracic and right ducts is about 10 cm and 2-5 cm of water, respectively. In the presence of physiological anastomoses, lymph would reach the same vertical height in both cannulae.

(b) Pressure was applied to the abdomen. This manoeuvre should result in an increase in thoracic duct flow for a short period of time.

(c) The animal was placed on a positive pressure respirator. In the absence of any physiological anastomoses, flow from the thoracic and right ducts should decrease, and increase, respectively.

Electrodes were placed on the dog's hind limbs in preparation for stimulation and, after a one hour control period, muscular exercise was simulated. Blood and lymph samples were collected and treated as in the preceding experiment.

After the experiment, Evans blue dye was injected into the lungs and the animal was placed on a respirator. In the non-anastomotic situation, dye should only appear in the right duct lymph.
8. *Simulated muscular exercise in the splenectomised dog*

Dogs were anaesthetised with sodium pentobarbital (27 mg/kg body weight) (n=2). The right common carotid artery, right cephalic vein, and trachea were cannulated. A midline laparotomy was performed, and the gastroepiploic artery and vein exposed. All bleeding points were ligated, and gauze sponges, moistened in saline, were applied to exposed tissue. All vessels arising from the gastroepiploic artery and vein were ligated and sectioned. The spleen was gently freed from the mesentery and removed. The abdomen was closed and the animal left for one hour, during which electrodes were positioned on the hind limbs in preparation for muscle stimulation. Following a further one hour control period, muscular exercise was simulated in the standard manner. Arterial blood samples were obtained at intervals as described in Section 2. The plasma was prepared and analysed for PCK, PGOT, PLDH, and protein. Data on cardiovascular and respiratory function were recorded as previously described.
9. **Chemical analyses**

All enzyme assays were performed on plasma or lymph. Freshly obtained blood or lymph samples (5 ml) were placed in a 15 ml heparinised centrifuge tube, and centrifuged at 1600 X g for 5 minutes. The supernatant plasma or lymph was separated and stored at 4°C.

Creatine phosphokinase (CPK) and glutamic-oxalacetic transaminase (GOT) were assayed within 12 and 24 hours of obtaining the sample respectively. The procedures of Rosalki (1967) and of Karmen (1955) were used for the determination of CPK and GOT, respectively. Reagents were obtained from Calbiochem, Los Angeles, California.

Lactic dehydrogenase (LDH) was assayed colorimetrically, within 48 hours of obtaining the sample, by the method of Babson and Phillips (1965). An estimation of the relative activities of the 'H' and 'M' subunits of LDH was made according to the procedure of Babson (1965). In this method, LDH was assayed colorimetrically as described above. Lactate and urea were added to test samples to inhibit H and M subunit activity respectively. The LDH activity of each test sample was read, and the ratio of the activities of
H-inhibited to M-inhibited samples recorded. The larger
this ratio, the greater was the ratio of slow to fast
migrating isoenzymes in the test sample. Reagents for
total and Profile LDH assays were obtained from Warner-
Chilcott Laboratories, Morris Plains, New Jersey.

Activities of all enzymes were expressed in
international units per litre (IU/L).

Lactate determinations were made on whole blood,
derproteinesed with 3% perchloric acid, according to the
method of Pfleiderer and Dose (1955).

The procedure of Henry (1957) was used to estimate
the protein concentration of lymph and plasma.

Full details of all assays are given in Appendix 1.

10. **Statistical methods**

In the comparison of data, statistical evaluation
of the difference between means was performed using the
Student's *t*-test. The null hypothesis was rejected at the
5% level. Other statistical tests were applied where
appropriate and these are described at relevant points
in the text. (Refer also to Appendix 4.)
RESULTS

1. The effect of exercise on PLDH, PGOT, and PCPK in the conscious dog

   The results reported here were obtained from 6 animals in experiments conducted as outlined in Methods. After preliminary familiarisation with the treadmill, all dogs ran without any evidence of anxiety or stress.

   The data obtained from 3 animals (J, S, and C) each performing 4 separate trials, are presented in Figure 3 and Table 1. A steady-state exercise heart rate of over 200 beats/minute was achieved in most trials. From the data of Cerretelli et al. (1964b) and the steady-state exercise heart rate it can be estimated that during these runs, oxygen consumption, cardiac index, and mean arterial blood pressure were 40 to 50 ml/kg/min, over 400 ml/kg/min, and in excess of 160 mmHg, respectively.

   The changes in forelimb venous haematocrit during exercise were inconsistent and unrelated to work intensity or duration. In two animals, all trial runs brought about increases in rectal temperature of over 1°C. In the third dog, there were increases of between 0.4°C and 0.7°C.

   Elevated levels of the plasma enzymes studied were seen following 10 of the 12 trials. Following a
FIGURE 3

Plasma enzyme changes elicited by running.

Each of three dogs (J, C and S) performed 4 separate trials. Results are presented, from left to right, in terms of a progressively larger exercise-induced increment in PLDH. The height of the rectangle reflects the absolute increase in enzyme activity (IU/L), and the number over this is the increase calculated as a percent of the pre-exercise value.

Not recorded ..... X
DOG:  J  C  J  S  S  J  C  C  S  J  S  C

HR bpm  207  156  235  225  X  236  X  213  225  252  X  207
DURATION min  30  30  30  30  30  15  15  15  15  15  15  30
SPEED mph  4  4  6  6  6  6  6  10  10  10  6  10
ELEVATION %  0  0  10  0  10  10  10  10  10  10  10
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<th>J</th>
<th>S</th>
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<td>+1.2</td>
<td>+1.8</td>
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<td>+0.7</td>
<td>----</td>
<td>+0.4</td>
<td>----</td>
<td>+1.4</td>
<td>+1.1</td>
<td>+1.4</td>
</tr>
<tr>
<td>Change in Hct (%)</td>
<td>+1.2</td>
<td>-6.0</td>
<td>-8.5</td>
<td>-5.9</td>
<td>+2.5</td>
<td>+3.4</td>
<td>-2.4</td>
<td>+7.6</td>
<td>-16.0</td>
<td>-4.0</td>
<td>+4.4</td>
<td>+5.4</td>
</tr>
<tr>
<td>Increase in blood lactate (mg%)</td>
<td>7.3</td>
<td>32.2</td>
<td>21.2</td>
<td>58.7</td>
<td>20.6</td>
<td>18.3</td>
<td>4.9</td>
<td>33.4</td>
<td>9.8</td>
<td>59.7</td>
<td>89.6</td>
<td>20.6</td>
</tr>
<tr>
<td>Increase in PCPK (IU/L)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Increase in PCPK (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>86</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td>Increase in PGOT (IU/L)</td>
<td>0.0</td>
<td>1.8</td>
<td>0.0</td>
<td>7.2</td>
<td>2.2</td>
<td>2.1</td>
<td>0.0</td>
<td>1.8</td>
<td>0.0</td>
<td>0.0</td>
<td>6.7</td>
<td>9.0</td>
</tr>
<tr>
<td>Increase in PGOT (%)</td>
<td>0</td>
<td>21</td>
<td>00</td>
<td>46</td>
<td>14</td>
<td>10</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>56</td>
<td>60</td>
</tr>
<tr>
<td>Increase in PLDH (IU/L)</td>
<td>0.0</td>
<td>0.0</td>
<td>3.5</td>
<td>5.8</td>
<td>2.1</td>
<td>2.1</td>
<td>13.1</td>
<td>8.1</td>
<td>0.0</td>
<td>3.5</td>
<td>6.7</td>
<td>14.7</td>
</tr>
<tr>
<td>Increase in PLDH (%)</td>
<td>0</td>
<td>0</td>
<td>39</td>
<td>72</td>
<td>11</td>
<td>12</td>
<td>111</td>
<td>86</td>
<td>0</td>
<td>31</td>
<td>68</td>
<td>171</td>
</tr>
</tbody>
</table>
trial run, PLDH was elevated more frequently than PGOT or PCPK (Figure 3). The changes in plasma enzyme activity did not correlate with the change in haematocrit, elevation in rectal temperature, increase in blood lactate, or the steady-state exercise heart rate. However, there did appear to be a relationship between the intensity of the exercise and the magnitude of the plasma enzyme response. In general, the greater the speed and grade of the run, the larger the increase in PLDH and PGOT. Furthermore, with one exception, a post-exercise elevation of PCPK was seen only after the most intense exercise.

Table 2 summarises the data obtained from 6 dogs performing a standard run at 10 mph and 10% elevation for 15 minutes. All 6 animals achieved a high steady-state exercise heart rate. As previously noted, there was a large variability in the post-exercise change in haematocrit. Both rectal temperature and blood lactate were elevated following the run. The changes in these parameters were not related to one another, nor to the steady-state exercise heart rate.

At the end of the run, PLDH, PGOT, and PCPK were elevated in all but one animal. The dogs in which the largest changes in PLDH occurred also demonstrated the largest alterations in PGOT and PCPK. The mean
Table 2. Heart rate, rectal temperature, venous haematocrit, blood lactate and plasma enzyme responses to running exercise in the dog (15 min, 10 mph, 10% elev)

<table>
<thead>
<tr>
<th>Dog</th>
<th>J</th>
<th>C</th>
<th>K</th>
<th>D</th>
<th>S</th>
<th>E</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (bpm)</td>
<td>252</td>
<td>213</td>
<td>210</td>
<td>208</td>
<td>225</td>
<td>266</td>
<td>229 ± 10</td>
</tr>
<tr>
<td>Final Rect. Temp (°C)</td>
<td>40.6</td>
<td>40.7</td>
<td>40.8</td>
<td>41.6</td>
<td>39.8</td>
<td>39.8</td>
<td>40.6 ± 0.3</td>
</tr>
<tr>
<td>Rect. Temp (°C)</td>
<td>+1.8</td>
<td>+1.1</td>
<td>+1.4</td>
<td>+2.1</td>
<td>+0.4</td>
<td>+0.7</td>
<td>+1.3 ± 0.3</td>
</tr>
<tr>
<td>Change in Hct (%)</td>
<td>5.9</td>
<td>4.4</td>
<td>-5.8</td>
<td>+1.0</td>
<td>+7.6</td>
<td>0.0</td>
<td>+0.2 ± 2.2</td>
</tr>
<tr>
<td>Increase in blood lactate (mg%)</td>
<td>58.7</td>
<td>89.6</td>
<td>87.5</td>
<td>30.8</td>
<td>33.4</td>
<td>46.9</td>
<td>57.8 ± 10.5</td>
</tr>
<tr>
<td>Increase in PCKP (IU/L)</td>
<td>12</td>
<td>0</td>
<td>4</td>
<td>19</td>
<td>11</td>
<td>29</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>(%)</td>
<td>86</td>
<td>0</td>
<td>21</td>
<td>237</td>
<td>33</td>
<td>57</td>
<td>72 ± 35</td>
</tr>
<tr>
<td>Increase in PGOT (IU/L)</td>
<td>7.2</td>
<td>6.7</td>
<td>4.9</td>
<td>9.5</td>
<td>1.8</td>
<td>9.8</td>
<td>6.7 ± 1.2</td>
</tr>
<tr>
<td>(%)</td>
<td>46</td>
<td>56</td>
<td>52</td>
<td>108</td>
<td>11</td>
<td>82</td>
<td>59 ± 14</td>
</tr>
<tr>
<td>Increase in PLDH (IU/L)</td>
<td>5.8</td>
<td>6.7</td>
<td>5.1</td>
<td>12.9</td>
<td>8.1</td>
<td>18.6</td>
<td>9.5 ± 2.1</td>
</tr>
<tr>
<td>(%)</td>
<td>72</td>
<td>68</td>
<td>82</td>
<td>451</td>
<td>86</td>
<td>146</td>
<td>151 ± 61</td>
</tr>
<tr>
<td>PLDH Ratio</td>
<td>before</td>
<td>2.6</td>
<td>2.6</td>
<td>2.7</td>
<td>2.4</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>after</td>
<td>2.6</td>
<td>3.0</td>
<td>2.7</td>
<td>2.4</td>
<td>2.8</td>
<td>2.4</td>
<td>2.7 ± 0.1</td>
</tr>
</tbody>
</table>
increase in activity of a particular plasma enzyme, during exercise, showed less variance when expressed as the absolute value than when calculated as the percent change from the resting value. From this it was concluded that the magnitude of an exercise-induced change in plasma enzyme activity was probably independent of the resting plasma enzyme activity. Consequently, in this and subsequent studies, the absolute change in enzyme activity, rather than the percent change, was used in statistical analysis. There was no alteration in the PLDH isoenzyme ratio following exercise although there was about a 2-fold rise in total PLDH. This means that there was a similar proportional increase in the H and M subunit activity of LDH in the plasma during exercise.

2. The effect of right atrial pacing on PGOT and PCPK

The results reported here were obtained on anaesthetised animals in which cardiac pacing was attempted using a transvenous unipolar electrode. The cardiac rhythm was readily captured in all animals that were anaesthetised with sodium pentobarbital. At autopsy, the site of the electrode was invariably found to be in the region of the caval-right atrial junction.
Heart rates, and systolic and diastolic arterial blood pressures in cardiac-paced and control animals are given in Table 3. Although during pacing, the mean heart rate was almost 70 beats/minute above control, neither the systolic nor diastolic arterial blood pressure of the paced group was significantly different from control. The cardiac-paced group had a lower heart rate than the control group at 4 hours after pacing (P<0.01), whereas arterial blood pressure was the same in both groups. While under pentobarbital anaesthesia, the dog has an elevated heart rate which may fall within a wide range of values. In contrast, arterial blood pressure is only slightly raised with small inter-individual variation (Nash, Davis, and Woodbury, 1956).

During cardiac pacing there was an increase in arterial PO2, and a concomitant decrease in arterial PCO2 (P<0.05, P<0.05) (Table 4). Blood gas tensions had returned to control levels by 30 minutes after pacing. Accidental stimulation of the phrenic nerve occasionally occurs during placement of transvenous cardiac pacing catheters in man (Furman and Escher, 1970). In the supine dog, this situation is aggravated by the proximity of the phrenic nerve to the right atrium. Periodic stimulation of the phrenic nerve by the pacing current,
Table 3. Heart rate and arterial blood pressure in control and cardiac-paced animals

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate (beats/min)</th>
<th>Systolic Pressure (mmHg)</th>
<th>Diastolic Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>paced</td>
<td>control</td>
</tr>
<tr>
<td>1 hour before pacing</td>
<td>184 ± 18*</td>
<td>174 ± 7</td>
<td>174 ± 18</td>
</tr>
<tr>
<td>before pacing</td>
<td>182 ± 12</td>
<td>171 ± 7</td>
<td>174 ± 18</td>
</tr>
<tr>
<td>during pacing</td>
<td>178 ± 14</td>
<td>240 ± 0</td>
<td>172 ± 18</td>
</tr>
<tr>
<td>5 minutes after pacing</td>
<td>176 ± 15</td>
<td>174 ± 9</td>
<td>171 ± 18</td>
</tr>
<tr>
<td>30 minutes after pacing</td>
<td>169 ± 13</td>
<td>165 ± 6</td>
<td>171 ± 13</td>
</tr>
<tr>
<td>1 hour after pacing</td>
<td>175 ± 15</td>
<td>153 ± 10</td>
<td>172 ± 15</td>
</tr>
<tr>
<td>2 hours after pacing</td>
<td>170 ± 5</td>
<td>144 ± 11</td>
<td>168 ± 18</td>
</tr>
<tr>
<td>4 hours after pacing</td>
<td>169 ± 7</td>
<td>129 ± 12</td>
<td>167 ± 16</td>
</tr>
</tbody>
</table>

* Mean ± S E M

n is equal to 6 for both control and paced groups
Table 4. **Arterial PO₂ and PCO₂ in control and cardiac-paced animals**

<table>
<thead>
<tr>
<th></th>
<th>Arterial PO₂ (mm Hg)</th>
<th>Arterial PCO₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>paced</td>
</tr>
<tr>
<td>Before pacing</td>
<td>71 ± 2*</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>end of pacing</td>
<td>69 ± 5</td>
<td>80 ± 4</td>
</tr>
<tr>
<td>30 minutes after pacing</td>
<td>69 ± 5</td>
<td>73 ± 7</td>
</tr>
<tr>
<td>1 hour after pacing</td>
<td>72 ± 4</td>
<td>73 ± 7</td>
</tr>
<tr>
<td>2 hours after pacing</td>
<td>70 ± 6</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>4 hours after pacing</td>
<td>77 ± 3</td>
<td>77 ± 6</td>
</tr>
</tbody>
</table>

* Mean ± S.E.M

n is equal to 6 for both control and paced groups.
resulting in a slight increase in ventilation, may have been responsible for the observed changes in blood gases. Neither minute ventilation nor respiratory rate were determined in these dogs.

The effect of cardiac pacing on PCPK and PGOT is illustrated in Figure 4 and in Tables 5 and 6. There was a small but significant increase in PGOT during pacing. Following the termination of pacing PGOT remained elevated for a further 2 hours, but had returned to control values by 4 hours. No significant change in PCPK was observed in response to pacing. However, there was a gradual rise in PCPK in both paced and control groups throughout the course of the experiment.

Similar experiments were attempted in α-chloralose-anaesthetised dogs. It was found that atrial pacing at rates above 170 to 180 beats/minute was not possible. The control heart rate ranged from 90 to 135 beats/minute, which was considerably lower than the control heart rate of pentobarbital-anaesthetised dogs. In the resting unanaesthetised dog the author has been unable to perform atrial pacing at rates above 150 beats/minute. The heart rate of the resting dog was in the range of 40 to 60 beats/minute. The different resting heart rates of the above
The responses of PCPK and SGOT to a 30-minute period of cardiac pacing (240 beats/minute).

Enzyme values are expressed as the difference in activity from the pre-pacing value (B).

- pacing period ...........
- paced group ...........
- control group ...........
<table>
<thead>
<tr>
<th></th>
<th>PCPK (IU/litre)</th>
<th>PGOT (IU/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>paced</td>
</tr>
<tr>
<td>1 hour before</td>
<td>14.0 ± 1.2*</td>
<td>17.8 ± 2.4</td>
</tr>
<tr>
<td>pacing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>before pacing</td>
<td>14.5 ± 0.7</td>
<td>17.7 ± 1.9</td>
</tr>
<tr>
<td>end of pacing</td>
<td>15.0 ± 0.7</td>
<td>19.8 ± 2.9</td>
</tr>
<tr>
<td>30 minutes after</td>
<td>16.8 ± 0.9</td>
<td>21.7 ± 3.4</td>
</tr>
<tr>
<td>pacing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hour after</td>
<td>16.0 ± 1.8</td>
<td>22.6 ± 3.2</td>
</tr>
<tr>
<td>pacing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 hours after</td>
<td>17.4 ± 0.6</td>
<td>23.8 ± 3.4</td>
</tr>
<tr>
<td>pacing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 hours after</td>
<td>19.8 ± 1.1</td>
<td>25.2 ± 3.6</td>
</tr>
<tr>
<td>pacing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SEM

n is equal to 6 for both control and paced groups
<table>
<thead>
<tr>
<th>Time after Pacing</th>
<th>PCPK (IU/litre)</th>
<th>PGOT (IU/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>paced</td>
</tr>
<tr>
<td>1 hour before pacing</td>
<td>$-0.5 \pm 0.6^*$</td>
<td>$+0.2 \pm 0.9$</td>
</tr>
<tr>
<td>before pacing</td>
<td>$0.0 \pm 0.0$</td>
<td>$0.0 \pm 0.0$</td>
</tr>
<tr>
<td>end of pacing</td>
<td>$+0.5 \pm 0.3$</td>
<td>$+2.2 \pm 1.3$</td>
</tr>
<tr>
<td>30 minutes after pacing</td>
<td>$+2.3 \pm 0.5$</td>
<td>$+4.0 \pm 1.6$</td>
</tr>
<tr>
<td>1 hour after pacing</td>
<td>$+1.5 \pm 1.3$</td>
<td>$+5.0 \pm 1.4$</td>
</tr>
<tr>
<td>2 hours after pacing</td>
<td>$+2.8 \pm 0.5$</td>
<td>$+6.2 \pm 1.8$</td>
</tr>
<tr>
<td>4 hours after pacing</td>
<td>$+4.8 \pm 0.9$</td>
<td>$+7.2 \pm 2.5$</td>
</tr>
</tbody>
</table>

* Mean ± SEM

n is equal to 6 for both control and paced groups.
three preparations probably reflect differences in tonic activity of the cardiac branches of the parasympathetic and sympathetic nervous systems. Such preparation differences in cardiac autonomic nervous activity might likewise determine the atrio-ventricular conduction velocity, and determine the maximum rate at which the heart could follow an atrial pacemaker (Marshall, 1968).

