FACTORS INFLUENCING MUSCLE FIBRE COMPOSITION IN THE HORSE

A THESIS

PRESENTED FOR THE DEGREE

OF

DOCTOR OF PHILOSOPHY

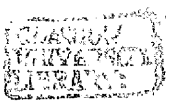
BY

PAUL SCOTT GUY BSc(HONS.)

DEPARTMENT OF VETERINARY PHARMACOLOGY

UNIVERSITY OF GLASGOW

JANUARY 1978.



TO MY PARENTS

Acknowledgements

I would like to take this opportunity of thanking the following people for their help. My supervisor, Dr. D.H. SNOW for initiating the project, taking the biopsies and giving advice and helpful criticism of my ideas, both good and bad during the last three years. Dr. J.A. BOGAN, head of the department of Veterinary Pharmacology for the use of the facilities in the department and his advice, Professor T.A. DOUGLAS of the department of Veterinary Biochemistry and Professor N. WRIGHT of the department of Veterinary Anatomy for the use of facilities in their respective departments.

I should further like to thank Miss A. GRAHAM and Mrs. B. WESTON for looking after the horses, Mr. J. MURPHY for access to post-mortem horses, Mr. P. BAXTER for technical assistance, Miss E. SOMERVILLE for help with diagrams, Dr. P. ROSENBERG and Mr. M. MOORE of the University of Glasgow Computing Service for statistical and computing advice. A further debt of gratitude is due to Mr. A. FINNEY and the staff of the department of photography of the Veterinary Hospital for the excellent photographic prints used in this thesis. Fig. 2.4 was taken by Dr.D.H.Snow.

Finally I should like to thank Mrs. A. STRACHAN for carefully typing this thesis.

This work was undertaken whilst I was a research assistant on a grant from the Horse Race Betting Levy Board (Project No. 169). Quarterhorse and Arab horse samples were obtained by Dr. D.H. SNOW whilst he was under a Carnegie Travelling Fellowship.

Finally, I would like to thank Dirk, Adam, Ryne, Astryl, Oscar, Gertie, Sunny and all of the unknown horses without whom this project could not have been carried out.

This thesis is based in part on the following papers.

1) Percutaneous needle muscle biopsy in the horse: Snow D.H. and Guy P.S. Equine vet. J. 8, 150-155 (1976).

2) The effect of training and detraining on Lactate Dehydrogenase isoenzymes in the horse: Guy P.S. and Snow D.H. Biochem. Biophys. Res. Comm. 75, 863-869 (1977).

3) The effect of training and detraining on muscle composition in the horse: Guy P.S. and Snow D.H. J. Physiol. 269, 33-51 (1977).

4) The structure and biochemistry of equine skeletal muscle: Snow D.H. and Guy P.S. Proc. of Am. Ass. Equine Practitioners Conference, December 1976, 199-210 (1977).

5) A preliminary survey of skeletal muscle fibre types in equine and canine species: Guy P.S. and Snow D.H. J. Anat. 124, 499-500 (1977).

6) The actions of the β-adrenoceptor blocking agents propranolol and metaprolol in the maximally exercised horse: Snow D.H., Summers R.J. and Guy P.S. Submitted to Eur. J. appl. Physiol.

Summary

1) Horse skeletal muscle was found to consist of three distinct fibre types.

a) A high myosin ATPase activity at pH 9.4, high oxidative, high glycolytic capacity fibre (FTH).

b) A high myosin ATPase activity at pH 9.4, low oxidative, high glycolytic capacity fibre (FT).

c) A low myosin ATPase activity at pH 9.4, high oxidative, low glycolytic capacity fibre (ST).

2) Most dog skeletal muscle examined also contained these fibre types but identification was more difficult. Some mongrels had an unusual fibre type profile, with only two types being distinguished, one having high oxidative, high myosin ATPase at pH 9.4 activity and the other having low oxidative, low myosin ATPase at pH 9.4 activity.

3) In the muscles examined in the horse, fibre type percentages were similar in both deep and superficial regions of the muscle. In the gluteus medius variation within a muscle and between contralateral muscles was small for enzyme activities, glycogen and protein. Triglyceride variation was much larger and results were impossible to interpret.

4) The percentages of the three fibre types in the horse varied from muscle to muscle, being generally lower in the fore limb muscles. This variation between muscles was not as great in the dog.

5) In the gluteus medius muscle of the horse, the fastest breed had the highest percentage of high myosin ATPase activity at pH 9.4 fibres. This relationship was less obvious in the other muscles examined.

6) The greyhound dog had the highest percentage of high myosin ATPase activity at pH 9.4 in all of the muscles examined when compared to the foxhound and cross-bred mongrel. This percentage reached 100% in the deltoideus of several of the greyhounds.

7) In the horse, the Quarterhorse had the largest Lactate Dehydrogenase and Aldolase activities and the lowest Citrate Synthase and 3-Hydroxy-acyl CoA Dehydrogenase activities indicating a high capacity for anaerobic metabolism. Similarly, the breeds best suited to endurance exercise had the highest capacity for aerobic metabolism. The donkey, in general, had the lowest enzyme activities.

8) In the dog, greyhound muscle was highest in aerobic and glycolytic carbohydrate metabolism enzyme activities. The mongrel had higher activities in fatty acid oxidation enzymes.

9) During a training programme involving both aerobic and anaerobic work, the activity of several enzymes increased progressively but at different rates, with an approximately twofold increase in Aldolase, Lactate Dehydrogenase, Alanine Aminotransferase, Aspartate Aminotransferase, Citrate Synthase and 3-Hydroxy-acyl CoA Dehydrogenase and a 30% increase in Creatine Phosphokinase. When the isoenzyme content of Lactate Dehydrogenase was examined, an increase in the percentage of the H subunit was found. When the relative activity of the subunits were determined, an increase in both subunits occurred. Hexokinase, Glycogen Synthetase Glycerol-3-Phosphate Dehydrogenase and protein were unaffected by training. Glycogen increased by about 33% with training.

10) There was a significant increase in the percentage of FTH fibres at the expense of both the percentage of ST and FT fibres after ten weeks training.

11) A decrease in the activity of those enzymes which increased with training occurred after five weeks detraining. By ten weeks detraining, the activity of those enzymes had increased again to values above those at the end of training. Enzymes which were unchanged with training were similarly unaffected by detraining. Glycogen remained elevated above pre-training levels after ten weeks detraining.

12) Glycogen depletion occurred in horse skeletal muscle after both maximal and sub-maximal exercise. The β_1, β_2 adrenoreceptor blocker dl propranolol (0.2 $mg.kg^{-1}$) decreased the rate of glycogen depletion during maximal exercise. During submaximal exercise, glycogen depletion was unaffected by dl propranolol (0.2 $mg.kg^{-1}$), the biologically inactive d propranolol (0.2 $mg.kg^{-1}$) or the β_1 blocker dl metoprolol (0.2 $mg.kg^{-1}$).

ABBREVIATIONS

The following abbreviations were used in this thesis.

LDH	Lactate Dehydrogenase
CPK	Creatine Phosphokinase
ALD	Aldolase
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
CS	Citrate Synthase
HK	Hexokinase
GS	Glycogen Synthetase
GDH	Glycerol-3-Phosphate Dehydrogenase
HAD	3-Hydroxyacyl Coenzyme A Dehydrogenase
TEMED	N,N,N,N, Tetramethylethylene diamine
R.Q.	Respiratory Quotient
MK	Myokinase

TABLE OF CONTENTS

SECTION 1

GENERAL INTRODUCTION

INTRODUCTION

Muscle has fascinated man since the dawn of time and the first detailed anatomical studies were performed by the ancient Greeks such as Hippocrates and Galen. This work was later supplemented by Leonardo da Vinci with his sketches of working muscles. Until the 16th century, however, little progress was made into the understanding of muscle and the work of Galen remained virtually unchallenged. By this time, several workers including Croone, Borelli, Willis, Stensen and Mayow (Needham, 1971) were looking at muscle in a scientific manner. Mayow discovered that a substance, now known to be oxygen, was fundamental in muscle contraction and that body constituents were utilised in the process (Needham, 1971). Unfortunately, this work was largely ignored and little else was done until the discovery of oxygen by Priestley.

The first detailed description of skeletal muscle was by Lorenzini, who in 1678 noted the striking differences in certain muscles of the rabbit (see Paukul, 1904). He divided muscle into "red" and "white", a classification still accepted, and suggested that the colour was due to differing blood supplies in the two types of muscle. Later Kolliker in 1850 (see Busk and Huxby, 1853) postulated that it was something in the contractile machinery which gave muscles their distinctive colour and this was corroborated by Kühne (1865). Mörner (1894) discovered that the pigmentation had a different spectral analysis from haemoglobin in the blood and it was eventually called myoglobin by Günther (1921).

Lavosier measured the "air vital" or oxygen used during work and he was the first to show that the human organism increased combustion

during work (Asmussen, 1971). At the same time, Crawford in Glasgow (Asmussen, 1971) measured the heat output of small animals and showed that there was a close relationship between heat output and carbon dioxide production.

By the second half of the 19th century, it had become generally accepted that the metabolic processes during work were combustions, but what was being utilised - fat and carbohydrate or just carbohydrate? There were two schools of thought concerning this problem, both based on results obtained by measuring the respiratory quotient (R.Q.). Zuntz and coworkers (Asmussen, 1971) claimed that the same mixture of fat and carbohydrate was used during exercise as at rest, whereas Chauveau and his colleagues (Asmussen, 1971) maintained that carbohydrate exclusively was used during exercise. As it turned out, both results were correct, the discrepancy occurring because Zuntz used long-term, non-exhaustive work whereas Chauveau experimented with heavy work for 1 hour. This problem was not solved until Christensen et al. (1939), building on the work of Krogh and Lindhard (1920) concluded that:

i) Both fat and carbohydrate are used as fuel.

ii) The ratio of fat/carbohydrate depends on several factors.

   a) In light to moderate exercise of short duration, diet is more important.

   b) In long continuous moderate exercise, the relative fat utilisation increases gradually due to decreased carbohydrate stores.

   c) With exercise of increasing intensity, the relative carbohydrate utilisation increases at a certain intensity until approaching 100% at maximum work.

As well as the studies in man, the turn of the 20th century saw the beginning of biochemical research using isolated muscles and "muskelbrei" - minced muscles. It had been known for a long time that lactic acid was a metabolite in muscle, but its origin was unknown. Hermann (1874) had suggested that a giant molecule he called inogen, gave rise to lactic acid and carbon dioxide by an explosive combination with inbuilt oxygen when muscle contracted. During recovery, oxygen was again taken up and inogen formed. Fletcher and Hopkins (1907) showed that in amphibian muscle, lactic acid was formed from glycogen and this observation was followed by others until ultimately the Hill-Meyerhoff theory was developed. This theory which was to dominate the field for several years stated: During muscular work,

a) Glycogen disappears,

b) Lactic acid appears in equivalent amounts,

c) Preformed carbon dioxide is driven off,

d) Heat is produced in proportion to the lactic acid formed,

e) Hydrogen ion concentration increases.

The years between 1927 and the early thirties were momentous in the study of muscle metabolism. Creatine phosphate (CP) was discovered simultaneously by Eggleton and Eggleton (1927) and Fiske and Subbarow (1927) and shown to decrease during contraction and increase again during relaxation. Adenosine triphosphate (ATP) was discovered by Lohmann (1931), and the Hill-Meyerhoff theory was destroyed by the discovery of Lundsgaard (1930,a,b) that muscle poisoned by iodoacetate could still contract without glycolysis taking place and lactic acid being formed. Lohmann (1934, 1935) found that CP would not split into two unless adenosine diphosphate (ADP)

was present in the muscle to accept the phosphate (Pi). This meant that CP could not deliver energy directly into the contractile machinery until ATP had been split into ADP and Pi and this suggested that ATP was the immediate source of energy (The Lohmann Reaction).

These discoveries led to the development of the terms lactacid energy - derived from glycolysis going to lactate, and alactacid energy - derived from the splitting of ATP and CP, for anaerobic energy output (Lundsgaard, 1932). Margaria et al. (1933) then developed the hypothesis of a lactacid and alactacid portion of the oxygen debt in man and they were able to calculate the alactacid portion by making the assumption that blood lactate concentration was representative of the concentration in the total body water. Finally, Engelhardt and Ljubimowa (1939) supplied the link between muscle contraction and biochemical events when they discovered that myosin, a known constituent of muscle fibres, acted as an ATPase, thus allowing energy production from CP to ADP to take place consecutively to muscle contraction.

Whilst these discoveries were taking place, other workers were mapping out the various metabolic pathways and establishing the importance of the energy rich phosphate bonds (Kalckar, 1941; Lipmann, 1941; Krebs, 1943). From these studies the following sequence of events in muscle can be described. ATP delivers energy directly to the contractile mechanism by splitting to ADP and Pi. It is resynthesised either anaerobically from CP; from glycolysis leading to lactic acid formation or aerobically from the oxygenation of fuels in the muscle fibre.

Since 1945, great advances have been made in the fields of muscle and exercise physiology in both man and animals. These have been possible due to the development of new techniques such as catheterization and the reintroduction of old techniques such as the percutaneous needle muscle biopsy of Bergström (1962) first used by Duchennes in the last century (Duchennes, 1868). In this way, it has been proved conclusively, in man, that glycogen (Ahlborg et al., 1967) and ATP and CP depletion (Hultman et al., 1967) are important in the onset of fatigue. At the same time, as better techniques were being developed to enable more information to be obtained in the living animal, more sophisticated analytical techniques were being evolved for physiological measurements - with the advent of electronics - and in histochemistry and biochemistry - with the production of radiochemicals and purer biochemicals.

It had been known for about a century (Ranvier, 1873) that the red muscle fibres contracted and relaxed more slowly than the white coloured fibres and work at this time had led to the general view that "red" muscles were associated with sustained, repeated work (tonic) and "white" muscles with rapid vigorous contractions of shorter duration (phasic). With the development of more specific histochemical techniques, it became apparent that there could possibly be more than two fibre types. Ogata (1958,a,b,c) found that on the basis of the staining intensity for oxidative enzymes, there were three different fibre types in several different animals. "Red" muscles stained intensely for oxidative enzymes whereas "white" muscles stained lightly. A third fibre type stained intermediate between the other two but, as most red muscles are

composed of some intermediate as well as some white fibres, the intermediate fibre was regarded as a variant of the red fibres (Barnard et al., 1971).

One of the major questions which has long been asked was what determined the properties of the muscle fibre? Buller et al. (1960a) found that cross-innovation between two muscles, representing fast and slow contracting times led to a reversal of properties. These authors concluded that there was a trophic influence in the nerve which determined whether the muscle was fast or slow. Later, several groups (Salmons and Vrbova, 1969; Pette et al., 1976) produced evidence that the pattern of stimulation was more important. Slow motorneurons fire continuously at a lower frequency than fast motorneurons which fire in short sharp bursts and increase in frequency as tension increases. By electrical stimulation it is possible to impose the frequency of slow muscle stimulation on a fast muscle and using this technique, fast muscles such as the tibialis anterior or extensor digitorum longus of the rabbit have been changed physiologically and biochemically to the characteristics of a slow muscle (Salmons and Vrbova, 1969; Pette et al., 1976).

In 1967, Barany (1967) showed that the activity of myosin ATPase is directly proportional to the intrinsic speed of sarcomere shortening in normal muscle with widely varying speeds of contraction. Guth and Samaha (1969) then proved that the high activity myosin ATPase fibres in the histochemical reaction were fast contracting and that low activity fibres were slow contracting. At the same time, Edgerton and Simpson (1969) found that the soleus muscle of rats was composed

predominately of "intermediate" fibres and the soleus of guinea pigs solely of these fibres. Both of these muscles are slow contracting and so they suggested that there were in fact three types, the "intermediate" fibre being different from the "red" fibre and slow contracting, whereas the "red" and "white" were both fast contracting. Conclusive proof that the histochemical properties of a muscle fibre could be correlated with the physiological and metabolic properties was eventually produced by Burke et al. (1973). Until then conclusions had been drawn from whole muscles or muscle segments which were not homogeneous in a fibre type but composed mainly of a given fibre type. Burke et al. (1973) using the glycogen depletion technique of Edstrom and Kugelberg (1968) were able to identify the muscle fibres innervated by a single motorneuron. The motorneuron was stimulated through a microelectrode and its contraction parameters and time to fatigue measured. Sections for histochemical analysis were later cut and the fibres that were stimulated by the motorneuron were marked by the fact they had been depleted of glycogen. In this way, these authors found three main fibre types in the cat gastrocnemius, two with relatively short contraction times designated FF (fatigue sensitive) and FR (fatigue resistant) and one with a relatively long contraction time designated S (fatigue resistant). These three types correspond to those of several other authors using only histochemical techniques. Table 1.1 gives the various nomenclature used by other authors and they are compared to that used in the present study which is based on that of Lindholm and Piehl (1974). Over the last few years, much work has been undertaken to further classify the fibre types quantitatively rather than qualitatively. Perhaps the most important technique developed is the "single fibre" dissections of Essen et al. (1975).

Authors	Species	Nomenclature		
Lindholm and Piehl (1974)	Horse	FT	FTH	ST
Ogata (1958,a,b,c)	Rabbit	white	red	intermediate
Stein and Padykula (1962)	Rat	A	C	B
Romanul (1964)	Rat	I	II	III
Kugelberg and Edström (1968)	Rat	A	B	C
Engel (1970)	Man	II	II	I
Brooke and Kaiser (1970 a,b)	Man	IIB	IIA	I
Yellin and Guth (1970)	Rat	Aα	Cαβ	Bβ
Ashmore and Doerr (1971)	Various	αW	αR	βR
Barnard et al. (1971)	Guinea Pig	FT white	FT red	ST intermediate
Peter et al. (1972)	Guinea Pig	FG	FOG	SO
Burke et al. (1973)	Cat	FF	FR	S

Table 1.1. Nomenclature used by various authors in classifying various types of mammalian skeletal muscle fibres as compared to that used in the present study as suggested by Lindholm and Piehl (1974).

