THE EFFECTS OF CHRONIC HYPOXIA ON THE ENZYME ACTIVITIES IN CARDIAC AND SKELETAL MUSCLE

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ABSTRACT

This study investigated the influence of chronic hypoxia on enzyme activities in cardiac and skeletal muscle. Guinea pigs and rabbits indigenous to high and low altitudes were studied and also guinea pigs subjected to chronic hypoxia in a hypobaric chamber.

The study was designed to see whether changes in metabolism occurring in response to hypoxia would be associated with changes in enzyme activity. The enzymes studied were from different catabolic pathways and were of cytoplasmic and mitochrondrial origin. The myocardium and sartorius muscle were studied as examples of muscles which, respectively, are dependent on or independent of oxygen.

The indigenous high altitude guinea pigs displayed enzyme activities which were similar to those of the sea level animals, while the high altitude rabbits possessed higher glycolytic enzyme activities in both the myocardial and sartorius muscles, but equivalent mitochondrial enzyme activities.

Acclimatization of guinea pigs to simulated high altitude had no effect on the sartorius muscle. However, in the myocardium there was a marked increase in glycolytic enzyme activity but no alteration in mitochondrial activities except at an early stage of acclimatization. Young, but not old, animals displayed an altered lactate dehydrogenase isoenzyme

pattern, as indicated by the ratio of αhydroxybutyrate dehydrogenase activity to total lactate dehydrogenase activity.

Changes in enzyme activities were followed in the myocardium during the postnatal development of guinea pigs under normobaric and hypobaric conditions. The normobaric animals displayed a falling glycolytic capacity throughout development, while the mitochondrial activity greatly increased to adult levels during the last days of gestation and remained constant thereafter. The developmental trends shown by the hypobaric animals were similar to those of the normobaric animals.

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ABBREVIATIONS

AMP	adenosine 5' monophosphate
ADP	adenosine 5' diphosphate
ATP	adenosine 5' triphosphate
DTNB	5,5' dithiobis (2 Nitrobenzoic acid)
EDTA	ethylenediamine tetraacetic acid
$NAD^{+}(P)$	β nicotinamide adenine dinucleotide (phosphate)
NAD(P)H	reduced β nicotinamide adenine dinucleotide (phosphate)
PPO	2,5-diphenyloxazole
POPOP	1,4-di-2-(5-phenyloxazolyl) benzene

INTRODUCTION

METHODS OF INDUCING TISSUE HYPOXIA

The supply of oxygen to the tissues is a complex process which can be considered as occuring in 3 stages: the passage of oxygen from the air into the blood, the transport by the blood to the tissues, and the uptake by the tissues (Hurtado 1964). Under normal conditions the supply of oxygen exceeds its utilization by the tissues, but situations can arise whereby the oxygen supply is reduced to such an extent that supply governs utilization and tissue hypoxia results. Alterations at any stage of the supply process could give rise to tissue hypoxia, and this complexity has allowed several experimental models to be developed in which one or more tissues are exposed to a hypoxic environment. Their suitability for different types of investigation varies; and in most situations alterations in other factors within the supply process occur, partially or completely compensating for the initial change.

For instance, tissue hypoxia can be produced by a reduction in the oxygen capacity of the blood. When this is caused by the inspiration of carbon monoxide (Penney, Dunham & Benjamin 1974, Penney, Benjamin & Dunham 1974), or by the withdrawal of red blood cells, there is a compensatory increase in the

concentration of haemoglobin in the blood, thus restoring the oxygen supply to normal. However, the reduction in oxygen capacity found in sideropenic anaemia is not associated with such compensation owing to the lack of iron (Penney, Bugaisky, Mieszala 1974, Poupa 1971). Although this last method provides a more permanent hypoxic state the effects of hypoxia are complicated by those caused by the general reduction in all iron-containing proteins which also occurs.

Reduction in the blood supply to a tissue can render the tissue hypoxic. Experimentally it can be produced by the ligation of an artery (Ferrans & Roberts 1971), but the degree of hypoxia in the surrounding tissue is variable, being dependent on the degree of constriction as well as the supply from collaterals. Compensation usually takes the form of development of other branches of the artery to supply the affected area. When the constriction is severe or complete it provides a model for ischaemia. In this situation the reduction in blood supply is so great that severe hypoxia develops, and in addition there is little or no supply or removal of metabolites, which has important repercussions (Kübler & Spieckermann 1970). At the onset of both hypoxia and ischaemia there is an increase in glycolytic flux which provides

the ATP required for continued function and structural integrity. In ischaemia this flux ceases quickly due to inhibition of phosphofructokinase activity, not substrate depletion. In the absence of blood flow the products of glycolysis accumulate causing a drop in tissue pH to which phosphofructokinase is very sensitive. In hypoxia, the presence of blood flow allows removal of the products and so increased glycolytic flux continues for longer. Consequently in ischaemia irreversible damage occurs more rapidly than in hypoxia.

The most suitable method of producing tissue hypoxia for studies of chronic effects is by reducing the partial pressure of oxygen in the inspired air. This can be achieved by hypobaric conditions which simulate high altitude (Miller & Hale 1968, Mager et al. 1968), or by mixing air or oxygen with an inert gas while maintaining normal atmospheric pressure (Smialek & Hamberger 1970, Koob et al. 1974, Ballard 1971). As with other situations compensatory changes occur including hyperventilation and erythropoiesis. Hyperventilation reduces the difference in the partial pressure of oxygen between the atmosphere and the blood, while the increase in the haemoglobin concentration of the blood raises the oxygen capacity of the blood. As a result of the changes, the oxygen content of the blood of high

altitude residents may be the same as that of the sea level residents, although the partial pressure of oxygen remains lower. This results in a reduced partial pressure gradient along which the transfer of oxygen from the blood to the tissues must occur. Further compensation is achieved by increased vascularization (Valdivia 1958, Kerr 1965, Tenney & Ou 1967), which reduces the diffusion distance from the capillary to the mitochondria and so increases the partial pressure gradient, and by an increase in the tissue content of myoglobin (Tappan & Reynafarje 1957, Costa & Taquini 1970, Reynafarje & Morrison 1962, Anthony et al. 1959, Reynafarje 1962) which increases the solubility of oxygen in the tissue and the rate of diffusion (Hemmingsen 1963, Wyman 1966). The extent to which these changes compensate for the low Po_2 in the blood is uncertain and will depend on the severity of the conditions and the requirement of the tissue. No reports of tissue Po_2 in acclimatized animals under hypoxic conditions are known.

RESPONSE OF TISSUES TO HYPOXIA

The response of a tissue to hypoxia can be considered as occurring in 2 stages: the acute response and the chronic response. A great deal of work has been done to investigate the changes occurring

during the acute phase (Morgan & Parmeggiani 1964, Williamson 1966, Kübler & Spieckermann 1970). In the well oxygenated heart oxidative phosphorylation by the mitochondria is regulated by the ATP/ADP ratio such that the oxygen utilization is governed by the utilization of ATP (Lubbers & Kessler 1968). With the transfer from a normoxic to a hypoxic state, the need for high energy phosphates continues but, despite the high affinity of cytochrome oxidase for oxygen, the regeneration by the mitochondria fails to maintain the normal levels. Initially the ATP level is maintained at the expense of creatine phosphate. The breakdown of creatine phosphate leads to an increase in the concentration of inorganic phosphate which stimulates glycogen phosphorylase b and so the breakdown of glycogen. In addition, the proportion of phosphorylase a increases as a result of the phosphorylation of phosphorylase b by a specific kinase. The increased supply of glucose-1-phosphate and, by isomerization, glucose-6-phosphate leads to an increased flux through the glycolytic pathway. This is greatly accelerated by the activation of phosphofructokinase by AMP. The increase in AMP results from a small decrease in ATP through the action of myokinase, but, with the normal concentration of AMP considerably less than that of ATP, the relative change in AMP is much greater than

that in ATP. Subsequently other enzymes appear to be activated and the control of the pathway moves to other reactions. In skeletal muscle the maximum rate of glycolysis is comparable to the activity of phosphofructokinase as measured in vitro. In contrast, in the heart the maximum rate is much lower and the generation of ATP by glycolysis is insufficient to maintain normal functioning, with the result that contraction soon ceases although viability continues for longer. With milder forms of hypoxia the transfer from oxidative to anaerobic metabolism is less marked, and the tissue may be able to survive and function.

In the chronic situation the whole animal must acclimatize so that despite the lower supply of oxygen the tissues can function normally. The ability to survive conditions of chronic hypoxia differs between species (Reeves, Grover, Grover 1963a,b) and between strains of a species (Gold, Johnson & Costello 1973). Whereas during acute exposure an altered pattern of metabolism is achieved by alterations in effector concentrations, previous work has suggested that changes in enzyme concentration occur as a result of chronic exposure to hypoxia (Barbashova 1967, Ou & Tenney 1970, Walpurger, Schlaak & Jipp 1970, Vergnes 1973) and it was on this aspect that the present work was to concentrate.

Survival of a tissue under conditions of hypoxia will depend on its capability to generate enough

"high energy" phosphates for its essential functions, and so on its metabolic rate. The various methods of ATP generation are present in all muscles but the importance of a particular pathway varies from tissue to tissue. The capability of a tissue to survive periods of anoxia varies and correlates with its capacity for anaerobic metabolism (Gesser & Poupa 1974, Poupa 1971, McGrath & Bullard 1968, McGrath, Bullard & Komives 1969). Although it would be probable that those muscles displaying predominantly aerobic metabolism would be more affected than those displaying anaerobic metabolism, the effects of chronic hypoxia on different muscle types is not known. This was one of the aspects studied here in both indigenous and acclimatized animals.

ACCLIMATIZATION AND ADAPTATION

In studies on the alterations shown by indigenous high altitude animals or animals taken from sea level to high altitude, the words 'acclimatization' and 'adaptation' have often been used without definition and seemingly interchangeably. However, here the term 'acclimatization' will be used to describe the acquisition of reversible and non-inheritable attributes in response to exposure to an altered environment, while the term 'adaptation' will be reserved for the acquisition of permanent, genetically inherited attributes. Thus when a sea level animal

is taken to high altitude, the initial responses are those of acclimatization. Upon return to sea level these changes will disappear. However, if the animal continues to live and breed at high altitude, over the course of generations offspring better able to survive the hypoxic conditions would be naturally selected. These animals might display genetically determined alterations, that is to say adaptations, which should persist if the animals were taken to sea level.

There has been much interest in the indigenous high altitude Indians of South America as they are capable of strenuous physical activity at high altitudes (Hurtado 1964). This could be because the natives are adapted to their environment or simply because they are well acclimatized. The comparative studies have inherent problems as often the background and life style of the groups chosen for study are very different (Kollias et al. 1968), and this may lead to differences other than those resulting from adaptation to the environment. One response to high altitude that has been shown to have a genetic determinant is the development of pulmonary hypertension. This response is shown by several but not all species (Dean et al. 1968, Grover et al. 1963, Reeves, Grover & Grover 1963a,b), and Will and co-workers (1970) have shown that in cattle this tendency is inherited. Several

workers have compared different biochemical properties in indigenous high and low altitude animals (Tappan et al. 1957, Reynafarje 1961, 1971, Harris et al. 1970). The study presented here was designed to extend this work and to compare the results with those of acclimatization studies.

INFLUENCE OF THE STAGE OF ONTOGENY

One important determinant of the response to hypoxia is the age and stage of development of the animal when the exposure occurs (McGrath et al. 1973, Flückiger & Verzar 1955, Frisancho et al. 1973). The foetus develops in an environment of low Po_2, and young animals are often able to withstand severe hypoxia better than older members of the species (Atland & Highman 1964, Ferrans & Roberts 1971). This suggests that during postnatal development the ability to survive hypoxic stress is lost. This may be associated with the permanent repression of certain genes which were active in the hypoxic situation of intrauterine life. If this is the case, then different responses to hypoxia might be shown if the stress was applied before or after the repression of these genes. It was, therefore, of interest to compare the responses of animals of different ages.

SEVERITY AND DURATION OF HYPOXIA

Other important determinants include the severity and duration of the hypoxic stress. Several workers have utilized very severe conditions using simulated altitudes of 24000' or higher (Gold, Johnson & Costello 1973, Widimský et al. 1973, Poupa 1971, Pelouch & Poupa 1969, Atland & Highman 1951). Exposure to this degree of stess results in considerable ultrastructural damage to the myocardium (Ferrans & Roberts 1971). However, even exposure to the altitude of 14000' has been shown to result in some changes (Bishoff et al. 1969), the degree of which depends on the species of animal used.

INFLUENCES FROM OTHER ORGANS

In addition, the complex interactions between tissues that are present in all experiments involving whole animals will affect the response seen. Guinea pigs and especially rats become anorexic upon exposure to low barometric pressure and this affects the metabolism of the animals (Gloster, Heath & Harris 1972, Gold & Costello 1974). Several authors have reported this response but few have considered its significance for their results (Strickland, Ackerman & Anthony 1962, Gold & Costello 1974, Gold, Johnson & Costello 1973). The stress of the new

conditions alone will cause changes in the hormone balance in the animal, and this is more difficult to control, although it can be minimized. At altitude the temperature and humidity may be different from those at sea level, and these factors may be responsible for some of the differences found (Petropoulos & Timiris 1974). The development of pulmonary hypertension leads to right ventricular hypertrophy which adds a further complication unless the different portions of the heart are distinguished. It was with these many factors in mind that the study into the effects of chronic hypoxia was designed.

EXPERIMENTAL DESIGN

This study was designed to investigate some of the responses to chronic hypoxia already mentioned, namely: (1) the adaptation and/or acclimatization of indigenous high altitude animals;

(2) the acclimatization of sea level animals to hypobaric hypoxia, and the influence of the tissue type, the duration of exposure, and the age of the animal. As the intention was to compare results obtained from acclimatization studies with those obtained from the studies of indigenous high and low altitude animals, the conditions and the species of animal to be used needed to be carefully chosen.

NATURE OF THE HYPOXIA TO BE STUDIED

A hypobaric chamber can simulate only certain of the environmental factors characteristic of natural high altitude. It provides a condition of hypobaric hypoxia suitable for chronic studies without the reduced gravitational force, the increased cosmic radiation and other factors normally associated with high altitude. However, this is an advantage when trying to understand the means by which animals adjust to life at high altitudes, as it allows analysis of the response to two variables only,

the atmospheric pressure and the partial pressure of oxygen. In the natural environment, the other factors will also influence the animal, but it is unlikely that this influence will completely alter the response to hypobaric hypoxia studied here. As a result it is hoped that the information gained will contribute to further understanding of the processes occurring in the natural environment.

The pressure of 400 torr was chosen as it simulates an altitude of 15000' at which humans and animals naturally live. The conditions then correspond to a physiological rather than pathological state and comparisons can be made between the acclimatized and indigenous animals.

ANIMALS CHOSEN

The species of animal chosen for study needed to be indigenous to both high and low altitudes, and to be suitable for laboratory investigations involving a hypobaric chamber. It was therefore decided to study guinea pigs and rabbits, with the work concentrating on the former species.

The choice of control animals is important. In the study of indigenous animals, outbred animals were obtained from high and low altitudes. In this way any differences other than those arising from altitude were minimised. This arrangement was considered preferable to the use of laboratory animals available

in London as the sea level controls. In the acclimatization studies the control animals were of two different types. One type was allowed food ad libitum and is referred to herein as "free fed", while the other was given only that amount of food taken by the hypobaric animals in the corresponding period and is referred to as "restricted diet". In this way the effects of the reduced food intake could be assessed and distinguished from those of the hypoxia.

In an attempt to improve the nature of the control samples, an investigation was carried out into the possibility of using the skeletal muscles from one side of an animal as the "initial state" controls for the corresponding muscles of the other side. It was found to be possible in practice for the muscles to be removed and the animal to recover completely. The procedure had no effect on the enzyme activities of the muscles subsequently taken. However, there was no difference in the variability between samples from the same animal and samples from different animals, and so the procedure was discontinued.

EFFECT OF AGE

The acclimatization of adult guinea pigs was studied initially, with exposure for 2 and 4 weeks. To ascertain the effect of age, young animals of 6 weeks were subjected to the same conditions for 4 weeks, and the same was intended for newborn animals.

To achieve this the pregnant sows were gradually acclimatized and it was hoped that parturition would then occur successfully under the hypobaric conditions. However, there was a high mortality rate among the pregnant sows and the majority of the young were stillborn. Consequently the conditions were ameliorated so that the final pressure was 460 torr, corresponding to 13000'. Further details are given in the appropriate section.

TISSUE TYPES

The effect of hypoxia on different tissues was investigated. The heart was studied because this tissue, under normal conditions, utilizes predominantly free fatty acids and ketone bodies (Shipp, Opie & Challoner 1961, Williamson & Krebs 1961) and has an absolute requirement for oxygen for continued function. In contrast the sartorius muscle, predominantly dependent on anaerobic glycolysis for its energy production (Czok & Bücher 1960, Ogata 1960, Wirsén 1965), was also studied. Preliminary work also included studies of the gastrocnemius muscle, which was chosen as an example of a skeletal muscle with a higher capacity for fatty acid oxidation and a lower capacity for glycolysis than the sartorius. This was not pursued as it was considered preferable to concentrate on the heart and sartorius alone.

ENZYME ANALYSIS

The activity of an enzymic reaction depends on the concentration of the enzyme, its substrates and cofactors, the effectors to which it is sensitive and the ionic composition of the medium in which it acts. In vitro, measurements are made under optimal or near optimal conditions and concern only the forward or reverse reactions individually. In vivo, optimal conditions may not exist and, although some enzymes catalyse unidirectional reactions, most seem to catalyse forward and backward reactions simultaneously under near equilibrium conditions. Despite the discrepancy between the enzymic environments in vitro and in vivo, the comparison of the enzyme activity patterns of different tissues correlates well with the observed metabolic differences (Bass et al. 1969, Pette 1971, Standte & Pette 1972). Thus the enzyme activity pattern provides an indication of the metabolic pattern of the tissue.

The metabolic flux through a pathway is controlled at certain points by the activity of regulatory enzymes (Newsholme & Start 1973). Alterations in the activity of these enzymes can be brought about not only by changes in the substrate and cofactor concentrations but also by changes in the concentrations of effectors which may or may not be involved in the metabolic pathways in which the enzymes function. The action of the effectors provides a fine and rapid

regulation of the enzyme potential allowing the integration of the complex metabolic processes within the tissue to occur. A change in the metabolic pattern of a tissue could be achieved by alterations in the concentrations of the appropriate enzymes or by alterations in the activities of the regulatory enzymes by alterations in the concentrations of their effectors. The former method is relatively slow but is specific, and so would seem suitable for long term changes. The latter method, however, is rapid but lacks specificity as one effector may regulate several enzymes. In addition alterations in the concentrations of effectors might limit the response of the enzyme to subsequent changes in the effector concentrations. It was hoped that any alterations in metabolism occurring in response to hypobaric hypoxia would be reflected in appropriate changes in the activities of the enzymes as measured under standardized conditions.

In the interpretation of any changes in activity, it must be remembered that an increase in activity might indicate an increase in the metabolic flux through the enzyme reaction, but it might also enable normal flux to occur despite decreased substrate or cofactor concentrations. For instance, the same rate of oxidative phosphorylation could be achieved at a lower Po_2 if the concentration of the electron transport chain components were increased (Lubbers & Kessler 1968).

The enzymes chosen for study were from different catabolic pathways and were of cytoplasmic and

mitochondrial origin. The pathways concerned and the specific enzymes chosen were:

(1) glycolytic pathway : hexokinase, glycogen phosphorylase, phosphoglucomutase, phosphofructokinase, glucose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase, pyruvate kinase, lactate dehydrogenase.

(2) pentose phosphate pathway : glucose-6-phosphate dehydrogenase.

(3) fatty acid oxidation : βhydroxyacylcoenzyme A dehydrogenase, palmityl carnitine transferase.

(4) tricarboxylic acid cycle : succinate dehydrogenase, malate dehydrogenase.

(5) shuttles for transferring reducing equivalents across the mitochondrial membrane: mitochondrial glycerol-3-phosphate dehydrogenase, aspartate aminotransferase, malate dehydrogenase.

Not all these enzymes were measured in each experiment, and the selection of those to be studied was made on the basis of earlier results.

The aim of an enzyme assay is to give a measure of the rate of the reaction under study. Conditions are chosen to give maximal or near maximal activity, and are usually very different from those in vivo, especially with respect to the concentrations of the reactants including the enzyme itself (Wuntch, Chen, & Vesell 1970). Where the reaction under investigation utilizes NAD(P)+ or an artificial electron

acceptor, the oxidation - reduction of these compounds can be monitored and the rate of the reaction directly measured. Where this is not the case, the reaction under investigation may be linked to one which does so act, and the linkage between the test reaction and the indicator reaction may be direct or involve several intermediary reactions. Conditions are sought whereby the rate of the indicator reaction is the same as that of the test reaction. Bücher et al. (1964) have explained many of the problems associated with coupled systems and indicated why the rate of the indicator reaction may not be equal to that of the reaction under investigation. For this reason, and because the results obtained here were for comparative analysis, the assays were primarily designed to give a relative measure of the activity rather than necessarily the maximum activity.

The important features of the assays were:

(1) that the initial rate of change of absorbance be proportional to the enzyme concentration;

(2) that the change of absorbance be proportional to time for as long as possible;

(3) that the assay be sensitive and yet convenient. This aspect was important as it was intended that many assays should be performed on the same sample.

The assays give a measure of enzyme ACTIVITY, not directly of concentration. The rate of the reaction

measured depends on various parameters: enzyme concentration, substrate and coenzyme concentrations, and the kinetic characteristics of the enzyme. The kinetic characteristics are affected by the pH, ionic strength and composition of the medium, and by the temperature. In addition, for several enzymes the characteristics are altered by the presence or absence of effector molecules. Knowledge of these features is important in the design and interpretation of the assays; and where possible activators were added to the reaction medium. These additions increased the sensitivity of the assay and removed the problem of small quantities of endogenous activators.

To investigate whether the changes in activity measured were the result of changes in enzyme concentration or the result of changes in activator or inhibitor concentrations, mixing studies were carried out. With this method, the activity of the mixture of two samples will not be the same as that calculated from the individual activities if the concentrations of activators or inhibitors are different in the samples. This method is limited in that it can only distinguish changes due to alterations in the concentration of an effector if it is freely dissociable, and not if it is tightly bound to the enzyme in the form of a prosthetic group.

The order in which the assays are carried out is

important as the stabilities of the enzymes vary. The order was the same within any experiment and always started with those for glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, phosphofructokinase, and enolase as recommended by Shonk & Boxer (1964). The assays were always completed before the time at which inactivation could be detected.

METHODS

ANIMALS

The laboratory animals were male Dunken Hartley guinea pigs obtained from S.A.C.I. Ltd., Braintree, Essex and Redfearn Animal Breeders Ltd., Brenchley. They were maintained on a diet SGIV from E. Dixons and Sons (Ware) Ltd., Ware, Herts, supplemented with hay.

Indigenous rabbits and guinea pigs were obtained at Cerro de Pasco, Peru, altitude 4300 m, and at Lima, Peru, altitude 50 m. They were maintained on a laboratory diet supplemented with fresh green vegetables provided ad libitum.

HYPOXIA

This was produced in a hypobaric chamber obtained from Edwards Vacuum Ltd., Crawley, Sussex. The pressure was regulated by means of an inlet valve to a value of 400 torr, corresponding to an altitude of 15000'. The chamber was housed in a room in which the cycle of light and dark, and the temperature were controlled. The control animals were housed under similar conditions. The pressure of the chamber was returned to normal for a short period each day to allow for the weighing of the animals and food, and for the cleaning of the cages.

For the study of newborn animals, the final pressure of 460 torr was utilised owing to the great difficulty in obtaining live young with the pressure at 400 torr. As it was not possible to ascertain the amount of milk consumed by the hypobaric young, both groups

were allowed to feed ad libitum.

TISSUES

The sartorius and ventricular muscles were studied. The ventricles were dissected from from the septum after the atria and great vessels had been removed from the heart, and were treated individually.

Biopsy Method

This technique was developed whereby the skeletal muscles from one side of the animal could serve as controls for those from the other side, which would be investigated after the recovery of and the experimentation on the animal.

Operating Procedure

Guinea pigs were given atropine to reduce mucal secretions and the possibility of suffocation during anaesthesia. Nembutal was given by intraperitoneal injection at a dose of 0.57 ml/kg. This is about 30% higher than generally recommended but was found to be necessary. When required, the level of anaesthesia could be further deepened by the use of ether. The sartorius and gastrocnemius muscles were removed from one hind leg under aseptic conditions. After 2 weeks the process was repeated and the muscles from the other hind leg removed. The activities of the glycolytic enzymes were measured in homogenates of the tissues immediately after removal from the

animal. The results are expressed in Table 1 as % of the initial values ± S.D. for three animals. Although there appeared to be little difference between the paired samples, the variability within an animal was as great as that between animals and so the method gave no benefit.

The same technique was developed for rabbits. Anaesthesia was induced by intravenous injection of Nembutal into an ear vein. Again no advantage was gained with this technique and so it was discontinued.

PREPARATION OF HOMOGENATES

The guinea pigs were killed by cervical dislocation. The heart was removed while still beating, was washed in ice-cold saline, and then dissected into left and right ventricles, which were subsequently treated separately. Skeletal muscle was also immersed in the saline after removal from the animal. The tissues were blotted dry with filter paper, weighed and homogenized in 20 volumes of ice-cold 0.1 M potassium phosphate buffer containing 6.0 mM EDTA, pH 7.5. The ionic strength of this buffer is greater than 0.1, preventing the adsorption of the glycolytic enzymes onto the muscle proteins (Arnold & Pette 1968). Homogenization was carried out using an Ultra Turrex homogenizer with a 18K shaft running at maximum speed for 35 s. The process was completed in 3 bursts to

TABLE 1

Mean activities of glycolytic enzymes in the biopsy samples taken from 3 guinea pigs at zero and 2 weeks, expressed / mg protein ± S.D. and as a % of the initial value.

	SARTORIUS		GASTROCNEMIUS	
	0 weeks	2 weeks	0 weeks	2 weeks
Hexokinase	-	-	100±37	59±16
Glucosephosphate isomerase	100±25	145±14	100±12	102±15
Phosphoglucomutase	100±13	130±40	100±30	104±15
Phosphofructokinase	100±15	91± 8	100±48	133±67
Aldolase	100± 6	92±15	100±23	70±25
Phosphoglyceromutase	100± 7	97± 4	100±20	83±12
Enolase	100±13	110±18	100±18	115±51
Pyruvate kinase	100±19	117±10	100±10	117±34
Lactate dehydrogenase	100± 5	97±16	100±35	119±50

allow adequate time for the heat generated to be transferred to the surrounding ice, and so prevent the temperature of the homogenate from rising. Electron micrographs showed that this process caused complete disruption of the cells (Plate 1, 2).

In the investigation of newborn guinea pigs there was not sufficient tissue for the Ultra Turrex to operate, and so an ILA homogenizer with a 10T shaft was used. The principle of action was the same for both homogenizers, and with the small rotor volumes of 1.0 ml were sufficient, enabling analyses to be made on tissue samples of 50 mg. However, with the small volume the rate of loss of heat from the homogenate to the surrounding ice became of critical importance. The homogenization pattern used with the Ultra Turrex was unsuitable as it yielded a discolored homogenate with low enzymic activity. To ascertain the most suitable duration and speed of homogenization such that the resulting homogenate was comparable to that obtained with the Ultra Turrex, the activities of several enzymes were measured in homogenates produced by different homogenization patterns. The enzymes studied were αhydroxybutyrate dehydrogenase, succinate dehydrogenase, βhydroxyacyl coenzyme A dehydrogenase

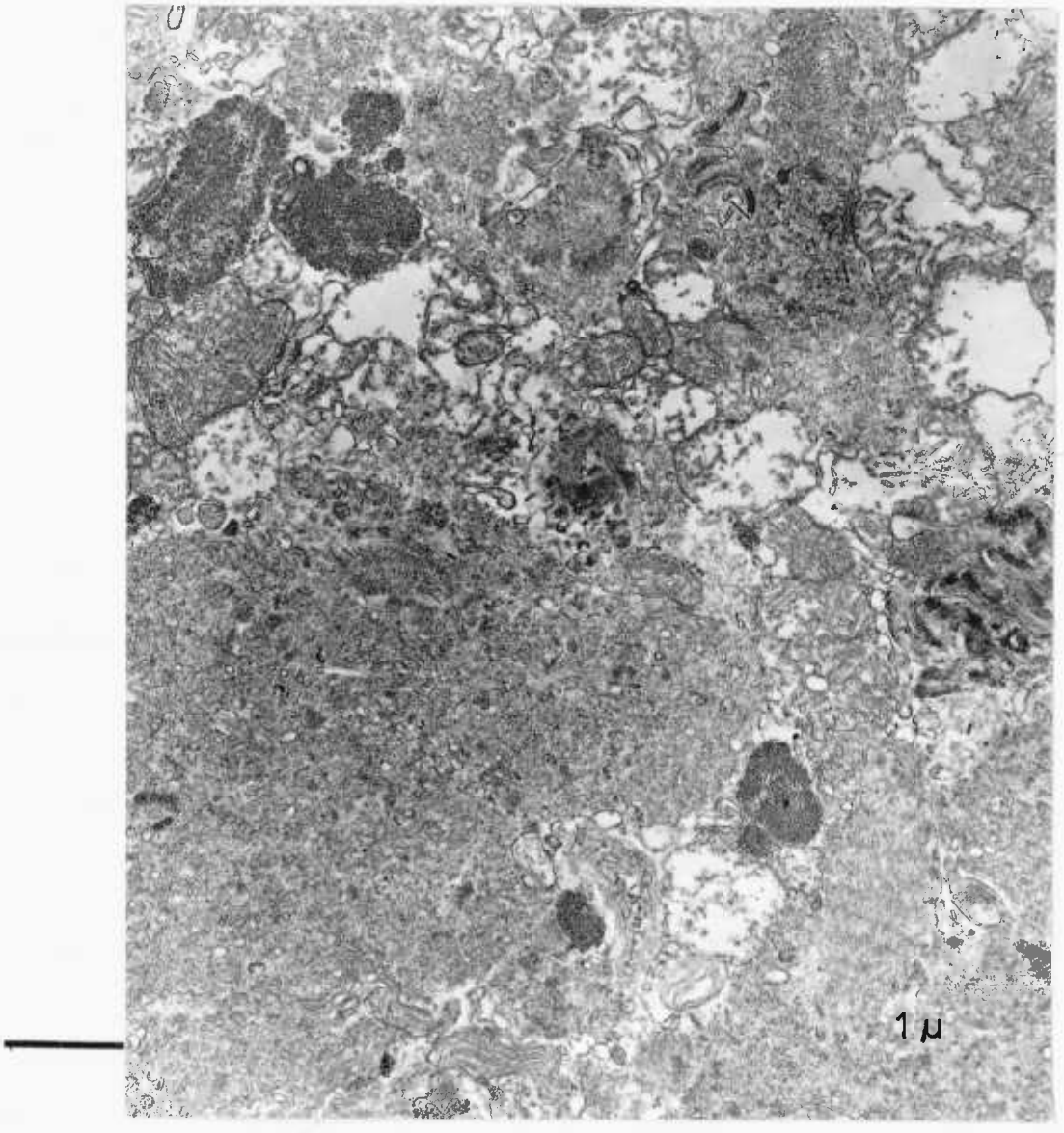


Plate 1

Electron micrograph of a ventricular homogenate prepared with the Ultra Turrex homogenizer.

Magnification x 15,000

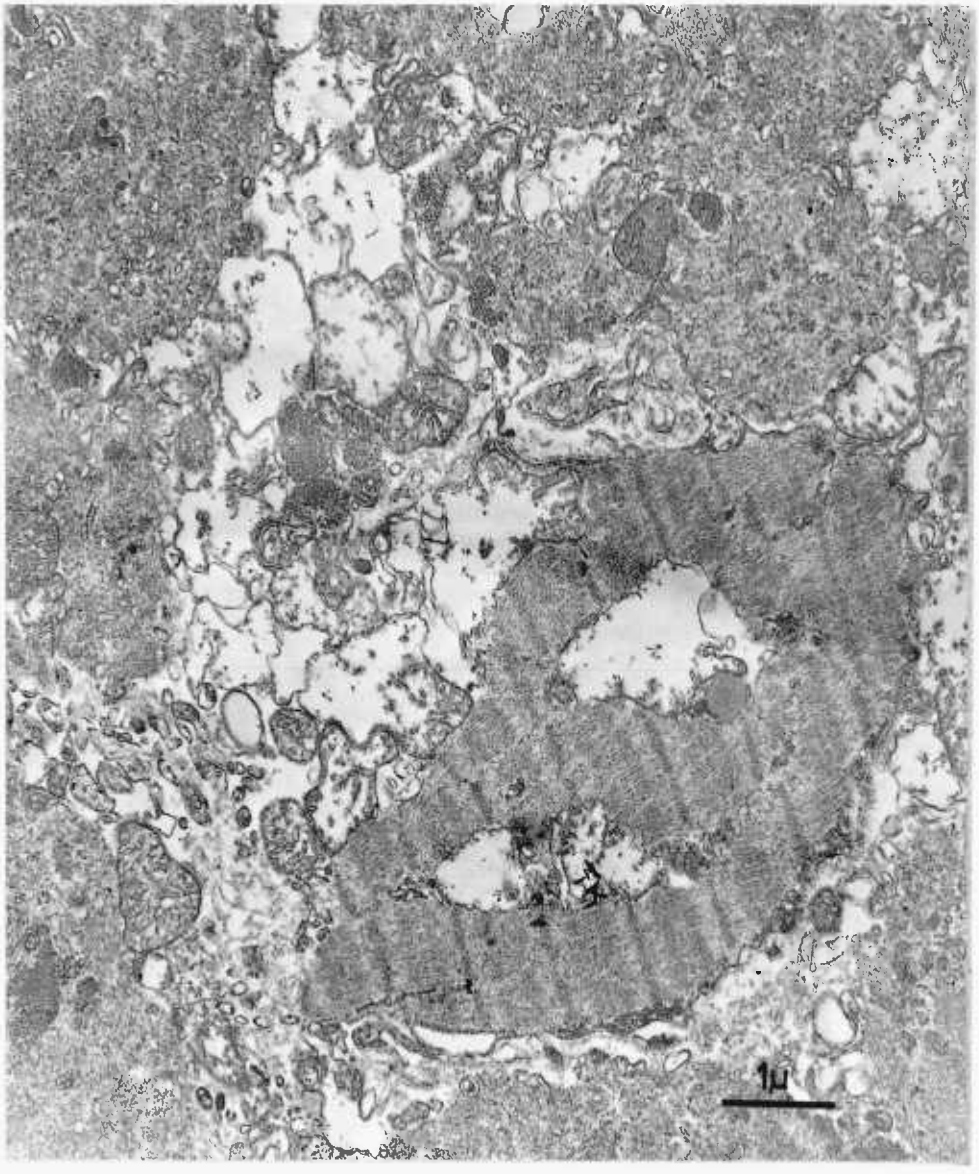


Plate 2
Electron micrograph of a ventricular homogenate prepared with the Ultra Turrex homogenizer.
Magnification x 15,000

and palmityl carnitine transferase, and the results are given in Table 2. The procedure adopted was C, consisting of 7 bursts of 10 s each at the rheostat setting number 7. Electron micrographs of the resulting homogenate are shown in Plates 3 and 4, and indicate complete disruption of the myocardium.

It was necessary for the homogenates from adult and young, but not newborn, guinea pigs to be strained through terylene netting to remove any pieces of fibrous tissue. The homogenates were stored on ice, and further dilutions were achieved using the homogenization medium.

MATERIALS

The biochemicals were obtained from Boehringer Corp. (London) Ltd. and Sigma (London) Chemical Co., and the inorganic chemicals from B.D.H. Chemicals. All were of a high degree of purity. The water used was all glass distilled.

STATISTICS

Statistical analysis was carried out on an Olivetti Programma 102 using the students "t" and paired "t" tests where applicable.

TABLE 2

Effect of homogenization pattern on measured enzyme activities, given as relative values, with the figures in parentheses representing the values relative to those for αhydroxybutyrate dehydrogenase.

Pattern A : 35 s with the Ultra Turrex at maximum speed.

Pattern B : 70 s with the ILA homogenizer at rheostat setting 6.

Pattern C : 70 s with the ILA homogenizer at rheostat setting 7.

Pattern D : 70 s with the ILA homogenizer at rheostat setting 8.

	A	B	C	D
αhydroxybutyrate dehydrogenase	7.22 (1.00)	6.85 (1.00)	6.96 (1.00)	6.33 (1.00)
βhydroxyacyl-coenzyme A dehydrogenase	2.84 (0.394)	3.09 (0.451)	2.58 (0.370)	3.35 (0.529)
succinate dehydrogenase	19.1 (2.64)	18.3 (2.68)	18.1 (2.60)	20.3 (3.20)
palmityl carnitine transferase	24.6 (3.41)	20.4 (2.98)	27.1 (3.89)	18.8 (2.90)

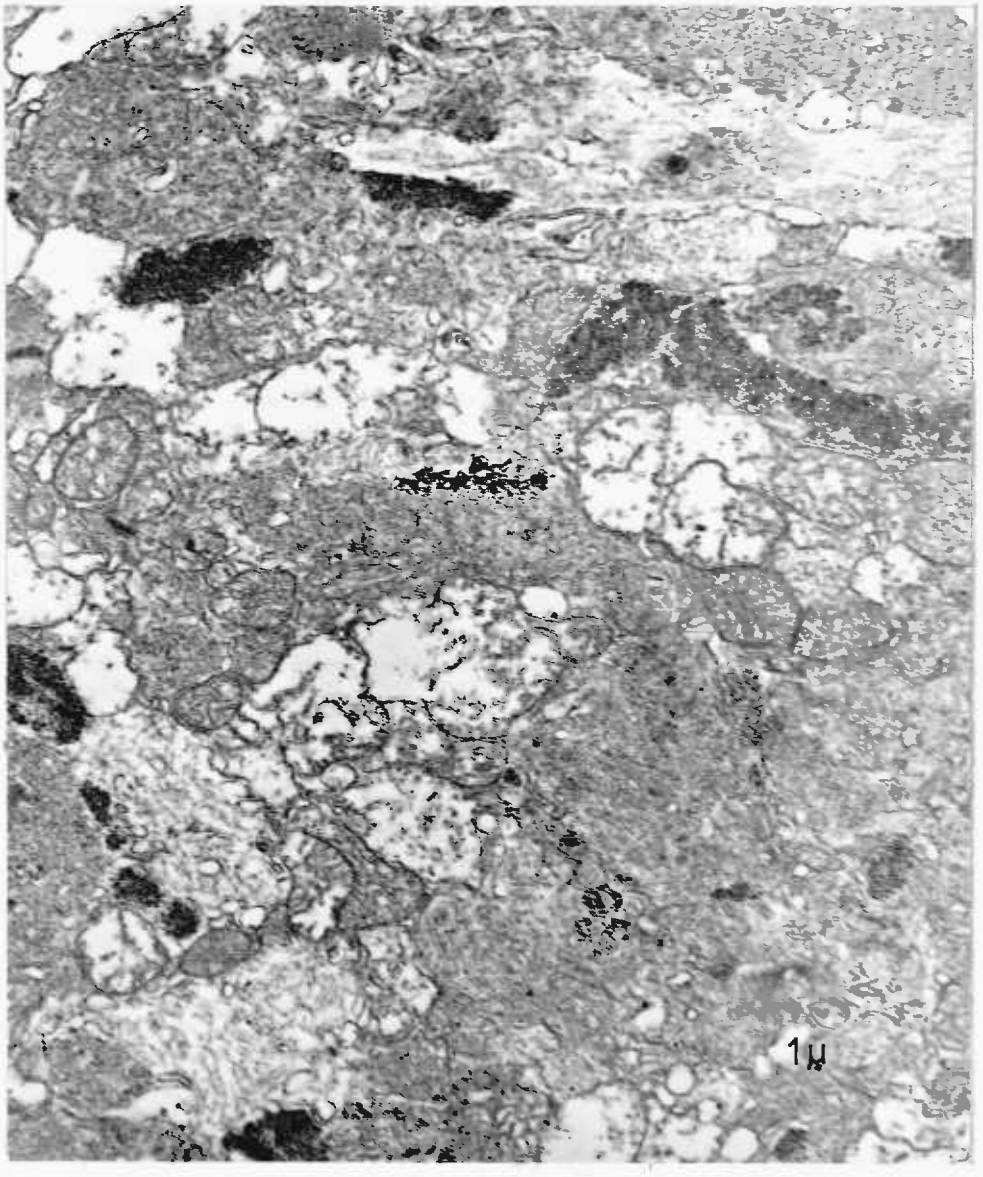


Plate 3

Electron micrograph of a ventricular homogenate prepared with the ILA homogenizer, and homogenization pattern C (page 35).
Magnification x 15,000

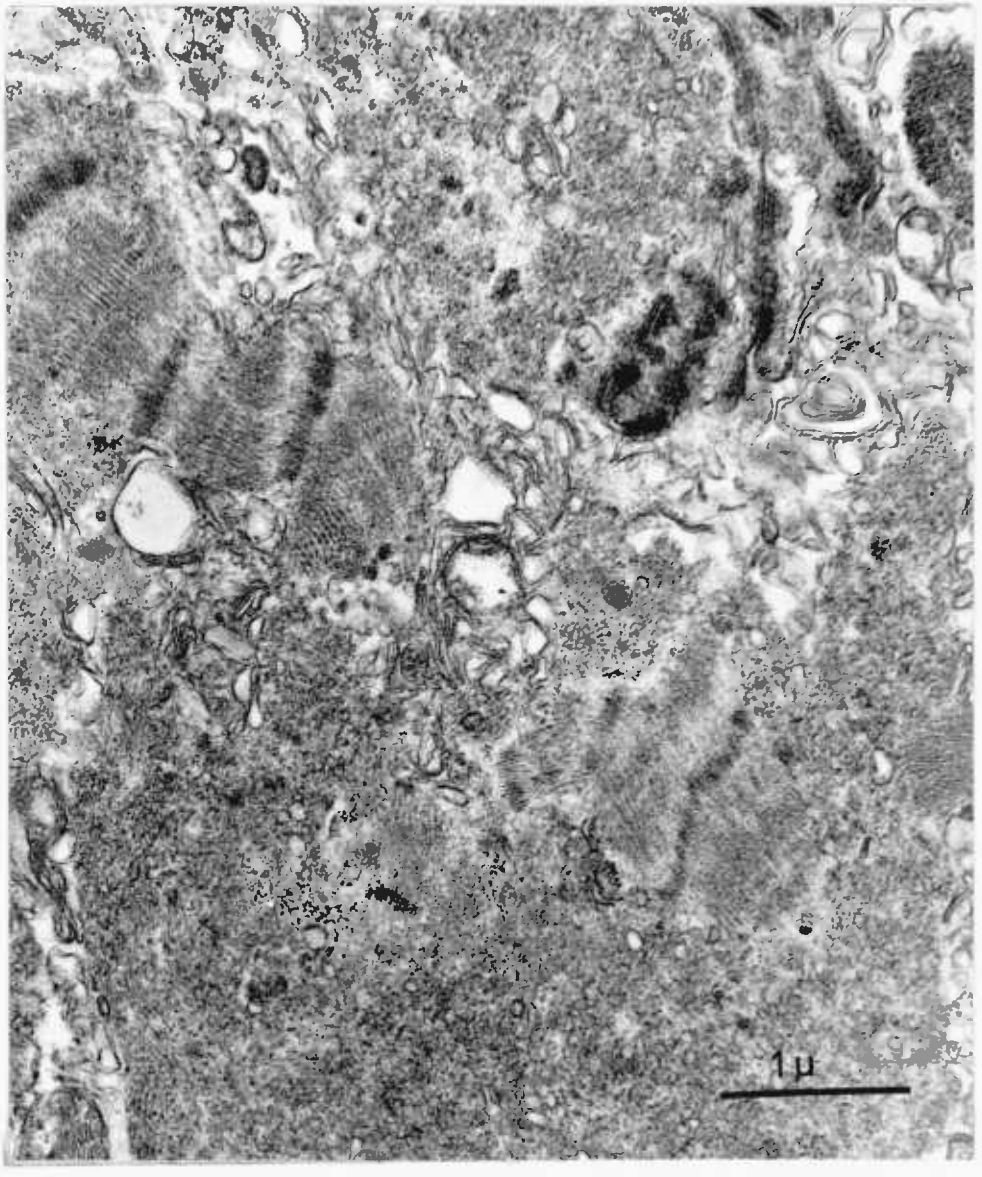


Plate 4

Electron micrograph of a ventricular homogenate prepared with the ILA homogenizer, and homogenization pattern C (page 35).
Magnification x 25,000

ENZYME ASSAYS

In the acclimatization studies the spectrophotometric assays were performed at 25° C using a Gilford 2400 recording spectrophotometer, and a total volume of 1 or 3 ml depending on the quantity of tissue available. In the investigation of indigenous animals carried out in Peru, a Zeiss PMQII spectrophotometer was used for the assays using $NAD(P)^+$ with readings taken every minute, and a Coleman Junior II spectrophotometer was used for the assays involving dichlorophenolindophenol, with readings taken every 2 minutes.