3. PGOT and PCPK during simulation of the cardiovascular response to exercise by diencephalic stimulation

In these experiments, exercise-type systemic cardiovascular responses were obtained by electrical stimulation of the diencephalon in chloralose-anaesthetised dogs. A maintained cardiovascular response (cardioacceleration and blood pressure elevation) similar to that which has been observed in spontaneously exercising dogs (Ceretelli et al., 1964), was obtained in 9 animals by stimulating within either the hypothalamic periventricular grey, or the H2 Field of Forel (Figure 5). The mean heart rate before and during stimulation was 127 ± 11, and 225 ± 11 beats/minute, respectively. The mean systolic arterial blood pressure before and during stimulation was 160 ± 5 and 183 ± 8 mmHg, and the mean diastolic arterial blood pressure at these
FIGURE 5

Drawing of the transverse section of a dog's brain at RL7, showing the extent of sites from which the desired cardiovascular responses were elicited.

3V : third ventricle
CP : cerebral peduncle
MM : medial mammillary nucleus
MT : mammilothalamic tract
OT : optic tract

Regions stimulated ............

1 : a portion of the periventricular grey (right)
2 : H₂ Field of Forel (right)
times was $110 \pm 3$ and $122 \pm 6$ mmHg (Table 7).

There was an immediate increase in heart rate and blood pressure on application of the stimulus. Both parameters attained a steady value within 10 to 20 seconds (Figure 6). The cardiovascular responses were maintained for the full 30 minutes of stimulation in the majority of animals. In 4 dogs, the intensity of the stimulus was increased in the latter half of the period of stimulation to counteract a tendency of the cardiovascular response to attenuate. There was an immediate decrease in heart rate and blood pressure upon the termination of stimulation. These parameters reached steady values within 10 minutes after stimulation, and then remained unchanged for the remainder of the experiment.

Other responses associated with increased sympathetic activity were observed during diencephalic stimulation. Pupillary dilation and a flow of viscous saliva were observed in some dogs. Smith, Rushmer, and Lasher (1960) have elicited somatic activity in the form of rhythmic limb movements by stimulating in the H2 Fields of Forel. In the present study, such movements were not seen. An extension of the forelimbs occurred in 4 dogs when the stimulus was applied.
<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats/min)</th>
<th>Systolic Pressure (mmHg)</th>
<th>Diastolic Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour before stimulation</td>
<td>120 ± 8*</td>
<td>160 ± 7</td>
<td>101 ± 5</td>
</tr>
<tr>
<td>before stimulation</td>
<td>127 ± 11</td>
<td>160 ± 5</td>
<td>108 ± 3</td>
</tr>
<tr>
<td>during stimulation</td>
<td>225 ± 11</td>
<td>183 ± 8</td>
<td>122 ± 6</td>
</tr>
<tr>
<td>5 minutes after stimulation</td>
<td>169 ± 8</td>
<td>156 ± 6</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>10 minutes after stimulation</td>
<td>155 ± 9</td>
<td>154 ± 5</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>157 ± 10</td>
<td>150 ± 6</td>
<td>104 ± 5</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>153 ± 13</td>
<td>153 ± 4</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>156 ± 10</td>
<td>160 ± 4</td>
<td>110 ± 5</td>
</tr>
<tr>
<td>4 hours after stimulation</td>
<td>155 ± 13</td>
<td>159 ± 2</td>
<td>112 ± 2</td>
</tr>
</tbody>
</table>

* Mean ± S E M
n is equal to 9
A record illustrating the cardiovascular response to 30 minutes continuous electrical stimulation of the right H₂ Field of Forel.
Control and stimulus application

At 15 minutes of stimulation

At termination of stimulation

One minute later

10 seconds
Relaxation followed the initial contraction.

There was an increase in ventilation in 8 dogs which resulted in significant changes in arterial blood gas tensions (Table 8). The magnitude of the change in ventilation did not appear to be related to the magnitude of the cardiovascular response. During the 30 minutes of stimulation, arterial PO₂ increased from 75 ± 3 to 82 ± 3 mmHg (P<0.05, while arterial PCO₂ decreased from 41 ± 3 to 31 ± 4 mmHg (P<0.01). Arterial blood gas tensions returned to pre-stimulation values within 30 minutes after the termination of stimulation.

There was no change in the plasma enzyme activities following the limited surgery. This observation corresponds with the recent report by Loehering and Critz (1971) that minor surgery and prolonged anaesthesia did not affect plasma enzyme levels.

Both PCPK and PGOT increased significantly during stimulation (P<0.05; P<0.05). However, PGOT returned to control values within one hour, while PCPK remained elevated throughout the post-stimulation period (Tables 9 and 10, Figure 7). There was a high correlation between the magnitude of the increase in PGOT and the increase in PCPK during stimulation (r = 0.90, P<.01).

The changes in the plasma levels of PCPK and PGOT have been plotted as a function of either heart rate,
Table 8. Arterial blood PO\textsubscript{2} and PCO\textsubscript{2} during diencephalic stimulation

<table>
<thead>
<tr>
<th></th>
<th>Arterial PO\textsubscript{2} (mmHg)</th>
<th>Arterial PCO\textsubscript{2} (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before stimulation</td>
<td>75 ± 3*</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>end of stimulation</td>
<td>83 ± 3</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>30 minutes after</td>
<td>79 ± 2</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hour after</td>
<td>80 ± 3</td>
<td>39 ± 4</td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 hours after</td>
<td>79 ± 5</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 hours after</td>
<td>79 ± 4</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SEM

n is equal to 9
<table>
<thead>
<tr>
<th></th>
<th>PCPK</th>
<th>PGOT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(IU/litre)</td>
<td>(IU/litre)</td>
</tr>
<tr>
<td>1 hour before stimulation</td>
<td>16.3 ± 1.3</td>
<td>9.7 ± 0.8</td>
</tr>
<tr>
<td>before stimulation</td>
<td>15.3 ± 1.2</td>
<td>9.7 ± 0.9</td>
</tr>
<tr>
<td>end of stimulation</td>
<td>19.4 ± 1.5</td>
<td>12.0 ± 0.8</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>20.4 ± 1.6</td>
<td>11.5 ± 0.8</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>18.9 ± 2.0</td>
<td>10.6 ± 0.9</td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>20.6 ± 2.2</td>
<td>9.4 ± 1.0</td>
</tr>
<tr>
<td>4 hours after stimulation</td>
<td>18.5 ± 2.1</td>
<td>9.2 ± 1.1</td>
</tr>
</tbody>
</table>

* Mean ± SEM

n is equal to 9
Table 10. Changes in PCPK and PGOT during dienecephalic stimulation

<table>
<thead>
<tr>
<th></th>
<th>PCPK</th>
<th></th>
<th>PGOT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(IU/litre)</td>
<td></td>
<td>(IU/litre)</td>
<td></td>
</tr>
<tr>
<td>1 hour before stimulation</td>
<td>+1.0 ± 0.9*</td>
<td>0.0 ± 0.6</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>before stimulation</td>
<td>0.0 ± 0.0</td>
<td></td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>end of stimulation</td>
<td>+4.1 ± 1.2</td>
<td></td>
<td>+2.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>+5.1 ± 1.4</td>
<td></td>
<td>+1.8 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>+3.6 ± 2.1</td>
<td></td>
<td>+0.9 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>+5.3 ± 2.0</td>
<td></td>
<td>-0.3 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>4 hours after stimulation</td>
<td>+3.2 ± 1.9</td>
<td></td>
<td>-0.5 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± S E M
n is equal to 9
PCPK and PGOT responses to a 30-minute period of diencephalic stimulation.

Enzyme values are expressed as the difference in activity from the pre-stimulation value (B).

diencephalic stimulation ....
systolic blood pressure, or the product of heart rate and systolic blood pressure, during the period of stimulation (Figures 8 to 11). The product of heart rate and systolic pressure, termed cardiac effort, has been shown by Katz and Feinberg (1958) to behave as a reasonable index of myocardial oxygen consumption.

There was a low correlation between the heart rate during stimulation and the increases in PGOT and PCPK ($r = 0.22$, $P<0.05$; $r = 0.07$, $P<0.05$). A higher correlation existed between the absolute increase in both PGOT and PCPK and the cardiac effort during stimulation ($r = 0.68$, $P<0.05$; $r = 0.58$, $P<0.05$) (Figures 8 and 9). The highest correlation was observed between systolic blood pressure during stimulation and the changes in PGOT and PCPK ($r = 0.91$, $P<0.01$; $r = 0.77$, $P<0.01$) (Figures 10 and 11).

In 3 preparations, the diencephalon was stimulated within a region from which no systemic cardiovascular response was elicited. No changes in PGOT or PCPK were observed at any time during the course of these experiments. Essentially similar results were obtained in animals in which only small elevations in blood pressure and heart rate were elicited. Together these observations were considered to stand as adequate controls; that the plasma enzyme changes occurring during diencephalic stimulation were related to positive responses of the cardiovascular system.
FIGURE 8

Increase in PGOT as a function of cardiac effort (heart rate x systolic arterial pressure) during diencephalic stimulation.
Increase in PCPK as a function of cardiac effort (heart rate × systolic arterial pressure) during diencephalic stimulation.
FIGURE 10

Increase in PGOT as a function of systolic arterial blood pressure during diencephalic stimulation.
Increase in PCPK as a function of systolic arterial blood pressure during diencephalic stimulation.
4. **Continuous collection (drainage) of thoracic duct lymph**

In order to study enzyme activity in lymph, baseline values of thoracic duct lymph flow and enzyme activity were established in 6 dogs from which lymph was continuously collected.

Over a period of 5½ hours the mean volume of lymph collected was 108 ± 25 ml (range 41 to 193 ml). In 4 of these dogs, the volume of lymph collected fell within a far narrower range: 94 to 117 ml. These figures are similar to those obtained by Drinker and Field (1933), who have previously reported that thoracic duct lymph flow in the dog lies in the range of 20 to 25 ml/hour, and is independent of body weight.

Over the duration of the experiment, neither heart rate nor arterial systolic or diastolic blood pressure differed significantly from values obtained in control animals with an intact thoracic duct (Table 11). Thus the removal of between 40 and 50 ml of blood for chemical determinations, and the removal of approximately 100 ml of lymph had no appreciable effect on the cardiovascular parameters recorded.

Continuous drainage of thoracic duct lymph had different effects on PCPK, PGOT, and PLDH (Tables 12 and 13, Figure 12). In animals where lymph was continuously
<table>
<thead>
<tr>
<th></th>
<th>Heart Rate (beats/minute)</th>
<th>Systolic Pressure (mmHg)</th>
<th>Diastolic Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>drained</td>
<td>control</td>
</tr>
<tr>
<td>1 hour before</td>
<td>184 ± 13*</td>
<td>168 ± 11</td>
<td>174 ± 18</td>
</tr>
<tr>
<td>Before (B)</td>
<td>182 ± 12</td>
<td>171 ± 10</td>
<td>174 ± 18</td>
</tr>
<tr>
<td>After (A)</td>
<td>176 ± 15</td>
<td>173 ± 12</td>
<td>171 ± 18</td>
</tr>
<tr>
<td>30 minutes after</td>
<td>169 ± 13</td>
<td>179 ± 9</td>
<td>171 ± 13</td>
</tr>
<tr>
<td>1 hour after</td>
<td>175 ± 15</td>
<td>175 ± 9</td>
<td>172 ± 15</td>
</tr>
<tr>
<td>2 hours after</td>
<td>170 ± 5</td>
<td>175 ± 5</td>
<td>168 ± 18</td>
</tr>
<tr>
<td>4 hours after</td>
<td>169 ± 7</td>
<td>179 ± 5</td>
<td>167 ± 16</td>
</tr>
</tbody>
</table>

* Mean ± S.E.M

n is equal to 6 for both groups
Table 12. PCPK, PGOT, and PLDH in control and thoracic duct lymph-drained experiments

<table>
<thead>
<tr>
<th>Time</th>
<th>PCPK (IU/litre)</th>
<th>PGOT (IU/litre)</th>
<th>PLDH (IU/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>drained</td>
<td>control</td>
</tr>
<tr>
<td>1 hour before</td>
<td>14.0 ± 1.2*</td>
<td>25.6 ± 3.9</td>
<td>7.9 ± 1.2</td>
</tr>
<tr>
<td>Before (B)</td>
<td>14.5 ± 0.7</td>
<td>23.8 ± 4.3</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>After (A)</td>
<td>15.0 ± 0.7</td>
<td>23.0 ± 3.8</td>
<td>7.1 ± 1.2</td>
</tr>
<tr>
<td>30 minutes after</td>
<td>15.0 ± 0.9</td>
<td>22.8 ± 4.1</td>
<td>7.1 ± 1.0</td>
</tr>
<tr>
<td>1 hour after</td>
<td>14.9 ± 1.8</td>
<td>22.0 ± 3.6</td>
<td>7.0 ± 1.0</td>
</tr>
<tr>
<td>2 hours after</td>
<td>15.1 ± 0.6</td>
<td>20.6 ± 3.1</td>
<td>6.8 ± 1.0</td>
</tr>
<tr>
<td>4 hours after</td>
<td>15.2 ± 1.1</td>
<td>20.6 ± 3.3</td>
<td>6.3 ± 1.0</td>
</tr>
</tbody>
</table>

* Mean ± SEM

n is equal to 6 for both control and drained groups
Table 13. Changes in PCPK, PGOT, and PLDH in control and thoracic duct lymph-drained animals

<table>
<thead>
<tr>
<th></th>
<th>PCPK (IU/litre)</th>
<th>PGOT (IU/litre)</th>
<th>PLDH (IU/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>drained</td>
<td>control</td>
</tr>
<tr>
<td>1 hour before</td>
<td>-0.5 ± 0.6*</td>
<td>+1.8 ± 0.1</td>
<td>+0.4 ± 0.7</td>
</tr>
<tr>
<td>Before (B)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>After (A)</td>
<td>+0.5 ± 0.3</td>
<td>-0.8 ± 0.7</td>
<td>-0.2 ± 0.3</td>
</tr>
<tr>
<td>30 minutes after</td>
<td>+0.5 ± 0.5</td>
<td>-1.0 ± 0.5</td>
<td>-0.1 ± 0.2</td>
</tr>
<tr>
<td>1 hour after</td>
<td>+0.4 ± 0.8</td>
<td>-2.0 ± 0.7</td>
<td>-0.2 ± 0.4</td>
</tr>
<tr>
<td>2 hours after</td>
<td>+0.6 ± 0.5</td>
<td>-3.2 ± 1.4</td>
<td>-0.5 ± 0.4</td>
</tr>
<tr>
<td>4 hours after</td>
<td>+0.8 ± 0.9</td>
<td>-3.2 ± 1.6</td>
<td>-0.9 ± 0.6</td>
</tr>
</tbody>
</table>

* Mean ± S E M

n is equal to 6 for both control and drained groups
FIGURE 12

PLDH, PCPK and PGOT levels in control and thoracic duct lymph-drained animals.

Enzyme values are expressed as the difference in activity from the value at B.

control group ........... ————
lymph-drained group ... ————
drained, PCPK gradually fell throughout the experiment, reaching a value significantly lower than control during the last 2 hours (P<0.05). PGOT levels fell in both control and lymph-drained animals. However, PGOT declined faster in the latter group, although the difference between the groups never became statistically significant. In contrast to the behaviour of these two plasma enzymes, PLDH did not change significantly from its initial value in either the control or lymph-drained animals.

Data on the enzyme activity of the lymph drained from the thoracic duct helps explain the plasma enzyme responses observed in lymph-drained animals. Lymph GOT activity (L GOT) was greater than PGOT activity; lymph CPK activity (LCPK) was approximately equal to PCPK activity; and lymph LDH activity (LLDH) was considerably less than PLDH activity (Refer to the control period in Figures 16, 17 and 18). Thus while thoracic duct lymphatic delivery of CPK and GOT must play a role in the maintenance of the levels of these enzymes in the circulating blood, this is probably not true of LDH.

These realtionships were demonstrated in another manner. The ratios of the lymph to plasma protein concentrations and of CPK, GOT, and LDH activities were calculated from the data obtained during the 1 hour control period of 20 separate experiments. The results are
presented in the form of a histogram (Figure 13). The mean lymph/plasma ratio for protein was 0.69 ± 0.02 (median value 0.70). The mean lymph/plasma ratio for LDH was 0.55 ± 0.13 (median 0.39). In 4 dogs, no LDH could be detected in the lymph. The mean values of the lymph/plasma ratios for CPK and GOT were 0.97 ± 0.08 (median 0.90) and 1.22 ± 0.10 (median 1.20), respectively. Using the Chi-square test, it was shown that the lymph/plasma ratios for LDH<Protein<CPK<GOT (P<0.05; P<0.05; P<0.05). The significance of these observations will be considered in the discussion.

In the intact animals enzyme activity in the thoracic duct lymph would contribute to the maintenance of enzyme activity in the circulating plasma. Thus it can be assumed that in the lymph-dained animal there is a decrease in plasma enzyme activity proportional to the volume and enzyme activity of the drained lymph. The product of lymph flow and lymph enzyme activity has been termed lymphatic enzyme delivery, and was expressed in International Units/unit of time (e.g. IU/30 minutes). An estimate of the effect of the removal of thoracic duct lymph on plasma enzyme activity may be determined from figures on enzyme delivery and an assumed plasma volume of 50 ml per kg of body weight (Hoff, Deavers and Huggins, 1966). There are several simplifications in this calculation.
FIGURE 13

Histogram presenting the lymph/plasma ratios of LDH, protein, CPK and GOT.

Numbers in parentheses represent the number of animals in the sample.

Note that there is a '0' class of lymph/plasma ratios for LDH.
The value obtained represents the change in plasma enzyme activity that would occur if all the enzyme activity in the drained lymph was suddenly added to the circulating plasma. Under normal circumstances, this enzyme activity would have entered the blood gradually, and various factors, including the clearance of circulating enzyme should be taken into account. However, this calculation provides an estimate of the 'effective loss' of enzyme activity to the circulating plasma (in IU/L of plasma). Data on the effective loss of enzyme to the plasma are presented in Table 14. Similar calculations are presented in Section 5 of Results.

5. **Effect of skeletal muscle stimulation on thoracic duct lymph flow and enzyme activity**

Skeletal muscle stimulation was performed for 30 minutes in 8 pentobarbital-anaesthetised dogs. The heart rates, systolic, diastolic, and mean arterial blood pressures in muscle stimulated and control animals are presented in Table 15. During stimulation the mean heart rate increased by 28 beats/minute (P<0.05). No significant changes were observed during the experiment in any of the other cardiovascular parameters recorded.

Rectal temperature increased rapidly during muscle stimulation, with a gradual return towards
Table 14. The effect of drainage (4½ hours) of thoracic duct lymph on PCPK, PGOT and PLDH

<table>
<thead>
<tr>
<th></th>
<th>Difference between plasma enzyme activity in drained and control animals</th>
<th>Estimated loss through removal of lymph</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCPK</td>
<td>-4.0</td>
<td>1.9 ± 0.5*</td>
</tr>
<tr>
<td>PGOT</td>
<td>-0.8</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>PLDH</td>
<td>-0.5</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

* Mean ± S E M

n is equal to 6 for both control and lymph-drained groups

All values are in IU/L
Table 15. Heart rate and arterial blood pressure responses to skeletal muscle stimulation

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate (beats/minute)</th>
<th>Systolic Pressure (mmHg)</th>
<th>Diastolic Pressure (mmHg)</th>
<th>Mean Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>stim</td>
<td>control</td>
<td>stim</td>
</tr>
<tr>
<td>1 hour before stimulation</td>
<td>168 ± 11*</td>
<td>173 ± 6</td>
<td>157 ± 3</td>
<td>154 ± 9</td>
</tr>
<tr>
<td>before stimulation</td>
<td>171 ± 11</td>
<td>171 ± 8</td>
<td>161 ± 3</td>
<td>159 ± 8</td>
</tr>
<tr>
<td>end of stimulation</td>
<td>173 ± 12</td>
<td>198 ± 12</td>
<td>159 ± 5</td>
<td>159 ± 5</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>179 ± 9</td>
<td>188 ± 11</td>
<td>158 ± 4</td>
<td>159 ± 6</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>175 ± 9</td>
<td>180 ± 10</td>
<td>152 ± 8</td>
<td>159 ± 6</td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>175 ± 5</td>
<td>166 ± 10</td>
<td>150 ± 8</td>
<td>156 ± 7</td>
</tr>
</tbody>
</table>

* Mean ± S.E.M

n is equal to 6 and 8 for control and stimulated groups respectively.
pre-stimulation values during the following 2 hours (Table 16). In 3 animals, rectal temperature was initially above 39.0°C, and these animals were cooled using ice-water. In one animal in which rectal temperature eventually reached 42.8°C, panting began at 41.8°C. Since tracheostomy prevented the normal hydration of inspired air, and could have led to large evaporative loss of water from the pulmonary surfaces, an attempt was made to maintain rectal temperature below threshold for panting during the period of muscle stimulation. Two dogs were cooled during muscle stimulation in an attempt to prevent panting. One of these animals panted when rectal temperature reached 41.3°C. In the 6 animals that did not pant, the respiratory rate approximately doubled during muscle stimulation (Table 16).