This allows single fibres to be dissected out of muscle biopsy material, typed histochemically and characterised biochemically for a range of substrates and enzymes.

At the same time as all of this activity was being devoted to determining the physiological and biochemical properties of muscle, work was also in progress on the effects of training in various species. The first reports of endurance training in rats found no significant biochemical changes (Hearn and Wainio, 1956; Gould and Rawlinson, 1959) and it was not until a decade later that an increase in the respiratory capacity of muscle was reported with training (Holloszy, 1967). The discrepancy between the various reports was found to be due to the fact that the exercise regime given in the earlier studies was not sufficiently strenuous to induce an adaptive change in the muscle. Since then, many studies have been performed on several species including man (Varnauskas et al., 1970; Gollnick et al., 1973a), guinea pig (Barnard et al., 1970), cat (Gonyea et al., 1977), miniature pig (Fitts et al., 1973), pig (Fogd Jorgensen and Hildgaard-Jensen, 1975) and the lesser bush baby (galago senegalensis (Edgerton et al., 1972) to observe the effects of training on many different parameters. The results of these studies have been many and varied with not only species variation having to be taken into account, but also the type of training programme given to the various subjects (Holloszy and Booth, 1976).

The horse is a species which has been specifically bred by man for speed and endurance and as such is extremely suitable for studies into exercise physiology. The present study was therefore undertaken with several aims.

1) To examine several parameters in normal equine skeletal muscle to establish baseline values for future studies.

2) To examine the differences between several breeds of horses and to try and establish a link between their muscle composition and the type of exercise to which they are best suited. To aid in this part of the study, the greyhound dog, another sub-species specially bred for exercise, will also be examined and compared to other dogs.

3) To examine the effects of training on various parameters in equine muscle. This may be important from the point of view of the racing industry in determining new training techniques. It will also extend basic physiological knowledge in the horse.

4) To examine the effect of exercise on skeletal muscle and the effect of drugs on performance and muscle fuels.

SECTION 2

MATERIALS AND METHODS

Animals

(a) Equine. The animals used were obtained from various sources. Several were cases which had been treated in the hospital but for some reason had to be destroyed and the rest, except for the Quarterhorses and Arabs, were experimental animals that belonged to departments in the Veterinary Hospital. The Quarterhorses and Arabs were obtained from sources in the United States of America. All of the animals had, as far as could be determined, no illnesses directly connected with their musculo-skeletal system. Details of breed, age and sex of the horses used are given in the appendix.

(b) Canine. The dogs were all purchased as experimental animals and were of three types, greyhound, foxhound and mongrel. They were all considered to be healthy and free from disease.

Statistics

Conventional statistical methods were used to calculate means ($\bar{x}$), standard deviation (S.E.), standard error of the mean (S.E.M.) and correlation coefficients. Intraindividual differences were tested for significance using the paired "t" test (Snedecor, 1956).

The error of the methods (Precision, P) was calculated according to the formula

$$P = \frac{\Sigma d^2}{2n}$$

where d is the difference between duplicate determinations and n the number of determinations. Coefficients of variation (C.V.) are given as per cent of mean values. C.V.s within a muscle and between contralateral muscles were calculated from the formula

$$\text{C.V.} = (\text{SD of the difference between double values}) \cdot \frac{1}{2} \cdot \frac{100}{\bar{x}}$$

Muscle Sampling Techniques

Two techniques of obtaining muscle samples were used.

a) Percutaneous Needle Muscle Biopsy

The method used was an adaptation of that of Bergström (1962). An area of skin about 10 cm^2, was closely shaved and cleansed with surgical spirits. 1-2 ml of lignocaine (2% Xylocaine-Astra) was injected subcutaneously using a 25 gauge needle along the line of the proposed incision and into the overlying facia of the muscle. Care was taken not to inject the local anaesthetic into the muscle itself. An incision of about 1 cm long was made through the skin and where necessary, through the facia. The biopsy needle (Stille-Eschmann) (Fig 2.1) consisted of

i) a sharp tipped, hollow outer needle with a small opening ("window") near the tip

ii) a cylinder with a sharp edge fitting tightly into the needle

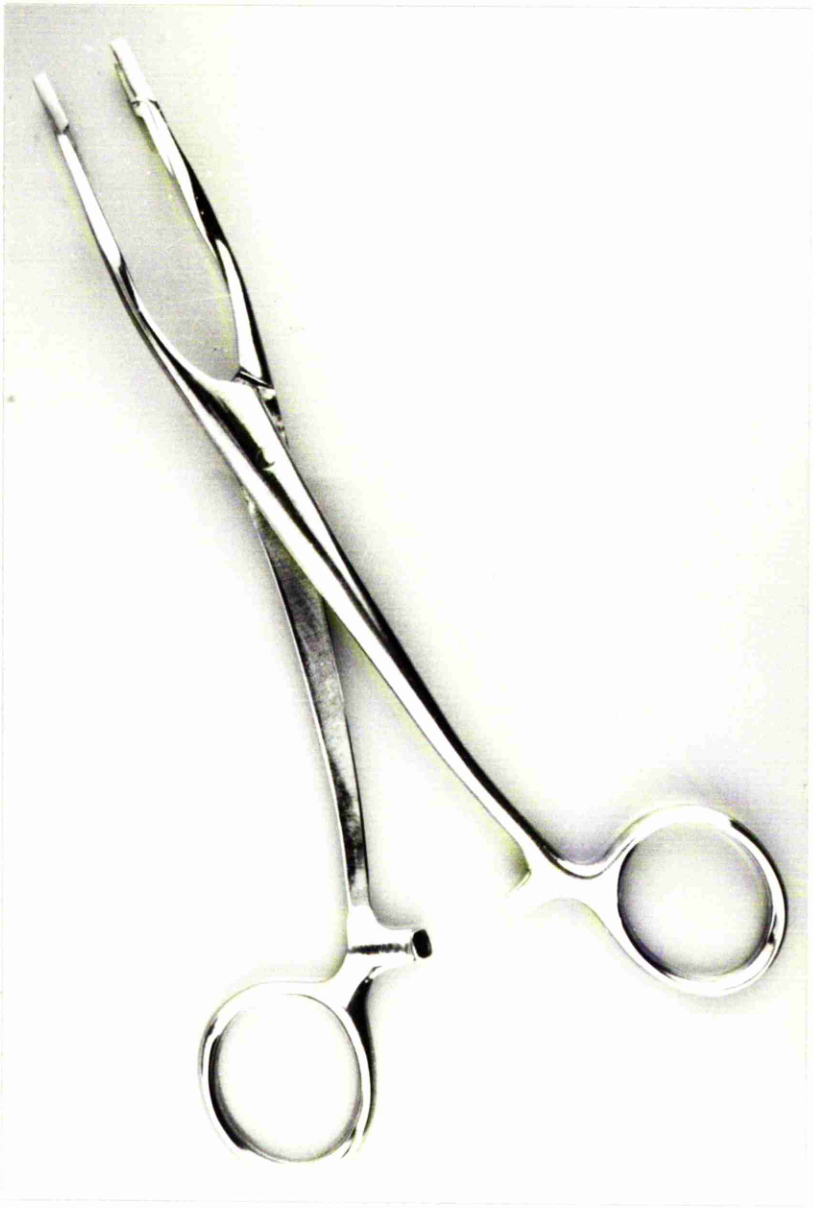
iii) a stylet to remove the specimen from the needle.

The needle, together with the cutting cylinder was inserted into the muscle through the incision. The window of the needle was positioned facing upwards or sidewards and pressed firmly against the muscle in order to allow a small piece of muscle to be caught within the needle. The cutting cylinder was then pushed down trapping a piece of muscle in its hollow interior, and the needle withdrawn. The muscle could then be removed with the stylet. Good technique allowed a cylindrical shaped piece of muscle 50-100 mg in weight to be obtained. This muscle

Fig 2.1: The percutaneous muscle biopsy needle
(a) outer needle, (b) cutting cylinder,
(c) stylette.


a
b
c

Fig 2.2: The biopsy clamp.



was then used for histochemistry or biochemistry. The incision was allowed to heal without suturing.

b) Biopsy Clamps

Samples obtained by clamps were from animals which were under a general anaesthetic or had undergone euthanasia. A large incision was made in the skin, exposing the underlying muscle. Two incisions were made 2 cm apart and the strip of muscle between trapped in the jaws of the clamp (Fig 2.2). The muscle was then incised out and used for histochemistry and biochemistry.

Due to Home Office regulations, biopsies obtained from live animals were taken by Dr. D.H. Snow.

Muscle obtained from the biopsy procedures was divided into two pieces, the part for histochemistry being treated as described in section 2. . The specimen for biochemistry was immediately frozen and stored in liquid nitrogen until it was next required.

Biochemistry

Enzyme activity is normally measured by monitoring changes in the concentration of the enzyme substrate or its reaction product. There are many ways of doing this, but perhaps the easiest and certainly the most common is to measure the change in optical density that occurs at a given wavelength as the reaction proceeds. If the enzyme under examination does not lend itself easily to this type of measurement, auxilliary enzymes or substrates or reagents which react with the product as it appears, can be added to produce changes in optical density which can be followed. The activity of the enzyme is therefore proportional to the rate of change of the optical density.

i.e. $E.A. = \Delta E.min^{-1} . 1000 .(M.E.C.)^{-1} .$ total tissue dilution

where E.A. is enzyme activity, ΔE is the change in optical density and M.E.C. is the molar extinction coefficient of the compound being measured.

Many of the enzyme assays used in this study were based on the principle that oxidised NAD does not absorb at 340 nm whereas reduced NADH absorbs strongly at this wavelength. As NAD is used in many of the reactions measured or can easily be incorporated into a system which does, the enzyme activities could easily be obtained. At 340 nm, the M.E.C. of NAD is 6.22 $cm^2.mole^{-1}$.

All of the enzyme assays were measured at 37°C as recommended by the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974) rather than at 25°C as recommended by the German Society for Clinical Chemistry (1970). As enzyme kinetics, like most other chemical reactions follow the Arrhenius equation

$$\log f = \Delta E \cdot \text{minute}^{-1} \cdot T^{-1} \cdot (Tx - T)^{-1}$$

where ΔE is the change in extinction, T is the temperature in ^{o}K, Tx is absolute temperature in ^{o}K and f is an activity factor, at least up to about $45^{o}C$, the higher the temperature, the faster the enzymatic reaction will proceed. This facilitates the measurement of enzyme activity and allows greater precision. The major objection to using $37^{o}C$ as opposed to $25^{o}C$ was that thermal degradation may occur. Preliminary results indicated that it was not a problem for the enzymes studied.

The enzyme activities which were determined optically were measured in the case of LDH, CPK, AST, ALT and HAD on an LKB 8600 Reaction Rate Analyser and in the case of GDH, ALD, HK and CS on a Pye Unicam SP 8000 UV Spectrophotometer. All assays were carried out in cuvettes of pathlength 10 mm. The radiochemical estimation of the activity of GS was performed on a Packard Tri-Carb 3255 Liquid Scintillation Spectrophotometer.

Chemicals were obtained from 3 main sources, Boehringer Mannheim (London), Sigma Company (London) and British Drug Houses (Poole). Wherever possible, commercial kits were used to standardise reaction conditions and improve the precision and reproducibility of the assay. The precision and batch to batch variation are given in Table 2.1.

The enzymes assayed in the present study were chosen as they were representative of various metabolic pathways important in muscle metabolism. A short description of their function and location

is shown in Table 2.1. A more detailed description of the reaction which they catalyse and the assay method chosen is described below.

Homogenation Solutions

The muscle samples for enzyme analysis were homogenised in all cases except that of GS, in a solution of 150 mM KCl, 50 mM $KHCO_3$ and 6 mM EDTA in water. Protein concentration was enhanced with 1% horse serum albumin.

Samples for the assay of GS activity were homogenised in 50 mM Tris-HCl buffer (pH 7.8) containing 5 mM EDTA.

Enzyme	Pathway/Function	Location
Aldolase	Glycolysis	Possibly bound to Actin in the I-band (1).
Lactic Dehydrogenase	Reduction of pyruvate	M-subunit predominately in S.R. H-subunit predominately bound to inner mitochondrial membrane (2).
Citrate Synthase	T.C.A. cycle	Mitochondrial matrix (3).
3-Hydroxyacyl CoA Dehydrogenase	Fatty acid oxidation	Mitochondrial matrix (3).
Glycerol-3-Phosphate dehydrogenase	NADH Shuttle	Cytosol and inner mitochondrial membrane (3).
Aspartate Amino-transferase	NADH Shuttle	Cytosol and mitochondrial matrix (3).
Hexokinase	Phosphorylation of glucose	Outer mitochondrial membrane and S.R. membrane (1).
Glycogen Synthase	Synthesis of glycogen	Cytosol (3).
Creatine Phosphokinase	Replenishment of ATP from ADP and CP	Z-band and mitochondria (4).
Alanine Aminotransferase	Conversion of pyruvate to alanine	Cytosol and mitochondria (3).

Table 2.1. The function and location of the enzymes assayed in the present study.

1. Pette (1975)
2. Baba and Sharma (1971)
3. Lehninger (1975)
4. Turner et al. (1973)

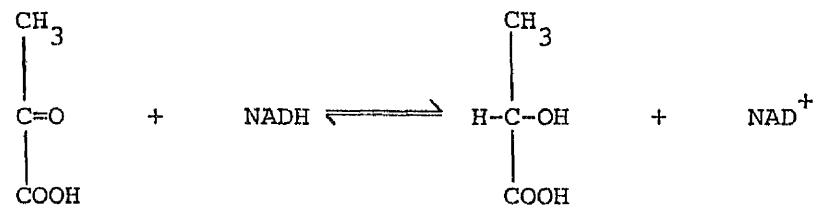
Enzyme	Buffer	pH	Reagents	Amount of tissue (mg)	Precision $\mu moles.min^{-1}$.	Daily Variation (gm dry wt. tissue)$^{-1}$
LDH	50 mM Phosphate	7.5	0.18 mM NADH, 0.6 mM pyruvate	0.002	1777 ±80 (4.5%)	2263 ±103 (4.6%)
CPK	100 mM Tri-ethanolamine	7.0	20 mM glucose, 10 mM magnesium acetate, 1 mM ADP, 10 mM AMP, 0.6 mM $NADP^+$, 35 mM creatine phosphate, 9 mM glutathione, HK > 1.2 $U.ml^{-1}$ and GPDH > 1.2 $U.ml^{-1}$	0.0005	20090 ±1217 (6.0%)	19312 ±1344 (6.9%)
AST	80 mM Phosphate	7.4	200 mM L-aspartate, 0.18 mM NADH, MDH > 0.6 $U.ml^{-1}$, LDH > 1.2 $U.ml^{-1}$ and 12 mM α-oxoglutarate	0.02	965 ±3.8 (3.9%)	949 ±6.5 (6.9%)
ALT	80 mM Phosphate	7.4	800 mM L(+)-alanine, 0.18 mM NADH, 18 mM α-oxoglutarate and LDH > 1.2 $U.ml^{-1}$.	0.10	58 ±2.0 (3.5%)	66 ±6.1 (9.2%)
ALD	100 mM Phosphate	7.0	5 mM fructose-1,6-diphosphate, 0.3 mM iodoacetate, 0.12 mM NADH, GDH > 0.13 $U.ml^{-1}$ and TIM > 4 $U.ml^{-1}$	0.05	406 ±24 (6.0%)	315 ±22 (7.1%)
CS	100 mM Tris-HCl	8.1	0.1 mM DTNB, 1.0 mM acetyl CoA and 0.5 mM oxaloacetate	0.05	20.2 ±0.9 (4.5%)	29.8 ±2.0 (6.7%)
HK	100 mM Tris-HCl	7.6	5 mM ATP, 10 mM magnesium chloride, 0.5 mM EDTA, 0.47 mM $NADP^+$, GPDH > 5 $U.ml^{-1}$, 2 mM glucose	0.20	10.4 ±0.4 (3.8%)	9.1 ±0.6 (6.6%)
HAD	50 mM Imidizole	7.0	0.5 mM EDTA, 0.124 mM NADH and 0.0275 mM acetoacetyl CoA	0.01	181 ±10 (5.5%)	162 ±12 (7.4%)
GDH	50 mM Tris-HCl	7.6	0.5 mM EDTA, 0.124 mM NADH and 1.25 mM dihydroxyacetone phosphate	0.01	75.1 ±4.7 (6.2%)	92.0 ±6.3 (6.9%)
GS	50 mM Tris-HCl	7.8	5 mM EDTA, 6.7 mM uridine diphosphoglucose (H^3 glucose) and 10 $mg.ml^{-1}$ glycogen ± 6.7 mM glucose-6-phosphate for a-form (without) or a+b forms (with)	a-form 0.20 a+b-forms 0.20	2.2 ± 0.21 (9.6%) 19.4 ± 1.8 (9.1%)	- -

Table 2.2: Concentrations of substances used in the various enzyme assays, their precision and their daily variation.

Lactate Dehydrogenase (LDH ; E.C.1.1.1.27)

(L-lactate : NAD oxidoreductase)

LDH catalyses the reversible reduction of pyruvate to lactate with the concommitant oxidation of NADH to NAD^+



pyruvate lactate

In this study, pyruvate was used as the substrate, as the equilibrium favours production of lactate. The problem of removing any product is not applicable. The method employed was based on that of Wroblewski and La Due (1955) and a Boehringer commercial kit was used. As with all of the following enzyme assays, the final concentrations in the test solutions are shown in Table 2.2.

Creatine Phosphokinase (CPK ; E.C.2.7.3.2.)