Details of the individual assays follow with the enzymes arranged in alphabetical order. The activities and concentrations of the test enzymes are given as relative values not absolute values in this section. In all subsequent sections the results are expressed as enzyme units* / mg. protein at 25° C under the conditions specified in the analysis of the methods. The activities of auxiliary enzymes in the methods are given as units* / ml. assay medium as calculated from information supplied by the manufacturer.

* 1 enzyme unit catalyses the conversion of 1 μmole substrate / min under specified conditions (Report of the Commission on Enzymes 1961). This unit is equivalent to 16.7 nkat (S.I. units) (Enzyme Nomenclature 1972).

ALDOLASE E.C. 4.1.2.13

D-Fructose 1,6 diphosphate $\rightleftharpoons$ Dihydroxyacetonephosphate + D-Glyceraldehyde-3-phosphate

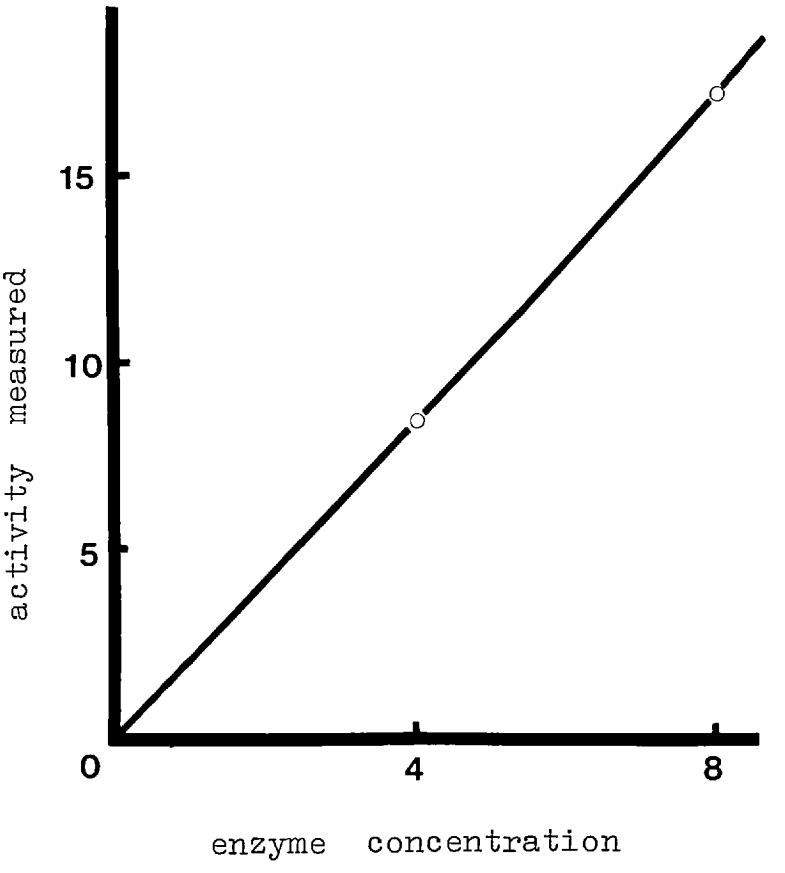
The assay was based on that of Bücher, Luh & Pette (1964), in which the interconversion of the products DHAP and GAP is catalysed by triosephosphate isomerase, and the rate of the test reaction indicated by the reduction of DHAP by NADH catalysed by glycerol-3-phosphate dehydrogenase. The concentrations of the auxiliary enzymes were the same as in the phosphofructokinase assay. The final assay gave changes of absorbance at 340 nm which were linear with time and rates of change which were proportional to the concentration of aldolase present over the range employed in the assays (Fig. 1).

Assay System

Triethanolamine 60 mM, EDTA 6.0 mM, NADH 0.19 mM, Fructose 1,6 diphosphate 3.2 mM, Triosephosphate isomerase 4 U/ml, Glycerol-3-phosphate dehydrogenase 1.3 U/ml, pH 7.5.

FIGURE 1 .

Relative activity measured v. relative concentration of aldolase .



ASPARTATE AMINOTRANSFERASE E.C. 2.6.1.1.

L-Aspartate + αketoglutarate ⇌ oxaloacetate + L-glutamate

The colorimetric method of Tonhazy et al (1950) as modified by Reitmann & Frankel (1957), and by Mohun & Cook (1957) was unsatisfactory for this work. The assay of Bücher, Luh & Pette (1964) was modified instead. In this assay the reduction of oxaloacetate to malate by NADH in the presence of malate dehydrogenase gives the measure of the test reaction.

There was no difference in the activity as measured using 0.1 M potassium phosphate buffer or 60 mM triethanolamine buffer, both with pH 7.5 (Table 3). The activity was not increased by utilising a higher aspartate concentration although lower concentrations reduced the activity measured (Table 4). Higher αketoglutarate concentrations did not increase the activity measured (Table 5). The assay was not improved by higher concentrations of malate dehydrogenase (Table 6) which also gave higher homogenate blank rates, as this enzyme also catalyses the reduction of αketoglutarate as well as of oxaloacetate. Using the method developed the change in absorbance 340 nm was linear with time and the rate of change was proportional to the amount of aspartate aminotransferase present (Fig. 2).

Assay System

Triethanolamine 60 mM, EDTA 6.0 mM, NADH 0.19 mM, aspartate 35 mM, αketoglutarate 7.0 mM, malate dehydrogenase 6 U/ml, pH 7.5.

TABLE 3

The effect of triethanolamine or phosphate buffer pH 7.5 on the measured activity of aspartate aminotransferase.

	Relative Activity				
Triethanolamine 60 mM	0.92	1.76	4.42	5.00	9.19
Phosphate 100 mM	1.09	1.91	3.75	4.96	9.06

TABLE 4

The effect of aspartate concentration on the measured activity of aspartate aminotransferase.

Aspartate Concentration	Relative Activity			
13.5 mM	1.72	2.97	5.72	18.52
35 mM	1.92	3.10	5.90	20.36
54 mM	1.98	3.21	5.94	20.88

TABLE 5

The effect of αketoglutarate concentration on the measured activity of aspartate aminotransferase.

αketoglutarate Concentration	Relative Activity			
7 mM	1.92	4.20	8.24	21.36
14 mM	1.94	4.09	8.72	21.36

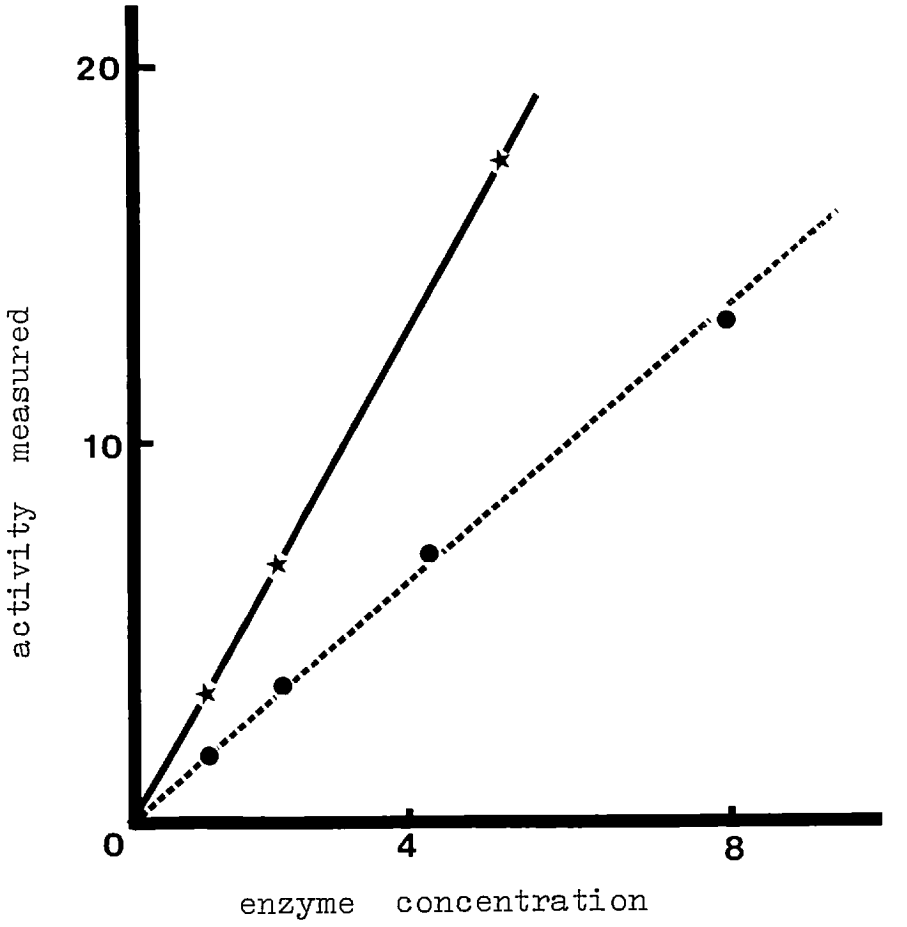
TABLE 6

The effect of malate dehydrogenase concentration on measured activity of aspartate aminotransferase

MDH Concentration				
6 U/ml	3.59	7.41	7.73	18.00
16 U/ml	3.27	6.91	7.21	17.74

FIGURE 2 .

Relative activity measured v. relative concentration of aspartate aminotransferase .



ENOLASE E.C. 4.2.1.11

2-phospho-D-glycerate $\rightleftharpoons$ phosphoenolpyruvate + H_2O

The assay developed was based on that of Bücher, Luh & Pette (1964) in which the test reaction is coupled to the indicator reaction of lactate dehydrogenase by pyruvate kinase. The activity measured was comparable using a wide range of concentrations of 2-phosphoglycerate (Table 7). The concentrations of auxiliary enzymes used were sufficient to give linear changes of absorbance at 340 nm with time and rates of change which were proportional to the enolase concentration for the range utilised in the experiments (Figure 3).

Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, $MgSO_4$ 8.0 mM, KCl 75 mM, NADH 0.19 mM, ADP 0.84 mM, 2-phosphoglycerate 1.0 mM, pyruvate kinase 1.0 U/ml, lactate dehydrogenase 6 U/ml, pH 7.5.

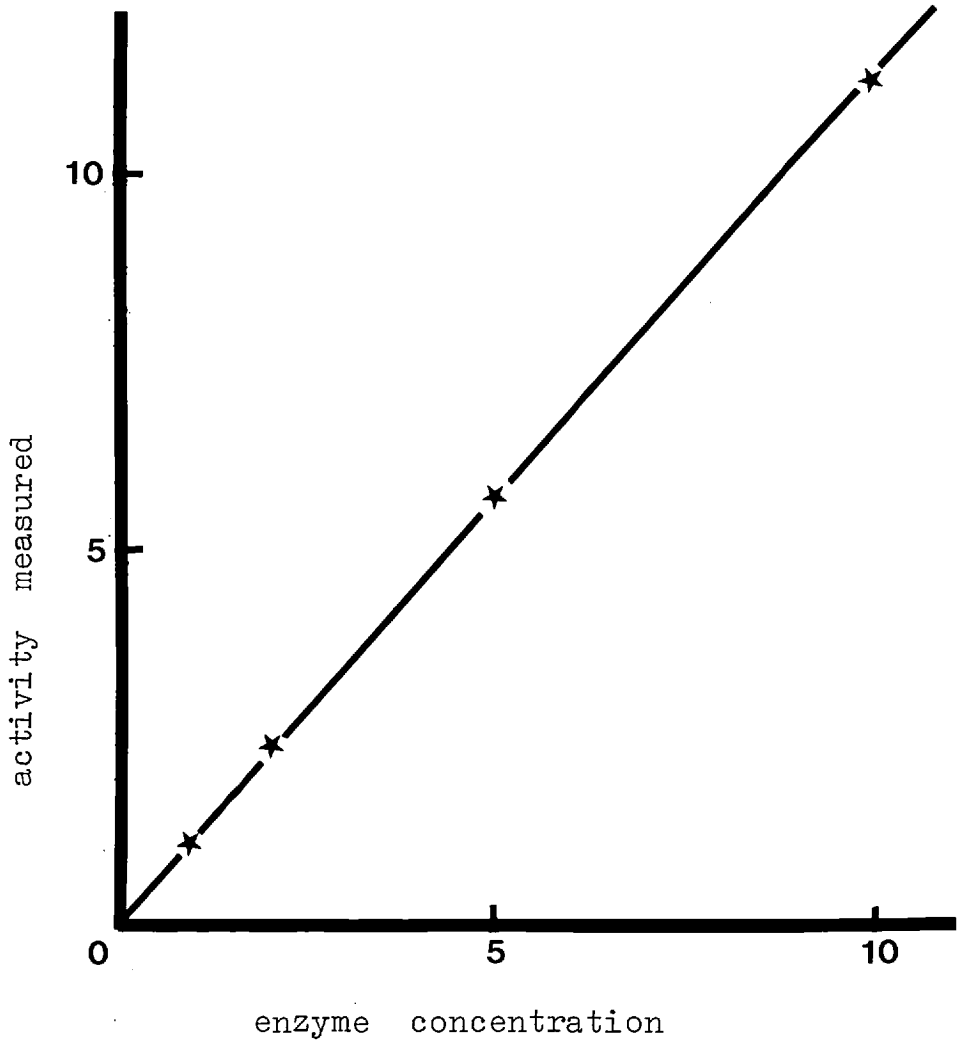
TABLE 7

The effect of the concentration of 2-phosphoglycerate on the measured activity of enolase

2PGA Concentration	Relative Activity			
0.75 mM	1.11	2.34		
1.5 mM	1.10	2.42	5.76	11.20
3.0 mM	1.16	2.31	5.76	11.06

FIGURE 3

Relative activity measured v. relative concentration of enolase



GLUCOSE 6 PHOSPHATE DEHYDROGENASE E.C. 1.1.1.49

D-Glucose-6-phosphate + $NADP^+$ $\rightleftharpoons$ D-glucono-δ-lactone-6-phosphate + NADPH

The assay was based on that of Bücher, Luh & Pette (1964). Sodium fluoride was not found to be advantageous and so was not included. The assay gave activities proportional to the concentration of the enzyme present (Figure 4). In tissues with an active pentose phosphate cycle the assay used might overestimate the activity of glucose-6-phosphate dehydrogenase, as the product 6-phosphogluconate might be further oxidised by its specific dehydrogenase which would also be present in the homogenate. Addition of exogenous 6-phosphogluconate dehydrogenase ensures the oxidation and so removes this possibility of error. In the adult myocardium this was not found to necessary, but in the study of newborn tissue this precaution was taken.

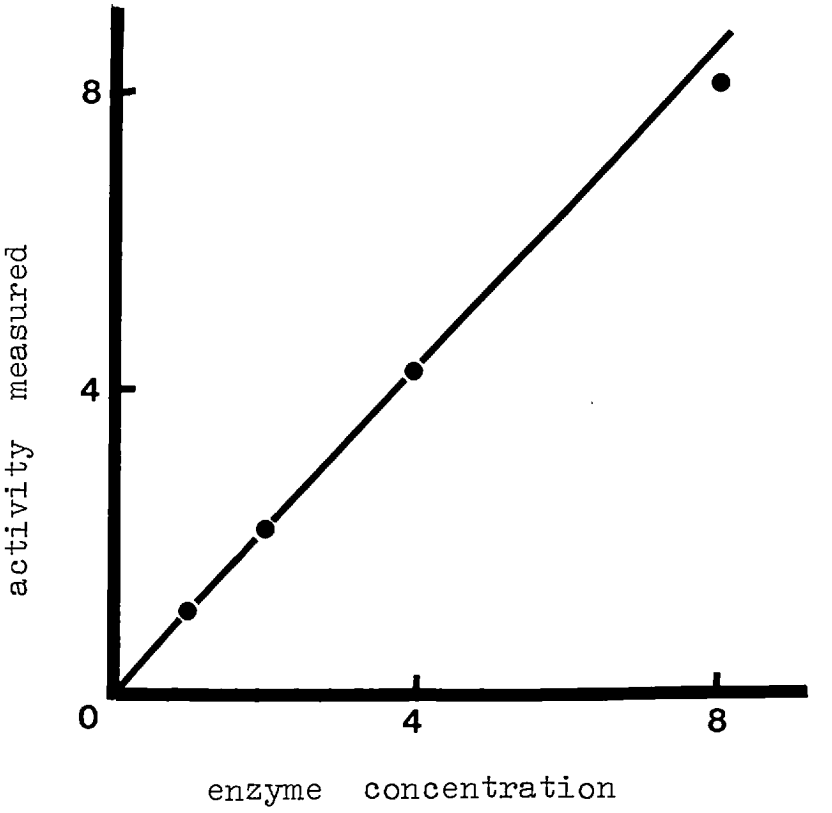
Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, $MgSO_4$ 8.0 mM, $NADP^+$ 0.52 mM, glucose-6-phosphate 3.0 mM, pH 7.5.

For newborn tissue the assay was as above with 6 phosphogluconate dehydrogenase 0.24 U/ml.

FIGURE 4 .

Relative activity measured v. relative concentration of glucose 6 phosphate dehydrogenase .



GLUCOSEPHOSPHATE ISOMERASE E.C. 5.3.1.9.

D-glucose-6-phosphate $\rightleftharpoons$ D-fructose-6-phosphate

The assay used was based on that of Bücher, Luh & Pette (1964) in which the rate of the conversion of fructose-6-phosphate to glucose-6-phosphate is indicated by the rate of oxidation of the product by $NADP^+$ as catalysed by glucose-6-phosphate dehydrogenase. The activity was not increased by higher concentrations of fructose-6-phosphate (Table 8), or by higher concentrations of glucose-6-phosphate dehydrogenase (Table 9) although lower concentrations reduced the measured activity. Magnesium sulphate was included to stimulate the auxiliary enzyme and to improve the linearity of the change in absorbance at 340 nm with time, although it appears inhibitory towards the test enzyme (Table 10). The resulting assay gave rates of reduction of $NADP^+$ which were proportional to the concentration of glucosephosphate isomerase present (Figure 5).

Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, $MgSO_4$ 4.0 mM, $NADP^+$ 0.52 mM, fructose-6-phosphate 1.3 mM, glucose-6-phosphate dehydrogenase 2.3 U/ml, pH 7.5.

TABLE 8

The effect of fructose-6-phosphate concentration on the measured activity of glucose phosphate isomerase.

F6P Concentration	Relative Activity			
0.63 mM	3.20	4.19	7.48	9.80
1.3 mM	3.47	4.56	8.30	10.60
2.5 mM	3.37	4.64	8.58	10.59

TABLE 9

The effect of the glucose-6-phosphate dehydrogenase concentration on the measured activity of glucose phosphate isomerase.

G6PDH Concentration	Relative Activity			
2.3 U/ml	2.08	4.33		19.89
5.8 U/ml	2.10	4.32	10.14	19.63
11.7 U/ml	2.04	4.23	9.98	19.64

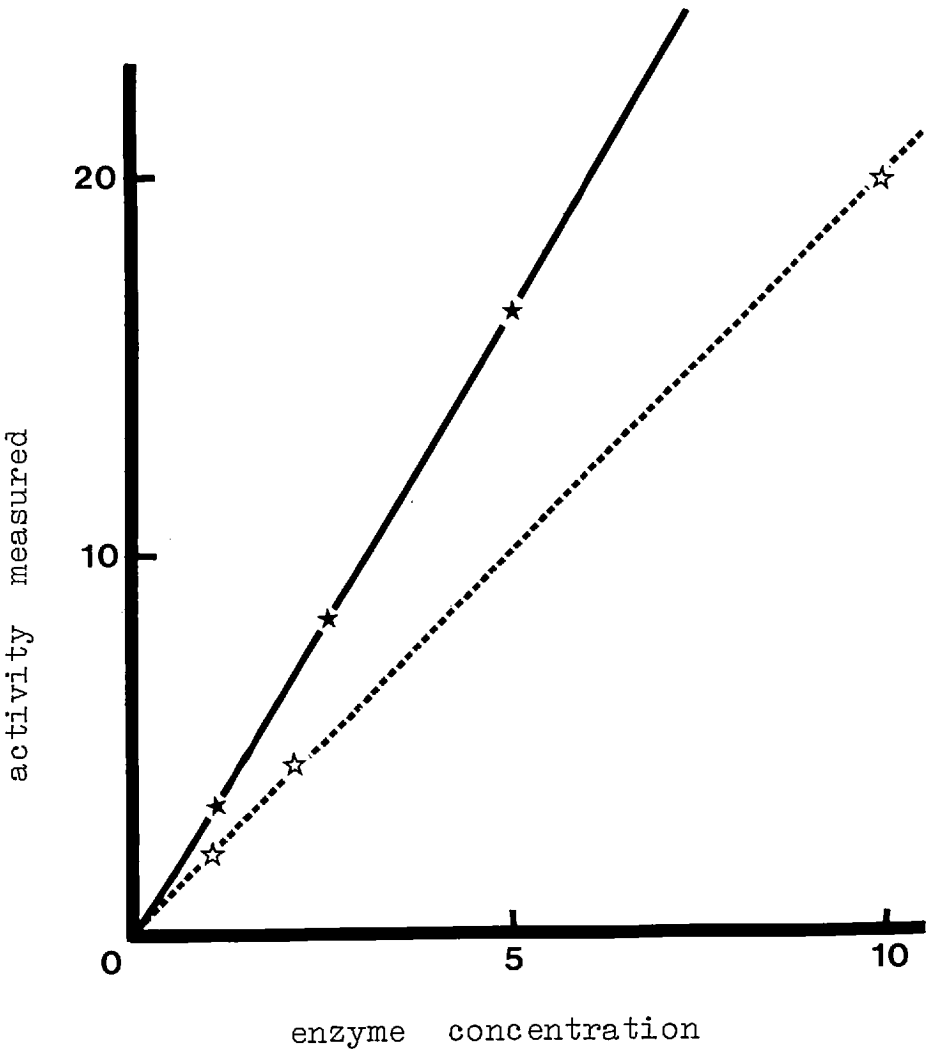
TABLE 10

The effect of the magnesium concentration on the measured activity of glucose phosphate isomerase.

$MgSO_4$ Concentration	Relative Activity		
0 mM	4.18	10.40	21.12
4.0 mM	3.42	8.14	16.20
8.0 mM	2.50	5.92	12.64

FIGURE 5 .

Relative activity measured v. relative concentration of glucose phosphate isomerase .



GLYCERALDEHYDE 3 PHOSPHATE DEHYDROGENASE E.C. 1.2.1.12

D-glyceraldehyde-3-phosphate + orthophosphate + NAD^+ $\rightleftharpoons$ 3-phospho-D-glyceroyl phosphate + NADH

The assay used was based on that of Bücher, Luh & Pette (1964) in which the substrate for reduction, diphosphoglycerate, is generated by the action of phosphoglycerate kinase on 3-phosphoglycerate and ATP. Thus the reverse reaction to that operating in glycolysis is measured.

The enzyme GAPDH is rapidly inactivated especially in dilute solutions. This can be prevented for a while by the presence of sulphydryl agents. With adult myocardial tissue 2.5 mM glutathione was insufficient to achieve this while 10 or 20 mM were found to give rise to maximal activity (Table 11). Other sulphydryl agents, for instance cysteine or dithiothreitol, were also effective at the concentration of 20 mM (Table 12). Comparison of the activities in the presence of 2.5 mM glutathione or 20 mM dithiothreitol indicates that the loss of activity was greatest with the least concentrated solutions. With the tissue from newborn guinea pigs, however, 2.5 mM glutathione was sufficient to give full activity and was used as initial work had suggested that higher concentrations were slightly inhibitory (Table 13).

The concentration of PGAK used was adequate for the range of activities measured (Table 14). The assay was not improved by higher concentrations of

3-phosphoglycerate (Table 15). The assay gave changes of absorbance at 340 nm which were linear with time and rates of change which were proportional to the enzyme concentration (Figure 6). By initiating the reaction by the addition of homogenate rather than of substrate, a higher activity was obtained, and so this was done. The assay was always complete within 1 hour of the homogenization of the tissue, which was before the time at which inactivation could be detected.

Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, $MgSO_4$ 4.0 mM, cysteine 20 mM (with newborn tissue glutathione 2.5 mM was used instead)
NADH 0.19 mM, ATP 1.5 mM, 3-phosphoglycerate 5.9 mM, phosphoglycerate kinase 2.7 U/ml, pH 7.5.

TABLE 11

The effect of sulphydryl agents on the measured activity of glyceraldehyde-3-phosphate dehydrogenase in adult tissue.

GSH Concentration	Relative Activity					
2.5 mM	4.75	9.68	1.64	3.70	10.04	1.56
5.0 mM	4.16	11.00	1.89	4.42	10.24	1.85
10.0 mM	5.46	12.64	2.19	4.95	10.96	2.22
10.0 mM	5.53	2.62				
20.0 mM	5.65	2.63				

TABLE 12

The effect of other sulphydryl agents on the measured activity of glyceraldehyde-3-phosphate dehydrogenase in adult tissue.

	Relative Activity					
2.5 mM GSH	4.36	4.70	6.14	6.20	14.3	
20.0 mM dithiothreitol	8.92	7.20	8.90	8.24	16.7	
% inactivation	104	53	45	33	17	
20 mM dithiothreitol	1.82	3.56	3.80	6.92	7.60	10.06
20 mM cysteine	2.33	3.75	3.85	6.51	7.52	9.43
20 mM dithiothreitol	16.76	17.98				
20 mM cysteine	17.24	17.52				

TABLE 13

The effect of sulphydryl agents on the measured activity of glyceraldehyde-3-phosphate dehydrogenase in tissue from newborn animals.

GSH Concentration	Relative Activity			
2.5 mM	8.37	7.92	6.71	5.34
10.0 mM	8.20	7.56	6.82	5.60
2.5 mM	9.68	8.02	10.44	10.22
20.0 mM	9.13	7.59	10.41	10.41

TABLE 14

The effect of the concentration on phosphoglycerate kinase on the measured activity of glyceraldehyde-3-phosphate dehydrogenase.

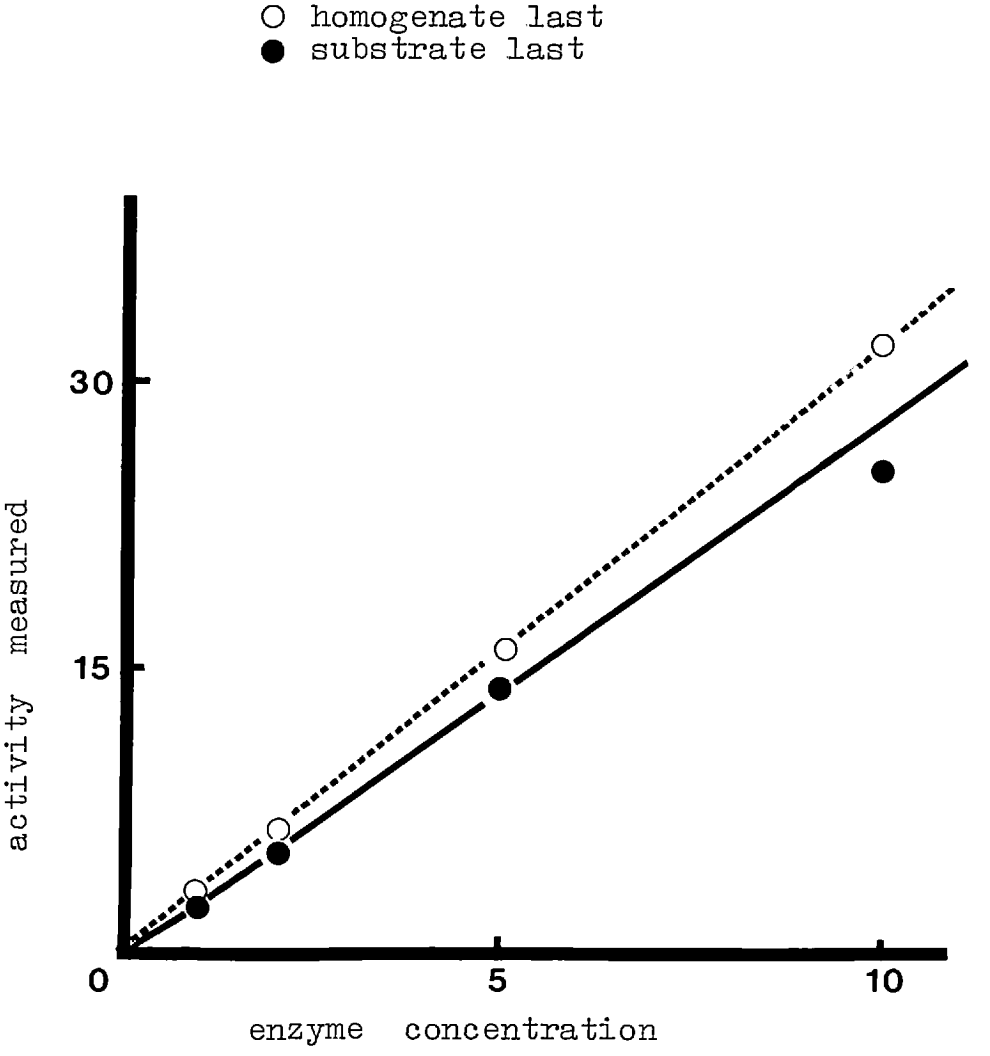
PGAK Concentration	Relative Activity			
2.7 U/ml	5.76	14.00	25.56	2.71
5.4 U/ml	5.50	13.54	26.64	2.59
6.7 U/ml	5.64	13.82	26.00	2.63

TABLE 15

The effect of the concentration of 3-phosphoglycerate on the measured activity of glyceraldehyde-3-phosphate dehydrogenase.

3PGA Concentration	Relative Activity			
2.95 mM	2.93	6.14	15.72	30.64
5.90 mM	3.16	6.52	16.08	32.40
11.8 mM	3.24	6.73	16.50	33.32

FIGURE 6.
Relative activity measured v. relative concentration of glyceraldehyde-3-phosphate dehydrogenase .



GLYCEROL 3 PHOSPHATE DEHYDROGENASE (MITOCHONDRIAL)
E.C. 1.1.99.5

glycerol-3-phosphate + flavoprotein ⟶ dihydroxyacetone phosphate + reduced flavoprotein

The assay of Crabtree & Newsholme (1970) was tried but unacceptably high blank values made the method unsuitable. The method of Bass et al. (1969) using potassium ferricyanide was tried but with the low extinction coefficient of the ferricyanide the method was not sensitive enough to measure the low activity present in the homogenates used. Instead of potassium ferricyanide as the electron acceptor 2,6 dichlorophenol indiphenol was used (Ling et al. 1957). Two millimolar potassium cyanide was used as it gave slightly higher activities (Table 16). The concentration of glycerol-3-phosphate affected the activity measured (Table 17). The change of absorbance at 600 nm was not linear with time owing to the nonlinear change in the homogenate blank. Automatic correction for this by the spectrophotometer allowed the net change due to the homogenate to be measured directly: this was linear with time, and the rate was proportional to the concentration of enzyme present (Figure 7).

Assay system

Potassium phosphate 92 mM, 5.5 mM EDTA, 2.0 mM KCN, 20 µg/ml 2,6 dichlorophenol indophenol, 12 mM glycerol-3-phosphate, pH 7.5.

TABLE 16

The effect of the concentration of potassium cyanide on the measured activity of mitochondrial glycerol-3-phosphate dehydrogenase.

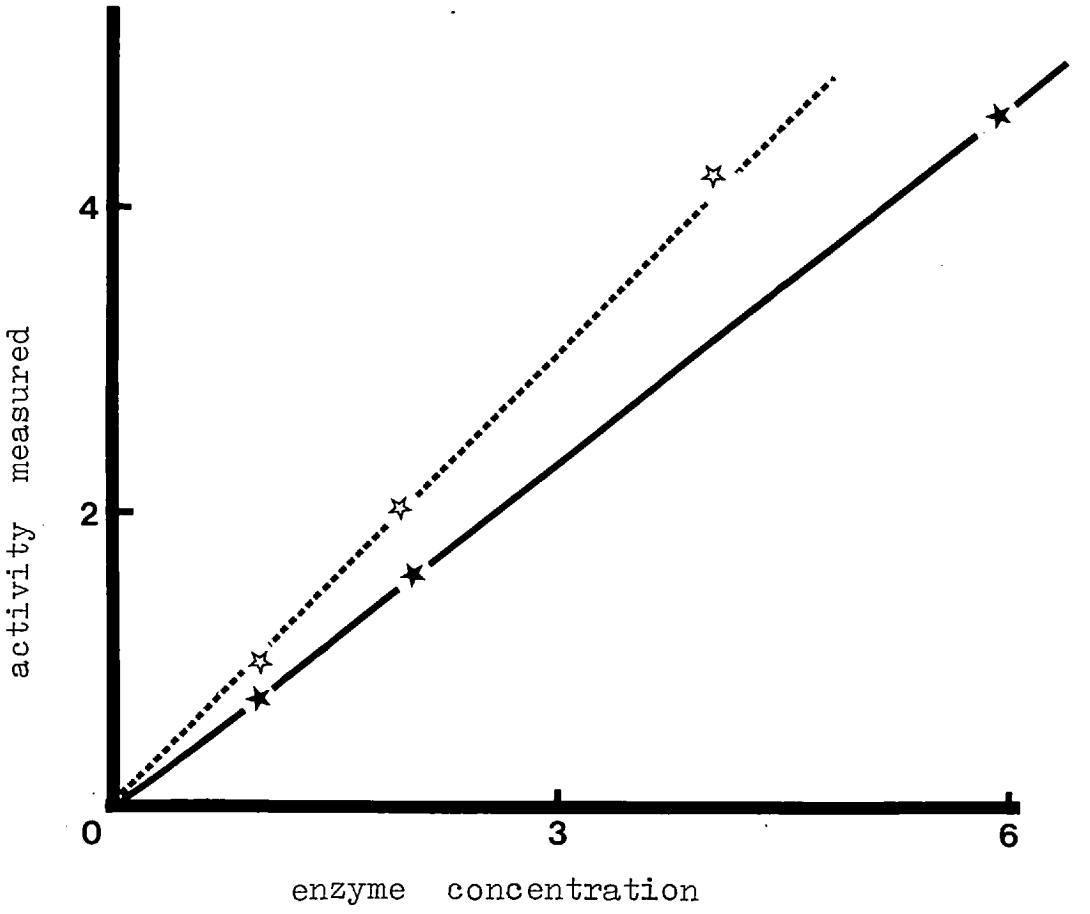
KCN Concentration	Relative Activity						
1.0 mM	0.78	0.85	1.10	1.57	1.85	2.33	3.13
2.0 mM	0.70	0.86	1.31	1.80	2.19	2.72	3.86

TABLE 17

The effect of the glycerol-3-phosphate concentration on the measured activity of mitochondrial glycerol-3-phosphate dehydrogenase.

Glycerol 3 phosphate Concentration	Relative Activity				
6.25 mM	0.63	0.67	1.87	1.20	2.96
12.5 mM	1.01	0.95	2.22	1.85	4.20
25.0 mM	1.08	1.32	2.75	2.40	4.80

FIGURE 7.
Relative activity measured v. relative concentration of mitochondrial glycerol-3-phosphate dehydrogenase .



GLYCOGEN PHOSPHORYLASE E.C. 2.4.1.1.

$$(1,4\text{-}\alpha\text{-D-glucosyl})_n + \text{orthophosphate} \rightleftharpoons (1,4\text{-}\alpha\text{-D-glucosyl})_{n-1} + \alpha\text{-D-glucose-1-phosphate}$$

The enzyme exists in 2 forms, phosphorylase a and b, which are interconvertible by dephosphorylation and phosphorylation involving a specific phosphatase and kinase. Phosphorylase b is inactive in the absence of AMP, while the activity of phosphorylase a is independent of AMP (Morgan & Parmeggiani 1964).

The assay was based on that of Bass et al. (1969) in which the test reaction is linked by phosphoglucomutase to the indicator reaction, which is the oxidation of glucose-6-phosphate to 6-phosphogluconate catalysed by glucose-6-phosphate dehydrogenase. The concentration of AMP used gave maximal activity (Table 18). Cysteine was not found to be necessary and so was excluded (Table 19). Other assay systems utilise different buffers and pHs from Bass, and some were tried (Table 20). Potassium phosphate 50 mM containing 6.0 mM EDTA pH 6.8 was found to be preferable and was subsequently used. Higher concentrations of auxiliary enzymes did not seem advantageous (Table 21): the amounts used were sufficient to give changes of absorbance at 340 nm that were linear with time, and rates which were

proportional to the concentration of phosphorylase present (Figure 8).

Assay system

Potassium phosphate 50 mM, EDTA 6.0 mM, $MgSO_4$ 8.0 mM, $NADP^+$ 1.0 mM, AMP 2.3 mM, glucose-1,6-diphosphate 17μM, glycogen 1 mg/ml, phosphoglucomutase 0.67 U/ml, glucose-6-phosphate dehydrogenase 1.2 U/ml, pH 6.8.

TABLE 18

The effect of the concentration of AMP on the measured activity of glycogen phosphorylase.

AMP Concentration	Relative Activity				
1.0 mM	1.96	2.07	3.24	4.00	4.26
2.0 mM	2.14	1.91	3.13	4.36	3.67

TABLE 19

The effect of 10 mM cysteine on the measured activity of glycogen phosphorylase.

	Relative Activity				
- cysteine	1.19	2.07	3.67	4.24	6.06
+ cysteine	2.14	1.96	4.36	4.00	5.05

TABLE 20

The effect of the buffer on the measured activity of glycogen phosphorylase.

		Relative Activity			
Triethanolamine + Phosphate	pH 7.5	2.07	4.24		
Phosphate	pH 6.4	3.05	5.62	5.00	9.38
Phosphate	pH 6.8			6.40	10.20

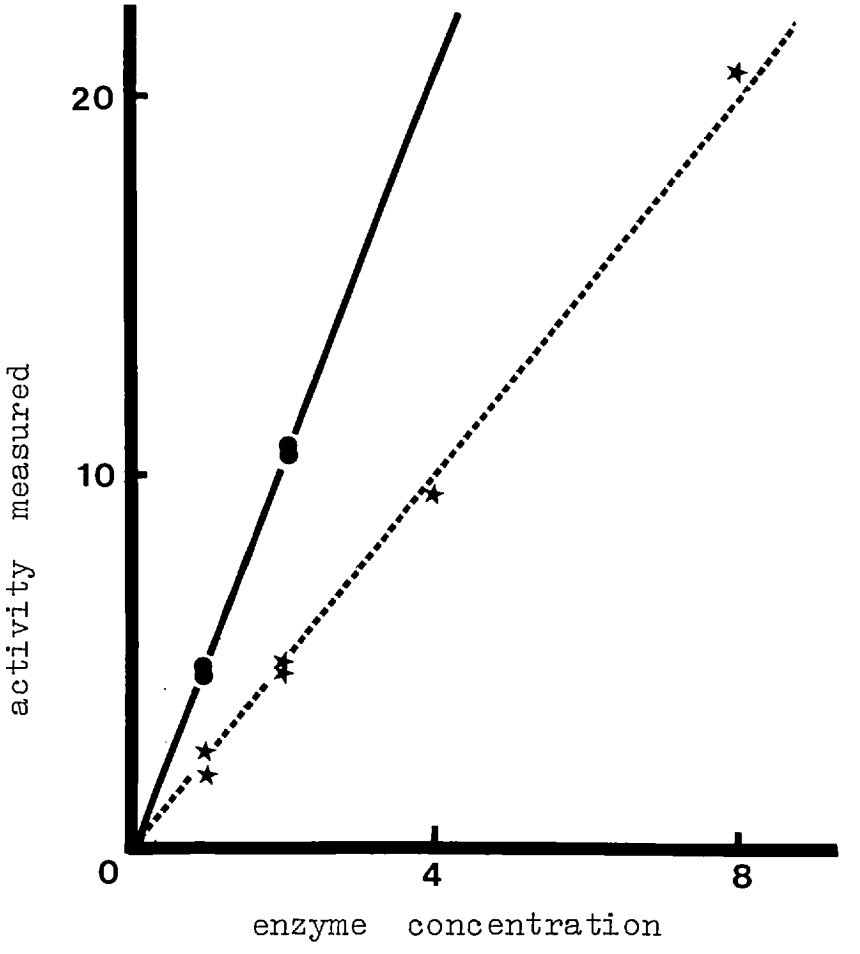
TABLE 21

The effect of different auxiliary enzyme concentrations on the measured activity of glycogen phosphorylase.

Concentrations		Relative Activity			
PGM	G6PDH				
0.67 U/ml	1.2 U/ml	6.00	10.08	11.06	12.16
0.67 U/ml	5.8 U/ml	5.38	10.40	11.44	11.96
1.0 U/ml	1.75 U/ml	6.22	7.97		
2.0 U/ml	3.5 U/ml	5.14	8.74		

FIGURE 8 .

Relative activity measured v. relative concentration of glycogen phosphorylase



HEXOKINASE E.C. 2.7.1.1.