In control animals, there was a gradual increase in haematocrit throughout the experiment (Table 16). Arterial haematocrit in the muscle-stimulated group increased markedly during stimulation in comparison with the control group (P 0.01). Following stimulation, arterial haematocrit fell towards pre-stimulation values and then increased slightly near the termination of the experiment.

Data on the plasma enzyme activity and plasma protein concentration of muscle-stimulated and control groups are presented in Tables 17 and 18, and in Figure 14.
Table 16. Respiratory rate, arterial haematocrit, and rectal temperature during skeletal muscle stimulation

<table>
<thead>
<tr>
<th></th>
<th>Respiratory Rate (breaths/min)</th>
<th>Haematocrit (%)</th>
<th>Rectal Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>stimulated</td>
<td>control</td>
</tr>
<tr>
<td>1 hour before stimulation</td>
<td>6 ± 1*</td>
<td>10 ± 2</td>
<td>43.4 ± 3.9</td>
</tr>
<tr>
<td>before stimulation</td>
<td>7 ± 1</td>
<td>11 ± 4</td>
<td>44.8 ± 3.8</td>
</tr>
<tr>
<td>end of stimulation</td>
<td>8 ± 2</td>
<td>25 ± 5</td>
<td>45.9 ± 3.5</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>10 ± 1</td>
<td>13 ± 3</td>
<td>46.2 ± 3.4</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>9 ± 1</td>
<td>13 ± 3</td>
<td>47.5 ± 3.7</td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>10 ± 2</td>
<td>15 ± 5</td>
<td>48.0 ± 3.8</td>
</tr>
</tbody>
</table>

*Mean ± S E M

n is equal to 6 and 8 for control and stimulated groups respectively.
Table 17. PCPK, PGOT, PLDH, and plasma protein concentration during skeletal muscle stimulation

<table>
<thead>
<tr>
<th></th>
<th>.1 hour before stimulation</th>
<th>before stimulation</th>
<th>end of stimulation</th>
<th>30 minutes after stimulation</th>
<th>1 hour after stimulation</th>
<th>2 hours after stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCPK (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>25.6 ± 3.9*</td>
<td>23.8 ± 4.3</td>
<td>23.0 ± 3.8</td>
<td>22.8 ± 4.1</td>
<td>22.0 ± 3.6</td>
<td>20.6 ± 3.1</td>
</tr>
<tr>
<td>stimulated</td>
<td>25.3 ± 1.3</td>
<td>23.9 ± 1.3</td>
<td>23.4 ± 1.3</td>
<td>22.6 ± 1.3</td>
<td>23.3 ± 1.4</td>
<td>26.3 ± 2.5</td>
</tr>
<tr>
<td><strong>PGOT (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>11.1 ± 0.7</td>
<td>10.7 ± 0.8</td>
<td>10.3 ± 0.9</td>
<td>10.0 ± 0.8</td>
<td>9.4 ± 0.9</td>
<td>9.4 ± 1.0</td>
</tr>
<tr>
<td>stimulated</td>
<td>7.7 ± 0.3</td>
<td>7.9 ± 0.4</td>
<td>8.8 ± 0.5</td>
<td>7.9 ± 0.6</td>
<td>7.0 ± 0.5</td>
<td>7.1 ± 0.3</td>
</tr>
<tr>
<td><strong>PLDH (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>8.0 ± 1.3</td>
<td>8.7 ± 1.0</td>
<td>8.1 ± 0.9</td>
<td>7.6 ± 0.6</td>
<td>8.3 ± 0.8</td>
<td>8.3 ± 1.1</td>
</tr>
<tr>
<td>stimulated</td>
<td>4.6 ± 1.0</td>
<td>4.3 ± 1.0</td>
<td>10.8 ± 1.3</td>
<td>8.8 ± 1.1</td>
<td>6.8 ± 1.3</td>
<td>6.6 ± 0.6</td>
</tr>
<tr>
<td><strong>Plasma protein (gm%)</strong></td>
<td>6.93 ± 0.28</td>
<td>6.72 ± 0.24</td>
<td>6.94 ± 0.22</td>
<td>6.81 ± 0.21</td>
<td>6.73 ± 0.21</td>
<td>6.90 ± 0.25</td>
</tr>
<tr>
<td>control</td>
<td>7.12 ± 0.30</td>
<td>7.05 ± 0.28</td>
<td>7.23 ± 0.27</td>
<td>7.20 ± 0.30</td>
<td>7.05 ± 0.27</td>
<td>6.96 ± 0.26</td>
</tr>
<tr>
<td>stimulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± S E M

n is equal to 6 and 8 for control and stimulated groups respectively
Table 18. Changes in PCPK, PGOT, and PLDH during skeletal muscle stimulation

<table>
<thead>
<tr>
<th></th>
<th>1 hour before stimulation</th>
<th>before stimulation</th>
<th>end of stimulation</th>
<th>30 minutes after stimulation</th>
<th>1 hour after stimulation</th>
<th>2 hours after stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCPK (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>+1.8 ± 0.1*</td>
<td>0.0 ± 0.0</td>
<td>-0.8 ± 0.7</td>
<td>-1.0 ± 0.5</td>
<td>-2.0 ± 0.7</td>
<td>-3.2 ± 1.4</td>
</tr>
<tr>
<td>stimulated</td>
<td>+0.9 ± 0.9</td>
<td>0.0 ± 0.0</td>
<td>-0.4 ± 0.4</td>
<td>-1.3 ± 0.8</td>
<td>-0.6 ± 0.9</td>
<td>+2.3 ± 2.3</td>
</tr>
<tr>
<td><strong>PGOT (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>+0.4 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>-0.4 ± 0.4</td>
<td>-0.5 ± 0.3</td>
<td>-1.3 ± 0.3</td>
<td>-1.3 ± 0.4</td>
</tr>
<tr>
<td>stimulated</td>
<td>+0.6 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>+0.9 ± 0.3</td>
<td>0.0 ± 0.5</td>
<td>-0.8 ± 0.5</td>
<td>-0.1 ± 0.4</td>
</tr>
<tr>
<td><strong>PLDH (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-0.5 ± 1.4</td>
<td>0.0 ± 0.0</td>
<td>-0.5 ± 0.3</td>
<td>-1.1 ± 1.2</td>
<td>-0.3 ± 0.6</td>
<td>-0.4 ± 0.5</td>
</tr>
<tr>
<td>stimulated</td>
<td>+0.3 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>+6.5 ± 0.9</td>
<td>+4.5 ± 0.9</td>
<td>+2.5 ± 1.1</td>
<td>+2.3 ± 0.7</td>
</tr>
</tbody>
</table>

*Mean ± SEM

n is equal to 6 and 8 for control and stimulated groups respectively
Effect of skeletal muscle stimulation on

PCPK, PGOT and PLDH in thoracic duct lymph-drained animals.

Enzyme values are expressed as the difference in activity from the pre-stimulation value (B)

- muscle stimulation
- muscle-stimulated group
- control group
In both control and muscle-stimulated groups, the plasma protein concentration remained steady throughout the experiment. The stimulation-induced changes in plasma enzyme activity were very similar to those reported by Loegering and Critz (1971). There was no change in PCPK during muscle stimulation. However, whereas in control animals PCPK gradually decreased throughout the experiment, in the stimulated animals the highest PCPK activity was reached at the termination of the experiment. PGOT showed a small but significant increase following muscle stimulation (mean increase = 0.9 IU/L; range 0.2 to 2.3 IU/L) (P<0.01). PGOT returned to pre-stimulation values within 30 minutes after stimulation. In control animals, PGOT levels dropped throughout the experiment. PLDH also increased during muscle stimulation (mean increase = 6.5 IU/L; range 2.3 to 9.9 IU/L) (P<0.01). Following stimulation, PLDH returned towards pre-stimulation values.

The effect of muscle stimulation on thoracic duct lymph flow is shown in Table 19 and Figure 19. During muscle stimulation there was a 2- to 3-fold increase in lymph flow, which was maintained throughout stimulation. During the first 30 minutes of recovery, lymph flow was reduced to approximately 50% of the pre-stimulation value, and gradually returned to control values over the next 1 hour.
Table 19. The response of thoracic duct lymph flow to skeletal muscle stimulation

<table>
<thead>
<tr>
<th>Volume of Lymph (ml)</th>
<th>control</th>
<th>stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-30 min before stimulation</td>
<td>11.7 ± 3.8*</td>
<td>9.7 ± 1.3</td>
</tr>
<tr>
<td>30-0 min before stimulation</td>
<td>11.8 ± 3.8</td>
<td>9.8 ± 0.9</td>
</tr>
<tr>
<td>0-10 min of stimulation</td>
<td>8.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>10-20 min of stimulation</td>
<td>8.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>20-30 min of stimulation</td>
<td>7.6 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>0-30 min of stimulation</td>
<td>9.9 ± 2.7</td>
<td>24.1 ± 1.8</td>
</tr>
<tr>
<td>0-30 min after stimulation</td>
<td>11.2 ± 2.7</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>30-60 min after stimulation</td>
<td>9.6 ± 2.4</td>
<td>6.0 ± 1.4</td>
</tr>
<tr>
<td>60-90 min after stimulation</td>
<td>10.5 ± 1.4</td>
<td>6.9 ± 0.8</td>
</tr>
<tr>
<td>90-120 min after stimulation</td>
<td>9.4 ± 1.9</td>
<td>6.8 ± 0.9</td>
</tr>
</tbody>
</table>

*Mean ± S E M

n is equal to 6 and 8 for control and stimulated groups respectively.
The data on thoracic duct lymph enzyme activity and protein concentration is presented in Table 20 and in Figures 15, 16, 17, and 18. Lymph protein concentration decreased throughout muscle stimulation, reaching its lowest value during the first 30 minutes of recovery, and returning to pre-stimulation values during the remainder of the experiment. (Figure 15).

As previously noted, thoracic duct LGOT activity was significantly greater than PGOT activity during the control period, LCPK activity was similar to PCPK activity, and LLDH activity was usually considerably less than PLDH activity (Figures 16, 17, and 18). There was a marked increase in LCPK activity during muscle stimulation (pre-stimulation range, 16 to 56 IU/L; end of stimulation range 28 to 252 IU/L) (Figure 16). The lymph collected during the first 30 minutes after stimulation still possessed high CPK activity. The thoracic duct and large abdominal lymphatic vessels contain several millilitres of lymph, and the small volume of lymph collected during the 30 minute period following stimulation probably consisted of lymph formed during the period of stimulation. LCPK activity had returned to control values by the second 30 minute period of recovery. The changes in LGOT were similar but smaller than those in LCPK (pre-stimulation
<table>
<thead>
<tr>
<th>Time Period</th>
<th>CPK (IU/litre)</th>
<th>GOT (IU/litre)</th>
<th>LDH (IU/litre)</th>
<th>Protein (g%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>stim</td>
<td>control</td>
<td>stim</td>
</tr>
<tr>
<td>60-30 min before stimulation</td>
<td>25.5±5.8*</td>
<td>30.5±4.2</td>
<td>16.1±3.4</td>
<td>11.4±1.6</td>
</tr>
<tr>
<td>30-0 min before stimulation</td>
<td>23.8±4.6</td>
<td>31.3±5.1</td>
<td>16.5±3.2</td>
<td>11.9±2.6</td>
</tr>
<tr>
<td>0-10 min of stimulation</td>
<td>37.3±5.8</td>
<td>12.4±2.0</td>
<td>5.0±1.2</td>
<td>5.02±0.38</td>
</tr>
<tr>
<td>10-20 min of stimulation</td>
<td>22.2±6.0 a</td>
<td>52.8±9.9</td>
<td>18.1±3.5</td>
<td>15.4±1.2</td>
</tr>
<tr>
<td>20-30 min of stimulation</td>
<td>91.4±28.5</td>
<td>18.7±2.1</td>
<td>8.7±0.6</td>
<td>4.71±0.41</td>
</tr>
<tr>
<td>0-30 min after stimulation</td>
<td>24.2±4.8</td>
<td>75.6±19.3</td>
<td>17.1±3.2</td>
<td>17.1±1.9</td>
</tr>
<tr>
<td>30-60 min after stimulation</td>
<td>24.0±5.2</td>
<td>38.8±7.1</td>
<td>18.4±3.6</td>
<td>11.4±1.2</td>
</tr>
<tr>
<td>60-90 min after stimulation</td>
<td>21.4±4.6</td>
<td>35.5±6.7</td>
<td>16.7±3.0</td>
<td>11.8±1.2</td>
</tr>
<tr>
<td>90-120 min after stimulation</td>
<td>19.6±5.1</td>
<td>34.6±5.5</td>
<td>16.6±3.2</td>
<td>11.1±1.5</td>
</tr>
</tbody>
</table>

*Mean ± SEM
n is equal to 6 and 8 for control and stimulated groups respectively
a control value obtained on lymph collected over 0-30 minutes of stimulation
Protein concentration in the plasma and thoracic duct lymph of control and muscle-stimulated animals.

muscle stimulation

Plasma control ($P_c$)
Plasma stimulated ($P_s$)
Lymph control ($L_c$)
Lymph stimulated ($L_s$)
CPK activity in the plasma and thoracic duct lymph of control and muscle-stimulated animals.

- muscle stimulation
- Plasma control \( (P_c) \)
- Plasma stimulated \( (P_s) \)
- Lymph control \( (L_c) \)
- Lymph stimulated \( (L_s) \)
GOT activity in the plasma and thoracic duct lymph of control and muscle-stimulated animals.

- Muscle stimulation
- Plasma control ($P_c$)
- Plasma stimulated ($P_s$)
- Lymph control ($L_c$)
- Lymph stimulated ($L_s$)
FIGURE 18

LDH activity in the plasma and thoracic duct lymph of control and muscle-stimulated animals.

- muscle stimulation .......... ______

- Plasma control ($P_c$) .......... ------
- Plasma stimulated ($P_s$) ......... ______
- Lymph control ($L_c$) .......... ............
- Lymph stimulated ($L_s$) .......... ............
range, 5.7 to 18.3 IU/L; end of stimulation range, 13.2 to 27.6 IU/L) (Figure 17). Lymph collected in the first 30 minutes of recovery still demonstrated elevated levels of GOT. However, LGOT had returned to pre-stimulation values within 1 hour after stimulation. LLDH activity changed considerably during muscle stimulation (pre-stimulation range, 0 to 7.1 IU/L; end of stimulation range 7.4 to 12.2 IU/L) (Figure 18). In all animals, LDH activity in the lymph during stimulation rose to values just less than those observed in the plasma. LLDH remained elevated for the first 30 minutes of recovery, and returned to control values over the next 90 minutes. In 3 animals in which no LDH could be detected in pre-stimulation lymph samples, LLDH reached 7.8, 8.5 and 12.2 IU/L during stimulation, and had returned to 0, 0.9, and 0.9 IU/L at the termination of the experiment.

Delivery of enzyme was determined as the product of lymph flow and enzyme activity in the lymph. Since there was a 2- to 3-fold increase in lymph flow during stimulation, an increase in enzyme delivery of similar magnitude would be expected if lymph enzyme activity were to remain constant. However, since lymph activity of all three enzymes studied increased during
stimulation, considerable increases in enzyme delivery occurred. This is demonstrated in Table 21 and in Figure 19. All three enzymes showed a progressive increase in their delivery during muscle stimulation. CPK delivery increased almost 5-fold during muscle stimulation (pre-stimulation range 12 to 53 IU.10^-2; end of stimulation range, 78 to 528 IU.10^-2). GOT delivery increased over 3-fold during muscle stimulation (pre-stimulation range, 4 to 29 IU.10^-2; end of stimulation range, 22 to 56 IU.10^-2). LDH showed a 7½-fold increase in delivery during stimulation (pre-stimulation range, 0 to 7 IU.10^-2; end of stimulation range, 10 to 36 IU.10^-2). While this was a large proportional increase in LDH delivery, it was quantitatively insignificant as will be shown in the following paragraph. Although all three enzymes maintained elevated lymphatic activities during the first 30 minutes of recovery, their delivery returned to control values at this time as a result of the small volumes of lymph produced during this period.

As described earlier, an estimate of the effect of the removal of thoracic duct lymph on plasma enzyme activity may be made from figures on enzyme delivery and an assumed plasma volume (50 ml/Kg of body weight) (Hoft, Deavers, and Huggins, 1966).
Table 21. The delivery of CPK, GOT and LDH in thoracic duct lymph during skeletal muscle stimulation

<table>
<thead>
<tr>
<th></th>
<th>CPK</th>
<th>GOT</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(IU/30 min x 10^{-2})</td>
<td>(IU/30 min x 10^{-2})</td>
<td>(IU/30 min x 10^{-2})</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>stimulation</td>
<td>control</td>
</tr>
<tr>
<td>60-90 min before stimulation</td>
<td>22 ± 7*</td>
<td>33 ± 6</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>30-60 min before stimulation</td>
<td>20 ± 7</td>
<td>28 ± 5</td>
<td>14 ± 6</td>
</tr>
<tr>
<td>0-10 min of stimulation</td>
<td>82 ± 11</td>
<td></td>
<td>27 ± 5</td>
</tr>
<tr>
<td>10-20 min of stimulation</td>
<td>118 ± 23</td>
<td></td>
<td>35 ± 4</td>
</tr>
<tr>
<td>20-30 min of stimulation</td>
<td>195 ± 64</td>
<td></td>
<td>39 ± 5</td>
</tr>
<tr>
<td>0-30 min of stimulation</td>
<td>21 ± 7</td>
<td>132 ± 29</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>0-30 min after stimulation</td>
<td>21 ± 8</td>
<td>37 ± 10</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>30-60 min after stimulation</td>
<td>25 ± 7</td>
<td>22 ± 4</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>60-90 min after stimulation</td>
<td>20 ± 4</td>
<td>25 ± 5</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>90-120 min after stimulation</td>
<td>18 ± 5</td>
<td>22 ± 3</td>
<td>10 ± 1</td>
</tr>
</tbody>
</table>

* Mean ± S E M

n is equal to 6 and 8 for control and stimulated groups respectively.
Thoracic duct lymph flow, and lymphatic delivery of GOT, CPK and LDH in control and muscle-stimulated animals.

control group .......... -----
stimulated group ...... ----

(Full data on CPK delivery can be found in Table 21.)
muscle stimulation ..... ----
During the control, pre-stimulation period, estimated values of effective enzyme loss to the plasma were very similar to those previously recorded (See Table 14). In 8 dogs, the removal of thoracic duct lymph brought about an estimated decrease in plasma activities of CPK, GOT, and LDH of 0.88, 0.25, and 0.05 IU/L/hour, respectively. Over the 30 minute period of muscle stimulation, removal of lymph brought about an estimated loss in PCPK of 1.8 IU/L (0.7 to 3.6), in PGOT of 0.5 IU/L (0.3 to 0.9), and in PLDH of 0.3 IU/L (0.1 to 0.7). By adding these calculated losses to the plasma enzyme changes observed during stimulation, the changes which would have been observed in the intact animal were estimated (Table 22).

6. **Effect of repeated bouts of skeletal muscle stimulation on thoracic duct lymph flow and enzyme activity**

Three dogs were subjected to two, 30 minute periods of muscle stimulation, separated by a 2 hour recovery interval.

There was a significant increase in heart rate during both periods of muscle stimulation ($P<0.01$) (Table 23). Arterial blood pressure remained steady throughout the experiment.
Table 22. The effect of drainage of thoracic duct lymph on the changes in PCPK, PGOT, and PLDH during 30 minutes of muscle stimulation

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Measured change</th>
<th>Estimated loss through removal of lymph</th>
<th>Adjusted change</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCPK</td>
<td>$-0.4 \pm 0.4^*$</td>
<td>$+1.8 \pm 0.5$</td>
<td>$+1.4 \pm 0.5$</td>
</tr>
<tr>
<td>PGOT</td>
<td>$+0.9 \pm 0.3$</td>
<td>$+0.5 \pm 0.1$</td>
<td>$+1.4 \pm 0.4$</td>
</tr>
<tr>
<td>PLDH</td>
<td>$+6.5 \pm 0.9$</td>
<td>$+0.3 \pm 0.7$</td>
<td>$+6.8 \pm 0.9$</td>
</tr>
</tbody>
</table>

* Mean ± SEM

n is equal to 8

All values are in IU/L
<table>
<thead>
<tr>
<th>Time Point</th>
<th>Heart Rate (beats/minute)</th>
<th>Systolic Pressure (mmHg)</th>
<th>Diastolic Pressure (mmHg)</th>
<th>Mean Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour before 1st stimulation</td>
<td>172 ± 12*</td>
<td>153 ± 11</td>
<td>125 ± 5</td>
<td>141 ± 8</td>
</tr>
<tr>
<td>before 1st stimulation</td>
<td>167 ± 13</td>
<td>158 ± 9</td>
<td>125 ± 5</td>
<td>140 ± 8</td>
</tr>
<tr>
<td>end of 1st stimulation</td>
<td>196 ± 23</td>
<td>167 ± 3</td>
<td>123 ± 2</td>
<td>139 ± 4</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>200 ± 24</td>
<td>160 ± 10</td>
<td>133 ± 9</td>
<td>143 ± 9</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>170 ± 14</td>
<td>160 ± 9</td>
<td>130 ± 9</td>
<td>142 ± 9</td>
</tr>
<tr>
<td>2 hours after; before 2nd stimulation</td>
<td>151 ± 12</td>
<td>158 ± 13</td>
<td>127 ± 12</td>
<td>137 ± 12</td>
</tr>
<tr>
<td>end of 2nd stimulation</td>
<td>206 ± 30</td>
<td>162 ± 7</td>
<td>113 ± 9</td>
<td>130 ± 12</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>171 ± 10</td>
<td>157 ± 14</td>
<td>127 ± 12</td>
<td>138 ± 11</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>180 ± 12</td>
<td>157 ± 9</td>
<td>125 ± 8</td>
<td>137 ± 9</td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>181 ± 13</td>
<td>158 ± 12</td>
<td>126 ± 7</td>
<td>137 ± 7</td>
</tr>
</tbody>
</table>

* Mean ± SEM
n is equal to 3
Following the first period of muscle stimulation, all animals were cooled to bring rectal temperature back to the initial pre-stimulation value before the second period of muscle stimulation. Over the two periods of muscle stimulation, mean rectal temperature increased by 2.6° and 2.9°C, respectively (Table 24).