(ATP : creatinephosphotransferase)

CPK catalyses the reversible reaction

creatine + ATP $\rightleftharpoons$ creatine phosphate + ADP

and can therefore be measured in both the creatine → creatine phosphate direction (Tanzer and Gilvarg, 1959) or as in the case of this study, in the opposite direction (Oliver, 1955). The activity is measured by using coupling reactions to follow the reduction of $NADP^+$.

creatine phosphate + ADP $\xrightarrow{CPK}$ creatine + ATP

ATP + glucose $\xrightarrow{HK}$ glucose-6-phosphate + ADP

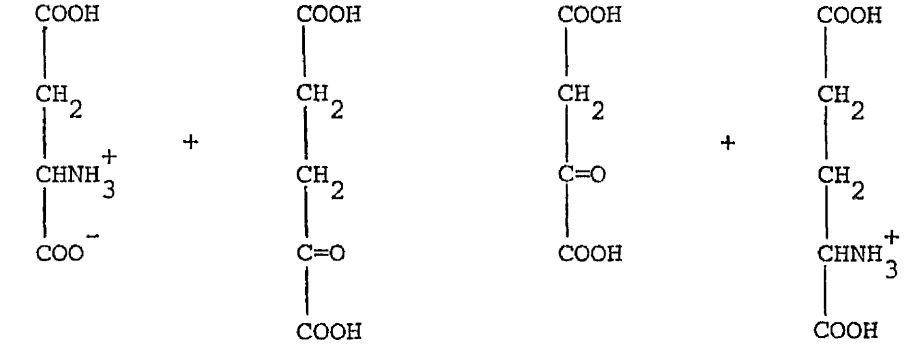
glucose-6-phosphate + $NADP^+$ $\xrightarrow{GPDH}$ 6-phosphogluconic acid + NADPH

A Boehringer commercial kit was used. Magnesium is required as a metal co-factor in the kinase reactions. AMP was added to inhibit myokinase and glutathione was included as thiol compounds have been shown to reactivate CPK.

Aspartate Aminotransferase (AST ; E.C.2.6.1.1.)

(L-aspartate:2-oxoglutarate aminotransferase)

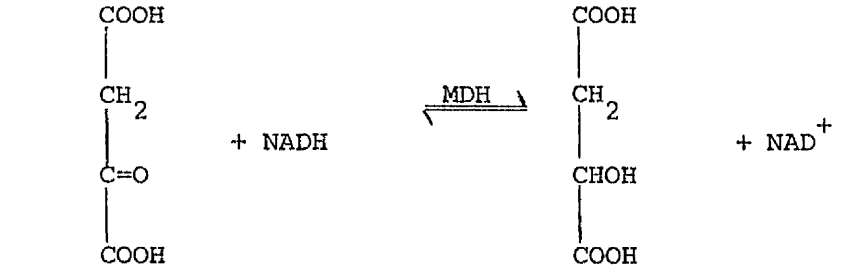
AST catalyses the reversible reaction



aspartate 2-oxoglutarate oxaloacetate glutamate

The most accurate and sensitive method of assay for AST is the U.V. method of Bergmeyer (1970). Colourimetric methods have also been used for the assay of AST but these methods have major problems such as product inhibition, high blanks and the colour developed does not obey Beer's Law (Varley, 1967). The method of choice in this study was therefore the U.V. measurement of the oxidation of NADH, as one of the

products, oxaloacetate, is converted to malate by the enzyme malate dehydrogenase (MDH).



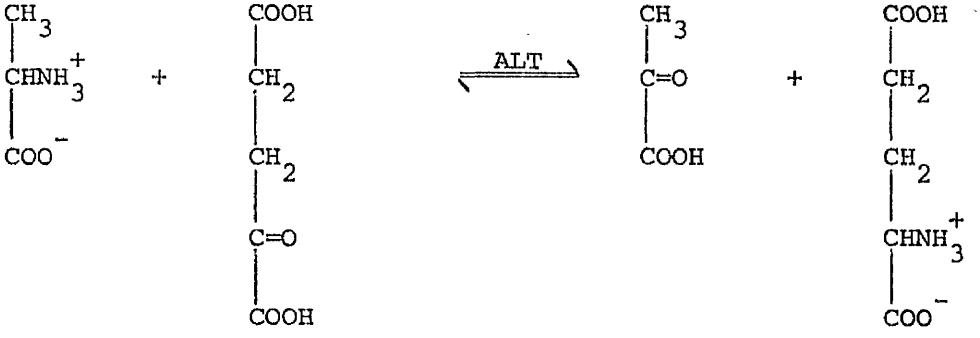
oxaloacetate malate

A Boehringer commercial kit was used.

Alanine Aminotransferase (ALT ; E.C.2.6.1.3.)

(L-alanine:2-oxoglutarate aminotransferase)

The reaction catalysed by the enzyme ALT is similar to that for AST in that transamination is involved in both.



L(+)alanine 2-oxoglutarate pyruvate glutamate

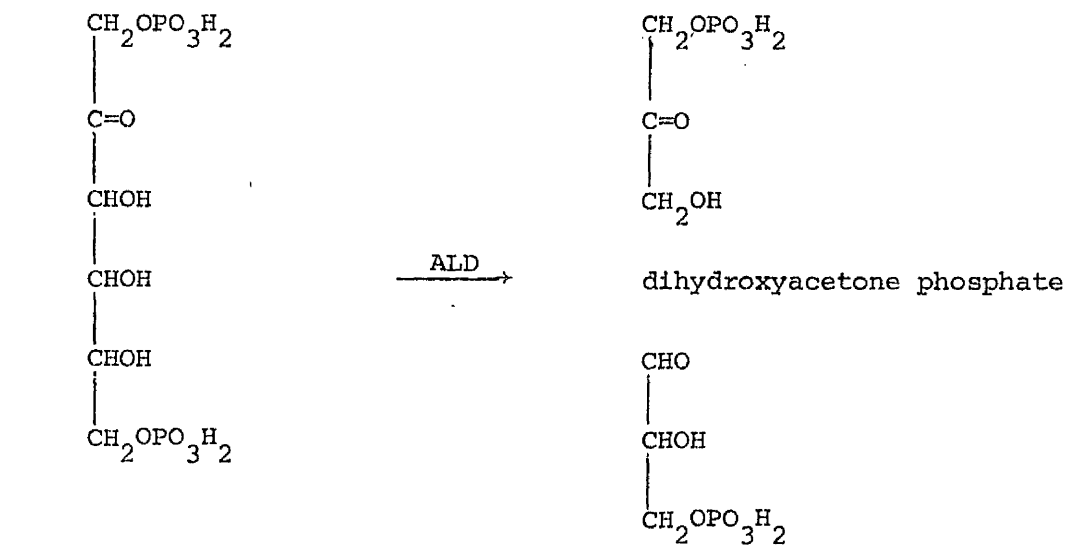
Colourimetric methods have also been applied in the assay of this enzyme but, the same problems as with AST exist. The U.V. method based on that of Bergmeyer (1970) was therefore used. The enzyme was assayed by measuring the decrease in absorbance as NADH is oxidised to NAD^+

during the conversion of one of the products, pyruvate, to lactate by the enzyme LDH. A Boehringer kit was used.

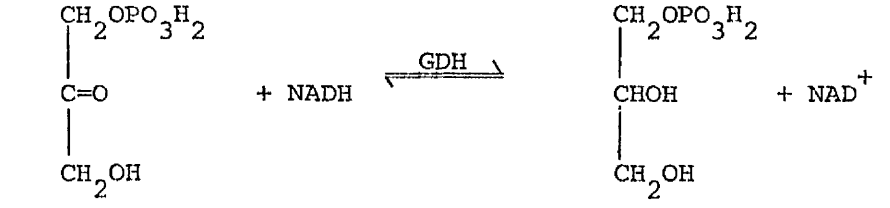
Aldolase (ALD ; E.C.4.1.2.7.)

(fructose-1,6-diphosphate D-glyceraldehyde-3-phosphate-lyase)

ALD catalyses the reaction



The method was based on that of Anderson (1975) and coupling reactions were used to follow the breakdown of fructose-1,6-diphosphate. The glyceraldehyde produced was converted to the other product of fructose-1,6-diphosphate cleavage, dihydroxyacetone phosphate, by the enzyme triose phosphate isomerase. The activity of ALD can then be monitored by following the oxidation of NADH during the reaction converting dihydroxyacetone phosphate to glycerol-3-phosphate by the enzyme GDH.



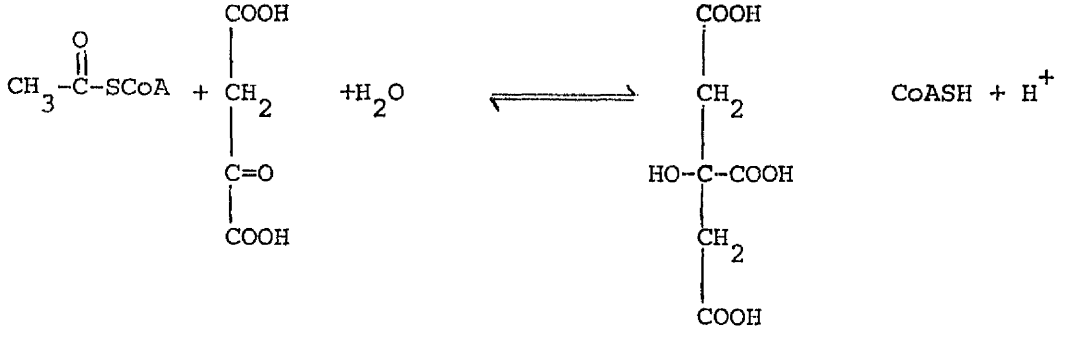
dihydroxyacetone phosphate glycerol-3-phosphate

Iodoacetate was included in the test solution although as pointed out by Anderson (1975) it has no effect on the activity of ALD. This was to allow a direct comparison of ALD activities in this study and other studies.

Citrate Synthase (CS ; E.C.4.1.3.7.)

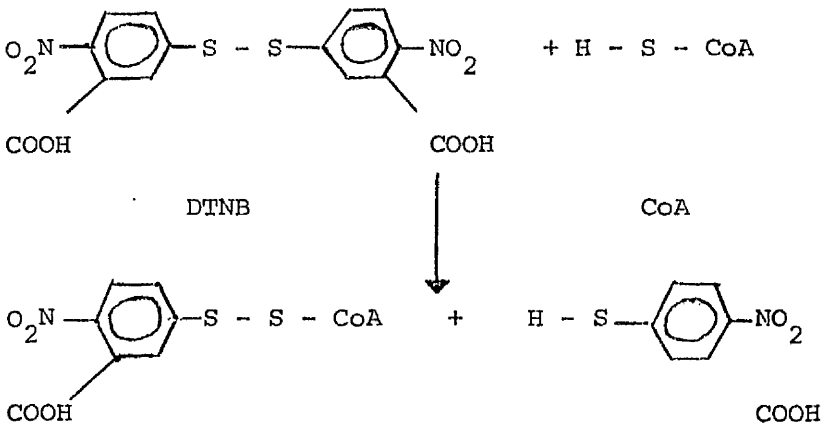
(Citrate oxaloacetate-lyase - CoA-acetylating)

CS catalyses the reaction



acetyl CoA oxaloacetate citrate CoA

It can be assayed directly by following the decrease in absorption at 233 nm caused by the breakdown of the acetyl-S-CoA thioester bond. This method cannot be used with crude homogenates as protein and nucleic acids also absorb at this wavelength. The method of assay used in this study was therefore that of Srére (1969) and is based on the reaction of coenzyme A with 5,5,dithiobis(-2-nitrobenzoate) - Ellman's Reagent or DTNB.



The reaction is followed readily at 412 nm where the mercaptide ion absorbs strongly. To convert the extinction to μmoles of acetyl CoA broken down a standard curve was prepared using known standards of cysteine to supply the sulphydryl group. These standards were incubated with the usual test mixture minus the homogenate. A standard curve of μmoles of cysteine (i.e. μmoles of -SH groups) against extinction was then plotted and a conversion factor calculated. A typical curve is shown in Fig 2.3.

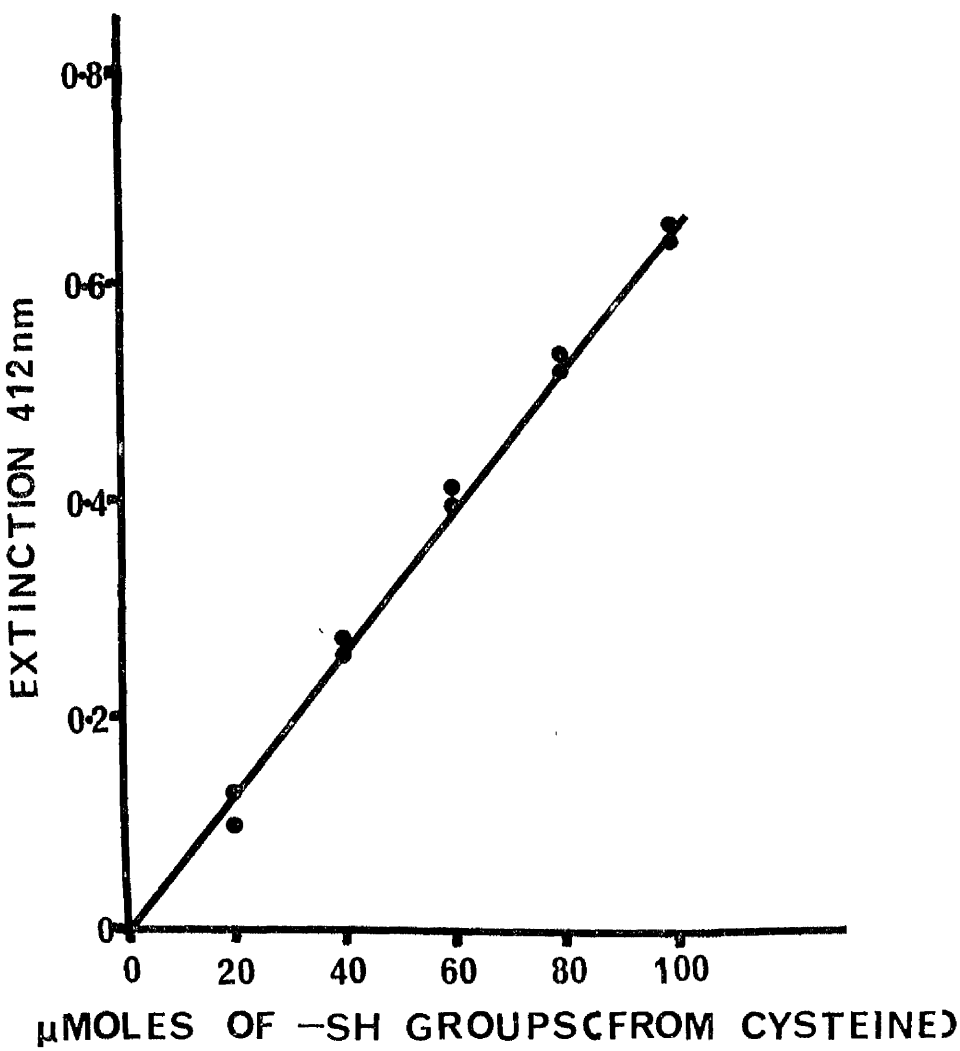
As CS is found only in the mitochondria, it was thought that disruption of the mitochondrial membrane might affect the activity of the enzyme. Disruption of the membrane can be achieved by several methods but the one of choice in this study was freezing and thawing of the homogenate three times followed by ionic oscillation (Holloszy et al., 1970). Results can be seen for duplicate samples of 4 homogenates (Table 2.3). No significant difference was found and so in all future assays attempts at disruption of the mitochondrial membrane were discontinued.

Sample	Intact*	Disrupted*	Different (Intact - Disrupted)
1	6.50	6.60	- 0.10
2	8.00	6.75	+ 1.25
3	8.25	8.40	- 0.15
4	10.60	10.00	+ 0.60

* $\mu moles.min^{-1}.gm$ dry weight of $tissue^{-1}$

Table 2.3. The effect on citrate synthase activity of freezing and thawing a homogenate three times followed by three 15 second periods of sonic oscillation 45 seconds apart to disrupt the mitochondrial membrane.

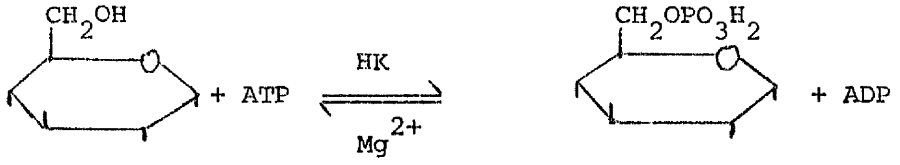
Fig 2.3: Standard curve of concentration of sulphydryl groups against extinction at 412 nm.


0·8
0·6
0·4
0·2
0
EXTINCTION 412nm
0
20
40
60
80
100
µMOLES OF –SH GROUPS(FROM CYSTEINE)

Hexokinase (HK ; E.C.2.7.1.1.)

(ATP:D-hexose-6-phosphotransferase)

The enzyme HK catalyses the reaction



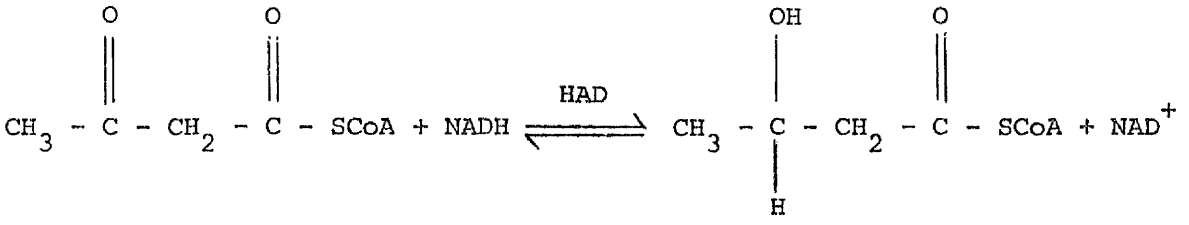
glucose glucose-6-phosphate

The spectrophotometric method of assay is based on that of Uyeda and Racker (1965) and involves the conversion of one of the products, glucose-6-phosphate to 6-phosphogluconate by the enzyme glucose-6-phosphate dehydrogenase. Simultaneously $NADP^+$ is reduced to NADPH. Magnesium is required as a metal cofactor for the kinase.