D-hexose + ATP ⟶ D-hexose-6-phosphate + ADP

The assay used was based on that of Bücher, Luh & Pette (1964) in which the rate of the reaction is indicated by that of the oxidation of the product to 6-phosphogluconate by glucose-6-phosphate dehydrogenase. The presence of fluoride was not considered advantageous as it did not decrease the change in absorbance of the blanks or give increased activity (Table 22), and so it was not included. The activity measured was not increased by higher or lower ATP concentrations (Table 23). Higher or lower glucose concentrations were not advantageous (Table 24). It is of great importance for the activity of glucose-6-phosphate dehydrogenase to be high enough to prevent any accumulation of glucose-6-phosphate as this compound inhibits hexokinase. The activity measured was not increased by utilising higher concentrations of the auxiliary enzyme (Table 25). The resulting assay gave rates of change of absorbance at 340 nm which were proportional to the concentration of hexokinase present (Figure 9).

Assay system:

Triethanolamine 60 mM, EDTA 6.0 mM, $MgSO_4$ 8.0 mM, $NADP^+$ 0.52 mM, ATP 3.0 mM, glucose 0.49 mM, glucose-6-phosphate dehydrogenase 1.2 U/ml, pH 7.5.

TABLE 22

The effect of 3 mM fluoride on the measured activity of hexokinase.

	Relative Activity					
- fluoride	0.97	1.24	1.80	2.79	4.99	7.84
+ fluoride	0.48	1.08	2.35	2.47	4.60	8.68

TABLE 23

The effect of ATP concentration on the measured activity of hexokinase.

ATP Concentration	Relative Activity			
1.5 mM	1.41	2.81	4.98	11.92
3.0 mM	1.58	2.95	5.30	13.40
6.0 mM	1.32	2.75	5.22	10.20

TABLE 24

The effect of the glucose concentration on the measured activity of hexokinase.

Glucose Concentration	Relative Activity			
0.25 mM	1.18	2.53	4.22	11.04
0.49 mM	1.32	2.49	4.44	11.36
0.98 mM	1.33	2.72	4.72	10.88

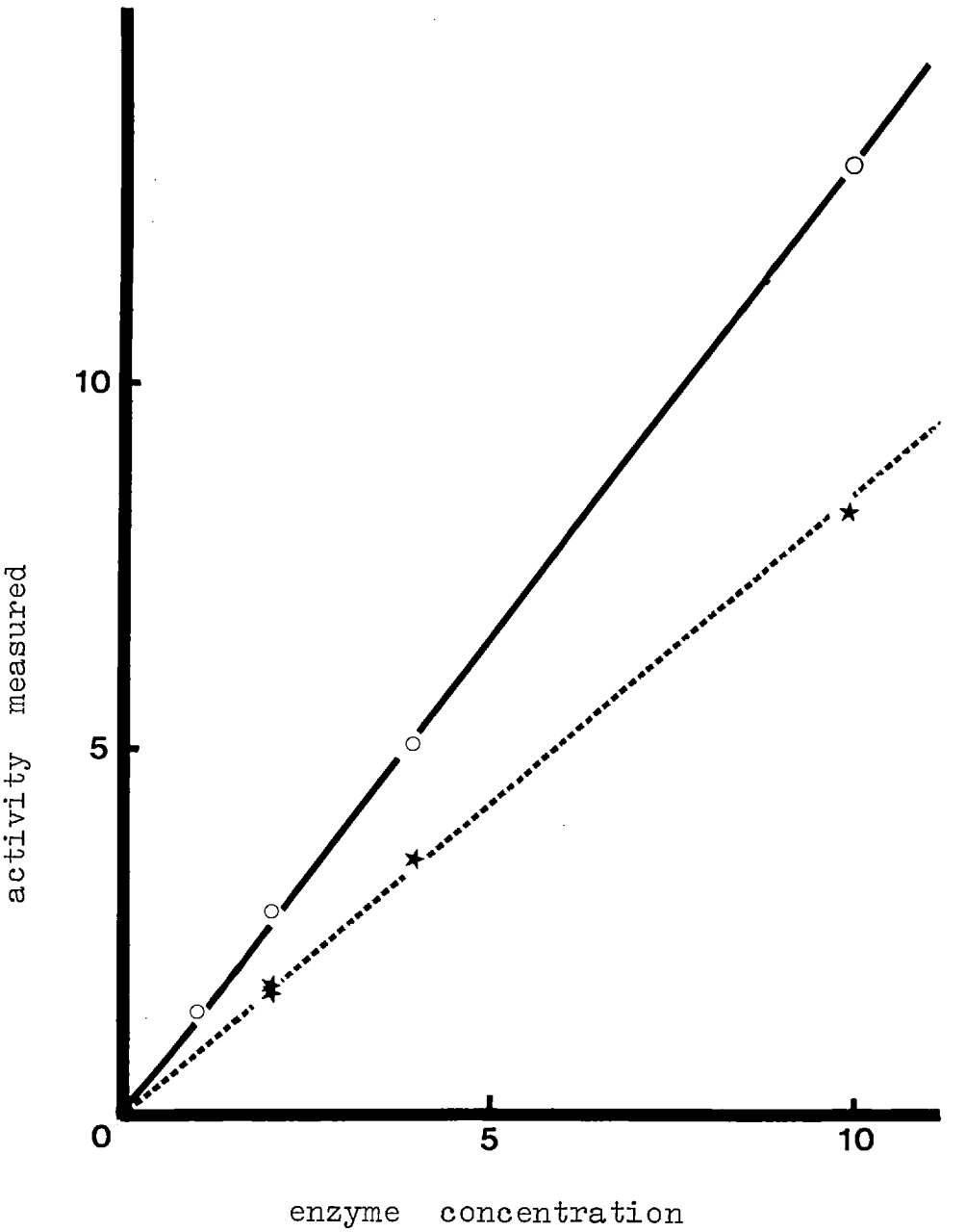
TABLE 25

The effect of the concentration of glucose-6-phosphate dehydrogenase on the measured activity of hexokinase.

G6PDH Concentration	Relative Activity			
1.2 U/ml	1.53	2.95	5.22	13.92
2.3 U/ml	1.53	2.96	5.42	14.72
5.8 U/ml	1.52	2.91	4.62	13.52

FIGURE 9 .

Relative activity measured v. relative concentration of hexokinase .



3-HYDROXYACYL-CoA DEHYDROGENASE E.C.1.1.1.35.

L-3-hydroxyacyl-CoA + NAD $\rightleftharpoons$ 3-oxoacyl-CoA + NADH

The assay was based on that of Lynen & Wieland (1952) which utilizes an analogue of the natural substrate. Phosphate and triethanolamine buffers were tried and were found to give low activities With sodium pyrophosphate buffer, the activity was higher at pH 6.8 than at pH 7.5 (Table 26), as anticipated, since the pH optimum for the reaction is given as between 6 and 7 (Mahler 1971). As higher activity was obtained by using a higher concentration of the substrate than in the original assay this was done (Table 27). The assay developed gave changes in absorbance at 340 nm that were linear with time, and rates of change that were proportional to the concentration of enzyme present (Figure 10).

Assay system

Sodium pyrophosphate 25 mM, NADH 0.19 mM, EDTA 6.0 mM, N-acetyl-S-acetoacetyl-cysteamine 5 mM, pH 6.8.

TABLE 26

Effect of the pH on the measured activity of βhydroxyacyl coenzyme A dehydrogenase.

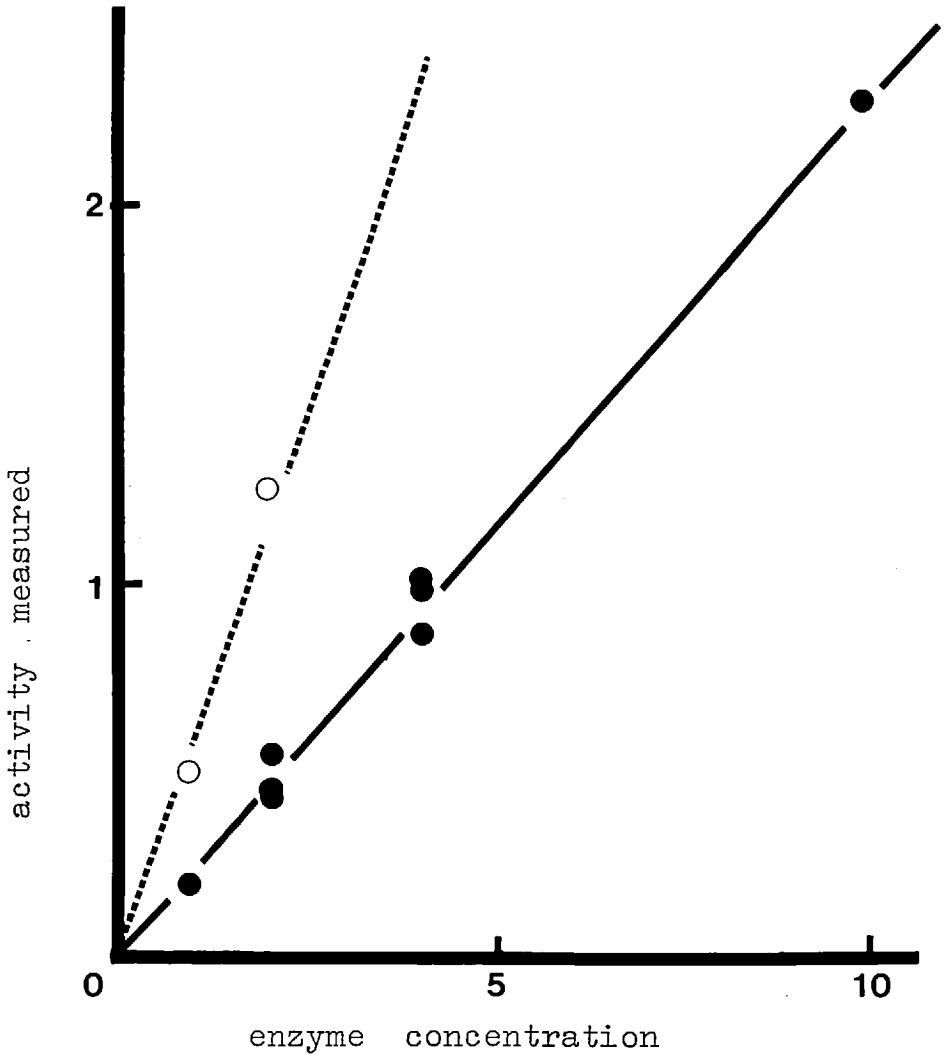
Pyrophosphate buffer pH	Relative Activity		
7.5	2.68	4.80	11.70
6.8	2.82	7.38	13.86

TABLE 27

Effect of the concentration of N-acetyl-S-acetoacetyl-cysteamine on the measured activity of βhydroxyacylcoenzyme A dehydrogenase.

	Relative Activity					
2.5 mM	1.51	2.56	1.12	2.60	4.23	7.59
5.0 mM	2.82	7.38	2.11	4.65	6.53	13.86

FIGURE 10.
Relative activity measured v. relative concentration of βhydroxyacylcoenzyme A dehydrogenase .



LACTATE DEHYDROGENASE E.C. 1.1.1.27.

$$\begin{array}{l} \text{L-lactate} \\ + \\ NAD^+ \end{array} \rightleftharpoons \begin{array}{l} \text{pyruvate} \\ + \\ NADH \end{array}$$

This enzyme is a tetramer composed of 2 types of subunit - M or A type, and H or B type - random combination of which gives rise to 5 isoenzymes. The subunits function independently (Whitaker et al. 1974) and display different catalytic properties which can be used to distinguish them. The proportion of one subunit type within a tissue varies, with M predominating in skeletal muscle and H predominating in the adult heart (Markert & Ursprung 1962). The assay for total lactate dehydrogenase activity was based on that of Bücher, Luh & Pette (1964). With the H_4 or LDH-1 isoenzyme higher pyruvate or NADH resulted in lower rates of reduction of pyruvate, while lower concentrations reduced the linearity of the assay (Figures 11, 12). It was very important to keep the sample volume the same in all assays as the homogenization medium affected the activity. The change of absorbance at 340 nm with time was linear with the M_4 isoenzyme or skeletal muscle homogenate, but was curved with the H_4 isoenzyme or myocardial homogenate. However, the initial rates were proportional to the concentration of the enzyme present, when this was low (Figure 13).

Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, NADH 0.19 mM, pyruvate 2.4 mM pH 7.5.

∝HYDROXYBUTYRATE DEHYDROGENASE

∝ketobutyrate + NADH $\rightleftharpoons$ ∝hydroxybutyrate + NAD^+

∝ketobutyrate is utilised more readily by the H subunits than by the M subunits and so the combination of the lactate dehydrogenase and ∝hydroxybutyrate dehydrogenase assays provides a measure of the proportion of the different subunits in a tissue. Using pig LDH-1 and LDH-5 supplied by the Boehringer Corporation (London) Ltd. the ratio of the activities ∝HBDH / LDH was 1.05 for LDH-1 and 0.13 for LDH-5 The activity of ∝hydroxybutyrate dehydrogenase measured was proportional to the concentration of enzyme present (Figure 14).

Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, NADH 0.19 mM, ∝ketobutyrate 1.5 mM pH 7.5.

FIGURE 11 .

Effect of the NADH concentration on the measured activity of LDH-1 .

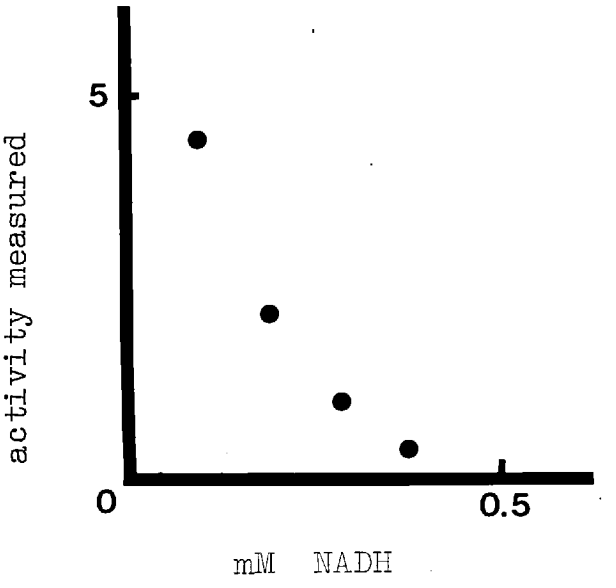


FIGURE 12 .

Effect of the pyruvate concentration on the measured activity of LDH-1 .

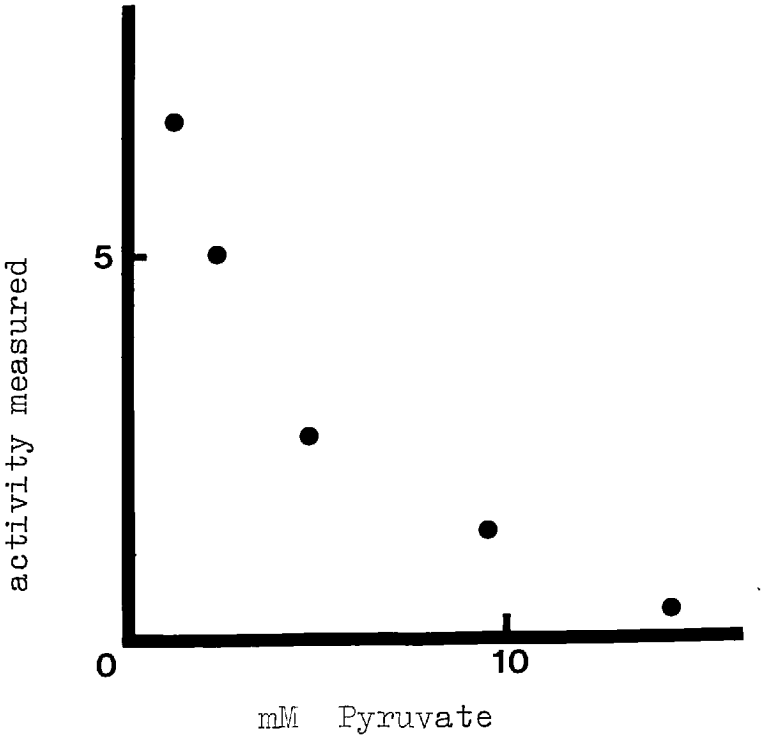


FIGURE 13 .

Relative activity measured v. relative enzyme concentration for lactate dehydrogenase .

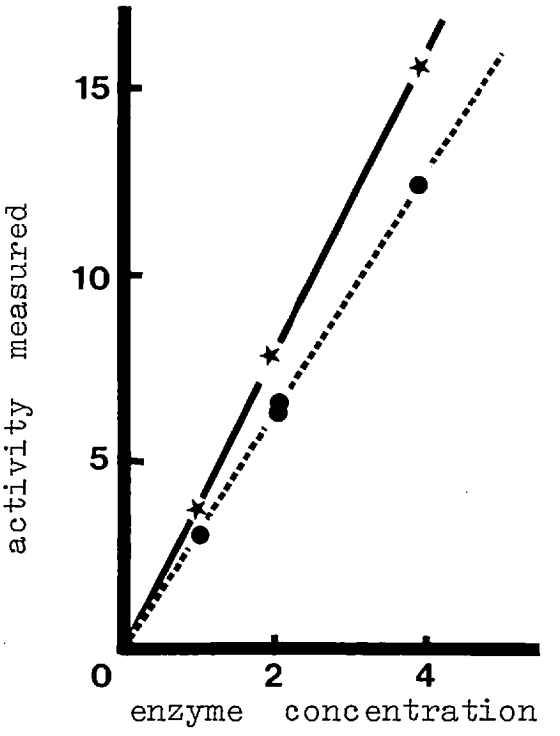
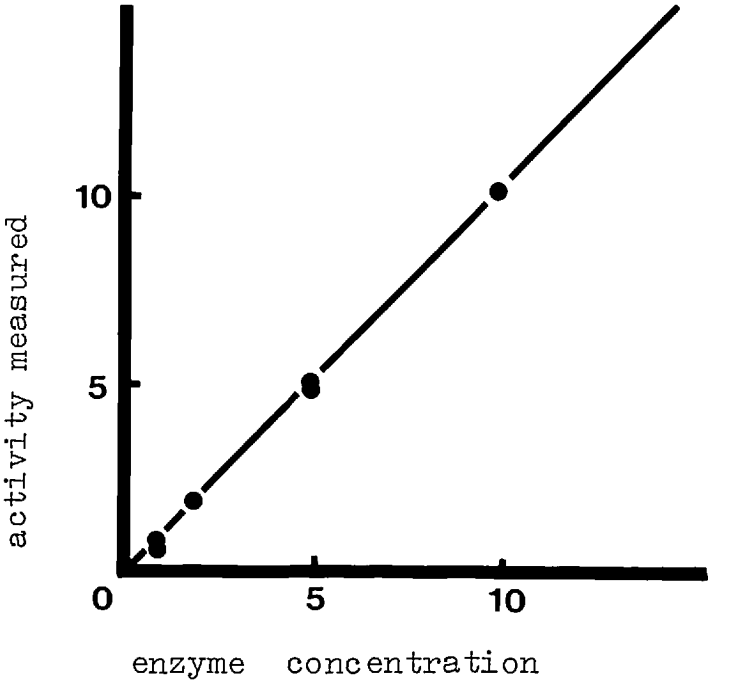


FIGURE 14 .

Relative activity measured v. relative enzyme concentration for
αhydroxybutyrate dehydrogenase



MALATE DEHYDROGENASE E.C. 1.1.1.37.

L-malate + NAD^+ $\rightleftharpoons$ oxaloacetate + NADH

Initially the enzyme was assayed by following the reduction of oxaloacetate. However the rapid contamination of the oxaloacetate by pyruvate as a result of decarboxylation proved to be a problem owing to the high activity of lactate dehydrogenase in the homogenate. As a result, the enzyme was assayed by following the oxidation of malate, based on the method of Wolfe & Neilands (1956). Despite the lower activity at the high pH required (Wolfe & Neilands 1956) the assay was preferable as the substrates were more stable. The concentration of malate was increased to 6.6 mM as this gave higher activities while further increases in concentration had no effect (Table 28). The change in abosrbance at 340 nm with time was not linear, but the initial rates were proportional to the concentration of the enzyme present (Figure 15) for the range of activities measured in the experiments.

Assay system

Glycine 0.1 M, NAD^+ 1.3 mM, malate 6.6 mM, pH 10.0.

Malate dehydrogenase occurs within the cytoplasm and mitochondria, and Shonk & Boxer (1966) distinguished between these compartments by inactivating the

cytoplasmic enzyme with ethanol. This was found to give variable results even when the duration of incubation with ethanol was strictly controlled. As a result only total malate dehydrogenase activity could be measured.

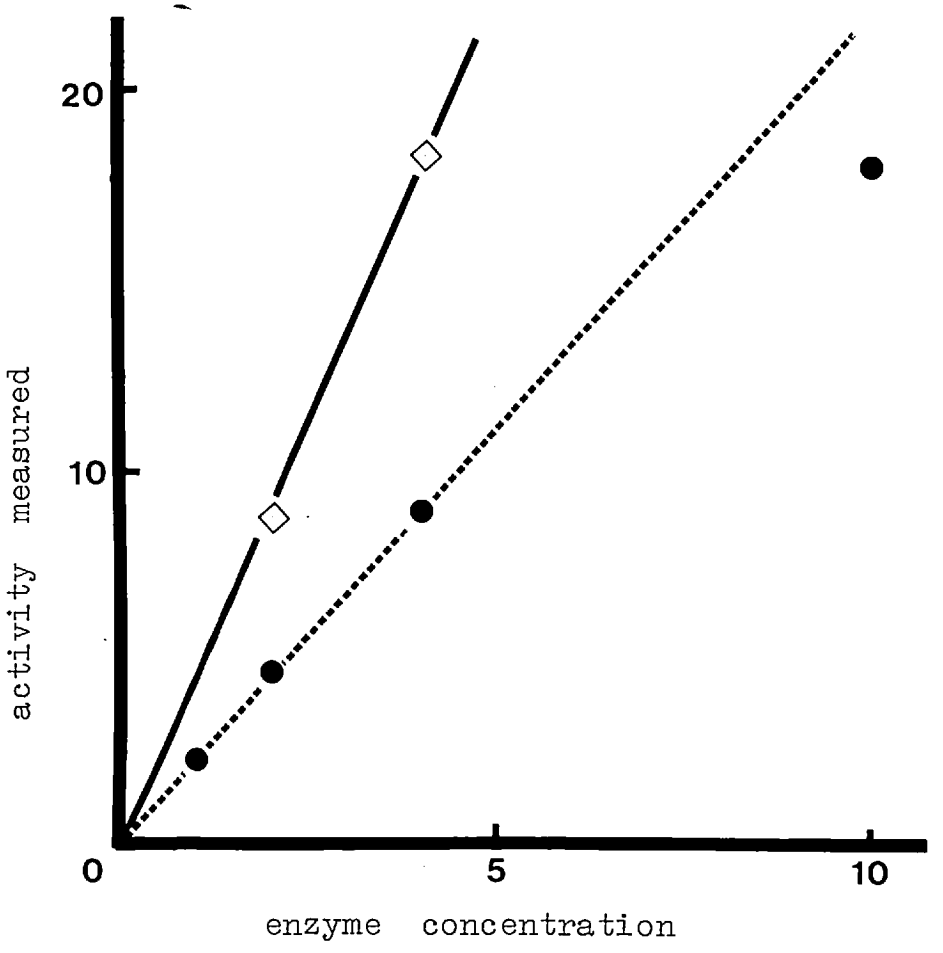
TABLE 28

Effect of the malate concentration on the measured activity of malate dehydrogenase.

Malate Concentration	Relative Activity			
3.3 mM	2.38	4.17	8.16	16.56
6.6 mM	2.50	4.61	8.80	18.20
13.2 mM	2.63	4.69	8.82	18.92

FIGURE 15

Relative activity measured v. relative concentration of malate dehydrogenase .



PALMITYL CARNITINE TRANSFERASE E.C. 2.3.1.21.

Palmityl coenzyme A + Carnitine $\rightleftharpoons$ Palmityl carnitine + Coenzyme A

This enzyme is responsible for the passage of fatty acids from the cytosol into the mitochondria prior to oxidation. Various assay methods have been developed but usually for mitochondrial preparations. It was therefore necessary to ascertain which was most suitable for crude homogenates.

The spectrophotometric assay of Norum (1964) measures the change in absorbance at 232 nm caused by the splitting of the thio-ester group of palmityl coenzyme A. This method was found not to be suitable for use with crude homogenates.

The assay of Bieber, Abraham & Helmrath (1972) involves the reaction of 5,5' dithiobis (2-Nitro benzoic acid) with the sulphydryl group of the liberated coenzyme A molecule. The carnitine blank measuring hydrolase activity was high with respect to the transferase activity. However, after correcting for this the change in absorbance at 412 nm with time was linear if the activity was low, and the rate of change was proportional to the enzyme concentration (Figure 16).

Assay conditions

Tris 0.1 M, palmityl coenzyme A 0.042 mM, DL carnitine 2.0 mM, 5,5' dithiobis (2-Nitrobenzoic acid) (DTNB) 0.12 mM pH 7.5.

With the low activity of this enzyme in adult myocardium and the lower activity in the newborn myocardium, an assay based on that of Norum (1964) was developed. This involves the exchange of ^{14}C carnitine with that of palmityl carnitine, with subsequent extraction of palmityl carnitine. At 25°C the incorporation of ^{14}C carnitine was linear with time for 20 min (Figure 17) and a 10 min incubation was subsequently used. The rate of incorporation was also proportional to the concentration of enzyme present (Figure 18). The method of Norum involves evaporation of the butanol extract with subsequent dissolution of the residue. This procedure could be avoided by using other scintillants. The results obtained using Bray's solution and a scintillant containing Triton X-100 are compared with those of the original method in Table 29. Quenching was estimated by means of the external standard ratio calibrated with a series of standards prepared using the scintillant and "cold" butanol extract.

The counting was carried out in a Packard liquid scintillation counter, and, using the scintillant containing Triton X-100, the efficiency of counting was between 86 and 88%.

Assay conditions

Tris 0.1 M, DL palmityl carnitine 0.5 mM, coenzyme A 0.12 mM, glutathione 5.0 mM, ^{14}C carnitine 0.20 mM, pH 7.5.

Scintillant

PPO 5g, POPOP 0.05g, Triton X-100 300 ml toluene 700 ml.

FIGURE 16 .

Relative activity measured v. relative concentration of palmityl carnitine transferase (DTNB assay).

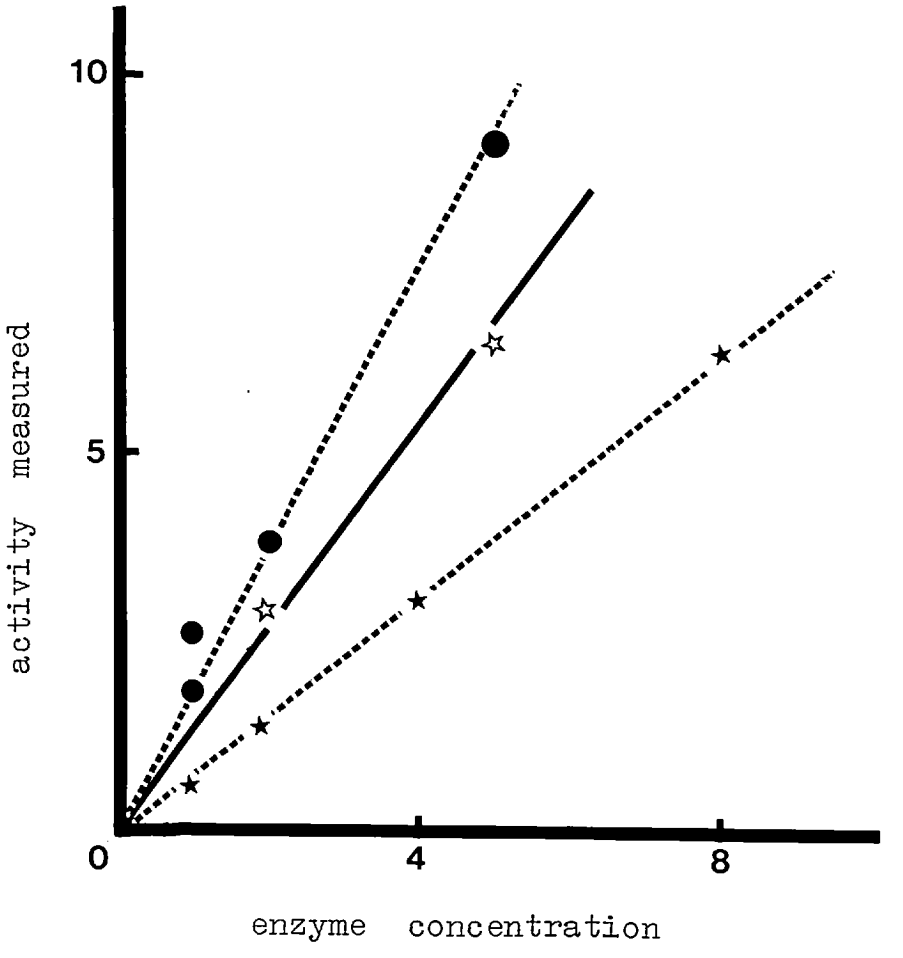


FIGURE 17 .

Incorporation of ^{14}C carnitine (c.p.m.) v. time of incubation at $25^{o}C$.

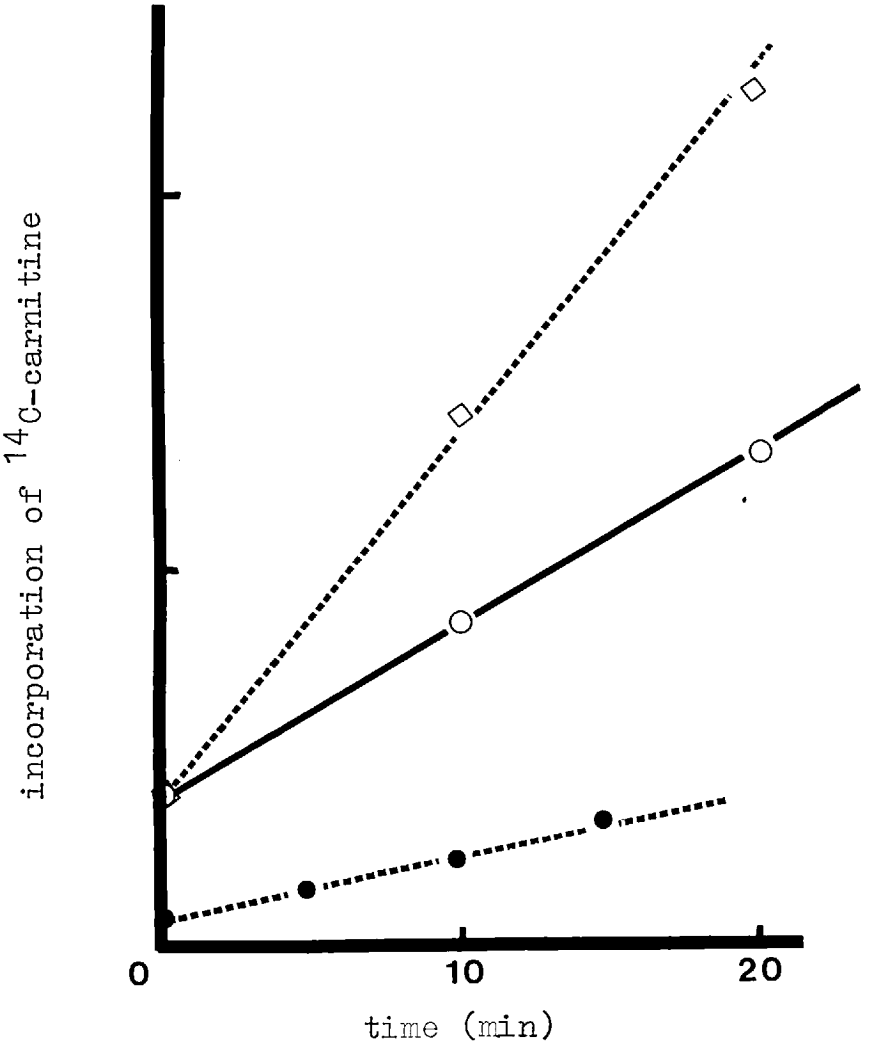


FIGURE 18 .

Relative activity measured (c.p.m. incorporated/min) v. relative concentration of palmityl carnitine transferase .

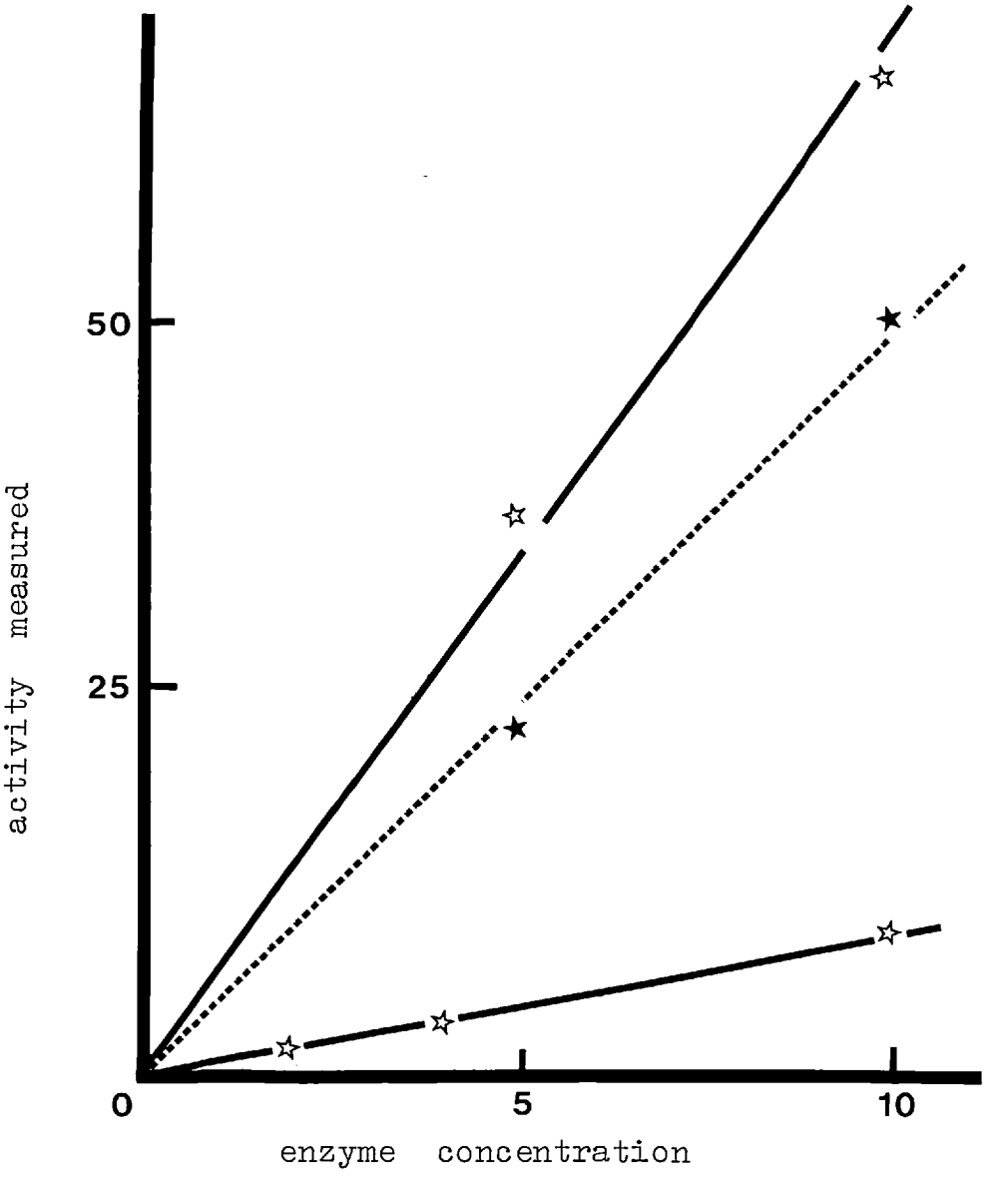


TABLE 29

Incorporation of ^{14}C carnitine as measured with different scintillants.

Scintillant A : as in Norum (1964)

Scintillant B : Bray's solution

Scintillant C : PPO 5g, POPOP 0.05g, Triton X-100 300ml, toluene 700ml.

Time of incubation	A	B	C
0 min	115	90	111
10 min	623	604	620
20 min	1068	1042	1066
rate of incorporation (cpm/min)	47.6	47.6	47.7
0 min	110	86	110
10 min	349	329	351
20 min	573	714	593
rate of incorporation (cpm/min)	23.1	31.4	24.1

PHOSPHOFRUCTOKINASE E.C. 2.7.1.11

D-fructose-6-phosphate + ATP ⟶ D-fructose-1,6-diphosphate + ADP

The assay was based on that of Bücher, Luh & Pette (1964) in which the test reaction is linked to the indicator reaction - the reduction of dihydroxyacetone phosphate to glycerol-3-phosphate by NADH as catalysed by glycerol-3-phosphate dehydrogenase - by aldolase and triose phosphate isomerase. Phosphofructokinase is subject to allosteric regulation by many effectors, and it was considered adviseable to add known activators to the assay medium to increase the measured activity and to reduce the problems from low concentrations of endogenous activators. The additions of AMP and KCl caused slight increases in activity (Tables 30, 31). With adult myocardial tissue, sulphydryl agents were not required. With the tissue from newborn animals, 2.5 mM glutathione was included in the assay as preliminary work had suggested this to be beneficial. Subsequent work indicated that this addition had no marked effect and that higher concentrations are inhibitory (Table 32). Higher concentrations of the substrate fructose-6-phosphate did not give higher activities (Table 33). Increasing the concentrations of the auxiliary enzymes would have caused a slight increase in the measured activity (Tables 34, 35, 36), but the levels used were sufficient to give linear changes of absorbance at 340 nm with time, and rates proportional to the

concentration of phosphofructokinase present over the range used in the experiments (Figure 19).

Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, $MgSO_4$ 8.0 mM, KCl 75 mM, NADH 0.19 mM ATP 1.5 mM, AMP 2.3 mM, fructose-6-phosphate 1.2 mM, aldolase 0.9 U/ml triosephosphate isomerase 4 U/ml, glycerol-3-phosphate dehydrogenase 1.3 U/ml, pH 7.5.

TABLE 30

Effect of 2.3 mM AMP on the measured activity of phosphofructokinase.

	Relative Activity			
- AMP	0.61	1.87	3.68	10.70
+ AMP	1.02	2.44	4.91	12.10

TABLE 31

Effect of 75 mM KCl on the measured activity of phosphofructokinase.

	Relative Activity			
- KCl	0.91	2.27	4.43	10.86
+ KCl	1.02	2.44	4.91	12.10

TABLE 32

Requirement for glutathione in the assay for phosphofructokinase with myocardial homogenates from newborn animals.

GSH Concentration	Relative Activity					
0.0 mM	2.42	2.44	13.56	15.38	10.18	10.56
2.5 mM	2.70	2.51	13.88	15.84	10.01	10.74
2.5 mM	7.32	5.87	7.48	6.38	6.27	6.80
5.0 mM	6.63	5.02				
10.0 mM			6.52	5.36		
20.0 mM					4.43	5.44

TABLE 33

Effect of the concentration of fructose-6-phosphate on the measured activity of phosphofructokinase .

F6P Concentration	Relative Activity			
0.65 mM	6.12	3.07	5.92	2.45
1.30 mM	6.13	3.13	5.96	2.37
2.60 mM	5.93	3.06	5.80	2.42

TABLE 34

Effect of the concentration of aldolase on measured activity of phosphofructokinase.

Aldolase Concentration	Relative Activity			
0.9 U/ml	5.06	3.04	2.58	5.67
1.8 U/ml	5.82	3.27	2.70	5.66
2.25 U/ml	5.88	3.55	2.78	5.89

TABLE 35

Effect of the concentration of triosephosphate isomerase on the measured activity of phosphofructokinase.

TIM Concentration	Relative Activity			
4 U/ml	4.80	2.48	5.71	4.81
8 U/ml	4.95	2.57	5.27	4.62
10 U/ml	5.00	2.52	5.67	4.58

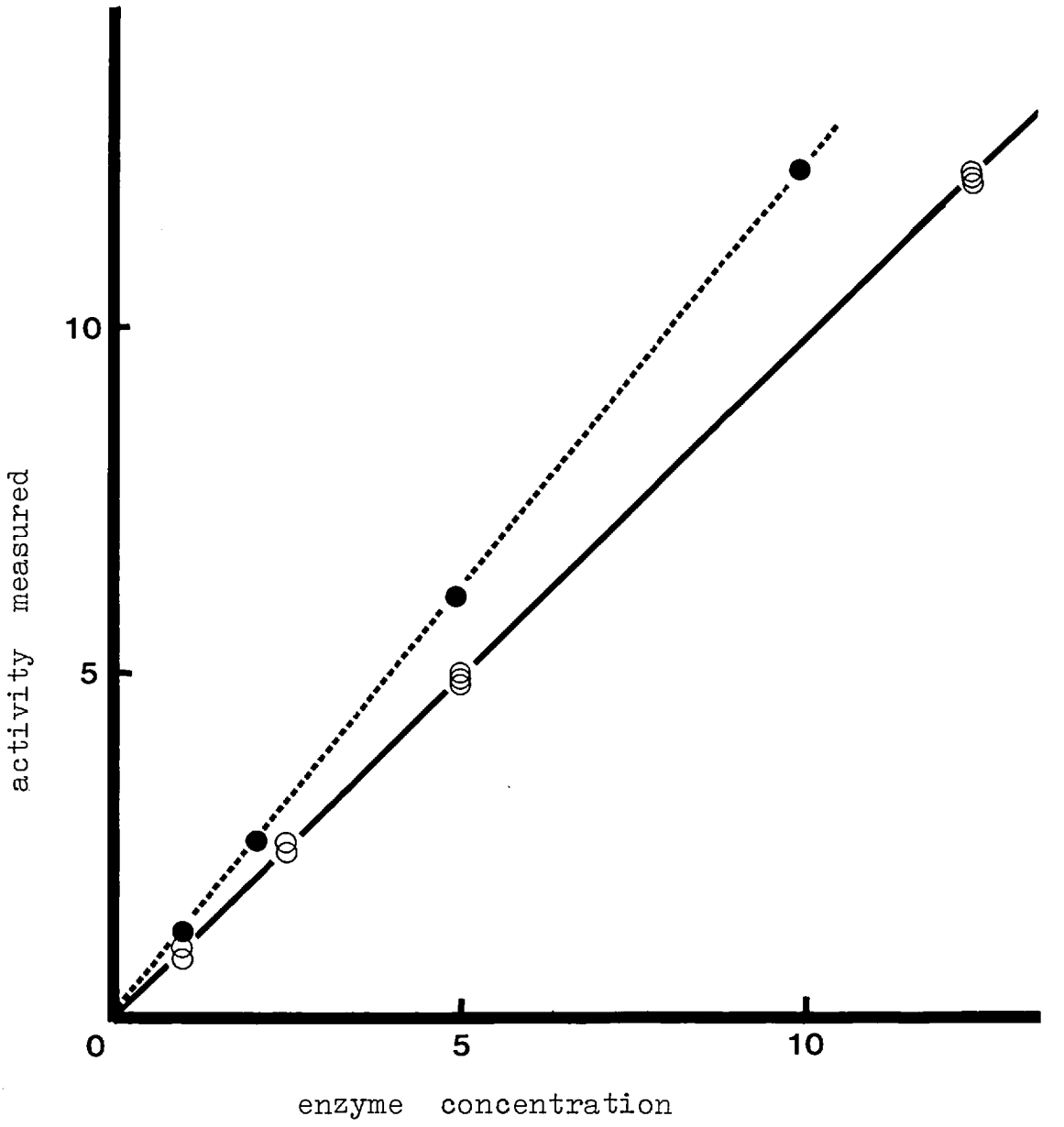
TABLE 36

Effect of the concentration of glycerol-3-phosphate dehydrogenase on the measured activity of phosphofructokinase.