Arterial blood pH was 7.34 prior to the first period of muscle stimulation (Table 24). At the end of muscle stimulation, arterial pH had increased to 7.39, with a further increase to 7.43 occurring during the first 30 minutes of recovery. During the 2 hours of recovery, arterial pH fell to 7.28. The second period of muscle stimulation raised the arterial pH to 7.35. It had fallen to 7.33 by the 30th minute of recovery.

Oxygen consumption increased to over 3-fold the control value during both periods of muscle stimulation (Table 24). There was no significant difference between the oxygen consumption achieved during the two separate periods of muscle stimulation.

The data on plasma protein concentrations and plasma enzyme activities are presented in Tables 25 and 26, and in Figure 20. In Table 26, the changes in plasma enzyme activity with respect to pre-stimulation values are given. To enable comparison between the
<table>
<thead>
<tr>
<th></th>
<th>Haematocrit (%)</th>
<th>Rectal Temperature (°C)</th>
<th>Arterial pH</th>
<th>Oxygen Consumption (ml/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour before lst stimulation</td>
<td>43.7 ± 5.7*</td>
<td>37.4 ± 0.1</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>before lst stimulation</td>
<td>45.2 ± 5.7</td>
<td>37.5 ± 0.3</td>
<td>7.34 ± 0.02</td>
<td>5.8 ± 0.7</td>
</tr>
<tr>
<td>end of lst stimulation</td>
<td>49.5 ± 5.0</td>
<td>40.1 ± 0.3</td>
<td>7.39 ± 0.02</td>
<td>20.1 ± 1.8</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>48.3 ± 4.6</td>
<td>39.3 ± 0.1</td>
<td>7.43 ± 0.02</td>
<td>7.7 ± 1.3</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>48.7 ± 4.4</td>
<td>38.5 ± 0.3</td>
<td>- - -</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>2 hours after; before 2nd stimulation</td>
<td>48.8 ± 5.1</td>
<td>37.9 ± 0.5</td>
<td>7.28 ± 0.04</td>
<td>6.8 ± 0.8</td>
</tr>
<tr>
<td>end of 2nd stimulation</td>
<td>52.5 ± 4.8</td>
<td>40.8 ± 0.2</td>
<td>7.35 ± 0.02</td>
<td>22.2 ± 3.1</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>51.0 ± 4.0</td>
<td>40.1 ± 0.3</td>
<td>7.33 ± 0.05</td>
<td>8.5 ± 0.4</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>51.3 ± 4.9</td>
<td>39.6 ± 0.4</td>
<td>- - -</td>
<td>8.4 ± 0.7</td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>52.2 ± 4.7</td>
<td>39.1 ± 0.3</td>
<td>- - -</td>
<td>7.5 ± 0.8</td>
</tr>
</tbody>
</table>

*Mean ± S.E.M
n is equal to 3
Table 25. PCPK, PGOT, PLDH and plasma protein concentration during repeated bouts of skeletal muscle stimulation

<table>
<thead>
<tr>
<th>Time after Stimulation</th>
<th>PCPK (IU/L)</th>
<th>PGOT (IU/L)</th>
<th>PLDH (IU/L)</th>
<th>Plasma Protein (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour before 1st stimulation</td>
<td>25.7 ± 1.2*</td>
<td>7.8 ± 0.5</td>
<td>3.4 ± 0.4</td>
<td>7.27 ± 0.53</td>
</tr>
<tr>
<td>before 1st stimulation</td>
<td>26.7 ± 1.7</td>
<td>7.2 ± 0.8</td>
<td>3.4 ± 0.3</td>
<td>7.10 ± 0.32</td>
</tr>
<tr>
<td>end of 1st stimulation</td>
<td>26.0 ± 1.5</td>
<td>8.4 ± 0.6</td>
<td>9.9 ± 2.5</td>
<td>7.37 ± 0.54</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>23.3 ± 1.3</td>
<td>8.3 ± 1.1</td>
<td>8.1 ± 2.1</td>
<td>7.65 ± 0.53</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>24.0 ± 2.7</td>
<td>7.8 ± 0.7</td>
<td>6.5 ± 2.5</td>
<td>7.33 ± 0.46</td>
</tr>
<tr>
<td>2 hours after 2nd stimulation</td>
<td>25.0 ± 4.9</td>
<td>7.3 ± 0.9</td>
<td>6.5 ± 0.4</td>
<td>7.32 ± 0.50</td>
</tr>
<tr>
<td>end of 2nd stimulation</td>
<td>27.3 ± 6.6</td>
<td>8.9 ± 1.0</td>
<td>17.9 ± 4.8</td>
<td>7.23 ± 0.42</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>27.7 ± 6.8</td>
<td>- - -</td>
<td>- - -</td>
<td>7.10 ± 0.39</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>30.0 ± 7.5</td>
<td>8.1 ± 0.8</td>
<td>9.7 ± 2.7</td>
<td>7.08 ± 0.30</td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>36.0 ± 4.6</td>
<td>7.9 ± 0.7</td>
<td>- - -</td>
<td>7.35 ± 0.40</td>
</tr>
</tbody>
</table>

* Mean ± S E M
n is equal to 3
<table>
<thead>
<tr>
<th>Time Point</th>
<th>PCPK (IU/L)</th>
<th>PGOT (IU/L)</th>
<th>PLDH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour before 1st stimulation</td>
<td>-1.0 ± 0.6*</td>
<td>+0.6 ± 0.1</td>
<td>-0.1 ± 0.2</td>
</tr>
<tr>
<td>before 1st stimulation</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>end of 1st stimulation</td>
<td>-0.7 ± 0.3</td>
<td>+1.2 ± 0.6</td>
<td>+6.4 ± 2.2</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>-2.4 ± 0.3</td>
<td>+1.0 ± 0.9</td>
<td>+4.6 ± 2.0</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>-2.7 ± 1.2</td>
<td>+0.4 ± 0.4</td>
<td>+3.0 ± 2.2</td>
</tr>
<tr>
<td>2 hours after; before 2nd stimulation</td>
<td>-1.7 ± 3.5</td>
<td>-0.1 ± 0.3</td>
<td>+3.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>end of 2nd stimulation</td>
<td>+2.3 ± 1.9</td>
<td>+1.6 ± 1.0</td>
<td>+11.3 ± 4.4</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>+2.7 ± 2.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>+5.0 ± 3.5</td>
<td>+0.9 ± 0.6</td>
<td>+3.1 ± 2.3</td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>+11.0 ± 1.2</td>
<td>+0.6 ± 0.2</td>
<td>--</td>
</tr>
</tbody>
</table>

*Mean ± S E M
n is equal to 3
FIGURE 20

The effect of repeated bouts of muscle stimulation on the activities of CPK, GOT and LDH in the plasma and thoracic duct lymph.

(For full details on the mean values ± SEM refer to Tables 25 and 27.)

 muscle stimulation ...........

 enzyme activity in plasma .......

 enzyme activity in lymph .......
plasma enzyme responses to the first and second periods of muscle stimulation, the enzyme levels following the second period of muscle stimulation are compared to the enzyme levels directly before this period. There were no consistent changes in plasma protein concentration during the experiment (Table 25). Likewise, no significant changes in PCPK were observed following either period of muscle stimulation. However, considerable increases in PCPK occurred in all 3 animals towards the end of the experiment (Table 26). PGOT showed approximately the same, small increase during both periods of muscle stimulation (Table 26). These small elevations in PGOT were followed by a decrease towards control values. In contrast to PGOT, PLDH responded to the second period of muscle stimulation with an increase in activity almost twice that in response to the first (6.4 and 11.3 IU/L, respectively) (Table 26). After both periods of muscle stimulation, PLDH fell rapidly towards pre-stimulation values.

Data on the changes in thoracic duct lymph flow, and thoracic duct lymph protein and enzyme levels during repeated muscle stimulation can be found in Table 27. Lymph flow increased almost 3-fold during both periods of stimulation. Toward the end of the experiment, lymph
Table 27. Thoracic duct lymph flow, protein, CPK, GOT, and LDH responses to repeated bouts of skeletal muscle stimulation

<table>
<thead>
<tr>
<th>Values of lymph (ml)</th>
<th>Protein (g%)</th>
<th>CPK (IU/L)</th>
<th>GOT (IU/L)</th>
<th>LDH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-30 min before stimulation</td>
<td>10.1 ± 1.3*</td>
<td>5.38 ± 0.36</td>
<td>32.3 ± 2.3</td>
<td>10.3 ± 2.7</td>
</tr>
<tr>
<td>30-0 min before stimulation</td>
<td>9.2 ± 1.1</td>
<td>5.61 ± 0.59</td>
<td>32.7 ± 8.2</td>
<td>13.5 ± 6.2</td>
</tr>
<tr>
<td>0-10 min of stimulation</td>
<td>7.9 ± 1.3</td>
<td>5.54 ± 0.62</td>
<td>32.7 ± 4.6</td>
<td>12.2 ± 4.7</td>
</tr>
<tr>
<td>10-20 min of stimulation</td>
<td>9.3 ± 0.8</td>
<td>5.34 ± 0.60</td>
<td>57.3 ± 11.4</td>
<td>15.2 ± 2.2</td>
</tr>
<tr>
<td>20-30 min of stimulation</td>
<td>8.1 ± 1.2</td>
<td>5.11 ± 0.90</td>
<td>106.7 ± 36.0</td>
<td>20.4 ± 3.7</td>
</tr>
<tr>
<td>0-30 min after stimulation</td>
<td>5.4 ± 0.4</td>
<td>4.81 ± 0.56</td>
<td>81.0 ± 26.2</td>
<td>19.5 ± 4.1</td>
</tr>
<tr>
<td>30-60 min after stimulation</td>
<td>4.8 ± 0.1</td>
<td>4.80 ± 0.29</td>
<td>40.0 ± 6.6</td>
<td>11.4 ± 2.5</td>
</tr>
<tr>
<td>60-90 min after stimulation</td>
<td>5.6 ± 0.5</td>
<td>4.95 ± 0.34</td>
<td>36.7 ± 3.8</td>
<td>14.8 ± 5.0</td>
</tr>
<tr>
<td>90-120 min after stimulation</td>
<td>7.2 ± 1.7</td>
<td>5.27 ± 0.43</td>
<td>36.3 ± 9.8</td>
<td>14.5 ± 7.1</td>
</tr>
<tr>
<td>0-10 min of stimulation</td>
<td>7.7 ± 0.8</td>
<td>5.19 ± 0.25</td>
<td>44.7 ± 8.0</td>
<td>16.6 ± 4.6</td>
</tr>
<tr>
<td>10-20 min of stimulation</td>
<td>6.8 ± 0.9</td>
<td>4.96 ± 0.36</td>
<td>92.3 ± 16.0</td>
<td>17.1 ± 3.0</td>
</tr>
<tr>
<td>20-30 min of stimulation</td>
<td>5.6 ± 0.7</td>
<td>5.07 ± 0.29</td>
<td>146.7 ± 48.0</td>
<td>20.4 ± 2.0</td>
</tr>
<tr>
<td>0-30 min after stimulation</td>
<td>6.2 ± 0.4</td>
<td>4.92 ± 0.57</td>
<td>98.3 ± 22.7</td>
<td>18.8 ± 3.9</td>
</tr>
<tr>
<td>30-60 min after stimulation</td>
<td>5.5 ± 0.6</td>
<td>4.80 ± 0.26</td>
<td>58.7 ± 18.0</td>
<td>15.7 ± 2.5</td>
</tr>
<tr>
<td>60-90 min after stimulation</td>
<td>5.5 ± 0.6</td>
<td>4.65 ± 0.13</td>
<td>43.0 ± 9.2</td>
<td>12.6 ± 3.4</td>
</tr>
<tr>
<td>90-120 min after stimulation</td>
<td>5.5 ± 1.1</td>
<td>4.64 ± 0.09</td>
<td>39.3 ± 10.2</td>
<td>-- -- --</td>
</tr>
</tbody>
</table>

* Mean ± S.E.M

n is equal to 3
flow was considerably less than control. Lymph protein concentration declined throughout the first $2\frac{1}{2}$ hours of the experiment, and then increased prior to the second period of muscle stimulation (Table 27). Following the second period of muscle stimulation, lymph protein concentration again declined. The marked changes in the lymph protein concentration seen in earlier experiments were not observed in these animals.

The changes in lymph enzyme activity observed during the second period of muscle stimulation were very similar to those occurring in the first episode (Table 27, Figure 20). By the end of the first period of muscle stimulation, thoracic duct LCPK had increased to 3.3-fold control (pre-stimulation range, 20 to 48 IU/L; end of stimulation range, 55 to 176 IU/L). By the termination of the second period of muscle stimulation, LCPK had increased 4-fold (pre-stimulation range, 26 to 56 IU/L; end of stimulation range, 62 to 228 IU/L). Lymphatic activity of CPK was high during the first 30 minutes of recovery, and then returned towards control values. LGOT had increased to 1.4-fold control by the end of the first period of muscle stimulation (pre-stimulation range, 5.9 to 25.8 IU/L; end of stimulation range, 15.0 to 27.6 IU/L). By the end of the second
episode of muscle stimulation, LGOT had increased to 1.4-fold control (pre-stimulation range, 4.4 to 29.7 IU/L; end of stimulation range, 16.5 to 23.0 IU/L). LLDH showed a 4.1-fold increase in activity by the end of the first period of muscle stimulation (pre-stimulation range, 0 to 6.2 IU/L; end of stimulation range, 7.8 to 9.2 IU/L). By the end of the second period of muscle stimulation LLDH had increased to 3.7-fold control (pre-stimulation range, 0 to 5.7 IU/L; end of stimulation range, 4.8 to 11.5 IU/L). GOT activity in the lymph was still elevated during the first 30 minutes of both recovery periods, returning to control values over the next 90 minutes. In contrast, LLDH levels had already fallen during the first 30 minutes of recovery.

Data on the delivery of enzyme via the thoracic duct are presented in Table 28 and Figure 21. Since lymph flow is a determinant of lymphatic enzyme delivery, the lower lymph flows during the second period of stimulation might have been expected to result in a lower delivery of enzyme during that period. However, this was almost entirely compensated for by increases in lymph enzyme activity. CPK delivery during the first period of muscle stimulation was 5.5-fold control (pre-stimulation range, 14 to 53 IU.10^{-2}; end of stimulation range, 96 to 528 IU.10^{-2}). During the second period
<table>
<thead>
<tr>
<th></th>
<th>CPK (IU/30 min x 10^2)</th>
<th>GOT (IU/30 min x 10^2)</th>
<th>LDH (IU/30 min x 10^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-30 min before stimulation</td>
<td>34 ± 4*</td>
<td>10 ± 3</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>30-0 min before stimulation</td>
<td>32 ± 20</td>
<td>14 ± 8</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>0-10 min of stimulation</td>
<td>80 ± 17</td>
<td>30 ± 13</td>
<td>13 ± 7</td>
</tr>
<tr>
<td>10-20 min of stimulation</td>
<td>162 ± 41</td>
<td>43 ± 3</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>20-30 min of stimulation</td>
<td>289 ± 128</td>
<td>50 ± 3</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>0-30 min of stimulation</td>
<td>175 ± 51</td>
<td>41 ± 4</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>0-30 min after stimulation</td>
<td>44 ± 15</td>
<td>11 ± 3</td>
<td>3 ± 0</td>
</tr>
<tr>
<td>30-60 min after stimulation</td>
<td>19 ± 4</td>
<td>5 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>60-90 min after stimulation</td>
<td>21 ± 3</td>
<td>9 ± 4</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>90-120 min after stimulation</td>
<td>29 ± 14</td>
<td>12 ± 9</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>0-10 min of stimulation</td>
<td>100 ± 10</td>
<td>38 ± 11</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>10-20 min of stimulation</td>
<td>184 ± 51</td>
<td>34 ± 6</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>20-30 min of stimulation</td>
<td>267 ± 104</td>
<td>34 ± 3</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>0-30 min of stimulation</td>
<td>183 ± 50</td>
<td>35 ± 6</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>0-30 min after stimulation</td>
<td>62 ± 18</td>
<td>11 ± 2</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>30-60 min after stimulation</td>
<td>31 ± 8</td>
<td>9 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>60-90 min after stimulation</td>
<td>23 ± 3</td>
<td>7 ± 2</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>90-120 min after stimulation</td>
<td>22 ± 7</td>
<td>- - -</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

* Mean ± S.E.M.

n is equal to 3.
FIGURE 21

The effect of repeated bouts of muscle stimulation on thoracic duct lymph flow, and lymphatic delivery of CPK, GOT and LDH.

(For full details on mean values ± SEM refer to Table 28.)

muscle stimulation ......
of muscle stimulation, CPK delivery increased to 6.3-fold control (pre-stimulation range, 12 to 57 IU.10^{-2}; end of stimulation range, 83 to 444 IU.10^{-2}). GOT delivery increased 2.9-fold during the first period of muscle stimulation (pre-stimulation range, 4 to 29 IU.10^{-2}; end of stimulation range, 46 to 56 IU.10^{-2}). During the second period of muscle stimulation, GOT delivery again increased 2.9-fold (pre-stimulation range, 3 to 30 IU.10^{-2}; end of stimulation range, 29 to 40 IU.10^{-2}). As previously observed, LDH delivery increased dramatically during stimulation. During the first period of muscle stimulation, delivery of LDH increased 8-fold (pre-stimulation range, 0 to 7 IU.10^{-2}; end of stimulation range, 16 to 36 IU.10^{-2}). In the second period of muscle stimulation, there was a 9-fold rise in LDH delivery (pre-stimulation range, 0 to 3 IU.10^{-2}; end of stimulation range, 9 to 21 IU.10^{-2}). However, in only one animal was there any significant lymphatic delivery of LDH during the control period.

During the first 30 minutes of recovery following both periods of stimulation, CPK and GOT delivery returned to control values. In the next 30 minutes of recovery, delivery of these two enzymes fell below control values. There was no significant difference between the delivery
of either CPK or GOT during the 2 separate periods of muscle stimulation. LDH delivery was significantly less during the second period of muscle stimulation (P<0.05).

The 'effective loss' of enzyme to the plasma compartment as a result of lymph drainage, was similar during the 2 separate periods of muscle stimulation. 'Loss' of PCPK was 1.8 and 2.1 IU/L, of PGOT was 0.5 and 0.5 IU/L; and of PLDH was 0.25 and 0.20 IU/L, during the first and second periods of muscle stimulation respectively (Table 29).