3-Hydroxyacyl Coenzyme A Dehydrogenase (HAD ; E.C.1.1.1.35)

(L-3-hydroxyacyl-CoA : NAD oxidoreductase)

HAD catalyses the reversible reaction



acetoacetyl CoA 3-hydroxyacyl CoA

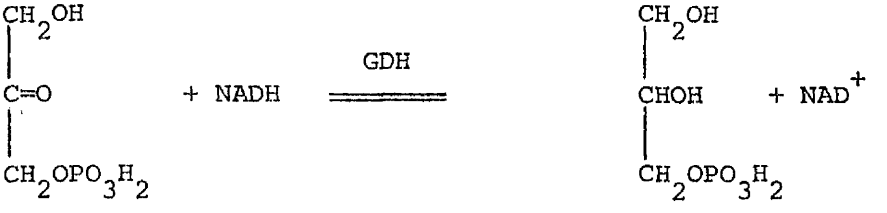
The reaction is very dependent on pH, favouring the formation of 3-hydroxyacyl CoA at pH 7.0 and the formation of acetoacetyl CoA at a pH greater than 9.0. In the present study, the activity was assayed in

the direction of acetoacetyl CoA ⟶ 3-hydroxyacyl CoA, using a modification of the method of Essen et al. (1975) that allowed the assay to be performed on an LKB Reaction Rate Analyser.

Glycerol-3-Phosphate Dehydrogenase (GDH ; E.C.1.1.1.8)

(L-glycerol-3-phosphate : NAD 2-oxidoreductase)

GDH catalyses the reaction



dihydroxyacetone phosphate glycerol-3-phosphate

The assay was based on that of Bucher et al. (1964) and followed the decrease in extinction at 340 nm as NADH was oxidised.

Glycogen Synthetase (GS ; E.C.2.4.1.11)

(UDP glucose glycogen α-4 glucosyltransferase)

This enzyme catalyses the reaction

$$\text{uridine diphosphoglucose} + \text{glycogen}_n \xrightarrow{\text{GS}} \text{glycogen}_{n+1} + \text{UDP}.$$

GS consists of 2 interconvertible forms, the b-form being dependent on glucose-6-phosphate for activity while the a-form is independent of this co-factor (Friedman and Larner, 1963). Several methods have been developed to assay GS activity including measurement of the disappearance of UDP^{14}C glucose (Schnid et al., 1959), the

production of UDP (Leloir and Goldemberg, 1960) and the incorporation of radioactivity from UDP^{14}-C-glucose into glycogen (Thomas et al., 1968). The first method is unspecific as UDP-glucose can be degraded by several enzymes (Ginsburg, 1964) and the second cannot be used to determine the activity of the a-form (Friedman and Larner, 1963). The third method, using the modification of Adolfsson (1972), was therefore the one of choice in this study.

The counts per minute measured by a scintillation counter are not always the same as the number of disintegrations occurring in the sample being assayed. This is because of quenching - the absorption of some of the energy being emitted by the sample, in this case, absorption in the filter paper. The efficiency of the procedure was therefore calculated using samples of known disintegrations per minute. Efficiency was counts per minute . (disintegrations per minute)$^{-1}$.100. In the present study, the efficiency was 8.4% $\pm$ 0.2% (mean $\pm$ S.E.M.).

Enzyme Stability

In order to ascertain that alteration in enzyme activities did not occur with storage in liquid nitrogen, a study was made of the stability of the enzymes under the storage conditions. This was done by splitting five muscle samples into two and assaying the enzyme activities approximately 12 to 18 months apart. Even after the length of time of storage used in this study, there was, at most, a 15% loss of activity with many of the enzymes remaining unaltered (Table 2.4).

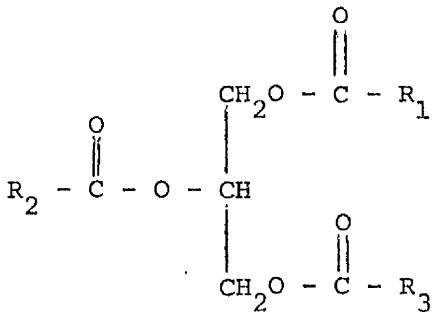
Further proof of the stability of the enzymes comes from the results obtained from the standard muscle run with each assay. No detectable decrease in the value of the activities was found during the time that it was used (approximately 6 months) (Table 2.2).

Months in Storage	LDH	CPK	ALD	AST	ALT	CS	GDH	HAD	HK
0	3194	19595	450	860	79	22.2	99.4	187	12.3
18	3189	18958	383	860	87	19.8	96.6	178	11.1
% difference	0.0	-3.3	-14.9	0.0	+9.2	-10.9	-2.8	-4.9	-9.8

Table 2.4 The effect of 18 months storage at -186^{o}C for several enzymes in horse skeletal muscle. Each value is the mean (μmoles.minute^{-1}.gm dry weight of tissue^{-1}) of 5 homogenates.

Triglycerides

Triglycerides, one of the main fuels in skeletal muscle metabolism, consist of a glycerol molecule and 3 long chain fatty acids.



where R_1, R_2 and R_3 are long-chain fatty acids

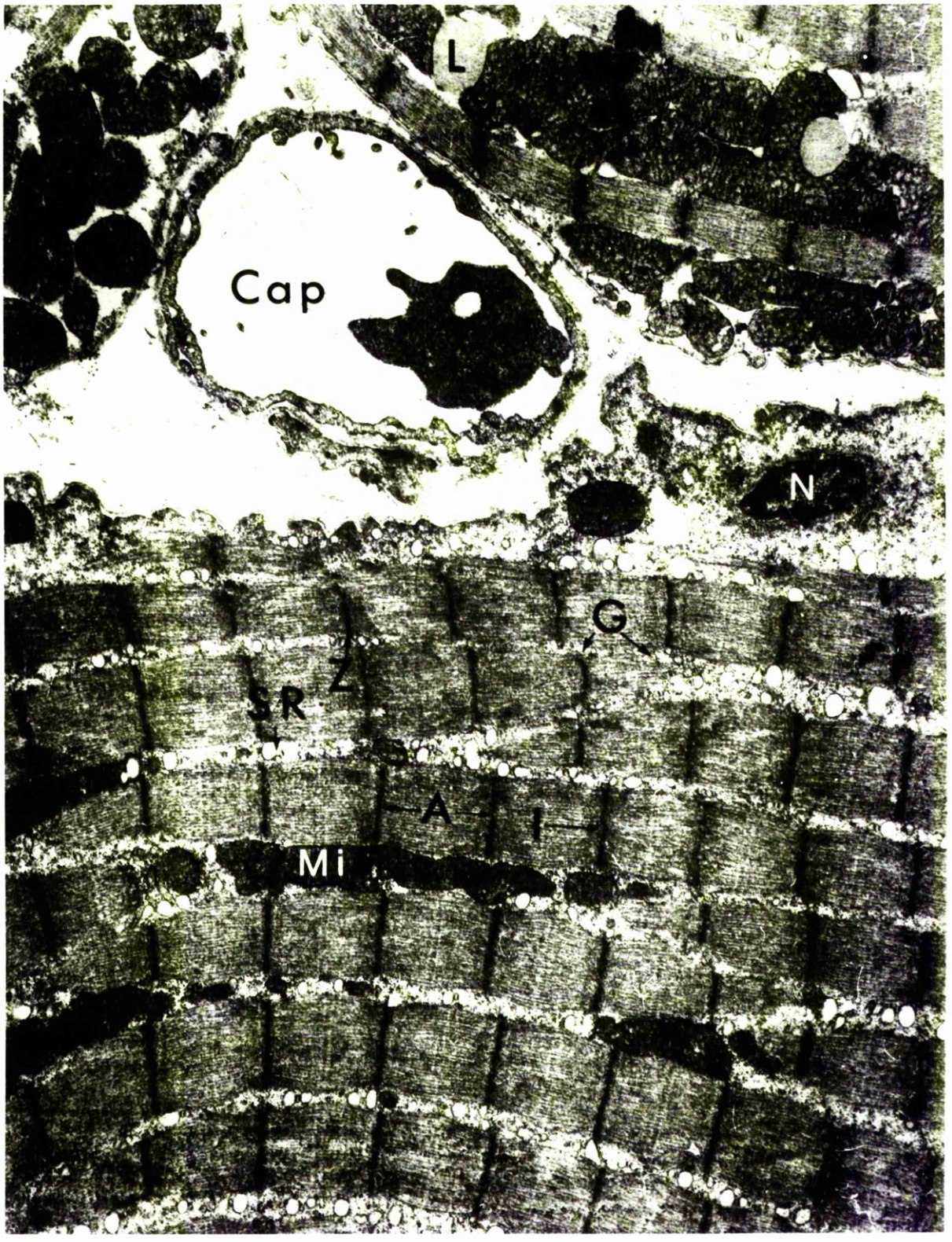
They form a large percentage of the family of lipids found in skeletal muscle and are found in droplets in close association with mitochondria being usually located between chains of intermyofibrillar mitochondria (Fig 2.4). It can be assayed by measuring the amount of glycerol released after hydrolysis of the triglyceride molecule, 1 mole of glycerol released being equivalent to 1 mole of triglyceride.

Method

Known amounts of muscle were homogenised in 0.5 ml of methanol and 1 ml of chloroform. Saline (1.5 ml) was added and mixed and the homogenate left for 24 hours. The saline/methanol layer was removed with a pasteur pipette and the chloroform layer containing free fatty acids, acyl glycerols and a small amount of phospholipid was evaporated to dryness, under a stream of nitrogen, in a water bath at $37^{o}C$.

The amount of triglyceride (acylglycerols) was determined by the method of Chernick (1968). They were gently hydrolysed in 1 ml of

Fig 2.4: Biopsy specimen obtained by needle. Note contracted I-band (I). Other labled structures are glycogen (G), lipid (L), A-band (A), Z-line (Z), M-line (M), sarcoplasmic reticulum (SR), nucleus (N), capillary (CAP) and mitochondria (Mi). Stained with uranyl acetate/lead citrate X 12,000.


L
Cap
N
G
Z
SR
A
I
Mi

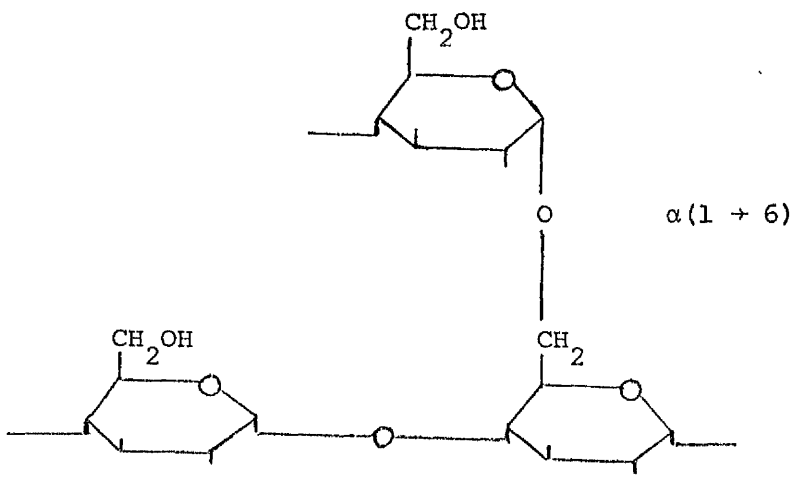
100 mM tetra-ethylammonium hydroxide (E_4 NOH) in ethanol (1:7 dilution of 10% E_4NOH:ethanol) at 60°C for 30 minutes. This hydrolyses the triglycerides while leaving the phospholipid component unchanged. While the tubes were still in the water bath at 60°C, 1.2 ml of 100 mM hydrochloric acid was added. Appropriate blanks and standards were treated in the same way. The glycerol released was assayed in a solution of 1M hydrazine, 200 mM glycine, 1 mM EDTA, 2.5 mM magnesium chloride, 0.2 mM NAD and 0.5 mM ATP. To a tube containing 200 µl of the above hydrolysate, 700 µl of assay mixture was added. Glycerol-3-phosphate dehydrogenase (50 µl of 20 $\mu.ml^{-1}$) was then added and 10 minutes later 50 µl of glycerol kinase (4 $\mu.ml^{-1}$) was added. This protocol removes any possible fluorescence due to contamination by glycerol-3-phosphate. In practice, this was not a problem and so the enzymes were added simultaneously. After 30 minutes at room temperature, the fluorescence was measured on an Aminco Bowman Fluorimeter (primary wavelength 340 nm, secondary wavelength 460 nm). Triglyceride content was determined from a graph of known standards.

Reproducibility

The precision of the method was for 10 duplicate samples 99 ± 5 µmoles.gram dry weight of $tissue^{-1}$ (C.V. = 5%).

Glycogen

Glycogen is an important fuel in muscle metabolism and is made up of long chains of glucose molecules joined in the $\alpha(1 \rightarrow 4)$ position with occasional branches in the $\alpha(1 \rightarrow 6)$ linkage.



$L\alpha(1 \rightarrow 4)^{-1}$

In the horse, glycogen is found widely distributed throughout the sarcoplasm of the fibre in both discrete granules and rosette formation. Granules can also be seen between the filaments of the myofibrils (Fig 2.4).

Method

The assay was based on that of Huijing (1970). Known amounts of muscle were homogenised in de-ionised water. Glycogen in the crude homogenate was specifically hydrolysed to glucose by mixing a suitable dilution of homogenate (200 µl) with a solution of $\alpha(1 \rightarrow 4)$, $\alpha(1 \rightarrow 6)$ amyloglucosidase (2.45 $U.ml^{-1}$) and α-amylase (20 $U.ml^{-1}$) in 100 mM sodium acetate buffer (pH 4.8; 200 µl). To ascertain the

optimum digestion time, incubations were carried out at 37°C for different lengths of time. By sixty minutes all of the glycogen had been converted to glucosyl units and so this was used in all subsequent assays.

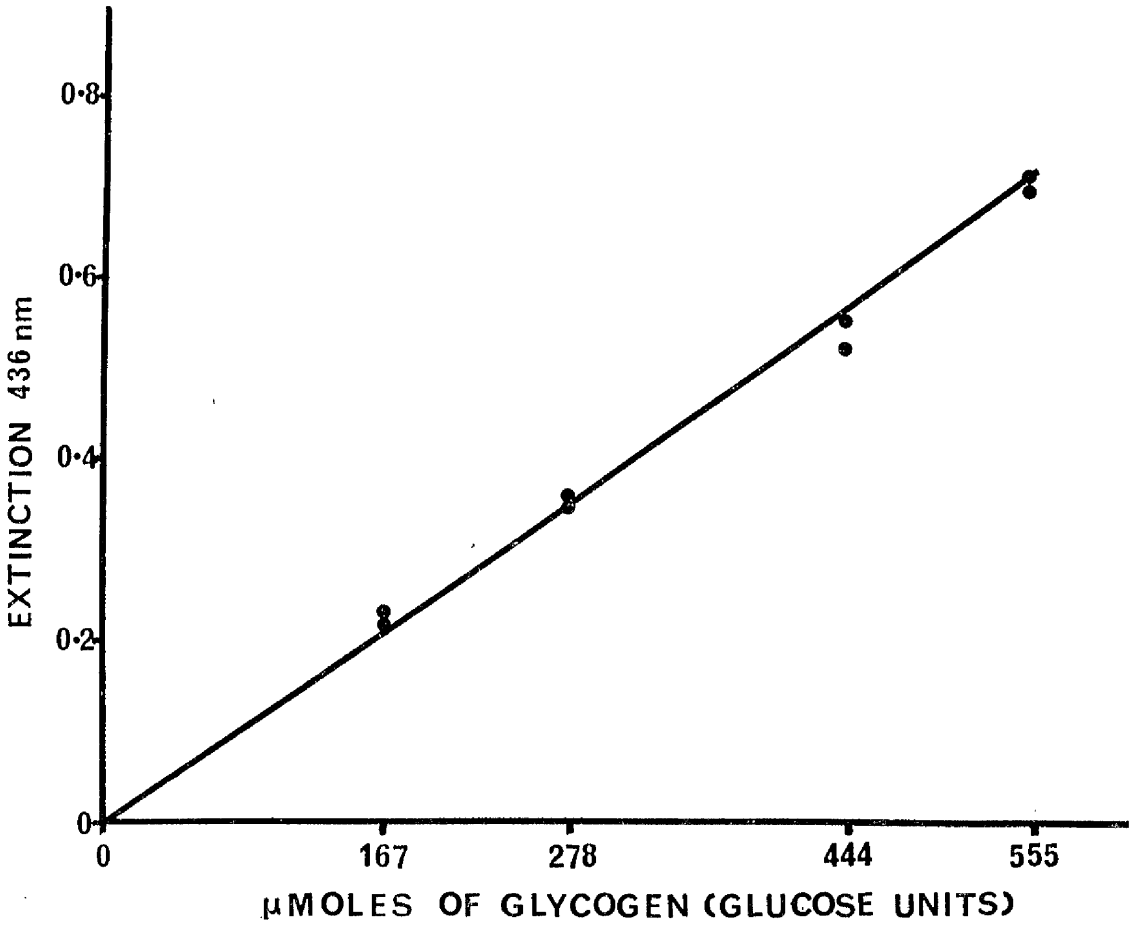
Glucose was measured, using a Boehringer test kit for blood sugar. A solution (500 µl) of 100 mM phosphate buffer (pH 7.0) containing glucose oxidase (> 10 $U.ml^{-1}$), peroxidase (> 0.8 $U.ml^{-1}$) and ABTS (1 $mg.ml^{-1}$) was added to each tube and incubated at 37°C for fifteen minutes. The reaction was stopped with 5M HCl (500 µl) and the extinction read at 436 nm.