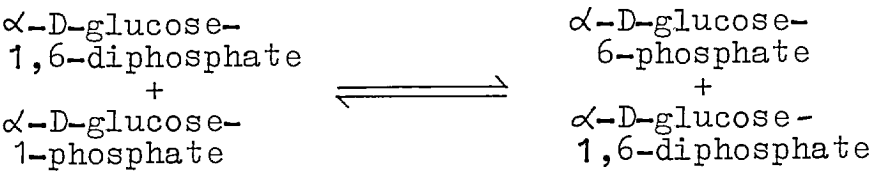
G3PDH Concentration	Relative Activity				
1.3 U/ml	4.87	2.63	5.49	2.35	5.67
2.6 U/ml	4.97	2.73	5.50	2.44	5.72
3.25 U/ml	5.27	2.80	5.83	2.50	5.86

FIGURE 19 .

Relative enzyme activity measured v. relative concentration of phosphofructokinase .



PHOSPHOGLUCOMUTASE E.C. 2.7.5.1.



The assay was based on that of Bücher, Luh & Pette (1964) in which the rate of the test reaction is indicated by that of the oxidation of the product glucose-6-phosphate by glucose-6-phosphate dehydrogenase. In the absence of added glucose-1,6-diphosphate the activity measured in heart muscle was low. The addition of 17 μM glucose-1,6-diphosphate increased the activity by about 50% while 34 μM gave a small further increase (Table 37). The effect of altering the magnesium sulphate concentration was most noticeable. The change from 4 mM to 8 mM magnesium sulphate resulted in a 20 fold increase in activity (Table 38), while higher concentrations had no further effect. There was no increase in activity with a higher concentration of glucose-1-phosphate (Table 39), nor with higher concentrations of glucose-6-phosphate dehydrogenase (Table 40): the concentration of indicator enzyme used was sufficient to give rates of change of absorbance at 340 nm which were proportional to the amount of phosphoglucomutase present (Figure 20)

Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, $MgSO_4$ 8.0 mM, $NADP^+$ 0.52 mM, glucose-6-phosphate dehydrogenase 1.2 U/ml, glucose-1-phosphate 2.7 mM, glucose-1,6-diphosphate 17 μM, pH 7.5.

TABLE 37

Effect of the concentration of glucose-1,6-diphosphate on the measured activity of phosphoglucomutase.

GDP Concentration	Relative Activity						
0.0 μM	1.78	2.20	0.90	3.66	28.6	48.6	29.4
17.0 μM	2.65	3.10	1.42	5.14	44.5	68.0	45.2
17.0 μM	2.94	8.80	18.24	9.30			
34.0 μM	3.80	9.78	19.20	9.74			

TABLE 38

Effect of the concentration of magnesium sulphate on the measured activity of phosphoglucomutase.

$MgSO_4$ Concentration	Relative Activity				
4.0 mM	0.80	0.32	0.00	0.16	0.28
8.0 mM	17.44	9.14	3.16	4.28	8.32
16.0 mM	17.20	9.08	3.10	3.59	7.42

TABLE 39

Effect of the concentration of glucose-1-phosphate on the measured activity of phosphoglucomutase.

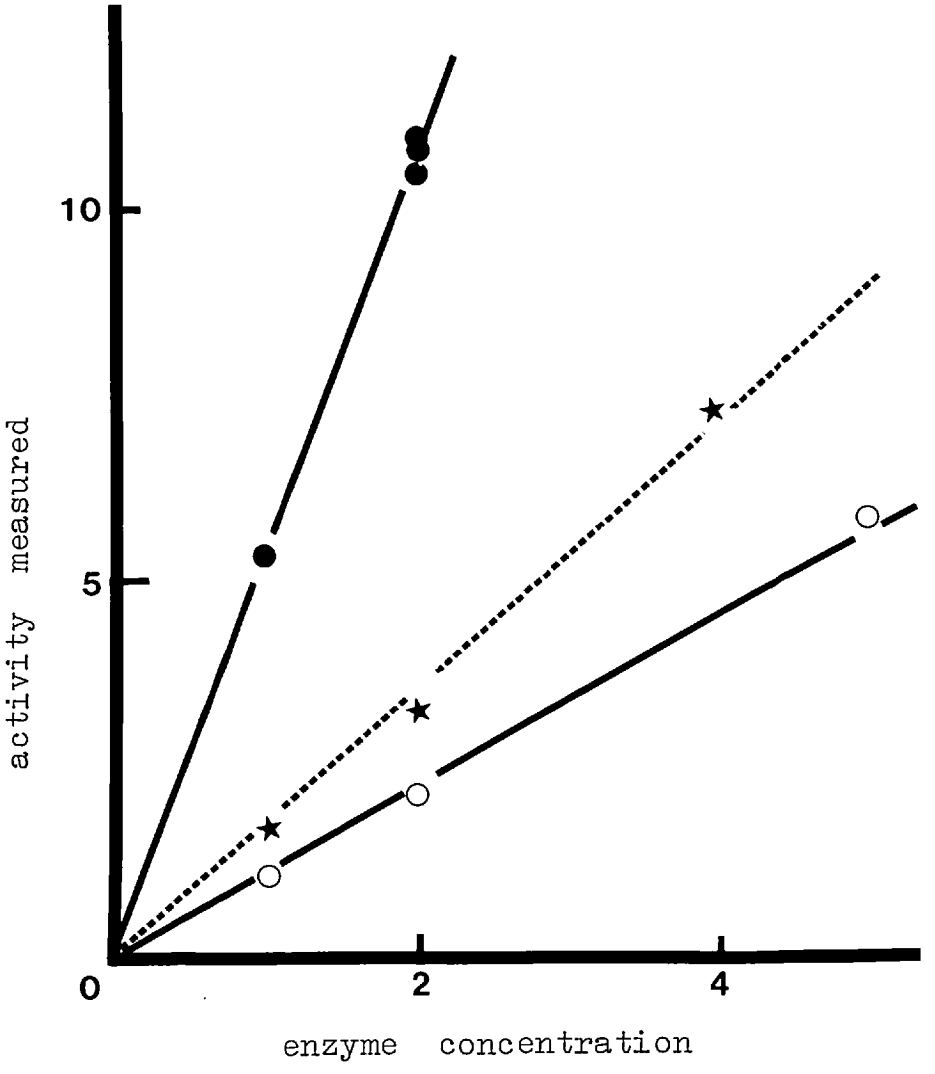
G1P Concentration	Relative Activity			
1.35 mM	4.20	4.47	8.36	10.50
2.7 mM	4.13	4.50	7.82	10.52
5.4 mM	3.80	4.50	7.14	10.70

TABLE 40

Effect of the concentration of glucose-6-phosphate dehydrogenase on the measured activity of phosphoglucomutase.

G6PDH Concentration	Relative Activity			
1.2 U/ml	2.12	4.09	8.20	18.84
2.9 U/ml	2.12	3.90	7.94	19.36
5.8 U/ml	1.94	3.52	7.16	18.00

FIGURE 20.
Relative activity measured v. relative concentration of phosphoglucomutase .



PHOSPHOGLYCERATE KINASE E.C.2.7.2.3.

3-phospho-D-glycerate + ATP $\rightleftharpoons$ 3-phospho-D-glyceroyl-phosphate + ADP

The assay used was based on that of Bücher, Luh & Pette (1964) in which the rate of utilization of the product 1,3 diphosphoglycerate by glyceraldehyde-3-phosphate dehydrogenase indicates the rate of the test reaction. The magnesium concentration was of great importance as the activity in the presence of 8 mM magnesium sulphate was about 3 fold higher than in the presence of 4 mM magnesium sulphate. Higher concentrations provided no advantage (Table 41). The concentration of 3-phosphoglycerate affected the activity measured (Table 42). Higher concentrations of ATP were not beneficial (Table 43). The concentration of the indicator enzyme used was sufficient to give linear changes of absorbance at 340 nm with time and rates of change which were proportional to the amount of phosphoglycerate kinase present (Figure 21), although not sufficient to give maximal values (Table 44).

Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, $MgSO_4$ 8.0 mM, cysteine 20 mM, NADH 0.19 mM, ATP 3.0 mM, 3-phosphoglycerate 5.9 mM, glyceraldehyde-3-phosphate dehydrogenase 2.3 U/ml, pH 7.5.

TABLE 41

Effect of the magnesium sulphate concentration on the measured activity of phosphoglycerate kinase.

$MgSO_4$ Concentration	Relative Activity					
4.0 mM	0.81	1.38	1.77	2.22	3.00	3.13
8.0 mM	3.70	7.81	7.19	8.10	12.76	11.67
8.0 mM	10.90	22.70				
12.0 mM	11.44	22.20				

TABLE 42

Effect of the 3-phosphoglycerate concentration on the measured activity of phosphoglycerate kinase.

3PGA Concentration	Relative Activity					
2.95 mM	3.14	6.46	15.60	3.90	7.24	7.50
5.90 mM	3.57	7.26	17.60	4.33	7.34	8.42
11.80 mM	3.96	7.76	19.08	4.76	8.86	9.28

TABLE 43

Effect of the ATP concentration on the measured activity of phosphoglycerate kinase.

ATP Concentration	Relative Activity			
1.5 mM	3.33	7.10	3.72	7.72
3.0 mM	3.56	7.40	4.17	8.48
6.0 mM	3.46	7.42	4.27	8.54

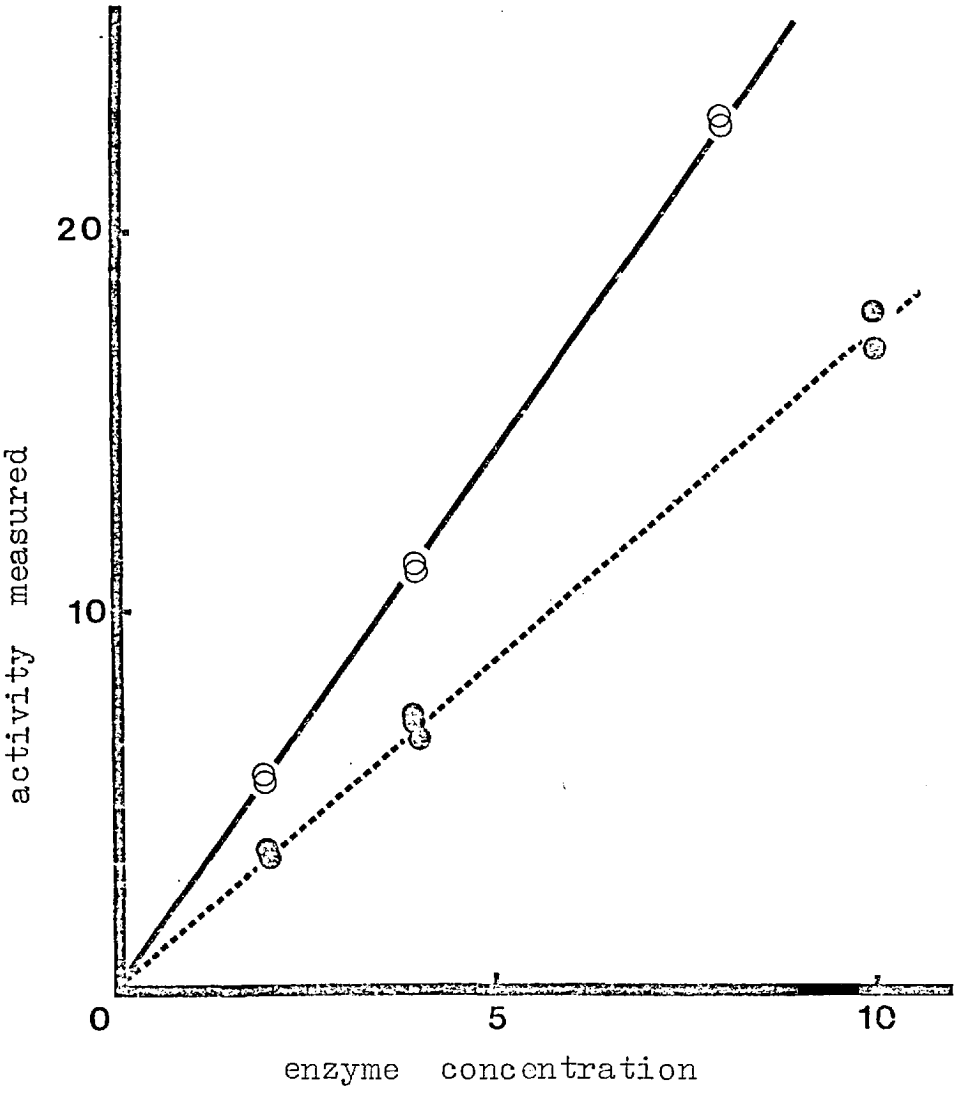
TABLE 44

Effect of the glyceraldehyde-3-phosphate dehydrogenase concentration on the measured activity of phosphoglycerate kinase.

GAPDH concentration	Relative activity				
2.3 U/ml	3.46	6.52	16.76	3.99	8.00
4.6 U/ml	3.66	7.48	18.08	4.25	8.64
5.75 U/ml	3.66	7.72	17.96	4.76	9.05

FIGURE 21

Relative activity measured v. relative concentration of phosphoglycerate kinase.



PHOSPHOGLYCEROMUTASE E.C. 2.7.5.3.

2,3-diphospho-D-glycerate + 2-phospho-D-glycerate ⇌ 3-phospho-D-glycerate + 2,3-diphospho-D-glycerate

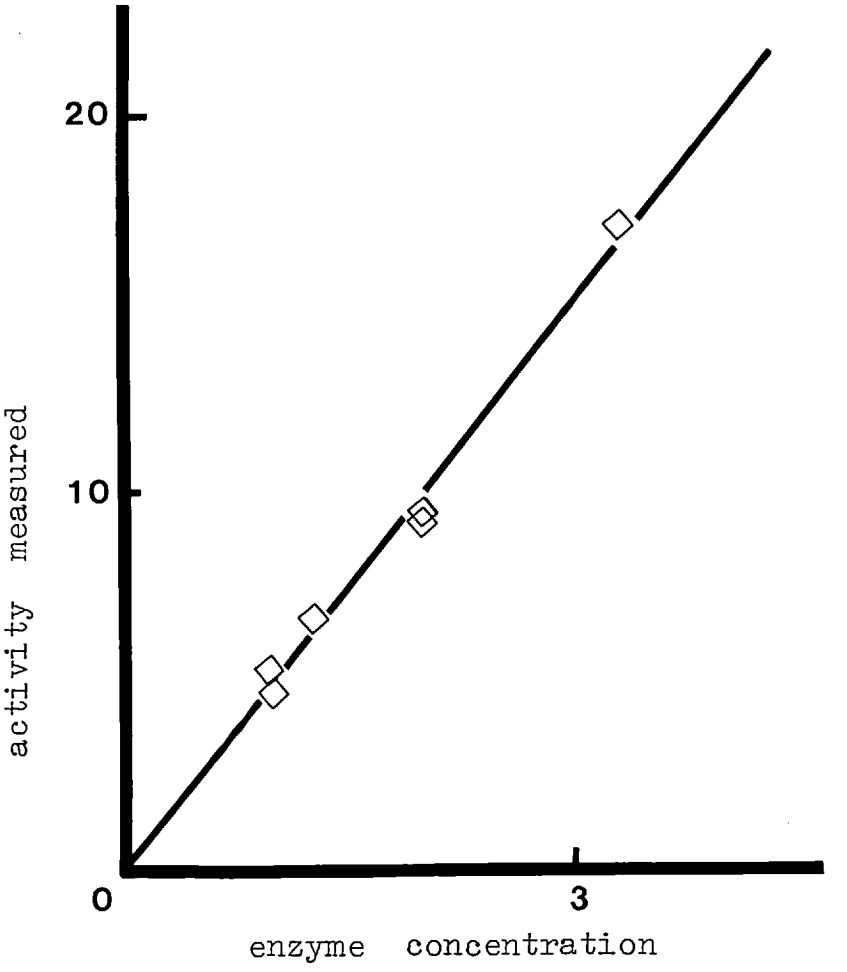
The assay was based on that of Bücher, Luh & Pette (1964) in which the test reaction is coupled by enolase and pyruvate kinase to the indicator reaction of lactate dehydrogenase. Added 2,3 diphosphoglycerate greatly accelerated the reaction. The auxiliary enzyme concentrations used were adequate to ensure that the changes in absorbance at 340 nm with time were linear and that the rates of change were proportional to the amount of phosphoglyceromutase present (Figure 22).

Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, $MgSO_4$ 8.0 mM, KCl 75 mM, ADP 0.84 mM, 2,3 diphosphoglycerate 0.55 mM, 3-phosphoglycerate 5.9 mM, NADH 0.19 mM, enolase 0.45 U/ml, pyruvate kinase 1.0 U/ml, lactate dehydrogenase 6.0 U/ml, pH 7.5.

FIGURE 22 .

Relative activity measured v. relative concentration of phosphoglyceromutase .



PYRUVATE KINASE E.C. 2.7.1.40.

ATP + pyruvate $\rightleftharpoons$ phosphoenolpyruvate + ADP

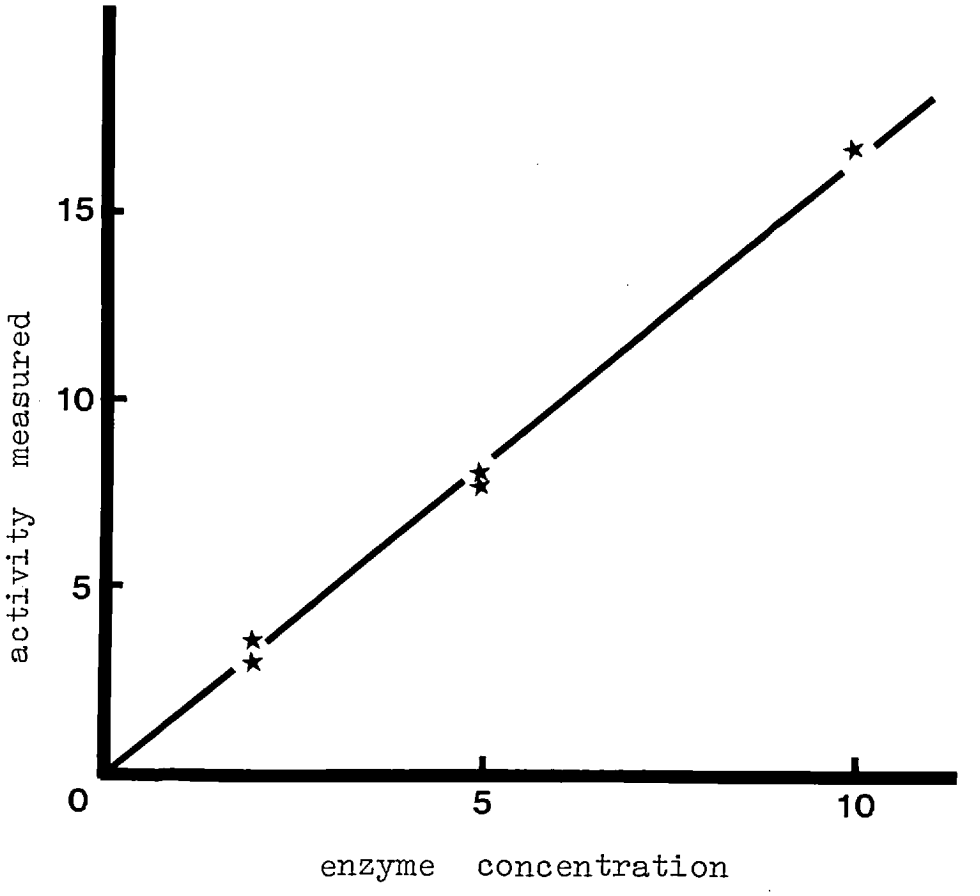
The assay was based on that of Bücher, Luh & Pette (1964) in which the rate of reduction of the product pyruvate by lactate dehydrogenase indicates the rate of the reaction. The enzyme is unstable and requires rapid assay (Shonk & Boxer 1966). The assay gave changes of absorbance at 340 nm that were linear with time and rates which were proportional to the amount of pyruvate kinase present (Figure 23).

Assay system

Triethanolamine 60 mM, EDTA 6.0 mM, $MgSO_4$ 8.0 mM, KCl 75 mM, NADH 0.19 mM, phosphoenolpyruvate 1.8 mM, ADP 1.7 mM, lactate dehydrogenase 6.0 U/ml, pH 7.5.

FIGURE 23 .

Relative activity measured v. relative concentration of pyruvate kinase .



SUCCINATE DEHYDROGENASE E.C. 1.3.99.1.

succinate + acceptor $\rightleftharpoons$ fumarate + reduced acceptor

The assay was developed from that of Green et al. (1955). The presence of bovine serum albumin did not prove advantageous and the assays were more regular in its absence (Table 45). 2,6-Dichlorophenol indophenol was found to be preferable to cytochrome c as, although the molar extinction coefficients were similar, the activity as measured with dichlorophenol indophenol was higher (Table 46). The activity measured was dependent on the amount of the electron acceptor present, especially at high activities (Table 47), while the concentrations of succinate had little effect (Table 48). Triethanolamine buffer was used as with the majority of assays, and the activity measured was the same as with the phosphate buffer (Table 49). The change in absorbance at 600 nm with time was not linear but the initial rate was proportional to the amount of enzyme present (Figure 24). The activity of the homogenate was found to increase with time and so the assay was always performed at the end of an experiment when such activation had occurred.

Assay system:

Triethanolamine 56 mM, EDTA 5.6 mM, KCN 2 mM, succinate 10 mM, 2,6 dichlorophenol indophenol 20 µg/ml, pH 7.5.

TABLE 45

Effect of the bovine serum albumim concentration on the measured activity of succinate dehydrogenase.

BSA Concentration	Relative Activity		
0.0 mg/ml	1.78 (178)	3.30 (165)	7.12 (178)
0.1 mg/ml	2.26 (226)	3.36 (168)	5.08 (127)
0.2 mg/ml	2.08 (208)		5.92 (148)
0.5 mg/ml	2.16 (216)		7.88 (197)

Value in parentheses denotes the activity per 100 arbitary enzyme units.

TABLE 46

Measured activity of succinate dehydrogenase using different electron acceptors.

		Relative Activity		
DCPIP	20 µg/ml	0.68	1.30	2.25
cytochrome c	17 µM	0.44	0.57	1.15

TABLE 47

Effect of the concentration of dicholorophenol indophenol on the measured activity of succinate dehydrogenase.

	Relative Activity				
20 µg/ml	1.65	3.61	8.36	17.4	23.4
16 µg/ml	1.67	3.69	8.22	15.1	20.1
12 µg/ml	1.65	3.49	7.62	13.5	18.3

TABLE 48

Effect of the succinate concentration on the measured activity of succinate dehydrogenase.

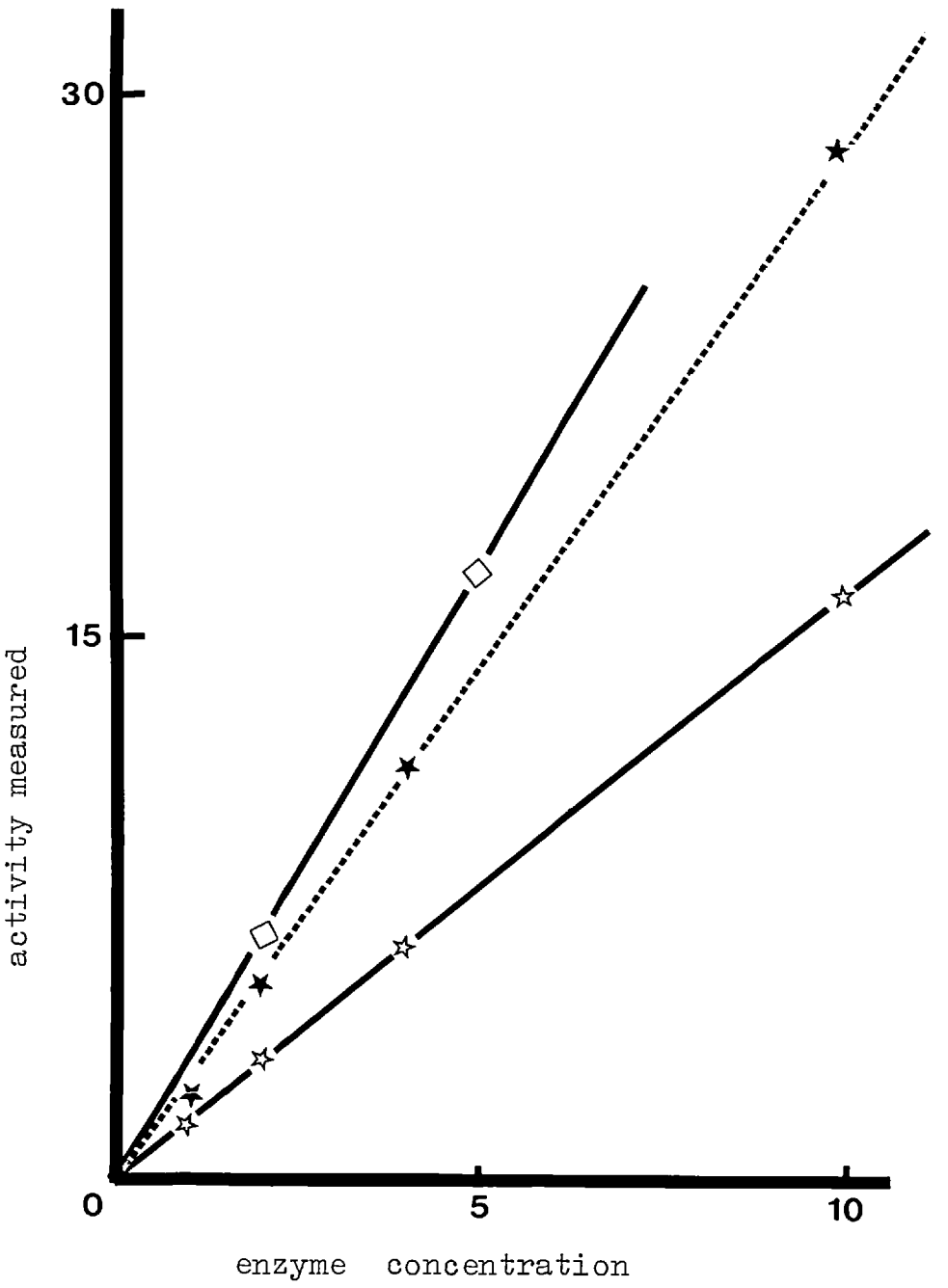
Succinate Concentration	Relative Activity			
5 mM	1.53	3.37	8.92	26.00
10 mM	1.56	3.34	8.96	26.64
20 mM	1.64	3.80	9.36	26.68

TABLE 49

Effect of the buffer on the measured activity of succinate dehydrogenase.

	Relative Activity					
Triethanol-amine pH 7.5	1.76	2.33	3.49	5.48	11.38	28.57
Phosphate pH 7.5	1.34	2.53	3.08	5.85	12.25	32.96

FIGURE 24.
Relative activity measured v. relative concentration of succinate dehydrogenase .



PROTEIN ESTIMATION

The biuret method was not used as its sensitivity was much lower than that of the method of Lowry et al. (1951). However, the method Lowry et al. was found to be sensitive to the homogenization medium resulting in a precipitate forming under certain conditions. The particular constituent that caused this was not identified, because if the sample volume was kept small then no precipitate formed and the estimation of protein content could be made. In all cases the standards contained the same concentration of the homogenization medium as did the samples, as sometimes the colour development was affected by the medium (Table 50).

TABLE 50

Influence of homogenization medium on protein standards.

Protein µg	0	25	50	75	100
0.0 mM phosphate	0	0.166	0.286		0.475
12.5 mM "	0	0.149	0.270	0.371	0.451
25.0 mM "	0	0.154	0.276	0.380	0.462
50.0 mM "	0	0.134	0.251	0.326	0.419

STUDIES ON INDIGENOUS HIGH AND LOW ALTITUDE ANIMALS

INTRODUCTION

The indigenous high altitude animal is suited to its environment in that it is able to survive and reproduce under the prevalent conditions. The differences between the animals of any species indigenous to high altitude and those of the same species indigenous to low altitude may be simply the result of lifelong acclimatization to their environments, or may be the result of adaptations arising during the course of many generations.

Since investigation of indigenous high and low altitude animals necessitates the sampling of two independent populations, there is the possibility that differences between the populations may be present that arise from factors other than the altitudes at which they live. Obvious factors to consider are the diet, and the degree of exercise undertaken, as these have been shown to affect the enzyme activities in different tissues (Knox & Greengard 1965, Schlimke 1962, Kraus 1971, York, Penney & Oscai 1975). With this problem in mind it was decided to use outbred animals from the same country but from different altitudes. The living conditions prior to purchase would probably be similar, and very different to those of the laboratory animals available in London. In this way differences

other than those arising from the altitude would be minimized, thus making the comparison more meaningful than in a previous study (Harris et al. 1970) in which laboratory animals obtained in London had represented the sea level populations.

Since the modifications shown by the indigenous high altitude population are present as a result of or despite natural selection acting over many generations, they provide an important reference against which to compare the results of later acclimatization studies.

METHODS

The animals, adult male outbred rabbits and guinea pigs, were obtained locally at Cerro de Pasco, Peru at an altitude of 4300 m, and at Lima, Peru at an altitude of 50 m. They are bred for food in both locations. They were maintained on a standard laboratory diet supplemented with fresh grass daily.

The tissues, the sartorius and ventricular muscles, were removed from the animals while under pentobarbitone anaesthesia and were subsequently treated as described in the previous section.

A selection of enzymes representing various metabolic pathways and of cytoplasmic and mitochondrial origin were studied. The methods were as described in detail in the previous section, except that the concentrations of the auxiliary enzymes were 50% higher owing to the total volume of the assay being 2.0 ml instead of 3.0 ml. This affects only two enzyme assays, those for phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase. In the former case the results obtained in this situation are not exactly comparable with those obtained in London, since the activity measured is slightly dependent on the activities of the auxiliary enzymes (see page 88). This reservation does not apply in the latter case (see page 53). The

measurements were made on a Zeiss PM Q11 spectrophotometer with readings taken every minute for the assays involving NAD^+, and on a Coleman Junior II spectrophotometer with readings taken every 2 min for assays involving dichlorophenol indophenol. With most of the assays the change in extinction with time was linear, and the regression line for the data was computed by the method of least squares using an Olivetti Programma 102. For succinate dehydrogenase, mitochondrial glycerol 3 phosphate dehydrogenase, and lactate dehydrogenase this extinction change was not linear with time. For these enzymes the data were fitted by computer by the method of least squares to the equation.

$$(E_o - E_i) = a(t_i - t_o)^b$$

where E_o and E_i were the extinctions at times t_o and t_i while t_o was the time of the first reading after the initiation of the reaction. This was 1 min for the lactate dehydrogenase assays, and 2 min for the succinate dehydrogenase and mitochondrial glycerol-3-phosphate dehydrogenase assays. The regression coefficient was always greater than 0.99. The value of ab, which is the slope at time $(t_o + 1)$, was taken as a measure of the enzyme activity. In this way a measurement of near initial rates of reaction could be made without the observer bias

inherent in curve drawing and in the estimation of tangents to curves. The activities expressed in μmoles substrate transformed/min/mg protein at 25°C were calculated using the molar extinction coefficients of $6.22\ 10^6$ for NADH and $21.0\ 10^6$ for dichlorophenol indophenol (Armstrong 1964).

Protein was estimated by the method of Lowry et al. (1951) as described, and the students "t" and paired "t" tests were used to calculate statistical significance.

RESULTS

The physiological parameters are given in Table 51. For the guinea pig the right ventricular weight, and the ratios of this to the body weight and to the weight of the left ventricle and septum, were significantly higher in the high altitude animals. In contrast, the weight of the left ventricle with septum was similar at both altitudes, as was the ratio of this to the body weight. The greater right ventricular size was the result of hypertrophy presumably caused by hypoxic pulmonary hypertension (Dean et al. 1968, Reeves et al. 1963 Ressl et al. 1974).

With the rabbit, there was a significant difference in the body weights in that those at high altitude were smaller. Despite this there was little difference in the weights of the right ventricle, and the ratio of this to the body weight was significantly higher at high altitude. The values obtained closely agree with those of Dean et al. (1968), and indicate that hypertrophy had occurred as with the guinea pigs, and as reported by Dean (1968), Van Liere, Krames & Northup (1965) and Burton & Smith (1969) for other species. The data for the left ventricle and septum indicate that slight hypertrophy had occurred. This had been found for rats (Sizemore et al. 1973).

TABLE 51

Physiological data for the high and low altitude guinea pigs and rabbits.

GUINEA PIGS

	Low Altitude		High Altitude
Body weight (gm)	527 ± 61		487 ± 69
Right ventricle (gm)	0.288 ± 0.053	P<0.02	0.381 ± 0.067
Left ventricle and septum (gm)	0.953 ± 0.157		1.000 ± 0.114
Right ventricle: body weight (mg:gm)	0.548 ± 0.087	P<0.05	0.791 ± 0.136
Left ventricle and septum: body weight (mg:gm)	1.83 ± 0.30		2.06 ± 0.12
Right ventricle: left ventricle and septum	0.302 ± 0.024	P<0.01	0.383 ± 0.066

RABBITS

	Low Altitude		High Altitude
Body weight (gm)	1635 ± 144	P<0.001	1003 ± 236
Right ventricle (gm)	0.651 ± 0.156		0.578 ± 0.200
Left ventricle and septum (gm)	2.17 ± 0.22	P<0.005	1.49 ± 0.39
Right ventricle: body weight (mg:gm)	0.399 ± 0.085	P<0.02	0.585 ± 0.147
Left ventricle and septum: body weight (mg:gm)	1.33 ± 0.11	P<0.05	1.49 ± 0.15
Right ventricle: left ventricle and septum	0.298 ± 0.059	P<0.02	0.390 ± 0.070

Data are expressed as mean ± S.D. for 8 observations per group except for the low altitude guinea pigs where there were 9 animals in the group.

ENZYME ACTIVITIES IN GUINEA PIGS

Table 52 shows the enzyme activities found in the sartorius muscle of guinea pigs. There was no difference between the activities in the high and low altitude animals. The ratio of glyceraldehyde-3-phosphate dehydrogenase to mitochondrial glycerol-3-phosphate dehydrogenase (GAPDH/mG3PDH), considered by Pette (1966) to be constant, was also similar at both altitudes (1900 $\pm$ 700 at low altitude and 1900 $\pm$ 400 at high altitude). The low ratio of αhydroxybutyrate dehydrogenase to lactate dehydrogenase (0.153 $\pm$ 0.021 at low altitude and 0.149 $\pm$ 0.009 at high altitude) corresponded to the high proportion of M-LDH subunits found in this type of tissue (Markert & Ursprung 1962, Gutierrez et al. 1974, Kaplan & Goodfriend 1964).

Tables 53 and 54 show the results obtained from the left and right ventricles of guinea pigs. In a comparison of the left and right ventricles, slight differences were found between the activities in the two ventricles at both high and low altitudes. Mitochondrial glycerol-3-phosphate dehydrogenase activity was significantly higher in the left ventricle than in the right ($P<0.02$, $P<0.05$ at low and high altitudes respectively, $P<0.005$ for the combined data), while the GAPDH/mG3PDH ratios were also similar for both ventricles at both altitudes

TABLE 52

Mean enzyme activities in sartorius of guinea pigs indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude	High Altitude
Phosphofructokinase	0.43 ± 0.10 (7)	0.35 ± 0.05 (8)
Glyceraldehyde-3-phosphate dehydrogenase	4.3 ± 1.1 (8)	4.0 ± 0.6 (8)
Lactate dehydrogenase	3.7 ± 0.9 (8)	3.2 ± 0.3 (8)
α-hydroxybutyrate dehydrogenase	0.56 ± 0.14 (8)	0.48 ± 0.03 (8)
Aspartate aminotransferase	0.15 ± 0.04 (8)	0.16 ± 0.04 (8)
β-hydroxyacyl coenzyme A dehydrogenase	0.0016 ± 0.0008 (8)	0.0013 ± 0.0003 (8)
Succinate dehydrogenase	0.0022 ± 0.0007 (8)	0.0016 ± 0.0009 (8)
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0024 ± 0.0009 (8)	0.0021 ± 0.0002 (8)

TABLE 53

Mean enzyme activities in left ventricle of guinea pigs indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude		High Altitude		
Phosphofructokinase	0.14 ± 0.02	(9)	0.15 ± 0.02	(8)	
Glyceraldehyde-3-phosphate dehydrogenase	0.86 ± 0.10	(9)	0.96 ± 0.05	(8)	
Lactate dehydrogenase	1.2 ± 0.2	(9)	1.1 ± 0.1	(8)	
α-hydroxybutyrate dehydrogenase	0.74 ± 0.1	(9)	0.61 ± 0.07	(8)	P<0.02
Aspartate aminotransferase	1.3 ± 0.1	(9)	1.2 ± 0.1	(8)	
β-hydroxyacyl coenzyme A dehydrogenase	0.037 ± 0.005	(9)	0.042 ± 0.006	(8)	
Succinate dehydrogenase	0.027 ± 0.005	(9)	0.022 ± 0.003	(4)	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0028 ± 0.0009	(9)	0.0024 ± 0.0005	(8)	

TABLE 54

Mean enzyme activities in right ventricle of guinea pigs indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude		High Altitude		
Phosphofructokinase	0.14 ± 0.03	(8)	0.14 ± 0.02	(8)	
Glyceraldehyde-3-phosphate dehydrogenase	0.90 ± 0.20	(8)	1.0 ± 0.1	(8)	
Lactate dehydrogenase	1.4 ± 0.2	(8)	1.2 ± 0.2	(8)	
α-hydroxybutyrate dehydrogenase	0.87 ± 0.13	(8)	0.72 ± 0.08	(8)	$P<0.05$
Aspartate aminotransferase	1.2 ± 0.1	(8)	1.2 ± 0.1	(8)	
β-hydroxyacyl coenzyme A dehydrogenase	0.033 ± 0.004	(8)	0.042 ± 0.008	(8)	$P<0.02$
Succinate dehydrogenase	0.024 ± 0.007	(8)	0.024 ± 0.003	(4)	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0017 ± 0.0004	(8)	0.0020 ± 0.0002	(8)	

ENZYME ACTIVITIES IN RABBITS

Table 55 shows the results for the sartorius muscle of rabbits. The activities of glyceraldehyde-3-phosphate dehydrogenase and phosphofructokinase were significantly higher in the high altitude animals ($P<0.005$, $P<0.02$ respectively), indicating a higher glycolytic capacity. The activity of mitochondrial glycerol-3-phosphate dehydrogenase was also significantly higher at altitude ($P<0.02$), while the GAPDH/mG3PDH ratios were similar (2200 ± 700 at low altitude and 1700 ± 400 at high altitude). The ratio αHBDH/LDH was again low, and was unaffected by altitude (0.093 ± 0.006 at low altitude and 0.094 ± 0.009 at high altitude).

Tables 56 and 57 show the enzyme activities for the left and right ventricles of rabbits. In a comparison of the left and right ventricles, the only difference found was in the activity of glyceraldehyde-3-phosphate dehydrogenase which was significantly higher in the left ventricle at low altitude only ($P<0.001$).

The differences between the activities in the high compared with the low altitude animals were similar for both ventricles, but were statistically more significant for the right ventricle. The glycolytic capacity was higher in the high altitude myocardium, as indicated by the higher glyceraldehyde-3-phosphate dehydrogenase and phosphofructokinase activities. Lactate dehydrogenase activity was also higher while αhydroxybutyrate dehydrogenase

(330 ± 100 and 560 ± 180 in the left and right ventricles respectively at low altitude, and 420 ± 140 and 520 ± 60 at high altitude). Both lactate dehydrogenase and αhydroxybutyrate dehydrogenase activities were higher in the right ventricle than in the left ($P<0.001$ for both enzymes using the combined data) but the ratio of αhydroxybutyrate dehydrogenase activity to total lactate dehydrogenase (αHBDH/LDH), which gives a measure of the proportion of the H-LDH subunits, was similar in both ventricles at both altitudes (0.629 ± 0.090 and 0.617 ± 0.072 in the left and right ventricles respectively at low altitude, and 0.584 ± 0.074 and 0.593 ± 0.108 at high altitude).

There were few differences between the myocardial enzyme activities of the high compared with low altitude guinea pigs. Lactate dehydrogenase activity was similar, while that of αhydroxybutyrate dehydrogenase was lower in the high altitude animals. However the ratio αHBDH/LDH was not significantly lower at high altitude indicating no significant change in the proportion of H-LDH subunits. The guinea pigs from high altitude had higher βhydroxyacyl coenzyme A dehydrogenase activity but this was statistically significant in the right ventricle only.

TABLE 55

Mean enzyme activities in sartorius of rabbits indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude (8)	High Altitude (8)	
Phosphofructokinase	0.5 ± 0.08	0.71 ± 0.18	P<0.02
Glyceraldehyde-3-phosphate dehydrogenase	6.2 ± 0.9	8.6 ± 1.7	P<0.005
Lactate dehydrogenase	5.5 ± 1.4	7.0 ± 1.5	
α-hydroxybutyrate dehydrogenase	0.52 ± 0.13	0.65 ± 0.14	
Aspartate aminotransferase	0.094 ± 0.029	0.12 ± 0.04	
β-hydroxyacyl coenzyme A dehydrogenase	0.0026 ± 0.0009	0.0034 ± 0.001	
Succinate dehydrogenase	0.0024 ± 0.0006	0.0031 ± 0.0012	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0031 ± 0.0014	0.0051 ± 0.0012	P<0.02

TABLE 56

Mean enzyme activities in left ventricle of rabbits indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude (8)	High Altitude (8)	
Phosphofructokinase	0.13 ± 0.01	0.15 ± 0.02	P<0.05
Glyceraldehyde-3-phosphate dehydrogenase	0.82 ± 0.04	0.95 ± 0.14	P<0.05
Lactate dehydrogenase	0.95 ± 0.16	1.2 ± 0.1	P<0.05
α-hydroxybutyrate dehydrogenase	1.2 ± 0.1	1.3 ± 0.2	
Aspartate aminotransferase	1.0 ± 0.1	1.3 ± 0.1	P<0.001
β-hydroxyacyl coenzyme A dehydrogenase	0.052 ± 0.01	0.073 ± 0.009	P<0.005
Succinate dehydrogenase	0.041 ± 0.007	0.044 ± 0.007	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0010 ± 0.0004	0.0013 ± 0.0004	

TABLE 57

Mean enzyme activities in right ventricle of rabbits indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude (8)	High Altitude (8)	
Phosphofructokinase	0.11 ± 0.01	0.14 ± 0.02	P<0.005
Glyceraldehyde-3-phosphate dehydrogenase	0.73 ± 0.05	0.91 ± 0.14	P<0.005
Lactate dehydrogenase	0.94 ± 0.16	1.3 ± 0.1	P<0.001
α-hydroxybutyrate dehydrogenase	1.2 ± 0.2	1.3 ± 0.1	
Aspartate aminotransferase	0.91 ± 0.07	1.3 ± 0.1	P<0.001
β-hydroxyacyl coenzyme A dehydrogenase	0.054 ± 0.008	0.076 ± 0.008	P<0.001
Succinate dehydrogenase	0.036 ± 0.006	0.041 ± 0.009	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0010 ± 0.0004	0.0011 ± 0.0003	

activity was unchanged. The αHBDH/LDH ratios were thus lower in the ventricles from the high altitude rabbit (1.32 ± 0.24 and 1.12 ± 0.12 at low and high altitude respectively for the left ventricle, and 1.32 ± 0.27 and 1.05 ± 0.07, $P<0.025$, for the right ventricle) indicating a higher proportion of the M-LDH subunits. The activities of aspartate aminotransferase and βhydroxyacyl coenzyme A dehydrogenase were also higher in the high altitude rabbit myocardium, while the activities of succinate dehydrogenase and mitochondrial glycerol-3-phosphate dehydrogenase were similar to those in low altitude rabbits. The GAPDH/mGPDH ratios were similar in both ventricles at both altitudes (890 ± 290 and 860 ± 460 in the left and right ventricles respectively at low altitude, and 760 ± 180 and 860 ± 270 at high altitude).