7. The effect of skeletal muscle stimulation upon the flow and enzyme activity of lymph from right and thoracic ducts

Following the observation that the drainage of thoracic duct lymph did not qualitatively alter the plasma enzyme response to muscle stimulation, it was decided to drain lymph from both the major lymphatic ducts concurrently during muscle stimulation. The right and thoracic ducts were cannulated and the flow and enzyme activity of lymph from each of these ducts were determined during muscle stimulation. The right lymph duct drains the heart, lungs, and some of the thoracic musculature, as well as a major part of excess peritoneal fluid. The only lymph that might have entered the circulating blood
Table 29. The effect of drainage of thoracic duct lymph on the changes in PCPK, PGOT, and PLDH during repeated 30-minute bouts of skeletal muscle stimulation

<table>
<thead>
<tr>
<th></th>
<th>Measured change</th>
<th>Estimated loss through removal of lymph</th>
<th>Adjusted change</th>
</tr>
</thead>
<tbody>
<tr>
<td>First period of stimulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCPK</td>
<td>$-0.7 \pm 0.3^*$</td>
<td>$+2.2 \pm 0.7$</td>
<td>$+1.5 \pm 0.6$</td>
</tr>
<tr>
<td>PGOT</td>
<td>$+1.2 \pm 0.6$</td>
<td>$+0.4 \pm 0.1$</td>
<td>$+1.7 \pm 0.4$</td>
</tr>
<tr>
<td>PLDH</td>
<td>$+6.4 \pm 2.2$</td>
<td>$+0.1 \pm 0.0$</td>
<td>$+6.5 \pm 2.2$</td>
</tr>
<tr>
<td>Second period of stimulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCPK</td>
<td>$+2.3 \pm 1.9$</td>
<td>$+2.1 \pm 0.8$</td>
<td>$+4.5 \pm 2.6$</td>
</tr>
<tr>
<td>PGOT</td>
<td>$+1.6 \pm 1.0$</td>
<td>$+0.5 \pm 0.0$</td>
<td>$+2.1 \pm 0.7$</td>
</tr>
<tr>
<td>PLDH</td>
<td>$+11.3 \pm 4.4$</td>
<td>$+0.0 \pm 0.0$</td>
<td>$+11.3 \pm 4.4$</td>
</tr>
</tbody>
</table>

* Mean $\pm$ SEM

n is equal to 3

All values are in IU/L.
in this preparation was that drained by the cervical and subclavian lymphatics. This lymph is formed primarily in the head, neck, and forelimbs, and its flow and composition would not be expected to change significantly during stimulation of hind limb skeletal muscle.

Direct cannulation of the right duct poses many problems, and an indirect approach was chosen. The venous segment into which the right lymph duct drained was isolated and used as a collection chamber. In the current study only 2 preparations out of 10 attempts were successful (success entailed the establishment and maintenance of a steady flow of lymph from the right duct for the full 6-hour duration of the experiment. In a further 2 animals, there was partial success and some data were obtained.

The main problems faced in establishing this preparation were (1) variable anatomy (including entry of the right duct into the azygos vein); (2) very low lymph flows (suggesting that only a branch of the right duct had been cannulated effectively); and (3) clotting of lymph. Of these problems the first was the most frequent and the least surmountable. Appendix 2 is a series of diagrams illustrating the various lymphovenous
arrangements of the right duct - jugular vein, in which a preparation was attempted.

The heart rate and arterial blood pressure changes during the experiment were similar to those previously observed and the results are given in Table 30. There was an increase in both heart rate (21 beats/minute) (P<0.01), and in systolic arterial pressure (13 mmHg) (P<0.01). Data on the respiratory rate, arterial haematocrit, and rectal temperature are given in Table 31. During muscle stimulation, respiratory rate increased over 4-fold. There was a large increase in arterial haematocrit during muscle stimulation (12.5% and 22% above pre-stimulation values). Rectal temperature increased by 2°C in both animals over the period of muscle stimulation.

Data on the plasma enzyme levels during the experiment are presented in Tables 32 and 33. PCPK levels did not differ significantly from pre-stimulation values at any time during the experiment. PGOT increased by a mean value of 7.7 IU/L during muscle stimulation (2.1 and 13.2 IU/L) (P<0.01). Two hours after muscle stimulation PGOT levels had returned to pre-stimulation values. PLDH also showed a large increase during muscle stimulation (11.5 and 42.5 IU/L) (P<0.01). PLDH returned towards pre-stimulation values during the 2-hour recovery
Table 30. Heart rate and arterial blood pressure during skeletal muscle stimulation in thoracic and right duct lymph-drained dogs.

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate (beats/minute)</th>
<th>Systolic Pressure (mmHg)</th>
<th>Diastolic Pressure (mmHg)</th>
<th>Mean Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>stim</td>
<td>control</td>
<td>stim</td>
</tr>
<tr>
<td>1 hour before stimulation</td>
<td>168 ± 11*</td>
<td>162 ± 6</td>
<td>157 ± 3</td>
<td>150 ± 5</td>
</tr>
<tr>
<td>before stimulation</td>
<td>171 ± 11</td>
<td>150 ± 6</td>
<td>161 ± 3</td>
<td>140 ± 5</td>
</tr>
<tr>
<td>end of stimulation</td>
<td>173 ± 12</td>
<td>171 ± 19</td>
<td>159 ± 5</td>
<td>153 ± 8</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>179 ± 9</td>
<td>175 ± 11</td>
<td>158 ± 4</td>
<td>145 ± 0</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>175 ± 5</td>
<td>166 ± 8</td>
<td>152 ± 8</td>
<td>140 ± 0</td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>175 ± 5</td>
<td>157 ± 17</td>
<td>150 ± 8</td>
<td>128 ± 3</td>
</tr>
</tbody>
</table>

* Mean ± S E M

n is equal to 6 and 2 for control and stimulated groups respectively.
Table 31. **Respiratory rate, arterial haematocrit, and rectal temperature during skeletal muscle stimulation in thoracic and right duct lymph-drained dogs**

<table>
<thead>
<tr>
<th>Time After Stimulation</th>
<th>Respiratory Rate (breaths/minute)</th>
<th>Arterial Haematocrit (%)</th>
<th>Rectal Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>stim</td>
<td>control</td>
</tr>
<tr>
<td>1 hour before stimulation</td>
<td>6 ± 1*</td>
<td>13 ± 4</td>
<td>43.4 ± 3.9</td>
</tr>
<tr>
<td>before stimulation</td>
<td>7 ± 1</td>
<td>11 ± 1</td>
<td>44.8 ± 3.8</td>
</tr>
<tr>
<td>end of stimulation</td>
<td>8 ± 2</td>
<td>47 ± 14</td>
<td>45.9 ± 3.5</td>
</tr>
<tr>
<td>30 minutes after stimulation</td>
<td>10 ± 1</td>
<td>13 ± 3</td>
<td>46.2 ± 3.4</td>
</tr>
<tr>
<td>1 hour after stimulation</td>
<td>9 ± 1</td>
<td>12 ± 2</td>
<td>47.5 ± 3.7</td>
</tr>
<tr>
<td>2 hours after stimulation</td>
<td>10 ± 2</td>
<td>11 ± 0</td>
<td>48.0 ± 3.8</td>
</tr>
</tbody>
</table>

*Mean ± S E M

n is equal to 6 and 2 for control and stimulated groups respectively.
<table>
<thead>
<tr>
<th></th>
<th>1 hour before stimulation</th>
<th>before stimulation</th>
<th>end of stimulation</th>
<th>30 minutes after stimulation</th>
<th>1 hour after stimulation</th>
<th>2 hours after stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCPK (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>25.6 ± 3.9</td>
<td>23.8 ± 3.8</td>
<td>23.0 ± 3.8</td>
<td>22.8 ± 4.1</td>
<td>22.0 ± 3.6</td>
<td>20.6 ± 3.1</td>
</tr>
<tr>
<td>stimulated</td>
<td>27.0 ± 5.0</td>
<td>25.0 ± 5.0</td>
<td>26.5 ± 4.5</td>
<td>26.5 ± 5.5</td>
<td>27.5 ± 2.5</td>
<td>26.0 ± 2.0</td>
</tr>
<tr>
<td><strong>PGOT (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>11.1 ± 0.7</td>
<td>10.7 ± 0.8</td>
<td>10.3 ± 0.9</td>
<td>10.0 ± 0.8</td>
<td>9.4 ± 0.9</td>
<td>9.4 ± 1.0</td>
</tr>
<tr>
<td>stimulated</td>
<td>10.1 ± 2.9</td>
<td>8.9 ± 2.2</td>
<td>16.6 ± 7.8</td>
<td>14.5 ± 6.2</td>
<td>13.3 ± 5.8</td>
<td>10.1 ± 4.9</td>
</tr>
<tr>
<td><strong>PLDH (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>8.0 ± 1.3</td>
<td>8.7 ± 1.0</td>
<td>8.1 ± 0.9</td>
<td>7.6 ± 0.6</td>
<td>8.3 ± 0.8</td>
<td>8.3 ± 1.1</td>
</tr>
<tr>
<td>stimulated</td>
<td>4.2 ± 1.7</td>
<td>3.9 ± 0.9</td>
<td>30.9 ± 16.4</td>
<td>21.6 ± 12.4</td>
<td>21.0 ± 9.5</td>
<td>11.3 ± 8.3</td>
</tr>
<tr>
<td><strong>Plasma Protein (g/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>6.93 ± 0.28</td>
<td>6.72 ± 0.24</td>
<td>6.94 ± 0.22</td>
<td>6.81 ± 0.21</td>
<td>6.73 ± 0.21</td>
<td>6.90 ± 0.25</td>
</tr>
<tr>
<td>stimulated</td>
<td>6.22 ± 0.16</td>
<td>6.42 ± 0.24</td>
<td>6.57 ± 0.27</td>
<td>6.63 ± 0.03</td>
<td>6.36 ± 0.02</td>
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</tr>
</tbody>
</table>

*Mean ± S E M

n is equal to 2 and 6 for stimulated and control groups respectively
Table 33. Changes in PCPK, PGOT, and PLDH during skeletal muscle stimulation in thoracic and right duct lymph-drained dogs

<table>
<thead>
<tr>
<th></th>
<th>1 hour before stimulation</th>
<th>before stimulation</th>
<th>end of stimulation</th>
<th>30 minutes after stimulation</th>
<th>1 hour after stimulation</th>
<th>2 hours after stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCPK (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>+1.8 ± 0.1*</td>
<td>0.0 ± 0.0</td>
<td>-0.8 ± 0.7</td>
<td>-1.0 ± 0.5</td>
<td>-2.0 ± 0.7</td>
<td>-3.2 ± 1.4</td>
</tr>
<tr>
<td>stimulated</td>
<td>+2.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>+1.5 ± 0.5</td>
<td>+1.5 ± 0.5</td>
<td>+4.5 ± 2.5</td>
<td>+2.0 ± 4.0</td>
</tr>
<tr>
<td><strong>PGOT (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-0.4 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>-0.4 ± 0.4</td>
<td>-0.5 ± 0.3</td>
<td>-1.3 ± 0.3</td>
<td>-1.3 ± 0.4</td>
</tr>
<tr>
<td>stimulated</td>
<td>1.2 ± 0.7</td>
<td>0.0 ± 0.0</td>
<td>+7.9 ± 5.6</td>
<td>+5.6 ± 4.0</td>
<td>+4.4 ± 3.6</td>
<td>+1.2 ± 2.7</td>
</tr>
<tr>
<td><strong>PLDH (IU/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-0.5 ± 1.4</td>
<td>0.0 ± 0.0</td>
<td>-0.5 ± 0.3</td>
<td>-1.1 ± 1.2</td>
<td>-0.3 ± 0.6</td>
<td>-0.4 ± 0.5</td>
</tr>
<tr>
<td>stimulated</td>
<td>+0.3 ± 0.8</td>
<td>0.0 ± 0.0</td>
<td>+27.0 ± 15.5</td>
<td>+17.7 ± 11.5</td>
<td>+17.1 ± 8.6</td>
<td>+7.4 ± 7.4</td>
</tr>
</tbody>
</table>

*Mean ± S E M

n is equal to 2 and 6 in stimulated and control groups respectively
period. The pre-stimulation levels of PGOT and PLDH in the second animal were not significantly different from those of other animals.

The data on lymph flow during the experiment are given in Table 34. Thoracic duct lymph flow increased almost 4-fold during muscle stimulation, falling to a value approximately 50% of control in the subsequent 2-hour recovery period. Flow of lymph from the right duct trebled during stimulation, and returned to pre-stimulation values immediately after. The behaviour of the post-stimulation flows of lymph from the right and thoracic ducts may indicate the origin of the stimulation-induced increases in lymph flow. The increase in lymph flow from the thoracic duct during muscle stimulation was probably due in part to the de novo production of lymph, and in part to the massage of lymph vessels and consequent propulsion of previously formed lymph along the thoracic duct. The larger volume of lymph flowing from the right duct during the period of muscle stimulation was probably newly filtered during stimulation.

The data on the enzyme activities in right and thoracic duct lymph are presented in Tables 35 and 36. In one animal the activities of all three enzymes in the thoracic duct lymph were considerably higher than had
<table>
<thead>
<tr>
<th></th>
<th>thoracic duct lymph</th>
<th>right duct lymph</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>volume (ml)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>stimulated</td>
</tr>
<tr>
<td>60-30 min before</td>
<td>11.7 ± 3.8</td>
<td>14.2 ± 3.0</td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-0 min before</td>
<td>11.8 ± 3.8</td>
<td>9.9 ± 0.4</td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 min of stimulation</td>
<td>10.1 ± 4.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-20 min of stimulation</td>
<td>9.0 ± 1.8</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-30 min of stimulation</td>
<td>7.5 ± 2.0</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-30 min of stimulation</td>
<td>9.9 ± 2.7</td>
<td>27.0 ±</td>
</tr>
<tr>
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<td>6.0 ±</td>
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<tr>
<td>0-30 min after</td>
<td>11.2 ± 2.7</td>
<td>5.3 ± 1.1</td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>30-60 min after</td>
<td>9.6 ± 2.4</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>60-90 min after</td>
<td>10.5 ± 1.4</td>
<td>5.1 ± 1.4</td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>90-120 min after</td>
<td>9.4 ± 1.9</td>
<td>6.1 ± 2.1</td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td>1.7 ± 0.2</td>
</tr>
</tbody>
</table>

*Mean ± SEM
n is equal to 2 and 6 for stimulated and control groups respectively
Table 35. Changes in protein concentration, CPK, GOT, and LDH in lymph from the thoracic duct during skeletal muscle stimulation in thoracic and right duct lymph-drained dogs

<table>
<thead>
<tr>
<th></th>
<th>60-30 min before stim</th>
<th>30-0 min before stim</th>
<th>0 to 10 min during stim</th>
<th>10 to 20 min during stim</th>
<th>20 to 30 min during stim</th>
<th>0-30 min after stim</th>
<th>30-60 min after stim</th>
<th>60-90 min after stim</th>
<th>90-120 min after stim</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>protein (gm%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
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<td>3.72</td>
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<td>3.85</td>
<td>3.58</td>
<td>4.15</td>
<td>3.93</td>
<td>3.64</td>
</tr>
<tr>
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<td>4.13</td>
<td>4.39</td>
<td>4.10</td>
<td>3.88</td>
<td>3.73</td>
<td>3.56</td>
<td>3.54</td>
<td>3.85</td>
<td>3.81</td>
</tr>
<tr>
<td>X</td>
<td>3.83</td>
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<td>3.87</td>
<td>3.79</td>
<td>3.57</td>
<td>3.84</td>
<td>3.89</td>
<td>3.73</td>
</tr>
<tr>
<td>±0.30</td>
<td>±0.34</td>
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<td>±0.01</td>
<td>±0.06</td>
<td>±0.01</td>
<td>±0.31</td>
<td>±0.04</td>
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<td><strong>CPK (IU/L)</strong></td>
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<td></td>
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<td></td>
<td></td>
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<td>±23</td>
<td>±30</td>
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<td><strong>GOT (IU/L)</strong></td>
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<tr>
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<td>±2.9</td>
<td>±5.1</td>
<td>±3.7</td>
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</tr>
</tbody>
</table>

Table 36. Changes in protein concentration, CPK, GOT, and LDH in lymph from the right duct during skeletal muscle stimulation in thoracic and right duct lymph-drained dogs.
previously been observed. In the other animal, extremely high levels of CPK and GOT were found in the lymph from the right duct. Since control values for right duct lymph activities of these two enzymes were low in 3 other animals, this animal was considered to be exceptional. The values of lymph enzyme activities for each animal are given separately in view of the large difference between individuals. As will be noted, however, the behaviour of the lymph enzymes during muscle stimulation was similar in both animals.

During muscle stimulation, thoracic duct lymph protein concentration remained at control values (Table 35). The effect of muscle stimulation on the enzymes in thoracic duct lymph was not entirely similar to that observed earlier (Table 35). CPK in thoracic duct lymph increased during muscle stimulation and was still elevated in the first 30 minutes of recovery, returning to pre-stimulation values during the last 90 minutes of the experiment. Both GOT and LDH in thoracic duct lymph behaved in a manner different from that seen in previous experiments. There was a small decrease in the activity of those enzymes in thoracic duct lymph during muscle stimulation, with a return to pre-stimulation values during recovery. The high initial activities of LDH and GOT in thoracic
duct lymph in one animal may have played a role in this response. However, the second animal with 'normal' levels of thoracic duct lymph enzyme activity also showed this anomalous response to muscle stimulation.

All three enzymes in the right duct lymph showed the same pattern of response to muscle stimulation (Table 36). During muscle stimulation the protein concentration in right duct lymph decreased, returning to pre-stimulation values during recovery. CPK, GOT, and LDH activities in right duct lymph all decreased during muscle stimulation, followed by an increase to pre-stimulation values during the first hour of recovery. The lower level of enzyme activity seen in the right duct lymph during muscle stimulation coincided with a decrease in lymph protein concentration. This suggests that the increase in flow of lymph from the right duct during muscle stimulation resulted from an increase in blood capillary pressure, or from an increase in the vascular surface available for plasma filtration, rather than from an increase in blood capillary permeability.

Unfortunately, the anomalous behaviour of GOT and LDH in thoracic duct lymph during muscle stimulation suggests that the lymphatic handling of these enzymes in the 2 animals studied may have been different
from that in animals used previously. However, it was evident that large changes in PGOT and PLDH elicited by muscle stimulation could occur while lymph from both the major lymphatic ducts was prevented from reaching the circulating blood. It was concluded that the transport of enzyme by the lymphatic route was not involved to any extent in the PGOT or PLDH response to muscle stimulation.

8. **The time course of the changes in plasma enzyme activity induced by skeletal muscle stimulation**

Although it was shown that consistent increases in PGOT and PLDH could be brought about by 30 minutes of muscle stimulation, the time course of these changes was not known. More particularly it was desirable to know (1) whether there was a gradual or step increase in the levels of PGOT and PLDH during the period of muscle stimulation, and (2) whether PGOT and PLDH would increase continuously provided muscle stimulation was maintained.

To investigate this, 3 pentobarbital-anaesthetised dogs were stimulated for either 1 hour (1 animal) or 30 minutes (2 animals), and arterial blood samples were taken at intervals before, during and after muscle stimulation. In addition, lymph was drained continuously from the thoracic duct.
The results are presented in Figures 22, 23, and 24. The 60 minute period of muscle stimulation brought about significant increases in both PGOT and PLDH, with enzyme activity reaching a plateau at the 10th minute of muscle stimulation (Figure 22). PCPK increased slightly at the beginning of muscle stimulation, and then returned to pre-stimulation values, rising again to a high level 1 hour after stimulation. Both arterial haematocrit and plasma protein concentration increased during muscle stimulation, reaching maximum values by the 10th minute of stimulation.

Very similar changes occurred in the dogs which were stimulated for 30 minutes (Figures 23 and 24). PCPK showed only small, insignificant changes during muscle stimulation. Arterial haematocrit, PGOT, and PLDH all increased during muscle stimulation, to reach a maximum value by the 10th to 15th minute of stimulation. In most cases this high value was maintained throughout the rest of the stimulation period. During the 1 hour of recovery, arterial haematocrit, PGOT and PLDH, all tended to return to pre-stimulation levels.

The results from these 3 experiments suggested that contraction of the spleen during muscle stimulation may play a part in the PGOT and PLDH responses. This
FIGURE 22

A diagram illustrating the changes in plasma protein concentration, arterial haematocrit, PGOT, PLDH and PCPK occurring during a 60-minute period of muscle stimulation.
A diagram illustrating the changes in arterial haematocrit, PGOT, PLDH and PCPK during a 30-minute period of muscle stimulation.(#1)

muscle stimulation ......
FIGURE 24

A diagram illustrating the changes in plasma protein concentration, arterial haematocrit, PGOT, PLDH and PCPK during a 30-minute period of muscle stimulation. (#2)

---

muscle stimulation ......
Plasma Protein

PCPK

PGOT

PLDH

Hematocrit

HOURS
possibility seemed attractive since the time course of the change in arterial haematocrit during muscle stimulation was very similar to that of PGOT and PLDH. Moreover, since earlier experiments had ruled out the lymphatic route as a source of the GOT and LDH introduced into the plasma during muscle stimulation, an intravascular origin for the source of this enzyme activity was indicated. In addition, all formed elements of the blood have very high activities of both LDH and GOT. By plotting the change in PLDH or PGOT during muscle stimulation against the change in arterial haematocrit, a fairly clear correlation between the changes in these parameters became evident (Figure 25).

9. The effect of muscle stimulation on the isoenzymes of LDH in the plasma

Arterial blood samples were obtained from two pentobarbital anaesthetised dogs before and at the end of a 30 minute period of muscle stimulation. The experimental procedure previously outlined was followed with the exception that neither the thoracic duct nor the right duct were cannulated. Plasma was prepared from the arterial blood samples, and the protein concentration and total LDH activity determined. The remaining
A diagram illustrating the relationship between the increases in PGOT and PLDH, and the increases in arterial haematocrit arising during a 30-minute period of muscle stimulation.
plasma was held under refrigeration (4°C) until electrophoretic separation of the isoenzymes of LDH was performed. The isoenzymes of LDH were separated by paper electrophoresis using the method of Blatt, Walker and Mager (1965). The proportional activity of each isoenzyme band was determined.