Appropriate blanks and standards were assayed at the same time and the glycogen content calculated from a standard curve (Fig 2.5).

Reproducibility

The precision of the assay was 313 $\pm$ 6.7 $\mu moles.gm^{-1}$ for 10 duplicate determinations (coefficient of variation = 2.1%). The daily variation was 420 $\pm$ 30 $\mu moles.gm^{-1}$ (coefficient of variation = 7.1%).

Fig 2.5: Standard curve of glycogen concentration against extinction at 436 nm.


0·8
0·6
0·4
0·2
0
EXTINCTION 436 nm
0
167
278
444
555
µMOLES OF GLYCOGEN (GLUCOSE UNITS)

Lactate

Lactate in the muscle was assayed by the method of Hohorst (1962) using a modified commercial kit (Boehringer Mannheim). A neutralised perchloric acid extract (100 µl) was added to a solution (1.1 ml) containing 500 mM glycine buffer and 400 mM hydrazine (pH 9.0 ; 1 ml) and 27 mM NAD^+ (100 µl). The reaction was started by the addition of >650 $U.ml^{-1}$ LDH (10 µl) and left for sixty minutes at 25°C whereupon, the extinction was read at 340 nm and the concentration of lactate calculated using the M.E.C. for NAD. Appropriate blanks and standards were assayed at the same time.

Reproducibility

The precision of the assay was 22.0 $\pm$ 1.5 µmoles.gram dry weight of $tissue^{-1}$ for 6 duplicate determinations (coefficient of variation = 6.7%). A known standard solution of lactate was assayed with each batch of samples and the coefficient of variation per batch was 4.2%.

Total Protein

Total protein was assayed according to the method of Lowry et al. (1951). The principle of the assay is based on a coloured complex forming between an alkaline copper-phenol reagent and the tyrosine and tryptophan residues of the protein.

The reagents used were

A) Sodium carbonate (4% w/v) in 100 mM sodium hydroxide.

B) Potassium Sodium Tartrate (1% w/v) in distilled water.

C) Copper sulphate (0.5% w/v) in distilled water.

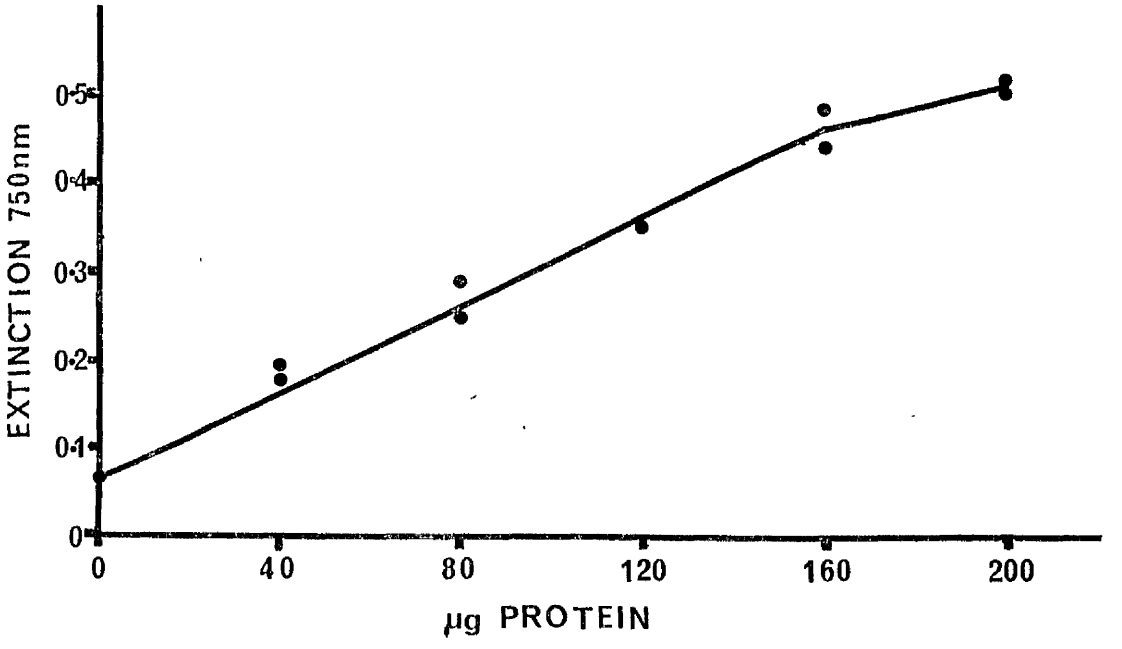
D) 1M Folin Ciocalteau Phenol Reagent.

Fifty parts of solution A, one part solutions B and C were mixed and added (2 ml) to a protein solution (1 ml) containing not more than one hundred and fifty micrograms of protein. The solutions were mixed and left for ten minutes at room temperature. Reagent D (0.5 ml) was added, the solutions mixed and left for at least thirty minutes. The optical density (750 nm) was read and unknowns calculated from a standard curve assayed at the same time (Fig 2.6). Horse serum albumin (Koch-Light) was used as the standard.

Reproducibility

The precision of the assay was 601 $\pm$ 28 mg protein.g dry weight of tissue^{-1} (coefficient of variation = 4.7%) for ten duplicate determinations. The daily variation was 622 $\pm$ 37 mg protein.g dry weight of tissue^{-1} (coefficient of variation = 5.9%).

Fig 2.6: Standard curve of protein concentration against extinction at 750 nm.


EXTINCTION 750nm
0·5
0·4
0·3
0·2
0·1
0
0
40
80
120
160
200
µg PROTEIN

Separation and Quantification of Lactate Dehydrogenase Isoenzymes

Several methods of electrophoresis have been applied for the separation of LDH isoenzymes including starch gel (Wroblewski et al., 1960), agar gel (van der Helm, 1962), cellulose acetate strips (Barnett, 1964) and poly-acrylamide gel (Dietz and Lubrano, 1967). This latter method has several advantages as the time for separation is short, the proteins are generally resolved into sharp bands and the resulting gel is clear permitting ready quantification of the bands. This was therefore the method of choice.

Method

Separation was obtained using the method of Anderson (1976). Samples were prepared by diluting tissue homogenates in 100 mM phosphate buffer pH 7.0 to activities around 500 μmoles.ml^{-1}.minute^{-1} and adding an equal volume of 40% sucrose. Gels were made up in the following manner. 15% acrylamide (Cyanogum 41 ; 10 ml) was added to 337 mM Tris-HCl (pH 8.9 ; 19.64 ml), TEMED (0.06 ml) and 7% ammonium persulphate (0.3 ml). This gave a final concentration of 5% acrylamide in 250 mM Tris-HCl buffer. Aliquots of this solution were pipetted into tubes of internal diameter 5 mm, overlayed with a small amount of water to prevent a meniscus forming on the gel and the tubes left for 30 minutes for the gel to polymerise. The gels were placed in a disc-electrophoresis tank and the cathode and anode compartments filled with 10 mM Tris-HCl, 50 mM glycine buffer (pH 8.5). To reduce background staining, 10 mM iodoacetate was added to the cathode buffer. The iodoacetate was flushed through the gels by electrophoresis at 2.5 mA.tube^{-1} for 30 minutes. The sample

was added simply by layering the sucrose/sample solution onto the gel through a syringe. The use of the sucrose solution avoided the need for spacer gels (Davis, 1964). Electrophoresis was carried out at 1 $mA.tube^{-1}$ for 15 minutes and then for 90 minutes at 4 $mA.tube^{-1}$. Gels were rimmed by the method of Reisfeld et al. (1962) and transferred to small tubes containing 3 ml of a staining solution. 1M sodium lactate (1 ml), 1 mg ml^{-1} NAD^{+} (1 ml), 100 mM NaCl (1 ml), 25 mM magnesium chloride (1 ml), 500 mM phosphate buffer pH 7.4 (2.5 ml), 1 $mg.ml^{-1}$ nitroblue tetrazolium (2.5 ml) and 1 $mg.ml^{-1}$ phenazine methosulphate (0.25 ml) in water (Dietz and Lubrano, 1967) .

The gel was incubated for 90 minutes at 37°C, then washed with tap water and placed in 5% trichloroacetic acid to destain the background. A blank was run with each batch of gels by omitting lactate from the staining solution.

The stain density can be used to evaluate the proportion of each isoenzyme, providing that all LDH isoenzymes have equal activity properties in the staining medium. Quantification of the staining density of the separated isoenzymes was performed using a densitometer (Kipp and Zonen, Delft, Holland). The relative quantity of the H and M subunits in the different separations can be calculated according to Thorling and Jensen (1966).

Histochemistry

Preparation of Samples

The pieces of muscle for histochemical analysis were orientated into transverse section under a dissection microscope, placed on filter paper and covered with talcum powder to prevent ice artefact when freezing. The muscle was then immersed in lqiuid nitrogen and stored at -90°C until required. Before cutting, the muscle samples were mounted on a chuck using a commercial embedding medium (Ames O.C.T. compound) which was hardened by placing in liquid nitrogen, thus keeping the muscle on the chuck while cutting. Serial sections 10μ thick were cut in a cryostat (Slee Ltd., London) and taken onto clean glass slides. The sections were air dried and stained by various procedures.

Myosin Adenosine Triphosphatase (Myosin ATPase)

This stain is used as an indicator of the contractile speed of a fibre. The two main methods of demonstrating myosin ATPase activity histochemically in a muscle fibre are precipitation of cobalt sulphide at an acid or alkali pH (Guth and Samaha, 1969) and precipitation of lead sulphide at a neutral pH (Meijer, 1970). There are several criticisms of the two methods and doubt has been expressed as to whether the intensity of the stain is actually indicative of the myosin ATPase activity. Biochemical and histochemical studies (Abraham et al., 1970; King Engel and Cunningham, 1970; Kar and Pearson, 1972) have revealed that in abnormal skeletal muscle fibres of patients with various neuromuscular disorders, alkaline phosphatase activity may be present. This enzyme can also hydrolyse ATP (Meijer and Vossenberg, 1977) and it was thought that some of the fibres may be giving false positive results. Studies with alkaline phosphatase inhibitors have shown no difference in sections of abnormal muscle incubated with and without the inhibitor and so it appears that false positive staining with alkaline phosphatase is not a problem (Meijer and Vossenberg, 1977).

At pH 9.4, a mitochondrial Ca^{2+} ion activated ATPase with a high pH optimum can also influence the reaction especially in young subjects (Guth and Samaha, 1972; Samaha and Yunis, 1973) suggesting that the cobalt sulphide method is unreliable. This finding has been refuted by Edgerton et al. (1975) who claim that the work of Guth and Samaha (1972) and Samaha and Yunis (1973) is actually supportive rather than against the use of the cobalt sulphide method as an indicator of a fibre's speed of contraction. Guth and Samaha (1972) used adult rat soleus and found

positive staining fibres for myosin ATPase at pH 9.4 and suggested that this was due to a mitochondrial ATPase. Close (1967) has found that in the soleus, 11% of the motor units are significantly faster contracting than the rest and Edgerton et al. suggest that those are the fibres which stain darker. The situation may be different in neonatal and extraocular muscles and so caution is required when using myosin ATPase stain as an indication of contractile speed in those muscles (Edgerton et al., 1975).

The technique using Pb^{2+} ions has also been subject to criticism as this ion may hydrolyse ATP (Moses and Rosenthal, 1968) but Novikoff (1967) and Meijer (1970) have not observed this under the conditions used. Comparison of the lead sulphide and cobalt sulphide methods has shown that they give essentially the same result in both normal and pathological muscle and therefore the criticisms outlined above do not appear to justify the discontinuation of this method of classification of contractile properties.

Pre-incubation at various pHs allows the distinction of three separate fibre types based on their histochemical staining properties and this method has been used along with the method of identifying only two main fibre types.

Method (Fig 2.7)

Pre-Incubation

Alkali (pH 10.2): Freshly cut cryostat sections were fixed for 5 minutes in phosphate buffered formaldehyde (pH 7.0) and then washed twice

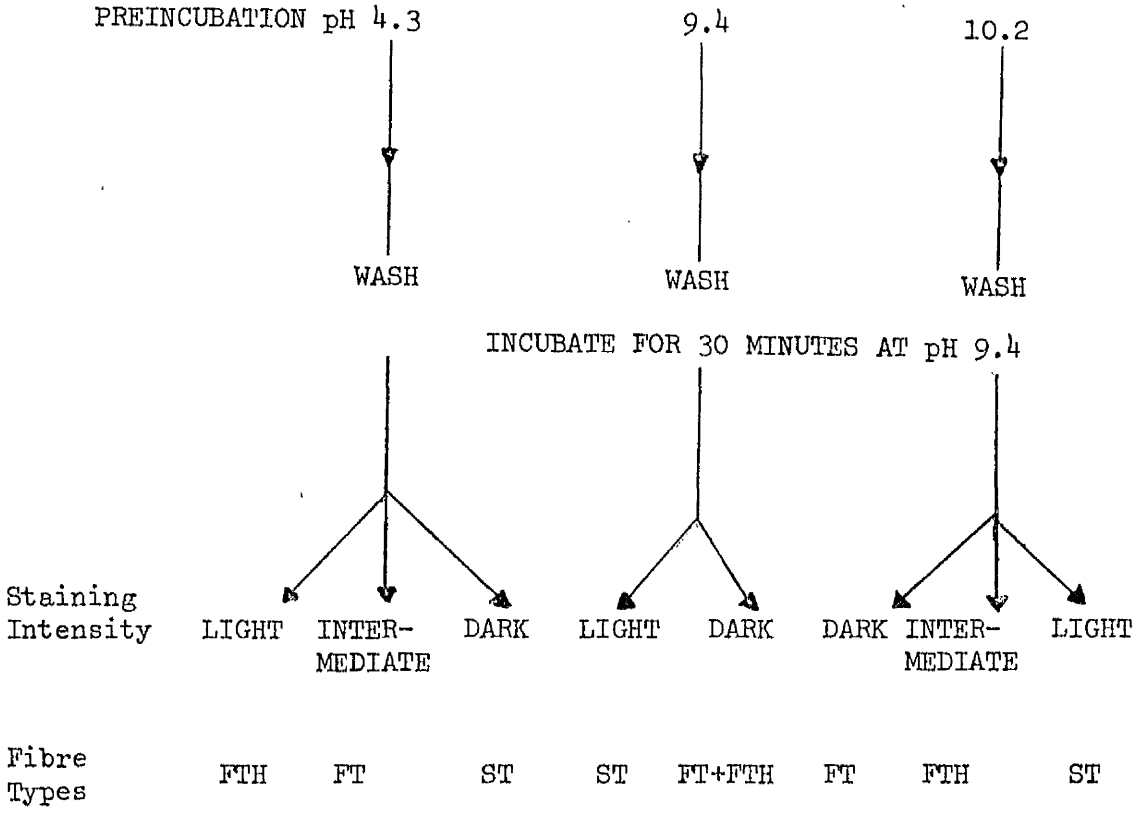


Fig 2.7: Flow diagram of methods of distinguishing fibre types using myosin ATPase staining activity at various pre-incubation pH's.

in 100 mM Tris-HCl containing 18 mM calcium chloride (pH 7.8). Alkaline pre-incubation was then carried out at pH 10.2 with 50 mM Tris-HCl buffer containing 18 mM calcium chloride. After 15 minutes, the solution was removed by washing twice in the 100 mM Tris-HCl containing 18 mM calcium chloride solution at pH 7.8. Failure to fix the tissues initially in buffered formaldehyde (pH 7.0) resulted in their destruction at pH 10.2.

Acid (pH 4.3): Freshly cut cryostat sections were incubated for 2.5 minutes in 100 mM sodium acetate buffer (pH 4.3) and then washed twice in the Tris-HCl/calcium chloride solution at pH 7.8.

The pre-incubated sections (or if desired, non pre-incubated sections) were incubated at room temperature for 30 minutes in a solution containing 18 mM calcium chloride in 50 mM Tris-HCl (pH 9.4). ATP (2 $mg.ml^{-1}$) was added to the buffer and the solution filtered just before use. After incubation the sections were washed three times for two minutes in 1% (w/v) calcium chloride followed by one 3 minute wash in cobalt chloride (2% w/v). The sections were washed three times in alkaline water (65 ml of distilled water + 15 ml of 100 mM sodium barbiturate) and developed in 1% ammonium sulphide. Sections were dried in absolute ethanol, cleared in xylol and mounted in DPX synthetic resin.

Succinate Dehydrogenase (SDH)

The enzyme succinic dehydrogenase is involved in the electron transport chain and the Kreb's cycle and it is therefore assumed that any measure of its activity is indicative of the oxidative capacity of the muscle or muscle fibre under examination. The histochemical visualisation of SDH is based on the reduction of a diformazan, nitro-blue tetrazolium (NBT), by the hydrogen ions released when succinate is converted to fumarate. NBT is selectively adsorbed on to the mitochondria and sarcoplasmic reticulum (Brooke and Engel, 1966) and as SDH is believed to be entirely intra-mitochondrial (Roodyn, 1967) a precise location for the enzyme can be found. This means, that the more mitochondria in a fibre, the more blue staining mitochondrial granules and the greater the oxidative capacity of the fibre. It is possible that deposition of the diformazan may occur outwith the sites of SDH activity, e.g. lipid, droplets (Hitzeman, 1963) but these should have little effect on the comparison between individual fibres. It is also possible that the SDH activity of mitochondria from different fibres may vary (Blancher, 1964) but the amount of diformazan deposited in a particular fibre after incubation for 20 minutes should depend primarily on the mitochondrial density, rather than on the actual level of SDH activity (Davies and Gunn, 1972). The activity of the SDH in the mitochondria does, however, become a problem in one instance. SDH is fairly unstable and if serial sections are cut and stained at different time intervals after cutting, it will be noticed that sections stained immediately stain much more intensely. This may make it difficult to determine whether a fibre is to be classified as high or low oxidative particularly if it is on the

borderline between the two classifications (Spamer and Pette, 1977). The staining intensity of a particular fibre decreases the longer the section is left. After about two hours, the rate of deterioration is much less, however, and little change occurs in the intensity of staining for the next twenty four hours. As all of the sections in this study were stained after two hours and before twenty four hours, this was not a problem.