DISCUSSION

The experimental design allows several comparisons to be made in addition to that on the basis of altitude. The enzyme activity patterns for the heart and sartorius show the expected variations and illustrate the well known differences in the metabolism of the tissues, with the sartorius having about 5 fold higher phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase activities reflecting the higher anaerobic glycolytic activity of this tissue. The marked difference in the αHBDH/LDH ratios of the tissues illustrates the different proportions of the lactate dehydrogenase subunit types, with the H subunit predominating in the myocardium, and the M subunit predominating in the sartorius muscle. In contrast, the activity of the mitochondrial enzymes succinate dehydrogenase and βhydroxyacyl coenzyme A dehydrogenase are about 15 fold lower in the sartorius than in the myocardium, illustrating the low mitochondrial content and the low capacity for fatty acid oxidation and aerobic metabolism. Of interest is the activity of mitochondrial glycerol-3-phosphate dehydrogenase in relation to that of succinate dehydrogenase. While in the sartorius they are comparable, in the myocardium the activity of

mitochondrial glycerol-3-phosphate dehydrogenase is considerably lower than that of succinate dehydrogenase, especially in the rabbit. This is consistent with the suggestion that the dihydroxyacetone phosphate - glycerol 3 phosphate shuttle is more important for the transfer of reducing equivalents across the mitochondrial membrane in white muscle than in red (Blanchaer 1964, Pette 1966). However, the importance of this cycle in vertebrate muscles has been questioned (Crabtree & Newsholme 1972). An alternative role that has been suggested for mitochondrial glycerol-3-phosphate dehydrogenase in skeletal muscle is in gluconeogenesis from glycerol-3-phosphate (Bass et al. 1969).

The enzyme patterns for each tissue differ between rabbit and guinea pig, and the differences between the patterns for the two tissues are more marked in the rabbit. Despite these differences the ratio GAPDH/mG3PDH is very similar in the two species for either tissue, but the ratio is not the same for both tissues. Pette (1966) proposed that the activity of mitochondrial glycerol-3-phosphate dehydrogenase was related to that of glyceraldehyde-3-phosphate dehydrogenase, having found that the ratio of their activities was the same in several skeletal

muscles from rabbits and rats. The results here support this but indicate that the relationship varies between tissue types.

Acclimatization to a changed environment can be said to be effective if the acclimatized members of the species can survive and reproduce in sufficient numbers to ensure the survival of that species in the new environment. Animals indigenous to high altitude are fully acclimatized members, but in addition, there is the possibility that genetically determined features would have arisen through natural selection operating over the course of many generations. Through selective breeding of cattle, Will and co-workers (1970) have found that the development of pulmonary hypertension at high altitude has a genetic component. It was in order to minimise differences other than those resulting from altitude, that outbred animals available in Lima, Peru were used as low altitude controls.

The larger cardiac size at high altitude was predominantly due to a greater right ventricle. This presumably resulted from the pulmonary hypertension shown by most species at high altitude which in turn increased the work of the right ventricle. Since this stress would be present from birth (Sime et al. 1963), the increase in right

ventricular weight might also be the result of hyperplasia since rat myocytes are capable of mitosis for a short period after birth (Zak 1973). The results of Tappan et al. (1957) suggest that hyperplasia may have occurred in indigenous high altitude guinea pigs as, despite the heart being larger at high altitude, the concentration of DNA per gram wet weight was higher than in the low altitude animals.

Since the two ventricles are subject to individual controls and responses, it would seem preferable to consider them separately. From the results obtained here, it is seen that the enzyme activities are similar in the right and left ventricles of the rabbit but that certain differences occur in the activities in the two ventricles in the guinea pigs. In this latter species it is, therefore, of great importance to consider the ventricles separately: otherwise alterations in activities could arise purely by an increase in the proportion of one ventricle relative to the other and yet be attributed to the stress giving rise to the shift in proportion of the ventricles.

Since the sartorius displays predominantly anaerobic metabolism, it might be considered less susceptible to changes in blood Po_2. It is thus

of interest to note the higher glycolytic activity in the high altitude rabbit sartorius compared with the muscle from low altitude animals, as exemplified by the higher activities of phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase and the correspondingly higher activity of mitochondrial glycerol-3-phosphate dehydrogenase. No differences were found between the guinea pig sartorius from high and low altitude animals and it is possible that those in the rabbit are the result of a different degree of physical activity (Short et al. 1969).

The myocardium is dependent on the presence of oxygen for continued function and so would be more susceptible to the low partial pressure of oxygen prevalent at high altitude. In the rabbit myocardium at high altitude the activities of the glycolytic enzymes were higher than in the sea level animals, which indicates a higher capacity for the production of ATP in the absence of oxygen. This might be of importance for the survival of the animal during stress or severe exercise when the supply of oxygen would be most likely to be limiting. The slightly lower αHBDH/LDH ratio indicates a shift in the proportion of LDH isoenzyme subunits in the high altitude rabbits towards more M-LDH. Sörensen,

Hellung-Larsen & Jensen (1972) studied the brains of two groups of humans, one from La Paz and the other from a low altitude, and found a higher proportion of M subunits in those from high altitude. However, it is not clear what advantage there is in these shifts.

The higher activity of aspartate aminotransferase suggests that the oxaloacetate-malate shuttle may be more active in the high altitude rabbit myocardium. This, coupled with the higher lactate dehydrogenase activity, represents an increase in the main pathways of cytoplasmic utilization of NADH. The higher βhydroxyacyl coenzyme A dehydrogenase activity might reflect a higher capacity for fatty acid oxidation. However, since this enzyme can be rate limiting for fatty acid oxidation under certain conditions (Hull & Radloff 1975) this increase could compensate for a higher $NADH/NAD^+$ ratio which might be prevalent in the mitochondria at high altitude, enabling normal rates of fatty acid oxidation to occur.

The guinea pig, on the other hand, does not display these differences. No significant alterations were found between the enzyme activities in the high and low altitude animals. This suggests that either the provision of oxygen was adequate for the tissue under all normal circumstances, or that the lack of oxygen had not given rise to any specific alterations in the enzymes investigated. Alterations in pathway

fluxes could still occur through variations in effector and substrate concentrations. Valdivia (1958) has shown an increased capillary density in the high altitude guinea pig, and tissue myoglobin was also higher in several species (Reynafarje & Morrison 1962, Tappan & Reynafarje 1957). These adaptations would increase the transfer of oxygen from the blood to the tissues, although no actual measurements are known to indicate how effective they are. In addition Reynafarje (1971) has reported that the kinetic characteristics of the mitochondria may be altered in the high altitude population.

It is thus clear that indigenous animals have adjusted to high altitude in different ways. The differences found between the indigenous high and low altitude populations may be the result of genetic adaptations selected over many generations as advantageous for the survival of the species. Alternatively, the indigenous high altitude animal may just display the responses of a low altitude animal that had developed throughout life at high altitude. Subsequent studies on the acclimatization of low altitude animals to high altitude may clarify this point.

SUMMARY

Indigenous high altitude guinea pigs and rabbits displayed right ventricular hypertrophy, presumeably

in response to hypoxic pulmonary hypertension. No differences between the high and low altitude groups were found in the guinea pig sartorius or myocardium. The activities of the glycolytic enzymes were greater in the high altitude rabbit muscles. In the high altitude rabbit myocardium, the proportion of M-LDH subunits was also higher. The differences between the high and low altitude animals may be the result of genetic adaptation or simply the result of lifelong acclimatization.

ACCLIMATIZATION TO SIMULATED HIGH ALTITUDE

INTRODUCTION

Having studied the fully acclimatized animal, the next aspect of the project was that of the response of low altitude animals to high altitude. So that a comparison could be made with the indigenous animals, guinea pigs were used and were kept under conditions simulating those at which they live naturally. Since during acclimatization the animals became anorexic (Gloster, Heath & Harris 1972, Gold & Costello 1974, Hunter et al. 1974, Koob et al. 1974) it was important to separate the effects of hypoxia from those of anorexia, and so the controls were restricted to that amount of food taken by the hypobaric animals. In addition free fed controls were included to ascertain the effect of the reduced food intake alone. Initially adult animals were used, as this removed the complication of developmental changes being superimposed on acclimatization changes. Subsequently, a study was carried out using immature animals to gain information as to the effect of age on the process of acclimatization. This has been shown to be great with regard to some morphological aspects (Frisancho 1975). The work concentrated on the heart, considering the ventricles separately, but also considered a contrasting muscle, the sartorius.

The methods were set out in an earlier section.

Acclimatization: ADULT GUINEA PIGS

RESULTS

All the animals exhibited anorexia when first exposed to the hypobaric conditions. This loss of appetite gradually lessened and consumption seemed to be normal after 7 - 10 days. The changes in body weight are shown in Figs. 25 and 26 for the adult guinea pigs in groups 1 and 2 which were acclimatized for 2 and 4 weeks respectively. The changes in body weight are expressed as percentages of the initial body weight. The animals in both groups showed a sharp fall in body weight over the first 4 - 5 days. Thereafter, however, those of group 1 continued to lose weight gradually while those of group 2 gained weight. The animals in group 1 were older than those in group 2 (approximately 5 months compared with 3½ months) and this might have been responsible for the difference as it was subsequently found that young guinea pigs acclimatized more rapidly and successfully than adults as judged by body weight changes. The restricted diet controls were given the same amount of food as that taken by the hypobaric animals, and their body weight changes closely parallel those of the experimental animals. Similar observations have been reported in rats (Gloster, Heath & Harris 1974).

FIGURE 25.

Mean percentage change in body weight ± S.E.M. of adult guinea pigs during hypobaric exposure or dietary restriction.

●——● restricted diet

✶——✶ hypobaric

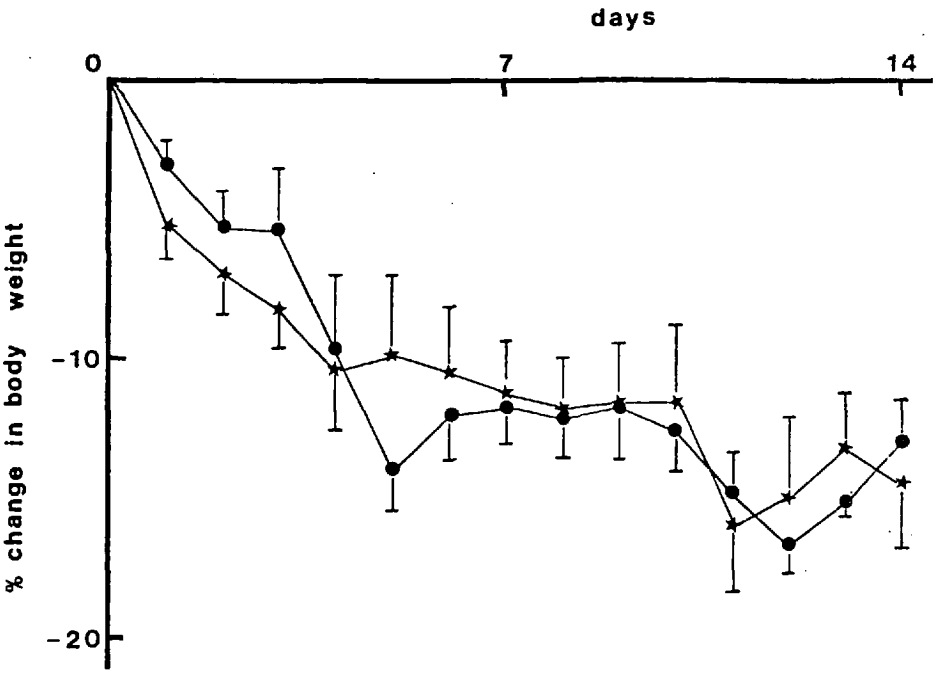
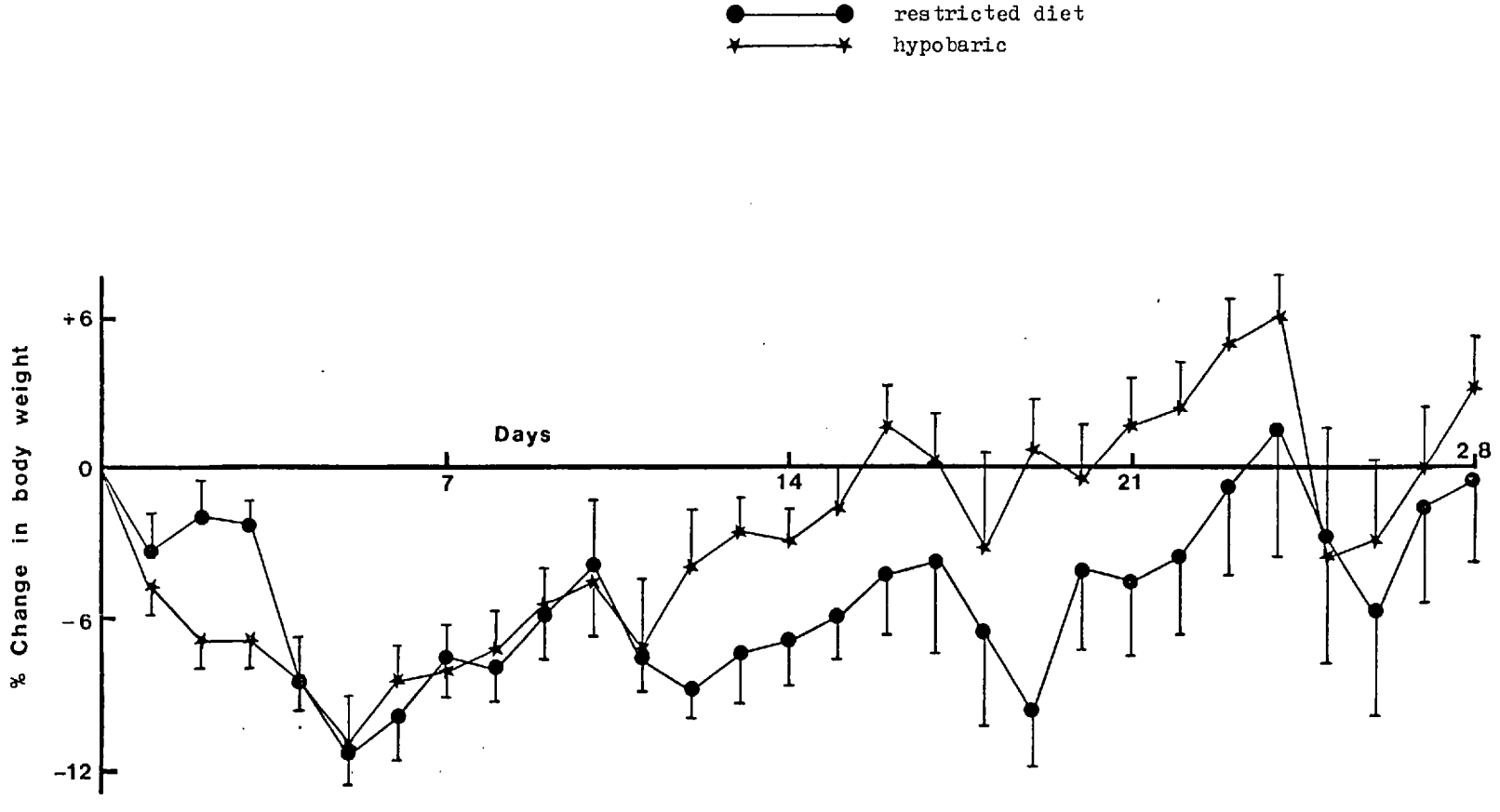


FIGURE 26.

Mean percentage change in body weight ± S.E.M. of adult guinea pigs during hypobaric exposure or dietary restriction.



Despite the parallel body weight changes, the heart weight of the hypobaric animals was higher than that of the controls. Table 58 shows the various parameters measured, and the values of those referred to body weight for comparative purposes. The restricted diet for 14 days caused a significant decrease in the ratio of the right ventricular weight to that of the left ventricle; this seemed to be predominantly due to a reduction in the size of the right ventricle. By 28 days these parameters had returned to normal. Relative to the restricted diet controls, there was a marked increase in the weight of the right ventricle in the hypobaric animals, both in absolute terms and with reference to the body weight. This was presumably due to the hypertrophy induced by the increase in work resulting from the pulmonary hypertension which is usually found in the presence of hypoxia (Dean et al. 1968, Sizemore et al. 1973, Ressl et al. 1974, Burton, Besch & Smith 1968, Grover et al. 1963). After 14 days' exposure to hypobaric conditions the weight of the left ventricle was increased but the ratio of this to the body weight was not significantly greater than that of the restricted diet controls. After 28 days, however, this ratio was higher in the hypobaric group than in the restricted diet controls, indicating that slight hypertrophy had occurred. This has been reported to occur in rats (Sizemore et al. 1973) where again the

TABLE 58

Physiological data for adult guinea pigs.

		2 WEEKS		4 WEEKS	
	Free Fed	Restricted Diet	Hypobaric	Restricted Diet	Hypobaric
Initial body weight (gm)	530 ± 21	726 ± 76	786 ± 55	589 ± 51	545 ± 35 P<0.02
Final body weight (gm)		652 ± 76	696 ± 88	592 ± 71	561 ± 36 P<0.02
Right ventricle (mg)	290 ± 20	310 ± 42	475 ± 61 P<0.001	293 ± 38	468 ± 57 P<0.001
Left ventricle (mg)	598 ± 30	759 ± 101	881 ± 91 P<0.05	626 ± 83	671 ± 68
Right ventricle: body weight (mg:gm)	0.548 ± 0.039	0.481 ± 0.036	0.683 ± 0.063 P<0.001	0.497 ± 0.046	0.834 ± 0.072 P<0.001
Left ventricle: body weight (mg:gm)	1.13 ± 0.07	1.16 ± 0.10	1.28 ± 0.15	1.06 ± 0.12	1.20 ± 0.13 P<0.005
Right ventricle: left ventricle	0.484 ± 0.030	0.418 ± 0.034 P<0.001	0.539 ± 0.038 P<0.001	0.471 ± 0.039	0.700 ± 0.109 P<0.001

Stastical significance was calculated between the free fed and restricted diet groups, and between the hypobaric and appropriate restricted diet groups. The numbers of observations are given in parentheses.

magnitude was less than in the case of the right ventricle.

DIET RESTRICTION

The effects of a restricted diet on the enzyme activities in the myocardium were related to the severity of the restriction. This corresponded to the anorexia shown by the hypoxic animals, being greater during the first 14 days than during the second 14-day period. After 14 days of the restricted diet (comparing Table 59 and Table 60) the ventricular activities of hexokinase, glycogen phosphorylase and succinate dehydrogenase were considerably lower than in the free fed controls ($P<0.001$ except for hexokinase in the right ventricle where $P<0.005$). After 28 days, (comparing Table 59 with Tables 61, 62) the activities of hexokinase and succinate dehydrogenase in the restricted diet group had returned to the normal free fed values, whereas the activity of glycogen phosphorylase had decreased further to a value of only 50% of that in the free fed controls ($P<0.001$). In addition the lactate dehydrogenase activity was higher in the restricted diet group for both ventricles ($P<0.005$) while the αHBDH/LDH ratio was decreased ($P<0.001$), indicating an increased proportion of the M-LDH subunits. Glucose phosphate isomerase activity was slightly higher in both ventricles of the restricted diet group ($P<0.05$ for the right ventricle, $P<0.005$ for the left ventricle),

TABLE 59

Enzyme activities in the ventricles of free fed, normobaric adult guinea pigs, expressed as μmoles substrate transformed/min/mg protein at 25°C, and given as mean ± S.D. with the number of observations in parentheses.

		Right Ventricle	Left Ventricle	
Glycogen phosphorylase	(8)	0.0419 ± 0.0038	0.0431 ± 0.0036	
Phosphoglucomutase	(8)	0.115 ± 0.008	0.132 ± 0.012	P<0.01
Hexokinase	(7)	0.0647 ± 0.0082	0.0684 ± 0.0072	
Glucose-6-phosphate dehydrogenase	(8)	0.00128 ± 0.00022	0.00102 ± 0.00025	
Glucose phosphate isomerase	(7)	0.512 ± 0.037	0.482 ± 0.033	
Phosphofructokinase	(7)	0.106 ± 0.010	0.103 ± 0.007	
Aldolase	(7)	0.0564 ± 0.0034	0.0501 ± 0.0021	P<0.005
Glyceraldehyde-3-phosphate dehydrogenase	(7)	0.701 ± 0.049	0.674 ± 0.045	
3-Phosphoglycerate kinase	(7)	0.737 ± 0.053	0.690 ± 0.046	
Phosphoglyceromutase	(7)	0.789 ± 0.120	0.801 ± 0.110	
Enolase	(7)	0.167 ± 0.014	0.167 ± 0.009	
Pyruvate kinase	(7)	0.577 ± 0.043	0.570 ± 0.046	
Lactate dehydrogenase	(7)	1.06 ± 0.10	0.874 ± 0.076	P<0.005
αHydroxybutyrate dehydrogenase	(7)	0.701 ± 0.037	0.580 ± 0.032	P<0.001
αHydroxybutyrate dehydrogenase / Lactate dehydrogenase	(7)	0.664 ± 0.032	0.667 ± 0.035	
Malate dehydrogenase	(8)	1.34 ± 0.17	1.27 ± 0.13	
Aspartate aminotransferase	(8)	1.10 ± 0.16	0.994 ± 0.131	
βHydroxyacyl-coenzyme A dehydrogenase	(8)	0.0239 ± 0.0032	0.0239 ± 0.0043	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	(8)	0.00201 ± 0.00024	0.00268 ± 0.00018	P<0.001
Succinate dehydrogenase	(8)	0.0314 ± 0.0024	0.0325 ± 0.0025	

TABLE 60

Effect of 14 days at 400 torr on the enzyme activities in the ventricles of adult guinea pigs.

LEFT VENTRICLE

	Hypobaric	Restricted Diet Controls	
Glycogen phosphorylase	0.0332 ± 0.0027	0.0296 ± 0.0031	
Hexokinase	0.0657 ± 0.0053	0.0543 ± 0.0028	P<0.001
Glucose-6-phosphate dehydrogenase	0.00137 ± 0.00072	0.00106 ± 0.00020	
Phosphofructokinase	0.129 ± 0.013	0.113 ± 0.015	
Glyceraldehyde-3-phosphate dehydrogenase	0.914 ± 0.092	0.617 ± 0.038	P<0.001
Lactate dehydrogenase	0.984 ± 0.119	0.939 ± 0.071	
∝Hydroxybutyrate dehydrogenase	0.593 ± 0.059	0.575 ± 0.028	
$\frac{\text{∝Hydroxybutyrate dehydrogenase}}{\text{Lactate dehydrogenase}}$	0.613 ± 0.048	0.616 ± 0.056	
Succinate dehydrogenase	0.0289 ± 0.0030	0.0257 ± 0.0022	

RIGHT VENTRICLE

	Hypobaric	Restricted Diet Controls	
Glycogen phosphorylase	0.0338 ± 0.0027	0.0298 ± 0.0037	
Hexokinase	0.0731 ± 0.0062	0.0530 ± 0.0036	P<0.001
Glucose-6-phosphate dehydrogenase	0.00183 ± 0.00034	0.00126 ± 0.00031	P<0.01
Phosphofructokinase	0.136 ± 0.013	0.108 ± 0.013	P<0.005
Glyceraldehyde-3-phosphate dehydrogenase	1.16 ± 0.11	0.671 ± 0.033	P<0.001
Lactate dehydrogenase	1.35 ± 0.12	1.14 ± 0.09	P<0.005
∝Hydroxybutyrate dehydrogenase	0.758 ± 0.044	0.692 ± 0.040	P<0.02
$\frac{\text{∝Hydroxybutyrate dehydrogenase}}{\text{Lactate dehydrogenase}}$	0.565 ± 0.050	0.611 ± 0.056	
Succinate dehydrogenase	0.0280 ± 0.0021	0.0254 ± 0.0013	P<0.02

Activities are expressed as μ moles substrate transformed/min/mg protein at 25°C, and given as mean ± S.D. for 8 observations.

while aldolase was higher only in the left ventricle of the restricted diet group ($P<0.01$).

EFFECTS OF HYPOBARIC EXPOSURE

With differences being found between the restricted diet and free fed controls, the assessment of the effects of hypobaric exposure was made by comparing the hypobaric group with the restricted diet group. Since both ventricles underwent hypertrophy, although of different degrees, the differences noted will represent the effects of hypertrophy as well as those of hypoxia per se. Table 60 shows the results obtained after exposure for 14 days to the hypobaric conditions. The activities of most of the enzymes measured were increased in the right ventricle of the hypobaric animals, the most notable changes being for hexokinase + 38%, phosphofructokinase + 26%, and glyceraldehyde-3-phosphate dehydrogenase + 73%, and for glucose-6-phosphate dehydrogenase + 45%. These changes suggest an increase in the ability to utilize extracellular glucose via the glycolytic pathway and the pentose phosphate pathway. The latter is important in rapidly synthesizing tissues and would be expected to be more active in a tissue undergoing hypertrophy. Despite the increase in both lactate dehydrogenase and αhydroxybutyrate dehydrogenase

TABLE 61

Effect of 28 days at 400 torr on the enzyme activities in the right ventricle of adult guinea pigs.

		Hypobaric	Restricted Diet Controls	
Glycogen phosphorylase	(8)	0.0271 ± 0.0024	0.0208 ± 0.0026	P<0.001
Phosphoglucomutase	(8)	0.104 ± 0.007	0.107 ± 0.014	
Hexokinase	(8)	0.0817 ± 0.0036	0.0596 ± 0.0049	P<0.001
Glucose-6-phosphate dehydrogenase	(8)	0.00125 ± 0.00048	0.00134 ± 0.00031	
Glucose phosphate isomerase	(8)	0.678 ± 0.044	0.558 ± 0.035	P<0.001
Phosphofructokinase	(8)	0.122 ± 0.007	0.119 ± 0.019	
Aldolase	(8)	0.0674 ± 0.0030	0.606 ± 0.0060	P<0.02
Glyceraldehyde-3-phosphate dehydrogenase	(8)	1.08 ± 0.08	0.697 ± 0.061	P<0.001
3 Phosphoglycerate kinase	(8)	1.07 ± 0.05	0.734 ± 0.054	P<0.001
Phosphoglyceromutase	(8)	0.752 ± 0.155	0.740 ± 0.107	
Enolase	(8)	0.209 ± 0.017	0.180 ± 0.018	P<0.01
Pyruvate kinase	(8)	0.746 ± 0.072	0.630 ± 0.095	
Lactate dehydrogenase	(16)	1.46 ± 0.16	1.26 ± 0.20	P<0.01
∝Hydroxybutyrate dehydrogenase	(16)	0.810 ± 0.105	0.703 ± 0.069	P<0.005
∝Hydroxybutyrate dehydrogenase / Lactate dehydrogenase	(16)	0.577 ± 0.062	0.548 ± 0.055	
Malate dehydrogenase	(8)	1.34 ± 0.15	1.50 ± 0.30	
Aspartate aminotransferase	(8)	0.925 ± 0.091	1.09 ± 0.23	
βHydroxyacyl coenzyme A dehydrogenase	(8)	0.0219 ± 0.0045	0.0267 ± 0.0017	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	(8)	0.00154 ± 0.00021	0.00185 ± 0.00018	P<0.02
Succinate dehydrogenase	(8)	0.0291 ± 0.0031	0.0322 ± 0.0038	

Activities are expressed as μmoles substrate transformed/min/mg protein at 25°C, and are given as mean ± S.D. with the number of observations in parentheses.

activities, the αHBDH/LDH ratio did not change significantly. Succinate dehydrogenase activity was slightly but significantly increased. In the left ventricle only the activities of hexokinase and glyceraldehyde-3-phosphate dehydrogenase increased significantly, and the magnitude of these changes was less than in the right ventricle (21, 48% respectively). Succinate dehydrogenase activity showed the same percentage increase as in the right ventricle, but this was not statistically significant in the left ventricle.

Table 61 shows the results obtained for the right ventricle after 28 days at 400 torr. As after 14 days exposure, hexokinase, glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase and αhydroxybutyrate dehydrogenase activities were increased relative to the restricted diet controls, although the increase in glyceraldehyde-3-phosphate dehydrogenase activity was less marked than after 14 days (54% compared with 73%). The αHBDH/LDH ratio was again unchanged indicating no alteration in the proportion of the LDH isoenzyme subunits. In addition glycogen phosphorylase, glucose phosphate isomerase, 3-phosphoglycerate kinase and enolase were increased suggesting an increased activity of the glycolytic pathway. On the other hand, phosphofructokinase activity was not altered.

TABLE 62

Effect of 28 days at 400 torr on the enzyme activities in the left ventricle of adult guinea pigs.

		Hypobaric	Restricted Diet Controls	
Glycogen phosphorylase	(8)	0.0248 ± 0.0027	0.0230 ± 0.0027	
Phosphoglucomutase	(8)	0.107 ± 0.011	0.123 ± 0.013	
Hexokinase	(8)	0.0737 ± 0.0050	0.0646 ± 0.0035	P<0.005
Glucose-6-phosphate dehydrogenase	(8)	0.00123 ± 0.00042	0.00096 ± 0.00023	
Glucose phosphate isomerase	(8)	0.586 ± 0.031	0.546 ± 0.032	
Phosphofructokinase	(8)	0.128 ± 0.006	0.120 ± 0.019	
Aldolase	(8)	0.0667 ± 0.0067	0.0559 ± 0.0041	P<0.005
Glyceraldehyde-3-phosphate dehydrogenase	(8)	0.871 ± 0.076	0.682 ± 0.056	P<0.001
3 Phosphoglycerate kinase	(8)	0.924 ± 0.096	0.723 ± 0.059	P<0.001
Phosphoglyceromutase	(8)	0.788 ± 0.119	0.784 ± 0.104	
Enolase	(8)	0.196 ± 0.012	0.180 ± 0.013	
Pyruvate kinase	(8)	0.702 ± 0.052	0.626 ± 0.059	P<0.025
Lactate dehydrogenase	(16)	1.14 ± 0.11	1.05 ± 0.11	
αHydroxybutyrate dehydrogenase	(16)	0.685 ± 0.098	0.578 ± 0.062	P<0.005
αHydroxybutyrate dehydrogenase / Lactate dehydrogenase	(16)	0.616 ± 0.067	0.553 ± 0.054	P<0.01
Malate dehydrogenase	(8)	1.34 ± 0.20	1.41 ± 0.21	
Aspartate aminotransferase	(8)	0.901 ± 0.171	0.913 ± 0.153	
βHydroxyacyl coenzyme A dehydrogenase	(8)	0.0214 ± 0.0052	0.0229 ± 0.0047	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	(8)	0.00218 ± 0.00034	0.00254 ± 0.00035	
Succinate dehydrogenase	(8)	0.0294 ± 0.0051	0.0309 ± 0.0047	

Activities are expressed as μmoles substrate transformed/min/mg protein at 25°C, and are given as mean ± S.D. with the number of observations in parentheses.

Glucose-6-phosphate dehydrogenase activity was no longer increased in the hypobaric group suggesting that a stable state of hypertrophy had been achieved. Mitochondrial glycerol-3-phosphate dehydrogenase activity was decreased in the hypobaric group ($P<0.02$) and there was a general trend for all enzymes associated with mitochondria to be lower although the individual differences were not statistically significant.

The results for the left ventricle are shown in Table 62. As before, the changes were less marked in the left than in the right ventricle. The activities of hexokinase and glyceraldehyde-3-phosphate dehydrogenase were again elevated although to a lesser extent than after 14 days (14% & 23% respectively compared with 21% & 48%). The activities of aldolase, 3-phosphoglycerate kinase and αhydroxybutyrate dehydrogenase were also higher and there was a less significant increase in pyruvate kinase activity. The ratio αHBDH/LDH was increased in this ventricle relative to the restricted diet controls but was still lower than in the free fed controls.

DISCUSSION

Gold and Costello (1974) stressed the importance of considering the effects of the anorexia exhibited during the initial period of acclimatization to high altitude. They found that changes in the function of rat heart mitochondria after 7 days hypoxia could be attributed to the low food intake of the animals. The results presented here indicate that the effects are more marked during the initial 2 weeks but that some are present even after 4 weeks. With longer acclimatization the extent and influence of the anorexia would be less but should still be considered in the interpretation of results.

In the present experiment, the choice of free fed rather than restricted diet controls would have greatly affected the conclusions reached regarding the lactate dehydrogenase isoenzyme pattern. When comparing the αHBDH/LDH ratio for individual ventricles from the hypobaric group with the corresponding values from the free fed control group, it appears that there was a significant fall in the ratio in the right ventricle ($P<0.005$, $P<0.001$ after 14, 28 days respectively) but not in the left ventricle. This distinction between the responses of the ventricles would suggest that the change was related to the hypertrophy which had clearly occurred in the right ventricle, but not to the

hypoxia which affects both ventricles. However, when the alterations in dietary intake are considered, no such significant changes are found. This indicates the necessity for considering the effects of alterations in appetite on the parameters measured. The extent to which dietary changes may have affected the myocardial lactate dehydrogenase isoenzyme pattern in earlier investigations on cardiac hypertrophy (Bishop & Altshuld 1970, Fox & Reed 1969, Sobel et al. 1970) or in the earlier acclimatization studies (Anderson & Bullard 1971, Mager et al. 1968, Miller & Hale 1968, Penney 1974) is not clear but may be considerable in the shorter experiments.

As in the study on indigenous guinea pigs, certain differences were consistently found between the enzyme activities in the two ventricles of the control animals. The left ventricle contained higher activities of phosphoglucomutase ($P<0.001$) and mitochondrial glycerol-3-phosphate dehydrogenase ($P<0.001$), and lower activities of lactate dehydrogenase and αhydroxybutyrate dehydrogenase ($P<0.001$ for both), while the ratios αHBDH/LDH were the same in the two ventricles. In addition aldolase activity was lower in the left ventricle ($P<0.01$ for the 28 day restricted diet controls,

$P<0.005$ for the free fed controls), as was glyceraldehyde-3-phosphate dehydrogenase ($P<0.01$ for free fed controls, $P<0.02$ for 14 day restricted diet controls, but $P>0.05$ for 28 day restricted diet controls). Since the ratio between the ventricular weights changed during the exposure to the hypoxic conditions, it was important to consider the ventricles separately especially in relation to these particular enzymes.

Most workers have expressed their results in terms of enzyme activity per gram wet weight, whereas here the results are given as activity per milligram protein. This was considered preferable as the homogenizing procedure tended to exclude fibrous tissue from the homogenate. To ascertain whether this influenced the results, calculations were made of the protein content of the homogenate per gram wet weight of tissue homogenized. These are shown in the Tables. It can be seen that there was little difference between the values for the hypobaric and control animals, except for the 4 week group. In this case expression of the activity in terms of gram wet weight would magnify the differences noted. Most striking is the lower protein content of the homogenates of the right compared with the left ventricle, and this may be related to the homogenizing procedure. Caspari et al. (1975) found

that the collagen concentration/gram dry weight in rabbits was higher in the right ventricle than in the left. This was also found in rats (private communication), and since Gloster et al. (1972) showed that the percentage of water in rat tissues was unaffected by hypoxia or dietary restriction, this would mean that the collagen concentration/gram wet weight was also higher in the right ventricle than in the left. Assuming this was the case for guinea pigs as well as rats, and since fibrous tissue is largely excluded from the homogenate, this could account for the difference in the protein contents of the homogenates. As to the effect this difference has on the activities within the ventricles, expression in terms of wet weight would magnify the distinction between the ventricles for lactate dehydrogenase, αhydroxybutyrate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and aldolase, but diminish the distinction with respect to mitochondrial glycerol-3-phosphate dehydrogenase and phosphoglucomutase.

Under hypobaric conditions both ventricles were subjected to the low partial pressure of oxygen in the blood with which they were perfused. In addition both ventricles underwent hypertrophy, but this was more marked in the right ventricle than in the left. As a result, during the initial stages of

acclimatization alterations in metabolism associated with active hypertrophy and hypoxia would occur whereas, once the hypertrophy had ceased and the tissue size was stable, it is probable that alterations associated with active hypertrophy would also cease and any alteration in metabolism still present would represent responses to hypoxia. Since both ventricles were subjected to equal hypoxic stress, alterations in metabolism in response to hypoxia would be expected to be the same in both ventricles, whereas alterations due to hypertrophy would be expected to be greater in the right ventricle than in the left.

To assess the effects of hypertrophy the ratios of the enzyme activities in the right to those in the left ventricles were calculated, and are shown in Tables 63, 64. The alterations in the ratios for the hypobaric animals compared with those for the normobaric restricted diet controls indicate alterations in enzyme activity specifically associated with hypertrophy. After 2 and 4 weeks exposure to hypobaric hypoxia, there was an increase in the activities of several glycolytic enzymes in the right ventricle relative to the values in the left. The enzymes specifically affected were those involved with the production or utilization of glucose-6-phosphate, together with glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase.

TABLE 63

Effect of 2 weeks at 400 torr on the ratios of enzyme activities in the right ventricle to those in the left ventricle in adult guinea pigs. Mean ± S.D. for 8 observations.

	Hypobaric	Restricted Diet Controls	
Glycogen phosphorylase	1.03 ± 0.12	1.00 ± 0.05	
Hexokinase	1.12 ± 0.11	0.976 ± 0.053	P<0.01
Glucose-6-phosphate dehydrogenase	1.75 ± 0.98	1.25 ± 0.43	
Phosphofructokinase	1.06 ± 0.10	0.964 ± 0.059	P<0.05
Glyceraldehyde-3-phosphate dehydrogenase	1.28 ± 0.13	1.09 ± 0.07	P<0.005
Lactate dehydrogenase	1.40 ± 0.15	1.22 ± 0.06	P<0.005
∝hydroxybutyrate dehydrogenase	1.29 ± 0.11	1.20 ± 0.06	
$\frac{\text{∝hydroxybutyrate dehydrogenase}}{\text{Lactate dehydrogenase}}$	0.922 ± 0.074	0.994 ± 0.043	P<0.05
Succinate dehydrogenase	0.973 ± 0.080	0.995 ± 0.098	

TABLE 64

Effect of 28 days at 400 torr on the ratios of enzyme activities in right ventricle to those in left ventricle of adult guinea pigs. Mean ± S.D. with number of observations in parentheses.

		Hypobaric	Restricted Diet Controls	
Glycogen phosphorylase	(8)	1.10 ± 0.08	0.909 ± 0.113	P<0.005
Phosphoglucomutase	(8)	0.975 ± 0.086	0.864 ± 0.057	P<0.01
Hexokinase	(8)	1.11 ± 0.09	0.924 ± 0.088	P<0.001
Glucose-6-phosphate dehydrogenase	(8)	0.961 ± 0.201	1.44 ± 0.37	P<0.01
Glucose phosphate isomerase	(8)	1.16 ± 0.05	1.02 ± 0.05	P<0.001
Phosphofructokinase	(8)	0.952 ± 0.066	0.999 ± 0.064	
Aldolase	(8)	1.02 ± 0.07	1.08 ± 0.05	
Glyceraldehyde-3-phosphate dehydrogenase	(8)	1.24 ± 0.08	1.02 ± 0.07	P<0.001
Phosphoglycerate kinase	(8)	1.17 ± 0.09	1.02 ± 0.07	P<0.005
Phosphoglyceromutase	(8)	0.947 ± 0.070	0.944 ± 0.053	
Enolase	(8)	1.07 ± 0.07	1.00 ± 0.04	
Pyruvate kinase	(8)	1.06 ± 0.05	1.00 ± 0.08	
Lactate dehydrogenase	(16)	1.28 ± 0.10	1.24 ± 0.09	
αhydroxybutyrate dehydrogenase	(16)	1.19 ± 0.09	1.22 ± 0.08	
$\frac{\alpha\text{hydroxybutyrate dehydrogenase}}{\text{Lactate dehydrogenase}}$	(16)	0.907 ± 0.069	0.994 ± 0.089	P<0.01
Malate dehydrogenase	(8)	1.00 ± 0.08	1.05 ± 0.09	
Aspartate aminotransferase	(8)	1.05 ± 0.13	1.19 ± 0.10	P<0.05
β hydroxyacyl coenzyme A dehydrogenase	(8)	1.03 ± 0.12	1.18 ± 0.26	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	(8)	0.723 ± 0.126	0.733 ± 0.071	
Succinate dehydrogenase	(8)	1.01 ± 0.11	1.06 ± 0.13	

Since glucose-6-phosphate and glyceraldehyde-3-phosphate are both metabolites of glycolysis and the pentose phosphate cycle, any alteration in the flux through either pathway might cause alterations in the enzymes producing or utilising these metabolites. Since not all glycolytic enzymes were affected in this way, it suggests that the alterations were due to an increase in the activity of the pentose phosphate cycle. The results for glucose-6-phosphate dehydrogenase at 2 weeks support this suggestion although the difference in the right to left ventricular ratios is not statistically significant. Unfortunately owing to the low activity of this enzyme in the heart, slight differences in assay blank values cause a relatively large difference in activity values, and this variation is magnified by calculating the activity ratios. Therefore, despite being a key enzyme in the pentose phosphate cycle, glucose-6-phosphate dehydrogenase does not seem to be a sensitive indicator of the cycle activity. After 4 weeks' hypobaric exposure its activity was normal, and the ventricular activity ratio was lower than for the controls, although the other data indicate that hypertrophy was still continuing.

Associated with hypertrophy there was a small shift in the lactate dehydrogenase isoenzyme pattern towards a higher proportion of M-subunits as

indicated by the ventricular ratio of the αHBDH/LDH ratios. This was not apparent by direct comparison of the hypobaric and control animal data, and illustrates the advantage of this type of analysis where the variations between animals are eliminated. It is also of interest that although the mean αHBDH/LDH ratios were affected by the reduction in the diet, the ventricular ratio of this was not, and was the same as in the free fed controls which was 0.996 ± 0.025.