The results of these experiments are presented in Table 37. Total LDH increased considerably during muscle stimulation (9.5 and 17.0 IU/L), while there was an inconsistent alteration in plasma protein concentration. The distribution of LDH isoenzymes in the plasma obtained before muscle stimulation was similar in both dogs. Furthermore, there was no significant change in the LDH isoenzyme distribution in the plasma following muscle stimulation. This means that during muscle stimulation there was a similar proportional increase in each LDH isoenzyme in the plasma.

10. **The effect of splenectomy on the plasma enzyme responses to skeletal muscle stimulation.**

Two animals were splenectomised following the method previously outlined. After stabilisation and a 1 hour control period, hind limb skeletal muscles were stimulated for 30 minutes. Lymph from the thoracic duct was continuously drained throughout the experiment.
<table>
<thead>
<tr>
<th>Total LDH (U/L)</th>
<th>Plasma protein (g/L)</th>
<th>Before stimulation</th>
<th>After stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD1</td>
<td>50</td>
<td>6.0</td>
<td>15.5</td>
</tr>
<tr>
<td>LD2</td>
<td>20</td>
<td>7.38</td>
<td>7.51</td>
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<tr>
<td>LD3</td>
<td>21</td>
<td>4.9</td>
<td>4.7</td>
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<tr>
<td>LD4</td>
<td>26</td>
<td>18</td>
<td>17</td>
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<td>LD8</td>
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<td>LD9</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>LD10</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 7: The effect of splenic muscle stimulation on the isoenzymes of LDH in the plasma.
During muscle stimulation, heart rate increased in both animals by over 30 beats/minute. Arterial blood pressure remained steady throughout the experiment.

Data on the arterial haematocrit, plasma protein concentration, and plasma enzyme levels in these animals are presented in Figure 26. In one animal, there was a small increase in haematocrit during the early stages of muscle stimulation. This coincided with an increase in the plasma protein concentration, indicating a loss of fluid from the circulating blood. During the later stages of muscle stimulation, both these parameters returned to pre-stimulation values. In the second dog, neither haematocrit nor plasma protein concentration changed significantly from pre-stimulation values at any time during the experiment.

In both animals there was a marked increase in PLDH and PGOT at the beginning of muscle stimulation, reaching a steady value at about the 15th minute of stimulation. PCPK behaved inconsistently, with no significant change during muscle stimulation. However, there was a marked increase in PCPK in one animal at one hour after stimulation. These responses were qualitatively quite similar to those observed in intact anaesthetised dogs during muscle stimulation.
A diagram illustrating the changes in plasma protein concentration, arterial haematocrit, PGOT, PLDH and PCPK during a 30-minute period of muscle stimulation. (n=2; A and B)

muscle stimulation ......
The increase of PLDH and PGOT to a plateau early in muscle stimulation suggests that a perturbation in the regulation of these plasma enzymes may have occurred, with a rapid adjustment to a new higher steady-state value. The full implications of this observation will be treated in depth in the Discussion.
Discussion

This discussion has been divided into two sections. In the first section, the results of each individual experiment are considered with respect to current knowledge in this field. The second section consists of a general discussion in which is presented a hypothesis accounting for the exercise-induced increases in plasma enzyme activity. This hypothesis is based on the results of this and earlier studies.

Section 1.

1. Experiments on the conscious exercising dog

These experiments provided the only comprehensive data on the plasma enzyme changes arising during running exercise in the dog. A comparison of the plasma enzyme changes observed in these experiments with those previously reported is difficult owing to differences in the intensity and duration of the exercise performed. Wagner and Critz (1968) found that running at 10 mph and 10° (17%) elevation for 1 hour resulted in an approximately 5-fold increase in SCPK. The dogs
achieved an exercise heart rate of less than 180 beats/minute. In the current study, an exercise heart rate of over 200 beats/minute was achieved in trial runs which lasted for 30 minutes. PCPK, however, increased in only 2 of these 6 trials. The low exercise heart rate of the dogs studied by Wagner and Critz is surprising in view of the high work-load. The larger size of the dogs used in this study (17.7 kg mean body weight) cannot account for this finding. Wagner and Critz did not analyse the serum for other enzymes since the post-exercise samples were frequently haemolysed (personal communication).

The formed elements of the blood do not contain detectable activity of CPK and on this basis, assay for CPK in haemolysed samples should not be affected. In some preliminary trial runs in the present study, post-exercise blood samples were haemolysed. In these samples, very high levels of CPK activity were found suggesting that haemolysis may in some way interfere with the assay for CPK.

The following pattern (of behaviour) of the three plasma enzymes studied was observed in the first group of experiments. PLDH increased most frequently during the exercise and PGOT increased fairly frequently.
PCPK increased only during runs at a greater speed and elevation. For all three enzymes, however, it appeared that the magnitude of the increase following exercise was determined more by the intensity of the exercise than by its duration.

The similarity between the plasma enzyme increases observed during running exercise (current study) and during simulated exercise (Loegering and Critz, 1971) was of great interest. PGOT increased 6.7 IU/L during a standard run (present study) and 3.5 IU/L during simulated exercise (electrical stimulation of hind limb skeletal muscles, Loegering and Critz). PLDH increased 9.5 IU/L during the run, and 11.5 IU/L during simulated exercise. PCPK increased 13 IU/L during the run, but did not change during simulated exercise. However, it should be remembered that in some dogs exercise, in which PCPK levels did not change, brought about marked increases in both PGOT and PLDH. Since the raw data from the simulated exercise experiments of Loegering and Critz indicate that in a few animals there was an increase in PCPK during muscle stimulation, there appears to be considerable correspondence between the results from these two experiments.
In comparison with the exercise-induced increases in plasma enzyme activity observed in other species, the dog shows several qualitative differences. In man, PGOT is more 'labile' than PCPK or PLDH, and is elevated during relatively light exercise. PCPK also increases following most forms of exercise, while PLDH requires heavy or prolonged exercise to bring about its increase (Hunter and Critz, 1971). In a recent study on the rat, Loegering has found that PLDH and PCPK show much larger responses to swimming exercise than PGOT (Loegering, personal communication, 1973).

2. The effect of pacing the heart on PGOT and PCPK

It is evident from the results obtained that cardiac pacing at a high frequency (240 beats/minute) can bring about small but consistent increases in PGOT, but has no effect on PCPK.

Pacing the heart at a high frequency brings about both metabolic and mechanical alterations. The heart rate is a primary determinant of myocardial oxygen consumption. Therefore during pacing at a high frequency there would be a considerable increase in myocardial oxygen consumption (Laurent et al., 1956; Boerth et al., 1969).
Carrier molecules, protein in nature, appear to be associated with cell membranes, and may transport small molecules through the cell membrane by a mechanism requiring a structural reorganisation of the membrane (Mitchell, 1957). Thus an increase in membrane transport, resulting from an increase in myocardial metabolism, may produce a more labile and permeable cell membrane.

Zierler (1958) suggested that the permeability of the sarcolemmal membrane to enzyme molecule might be due to the transient appearance of pores. One possibility is that an increase in myocardial metabolism could result in an increase in the size or rate of occurrence of such pores, and thus in an increase in the permeability of the myocardial cell membrane to enzyme molecules (Schmidt and Schmidt, 1969). Although both of the above mechanisms may have been involved during the pacing period, allowing an accelerated release of enzyme, their effect on plasma enzyme activity was minimal.

The intracellular locations of CPK and GOT may explain the difference in their response to cardiac pacing. CPK with a molecular weight of 83,000M (Eppenberger, Dawson and Kaplan, 1967), is bound, in the bovine heart, principally to the myofibrillar
elements of the muscle cell (Ottoway, 1967). GOT, with a molecular weight of 110,000M (Jenkins, Yphantis and Sizer, 1959), is distributed, in the rat heart, in several subcellular sites; mitochondria, T-tubules, and the sarcolemmal membrane (Papadimitriou and Van Duijn, 1970).

Patients with paroxysmal tachycardia (a ventricular rate: 140 beats/minute) often have elevated levels of SGOT, SGPT, and SOCT (Stasinski, Stasinska and Banaszkiewicz, 1965; Runde and Dale, 1966). The increase in the activities of these enzymes was dependent upon both the duration of the episode and the actual ventricular rate. No serum enzyme changes were observed in patients in whom the rhythm was of atrial origin. The authors concluded that the increase in serum activity of these enzymes of hepatic origin was dependent upon liver congestion resulting from cardiac insufficiency. During cardiac pacing in the dog, cardiac output and other principle haemodynamic parameters remain normal at pacing rates of up to 240 beats/minute (Maxwell et al., 1958; Sugimoto, Sagawa and Guyton, 1966).
3. **The effect of simulation of the cardiovascular response to exercise by diencephalic stimulation on PGOT and PCPK**

It was found that during a 30 minute period of diencephalic stimulation, which resulted in a maintained cardioacceleration and blood pressure elevation, increases in both PCPK and PGOT activity occurred. The high correlation between the magnitude of the increase in PCPK and in PGOT suggests that a common mechanism may have been responsible for their increase. The magnitude of the plasma enzyme responses were related to the cardiac effort and, more closely, to the absolute value of the systolic arterial blood pressure maintained during the period of stimulation.

While the possible effects of diencephalic stimulation on regions other than the systemic vascular system should not be ignored, the relationships noted above suggest that the increase in enzyme activity in the plasma during stimulation may be related primarily to the changes in systemic cardiovascular dynamics. Preliminary studies showed that there was no alteration in PGOT or PCPK during electrical stimulation of regions of the diencephalon from which no systemic circulatory
responses could be evoked. Thus it seems unlikely that neural damage, possibly arising during electrical stimulation, could be responsible for the observed changes in plasma enzyme activity.

The high correlation between the magnitude of the plasma enzyme increase and the systolic arterial blood pressure during stimulation, suggests that this latter factor may be an important determinant of the increase in plasma enzyme activity during stimulation. Left ventricular pressure is determined primarily by wall tension and radii of the left ventricle (Woods, 1892). These latter parameters were not measured in this experiment. However, changes in peak arterial pressure possibly reflect similar changes in peak left ventricular wall tension. As pointed out earlier, Zierler (1958) has suggested that the permeability of the sarcolemmal membrane to enzymes is due to the occurrence of transient pores. It is conceivable that an increase in myocardial tension during the period of stimulation could result in an increased rate of production of such pores and increase the permeability of the plasma membrane to enzyme molecules (Schmidt and Schmidt, 1969). Similarly, the increase in peak tension within the arterial walls during stimulation may have exaggerated
a similar process.

After stimulation, PGOT returned to control levels within 30 minutes, while PCPK remained elevated for the duration of the experiment. This suggests that release of GOT was restricted to the period of stimulation while CPK release persisted for some time. The relatively short half-lives of PGOT (6 to 14 hours) and PCPK (6 hours) in the dog support this conclusion (Wakim and Fleisher, 1963a; personal observation). Similar responses have been observed in the anaesthetised dog following periods of arterial hypoxemia (Loeffering and Critz, 1971). After ventilation for 30 minutes with 8.5% oxygen in inspired air, PGOT rose by 2 IU/L and then returned to control levels within 2 hours. PCPK increased by 5 IU/L and remained at this level for 6 hours.

As noted earlier in the Discussion, exercise in the dog which evoked a heart rate of over 200 beats/minute, was not always accompanied by changes in plasma enzyme activity. In this experiment, it was found that in preparations in which there was a high heart rate but only small arterial blood pressure elevation during stimulation, there was no increase in plasma enzyme activity.
The mean increases in PCPK during diencephalic stimulation (30 minutes) and a standard running exercise (15 minutes) were 4 and 13 IU/L, respectively. The mean increases in PGOT during the same two interventions were 2.3 and 6.7 IU/L. The mean heart rates during diencephalic stimulation and running exercise were 225 and 229 beats/minute. Thus it appears that a 30-minute stimulation of the cardiovascular responses to exercise brings about increases in PGOT and PCPK which are approximately one-third of the magnitude evoked by 15 minutes of running exercise. With much oversimplification it can be concluded that only a part of the increase in plasma enzyme activity occurring during exercise can be attributed to the cardiovascular adjustments.

4. The activities of CPK, GOT and LDH in the thoracic duct lymph and plasma of pentobarbital-anaesthetised dogs

The activity of a particular enzyme in lymph from the thoracic duct is determined by three factors (Refer to Figure 27): (1) the amount of activity of that enzyme which is filtered by the blood capillaries in the various organs whose interstitial fluid is eventually drained by the thoracic duct; (2) Further addition of that enzyme from the intracellular compartment directly into the
FIGURE 27

The movement of enzyme molecules between body compartments.

Factors determining the movement (A to E), and loss (F to I), of enzyme molecules in the extracellular fluid.

(A) (1) site and strength of binding of enzyme molecules within the cell. (2) dimensions of the enzyme molecule. (3) permeability of the cell membrane.

(B) There is no hindrance to enzyme transport. Lymph production determines rate of movement.

(C) The normal route of lymph to the circulating blood.

(D) Determined by the filtration of enzyme molecules at the blood capillary; depends upon capillary permeability.

(E) The reverse process to (D). This probably occurs only when there is considerable resorption of fluid by a blood capillary (e.g. when a pre-capillary sphincter is closed).

(F) Within each of the compartments, 1 to 4, enzyme activity is lost through the continual inactivation and breakdown of enzyme protein.

(G, G') The RE cells within the lymph nodes, and lining the walls of blood vessels in the liver, spleen, lungs and bone, are capable of clearing many, but not all enzymes from the circulating blood and lymph.

(H) In some species, certain enzymes are secreted into the bile or into the intestinal tract.

(I) Enzymes of sufficiently small dimension are excreted into the urine.
interstitial fluid; and (3) Removal or degradation of that enzyme en route from the interstitial space to the thoracic duct.

The first of these factors, filtration at the blood capillary level, is complex. The thoracic duct is the final lymphatic vessel into which drain lymphatics from tissues served by both highly permeable and highly impermeable vascular beds. As discussed in the Historical Review, the protein concentration and volume of lymph produced by a given tissue is related to the permeability of the vascular bed in that tissue. The ratio of albumin to globulin (A/G ratio) of lymph from a particular tissue is dependent on the permeability of the regional vascular bed. In the anaesthetised dog, the A/G ratio of lymph from the thoracic duct has been found to be only slightly greater than that for plasma (2.32 vs. 2.04) (Nix et al., 1951). Wasserman and Mayerson (1952) found lymph/plasma ratios of 0.70 and 0.68 for albumin and globulins, respectively, in the anaesthetised dog. These findings indicate that the vascular beds in tissues whose interstitial space is eventually drained by the thoracic duct, have a high permeability to globulins. This fact is not surprising since in excess of 70 percent of the thoracic duct lymph is formed in the liver and intestinal
tract (Nix et al., 1951). Several studies have indicated that the vascular beds in these organs are reasonably permeable to macromolecules. In the studies of Grotte, (1956) and of Mayerson et al., (1960) the capillary bed of the liver was found to be highly permeable to dextran molecules of up to $420 \times 10^3 \text{M}$. While the molecular dimensions rather than the molecular weight are probably the principle factors determining vascular permeability to macromolecules, these studies suggested that the vascular beds in both the liver and intestinal tract should demonstrate a high permeability to the enzymes studied in this investigation; GOT, CPK, and LDH (molecular weights of 110,000, 83,000 and 140,000 M (Jenkins, Yphantis and Sizer, 1959; Eppenberger, Dawson and Kaplan, 1967; Nisselbaum and Bodansky, 1961)).

Lymph is derived directly from interstitial fluid and can therefore be considered to have a similar composition. While lymphatic end-capillaries are highly permeable (e.g. to erythrocytes), all other lymphatic vessels are impermeable to protein. Thus once the lymph is formed, no further protein can be added to or lost from it by the process of diffusion (Patterson et al., 1958). Therefore, any variation in the activity of a particular enzyme in the lymph from the value
expected from the data on its activity in the plasma, and
the vascular permeability to that enzyme, must be explained
on the basis of addition or removal of enzyme to or from
the interstitial fluid or lymph. The author conceives
the following scheme (Refer to Figure 27). The maintenance
of a steady activity of an enzyme in the extracellular
compartment requires a steady introduction of enzyme from
the intracellular compartment at a rate related to the half-
life of that enzyme in the extracellular fluid (here
referred to as the plasma half-life). Therefore, for an
enzyme with a relatively short plasma half-life, (i.e. a
rapid turnover), one would expect to find that it possessed
a greater activity in the interstitial fluid (lymph) than
in the plasma.

To determine whether the activity of an enzyme in
the lymph was greater or less than would be expected solely
through filtration, the ratio of its activities in the
lymph and plasma were compared to lymph/plasma ratios
for total protein. The lymph/plasma ratios of both GOT
and CPK were significantly greater than the lymph/plasma
ratio for total protein. Morea, Furlan and Taboga (1960)
have previously reported that in the dog, the activities
of both GOT* and GPT* in lymph from the thoracic duct
were approximately 2-fold their activities in the serum.
These data imply that there is a considerable addition of both GOT and CPK to the newly filtered interstitial fluid before it enters the lymphatic capillaries. The large number of tissues drained by the thoracic duct prevents any definite conclusion about the source(s) of this added enzyme. In the present study, the lymph/plasma ratio for LDH was considerably less than that for total protein. No LDH could be detected in the thoracic duct lymph in four out of 16 animals. Fur- lan, Morea and Taboga (1960) made a similar observation; in four of six dogs, activity of LDH in thoracic duct lymph was much less than that of the plasma. Unfortunately there is no precise data available on the plasma clearance of LDH in the dog. A rapid return of LDH in the plasma to control levels following an increase evoked by muscle stimulation suggests a short plasma half-life (Loehering and Critz, 1971). Wakim and Fleisher (1963b) calculated the plasma half-life of the muscle-type isoenzyme of LDH to be approximately 30 minutes in the dog. They also provided evidence for a short plasma half-life of the fast-migrating, heart type isoenzymes of LDH. If the clearance of LDH from the plasma is as rapid as these figures suggest, there must be a continual rapid release of LDH from the intracellular into the extracellular compartment. It appears, however, that in the process of filtration at the blood capillary and in transport through the interstitial space, the extracellular fluid tends to lose
a great deal of its LDH activity. Therefore a steady activity of LDH in the extracellular fluid (plasma) must be maintained by the addition of LDH directly into the circulating blood. This possibility has been considered by Friedel and Mattenheimer (1970) who noted that in man the various formed elements of the blood possessed sufficient activity of all the isoenzymes of LDH to account for the normal LDH isoenzyme spectrum of the plasma.

Considerable LDH activity has been found in lymph obtained from peripheral lymphatics. In particular, lymph from the deep lymphatics of the hind limb of both the cat and the dog possessed a greater activity of LDH than the plasma (Lewis, 1967; personal observation). Since skeletal muscle has one of the least permeable vascular beds (Mayerson et al., 1960), one or both of the following points may be concluded: (1) Since the lymph/plasma ratio for protein in skeletal muscle lymph is in the region of 0.2 to 0.3, there must be a considerable amount of LDH released directly into the interstitial fluid from skeletal muscle in the resting anaesthetised dog; (2) that since one of the least permeable vascular beds may filter LDH in reasonable activity, LDH must be filtered by the more permeable vascular beds in other tissues drained by the thoracic duct. The important point, however, is that LDH is present in considerable activity in lymph from peripheral lymphatics, but in much
reduced activity in lymph from the thoracic duct. As mentioned earlier, blood capillaries in the liver and intestinal tract are highly permeable and should filter LDH.

Since it can be shown that there is a relatively high activity of LDH in interstitial fluid, LDH must be removed from the extravascular fluid somewhere on route to the thoracic duct. Inactivation and degradation of enzyme probably occurs in every body compartment, and there is no reason to presume that the clearance of LDH by these mechanisms is greater than that of any other enzyme. There is some evidence to support the concept of clearance of several enzymes from the extracellular fluid by the reticulo-endothelial system (RES) (Posen, 1970). At the present time, the status of the RES as a site of clearance of LDH is controversial. Studies on the dog by Wakim and Fleisher (1963b) indicate that the RES may clear both fast and slow isoenzymes of LDH. On the other hand, Mahy and Rowson (1965) found that in the mouse, only the fast isoenzymes of LDH were cleared by the RES. The picture is a little more complicated than some authors have assumed. It appears that when elevated to plasma levels of above three-fold normal value, the slowest isoenzyme of LDH is cleared by the RES, but the fastest isoenzyme is cleared by some other, slower mechanism. At plasma levels below three-fold normal, both fast and slow isoenzymes are cleared by the same slow mechanism, not involving the RES.
In summary the following conclusions were made:

(1) PGOT and PCPK levels are maintained by the continual release of enzymes from the intracellular compartment. This enzyme reaches the plasma principally by the thoracic duct.