Method

The method was based on that of Nachlas et al. (1957). Freshly cut cryostat sections were incubated for 20 minutes at 37^{o}C in a solution containing 100 mM phosphate buffer (pH 7.6), 100 mM sodium succinate and an equal volume of water containing 1 mg. ml^{-1} of nitro-blue tetrazolium. The sections were washed in saline and left to dry for 60 minutes. Fixing with 4% buffered formaldehyde (pH 7.0) for 10 minutes was followed by drying in 15% (v/v) ethanol for 5 minutes. The sections were mounted in glycerine jelly.

Blue diformazan deposits indicated sites of SDH activity.

Phosphorylase-(a) (Ph)

The enzyme phosphorylase consists of two interconvertible forms, the a-form being active and the b-form inactive. This latter form is converted to the a-form by the enzyme phosphorylase kinase using two ATP molecules. It catalyses the reversible reaction

$$n(\text{glucose-1-phosphate}) + (\text{glycogen})_m \rightleftharpoons (\text{glycogen})_{n+m} + nH_3PO_4$$

which is pH dependent, favouring glycogen synthesis at pH 6.0. Traces of AMP and cysteine or EDTA are required for maximum activity.

The histochemical method involves supplying the section with glucose-1-phosphate and a little glycogen as primer. The tissue phosphorylase increases the size of the glycogen by addition of glucose units from glucose-1-phosphate. It is hoped that glycogen will be bound to the tissue. The glycogen is visualised by its reaction with iodine. Differences in the colour of the iodine staining have been attributed to the progressive increase in chain length during synthesis of the glycogen polymer, blue indicating over thirty glucosyl units and red indicating seven to thirteen units (Swanson, 1948). The result may be modified by a debranching enzyme present in the tissue and so this is inactivated by the addition of ethanol to the incubation medium. The activity of phosphorylase can be enhanced by the addition of insulin.

Method

The method is based on that of Takeuchi (1956). Sections were incubated at 37°C in a medium containing glucose-1-phosphate (16.7 mg),

AMP (3.3 mg), glycogen (0.6 mg), distilled water (5 ml), 100 mM sodium acetate buffer (pH 5.8; 3.3 ml), 1 drop of insulin (20 $\mu.ml^{-1}$) and absolute alcohol (1.7 ml). After ninety minutes, the sections were rinsed in distilled water and dried at $37^{o}C$ for two minutes, fixed in absolute alcohol for two minutes before being dried in air. The colour was developed in dilute Grams iodine (1:10 with distilled water) until the blue colour appeared. Finally, the sections were drained and amounted in iodine glycerol (1:1) and the coverslips sealed with nail-polish.

A positive result indicated glycolytic activity.

Lipid (Sudan Black)

Histochemically, lipid can be defined as fatty matter which has some of the chemical characteristics of lipid molecules - either molecules containing choline, unsaturated fatty acids or phosphatides. There are two main methods of defining the lipid content of a section.

a) Physical methods which depend on the readiness of lipophyllic molecules to partition between their solvent and the lipid.
b) Chemical methods like the acid haematin test or the use of Nile blue.

The physical methods are more important and depend on the ability of lipid to concentrate Sudan Black or benzpyrene from semi-aqueous solutions in which these compounds are not very soluble.

Method

The method was based on that of Davies and Gunn (1972). Freshly cut cryostat sections were fixed in formol calcium (1 gm of calcium chloride in 100 ml of buffered formaldehyde at pH 7.0) for five minutes, rinsed in distilled water followed by ethanol (70% v/v). The sections were stained in a freshly filtered saturated solution of Sudan Black in ethanol (70% v/v) for thirty minutes. Sections were rinsed in ethanol (70% v/v) and washed well in running water. Coverslips were mounted in glycerine jelly.

Granular deposits indicate sites of lipid concentration.

Haematoxylin and Eosin

This stain was used to identify the general morphology of the muscle sections. Freshly cut cryostat sections were stained as follows.

a) Delafield's haematoxylin - four minutes.

b) Rinsed in distilled water.

c) Stained in Scott's Tap Water Substitute (S.T.W.S.) until the section turned blue - normally thirty second.

d) Rinsed in distilled water.

e) Stain in Eosin (1%) for two minutes.

f) Rinsed three times in absolute ethanol.

g) Rinsed in carbol xylol.

h) Rinsed twice in xylol.

Sections were mounted in synthetic resin. Haematoxylin was made up as Delafield's haematoxylin (6.4 g), glycerol (160 ml), absolute ethanol (200 ml) and distilled water (600 ml). The solution was left for six weeks before use.

Scott's Tap Water Substitute consisted of sodium bicarbonate (3.5 g), magnesium sulphate (20 g) and tap water (1000 ml).

SECTION 3

INVESTIGATION OF THE VARIATION OF SEVERAL PARAMETERS IN HORSE SKELETAL MUSCLE

Introduction

Much of the sampling in this study was carried out by the technique of percutaneous needle biopsy with normally only thirty to a hundred milligrams of muscle being obtained. It was essential therefore to know how homogeneous a muscle was with respect to its fibre composition.

Methods

Histochemistry: Histochemical analysis of the variation within a muscle was performed in three ways.

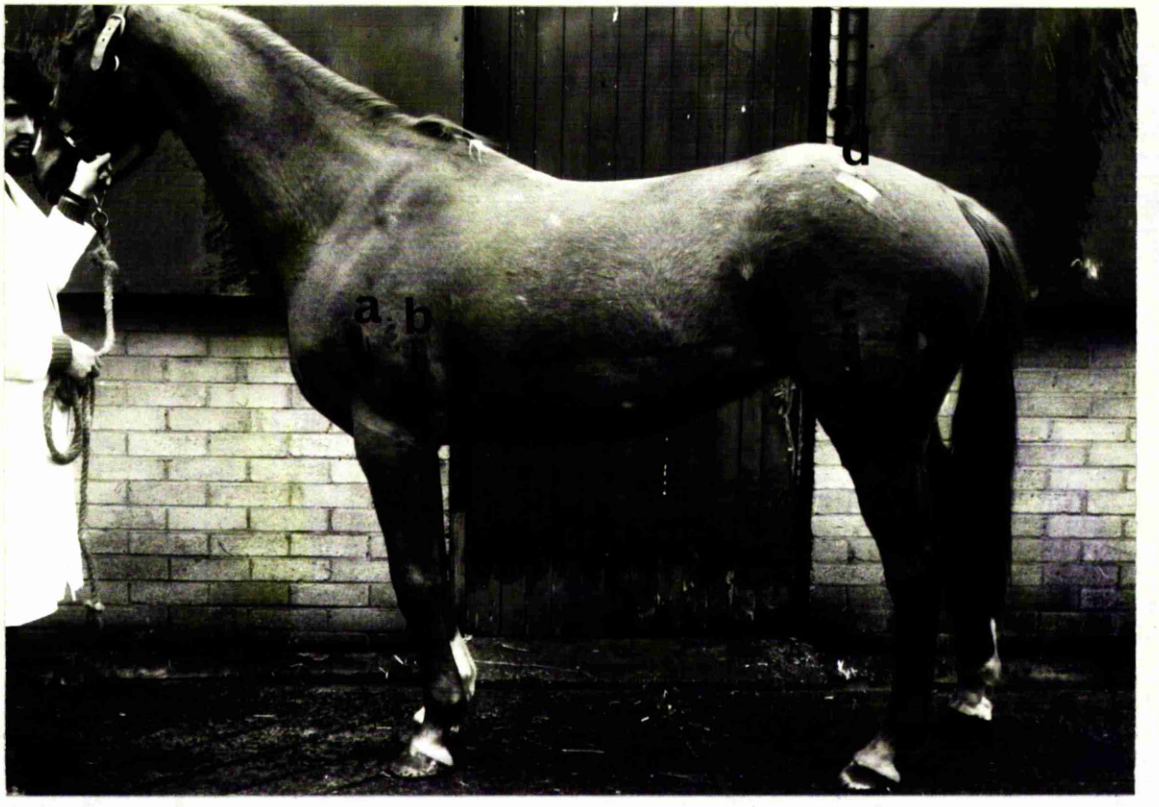
a) Analysis of the variation in the percentage fibre composition when 200 fibres as compared to 600 or 800 fibres were counted from the same muscle.

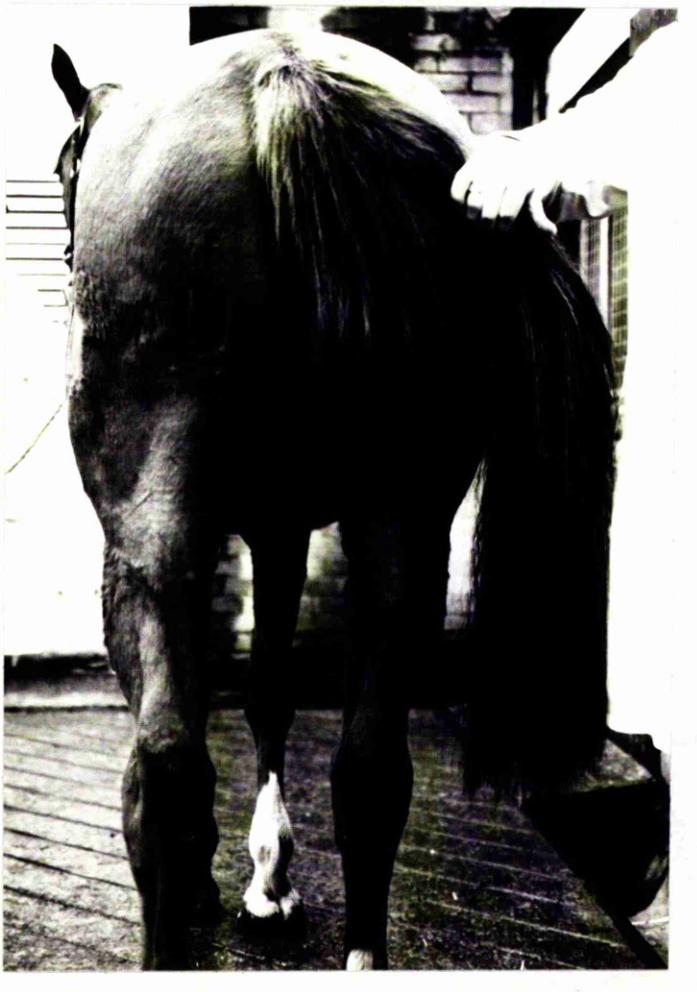
b) The difference between deep and superficial areas of the same muscle.

c) The error obtained when duplicate samples were taken from within a muscle and between the left and right muscles at the same time.

For parts (a) and (b), blocks of muscle approximately 1 cm^3 were obtained from the deep and superficial portions of six muscles, (Fig 3.1), deltoideus, long head of the triceps brachii, vastus lateralis, gluteus medius, biceps femoris and semitendinosus, of six horses freshly killed (8, 9, 10, 11, 12, 13). The blocks were frozen and processed as in section 2. Duplicate biopsies were obtained from a muscle and processed as in section 2 to determine the intra-muscular and contralateral variation as in part (c).

Fig 3.1: Muscle sites used in the investigation a) deltoideus, b) long head of the triceps brachii, c) vastus lateralis, d) gluteus medius, e) biceps femoris, f) semitendinosus.


a
b



Biochemistry: Biochemical analysis of the variation in the muscle was performed in three ways.

a) The effect of freeze-drying and powdering on the coefficient of variation in a muscle was examined.

b) The intra-biopsy variation was obtained by assaying ten homogenates from the same biopsy as in section 2.

c) The intra-muscular and contralateral muscle variation was determined from duplicate biopsies taken at the same time.

Results

Histochemistry: As will be explained more fully in section 4 three fibre types were found in the horse muscle in this study, two with a high myosin ATPase activity at pH 9.4 and one with a low activity at this pH. One of the high myosin ATPase fibres also had a high intensity stain with SDH and wasdesignatedFTH. The other high myosin ATPase fibre stained only slightly with SDH and was designated FT. The third fibre type, the low myosin ATPase activity fibre, also stained intensely for SDH and was called ST.

a) No significant difference was found in the percentage of fibres obtained when 200 fibres and 600-800 fibres were counted ($P > 0.5$).

b) In most of the muscles examined, only minor non-significant differences occurred between the deep and superficial portions of a muscle (Table 3.1). This applied to both the myosin ATPase properties and the oxidative properties. In those muscles where a significant difference did occur, no pattern was established where either the deep or superficial region was higher in a given property of the fibres.

	Horse 8		Horse 9		Horse 10		Horse 11		Horse 12		Horse 13	
	M	O	M	O	M	O	M	O	M	O	M	O
Deltoideus Superficial	67.9	56.1	65.6	64.5	66.9	-	79.3	52.4	55.7	64.7	64.6	58.1
Deep	69.8	58.2	71.4	63.9	71.1	-	66.6*	55.9	55.9	63.7	64.2	66.0
Triceps Brachii Superficial	79.1	48.0	85.4	54.7	67.8	68.1	77.2	64.8	78.6	61.1	70.1	62.3
Deep	68.1*	51.2	82.7	56.4	68.8	67.7	76.9	58.8	75.1	61.7	70.4	62.5
Vastus Lateralis Superficial	92.8	45.2	98.0	63.4	73.9	62.6	91.9	53.1	91.7	67.4	89.2	66.0
Deep	90.2	46.5	98.9	55.4*	67.3	60.0	86.2	52.9	94.3	64.4	83.9	66.0
Gluteus Medius Superficial	81.5	55.6	65.9	63.7	64.8	59.6	79.1	65.7	74.1	55.3	66.4	68.8
Deep	80.0	60.6	92.0*	52.6*	82.3*	60.6	68.3	66.6	75.1	63.9*	64.0	68.8
Biceps Femoris Superficial	73.9	60.9	86.5	65.2	76.2	63.0	74.4	58.6	82.3	71.6	81.6	69.1
Deep	72.6	59.4	79.9	63.9	77.8	73.8*	71.6	68.2	81.0	70.2	79.1	64.4
Semitendinosus Superficial	91.9	56.4	52.6	57.4	87.9	61.3	80.4	59.9	87.2	59.7	97.7	64.6
Deep	92.6	59.7	51.0	53.9	88.9	61.2	83.3	60.1	82.1	63.0	97.5	64.2

M = % of high myosin ATPase fibres; O = % of high oxidative fibres; * = $P < 0.05$ between deep and suprficial (χ^2 test).

Table 3.1. The Fibre Types in Deep and Superficial Regions of 6 Equine Skeletal Muscles (Individual Animals)

c) The coefficient of variation between duplicate biopsies is shown in Table 3.2. The greatest variation occurred in the FTH fibres but was within acceptable levels. (Sparapani and Berry, 1965). A similar variation was found in the contralateral muscles.

Biochemistry: a) Freeze drying and powdering reduced the coefficient of variation, within a muscle sample, from about 25% to about 8% (Table 3.3). There was little change in the enzyme activities or the glycogen concentration after freeze drying and powdering when compared to fresh tissue.

b) The variation within a biopsy was between 3% and 8% for all of the enzymes examined (Table 3.4).

c) The coefficient of variation between duplicate biopsies from the same and contralateral muscles are shown in Table 3.5. The variation tended to be higher for the enzymes than for glycogen or protein but was similar within a muscle and between the left and right muscles.

Triglycerides were also measured in the muscle but the variation within a muscle proved too large to produce meaningful results (128 ± 42.6 μmoles . (g dry weight of tissue)$^{-1}$. C.V.= 33.3%).

Discussion

Histochemistry: Normally, the technique of percutaneous needle biopsy yields sections for histochemical analysis of between 150 and 300 fibres. The finding that similar values were obtained whether 200 or 600 to 800 fibres were counted justifies the use of needle biopsy material in determining the fibre composition of a muscle. The use of this technique

	N	Coefficient of Variation (%)	
		Within a Muscle	Between Contra-lateral Muscles
FT fibres	7	1.4	1.7
FTH fibres	7	7.9	8.3
ST fibres	7	4.2	4.2

Table 3.2. Coefficient of variation for the three fibre types within a muscle and between contralateral muscles. Duplicate biopsies were taken from the left gluteus medius to calculate the within muscle variation. The mean of these two values was used along with the value obtained from the right gluteus medius muscle, to give the variation between contralateral muscles.

N = number of horses.

	Frozen		Freeze Dried	
	Activity or Concentration	C.V.	Activity or Concentration	C.V.
ALD+	45.5 ± 13.00	28.6	50.3 ± 4.8	9.4
CS+	3.0 ± 0.8	26.3	3.1 ± 0.3	8.0
AST+	82.6 ± 22.5	27.2	75.5 ± 5.3	7.1
Glycogen*	60.5 ± 11.5	19.0	63.9 ± 6.0	9.4

+μmoles.minute^{-1}.(g wet weight of tissue)$^{-1}$

*μmoles.(g wet weight of tissue)$^{-1}$.

Each value is the mean of 4 separate homogenates from the same muscle.

Table 3.3. The effect of freeze drying and powdering to remove connective tissue and fat, on enzyme activity and glycogen concentration and on reproducibility. (Values for freeze dried tissue have been converted to wet weight assuming a water content in the muscle of 75%.)