Although glucose-6-phosphate dehydrogenase activity does not appear to be a sensitive indicator of hypertrophy, it does appear that certain of the enzymes specifically altered by hypertrophy may be. The most suitable enzymes seem to be hexokinase, glucose phosphate isomerase and glyceraldehyde-3-phosphate dehydrogenase, especially the last one. After 2 weeks exposure, when active hypertrophy was occurring in the right ventricle, these enzymes were increased considerably more than the other glycolytic enzymes which were specifically affected by hypertrophy. This was also found after 4 weeks exposure although the percentage increases were less than after 2 weeks. In the left ventricle, a similar differential increase was found at 2 weeks. Although at this time there was no statistically significant increase in the left ventricular weight

to body weight ratio, by 4 weeks there was, indicating that hypertrophy had been occurring in the left ventricle although to a lesser degree than in the right ventricle. Given that these enzymes can indicate an activated pentose phosphate pathway, the lack of a differential increase in these enzymes in the left ventricle after 4 weeks suggests that, in this case, hypertrophy had ceased and that any alterations in enzyme activities still present would be in response to hypoxia.

At this time, the left ventricle of the hypobaric guinea pig showed an increased glycolytic capacity and no change in mitochondrial enzyme activities. However, there had been a small increase in succinate dehydrogenase activity after 2 weeks exposure but this had been statistically significant in the right ventricle only. Since there had been no alteration in the ventricular activity ratio, it suggests that the transient increase in activity was in response to hypoxia. This behaviour of succinate dehydrogenase is consistent with the reports of Vergnes (1973) and Walpurger et al. (1970) on the acclimatization of rats to hypobaric hypoxia. There was a slight shift in the isoenzyme pattern of lactate dehydrogenase that could be attributed to hypoxia. In contrast to previous reports, this was towards a greater proportion of H-LDH subunits in the adult animals.

SUMMARY

It is important to distinguish the effects of anorexia and hypertrophy from those of hypoxia when analysing the effects of exposure to hypobaric hypoxia. A reduced diet resulted in a decrease in the activities of most glycolytic enzymes in the myocardium and in the proportion of H-LDH subunits. The extent of the changes was dependent on the severity of the reduction in the diet.

Hypertrophy occured in both ventricles, but was more marked in the right ventricle. After 4 weeks hypobaric hypoxia, hypertrophy was still continuing in the right ventricle but seemed to have ceased in the left. The enzymes affected were those whose reactions involved metabolites common to the glycolytic and pentose phosphate pathways. No changes were found in the mitochondrial enzymes. Associated with hypertrophy was a shift in the lactate dehydrogenase isoenzyme pattern with an increase in the proportion of M-LDH subunits.

Hypoxia itself induced an increase in the activities of the glycolytic enzymes but did not increase the proportion of M-LDH subunits as did dietary restriction or hypertrophy. There was a transient increase in the activity of succinate dehydrogenase, but no other mitochondrial enzymes were affected.

Acclimatization: YOUNG GUINEA PIGS

RESULTS

The effect of exposure to the hypobaric conditions on the body weight of the animals is shown in Figure 27, with the changes expressed as percentages of the initial body weight. The initial fall was more marked than with the adults but the subsequent gain in weight occurred much earlier so that the minimal weight occurred at 2 days. The body weights of the restricted diet group did not fall as much as those of the hypobaric group, despite the identical food intake. It is possible that reduced fluid intake and increased fluid loss under the hypoxic conditions (Koob et al. 1974, Fregly,Lutherer & Tyler 1974, Picon-Réategui et al. 1953) were not matched in the controls. Thereafter the gain in weight was slightly higher in the controls with the result that there was a significant difference in body weight at the end of the experiment (Table 65). Both ventricles increased relative to the body weight in the hypobaric animals, but this was more marked for the right ventricle with the resulting alteration in the ratio of the ventricular weights (Table 65). The change in the right ventricle was considerably more evident in the young animals than in the adults after the same stress (c.f. Table 58).

The enzyme activities in the ventricles of the hypobaric and control guinea pigs are shown in

FIGURE 27.

Mean percentage change in body weight ± S.E.M. of young guinea pigs during hypobaric exposure or dietary restriction.

●——● restricted diet

✱——✱ hypobaric

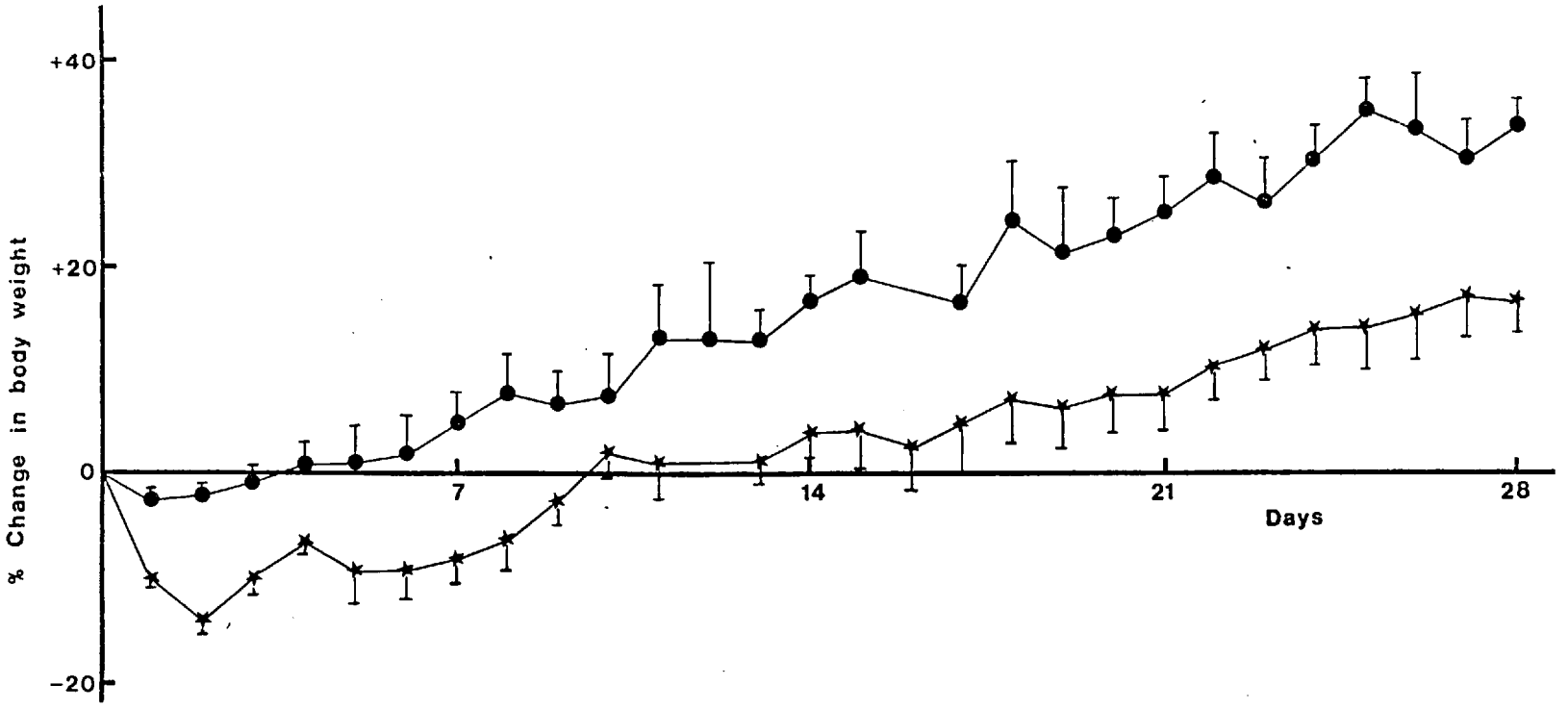


TABLE 65

Physiological data for young guinea pigs.

	Free Fed (5)	Restricted Diet (12)	Hypobaric (12)	
Initial body weight (gm)	277 ± 17	284 ± 20	284 ± 15	
Final body weight (gm)	393 ± 49	379 ± 27	331 ± 28	P<0.001
Right ventricle (mg)	210 ± 18	218 ± 43	449 ± 77	P<0.001
Left vetricle (mg)	496 ± 76	480 ± 70	525 ± 56	
Right ventricle: final body weight (mg:gm)	0.539 ± 0.037	0.572 ± 0.074	1.36 ± 0.24	P<0.001
Left ventricle: final body weight (mg:gm)	1.26 ± 0.07	1.26 ± 0.13	1.59 ± 0.12	P<0.001
Right ventricle: left ventricle	0.429 ± 0.044	0.454 ± 0.047	0.854 ± 0.121	P<0.001

Data are expressed as mean ± S.D. with the number of observations in parentheses. Statistical significance was calculated for the free fed or hypobaric groups compared with the restricted diet group.

Table 66. Lactate dehydrogenase activity was again higher in the right ventricle ($P<0.001$ for the controls) as was αhydroxybutyrate dehydrogenase activity ($P<0.001$) and glyceraldehyde-3-phosphate dehydrogenase ($P<0.001$). In addition the activity of phosphofructokinase was also higher ($P<0.001$). The effects of the hypobaric conditions were similar in both ventricles. In the right ventricle the activities of hexokinase and glyceraldehyde-3-phosphate dehydrogenase were significantly increased while those of phosphofructokinase and αhydroxybutyrate dehydrogenase were significantly reduced. The last result, coupled with a slight rise in total lactate dehydrogenase activity, resulted in a significantly lower ratio of αHBDH/LDH. In the left ventricle hexokinase activity was increased but this was not statistically significant. Glyceraldehyde-3-phosphate dehydrogenase activity was also increased but to a lesser extent than in the right ventricle. Lactate dehydrogenase activity was significantly higher while there was little change in the activity of αhydroxybutyrate dehydrogenase, again giving rise to a lower αHBDH/LDH ratio than in the controls.

The enzyme activities in the young animals were similar to those in the adults after corresponding experimentation. However, the activity of glyceraldehyde-3-phosphate dehydrogenase was lower in the young in each situation, although the increase with hypobaric hypoxia was similar to that shown by the adults. The activity of lactate dehydrogenase

TABLE 66

Effect of 28 days at 400 torr on the enzyme activities of the ventricles of young guinea pigs, expressed as μmoles substrate transformed/min/mg protein at 25°C, and given as mean ± S.D. for 8 observations.

RIGHT VENTRICLE	Hypobaric		Restricted Diet Control
Hexokinase	0.0703 ± 0.0063	P<0.005	0.0587 ± 0.0031
Phosphofructokinase	0.113 ± 0.008	P<0.01	0.132 ± 0.014
Glyceraldehyde-3-phosphate dehydrogenase	0.933 ± 0.136	P<0.001	0.599 ± 0.046
Lactate dehydrogenase	1.61 ± 0.17		1.45 ± 0.19
∝Hydroxybutyrate dehydrogenase	0.690 ± 0.039	P<0.001	0.792 ± 0.076
$\frac{\text{∝Hydroxybutyrate dehydrogenase}}{\text{Lactate dehydrogenase}}$	0.434 ± 0.035	P<0.001	0.549 ± 0.032

LEFT VENTRICLE	Hypobaric		Restricted Diet Control
Hexokinase	0.0676 ± 0.0047		0.0603 ± 0.0055
Phosphofructokinase	0.121 ± 0.012		0.114 ± 0.012
Glyceraldehyde-3-phosphate dehydrogenase	0.742 ± 0.073	P<0.001	0.560 ± 0.055
Lactate dehydrogenase	1.35 ± 0.10	P<0.025	1.17 ± 0.16
∝Hydroxybutyrate dehydrogenase	0.617 ± 0.030		0.639 ± 0.067
$\frac{\text{∝Hydroxybutyrate dehydrogenase}}{\text{Lactate dehydrogenase}}$	0.457 ± 0.025	P<0.001	0.551 ± 0.022

tends to be higher in the young throughout, while the ∝hydroxybutyrate dehydrogenase activity varied in accordance with the ratio of ∝HBDH/LDH.

Since changes in enzyme activity can result from changes other than in the enzyme content of the tissue (Greengard & Feigelson 1961, Kenney 1960, Susheela & Ramasarma 1973), mixing studies were carried out. The homogenates from the hypobaric and normobaric young animals were studied with respect to the enzymes hexokinase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, lactate and ∝hydroxybutyrate dehydrogenases. The results are shown in Table 67 and indicate that the activities measured in the mixtures were as predicted from the activities in the individual homogenates.

TABLE 67

Effect of mixing homogenates from hypobaric and control animals. Activities are expressed as μmoles substrate transformed/min/mg protein at 25°C, and are given as mean ± S.D. for 8 observations.

RIGHT VENTRICLE	Mixture	Calculated Mixture Activity
Hexokinase	0.0666 ± 0.0044	0.0660 ± 0.0049
Phosphofructokinase	0.123 ± 0.006	0.121 ± 0.006
Glyceraldehyde-3-phosphate dehydrogenase	0.722 ± 0.062	0.715 ± 0.058
Lactate dehydrogenase	1.55 ± 0.13	1.53 ± 0.15
αHydroxybutyrate dehydrogenase	0.738 ± 0.047	0.739 ± 0.044

LEFT VENTRICLE	Mixture	Calculated Mixture Activity
Hexokinase	0.0633 ± 0.0036	0.0640 ± 0.0043
Phosphofructokinase	0.118 ± 0.006	0.118 ± 0.006
Glyceraldehyde-3-phosphate dehydrogenase	0.623 ± 0.041	0.624 ± 0.045
Lactate dehydrogenase	1.29 ± 0.08	1.26 ± 0.10
αHydroxybutyrate dehydrogenase	0.624 ± 0.033	0.627 ± 0.032

DISCUSSION

The responses of several features to hypobaric hypoxia vary with the age of the animal, both in terms of the magnitudes and the time courses of the responses. Upon initial exposure to simulated high altitude, the body weight of the young guinea pigs dropped more markedly than that of the adults, but the subsequent gain in weight occurred more rapidly in the younger animals and seemed absent in the oldest group which were only 5 months old. The degree of hypertrophy of the right ventricle was greater in the young animals than in the adult ones, suggesting that the stimulus for hypertrophy was greater in the young guinea pigs, or that they were more responsive. In some species the young display greater pulmonary hypertension than the adults in response to the same hypoxic stimulus (Vogel et al. 1963, Sime et al. 1963), which might account for the distinction observed.

Flückiger and Verzar (1955) measured the rectal temperature of rats during chronic exposure to the simulated altitude of 6000 m and found that its restoration to normal was more rapid in the young and was absent in rats aged 10 months or more. They reported, however, that the haemopoietic response was the same in all ages, but Martin et al. (1975) have shown that this response had declined in rats

aged 40 months. Atland and Highman (1964) found that the survival of rats under conditions of acute severe hypoxia varied in a more complex manner, and increased with age within the range 2 - 10 months.

The changes in enzyme activities seen with the young animals (aged 6 weeks) were similar to those occurring with the adults. Comparison of the ventricular activity ratios of the hypobaric and control animals, shown in Table 68, again indicates an increase in glyceraldehyde-3-phosphate dehydrogenase activity specifically associated with hypertrophy. This ccupled with the slight increase in hexokinase activity and the decrease in phosphofructokinase activity suggests an activation of the pentose phosphate pathway.

The data indicate that, as with the adult animals, hypertrophy of the right ventricle had not ceased after 4 weeks of hypobaric exposure. In addition, since the activity of glyceraldehyde-3-phosphate dehydrogenase the left ventricle was more elevated than the activities of the other enzymes, it appeared that the hypertrophy of this ventricle although less marked than that of the right ventricle had also not ceased. Therefore, with the young animals neither ventricle displayed the effects of hypoxia alone.

TABLE 68

Effect of 4 weeks at 400 torr on the ratios of the enzyme activities in right ventricle to those in left ventricle of young guinea pigs. Mean ± S.D. for 8 observations.

	Hypobaric	Restricted Diet Controls	
Hexokinase	1.04 ± 0.07	0.978 ± 0.057	
Phosphofructokinase	0.935 ± 0.041	1.16 ± 0.07	P<0.001
Glyceraldehyde-3-phosphate dehydrogenase	1.26 ± 0.13	1.07 ± 0.04	P<0.005
Lactate dehydrogenase	1.19 ± 0.09	1.25 ± 0.08	
∝hydroxybutyrate dehydrogenase	1.12 ± 0.05	1.24 ± 0.07	P<0.001
$\frac{\text{∝hydroxybutyrate dehydrogenase}}{\text{Lactate dehydrogenase}}$	0.949 ± 0.047	0.996 ± 0.041	

As with the adult animals there was a decrease in the proportion of H-LDH in response to hypertrophy. However, in addition to this, there was a more pronounced difference for both ventricles between the proportions of the lactate dehydrogenase subunits within the hypobaric and control animals, as indicated by the αHBDH/LDH ratios. This difference may be related to the effects of hypoxia. However, since during postnatal development the proportions of H-LDH subunits normally increases in the heart (Mager et al. 1968, Markert & Ursprung 1962), it is possible that the change in subunit proportions occurring here was not due to an increase in the M-LDH subunits in response to hypobaric exposure but to the lack of a normal developmental decrease. However, as shown in the next section, the αHBDH/LDH ratios in the left and right ventricles of normobaric guinea pigs aged 1 month were 0.636 ± 0.090, and 0.594 ± 0.070 respectively, which is nearly as high as in the adult. It seems, therefore, reasonable to assume that the ratios in the myocardium of the animals used in this experiment were at least that of the month old animals. Consequently, it would appear that the restricted diet and hypertrophy caused a decrease in the ratio in the ventricles as was the case with the adult animals, and that hypoxia had resulted in a further

decrease in the ratio which was not found in the adults. It is not clear why the adult animals did not show this shift in the enzyme ratio. The adult heart certainly had the capacity to change the ratio as is shown by the effect of dietary restriction alone. The change in the ratio in the immature animals under hypobaric conditions occurred to approximately the same degree in both ventricles, suggesting that it was a response to the hypoxia and not associated specifically with hypertrophy of the myocardium. Such a response seems to be lost in the adult.

The mixing studies indicated that the changes in the enzyme activities were not due to changes in dissociable activator or inhibitor concentrations. Greengard & Feigelson (1961) showed that an increase in activity of tryptophan pyrrolase could be brought about by the increased saturation of the enzyme by an activator which was not freely dissociable. This was not indicated by mixing studies. In the case of the enzymes investigated here, there are no such activators, and so the changes in activity observed are most probably due to changes in the enzyme content of the tissue. This would seem the most suitable method for maintaining an altered capacity of a given pathway within a tissue for a period of time which is long in terms of the half-lives of the enzymes concerned (Dölken & Pette 1974), as it has the advantage of selectivity.

SUMMARY

The young animals exposed to the hypobaric environment displayed many of the responses shown by the adult animals. However, in addition there was a marked increase in the proportion of M-LDH subunits in response to hypoxia, which had not been shown by the adult animals. Mixing studies indicated that the changes in the enzyme activities found were the result of changes in the enzyme concentration in the tissue.

Acclimatization: SARTORIUS MUSCLE

RESULTS

This tissue was studied only in the adult guinea pigs, after 28 days exposure to the hypobaric conditions. Table 69 shows the enzyme activities measured. No alterations were found as a result of the hypobaric exposure.

DISCUSSION

The enzyme activities measured reflect the metabolic pattern of the tissue. The activities of the glycolytic enzymes were high in comparison with the values for the heart and especially in comparison with the values for the enzymes of or associated with the mitochondria, indicating the predominance of anaerobic glycolysis over oxidative metabolism.

No previous work is known on the effects of hypobaric hypoxia on "white" muscle although other skeletal muscles have been investigated. Adaptation of rats to hypoxia has been associated with a decrease in the oxygen consumption after 10 weeks to 10 months (Duckworth 1961) and an increase in glycolytic activity of the diaphragm after up to 26 days (McGrath, Bullard & Komives 1969), while Ullrick et al. (1956) reported that no change was present after 3 - 5 months. Altman et al. (1969) reported that the proportion of

TABLE 69

Effects of 28 days at 400 torr on the enzyme activities in the sartorius muscle of adult guinea pigs. Activities are expressed as μmoles substrate transformed/min/mg protein at 25°C, and given as mean ± S.D. with the number of observations in parentheses

	Hypobaric		Restricted Diet Controls	
Glucose-6-phosphate dehydrogenase	0.00038 ± 0.00029	(8)	0.00039 ± 0.00029	(5)
Phosphofructokinase	0.247 ± 0.032	(8)	0.234 ± 0.034	(5)
Glyceraldehyde-3-phosphate dehydrogenase	2.88 ± 0.23	(8)	2.63 ± 0.26	(5)
Lactate dehydrogenase	3.16 ± 0.51	(8)	3.22 ± 0.29	(5)
∝Hydroxybutyrate dehydrogenase	0.443 ± 0.065	(8)	0.428 ± 0.030	(5)
∝Hydroxybutyrate dehydrogenase / Lactate dehydrogenase	0.141 ± 0.008	(8)	0.134 ± 0.008	(5)
Malate dehydrogenase	0.0788 ± 0.010		0.0715 ± 0.0110	(5)
Aspartate aminotransferase	0.0792 ± 0.0099	(8)	0.0831 ± 0.0098	(5)
β Hydroxyacyl coenzyme A dehydrogenase	0.00118 ± 0.00038	(7)	0.00111 ± 0.00045	(5)
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.00186 ± 0.00040	(8)	0.00176 ± 0.00045	(5)
Succinate dehydrogenase	0.00162 ± 0.00040	(8)	0.00159 ± 0.00044	(5)
GAPDH/mG3PDH	1630 ± 170	(8)	1560 ± 100	(5)

M-LDH subunits was elevated in the skeletal muscle of humans suffering from the tetralogy of Fallot. None of these adaptations was observed in the sartorius of guinea pigs. Acclimatization to high altitude has also been associated with increased myoglobin in the skeletal muscles of rats (Costa & Taquini 1970, Anthony, Ackerman & Strother 1959, Criscuolo, Hale & Mefford 1958) although the time course of the adaptation varied. Possibly these alterations are associated with the "red" muscle fibres for which oxidative metabolism is normally more important (Peter et al. 1972, Bass et al. 1969, Staudte & Pette 1972).

Since in "white" fibres the energy is produced predominently by anaerobic glycolysis and the oxygen consumption of the fibres is small (Ogata 1960), it is not unexpected that a decreased supply of oxygen should have little or no effect, whereas a tissue which is more dependent on oxygen for normal energy production, for instance the "red" skeletal fibres or the heart, should be more affected.

SUMMARY

Unlike the myocardium, the sartorius muscle of adult guinea pigs displayed no alterations following acclimatization to the hypobaric environment.

ACCLIMATIZATION DURING POSTNATAL DEVELOPMENT

INTRODUCTION

In the preceding sections it has been shown that acclimatization of guinea pigs to hypobaric conditions is associated with increases in the activities of some glycolytic enzymes in the myocardium. Indigenous high altitude animals do not have elevated activities of these enzymes. The indigenous animals may be genetically adapted to the altitude, but the observations may be due to acclimatization from the moment of birth, since the response has been shown to depend on the age of the animal at which exposure occurs. To clarify this point experiments were designed in which guinea pigs were to be born and to develop postnatally under the hypobaric conditions within the chamber, and the activities of a range of enzymes were to be measured in the ventricles at different times after birth. Within the uterus the partial pressure of oxygen is low and the metabolism of the foetus is suited to a state of hypoxia. During hypoxic postnatal development this state might be maintained with the suppression of the changes normally occurring after birth in response to the normoxic environment. The enzyme activities were therefore also measured in the foetal ventricles at 59 days to ascertain the

changes occurring immediately prior to and after birth. It was considered important that the animals should be born at simulated high altitude and not placed under these conditions after birth as several developmental changes are triggered during the initial hours after birth (Greengard 1971, Hallman 1971). To avoid any effects of the hypobaric environment on the course of foetal development, the pregnant sows were placed in the chamber toward the end of their pregnancy. The exact stages of pregnancy at which this was carried out were varied in an attempt to obtain a high percentage of live births. As it was impossible to monitor the amount of milk taken by the hypobaric newborn animals, no attempt was made to restrict the amount taken by the normobaric ones.

METHODS

The homogenization procedure and enzyme assays were as described earlier. The pregnant sows were accustomed to the chamber and then the pressure was gradually lowered over a period of days. Normal atmospheric pressure was re-established for animal maintenance only every other day to reduce any stress of recompression and decompression on the animals.

RESULTS

Initially the animals were gradually acclimatized to 400 torr but the percentage of live births was low while the number of pregnant sows dying before term was high. The conditions were, therefore, ameliorated to 460 torr which simulates the altitude of 13000'. At both simulated altitudes, the animals were placed in the chamber at different stages of pregnancy, varying from 44 days to 56 days, in an effort to find the time most conducive to live birth. The data shown in table 70 indicate that, despite the longer more gradual acclimatization to simulated altitude, when the sows were placed in the chamber at the earlier stages of pregnancy the percentage of live births was lower than when the animals were placed in the chamber near to term.

Table 71 shows the data on the successful births. The duration of pregnancy was reduced under the hypobaric conditions ($P<0.05$ for the combined hypobaric groups). The pregnant sows exposed to 400 torr barometric pressure had been obtained from a different supplier from those exposed to 460 torr, and this may account for the smaller litter size in this group. The proportion of stillborn animals in the litters were higher under the hypobaric conditions than at normal barometric pressure. The death rate during the first 5 days was also higher at simulated

TABLE 70

Effect of hypobaric hypoxia on the outcome of pregnancy. Numbers in parentheses give the results as percentages of the number of sows used.

Stage of pregnancy upon exposure	Number of sows used	Number of sows dying	Number of sows producing only stillborn young	Number of sows producing live young
		400 TORR		
44 days	5	2 (40)	3 (60)	0 (0)
50 days	10	4 (40)	5 (50)	1 (10)
56 days	8	2 (25)	2 (25)	4 (50)
		460 TORR		
50 days	14	3 (21)	4 (29)	7 (50)
56 days	10	1 (10)	1 (10)	8 (80)
		760 TORR		
-	17	3 (18)	1 (6)	13 (76)

TABLE 71

Data on the births of litters containing live young.

	BAROMETRIC PRESSURE		
	760 torr	460 torr	400 torr
Number of litters	13	15	5
Duration of pregnancy (days)	64.2 ± 4.4	61.0 ± 2.7	61.6 ± 2.8
Mean litter size	4.5	4.1	2.2
Mean % stillborn per litter	16	32	18
Deaths before the age of 5 days (% live at birth)	16	69	44

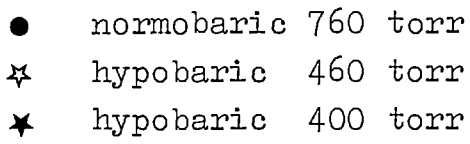
altitude, especially at 460 torr. In some cases the animals would suddenly deteriorate and die. With the normobaric group, there was a similar death rate during the first 5 days to that during the following 10 days.

The mean body weights at birth were similar at all barometric pressures (82.6 $\pm$ 14.4 at 760 torr, 84.4 $\pm$ 22.9 at 400 torr, and 75.8 $\pm$ 8.4 at 460 torr). The growth curves are shown in figure 28. The increase in body weight at 400 torr and at 460 torr was similar to that seen in those animals developing at 760 torr. The results for the hypobaric animals have subsequently been pooled.

The physiological data for the animals are shown in Tables 72 and 73. During normobaric development the left ventricle increased in weight more than the right ventricle, with a resulting fall in the ratio of the right ventricular weight to that of the left ventricle. The body weight increased proportionally more than either ventricle causing a decrease in the ratios of individual ventricular weight to body weight. Under hypobaric conditions, however, both ventricles increased equally with the result that the ventricular ratio was little changed, and was similar to that of the foetus. At birth there was no significant difference between the values for the normobaric and hypobaric animals but by 14 days of age the right

FIGURE 28 .

Mean gain in weight during postnatal development under normobaric and hypobaric conditions .



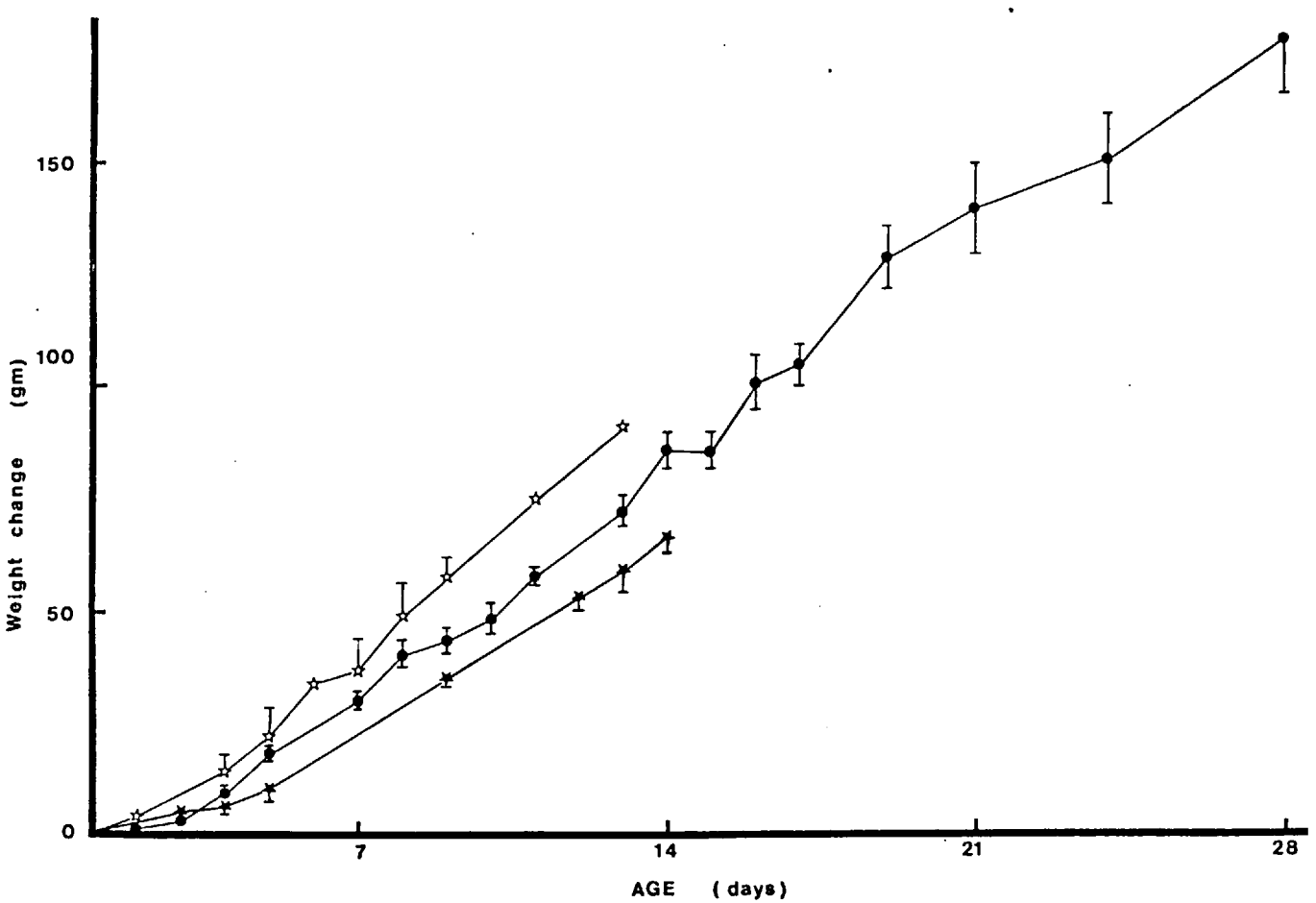

150
100
50
0
Weight change (gm)
7
14
21
28
AGE (days)

TABLE 72

Physiological data for guinea pigs during normobaric development.

	Foetal	Newborn	14 days	28 days
Body weight (gm)		82.6 ± 14.4 (30)	166.7 ± 24.3 (18)	240.8 ± 48.6 (10)
Right ventricle (mg)	92 ± 18 (6)	122 ± 22 (6)	128 ± 19 (6)	157 ± 34 (6)
Left ventricle (mg)	110 ± 19 (6)	150 ± 26 (6)	237 ± 72 (6)	336 ± 83 (6)
Right ventricle: body weight (mg:gm)		1.35 ± 0.23 (6)	0.709 ± 0.037 (6)	0.579 ± 0.029 (6)
Left ventricle: body weight (mg:gm)		1.64 ± 0.23 (6)	1.29 ± 0.29 (6)	1.23 ± 0.07 (6)
Right ventricle: left ventricle	0.829 ± 0.052 (6)	0.817 ± 0.068 (6)	0.569 ± 0.100 (6)	0.470 ± 0.024 (6)

Data are given as means ± S.D. with the number of observations in parentheses.

TABLE 73

Physiological data for guinea pigs during hypobaric postnatal development

	Newborn		14 days	
Body weight (gm)	79.7 ± 16.3	(11)	156.7 ± 31.6	(6)
Right ventricle (mg)	107 ± 18	(7)	214 ± 32	(4)
Left ventricle (mg)	126 ± 21	(7)	267 ± 44	(4)
Right ventricle: body weight (mg:gm)	1.51 ± 0.09	(7)	1.25 ± 0.30	(4)
Left ventricle: body weight (mg:gm)	1.80 ± 0.20	(7)	1.53 ± 0.13	(4)
Right ventricle: left ventricle	0.847 ± 0.081	(7)	0.822 ± 0.188	(4)

Data are given as means ± S.D. with the number of observations in parentheses.

ventricle was significantly greater in the hypobaric animals, as shown by the difference in the ratio of the right ventricle to the body weight ($P<0.005$). The development of the left ventricle was also greater in the hypobaric groups but to a lesser extent.

NORMOBARIC ANIMALS

FOETUS (59 DAYS)

The enzyme activities are shown for the left and right ventricles in Tables 74 and 75, while figures 29 - 32 display the data for the left and right ventricles expressed as percentages of the adult values obtained earlier. The pattern of developmental changes was similar for both ventricles. In the foetus the enzyme activities were similar in both ventricles and those of hexokinase and glycolytic enzymes were high relative to the adult state, while glycogen phosphorylase activity was somewhat lower. The ratio of αHBDH/LDH was low indicating a high proportion of M-LDH subunits. In contrast the mitochondrial enzyme activities were low, as were malate dehydrogenase and aspartate aminotransferase activities. Glucose-6-phosphate dehydrogenase activity was high as anticipated in a rapidly growing tissue.

NEWBORN

Certain changes in pathway capacity were apparent in the newborn. The glycolytic enzymes showed lower

TABLE 74

Enzyme activities in the left ventricle during normobaric development.

	Foetal (6)	Newborn (6)	14 days (6)	28 days (6)
Glycogen phosphorylase	0.0355 ± 0.0041	0.0326 ± 0.0029	0.0324 ± 0.0019	0.0338 ± 0.0027
Hexokinase	0.117 ± 0.019	0.120 ± 0.007	0.0918 ± 0.0069 P<0.005	0.0936 ± 0.0047 P<0.001
Phosphofructokinase	0.133 ± 0.014 P<0.05	0.110 ± 0.014	0.121 ± 0.007	0.130 ± 0.018
Glyceraldehyde-3-phosphate dehydrogenase	1.07 ± 0.15	0.918 ± 0.061	0.726 ± 0.061 P<0.001	0.735 ± 0.051 P<0.001
Lactate dehydrogenase	1.93 ± 0.31	1.64 ± 0.16	1.27 ± 0.16 P<0.005	1.18 ± 0.17 P<0.005
αHydroxybutyrate dehydrogenase	0.746 ± 0.096	0.836 ± 0.029	0.667 ± 0.028 P<0.001	0.716 ± 0.053 P<0.005
αHydroxybutyrate dehydrogenase / Lactate dehydrogenase	0.392 ± 0.063 P<0.01	0.513 ± 0.043	0.531 ± 0.051	0.636 ± 0.090 P<0.025
Glucose-6-phosphate dehydrogenase	0.00167 ± 0.00069	0.00135 ± 0.00016	0.00127 ± 0.00030	0.00113 ± 0.00018
Malate dehydrogenase	0.968 ± 0.132 P<0.005	1.35 ± 0.18	1.31 ± 0.13	1.34 ± 0.20
Aspartate aminotransferase	0.844 ± 0.136 P<0.005	1.16 ± 0.09	1.03 ± 0.07 P<0.025	1.03 ± 0.14
βHydroxyacyl coenzyme A dehydrogenase	0.0214 ± 0.0049 P<0.025	0.0321 ± 0.0075	0.0308 ± 0.0052	0.0369 ± 0.0094
Palmityl carnitine - DTNB transferase	0.00183 ± 0.00037	0.00291 ± 0.00118	0.00597 ± 0.00104 P<0.005 (5)	0.00496 ± 0.00175 (5)
^{14}C exchange	0.00135 ± 0.00043	0.00200 ± 0.00044 (3)	0.00359 , 0.00261 (2)	0.00344 , 0.00304 (2)
Succinate dehydrogenase	0.0193 ± 0.0035 P<0.001	0.0352 ± 0.0075	0.0314 ± 0.0026	0.0325 ± 0.0048
Protein (mg/gm wet wt.)	135 ± 15	141 ± 12	137 ± 8	142 ± 9

Statistical significance was calculated comparing the age groups with the newborn group.
Date are given as means ± S.D. with the number of observations in parentheses.

TABLE 75

Enzyme activities in the right ventricles of guinea pigs during normobaric development.

	Foetal (6)	Newborn (6)	14 days (6)	28 days (6)
Glycogen phosphorylase	0.0342 ± 0.0038	0.0321 ± 0.0024	0.0311 ± 0.0025	0.0325 ± 0.0037
Hexokinase	0.114 ± 0.009	0.112 ± 0.006	0.0889 ± 0.0020 P<0.001	0.0870 ± 0.0051 P<0.001
Phosphofructokinase	0.121 ± 0.011	0.0951 ± 0.0179	0.130 ± 0.006 P<0.005	0.152 ± 0.018 P<0.001
Glyceraldehyde-3-phosphate dehydrogenase	1.12 ± 0.05 P<0.02	1.03 ± 0.04	0.814 ± 0.042 P<0.001	0.780 ± 0.056 P<0.001
Lactate dehydrogenase	2.11 ± 0.34	1.85 ± 0.16	1.55 ± 0.18 P<0.02	1.43 ± 0.20 P<0.005
αHydroxybutyrate dehydrogenase	0.751 ± 0.115	0.819 ± 0.074	0.795 ± 0.053	0.832 ± 0.033
αHydroxybutyrate dehydrogenase / Lactate dehydrogenase	0.363 ± 0.049 P<0.05	0.453 ± 0.060	0.516 ± 0.051	0.594 ± 0.079 P<0.01
Glucose-6-phosphate dehydrogenase	0.00149 ± 0.00039	0.00138 ± 0.00019	0.00131 ± 0.00013	0.00146 ± 0.00023
Malate dehydrogenase	0.915 ± 0.146 P<0.01	1.27 ± 0.19	1.48 ± 0.22	1.42 ± 0.25
Aspartate aminotransferase	0.815 ± 0.142 P<0.01	1.08 ± 0.10	1.08 ± 0.06	1.13 ± 0.12
βHydroxyacyl coenzyme A dehydrogenase	0.0196 ± 0.0067 P<0.05	0.0318 ± 0.0089	0.0313 ± 0.0031	0.0366 ± 0.0062
Palmityl carnitine - DTNB transferase	0.00167 ± 0.00026 P<0.02	0.00284 ± 0.00080	0.00463 ± 0.00118 (5) P<0.025	0.00439 ± 0.00122 P<0.05
^{14}C exchange	0.00132 ± 0.00032	0.00193 ± 0.00042 (3)	0.00369 , 0.00195 (2)	0.00305 , 0.00337 (2)
Succinate dehydrogenase	0.0193 ± 0.0035 P<0.001	0.0355 ± 0.0055	0.0324 ± 0.0021	0.0325 ± 0.0048
Protein (mg/gm wet wt.)	135 ± 15	142 ± 10	130 ± 11	132 ± 8

Stastical significance was calculated comparing the age groups with the newborn group.
Data are given as means ± S.D. with the number of observations in parentheses.

Fig. 29 Developmental changes in enzyme activity in guinea pig left ventricle. Activities are expressed as percentages of the adult activities. Full data are given in Table 74.

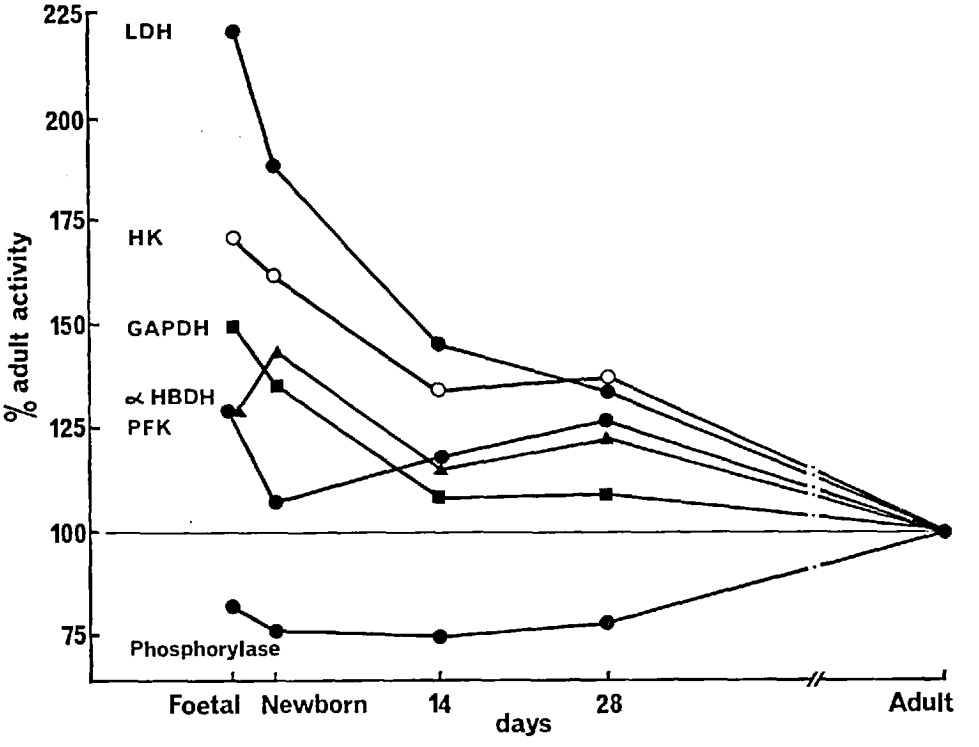


Fig. 30 Developmental changes in enzyme activity in guinea pig left ventricle. Activities are expressed as percentages of the adult activities. Full data are given in Table 74.