(2) PLDH levels are not affected by the continuous drainage of thoracic duct lymph. The entry of LDH into the blood directly from the intracellular compartment is indicated. The formed elements of the blood are suggested as a likely source of the plasma LDH activity.

5. The effect of skeletal muscle stimulation on thoracic duct lymph flow, and on the activities of LDH, GOT and CPK in the plasma and thoracic duct lymph.

As concluded earlier in the Discussion, the magnitude of the increases in PGOT, PCPK, and PLDH evoked by running exercise were quite similar to those evoked by skeletal muscle stimulation (Loegering and Critz, 1971). In the current study, the findings of Loegering and Critz were verified; skeletal muscle stimulation for 30 minutes brought about significant increases in PLDH and PGOT, but no change in PCPK. Taking into consideration the amount of enzyme lost to the plasma by the continuous collection of thoracic duct lymph, the changes observed in PGOT and PLDH were not significantly different from those reported by Loegering and Critz.
Thoracic duct lymph flow was increased to a level approximately three-fold control during the period of muscle stimulation. Since it was unlikely that muscle contraction was accompanied by an increase in vascular permeability (Arturson and Kjellmer, 1964) the increase in lymph flow must have been the result of either an increase in mean capillary pressure, or an increase in the surface area of the capillary beds in tissues drained by the thoracic duct.

The difference in the behaviour of CPK, GOT, and LDH in thoracic duct lymph was of particular interest. CPK reached very high activities in the lymph during muscle stimulation, while its activity in the plasma remained unchanged. There was also a considerable increase in the activity of GOT in the lymph, with a small increase in PGOT. In contrast, LDH demonstrated a larger increase in the plasma than in the lymph.

These findings suggest that CPK is released from the contracting muscle into the interstitial fluid, and finds its way directly into the lymphatics, with little or no activity entering the blood. If this is the case, one would also expect that under these conditions, molecules larger than CPK (83,000 M), and released from muscle tissue during muscle stimulation would follow this route. Since both GOT and LDH are considerably larger than CPK their behaviour, on release from muscle tissue, might be expected to parallel that of CPK.
These two enzymes may be released from some other tissue directly into the circulating blood during muscle stimulation. One possible source is the liver. However, as previously described, the flow of lymph from the liver increases considerably during exercise (Nix et al., 1951), and is probably the major contributor to the increase in thoracic duct lymph flow seen during skeletal muscle stimulation. It is unlikely, therefore, that any enzyme released from the liver cells during muscle stimulation would preferentially enter the blood directly. A second possible source of enzyme are the formed elements of the blood. While these may be the source of the plasma activity of GOT and LDH in the resting animal, it has yet to be demonstrated whether or not an accelerated release of these enzymes from the formed elements of the blood occurs during muscle stimulation.

The following hypothesis takes into account the facts available from this and previous studies. At rest there appears to be a considerable removal of LDH from the lymph on its passage to the veins. A similar mechanism may also be operating for GOT. Both enzymes have been shown to be cleared by the RES in the dog. The increase in the flow of lymph during skeletal muscle stimulation may result in an accelerated passage of lymph past the phagocytic cells within the lymph nodes, such that the fractional clearance of enzyme is reduced. As a result, there would be an increase in the
activity of enzyme in thoracic duct lymph without any change in the rate of release of enzyme from tissues into the interstitial space. It was found that during muscle stimulation, the activity of LDH in the lymph rose to a level just less than that achieved in the plasma, and returned to low levels immediately following stimulation. The possibility that there was some release of LDH from tissues into the interstitial fluid during skeletal muscle stimulation cannot be discounted. The changes in GOT activity in the lymph during muscle stimulation probably involve a similar mechanism to that described for LDH.

It remains to be explained how GOT and LDH become elevated in the plasma during skeletal muscle stimulation. A direct release of these enzymes into the blood from fixed tissues does not seem tenable in view of the evidence contrary to such a mechanism.

6. The effect of repeated bouts of skeletal muscle stimulation on LDH, GOT and CPK in plasma and thoracic duct lymph.

If the mechanisms responsible for the plasma enzyme changes evoked by skeletal muscle stimulation are entirely reversible, it would be expected that repeated bouts of skeletal muscle stimulation would result in identical plasma enzyme responses. On the other hand, if the plasma enzyme
response is a result of either damage at the cellular level, or release of enzyme from a depletable source, one would expect a different response following successive bouts of skeletal muscle stimulation. In the case of damage, one would not expect the plasma enzyme response to terminate abruptly at the end of stimulation, but to continue for some time. In addition one would expect a potentiation of the plasma enzyme response to a second bout of muscle stimulation, due to residual effects of the damage. If, however, the plasma enzyme response is due to the release of a depletable amount of enzyme, one would expect the plasma enzyme response to a second bout of muscle stimulation to be smaller.

The results obtained did not support either of these possibilities. There was an identical delivery of CPK in thoracic duct lymph in both bouts of stimulation. This implied that identical amounts of CPK were released into the interstitial space during both bouts of stimulation. It also indicated that the changes in permeability of the muscle cell membrane were similar in both bouts of stimulation, and that there was no potentiation of the second response by the preceding bout of stimulation.

Likewise there were similar changes in the activity of G0T in the plasma and thoracic duct lymph during both bouts of muscle stimulation. The increase in PLDH during
the second period of stimulation was almost twice that observed during the first. In previous discussion it was concluded that the PLDH changes evoked by muscle stimulation were unlikely to originate from either the stimulated muscle or from any fixed tissue. Delivery of LDH in the lymph during the second period of stimulation was much less than that during the first. This supports the concept of a clearance of LDH from the lymph by the phagocytic cells in the lymph nodes. Lymph flow was much less during the second period of stimulation, and this lower flow would have enabled the RES to achieve a greater fractional clearance of LDH.

It was concluded that there was no significant potentiation of the plasma or lymph enzyme response to skeletal muscle stimulation as a result of a prior period of stimulation. That is, that the mechanisms responsible for the plasma and lymph enzyme changes during muscle stimulation were readily reversible.

7. **The effect of skeletal muscle stimulation on right duct lymph flow, and on the activities of LDH, GOT and CPK in the plasma and right duct lymph.**

To further examine the possible involvement of the lymphatic route in the plasma enzyme changes evoked by skeletal muscle stimulation, the flow and enzyme activity of lymph from the right duct was studied. The results obtained
indicated that in the tissues drained by the right duct, there is an increase in lymph production during muscle stimulation, with a concomitant decline in lymph protein concentration. Changes in the lymph activities of the three enzymes studied paralleled the changes in lymph protein, implying that there was no increase in the release of enzyme from the tissues drained by the right duct during skeletal muscle stimulation. These observations eliminate the heart, lungs and peritoneal cavity as sources of enzyme for the plasma enzyme increases evoked by skeletal muscle stimulation.

The magnitude of the cardiovascular responses to muscle stimulation were considerably smaller than those observed during hard spontaneous exercise or diencephalic stimulation. Possibly, in these last two situations, there would have been some alteration in the enzyme content of the cardiac lymph.

8. The time course of the changes in PLDH and PGOT evoked by skeletal muscle stimulation.

Information on the time course of the changes in plasma enzymes during muscle stimulation was not available.

Frequent sampling of arterial blood during muscle stimulation provided the required data. It was observed that the activities of both PGOT and PLDH increased rapidly during the first 15 minutes of muscle stimulation, and then
remained at a steady value while the stimulation was maintained (30 or 60 minutes). It was noted that the changes in arterial haematocrit followed the same time course as the changes in PGOT and PLDH, suggesting that a single event, splenic contraction, may have been responsible for all these responses to muscle stimulation. This possibility was examined at a later stage of the study. Critz (1966) has previously found that in the rat, the increase in SGOT was similar following either 60 minutes or 120 minutes of swimming. In preliminary studies on man, the author has found that in hard prolonged exercise, PGOT increases to a new steady value after 15 minutes of exercise (Refer to Appendix 3).


It is possible that changes in the various isoenzymes of LDH in the plasma during skeletal muscle stimulation could indicate the origin of the newly introduced LDH. The level of an isoenzyme in the plasma is dependent on both the rate of influx and the rate of clearance of that isoenzyme from the plasma. It has been shown that, in the mouse and sheep, at least for high levels of circulating LDH isoenzymes, muscle-type isoenzymes are cleared at a faster rate than heart-type isoenzymes (Mahy and Rowson, 1965; Boyd,
1967). Wakim and Fleisher (1963b) found that both muscle and heart type isoenzymes of LDH have short plasma half-lives in the dog.

During skeletal muscle stimulation, all five isoenzymes of LDH showed similar proportional increases. Since the fast, heart-type isoenzymes predominated in the pre-stimulation plasma sample, these isoenzymes showed the larger absolute increases during stimulation. Allowing for possible differences in the clearance rates of fast and slow isoenzymes of LDH, the results may indicate that LDH was principally released from tissues containing the faster isoenzymes. These observations agree with those of Loegering and Critz (1971) who found no change in the ratio of slow to fast isoenzymes of LDH in the plasma following muscle stimulation. Similarly, in the present study, no change in the LDH isoenzyme ratio was observed following running exercise. Another possible interpretation is that the rate of clearance of all five isoenzymes of LDH from the plasma was reduced during skeletal muscle stimulation. This would have resulted in a similar proportional increase in all isoenzymes. However, regardless of the mechanism responsible, there must be a constant input of LDH into the vascular compartment, and so far the tissue origin of this "new" LDH has not been identified.
10. The effect of splenectomy on the response of PGOT, PLDH and PCPK to skeletal muscle stimulation.

The high correlation between the magnitude of the increases in PLDH, PGOT, and the arterial haematocrit during muscle stimulation suggested that splenic contraction might play some role in the plasma enzyme response. In addition, the time course of the changes in PLDH, PGOT, and arterial haematocrit were similar. However, a given quantity of LDH or GOT introduced into the circulating blood over a short period of time would result in an increase in the plasma levels of these enzymes, followed by a decrease to their control values at a rate determined by their plasma half-lives.

Initial experiments had indicated that during splenic contraction, the LDH and GOT activity in plasma from splenic venous blood reached values up to ten-fold their activity seen in arterial blood plasma. These results were surprising in view of the fact that it has been shown, in the cat and the dog, that blood cells but not plasma, are sequestered by the spleen (Song and Groom, 1971). In other words, the splenic venous blood obtained during contraction of the spleen contains cells, of which some had previously been sequestered and some had been circulating. The plasma fraction of this splenic venous blood was entirely of circulating origin, and would have been expected to have the same composition as arterial blood plasma.
It was calculated that despite the high GOT and LDH activities in the splenic venous blood during contraction, it was impossible that sufficient additional enzyme activity would be introduced into the circulating plasma to bring about the increases in PLDH and PGOT seen during muscle stimulation. This conclusion was substantiated by the observation that during muscle stimulation in acutely splenectomised dogs, increases in PLDH and PGOT occurred, which were indistinguishable from those observed in intact animals.

The achievement of a new high steady level of PGOT and PLDH during muscle stimulation indicated the nature of the mechanism behind the plasma enzyme responses. Briefly, when the activity of an enzyme in the plasma increases from one steady level to a higher steady level, there must be a change in the regulation of the plasma level of that enzyme. That is, that one or both of the following must occur:

1. An increase in the rate of influx of enzyme into the plasma compartment.

2. A decrease in the rate of clearance of that enzyme from the plasma compartment.

It should be evident that the time to achieve a new steady-state value will be dependent on the magnitude of the change in the rate of influx or clearance of enzyme. It is also important to realise that in the second case, a higher level of enzyme in the plasma can be realised, without any increase
in the rate of release of enzyme from the intracellular into the extracellular compartment. If this latter mechanism can be shown to be operating during muscle stimulation and exercise, it may not be necessary to invoke those mechanisms, such as cell damage and an increase in cell membrane permeability, which have previously been proposed to account for the plasma enzyme responses. It should be noted that those concepts have proved unsatisfactory in accounting for the plasma enzyme response to exercise, either spontaneous or simulated.

Section 2.

General Discussion - a consideration of the physiology of enzymes in the extracellular compartment as it pertains to a general hypothesis on the exercise-evoked plasma enzyme response.

The various experiments comprising the current study provided considerable data concerned with the physiology of enzymes in the blood and lymph. This study was principally designed to elucidate the mechanisms responsible for the exercise-evoked increase in plasma enzymes. The greater part of the information collected tended to rule out certain mechanisms that had previously been considered to be involved, rather than directly exposing the responsible mechanism. By eliminating several such possible mechanisms,
however, the data obtained indirectly provide a considerable amount of information about the mechanisms that must be involved.

In summary:

(1) The elevation in PCPK seen following exercise in the dog was probably due to CPK, released from skeletal muscle cells during the exercise, and reaching the blood via the lymphatics.

(2) The increases in PGOT and PLDH during exercise in the dog did not appear to be the result of the above mechanism. Splenic contraction has been eliminated as a major mechanism. GOT and LDH seem to enter the blood directly from some unidentified tissue(s) during muscle stimulation. However, the concept of a release of either of these enzymes from fixed tissues directly into the blood is not supported experimentally. A release of enzyme from the formed elements of the blood cannot be disproved at this point. The time course of the changes of these two enzymes in the plasma during muscle stimulation suggests that some perturbation in the regulation of their levels in the plasma had occurred. This perturbation resulted in a rapidly achieved and new, higher, steady-state value. With respect to the findings in the later part of this study, this observation proved to be the most important in determining the direction of the following arguments.
It can be readily appreciated that either of the following changes will bring about an increase in the plasma level of an enzyme.

1. An increase in the rate of influx of that enzyme into the extracellular from the intracellular compartment.

2. A decrease in the rate of clearance of that enzyme from the extracellular compartment.

1. Increase in the rate of influx of enzyme into the extracellular fluid.

Several mechanisms have been suggested to account for enzyme release from the intracellular compartment. Of these, overt damage to the cell membrane is readily perceived. However, marked changes in the plasma enzyme levels occur during forms of exercise in which no histologically demonstrable lesions occur (Highman and Altland, 1963). Also, no lesions are evident in the physically inactive animal in which a steady release of cellular enzymes must occur to maintain their "normal" extracellular activity. It has been suggested that "reversible damage" occurs. That is, that microscopic pores or holes appear in the cell membrane that are of a size sufficient to allow the egress of enzyme molecules, but which are readily repaired by the cell. This last type of membrane damage merges with the concept of a low permeability of the cell membrane to macromolecules. This concept is frequently cited to account for the loss of enzyme into the
perfusion or bathing medium of isolated in vitro tissue preparations (Zierler, 1958; Butterworth et al., 1972).

Overt damage to tissues does occur in certain forms of exercise. Muscle bruising and tearing, and tendon and ligamental injuries are associated with exercise, particularly when it involves violent muscle contractions or body contact. In several studies, a good correlation between the magnitude of the plasma enzyme response and the subjective soreness of the individual following exercise was noted (Nuttall and Jones, 1968; Baumann, Escher, and Richterich, 1962). In studies on both man and the dog, running exercise has been found to cause moderate haemolysis (Biorck, 1948; Malmstrom and Sjostrand, 1952; Critz, personal communication). Haemolysis would result in the release of erythrocyte enzymes directly into the circulating blood. While this may be an explanation for some of the exercise-induced plasma enzyme increases, it cannot account for the exercise-evoked increment in the plasma level of enzymes not present in the erythrocyte. Furthermore, similar increases in plasma enzymes are also seen following swimming and cycling exercise in which haemolysis is unlikely to occur. Of the other formed elements in the blood, platelets seem the most likely to be affected by exercise. Both increases and decreases in the platelet count have been recorded following exercise (Gerheim and Miller, 1949; Sarajas, Konttinen and Frick, 1961).
The difficulty in obtaining accurate counts, and the occurrence of local binding of platelets (e.g., within the pulmonary blood vessels), make it difficult to critically evaluate these studies.

The concept of a permeability of the cell membrane to macromolecules, in this instance of 100,000 to 200,000 M, is hard to accept. However, with an abundance of literature on the release of enzyme from *in vitro* incubated tissue, this concept requires acceptance, at least until a better hypothesis is advanced. In addition, there is a continual release of intracellular enzyme in the resting mammal, for which permeability of the cell membrane offers a suitable explanation. Since there has been no explanation of the physical structure of the cell membrane which would give it this permeability to macromolecules, it is difficult to suggest factors which may interact to alter this permeability. It is not likely that factors which alter the cell membrane permeability to ions would also alter its permeability to macromolecules, since this latter feature probably has nothing in common with ionic permeability. So, although factors such as hypoxia and a change in pH have been postulated to cause changes in the permeability of the cell membrane in the exercising mammal, the manner in which this change might be induced cannot be surmised.

*In vitro* studies suggest that an increase in the cell membrane permeability always accompanies a depression in
metabolism (Zierler, 1958). This can be interpreted to mean that the integrity of the cell membrane is necessary to keep enzymes within the intracellular compartment. It is difficult to reconcile this idea with the fact that most enzymes, cytoplasmic or mitochondrial, are bound with varying strength to the many unit membranes distributed throughout the cell (Roodyn, 1967). It is here suggested that an increase in the release of an enzyme from an in vitro preparation during metabolic depression might result, not from an increase in membrane permeability, but from an increase in the number of enzyme molecules free within the cell, and capable of passing through the cell membrane.

The idea of an increase in a cell/interstitial fluid gradient of enzyme activity has previously been suggested, but in a different context. During exercise there is an increase in the activity of several enzymes in skeletal muscle and liver tissue (Critz and Merrick, 1964; Maksimova, 1965; Sangster and Beaton, 1966). This increase has been attributed to a de novo synthesis rather than an activation of previously inactive enzyme. It has been suggested that an increase in the intracellular activity of an enzyme would increase its diffusion gradient from the intra- to extracellular compartments (Critz and Merrick, 1964; Maksimova, 1965). The size of such a gradient cannot be evaluated, since enzyme bound within the intracellular compartment
cannot contribute to it. However, if the increase in total intracellular activity is accompanied by an increase in enzyme that is free within the intracellular compartment, there may be an accelerated loss of this enzyme from the cell.

In the current study, CPK was found to achieve a high value in thoracic duct lymph during skeletal muscle stimulation. It seems reasonable that this enzyme was released from skeletal muscle during stimulation, as a result of an increase in the permeability of the muscle cell membrane. Of the enzymes studied, CPK had the lowest molecular weight (83,000 M). The other enzymes studied, GOT and LDH, did not appear to be released in any significant amount, from skeletal muscle into the lymph during stimulation. This difference in behaviour might be related to the different intracellular localisation and strength of binding of these enzymes within the muscle cell. There may be a threshold value for the permeability of the muscle cell membrane to macromolecules. In this last case, the threshold would lie between molecules of 83,000 and 110,000 M. The present study did not provide any information as to which of the alternative suggestions is the more likely.

2. **Decrease in the rate of clearance of enzyme from the extracellular fluid.**

    Based on the results obtained in this study, and on
arguments detailed in the preceding discussion, the following hypothesis is advanced; that the exercise-induced increases in the activity of several enzymes in the plasma are the result of a decrease in the rate of their clearance from the plasma, while the rate of their influx into the plasma is maintained.

The various mechanisms by which enzyme can be removed from the extracellular fluid compartment have already been described in the Historical Review. Of the various systems which carry out this function, the RES appears to be the most important. However, the biliary system, gut and kidneys all play minor roles in the clearance of enzymes from the extracellular compartment.

If the increase in plasma enzyme activity evoked by exercise is to be explained on the basis of a reduction in the rate of clearance of enzyme from the extracellular fluid, the hypothesis must comply with both the qualitative and quantitative data available. The manner in which a reduction of enzyme clearance during exercise can be effected must be considered. Since the RES is implicated, a depression of its activity during exercise is suggested.

The phagocytic activity of the RES resides primarily in certain cells lining the walls of blood vessels in the liver, spleen, lungs and bone, and within the lymph nodes (Saba, 1970). Of these sites the liver and spleen are by
far the most active in the clearance of blood-borne colloid material.

Experiments have shown that the phagocytic activity of the RES is greatest in the mouse, and in decreasing order in the rat, guinea-pig, rabbit and man. The greater RES activity associated with smaller mammals has been linked to the relatively larger size of the liver and spleen in these animals. In fact the global index of the activity of RES has been shown to bear a direct relationship to the ratio of the combined weight of the spleen and liver/total body weight.

Fundamentally there are two expressions of RES activity. The global phagocytic index, \( k \), is dependent on the phagocytic activity of the RES cells, the total amount of RES tissue relative to body weight, and the blood flow through the RES regions. The last component has been found relatively fixed in the mammal (Dobson and Jones, 1952). The corrected phagocytic index, \( \alpha \), is a measure of the activity of the RES tissue after correction for variations in liver and spleen weight, and in blood flow. \( \alpha \) does not vary appreciably from one mammalian species to another.

A depression of the RES during exercise might be associated with an inhibition of the phagocytic cells, or with a reduction in blood flow to the RES regions of the body.