	Coefficient of Variation (%)
LDH	3.9
CPK	5.4
AST	6.0
ALT	4.3
ALD	3.5
HAD	6.3
CS	5.7
GDH	5.1
HK	5.8
Glycogen	4.2
Protein	5.1

Table 3.4: The variation within a biopsy for several enzymes, glycogen and protein. Biopsy was divided into ten, homogenised and assayed.

Enzyme	N	Coefficient of Variation (%) Within a Muscle	Between Contralateral Muscles
ALD	8	8.9	8.7
CS	8	11.0	10.7
GDH	8	7.3	6.6
AST	8	12.0	9.1
ALT	8	11.5	7.2
LDH	8	9.5	7.9
CPK	8	6.4	5.2
HAD	8	9.1	5.6
HK	8	7.2	3.1
Glycogen	8	2.8	8.3
Protein	8	6.1	4.5

Table 3.5.: Coefficient of variation for enzymes, glycogen and protein within a muscle and between contralateral muscles. For details see table 3.2.
N = Number of horses.

for obtaining samples of tissue representative of the whole muscle was strengthened by the small coefficient of variation obtained when duplicate biopsies were analysed. A percentage coefficient of variation of less than 8% has been said to be acceptable (Sparapani and Berry, 1965) and the results in this study are within those limits.

It has been shown in several species (Baldwin et al., 1972; Ariano et al., 1973; Fitts et al., 1973) that the fibre composition of a muscle can vary from deep to superficial regions. The usual pattern is that the superficial regions contain more low oxidative fibres and high myosin ATPase activity (pH 9.4) fibres than the deeper regions. This would suggest that if a similar pattern existed in animals from which needle biopsies were taken, anomalous results may occur. The findings in this study suggest that equine skeletal muscle is homogeneous in fibre composition for at least the muscles studied and therefore the use of biopsy specimens was valid. In this respect, the horse is similar to man, where several reports have found no difference between the deep and superficial regions of many muscles (Susheela and Walton, 1969; Jennekens et al., 1971; Johnson et al., 1973; Edgerton et al., 1975). The variation between sites and between contralateral muscles, is much less than that reported for man where a coefficient of variation of 11.6% for the percentage of high myosin ATPase fibres (pH 9.4), was found using duplicate samples (Henriksson, 1976).

Biochemistry: When enzyme activities were measured initially in the biopsy samples, much variation was found, even within a single biopsy. A similar finding was reported in the dog gastrocnemius-plantaris muscle

group (Chapler and Moore, 1972) but not in man (Karlsson, 1971). The greatest potential source of error in small biopsy samples was the occurrence of variable amounts of connective tissue and blood as observed in this study and by other authors (Harris et al., 1974). These authors found that this could be minimised if the biopsy samples were freeze dried to extract water and powdered to enable the removal of dried blood and connective tissue. This treatment was also of use in this study as the coefficient of variation for several enzymes and glycogen was reduced by more than 100% to between 8% and 9%. At the same time, the activities of the enzymes or the concentration of glycogen were not greatly affected. Freeze drying and powdering, therefore was used in all subsequent studies.

Similar findings were obtained when duplicate biopsies from the same and contralateral muscles were examined. The coefficient of variation was between 3% and 12% depending on the parameter measured and only slightly higher than the variation within a biopsy.

Triglycerides were much more variable in the muscle. It appears that the distribution of lipid in the muscle is much less homogeneous than glycogen, the other main fuel. This assumption is made on the basis that the variation must be large as the precision of the assay was only 5.0%. Essen et al. (1975) and Lindholm et al. (1974) have already reported that this effect occurs in man and horse. Further evidence of the large variation in the location of triglyceride depots comes from ultrastructural studies (Snow, D.H. pers. comm.).

From these histochemical and biochemical results, the assumption that the small samples of muscle are representative of the whole muscle is

valid with the exception of triglyceride concentration. In all studies, however, biopsy samples were taken from a similar depth in case one of the subjects had an unusual fibre distribution.

SECTION 4

MUSCLE COMPOSITION IN VARIOUS BREEDS OF HORSE AND DOG

Introduction

The fibres of skeletal muscle can be sub-divided on the basis of their histochemical and biochemical properties as previously mentioned. The main sub-divisions are as follows.

a) A high myosin ATPase activity (pH 9.4), high oxidative, high glycolytic activity fibre.
b) A high myosin ATPase activity (pH 9.4), low oxidative, high glycolytic activity fibre.
c) A low myosin ATPase activity (pH 9.4), high oxidative, low glycolytic activity fibre.

Although these basic fibre types are found in most species, subtle differences exist between the various species with respect to the size of the fibre, which fibre has the highest oxidative capacity, the distribution of the mitochondria in the fibre and such like. These variations have in fact led to some confusion when classifying the types of fibres in the muscles. Padykula and Gauthier (1966) classified fibres on the basis of their oxidative properties as red, white and intermediate. Guth and Yellin (1971) demonstrated, however, that species differences occurred in the histochemically defined fibre types of skeletal muscle. The overall intensity of the slow contracting fibre may be greater than that of the fast contracting high oxidative fibre as in the cat and man or less than as in rat and mouse. These differences were further extended by work in the miniature pig (Fitts et al., 1973) and the horse (Lindholm and Piehl, 1974). This led to the suggestion that the red, white and intermediate terminology be prefixed by fast or slow depending on the

intensity of the histochemically demonstrated myofibrillar ATPase enzyme (Barnard et al., 1971). Unfortunately in the horse, the fast and slow high oxidative fibres tend to stain with a similar intensity in many fibres (Lindholm and Piehl, 1974), and so the classification that was used in this study was, as mentioned in section 3, FT, FTH and ST fibres.

Burke et al. (1973) were the first to show that in individual fibres, the histochemical profile could be correlated with the physical properties of the muscle fibre. A high myosin ATPase activity at pH 9.4 was indicative of a fast contraction speed whereas a low activity at the same pH meant that the fibre was slow contracting. This was an extension of the work of several workers who proved that the myosin ATPase activity was directly proportional to the intrinsic speed of sarcomere contraction (Barany, 1967) and that the histochemical technique of Padykula and Herman (1955) is specific for myosin ATPase. Burke et al. (1973) also demonstrated that the fatiguability of the fibre was related to the number of mitochondria in the fibre, the more mitochondria, the greater the resistance to fatigue. The low myosin ATPase activity (pH 9.4) fibres are predominately recruited during contractions of low tension output whereas the high myosin ATPase activity (pH 9.4) fibres appear to be recruited only when high tension and/or velocity is required (Burke and Edgerton, 1975). No direct evidence is available in the horse to show that high myosin ATPase activity (pH 9.4) is indicative of fast contractility but these assumptions have been made by several workers (Davies and Gunn, 1972; Lindholm and Piehl, 1974) and have been made in the present study with the proviso that a high myosin ATPase activity at pH 9.4 is only probably indicative of a fast contracting fibre. In this thesis therefore the

terms high myosin ATPase activity at pH 9.4 fibre and fast twitch fibre will be synonymous. Similarly slow twitch fibres will be the same as low myosin ATPase activity at pH 9.4 fibre. (It has also to be remembered that fast and slow are relative terms and that the properties of the fast twitch fibre in the horse are not necessarily identical to the fast twitch fibre in the rat.

Differences between the muscle fibre types can also be demonstrated biochemically. Using muscles that are relatively homogeneous in a given fibre type, several workers, prominent among them being Pette and his coworkers (see Pette and Staudte, 1973), have demonstrated that differences exist in the enzyme activities of the three main fibre types (Table 4.1).

It has been reported that histochemically, endurance athletes have a much lower percentage of fast twitch fibres in their limb muscles than athletes who excel at sprinting or power events (Gollnick et al., 1972). Those latter athletic types appear to have muscles which are composed predominantly of fast twitch fibres (Edstrom and Ekblom, 1972; Prince et al., 1976; Rusko, 1976; Thorstensson et al., 1977). These findings indicate that endurance athletes have muscles which contain a high proportion of high oxidative fibres whilst sprint/power athletes have a high proportion of fast contractile fibres.

The muscle fibre composition in humans is thought to be genetically determined (Komi et al., 1977b) and this is interesting when it is considered that horse breeds such as the Thoroughbred and Quarter-horse have been specifically bred over many generations for their ability to

	Soleus	Rectus	Vastus
	ST	FT	FTH
Glycogen Phosphorylase	2.8	8.8	8.6
Hexokinase	1.6	0.5	2.0
Triose Phosphate Dehydrogenase	80	350	390
mitochondrial-Glycerol Phosphate Dehydrogenase	0.4	1.1	2.3
Citrate Synthase	9.4	2.2	29.0
3-Hydroxyacyl-CoA Dehydrogenase	9.8	3.3	19.0
Creatine Phosphokinase	127	340	155
Lactate Dehydrogenase	100	550	430
Myoglobin (mg.(gm wet weight)$^{-1}$)	5.0	0.33	4.8

Table 4.1: Activity levels of several key enzymes (μmoles.(min.gm wet weight of tissue)$^{-1}$) in three muscles composed mainly of a given fibre type in the guinea pig (Pette and Staudte, 1973).

perform a specific type of exercise (Gunn, 1975). This raises the question as to whether the various breeds of horse have muscle fibre compositions which are adapted by selection over the years to be most suited for the type of exercise which they perform. A similar phenomenon has occurred with the greyhound which has been specifically bred for speed for over 3000 years. The aims of this section were therefore as follows.

1) To determine if there was a correlation between the type of exercise to which a breed of animal is best suited and the histochemical and biochemical properties of its muscles. This was undertaken by examining the muscles in several breeds of horse and dog.

2) At the same time, attempts were made to classify the metabolic profiles of the fibre types. This was extremely difficult as only muscles of mixed fibre types can be obtained in the horse and dog. The method used was therefore similar to that used by Gollnick et al. (1974b) where individuals with muscles of widely varying fibre composition and various muscles of different fibre composition were used.

Methods

The analytical methods used were as described in section 2. Muscle samples were obtained by the technique of percutaneous needle muscle biopsy (Bergström, 1962) from living animals or by muscle biopsies using clamps from autopsy specimens.

Animals: The horses and dogs used were those described in the appendix.

Muscles: For histochemical analysis, six muscles were examined, the deltoideus, long head of the triceps brachii, vastus lateralis, gluteus medius, biceps femoris and semitendinosus. Biochemical analysis was performed on the gluteus medius and the semitendinosus. The assays used were for ALD, LDH, ALT, AST, CPK, CS, HK, GDH, HAD, and glycogen.

Statistics: The results were analysed on a computer using conventional statistical methods (Snedecor, 1956).

Results (1)

Qualitative Histochemistry

Horse: As with most other studies, horse skeletal muscle was found to consist of three main fibre types as previously described (Fig 4.1). The main staining and contractile properties of the three types are as described in Table 4.2.

SDH: SDH activity as defined by the diformazan deposition occurred throughout the myofibre with the highest level of activity and hence diformazan deposition occurring in the sub-sarcolemmal region. Although the staining intensity was always much less in the FT fibres, the staining intensity in the FTH fibres was sometimes less than and sometimes equal to that in the ST fibres even within the same muscle (Fig 4.1).

Lipid: The density of staining of Sudan black B for lipids always corresponded to the activity of SDH (Figs 4.2; 4.3). Either stain could therefore be used to type the fibres but in practice only the SDH stain was used.

Myosin ATPase: Myosin ATPase activity at various pH's could be used to split the fibres into three distinct groups (Figs 4.4; 4.5; 4.6). At pH 9.4, only two fibre types were found, one showing a dense dark brown reaction which was indicative of a high activity and the other staining a light brown to white colour, demonstrating low myosin ATPase activity. If the section was pre-incubated at pH 10.2 before the normal pH 9.4 incubation, the fibres were split into three types with the high myosin ATPase activity at pH 9.4 fibres that stained dark without pre-incubation being split into

Stain or Property of Fibre	FT	FTH	ST
Speed of Contraction	Fast	Fast	Slow
Myosin ATPase pH 9.4	High	High	Low
Myosin ATPase pH 9.4 (Pre-Incubation pH 10.2)	High	Intermediate	Low
Myosin ATPase pH 9.4 (Pre-Incubation pH 4.35)	Intermediate	Low	High
Phosphorylase	High	High	Low
SDH	Low	High	High
NADH Diaphorase (Lindholm and Piehl, 1974)	Low	High	High
Sudan Black	Low	High	High
P.A.S. (Lindholm and Piehl, 1974)	High	High	Intermediate

Table 4.2: Staining intensity and contractile properties in the skeletal muscle fibres of the horse.

Fig 4.1; Serial section of horse gluteus medius muscle, stained for (a) myosin ATPase (pH 9.4) and (b) SDH. Three main fibre types evident, FT, FTH and ST. Note intensity of the FTH and ST fibres in some cases are similar. X 250.

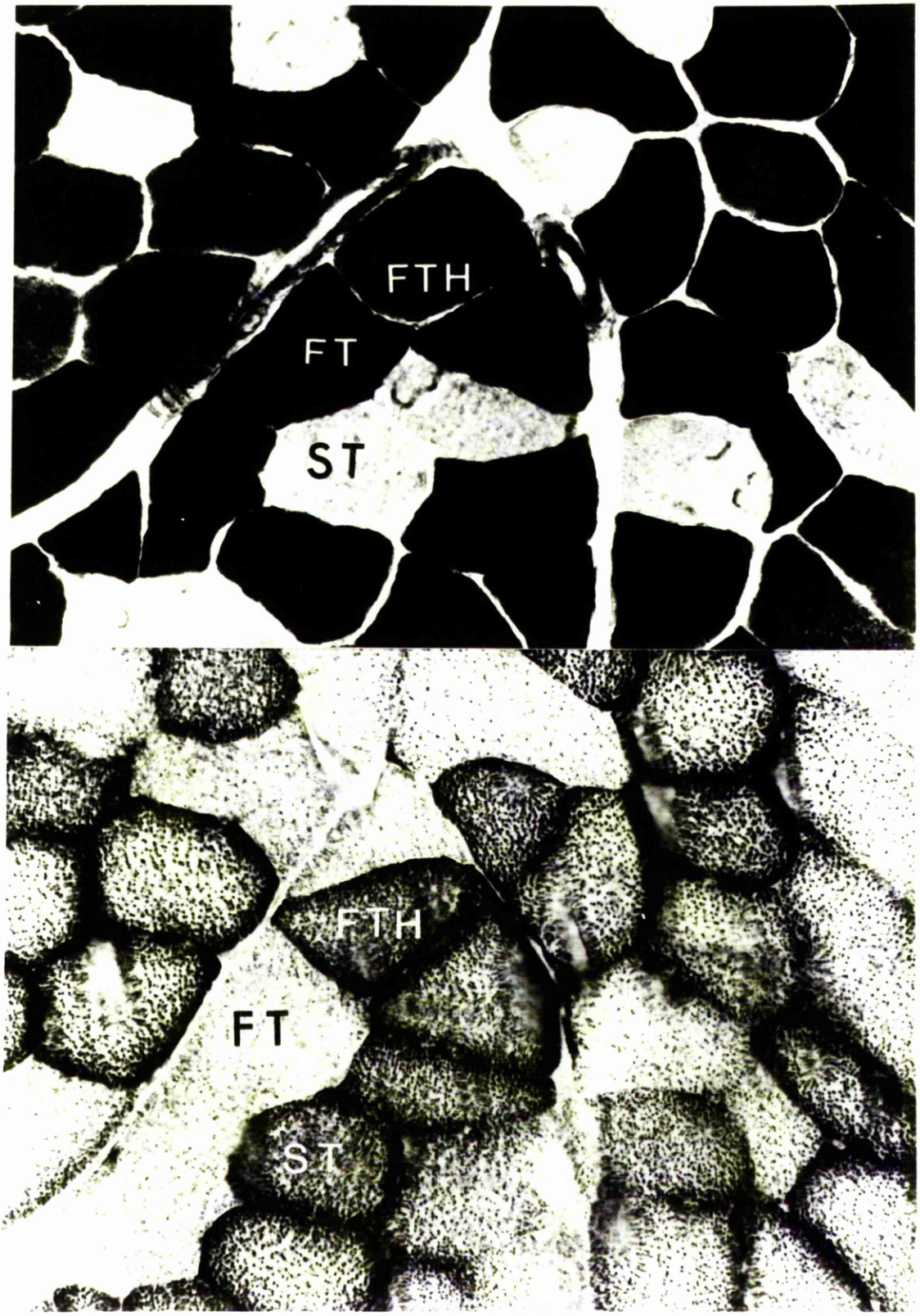
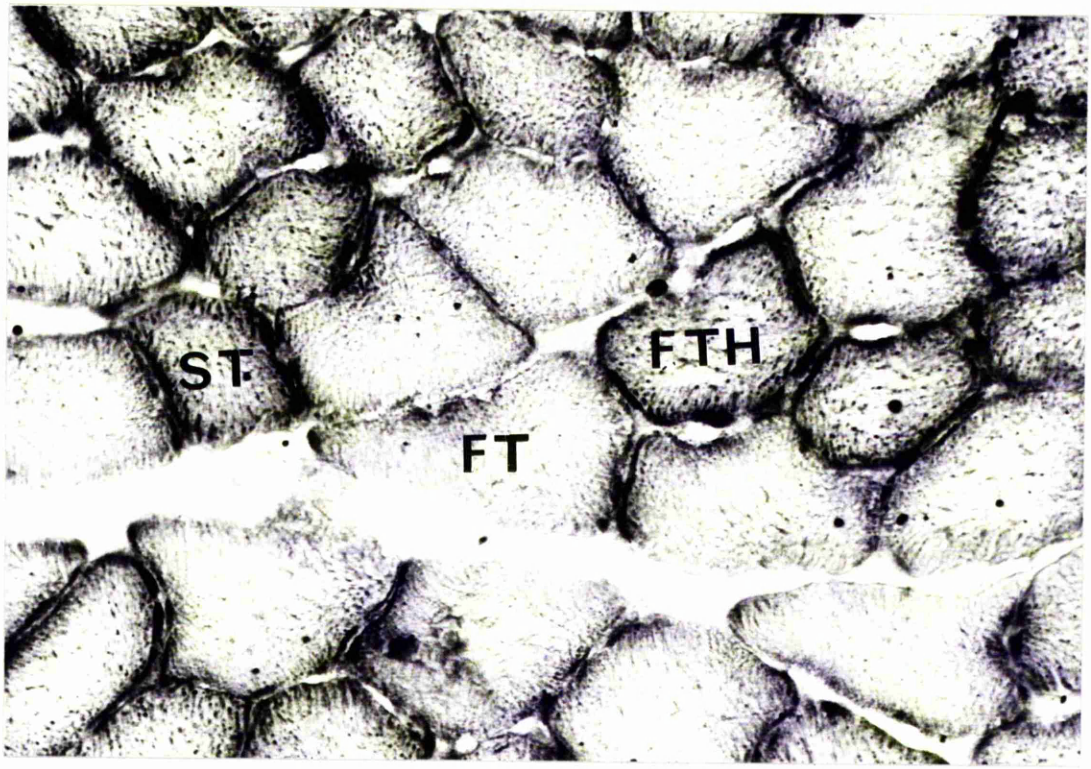

FTH
FT
ST
FTH
FT
ST

Fig 4.2: Sites of lipid depots in horse skeletal muscle.