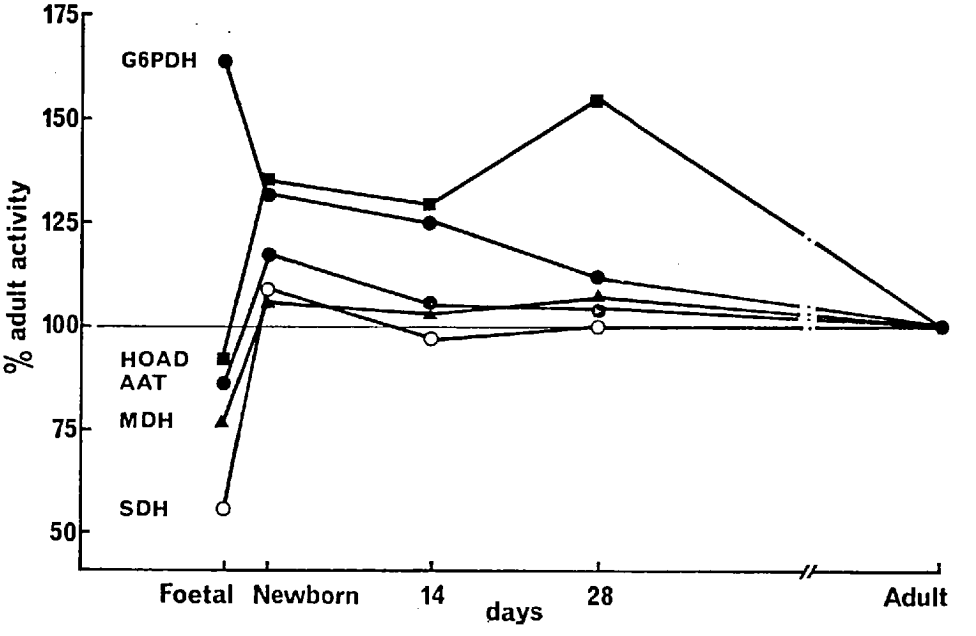


Fig. 31 Developmental changes in enzyme activity in guinea pig right ventricle. Activities are expressed as percentages of the adult activities. Full data are given in Table 75.

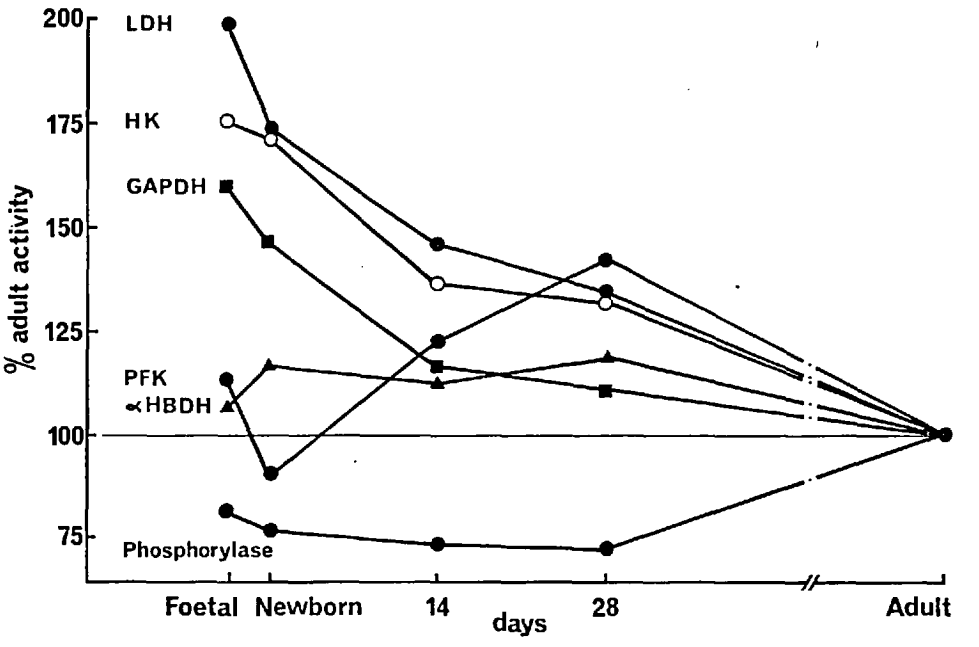
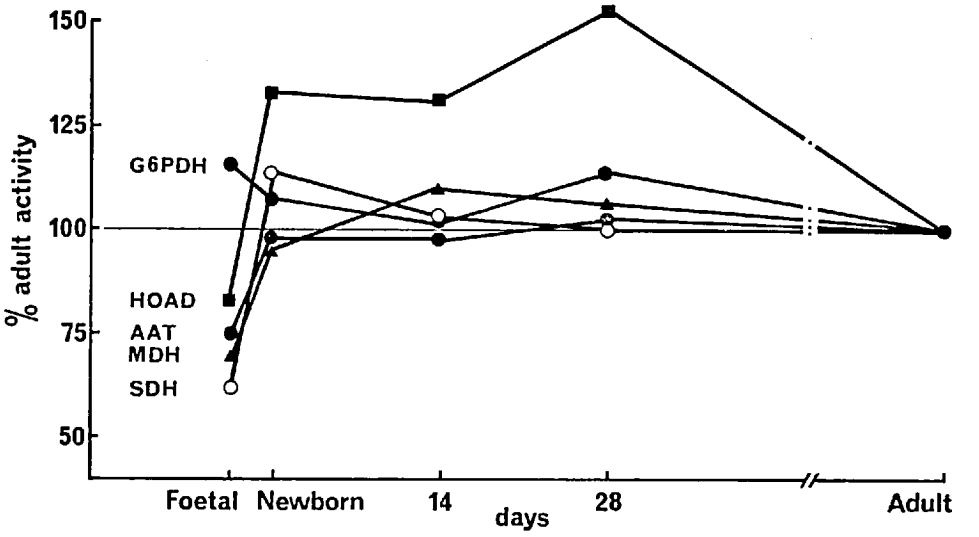


Fig. 32 Developmental changes in enzyme activity in guinea pig right ventricle. Activities are expressed as percentages of the adult activities. Full data are given in Table 75.



activities than in the foetus, although this was only significant for glyceraldehyde-3-phosphate dehydrogenase. The αHBDH/LDH ratio was higher indicating a shift in the LDH isoenzyme pattern towards a higher proportion of H-LDH. The most marked changes were in the activities of the mitochondrial enzymes which had increased considerably to about adult values. Malate dehydrogenase and aspartate aminotransferase activities showed similar significant changes.

POSTNATAL DEVELOPMENT

At 14 and 28 days the activity of hexokinase, glyceraldehyde-3-phosphate and lactate dehydrogenase had further decreased. The ratio αHBDH/LDH had increased and was nearly as high as in the adult by 28 days. During the postnatal development the distinction between left and right ventricles with respect to the lactate dehydrogenase activity became apparent ($P>0.05$ in foetus, $P<0.05$ in newborn, $P<0.001$ in 14 day old, $P<0.02$ in 28 day old). In contrast to these enzymes phosphofructokinase increased in activity during postnatal development, a trend more noticeable in the right ventricle.

The activities of the mitochondrial enzymes succinate dehydrogenase and βhydroxyacyl coenzyme A dehydrogenase and the activities of malate dehydrogenase and aspartate aminotransferase showed no further

change. Although the different assay methods gave different absolute activities, both methods indicated that after birth palmityl carnitine transferase activity had further increased by age 14 days and was unchanged at age 28 days.

The results suggest that the glycolytic capacity, which was high in the foetus, fell gradually throughout the development period studied, although the time course of phosphofructokinase activity was somewhat anomalous. The aerobic capacity of the foetal myocardium increased markedly during the last few days of gestation or immediately after birth, and thereafter remained constant in relation to the tissue size. However, the capability for fatty acid oxidation increased after birth as indicated by the activity of palmityl carnitine transferase, which is necessary for the transport of the long chain fatty acyl moieties across the mitochondrial membranes. The activity of glucose-6-phosphate dehydrogenase, which is a key enzyme in the pentose phosphate cycle, also gradually declined during development.

HYPOBARIC ANIMALS

The enzyme activities as measured in the newborn and 14 day old hypobaric animals are shown in Table 76, and are also depicted in Figures 33 - 37 for comparison with the normobaric data. Unfortunately it had not been possible to obtain any data on 28 day old guinea pigs. The activities were similar in both ventricles at both ages. No distinction for lactate dehydrogenase was apparent even after 14 days, in contrast to the normobaric animals.

NEWBORN

The values for glycogen phosphorylase, αhydroxybutyrate dehydrogenase, βhydroxyacyl coenzyme A dehydrogenase, succinate dehydrogenase, malate dehydrogenase, aspartate aminotransferase and palmityl carnitine transferase were similar in the hypobaric and normobaric newborn guinea pigs although there was a tendency for the enzymes associated with the mitochondria to be lower in the hypobaric group. The activities of hexokinase, phosphofructokinase, glyceraldehyde-3-phosphate, lactate, and glucose-6-phosphate dehydrogenases were higher in the hypobaric newborn, while the αHBDH/LDH ratio was lower; but these differences were not statistically significant in all cases. The enzyme activity pattern of the hypobaric newborn guinea pig resembled that of the normobaric newborn animal but with a higher glycolytic capacity.

TABLE 7b

Enzyme activities in the ventricles of guinea pigs during hypobaric postnatal development.

	RIGHT VENTRICLE		LEFT VENTRICLE	
	Newborn	14 days (4)	Newborn	14 days (4)
Glycogen phosphorylase	0.0301 ± 0.0038 (7)	0.0369 ± 0.0023 P<0.05	0.0319 ± 0.0063 (7)	0.0338 ± 0.0017
Hexokinase	0.131 ± 0.006 (6)	0.105 ± 0.010 P<0.005	0.121 ± 0.007 (6)	0.103 ± 0.009 P<0.02
Phosphofructokinase	0.138 ± 0.033 (6)	0.108 ± 0.006	0.151 ± 0.047 (6)	0.116 ± 0.008
Glyceraldehyde-3-phosphate dehydrogenase	1.22 ± 0.17 (6)	0.939 ± 0.120 P<0.05	1.19 ± 0.24 (6)	0.841 ± 0.057 P<0.05
Lactate dehydrogenase	2.13 ± 0.26 (6)	1.52 ± 0.15 P<0.01	1.98 ± 0.23 (6)	1.42 ± 0.08 P<0.005
αHydroxybutyrate dehydrogenase	0.854 ± 0.150 (6)	0.714 ± 0.088	0.870 ± 0.135 (6)	0.684 ± 0.047 P<0.05
αHydroxybutyrate dehydrogenase / Lactate dehydrogenase	0.399 ± 0.029 (6)	0.476 ± 0.080	0.436 ± 0.025 (6)	0.483 ± 0.031 P<0.05
Glucose-6-phosphate dehydrogenase	0.00160 ± 0.00022 (6)	0.00117 ± 0.00021 P<0.025	0.00157 ± 0.00036 (6)	0.00119 ± 0.00024
Malate dehydrogenase	1.21 ± 0.14 (7)	1.16 ± 0.11	1.25 ± 0.18 (7)	1.22 ± 0.11
Aspartate aminotransferase	1.04 ± 0.08 (7)	0.910 ± 0.088	1.21 ± 0.09 (7)	0.958 ± 0.084 P<0.05
βHydroxyacyl coenzyme A dehydrogenase	0.0281 ± 0.0093 (7)	0.0282 ± 0.0046	0.0325 ± 0.0098 (7)	0.0284 ± 0.0034
Palmityl carnitine - DTNB transferase	0.00215 ± 0.00054 (7)	0.00380 ± 0.00039 P<0.001	0.00219 ± 0.00075 (7)	0.00351 ± 0.00058 P<0.025
^{14}C exchange	0.00245 ± 0.00054 (6)	0.00345 , 0.00384(2)	0.00230 ± 0.00048 (6)	0.00352 , 0.00496(2)
Succinate dehydrogenase	0.0300 ± 0.0051 (6)	0.0313 ± 0.0027	0.0274 ± 0.0045 (6)	0.0322 ± 0.0022
Protein (mg/gm wet wt.)	140 ± 10 (7)	146 ± 1	141 ± 19 (7)	143 ± 4

Data are expressed as means ± S.D. with the number of observations in parentheses.

FIGURES 33 - 37 .

The changes in enzyme activity occurring during early postnatal development under normobaric and hypobaric conditions .

Activities are expressed as μmoles substrate transformed/min/mg protein at 25°C and given as mean ± S.E.M. and are shown in detail in Tables 74-76 .



normobaric development



hypobaric development

FIGURE 33

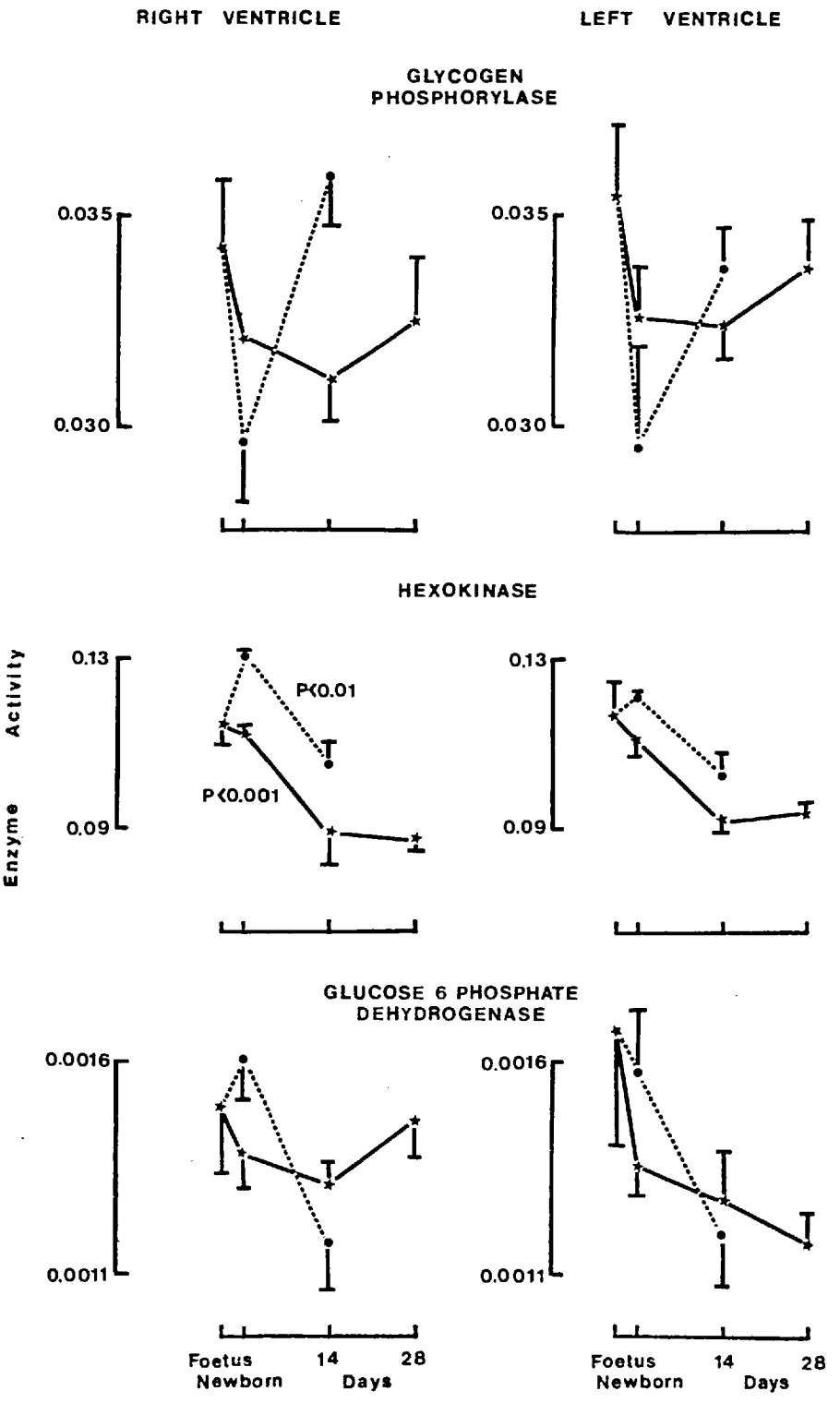

RIGHT VENTRICLE
LEFT VENTRICLE
GLYCOGEN PHOSPHORYLASE
0.035
0.030
0.035
0.030
HEXOKINASE
Enzyme Activity
0.13
0.09
P<0.01
P<0.001
0.13
0.09
GLUCOSE 6 PHOSPHATE DEHYDROGENASE
0.0016
0.0011
0.0016
0.0011
Foetus
Newborn
14
Days
28
Foetus
Newborn
14
Days
28

FIGURE 34

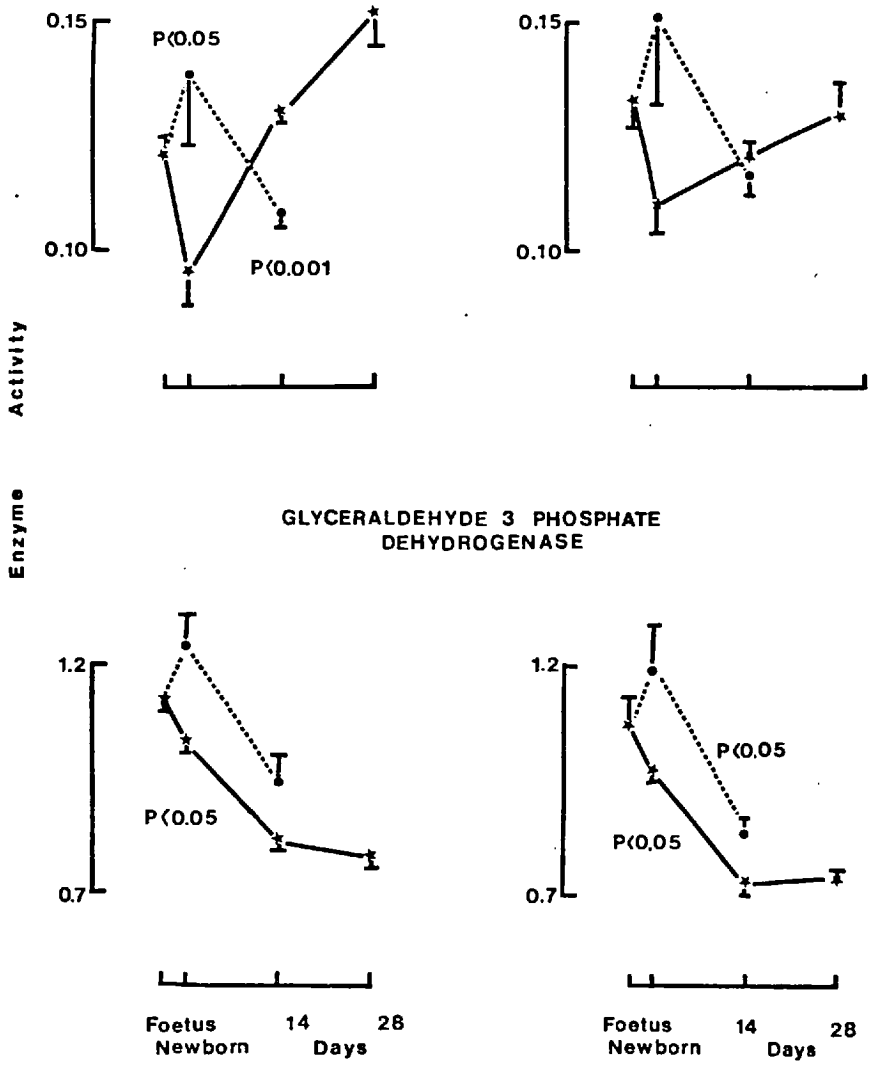

RIGHT VENTRICLE
LEFT VENTRICLE
PHOSPHOFRUCTOKINASE
0.15
0.10
P<0.05
P<0.001
Enzyme Activity
GLYCERALDEHYDE 3 PHOSPHATE DEHYDROGENASE
1.2
0.7
P<0.05
P<0.05
Foetus
Newborn
14
Days
28

FIGURE 35

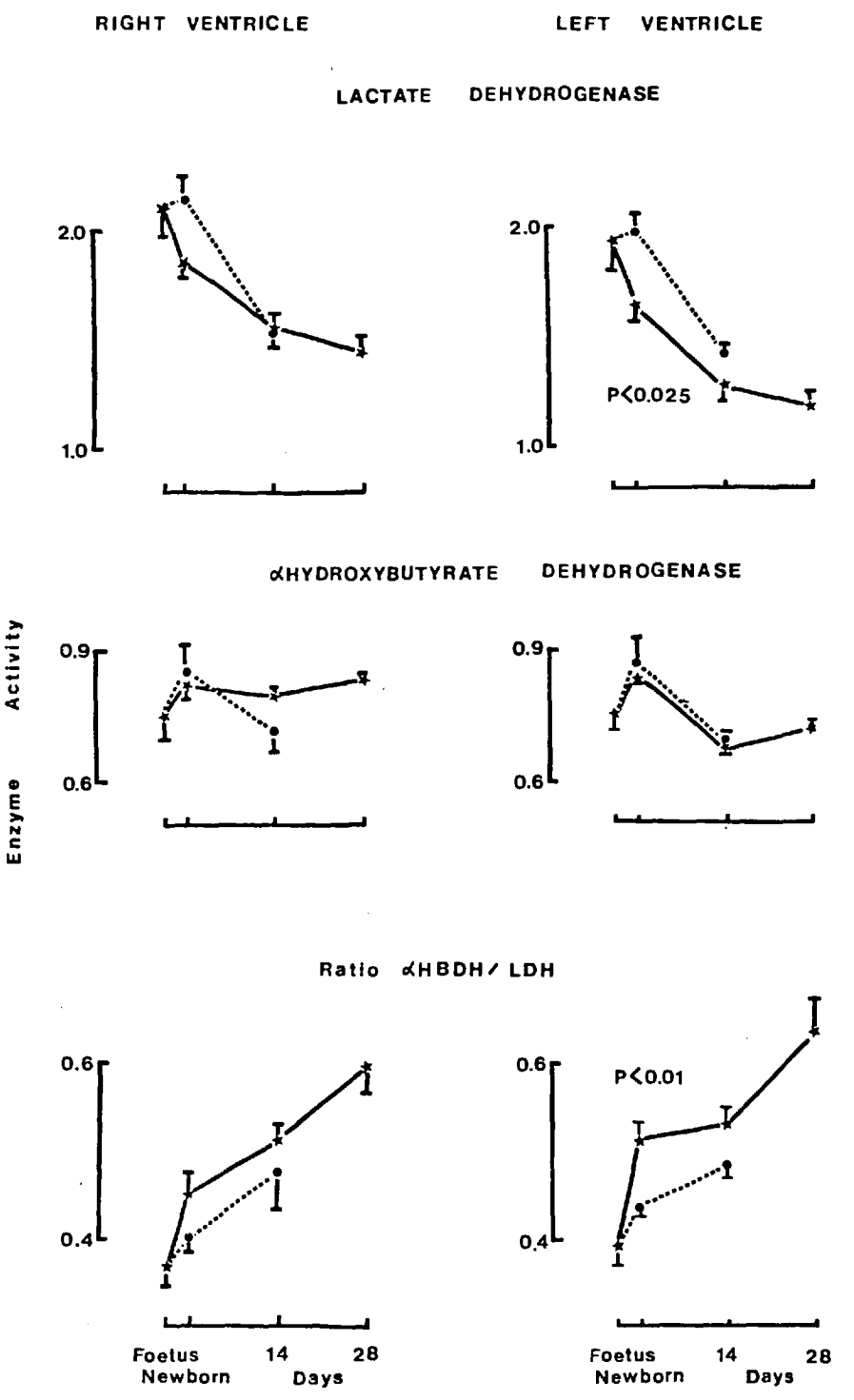

RIGHT VENTRICLE
LEFT VENTRICLE
LACTATE DEHYDROGENASE
2.0
1.0
P<0.025
αHYDROXYBUTYRATE DEHYDROGENASE
Enzyme Activity
0.9
0.6
Ratio αHBDH/ LDH
0.6
0.4
P<0.01
Foetus
Newborn
14
28
Days

FIGURE 36

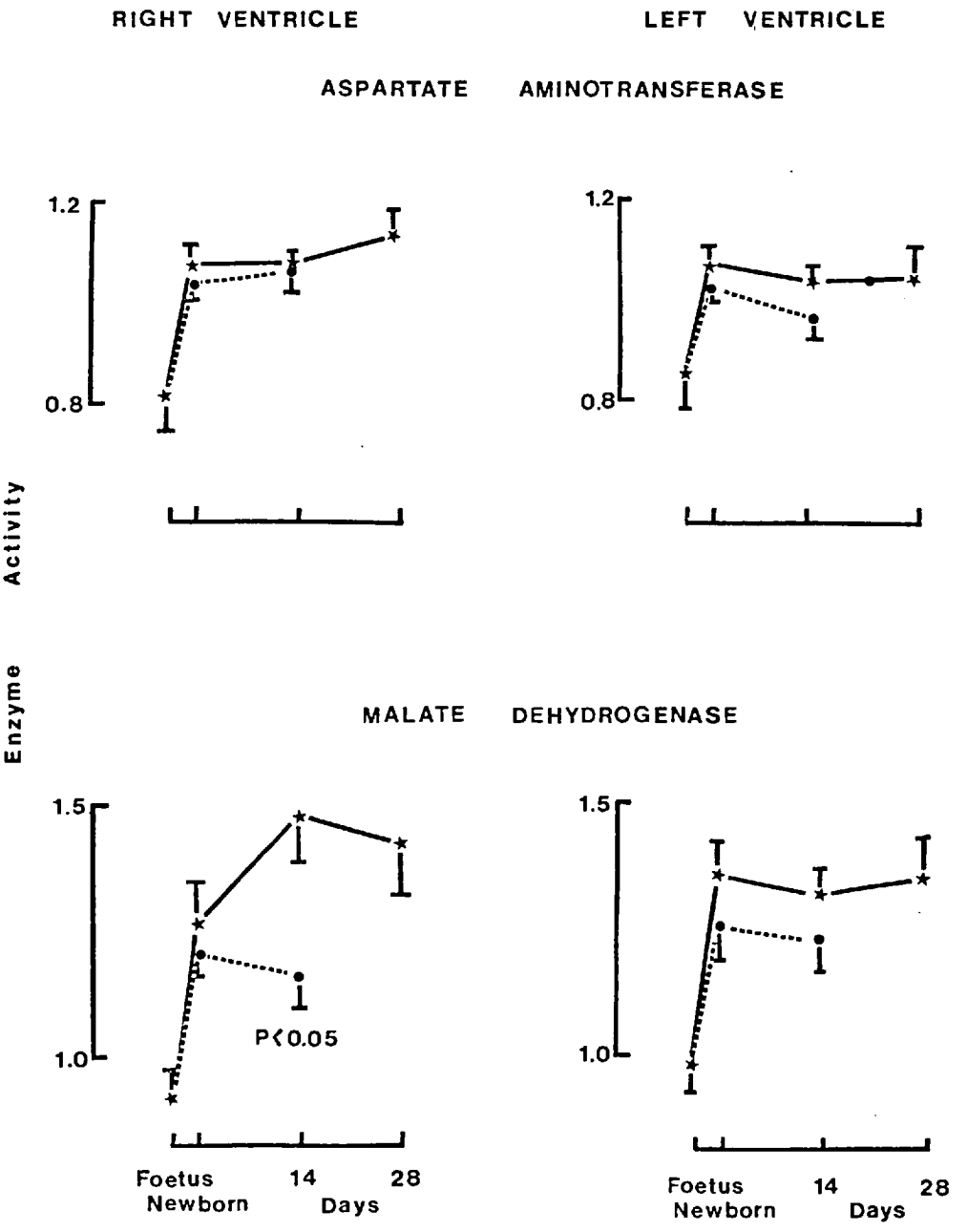
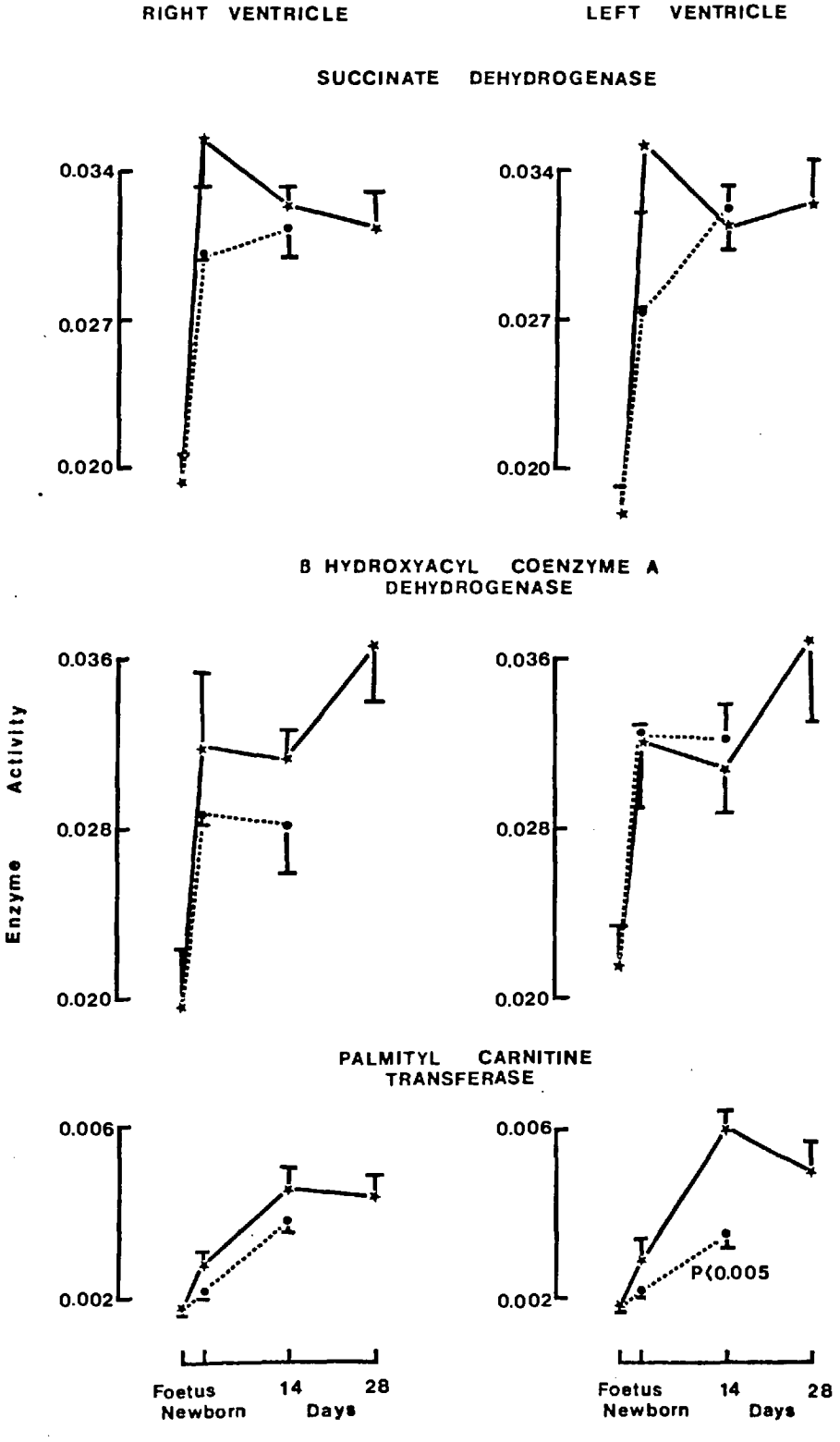

RIGHT VENTRICLE
LEFT VENTRICLE
ASPARTATE AMINOTRANSFERASE
1.2
0.8
Activity
Enzyme
MALATE DEHYDROGENASE
1.5
1.0
P<0.05
Foetus
Newborn
14
28
Days

FIGURE 37


RIGHT VENTRICLE
LEFT VENTRICLE
SUCCINATE DEHYDROGENASE
0.034
0.027
0.020
0.034
0.027
0.020
β HYDROXYACYL COENZYME A DEHYDROGENASE
Enzyme Activity
0.036
0.028
0.020
0.036
0.028
0.020
PALMITYL CARNITINE TRANSFERASE
0.006
0.002
0.006
0.002
P<0.005
Foetus
Newborn
14
28
Days
Foetus
Newborn
14
28
Days

POSTNATAL DEVELOPMENT

The differences between the hypobaric newborn and 14 day old guinea pigs were similar to those of the normobaric animals. Glycogen phosphorylase activity was increased at 14 days but there was a general decrease in the activity of the glycolytic enzymes. This included phosphofructokinase in the hypobaric group in contrast to the increase found in the normobaric animals. The αHBDH/LDH ratio was higher than in the newborn but was still lower than in the normobaric group. The activity of aspartate aminotransferase was lower at 14 days than in the newborn but the difference was statistically significant in the left ventricle only ($P<0.05$). As with the normobaric animals palmityl carnitine transferase activity was increased after birth but it was significantly lower than in the normobaric group ($P<0.005$) for the left ventricle. Other mitochondrial enzymes showed no alterations in activity and were similar to those in the normobaric animals.

DISCUSSION

It proved difficult to obtain live newborn guinea pigs under the hypobaric conditions chosen. Delaquerriere-Richardson, Forbes & Valdivia (1965) had exposed pregnant guinea pigs during the second half of gestation to a lower altitude than used here and were more successful. The stage of pregnancy at which the sows were placed in the chamber was of great importance. Flexner, Belknap & Flexner (1953) showed that for the differentiation of the nerve cells of the guinea pig the period of gestation between 41 - 45 days was critical, and that from this time the activities of cytochrome oxidase and succinate dehydrogenase in the brain increased considerably. It is possible that these developmental changes were upset when the pregnant sows were placed in the chamber at this time, leading to the death of the foetuses. Schnabel et al. (1974) calculated that damage would occur to the human foetus if the unadapted pregnant woman were exposed to altitudes higher than 2000m. The alterations in ventilation, acid - base balance, erythrocyte concentration and their content of 2,3 diphosphoglycerate upon acclimatization to altitude (Pace 1974, Martin et al. 1975) would raise this critical altitude, but the foetal Po_2 does fall following acute exposure of the mother to low Po_2 (Snyder 1942). Despite the

gradual acclimatization to simulated altitude, the stress of the new environment and of the low Po_2 may have been too great at this critical period for the survival of the foetus. When the pregnant sows were used at later stages of gestation, despite the shorter acclimatization period, the proportion of live births was higher.

The duration of pregnancy was shorter at altitude as reported by Delaquerriere-Richardson et al. (1965). However, unlike the animals of Delaquerriere-Richardson et al. (1965) or of Astrup et al. (1975) the birth weights were similar at both levels of barometric pressure. Since most of the young investigated here were born shortly after the sows had been exposed to the simulated altitude, and only a short time before normal term, this was not unexpected. With sheep, foetal growth is normal at altitude (Metcalfe et al. 1962, Barron et al. 1964).

The neonatal mortality rate was higher under conditions of low barometric pressure than under normobaric conditions. This was also reported in other studies (Delaquerriere-Richardson et al. 1965, Astrup et al. 1975). The cause of this is unclear. Rats are susceptible to fatty liver degeneration, but guinea pigs and rabbits are more resistant (Chiodi & Bass 1969). The growth rate of the hypobaric guinea pigs was similar to that of the

normobaric animals whereas Delaquerriere-Richardson et al. (1965) reported a lower growth rate for guinea pigs at 12000'. Rats exposed to simulated altitude after birth showed a decreased growth rate (Cheek, Graystone & Rowe 1969, Cunningham, Brody & Jain 1974), which was not solely related to malnutrition.

In the foetus and newborn guinea pig the left and right ventricles are of similar size. During normobaric postnatal growth the left increases considerably more than the right. This distinction must occur rapidly as the ventricular ratio at 4 days given by Lee, Taylor & Downing (1975) is similar to that found here after 14 days. The hypobaric newborn displayed a parallel increase in right and left ventricles, both of which were larger than in the normobaric guinea pigs. This increase may be the result of hyperplasia as well as of hypertrophy in response to the higher work load (Zak 1973).

The changes in enzyme pattern during development have been well studied in the liver and brain but less so in the heart (Stave 1964, Walpurger 1967), with most studies concerning rats and rabbits (Greengard 1971). These animals are relatively immature at birth in comparison with the guinea pig, and it is not unexpected that the enzyme activity patterns should be different during peri- and post-natal development. In the guinea pig the foetal

and neonatal heart contained high anaerobic glycolytic capacity which decreased during postnatal development, while in the rat and rabbit heart at this stage the glycolytic capacity was lower than in the adult (Stave 1964, Walpurger 1967, Heidger, Miller & Miller 1970). These developmental trends are surprising in view of the higher survival rate under anoxic conditions displayed by newborn rabbits compared with newborn guinea pigs and with adult rabbits. However, the functioning of the brain, and especially the respiratory centre, could be more important than that of the heart under these conditions, and the changes in pathway predominance may not be in parallel in both tissues (Heidger, Miller & Miller 1970). In addition, prolonged survival may be related to a lower rate of ATP utilization rather than to a higher rate of regeneration (Mäenpää & Räihä 1968).

The activities of hexokinase and glucose-6-phosphate dehydrogenase were higher in the neonate than in the adult in all the species. The former presumably relates to the predominant utilization of glucose in the foetus, while the latter, being a key enzyme in the pentose phosphate pathway, is generally elevated in rapidly growing tissues. The shift in the αHBDH/LDH ratio and so in the proportion of H-LDH subunits was marked during the

period of development studied. The transfer from predominantly M-LDH subunits to predominantly H-LDH subunits was almost complete by 28 days of age.

The activities of the mitochondrial enzymes succinate dehydrogenase and βhydroxyacyl coenzyme A dehydrogenase were low in the foetus and in the neonate of the rat and rabbit (Walpurger 1967, Cooper 1955, Heidger, Miller & Miller 1970, Hallman 1971), whereas in the guinea pig these activities, although low in the foetus, were at adult levels in the newborn. During the suckling period the capacity for fatty acid oxidation increases in the rat and rabbit (Lockwood & Bailey 1970). This is associated with an increase in the palmityl carnitine transferase activity and in the carnitine content of the rat heart (Wittels & Bressler 1965). Similar changes occurred in the guinea pig heart with the activities of βhydroxyacyl coenzyme A dehydrogenase and palmityl carnitine transferase increasing considerably. The observation that malate dehydrogenase and aspartate aminotransferase activities changed in parallel with that of succinate dehydrogenase indicates that, despite the fact that the measurements were of total activity, they reflect the close relationship between these enzymes (Pette 1966).

The enzyme activity pattern of the newborn hypobaric animals was similar to that of the normobaric newborn, but with a higher glycolytic capacity. This may be the result of premature delivery with the pattern being equivalent to that of a foetus aged 61 days. The anoxic tolerance of rabbits has been found to be dependent on the absolute age rather than on the age relative to birth (Glass, Snyder & Webster 1944). Alternatively the hypobaric conditions might have slowed the normal developmental changes. The age of the newborn varied between 1-20 hours and so the differences between the foetal and newborn patterns may have arisen during the final days of gestation, or within the first few hours of postnatal life. Changes in enzyme activities have been reported to occur within 6 hours of birth (Stave 1967, Cooper 1955).

Subsequent changes followed the pattern found in normobaric animals although at 14 days the glycolytic capacity of the hypobaric animals was still higher while the mitochondrial enzymes had lower activities than in the normobaric guinea pigs. There was a small but significant difference in the protein content of the hearts of the 14 day old animals. If this were taken into account, and the activities expressed relative to wet weight there would be a greater similarity between the activities

of the mitochondrial enzymes in the hypobaric and normobaric 14 day old animals, but a greater difference between those of the glycolytic enzymes. Unfortunately it is not clear from the available data whether, with further hypobaric postnatal development, the enzyme pattern of the hypobaric animals would have become equivalent to that of the normobaric ones, or whether these small differences would have persisted.

SUMMARY

The number of litters born alive, and the subsequent survival rate was reduced under hypobaric conditions. Under normobaric conditions, the activities of several mitochondrial enzymes, malate dehydrogenase, and aspartate aminotransferase in the myocardium increased markedly during the late prenatal period, and remained constant thereafter. Palmityl carnitine transferase activity, however, increased during the suckling period. The activities of the glycolytic enzymes fell gradually during these periods, and the distinctions between ventricles appeared. Hypobaric conditions did not greatly affect these developmental changes but the activities of the glycolytic enzymes were higher than in the myocardium of normobaric animals of the same age. It was not possible to decide whether this distinction would be maintained throughout life, or whether the developmental trend would continue and a normal pattern of activities be established.

GENERAL DISCUSSION

Brief periods of hypoxia are common events in the lives of mammals and for a species to survive its individual members must be able to endure these periods. Prolonged hypoxia is not a common event except in certain geographical locations and so the ability to survive these conditions is not likely to be an important determinant of evolutionary trends at low altitudes. Despite this, animals from these low altitude regions are able to acclimatize and live under conditions of chronic hypoxia. Whereas the response to acute hypoxia involves rapid changes in metabolism brought about by alterations in effector concentrations, the possibility that the response to chronic hypoxia involves changes in enzyme concentrations was investigated here. However, before assessing the effects of hypoxia per se, it is necessary to consider the effects of the anorexia and ventricular hypertrophy associated with the initial period of acclimatization. Having allowed for these factors the influence of the nature of the tissue, the duration of exposure and the age of the animal on the response to hypoxia can be assessed.

EFFECTS OF THE RESTRICTED DIET

This was shown in the comparisons of the free fed controls compared with the restricted diet controls

which received the same amount of food as taken by the hypobaric animals. The changes in enzyme activities occurring as a result of the restriction in dietary intake included the reduction in several enzyme activities and in the alteration of the lactate dehydrogenase isoenzyme pattern. The extent of the alterations was related to the severity of the restriction as would be anticipated. However, the fact that some differences were still present several days after the quantity of diet had been restored to normal, indicated the importance of including restricted diet controls in any experiment in which the appetite is affected, even long term ones. It was also clear that, if the influence of the anorexia was neglected, erroneous conclusions could be reached, as also reported by Gold & Costello (1974). This was exemplified by the lactate dehydrogenase isoenzyme pattern.

LACTATE DEHYDROGENASE

Several earlier studies on acclimatization to hypobaric hypoxia have concerned the lactate dehydrogenase isoenzyme pattern. This pattern varies markedly between tissues and during development (Markert & Ursprung 1962), and correlations have been made between the isoenzyme pattern and the functional characteristics of the tissue (Gutierrez et al. 1974,

Kaplan & Goodfriend 1964), the mitotic index of the cell (Bloom, Tsuchioka & Wajima 1967), and also different cellular localisations (Markert & Ursprung 1962). Although it is not clear what factor(s) may determine the pattern in vivo nor what biological significance it has, the pattern is sensitive to different situations and conditions (Dawson, Goodfriend & Kaplan 1964), and as shown here, to the effects of a restricted diet on a guinea pig.

Several but not all reports on experiments involving the acclimatization of rats to high altitude (real or simulated) have indicated a shift in the pattern (Anderson & Bullard 1971, Mager et al. 1968, Miller & Hale 1968, Penney 1974, Vergnes 1973). The conflicting results cannot be explained on the basis of the age of the animals when initially exposed, although this was found in the present study to be an important determinant of the response in the guinea pig myocardium. However, in these earlier experiments, no account was taken of the anorexia displayed by the experimental animals, which has been shown to be so important here. In fact, those workers reporting no change in the isoenzyme pattern (Miller & Hale 1968, Vergnes 1973) had exposed their animals for longer periods (2-4 months) than those workers reporting changes (less than 2 months). If the isoenzyme pattern in the rat responds to dietary alterations

in the same way as in the guinea pig, then a shift would be anticipated during early acclimatization at which time the animals are anorexic, and a normal pattern would be anticipated with longer periods of acclimatization during which time the animals recover from their transient loss of appetite. Thus the results reported could be explained solely on the basis of alterations in the amount of food taken, and this could account for the conflicting results.