A decrease in the phagocytic activity of the RES cells would presumably be initiated by a chemical agent. During
various forms of shock, the RES is depressed by circulating chemical factors, presumably released from ischemic splanchnic tissues (Lefer, 1973). There is very little known, however, about the chemical regulation of the RES under physiological conditions. Heller (1957) has found that in the mouse, cortisone can depress the RES. During exercise, there is an increase in the secretion of 17-hydroxysteroids in the dog (Suzuki, Yamashita and Mitamura, 1958), but in man the circulating levels of 17-hydroxysteroids decrease (Connell, Cooper and Redfearn, 1958). Whether an increase in the level of circulating corticosteroids in the dog could have a depressant effect on the RES is speculative.

It seems reasonable to assume that with the large circulatory adjustments occurring in exercise, there may be important alterations in the blood flow through the RES regions of the body. Benacerraf et al. (1955) demonstrated that the rate of blood flow through the liver is an important determinant of carbon clearance by the RES. Ligation of the inferior vena cava, in the rat, increased portal blood flow and resulted in an increase in the rate of clearance of colloidal carbon from the blood. By resecting the intestine, the blood flow through the liver was severely reduced, and the rate of carbon clearance was similarly affected.

It has been estimated, by use of an indocyanine green clearance method, that during exercise in man, there is a
decrease in splanchnic blood flow which is proportional to the exercise work-load (Wade et al., 1956; Rowell, Blackman and Bruce, 1964). During work at a maximum load, splanchnic blood flow fell to 20 to 30 percent of its value at rest. Rowell, Blackman and Bruce (1964) also noted that there was a close relationship between the percent reduction in splanchnic blood flow and the work-load (expressed as a percent of the Max VO$_2$). This meant that exercise at a given work-load resulted in a much greater reduction of splanchnic blood flow in an individual with a low physical work-capacity, than in an individual with a high capacity.

In this observation lies a possible explanation for the effect of training on the exercise-evoked plasma enzyme response. In man, in particular, the plasma enzyme response to a standard work-load is much smaller in the physically well-trained than untrained individual. The smaller plasma enzyme response in the well-trained subject has been attributed to biochemical adaptations in skeletal muscle (Hunter and Critz, 1971) and improved cardiorespiratory function (Fowler et al., 1968) resulting from training. How these adaptations might reduce the plasma enzyme response to exercise is not clear. A much larger decline in splanchnic blood flow in the physically untrained subject would be expected during a standard exercise. It is suggested here, that in comparison with the well-trained subject, a relatively larger reduction in splanchnic blood flow would occur in the
untrained subject, leading to a greater reduction in the clearance of enzyme by the RES, and resulting in a greater increase in plasma enzyme activity.

Such clear-cut changes in splanchnic blood flow during exercise have not been demonstrated in other mammals. In the dog, in particular, the situation is controversial. Herrick et al. (1939) measured superior mesenteric and renal artery flows using a thermostromuhr. The exercise was a 3 mph walk. They found either no change, or a very slight increase in mesenteric and renal flows at this exercise load. More recently, the effect of hard exercise on mesenteric and renal artery blood flows in the dog have been determined using implanted ultrasonic flowmeter probes. Healthy mongrel dogs and trained Alaskan sled dogs, exercising at speeds of up to 30 mph, with heart rates approaching 300 beats/minute, exhibited no alteration in renal or mesenteric arterial flow (Rusher et al., 1961; Van Citters and Franklin, 1969). In another recent report, Fronek and Fronek (1970) measured splanchnic flow using electromagnetic flowmeters. They found that exercise which elicited a heart rate of 170 beats/minute resulted in a 10% decrease in splanchnic flow.

In one report on the rat, Stevens (1971) stated that there was no changes in splanchnic blood flow during very light exercise.

It is difficult to provide substantial evidence to support the quantitative aspects of the hypothesis. The
increases in plasma enzyme activity in the exercising mammal are not so large as to rule out an increase in the rate of release of enzyme from tissues as the sole mechanism responsible (cf. the elevated plasma levels of LDH in mice infected with the Riley virus.). However, a few simple theoretical estimations can be made, that indicate the viability of this hypothesis.

Let it be assumed that a given enzyme has a steady plasma value of 10 IU/L, and a plasma half-life of 30 minutes. A marked reduction in the clearance of this enzyme, together with no alteration in the rate of entry of the enzyme into the plasma, would result in a new plasma level of 20 IU/L. The time course of the change in plasma activity would be such that most of the increase would occur within one hour. Using the data of Doty, Sobel, and Bloor (1971), it can be shown that such a mechanism may indeed be functioning in the rat. The fast disappearance of the LD₁ and LD₂ isoenzymes of LDH from the plasma following a 3 hour period of swimming indicates a plasma half-life of between 1 and 2 hours. During exercise, the plasma levels of LD₁ and LD₂ increased by four-fold above their control values. It can be calculated that a similar increase in LD₁ and LD₂ in the plasma would occur if the clearance of these isoenzymes was severely reduced for 3 hours.

Drigoli et al., (1969) studied the changes in plasma amylase in man during a 30-minute period of exercise. The
rapid decrease in the activity of amylase in the plasma following the exercise indicated a plasma half-life of about 10 minutes. During 7 minutes of exercise at 100 watts, plasma amylase increased by 65%. During the subsequent 23 minutes of exercise at 50 watts, this elevated level of plasma amylase was not completely sustained. In man, amylase is cleared from the plasma by filtration at the kidney. During exercise, renal blood flow is reduced. In hard exercise renal plasma flow may decrease to a level that is 20% of the resting value (White and Rolf, 1948). Harpuder, Lowenthal and Blatt (1957) found that at work-loads of 77 and 147 watts (the latter evoking a heart rate of 142 beats/minute), renal blood flow fell to 69% and 45% of its resting value. A reduction in the renal blood flow during exercise, with a consequent decrease in renal clearance of amylase, is here proposed by this author as an explanation for the results of Drigoli et al.

It should be noted that if the increase in plasma amylase was a result of a release of amylase from various tissues, skeletal muscle could not have been the source. This observation is not consistent with the conclusions of Fowler et al. (1968) and Dieter (1970) who considered the exercise-evoked increases in plasma enzymes to originate entirely from skeletal muscle.

The different patterns of response to exercise of the various enzymes in the plasma also give some support to this
hypothesis. In man, LDH has a very long plasma half-life (40 hours, Dawson et al., 1969). In the rat, dog, and mouse, the values for the plasma half-life of LDH fall between 30 minutes and 5 hours. Of the three enzymes measured in this study, LDH is the least responsive to exercise in man, and the most responsive to exercise in the rat and the dog. If tissue damage or enzyme release by means of an increase in membrane permeability were the mechanism behind the exercise-evoked plasma enzyme increases, one would expect a similar pattern of response in all mammals. Furthermore, the animals whose enzymes possess the longer plasma-half-lives might be expected to show the more persistent plasma enzyme responses. In fact, the animals for whom the plasma clearance of LDH is the faster, show the most marked PLDH responses to exercise. This indeed seems inconsistent. However, if in reality the increase in PLDH during exercise is the result of a reduction in the clearance of plasma LDH, then it would be expected that animals which have the shortest plasma half-lives for PLDH, will have the most marked exercise-evoked increments in PLDH.

Other evidence can be cited to support this hypothesis. GOT and GPT are both found primarily in the liver, heart and skeletal muscle. In these last two tissues, GOT has a considerably higher activity. However, GPT is principally cytoplasmic, while slightly-more than half the GOT activity is mitochondrial (Swick, Barnstein and Stonge,
1965; Sheid, Morris and Roth, 1965). Thus within the cell cytoplasm there are roughly equal amounts of GOT and GPT.

If exercise caused cell damage or an increase in cell membrane permeability, one would expect both enzymes to increase in the plasma (M.W. GOT = 110,000 : GPT = 115,000) (Jenkins, Xphantis and Sizer, 1959; Saier and Jenkins, 1967). However, in the man and the rat, only GOT shows a marked response to exercise. There are no data on the clearance of these two enzymes by the RES in man or the rat. In the mouse and in the dog, however, GOT, but not GPT, is cleared by the RES. This suggests that changes in the pattern of enzyme clearance, rather than enzyme release may be responsible for the changes in plasma enzyme activity during exercise.

There is not sufficient data on the dog to allow a quantitative estimate of the importance of such a mechanism in this species. However, the short plasma half-life of LDH would certainly make this mechanism feasible.
SUMMARY AND CONCLUSIONS

These experiments were performed to identify the mechanism(s) responsible for the increases in activity of several enzymes in blood plasma evoked by physical exercise.

Comprehensive data were obtained on changes of three enzymes, GOT, CPK and LDH in the plasma of the exercising dog. The activities of the isoenzymes of LDH in the plasma were measured before and after skeletal muscle stimulation. In order to assess the importance of the circulatory adjustments to exercise in the exercise-evoked plasma enzyme response, systemic cardiovascular changes were achieved by either cardiac pacing, or diencephalic stimulation in the anaesthetised dog. Further experiments were carried out in the anaesthetised dog, to identify the routes by which these enzymes entered the plasma. Both thoracic and right lymphatic ducts were cannulated and the activity of enzyme delivered through these ducts was determined during periods of rest and skeletal muscle stimulation. The effect of repeated bouts of skeletal muscle stimulation on enzyme activity was determined. Since
splenic contraction was a possible mechanism responsible for the enzyme response, its role was examined.

The principle observations in these experiments were:

(1) During running exercise in the dog, PLDH was elevated more frequently than PGOT or PCPK. The increases in PLDH and PGOT were related to the intensity of the run (speed and grade). PCPK was only elevated after the most intense exercise. The duration of exercise did not appear to be an important determinant of the plasma enzyme response.

(2) A thirty-minute period of cardiac pacing at 240 beats/minute in the pentobarbital-dog resulted in a small increase in PGOT, but not in PCPK. (PLDH was not measured.)

(3) A thirty-minute period of diencephalic stimulation in the chloralose-dog resulted in marked increases in both heart rate and arterial blood pressure. Both PCPK and PGOT were elevated at the end of stimulation. (PLDH was not measured.) The magnitude of these plasma enzyme changes was approximately one-third as large as the plasma enzyme response to normal running exercise which evoked a similar heart rate.
(4) In resting pentobarbital-dogs, in which thoracic duct lymph was continuously drained, both PGOT and PCPK decreased relative to levels in control dogs. The activities of CPK and GOT in thoracic duct lymph were considerably higher than would be expected from simple recirculation of protein. PLDH levels were not affected by continuous drainage of thoracic duct lymph. The activity of LDH in thoracic duct lymph was low, and in several animals was undetectable.

(5) In anaesthetised dogs, in which thoracic duct, or thoracic and right duct lymph was drained, the activities of PLDH and PGOT, but not PCPK, increased during a 30-minute period of skeletal muscle stimulation.

(6) The activities of CPK, GOT and LDH all increased in thoracic duct lymph during skeletal muscle stimulation. However, the increase in CPK activity was far greater than that of GOT or LDH. Of the three enzymes, CPK alone was of sufficiently high activity in the lymph to have brought about a significant increase in plasma activity in the intact dog. The activities of GOT, CPK and LDH in right duct lymph decreased during skeletal muscle stimulation.
(7) Similar changes in plasma and thoracic duct lymph enzyme activity were elicited by repeated bouts of skeletal muscle stimulation.

(8) Following splenectomy, the responses to muscle stimulation of all three enzymes in the plasma were indistinguishable from those elicited in the intact dog.

(9) During a 30-minute period of muscle stimulation, which brought about a significant increase in PLDH, there were no changes in the proportional activity of the five isoenzymes of LDH in the plasma.

From these observations the following conclusions were made:

(1) The changes in plasma enzyme activity evoked by running exercise in the conscious dog, and by skeletal muscle stimulation in the anaesthetised dog were sufficiently alike to be caused by the same mechanisms.

(2) Changes in the cardiovascular system such as increases in heart rate and blood pressure during exercise may be responsible for part, but not all, of the plasma enzyme responses evoked by exercise.
(3) In the anaesthetised dog, the normal levels of PGOT and PCPK were maintained by the continuous addition of enzyme from the intracellular compartment via the lymphatic system. The normal levels of PLDH were maintained by the addition of intracellular enzyme directly into the circulating blood.

(4) CPK, released from contracting muscle, and reaching the circulating blood via the lymphatics, may be responsible for the exercise-evoked increase in PCPK.

(5) The increases in PGOT and PLDH during skeletal muscle stimulation do not depend on the integrity of the lymphatic system. Two alternative mechanisms were proposed to account for the exercise- or muscle stimulation-evoked increases in PGOT and PLDH: (a) there may be an increase in the release of these enzymes directly into the plasma from the intracellular compartment. The formed elements of the blood appear to be one such possible source, and (b) there may be a decrease in the clearance of enzyme from the extracellular fluid, with or without a concomitant increase in the release of enzymes into the extracellular compartment.
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APPENDIX 1

Outline of the assay procedures used in this study

1. **Spectrophotometric assay of glutamic-oxalacetic transaminase (GOT)**

   The method of Karmen (1955) was followed for the determination of GOT in samples of plasma and lymph.

**Reagents and procedure**

1 cm-square cuvette containing 1.0 ml of 0.1M phosphate buffer, pH 7.4, 0.5 ml of 0.2M aspartate in buffer at pH 7.4, 0.2 ml of a solution of NADH (1 mg/ml), 0.1 ml of a solution of purified malic dehydrogenase (MDH) (50 ug of enzyme protein/ml), and 0.2 ml of 0.1 M alphaketo glutarate in buffer at pH 7.4 is brought to 30°C.

To this is added 0.2 ml of the test sample. After 6 minutes incubation at 30°C an initial reading of the absorbance at 340 nm is recorded (A₀). Exactly 5 minutes after the initial reading, the final absorbance is determined (A₅). The rate of decrease of absorbance is the measure of GOT activity in the sample. All readings are made using deionised water as a blank.
Reaction sequence

alpha-ketoglutarate + aspartate $\xrightarrow{(GOT)}$ glutamate
  + oxalacetate

oxalacetate + NADH $\xrightarrow{(MDH)}$ NAD + malate

2. Spectrophotometric determination of creatine phosphokinase (CPK)

The method followed was that described by Oliver (1955), as modified by Rosalki (1967).

Reagents

Substrate mixture: 0.0001M ADP, 0.01M CP, 0.02M glucose, 0.03M magnesium chloride, hexokinase (0.6 IU/ml), glucose-6-phosphate dehydrogenase (0.3 IU/ml), 0.0008M NADP, 0.01M AMP (to inhibit myokinase activity), and 0.005M cysteine hydrochloride, in 0.05M tris buffer adjusted to pH 6.5 at 25°C.

Procedure

0.1 ml of the test sample are added to 3.0 ml of the substrate mixture in a 1 cm-square cuvette, and brought to 30°C (6 minutes). The absorbance at 340 nm is read ($A_0$) and the sample incubated for a further 5 minutes. At exactly 5 minutes after the initial reading, the absorbance
is read again (A5). The rate of increase in absorbance is a measure of CPK activity. All readings are made using deionised water as a blank.

**Reaction sequence**

Creatine phosphate + ADP ←(CPK)→ creatine + ATP

ATP + glucose ←(Hexokinase)→ glucose-6-phosphate + ADP

glucose-6-phosphate + NADP ←(G6PDH)→ NADPH + 6-phospho-gluconate

4. **Colorimetric determination of lactic dehydrogenase (LDH)**

The assay follows the method of Babson and Phillips (1965), and was used to determine LDH activity in samples of both plasma and lymph.

**Reagents**

1. **Colour reagent.** In 15 ml of water, dissolve, in this order; 50.0 mg of 2-p-iodophenyl-3-p-nitrophenyl tetrazolium chloride (INT), 12.5 mg of NAD, 12.5 mg of phenazine methosulphate (PMS). Make up to 25 ml with water. Store in the dark under refrigeration.

2. **Buffer reagent.** In 10 ml of water dissolve 1.0 g of ethoxylated oleyl alcohol (at 95°C). Dilute to 50 ml with water and add 12.1 g of trisaminomethane. Adjust the pH to
8.2 with 3N HCl and dilute to 100 ml. Store under refrigeration.

3. **Substrate.** To 50 ml of water add 5.0 ml of a 20% solution of L(+)-lactic acid. Adjust the pH to 5.5 with 1N NaOH, and dilute to 120 ml. Add a few drops of chloroform and store under refrigeration.

4. **Control reagent.** In 100 ml of water dissolve 0.2 g of potassium oxalate and 0.2 g of ethylenediaminetetraacetic acid, disodium dihydrate.

5. 0.1N HCl.

**Procedure**

Into each of two test-tubes pipette 0.1 ml of plasma (lymph) and 0.2 ml of buffer reagent. Add 0.5 ml reagent to one tube, and 0.5 ml of control reagent to the other. Mix and warm to 37°C (10 minutes). At precisely timed intervals add 0.2 ml of colour reagent and mix. Exactly 5 minutes after adding the colour reagent, add 5 ml of 0.1N HCl. Determine the difference in absorbance between the control and test samples at 520 nm.

Standard curves are plotted using Versatol and Versatol-E lyophilised serum standards.
**Reaction sequence**

L-lactate + NAD $\xrightarrow{(LDH)}$ pyruvate + NADH

NADH + PMS $\rightarrow$ NADH + reduced-PMS

Reduced-PMS + INT $\rightarrow$ PMS + reduced-INT (coloured, at 520 nm)

4. **Colorimetric determination of heart and liver type lactic dehydrogenase isoenzymes**

The reagents and procedure are exactly the same as in the previous description except for the following alterations (Babson, 1967). Using a substrate of 0.02M L (+)-lactate and 2M urea adjusted to pH 5.5 effectively measures only heart type isoenzymes. Conversely, using 2M L(+) lactate as substrate determines only liver type LDH activity.

5. **Spectrophotometric assay of blood lactate**

The method of Pfleiderer and Dose (1955) as modified by Rosenberg and Rush (1966) was used to determine the lactate concentration of the supernate prepared from whole blood deproteinised with perchloric acid.
Reagents

1. 0.02M NAD. Dissolve 266 mg of NAD in 10 ml of water. Bring the pH to 6.0 by adding 4 to 5 drops of 2N NaOH. Discard if accidentally made alkaline. Bring final volume to 20 ml.

2. 0.2M glycine-semicolonbazide buffer. Dissolve 1.5 gm of glycine and 2.2 g of semicolonbazide hydrochloride in 80 ml of water. Bring pH to 10.0 with 2N NaOH. Dilute to 100 ml.

Procedure

To a 1-cm square cuvette add 2.8 ml of glycine-semicolonbazide solution, 0.6 ml of NAD solution and 0.2 ml of the sample (or blank or standard). Invert to mix and determine absorbance at 340 nm. Incubate at 30°C. and read absorbance again at 1 hour, by which time the reaction should be completed.

Calculate the net absorbance, A, of the NADH₂ formed as a result of the enzymatic dehydrogenation of lactate.

\[ A = (S_{60} - 0.9S_0) - (B_{60} - 0.9B_0), \]

where S and B are the absorbance of the sample and the blank respectively.
Lactate concentration in gm/100 ml is determined directly from the absorbance of NADH$_2$, or from a standard curve.

**Reaction sequence**

L-lactate + NAD $\rightleftharpoons$ (LDH) $\longrightarrow$ pyruvate + NADH

Pyruvate is trapped by the semicarbazide, and the reaction becomes irreversible and goes to completion.
APPENDIX 2

Anatomical variations of the right lymph duct

in which cannulation was attempted

For full details refer to the section on Methods and to Figure 2.
APPENDIX 3

An example of the time course of changes in
PLDH, PGOT, PCPK and venous haematocrit
evoked by exercise in man

The author ran 7 consecutive miles at 12 mph,
with approximately 30 to 60 seconds intervals
between each mile to allow for a sample of venous
blood to be drawn. Blood samples were also drawn
5 minutes before, and at 5, 15 and 30 minutes
following the exercise.

For this individual, the work-load was
approaching maximal (i.e. Max VO$_2$). Note that
while the PGOT levels reached a plateau after
15 minutes of exercise, both PCPK and PLDH
gradually increased throughout the 35 minutes of
exercise. In addition it appeared that these
last two plasma enzymes continued to increase for
some time following termination of the exercise.
APPENDIX 4

**t-test on correlated mean differences**

The following statistic was applied to test the difference in behaviour of a given parameter over a particular time interval in experimental and control animals.

Values of the parameter were obtained for both control and experimental animals before and after a timed interval. For each group, the mean difference and sum of squares (SS) was computed. The variance of the difference between the mean differences was estimated from the pooled SSs of both groups:

\[
s^2(\bar{d} - \bar{d}') = \frac{SS + SS'}{(N-1)(N'-1)} x \frac{(1 + 1)}{N \times N'}
\]

\[
t = \frac{(\bar{d} - \bar{d}')}{s(\bar{d} - \bar{d}')}
\]

Where \( N = N' \), the following shortened form was used:

\[
t = \frac{(\bar{d} - \bar{d}')}{\sqrt{s^2 \bar{d} + s^2 \bar{d}'}}
\]