CAUSES OF HYPERTROPHY

It was found that during exposure to hypobaric hypoxia, the right ventricle underwent more pronounced hypertrophy than the left ventricle. The hypertrophy of the right ventricle has been associated with the pulmonary hypertension found in conditions of hypoxia (Reeves, Grover & Grover 1963, Grover et al. 1963, Dean et al. 1968, Burton Besch & Smith 1968). The reason for the slight left ventricular hypertrophy is less clear. The increase in haematocrit reported during acclimatization to hypoxia would raise the viscosity of the blood (Whittaker & Winton 1933) which in turn would result in a higher work load for the left ventricle. Buckley, Edwards & Hiestand (1953) showed that discontinuous decompression alone caused an increase in the systemic blood pressure which would also affect the work of the left ventricle. However, the blood pressure of the

high altitude native is lower than that of the sea level native (Hurtado 1964, Rotta et al. 1966) and so the rise in blood pressure may reflect the stress of the experiment rather than the effects of the low pressure per se. However, since in the acclimatization studies carried out here regular atmospheric pressure changes were necessary some rise in the systemic blood pressure in the guinea pigs could have resulted, contributing to an increased work load for the left ventricle which may have initiated hypertrophy.

As regards the mechanism by which an increase in the work load initiates events leading to tissue hypertrophy, Meerson (1974) considered that the increase in the phosphorylation potential (ADP.Pi/ATP ratio) occurring during work overload triggers the activation of the genetic apparatus giving rise to an increase in mitochondrial and cellular mass. Hypertrophy would continue until a stage is reached in which the ADP.Pi/ATP ratio is restored and the ATP utilization / unit mass of tissue is normal. At this point the trigger for further change is absent and a stable state of hypertrophy envisaged. In the situation of work overload the normal level of ATP utilization / unit mass of tissue could be restored by a simple increase in mass with proportional increases in all tissue components. This does not imply that, during the development of the hypertrophic state, all components of the tissue should

increase in parallel, but only that in the final state the proportions should be normal.

The means by which the change in phosphorylation potential could stimulate cardiac hypertrophy are not known. Ingwall et al. (1974) have shown that creatine specifically stimulates myosin synthesis in cultured embryonic skeletal muscle cells. Since a fall in creatine phosphate occurs concommitantly with the increase in the phosphorylation potential, the accumulated creatine may play a part in the development of cardiac hypertrophy, although the initial biosynthetic response seems to be concerned with mitonchondrial structures not myosin. (Meerson 1975).

EFFECTS OF HYPERTROPHY

These effects were determined by comparing the ratios of the enzyme activities in the right ventricle with those in the left ventricle for hypobaric and control animals. In this way the effects of hypoxia are eliminated since they should be the same in both ventricles, and the variations between animals and between experimental days reduced.

Hypertrophy is associated with an increase in the activity of the pentose phosphate cycle, which would be anticipated in a growing tissue as this cycle provides necessary metabolites for biosynthesis. The activities of the enzymes catalysing reactions producing glucose 6 phosphate, were also increased, which is explicable as glucose-6-phosphate is the substrate for the pentose phosphate cycle as well as for glycolysis. Although

glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase were increased, phosphoglyceromutase and enolase were not. In many tissues these enzymes occur in constant proportions to each other (Pette 1966, Bass et al. 1969) but the results here suggest that this may be a characteristic of a normal state and not of a changing state as in hypertrophy, although in comparison to the variation in activities between tissues, the variation resulting from hypertrophy is small, and so may be considered as not contradicting the proposed constancy.

There was a small shift in the lactate dehydrogenase isoenzyme pattern towards an increased proportion of M-LDH subunits that is associated with hypertrophy. A similar shift has been reported by other workers (Bishop & Altshuld 1970, Fox & Reed 1969, Sobel et al. 1970) but as already pointed out, no account was taken of any alterations in the appetite of the experimental animals in these studies, and so it is not clear to what extent the alterations reported were due to hypertrophy and to what extent dietary reduction.

EFFECTS OF HYPOXIA

Meerson (1975) envisages that, since initial exposure to hypoxia is associated with an increase in the phosphorylation potential, the same sequence of events is initiated by hypoxia as by work overload. If this is the case, then the effects of hypobaric

hypoxia would be the sum of those due to the low oxygen tension alone and those due to the increased work load associated. It would follow that the time course of acclimatization to both stimuli would be the same, and that it would not be possible to distinguish the effects of the two stimuli.

However, although the effect of hypoxia and overload on the phosphorylation potential is the same the direct causes are different. In both situations the rate of utilization of ATP is greater than the rate of its production, but, whereas during work overload this is due to increased utilization, during exposure to hypoxia this is due to decreased production. Therefore, a general increase in tissue mass would not alleviate the problem of the low rate of ATP production, except in that it might lead to a reduction in the utilization / unit mass to a level where the reduced production of ATP would be sufficient to fulfill the demand. If, however, there was a selective increase in cell components involved in the production of ATP, for instance the electron transport chain or the enzymes of the glycolytic pathway, then the supply of ATP might be increased to meet normal levels of demand despite the low tissue Po_2.

An attempt was made to distinguish the effects of hypertrophy from those of hypoxia. If the extent of the changes associated with hypertrophy or hypoxia are related to the degree of hypertrophy or hypoxia, then

alterations occurring predominantly in the right ventricle could be attributed to the effects of hypertrophy, while those occurring equally in both ventricles could be attributed to hypoxia.

EFFECT OF TISSUE TYPE ON THE RESPONSE TO HYPOXIA

The sensitivity of a tissue to hypoxia would be anticipated to be related to the tissue's normal requirement for oxygen. Thus a high sensitivity to reduced availability of oxygen would be anticipated in tissues whose metabolism was dependent on oxygen, while tissues whose metabolism was independent of oxygen would be expected to be unaffected. Whereas some effects of hypoxia on oxygen dependent tissues have been investigated, no such investigation appears to have been carried out on oxygen independent tissues. In this work a study was made on two different muscle types as exemplified by the sartorius and ventricular muscles.

SARTORIUS

The enzyme activity pattern of the sartorius measured here indicated a high glycolytic capacity and a low mitochondrial capacity, which would suggest a low oxygen consumption in vivo. This tissue was unaffected during acclimatization to hypoxia.

VENTRICULAR MUSCLE

DISTINCTION BETWEEN VENTRICLES

During the course of the experiments it became clear that there were certain intrinsic differences in the enzyme activities within the two ventricles. The activities of lactate dehydrogenase and αhydroxybutyrate dehydrogenase were higher in the right ventricle, while the activities of glyceraldehyde-3-phosphate and mitochondrial glycerol-3-phosphate dehydrogenase were higher in the left ventricle. These differences were small but consistent in the adult animal, having been absent at birth and appearing during postnatal development.

Similar ventricular distinctions have been reported for rats (Cryer & Bartley 1974) but in this species the activity of lactate dehydrogenase was higher in the left ventricle. It has also been reported that distinctions exist between the different layers of the ventricular wall (Lundshaard-Hansen et al. 1967, Jedeiken 1961) suggesting that the glycolytic capacity was higher in the endocardium. This would correlate with the tissue Po_2 distribution pattern as measured by Moss (1968) and Whalen (1971), and with the oxygen supply to the various layers as reported by Kirk & Honig (1964) although not as measured by Flohr et al. (1973).

With these intrinsic differences in enzyme activity patterns, it was clear that the ventricles should be considered separately as there is a shift in the proportions of the individual ventricles in the heart under conditions of hypobaric hypoxia.

RESPONSE OF VENTRICLES TO HYPOXIA

After 4 weeks acclimatization the left ventricle of the adult guinea pigs seemed to have reached a stable level of hypertrophy, and the differences in enzyme activities present at that time may be regarded as being due to hypoxia. There was a general increase in the activity of all the glycolytic enzymes but no alterations in the activities of the mitochondrial enzymes, although succinate dehydrogenase had shown a transient increase in activity after 2 weeks, which could be attributed to hypoxia.

The lactate dehydrogenase isoenzyme pattern shifted slightly in the adult animals, but this was towards a higher proportion of H-LDH subunits. In the young animals, however, there was a marked drop in the proportion of H-LDH subunits in response to hypoxia. This indicates the importance of the age of the animal when initially exposed to hypoxia.

NATURE OF CHANGES IN ACTIVITY

Although most glycolytic enzymes showed increase in activity, little or no change was found in the activity

of phosphofructokinase. A similar situation was found in the case of glucose-6-phosphate dehydrogenase as the changes in activity of this enzyme showed little relation to the activity of the pentose phosphate pathway as indicated by the activity of glyceraldehyde-3-phosphate dehydrogenase relative to other glycolytic enzymes as explained earlier. Phosphofructokinase and glucose-6-phosphate dehydrogenase are the main regulatory enzymes for their respective pathways and are subject to control via alterations in effector concentrations (Newsholme & Start 1973b, Eggleston & Krebs 1974). It is thus possible that alterations in these effector concentrations could increase the flux through the pathways sufficiently to result in increases in the other enzymes of the pathway without necessitating changes in the regulatory enzymes themselves. In the heart, unlike in skeletal muscle, the maximum rate of glycolysis measured in vivo is only 20% of the vitro activity of phosphofructokinase (Kübler & Spieckermann 1970). This suggests that in vivo the enzyme operates under suboptimal conditions. If,during acclimatization to high altitude,these conditions altered to allow greater activity then an increased flux could be achieved without an increase in the absolute concentration of the enzyme.

The enzyme assays measure activity and not concentration but the mixing studies carried out indicated that, at least for the enzymes tested, the

changes in activity were due to changes in enzyme concentration.

The concentration of an enzyme represents the balance between its rate of synthesis and its rate of degradation, and a shift in the concentration of an enzyme can occur as a result of changes in either mechanism. It is not yet known how the balance is regulated and what triggers changes for particular enzymes. Pette and co-workers have analysed the activity patterns for several tissues in different species, and have shown that for certain groups of enzymes their activities bear a constant relationship to each other. To account for the constant proportions it has been suggested that these enzymes are coded for by one operon on the DNA, analogous to the lac operon in Escherishia Coli. However, Tepper & Hommes (1970) studied the changes in activity during the development of the rat liver and concluded that the control of the enzymic concentrations was more complex. Staudte & Pette (1972) also concluded that the constant proportions were not the result of polycistronic coding on the DNA. However, it was not within the scope of these investigations to study the control mechanisms operating.

In the case of lactate dehydrogenase there was a shift in the isoenzyme ratio towards a higher proportion of M-LDH subunits as a result of a restricted diet, hypertrophy and, in the young animals, hypoxia. This

could result from an alteration in the rate of synthesis of either or both subunit types, or in the rate of degradation of specific isoenzymes. Dehlinger and Schimke (1970) proposed that the degradation of the subunits of a multimeric protein only occurred when the subunits were dissociated. Therefore, any alteration in the environment facilitating dissociation of a particular isoenzyme would lead to increased rate of degradation of its subunits. Fritz, White & Vessell (1971) suggested that exchange of subunits between lactate dehydrogenase isoenzymes could occur and that this process was also affected by the environment. It would, therefore, seem possible for the lactate dehydrogenase isoenzyme pattern to shift solely by an alteration in the environment without necessitating any change in the basic process of synthesis or degradation. It would be interesting to know whether this mechanism was operating in any of the 3 experimental situations examined here.

INFLUENCE OF THE DURATION OF ACCLIMATIZATION

The time course of individual responses varies, with the result that it is difficult to ascertain when, if ever, acclimatization is complete. Pulmonary changes occur rapidly and persist throughout the sojourn at high altitude (Shields et al. 1967, Pace 1975). The experiments carried out here indicate that the anorexia

initially displayed was lost after a few days, whereas ventricular hypertrophy and its associated enzyme changes occurred for at least 4 weeks. Simanovskii and Chotoev (1971) showed that the rate of glycolysis and glycogenolysis in rat ventricles increased after 20-30 days acclimatization and that the changes were stable after 30-40 days. Vergnes (1973), however, found alterations in enzyme activity occurring after several months. It was therefore unfortunate that Tappan et al. (1957), when studying the acclimatization of low altitude guinea pigs to high altitude and visa versa, should have pooled their results which had been obtained over an interval as wide as 0-80 days.

INFLUENCE OF AGE ON THE RESPONSE TO HYPOXIA

The age at which an animal is exposed to natural or simulated high altitude affects the response shown, with the young animals acclimatizing more quickly and completely than the older ones. The increase in haematocrit in response to continuous hypobaric hypoxia is found in young and old rats (Flückiger & Verzar 1955) but not in the newborn rat (Contopoulos et al. 1954) although increases were induced in all cases by intermittent hypoxia (Trojan et al. 1974). Pulmonary hypertension is greater in the young than in the old (Sime et al. 1963) explaining the greater right ventricular hypertrophy found in the young guinea pigs. The experiments performed here showed

that the variation in body weight was age dependent, since the hypobaric newborn showed the same weight gain as the normobaric ones in contrast to other reports (Delaquierre-Richardson et al. 1965, Cheek et al. 1969, Cunningham, Brody & Jain 1974), while the other age groups all showed an initial drop in weight, the recovery of which was delayed more the older the animal.

The alterations in enzyme activities in the young and adult animals were similar in respect of the lactate dehydrogenase isoenzyme pattern, which was altered in favour of an increased proportion of M-LDH subunits in the young hypobaric animals. It is not clear why the response of lactate dehydrogenase differed with age. The pattern in the adults changed in response to a reduced diet and to hypertrophy so the capability of change was present and yet the hypoxia did not initiate the change in the adult animals.

It is more difficult to analyse the response to hypoxia shown by the newborn animals as any alterations are superimposed on the normal pattern of development. The gradual decrease in the glycolytic capacity of the myocardium of normobaric developing guinea pigs was also found in the hypobaric animals, although the actual enzyme activities were higher in the hypobaric ones. The shift in the lactate dehydrogenase isoenzyme pattern towards a high proportion of H-LDH subunits was also found in both developing groups. Thus it seems as if

hypoxia has little or no affect on the developing animal although it is not known what would be the situation after further hypobaric exposure, which is unfortunate as this information would further understanding of the indigenous high altitude guinea pig.

INDIGENOUS HIGH ALTITUDE ANIMALS : ACCLIMATIZATION OR ADAPTATION

The indigenous high altitude animal has lived under conditions of hypoxia throughout its life, and so could have acclimatized for this period. In addition, since the species has lived at high altitude for many generations, it is possible that genetic adaptations have been selected which make the species better suited for life at high altitude.

Development at high altitude seems to be the important determinant for several features. People migrating to high altitude during development showed the same ventilatory response to exercise at high altitude as the high altitude native while those migrating after development showed characteristically different responses (Frisancho et al. 1973). The capillary density of skeletal and cardiac muscle (Valdivia 1958, Becker 1955) in the high altitude animals is reported as higher than in sea level animals, but since Kerr, Pilato & Foster (1965)

reported an increase in the coronary arteries following discontinuous exposure to simulated high altitude, there is the possibility that this difference between indigenous high and low altitude animals also arises as a result of development under hypoxic conditions. The higher tissue myoglobin content in the indigenous high altitude populations (Reynafarje 1962, Reynafarje & Morrison 1962) is also matched by increases found during acclimatization (Costa & Taquini 1970, Anthony, Ackerman & Strother 1959). Thus several differences which are found between the indigenous high and low altitude populations may arise simply as a result of acclimatization from birth and not imply any intrinsic differences between the populations. However, this may not be the whole explanation as Will and co-workers (1970) have shown that the development of pulmonary hypertension has a genetic determinant.

The enzyme activity pattern of the hearts of the high altitude rabbits showed a higher glycolytic capacity similar to that found during the acclimatization of guinea pigs. The guinea pig, however, displayed no differences between the enzyme activities in the high and low altitude populations. This might represent the result of specific genetic adaptation or the result of acclimatization from the moment of birth. It was to clarify this point that the study of the effects of hypoxia on the postnatal development

of guinea pigs was carried out. Unfortunately, the lack of data on animals older than 14 days prevents any firm conclusion being drawn. If the glycolytic enzyme activities of the hypobaric animals remain higher throughout development, then the resulting pattern would be similar to that of the animals acclimatized later in life. This result would imply that the indigenous high altitude animals were intrinsically different from the low altitude ones, and that some factor(s) obviated the need for increased glycolytic capacity. However, should the enzyme pattern have continued following the normal trend and resulted in a normal pattern later in life, then the distinction, or in this case the lack of one, between the high and low altitude populations could be accounted for by the nature of the postnatal development.

For a species to survive in a new environment, it must be able to reproduce, and for sufficient young to survive to maintain the species. In this connection the low number of young born alive coupled with their low subsequent survival rate is of interest. Foetal development can be protected to some extent against a lower atmospheric Po_2 through combined alterations in the maternal and foetal circulations (Jepson 1974, Martin et al. 1975), but the foetal blood Po_2 still falls following a drop in the Po_2 of the maternal inspired air (Snyder 1942). The placental weight is increased at high altitude (Metcalfe et al. 1962,

Petropoulos & Timiris 1974) presumably aiding oxygen transfer between the two circulations. An important determinant of normal foetal growth may be the number of foetuses carried. A reduction in this number would reduce the total oxygen demand on the mother and possibly allow adequate oxygen to be supplied to the foetuses. Observations at Cerro de Pasco on two litters indicated one and two offspring per litter, which is considerably lower than in the sea level laboratory animals (mean 4.5 animals / litter). This may be due to the high foetal resorption rate reported in hypoxic conditions (Petropoulos & Timiris 1974). The incidence of twins in either human or sheep high altitude populations is very low (Petropoulos & Timiris 1974) which may be related. If the number of animals per litter is as important as it seems it may account for the higher proportion of pregnancies yeilding live young that occurred under the conditions of 400 torr compared with 460 torr, since the average litter size of the animals subjected to 400 torr was smaller than that of those animals subjected to 460 torr atmospheric pressure.

Subsequent development was associated with a high mortality in the experimental conditions used here and has been reported by others (Delaquerriere-Richardson Forbes & Valdivia 1965, Petropoulos & Timiris 1974), although data on indigenous animals are not known. Rats are susceptible to fatty liver degeneration during

hypoxic postnatal development, but guinea pigs and rabbits are said to be more resistant (Chiodi & Bass 1969). The high mortality rate could be contributed to by faulty maternal lactation since they were not fully acclimatized. However the young surviving showed normal growth rates, and other authors have reported this increased mortality even when using acclimatized mothers (Delaquerriere-Richardson, Forbes & Valdivia 1965). The fact that the hypobaric young were a few days premature may also have contributed to the high mortality. Kelley & Pace (1968) suggest that the reduced water vapour at natural high altitude contributes to the high mortality, and under simulated conditions this state would be present to some degree. Brain development has been found to be affected by hypoxia but the extent of disruption is dependent on whether the animals were exposed perinatally or were conceived and born at high altitude (Petropoulos & Timiris 1974). Despite these alterations in all aspects of metabolism induced by hypobaric hypoxia the guinea pig can survive under natural high altitude conditions and so is a suitable species for continued research.

CONCLUSION

Exposure to hypobaric hypoxia induces vascular and haemopoietic changes leading to hypertrophy of both ventricles. The hypertrophy is more marked in the right

ventricle, and this observation, coupled with the intrinsic differences in the enzyme activities between the ventricles, makes it important to consider the two ventricles separately. In the studies on acclimatization, it is most important to consider the duration and severity of the exposure, and to consider the effects of any dietary changes that might be displayed as these may directly influence the parameters studied. Alterations in enzyme activities indicate an increase in glycolytic capacity in the heart of acclimatizing but not indigenous guinea pigs. The sartorius muscle was not affected. The age of the animal at which exposure occurs is of great importance in determining the nature of the response, and if this occurs during the developmental period many of the characteristics of the high altitude native are acquired by the low altitude native. However, it was not possible to determine whether the myocardial activity pattern of the indigenous high altitude guinea pig was the result of acclimatization since birth, or genetic adaptation.

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Enzyme Activities in Red and White Muscles of Guinea-Pigs and Rabbits Indigenous to High Altitude

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The activities of several enzymes functioning in different areas of fuel catabolism were measured under standardized conditions, using crude homogenates of sartorius and ventricular muscle from outbred guinea-pigs and rabbits indigenous to high or low altitude. The activities of sartorius and myocardium were found to reflect the metabolic patterns known to be associated with white and red muscle.

Both species had right ventricular hypertrophy at high altitude. The enzyme activities in the high altitude guinea-pig were not significantly different from those in the sea level animals. In the high altitude rabbit, compared with the low altitude rabbit, the activities of glyceraldehyde-3-phosphate dehydrogenase and phosphofructokinase were greater in both the sartorius and myocardium. In addition, mitochondrial glycerol-3-phosphate dehydrogenase activity was greater in the sartorius at high altitude, while aspartate aminotransferase and β-hydroxyacylcoenzyme A dehydrogenase activities were greater in the myocardium at high altitude. Succinate dehydrogenase activity was comparable at the two altitudes for both tissues. There was a greater proportion of skeletal muscle type lactate dehydrogenase in the high altitude rabbit myocardium but no difference was found with the guinea-pig.

Animals and men indigenous to high altitude appear to have a normal capacity for physical activity (Hurtado 1964) despite the low ambient partial pressure of oxygen. Physiological differences affecting the transport of oxygen can account in part for the adaptation (Hurtado 1964). However, differences also occur at the cellular level. Previous investigations into the effect of altitude on cellular metabolism have each been confined to only a few aspects such as lactate dehydrogenase (Anderson & Bullard 1971, Mager et al. 1968, Miller & Hale 1968, Reynafarje 1962, Sorensen et al. 1972), certain mitochondrial enzyme activities (Harris et al. 1970, Reynafarje 1961, Tenney & Ou 1970) and myoglobin content (Reynafarje 1962, Reynafarje & Morrison 1962).

The present work investigates in muscle the activities of several enzymes which are important in different aspects of cellular catabolism concerned with energy production. The myocardium and sartorius muscles were used as their metabolic pat-

terns differ. The myocardium aerobically oxidizes free fatty acids and ketone bodies in preference to glucose (Shipp et al. 1961, Williamson & Krebs 1961) while the sartorius, a predominantly white muscle, is more dependent on anaerobic glycolysis for its energy production (Czok & Bücher 1960, Ogata 1960, Wirsen 1965). Guinea-pigs and rabbits were chosen since these animals are indigenous to both high and low altitudes. The experimental design allowed several comparisons of enzyme activities to be made: guinea-pig with rabbit; myocardium with sartorius; left ventricle with right ventricle; high altitude with low altitude.

Experimental Procedure

Materials

Domestic guinea-pigs and rabbits, which are bred for food, were obtained locally at Cerro de Pasco, Peru (altitude 4300 m) and at Lima, Peru (altitude 200 m). They were fed *ad libitum* on standard diet with fresh grass daily.

Succinate and glycerol-3-phosphate and anhydrous bovine serum albumin were obtained from Sigma (London) Chemical Co., London, S.W.6, United Kingdom; aspartate, α-ketoglutarate, α-ketobutyrate, cysteine, dichlorophenolindophenol and all inorganic chemicals were obtained from B.D.H. Chemicals, Poole, Dorset, United Kingdom. All other chemicals and enzymes were obtained from the Boehringer Corp. (London) Ltd., London, W.5, United Kingdom.

Preparation of Homogenates

The tissues were taken from the animals while under pentobarbitone anaesthesia, washed in ice-cold saline, blotted dry and weighed. The heart was dissected into right and left ventricles and these were treated separately. The tissue was homogenized in 20 volumes of ice-cold 0.1 M sodium phosphate buffer pH 7.5 containing 6.0 mM EDTA for 35 s with an Ultra Turrex homogenizer at maximum speed. The homogenate was strained through fine terylene netting and was further diluted with the same extraction medium. All procedures were carried out at 0°C and the homogenate was stored at this temperature until assayed.

Enzyme Assays

All assays were carried out at 25°C in a total volume of 2.0 ml. The oxidation of NADH was followed at 340 mμ with a Zeiss PM Q II spectrophotometer, with readings taken at 1-min intervals. The reduction of dichlorophenolindophenol was followed at 600 mμ with a Coleman Jr. II spectrophotometer, with readings taken at 2-min intervals.

The assay conditions were as follows:

Glyceraldehyde-3-phosphate dehydrogenase (EC. 1.2.1.12): 60 mM triethanolamine, 6.0 mM EDTA, 4.0 mM $MgSO_4$, 20 mM cysteine, 0.19 mM NADH, 1.5 mM ATP, 5.9 mM 3-phosphoglycerate, 8 units 3-phosphoglycerate kinase, pH 7.5.

Phosphofructokinase (EC. 2.7.1.11): 60 mM triethanolamine, 6.0 mM EDTA, 8.0 mM $MgSO_4$, 750 mM KCl, 0.19 mM NADH, 1.5 mM ATP, 2.3 mM AMP, 1.3 mM fructose-6-phosphate, 2.25 units aldolase, 12 units triosephosphate isomerase, 4.0 units glycerol-3-phosphate dehydrogenase, pH 7.5.

Aspartate aminotransferase (EC. 2.6.1.1): 60 mM triethanolamine, 6.0 mM EDTA, 0.19 mM NADH, 35 mM aspartate, 7 mM α-ketoglutarate, 18 units malate dehydrogenase, pH 7.5.

Lactate dehydrogenase (EC. 1.1.1.27): 60 mM triethanolamine, 6.0 mM EDTA, 0.19 mM NADH, 2.3 mM pyruvate, pH 7.5

α-hydroxybutyrate dehydrogenase: 60 mM triethanolamine, 6.0 mM EDTA, 0.19 mM NADH, 1.5 mM α-ketobutyrate, pH 7.5.

β-hydroxyacyl coenzyme A dehydrogenase (EC.1.1.1.35): 25 mM sodium pyrophosphate, 6.0 mM EDTA, 0.19 mM NADH, 5.0 mM *N*-acetyl-*S*-acetacetyl-cysteamine, pH 6.8.

Succinate dehydrogenase (EC. 1.3.99.1): 52 mM triethanolamine, 5.5 mM EDTA, 2.0 mM KCN, 40 μg dichlorophenolindophenol, 10 mM succinate, pH 7.5.

Mitochondrial glycerol-3-phosphate dehydrogenase (EC. 1.1.99.5): 0.092 mM sodium phosphate, 5.5 mM EDTA, 40 μg dichlorophenolindophenol, 2.0 mM KCN, 12 mM glycerol-3-phosphate, pH 7.5.

As the stability of the enzymes varied, the assays were always carried out in the sequence in which the assay conditions are listed for those assays involving NADH, while the succinate dehydrogenase and mitochondrial glycerol-3-phosphate dehydrogenase assays were performed simultaneously with, but independently of, the other assays. All assays were complete within 4 h of the removal of the tissue.

With most of the assays, the change in extinction with time was linear, and the regression line for the data was computed by the method of least squares using an Olivetti Programma 102. For the succinate dehydrogenase, mitochondrial glycerol-3-phosphate dehydrogenase, and myocardial lactate dehydrogenase assays, the extinction change was not linear with time. The data was fitted by computer by the method of least squares to the equation:

$$(Eo - Ei) = a\,(t_i - t_o)^b$$

where Eo and Ei are the extinctions at time t_o and t_i while t_o is the time of the first reading after the initiation of the reaction. This was 1 min for the lactate dehydrogenase assays, and 2 min for the succinate dehydrogenase and mitochondrial glycerol-3-phosphate assays. The regression coefficient was always greater than 0.99. The value of ab, which is the slope at time $(t_o + 1)$, was taken as a measure of the enzyme activity. In this way a measurement of near initial rates of reaction could be made without the observer bias inherent in curve drawing and in the estimation of tangents to curves. The activities in μmoles substrate transformed/min/mg protein at 25°C were calculated using the molar extinction coefficients of 6.22×10^6 for NADH, and 21.0×10^6 for dichlorophenolindophenol (Armstrong 1964).

Protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Student's '*t*' test was used to calculate statistical significance.

Results

The ratio of the weight of the left ventricle with septum to the weight of the right ventricle for the high altitude animals was 2.7 ± 0.5 for guinea-pigs, and 2.8 ± 0.4 for rabbits. The ratio was significantly higher for the low altitude animals, being 3.3 ± 0.2 for guinea-pigs, and 3.8 ± 0.7 for rabbits ($P < 0.01$ for both species). The difference was due to the greater right ventricular weight in the high altitude animals, resulting from hypertrophy presumably caused by hypoxic pulmonary hypertension (Dean et al. 1968, Reeves et al. 1963, Ressl 1974).

Guinea-pig Sartorius

Table 1 shows the enzyme activities found in the sartorius muscle of guinea-pigs. There was no difference between the activities in the high and low altitude animals. The ratio of glyceraldehyde-3-phosphate dehydrogenase activity to mitochondrial glycerol-3-phosphate dehydrogenase activity (GAPDH/mGPDH) (Pette 1966) was also similar at both altitudes, being 1900 ± 700 at low altitude and 1900 ± 400 at high altitude.

Table 1

Mean enzyme activities in sartorius of guinea-pigs indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude	High Altitude
Phosphofructokinase	0.43 ± 0.10 (7)	0.35 ± 0.05 (8)
Glyceraldehyde-3-phosphate dehydrogenase	4.3 ± 1.1 (8)	4.0 ± 0.6 (8)
Lactate dehydrogenase	3.7 ± 0.9 (8)	3.2 ± 0.3 (8)
α-hydroxybutyrate dehydrogenase	0.56 ± 0.14 (8)	0.48 ± 0.03 (8)
Aspartate aminotransferase	0.15 ± 0.04 (8)	0.16 ± 0.04 (8)
β-hydroxyacyl coenzyme A dehydrogenase	0.0016 ± 0.0008 (8)	0.0013 ± 0.0003 (8)
Succinate dehydrogenase	0.0022 ± 0.0007 (8)	0.0016 ± 0.0009 (8)
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0024 ± 0.0009 (8)	0.0021 ± 0.0002 (8)

Guinea-pig Myocardium

Tables 2 and 3 show the results obtained from the left and right ventricles of guinea-pig. Comparing the left to the right ventricles, there was little difference between the activities found either in the high or in the low altitude animals. Mitochondrial glycerol-3-phosphate dehydrogenase activity was significantly higher in the left ventricle than in the right of low altitude animals (0.0028 ±

Table 2

Mean enzyme activities in left ventricle of guinea-pigs indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude	High Altitude	
Phosphofructokinase	0.14 ± 0.02 (9)	0.15 ± 0.02 (8)	
Glyceraldehyde-3-phosphate dehydrogenase	0.86 ± 0.10 (9)	0.96 ± 0.05 (8)	
Lactate dehydrogenase	1.2 ± 0.2 (9)	1.1 ± 0.1 (8)	
α-hydroxybutyrate dehydrogenase	0.74 ± 0.1 (9)	0.61 ± 0.07 (8)	$P < 0.02$
Aspartate aminotransferase	1.3 ± 0.1 (9)	1.2 ± 0.1 (8)	
β-hydroxyacyl coenzyme A dehydrogenase	0.037 ± 0.005 (9)	0.042 ± 0.006 (8)	
Succinate dehydrogenase	0.027 ± 0.005 (9)	0.022 ± 0.003 (4)	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0028 ± 0.0009 (9)	0.0024 ± 0.0005 (8)	

Table 3

Mean enzyme activities in right ventricle of guinea-pigs indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude	High Altitude	
Phosphofructokinase	0.14 ± 0.03 (8)	0.14 ± 0.02 (8)	
Glyceraldehyde-3-phosphate dehydrogenase	0.90 ± 0.20 (8)	1.0 ± 0.1 (8)	
Lactate dehydrogenase	1.4 ± 0.2 (8)	1.2 ± 0.2 (8)	
α-hydroxybutyrate dehydrogenase	0.87 ± 0.13 (8)	0.72 ± 0.08 (8)	$P < 0.05$
Aspartate aminotransferase	1.2 ± 0.1 (8)	1.2 ± 0.1 (8)	
β-hydroxyacyl coenzyme A dehydrogenase	0.033 ± 0.004 (8)	0.042 ± 0.008 (8)	$P < 0.02$
Succinate dehydrogenase	0.024 ± 0.007 (8)	0.024 ± 0.003 (4)	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0017 ± 0.0004 (8)	0.0020 ± 0.0002 (8)	

0.0009 c.f. 0.0017 ± 0.0004; $P < 0.02$), but was similar in both ventricles in high altitude animals. The GAPDH/mGPDH ratios were also similar for both ventricles at both altitudes being 330 ± 100 and 560 ± 180 in the left and right ventricles, respectively, at low altitude, and 420 ± 140 and 520 ± 60 at high altitude. α-hydroxybutyrate dehydrogenase activity was comparable between the ventricles at low altitude, but was higher in the right ventricle than the left of the high altitude guinea-pigs (0.72 ± 0.08 c.f. 0.61 ± 0.07; $P <$ 0.02). In the α-hydroxybutyrate dehydrogenase assay the heart type lactate dehydrogenase isoenzyme (H-LDH) is considerably more active than the skeletal muscle type isoenzyme (M-LDH), and the ratio α-hydroxybutyrate dehydrogenase activity to total lactate dehydrogenase activity (α-HBDH/LDH) gives a measure of the proportion of the H-LDH subunits. This ratio was similar in both ventricles at both altitudes.

There were few differences between the myocardial enzyme activities of high compared with low altitude guinea-pigs. Lactate dehydrogenase activity was similar while the α-hydroxybutyrate dehydrogenase activity was lower in the high altitude animals. However the ratio α-HBDH/LDH was not significantly lower at high altitude indicating no significant change in the proportion of H-LDH subunits. The guinea-pigs from high altitude had higher β-hydroxyacyl coenzyme A dehydrogenase activity but this was statistically significant in the right ventricle only ($P < 0.02$).

Rabbit Sartorius

Table 4 shows the results for the sartorius muscle of rabbits. The activities of glyceraldehyde-3-phosphate dehydrogenase, and phosphofructokinase were significantly higher in the high altitude animals ($P < 0.005$, $P < 0.02$ respectively), indicating a higher glycolytic capacity. The activity of mitochondrial glycerol-3-phosphate dehydrogenase was also significantly higher at altitude ($P < 0.02$), while the GAPDH/mGPDH ratios were similar, being 2200 ± 700 at low altitude and 1700 ± 400 at high altitude.

Table 4

Mean enzyme activities in sartorius of rabbits indigenous to high or low altitude. Activities expressed as μ moles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude (8)	High Altitude (8)	
Phosphofructokinase	0.5 ± 0.08	0.71 ± 0.18	$P < 0.02$
Glyceraldehyde-3-phosphate dehydrogenase	6.2 ± 0.9	8.6 ± 1.7	$P < 0.005$
Lactate dehydrogenase	5.5 ± 1.4	7.0 ± 1.5	
α-hydroxybutyrate dehydrogenase	0.52 ± 0.13	0.65 ± 0.14	
Aspartate aminotransferase	0.094 ± 0.029	0.12 ± 0.04	
β-hydroxyacyl coenzyme A dehydrogenase	0.0026 ± 0.0009	0.0034 ± 0.001	
Succinate dehydrogenase	0.0024 ± 0.0006	0.0031 ± 0.0012	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0031 ± 0.0014	0.0051 ± 0.0012	$P < 0.02$

Rabbit Myocardium

Tables 5 and 6 show the enzyme activities for the left and right ventricles of rabbits. Comparing the left to the right ventricle, the only difference found was in the activity of glyceraldehyde-3-phosphate dehydrogenase which was significantly higher in the left ventricle at low altitude (0.82 ± 0.04 c.f. 0.73 ± 0.05, $P < 0.005$).

Table 5

Mean enzyme activities in left ventricle of rabbits indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude (8)	High Altitude (8)	
Phosphofructokinase	0.13 ± 0.01	0.15 ± 0.02	$P < 0.05$
Glyceraldehyde-3-phosphate dehydrogenase	0.82 ± 0.04	0.95 ± 0.14	$P < 0.05$
Lactate dehydrogenase	0.95 ± 0.16	1.2 ± 0.1	$P < 0.05$
α-hydroxybutyrate dehydrogenase	1.2 ± 0.1	1.3 ± 0.2	
Aspartate aminotransferase	1.0 ± 0.1	1.3 ± 0.1	$P < 0.001$
β-hydroxyacyl coenzyme A dehydrogenase	0.052 ± 0.01	0.073 ± 0.009	$P < 0.005$
Succinate dehydrogenase	0.041 ± 0.007	0.044 ± 0.007	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0010 ± 0.0004	0.0013 ± 0.0004	

Table 6

Mean enzyme activities in right ventricle of rabbits indigenous to high or low altitude. Activities expressed as μmoles substrate transformed/min/mg protein at 25°C, ± S.D. Number of observations in parentheses.

Enzyme	Low Altitude (8)	High Altitude (8)	
Phosphofructokinase	0.11 ± 0.01	0.14 ± 0.02	$P < 0.005$
Glyceraldehyde-3-phosphate dehydrogenase	0.73 ± 0.05	0.91 ± 0.14	$P < 0.005$
Lactate dehydrogenase	0.94 ± 0.16	1.3 ± 0.1	$P < 0.001$
α-hydroxybutyrate dehydrogenase	1.2 ± 0.2	1.3 ± 0.1	
Aspartate aminotransferase	0.91 ± 0.07	1.3 ± 0.1	$P < 0.001$
β-hydroxyacyl coenzyme A dehydrogenase	0.054 ± 0.008	0.076 ± 0.008	$P < 0.001$
Succinate dehydrogenase	0.036 ± 0.006	0.041 ± 0.009	
Glycerol-3-phosphate dehydrogenase (mitochondrial)	0.0010 ± 0.0004	0.0011 ± 0.0003	

The differences between the activities in the high compared to the low altitude animals were similar for both ventricles, but were statistically more significant for the right ventricle. The glycolytic capacity was higher in the high altitude myocardium, as indicated by the higher glyceraldehyde-3-phosphate dehydrogenase phosphofructokinase activities. Lactate dehydrogenase activity was also higher while α-hydroxybutyrate dehydrogenase activity was unchanged. The α-HBDH/LDH ratios were thus lower in the ventricles from high altitude rabbits, indicating a higher proportion of the M-LDH subunits. The activities of aspartate aminotransferase and β-hydroxyacyl coenzyme A dehydrogenase were also higher in the high altitude rabbit myocardium while the activities of succinate dehydrogenase and mitochondrial glycerol-3-phosphate dehydrogenase were similar to those in low altitude rabbits. The GAPDH/mGPDH ratios were similar in both ventricles at both altitudes, being 890 ± 290 and 860 ± 460 in the left and right ventricles respectively at low altitude, and 760 ± 180 and 860 ± 270 at high altitude.

Discussion

Comparison of Myocardium and Sartorius Muscles

'White' muscle is more dependent on glycolysis and less dependent on fatty acid oxidation for its supply of energy, than is 'red'

muscle (Ogata 1960, Shipp et al. 1961, Wirsen 1965). This is reflected in the enzyme activities measured here for both guinea-pigs and rabbits, with the sartorius muscle having 3- to 5-fold higher phosphofructokinase and lactate dehydrogenase activities, and a 20-fold lower β-hydroxyacyl coenzyme A dehydrogenase activity, than the myocardium. The oxidative capacity, as indicated by succinate dehydrogenase activity, was 10- to 15-fold lower in the sartorius muscle. These results agree with previous work (Czok & Bücher 1960, Pette et al. 1962).

It has been suggested that the oxaloacetate-malate shuttle is less important and the dihydroxyacetone phosphate - glycerol-3-phosphate shuttle more important in white muscle than in red muscle for transferring reducing equivalents across the mitochondrial membrane (Blanchaer 1964, Pette 1966). The present results are consistent with this suggestion as the activity of glycerol-3-phosphate dehydrogenase was comparable to that of succinate dehydrogenase in the sartorius but 10-fold lower in the myocardium of both species. However, quantitatively, the importance of this cycle in vertebrate muscles is unclear (Crabtree & Newsholme 1972).

Comparison of Guinea-pig and Rabbit

Ventricular muscle specimens from rabbit and guinea-pig were similar with respect to the activities of the glycolytic enzymes and of aspartate aminotransferase, while the rabbit ventricle had higher β-hydroxyacyl coenzyme A dehydrogenase, succinate dehydrogenase and lower mitochondrial glycerol-3-phosphate dehydrogenase activities. This suggests that the rabbit myocardium had a higher oxidative metabolic capacity and a higher fatty acid oxidative capacity, and that the dihydroxyacetone phosphate - glycerol-3-phosphate shuttle was less important than in the guinea-pig myocardium. Considering the sartorius muscles, the enzyme activities suggest that the glycolytic capacity was higher in the rabbit muscle, as was the fatty acid oxidative capacity; while succinate dehydrogenase was comparable in the two species. Mitochondrial glycerol-3-phosphate dehydrogenase activity was also higher in the rabbit sartorius. Pette (1966) suggested that the activity of mitochondrial glycerol-3-phosphate dehydrogenase was related to that of glyceraldehyde-3-phosphate dehydrogenase, having found that the ratio of their activities was the same in several skeletal muscles from rabbits and rats. These results partially support this suggestion in that the ratio (GAPDH/mGPDH) is similar at both altitudes, and in the two species for either heart or sartorius muscle, despite differing absolute enzyme activities. However the ratio in the sartorius muscle is significantly higher than that in the heart muscle.

Effects of Altitude

Indigenous animals represent animals completely adapted to their environment. Such adaptation may involve the results of two distinct biological processes: physiological alterations which would occur in sea level animals moved to high altitude, and possibly differences which had arisen over many generations through natural selection. Natural selection has, for instance, been shown to play a part in the adaption of the pulmonary circulation in high altitude cattle (Will et al. 1970). In this study outbred animals indigenous to either high or low altitude were used to minimize differences arising except as a result of the altitude. Earlier work (Harris et al. 1970) had compared indigenous high altitude animals to laboratory strain animals and this may explain the different results obtained.

The high altitude guinea-pig showed no significant difference in enzyme activities from the low altitude ones. This contrasts with unpublished results obtained in our labo-

ratory which showed marked changes in the activity of some glycolytic enzymes in the guinea-pig myocardium during short-term acclimatization to hypobaric conditions. It would seem that, as a result of long-term adaptation, other factors must have altered sufficiently to allow adequate energy production to occur without elevated enzyme activities. Capillary density in the myocardium is increased (Valdivia 1958) thus reducing the diffusion distance for oxygen and substrates from the blood to the cells if the capillaries are normally open. Increased myoglobin concentration (Reynafarje 1962, Reynafarje & Morrison 1962) may aid the diffusion of oxygen through the cell (Hemmingsen 1963). Reynafarje (1971) has suggested that the kinetic characteristics of mitochondria may be altered in the high altitude guinea-pigs.

The high altitude rabbit showed increased glycolytic enzyme activity in both sartorius and myocardial muscle suggesting an increased glycolytic capacity, which might be important to allow adequate ATP production in these tissues in times of severe exercise when aerobic production of ATP might be insufficient. The slight increase in the proportion of M-LDH isoenzyme subunits is interesting in this context, although the significance is unclear as the pyruvate inhibition of the H-LDH isoenzyme found *in vitro* has not been shown *in vivo* (Garland et al. 1964). Alterations in the isoenzyme pattern of the myocardium have been found in some, but not all, acclimatization studies (Anderson & Bullard 1971, Mager et al. 1968, Miller & Hale 1968). Sorensen (1972) reported differences in the lactate dehydrogenase isoenzyme pattern of the brain of people who were indigenous to high altitude compared to those indigenous to low altitude.

The results indicate the different means by which these species have adapted to the low ambient partial pressure of oxygen, and should be considered when studying short-term acclimatization.

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