Fig 4.3: Sites of SDH activity in horse skeletal muscle. Serial section of Fig 4.2 with the same fibres indicated in both figures.


ST
FTH
FT

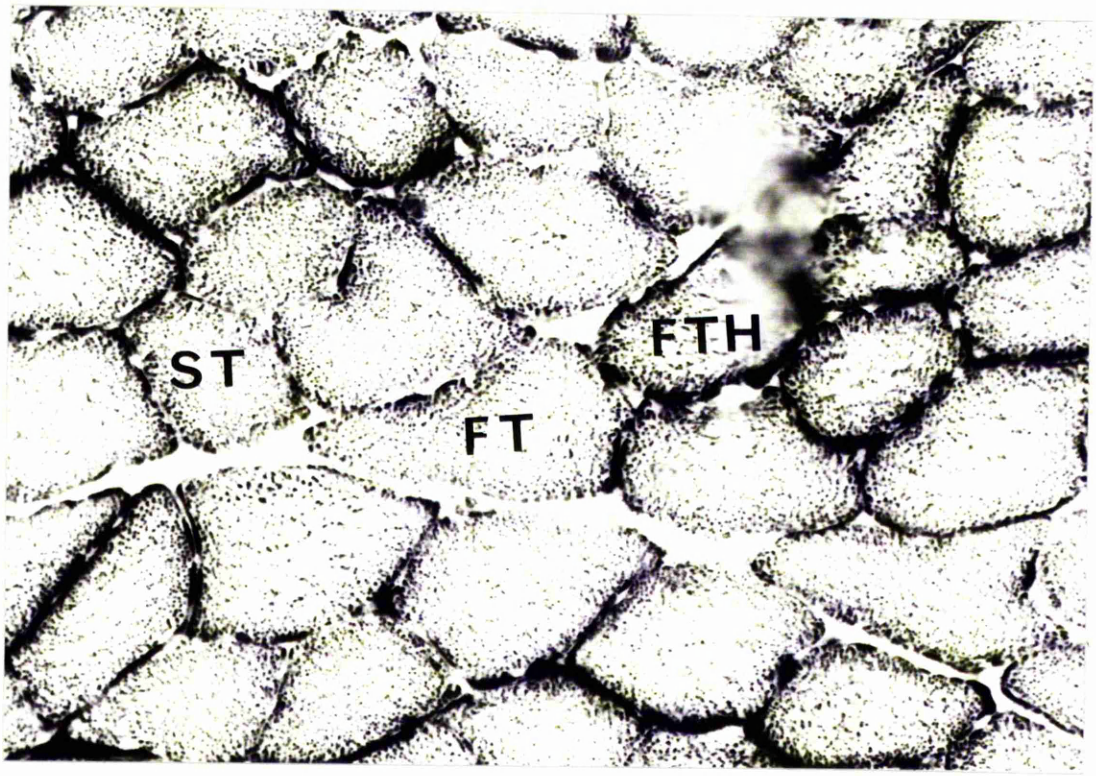
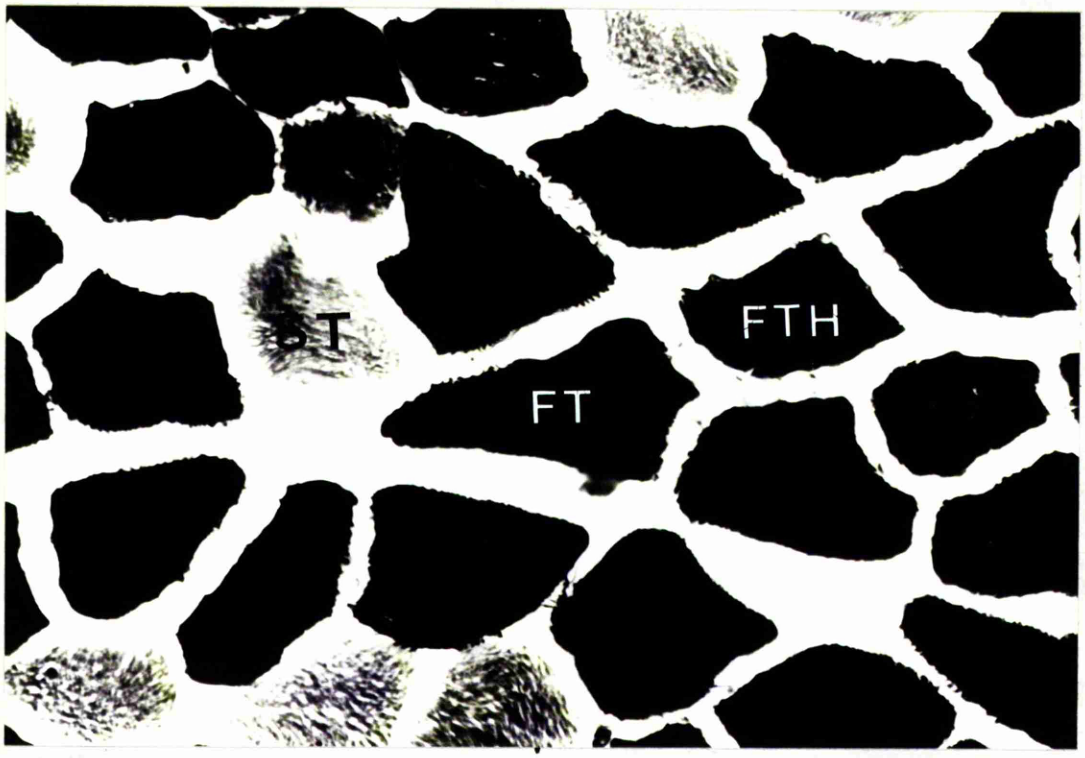

ST
FTH
FT

Fig 4.4: Serial section of horse gluteus medius muscle stained for (a) myosin ATPase (pH 9.4) and (b) SDH. X 250.


ST
FTH
FT

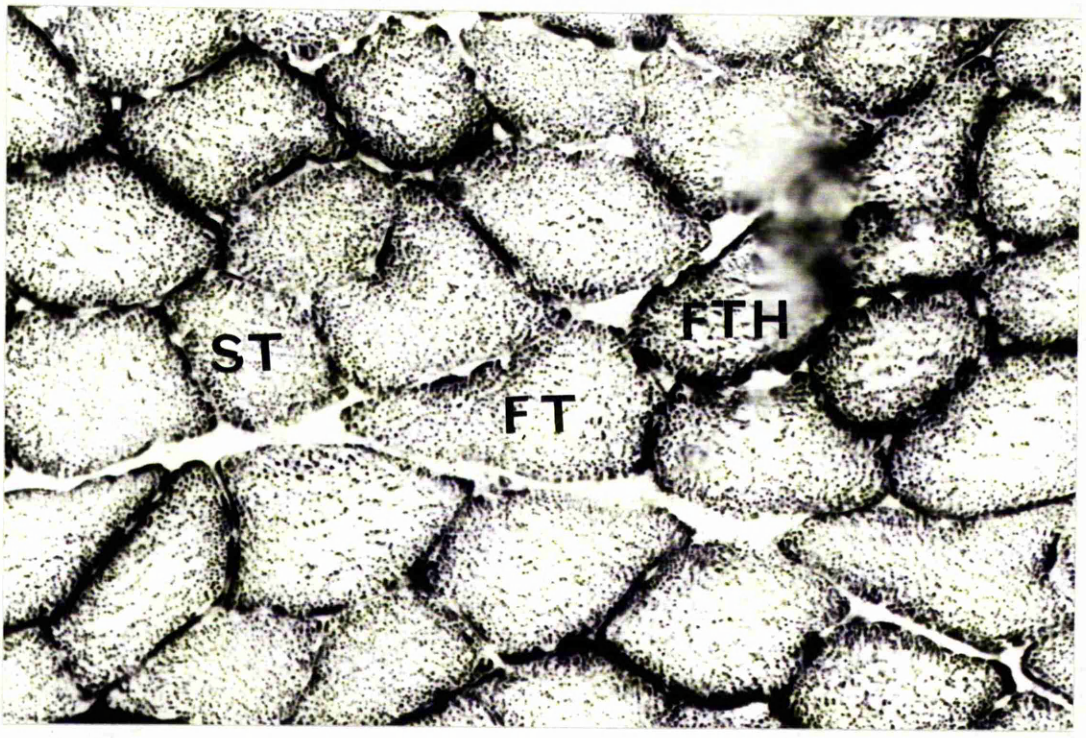
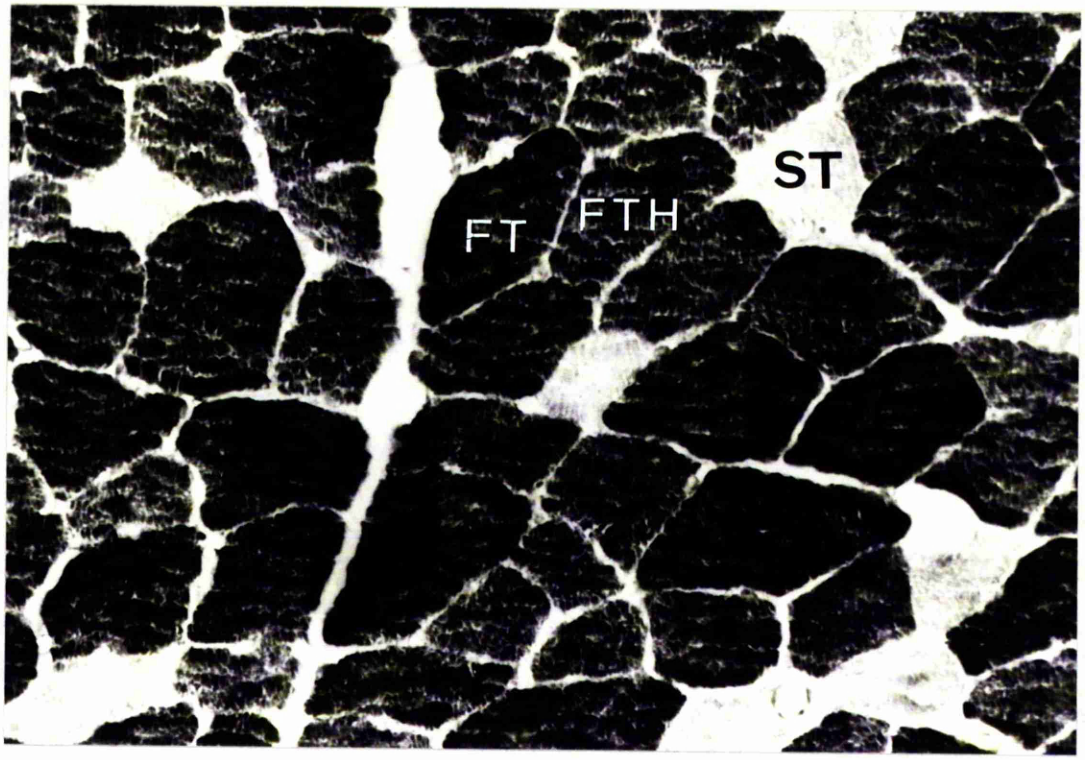

ST
FTH
FT

Fig 4.5: Serial section of horse gluteus medius muscle stained for (a) myosin ATPase (pre-incubation pH 10.2) and (b) SDH. FTH fibres staining intermediate. X 110.


ST
FT
FTH

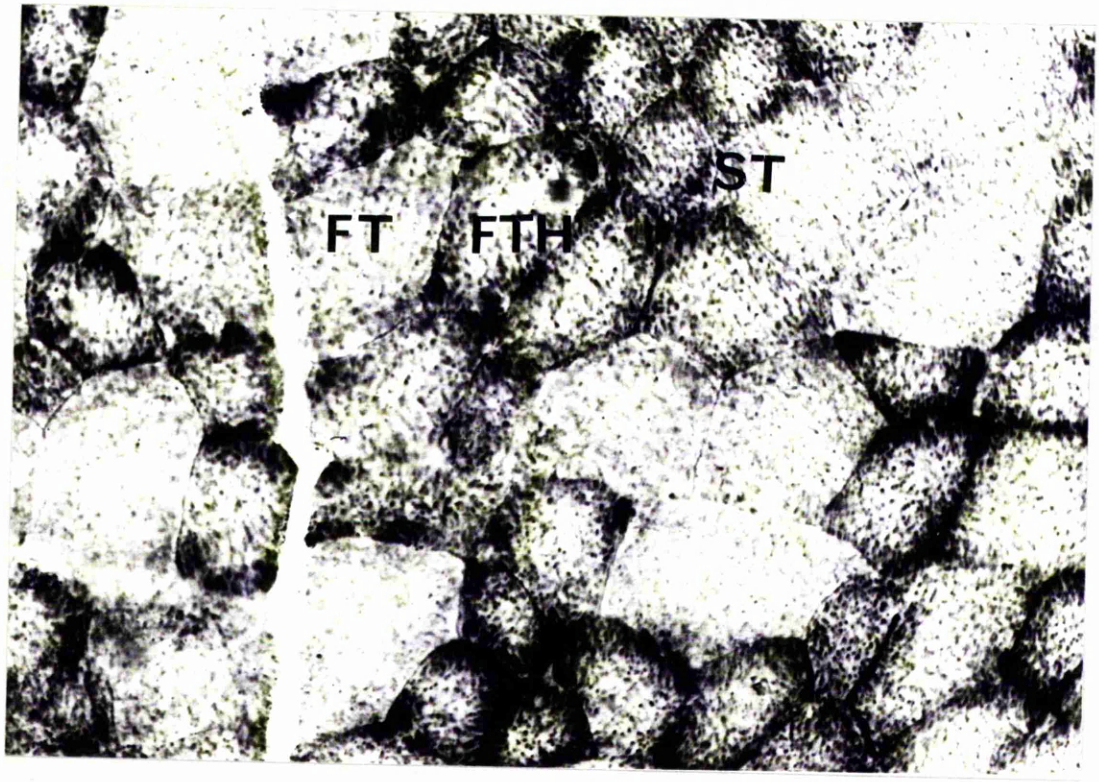
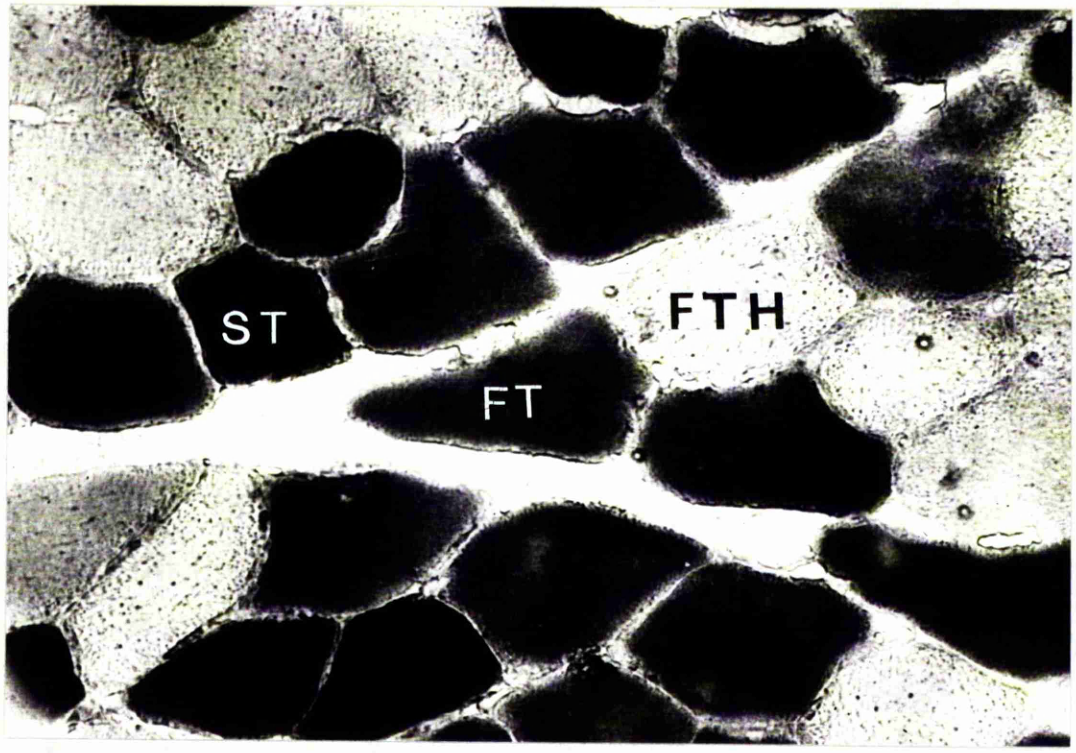
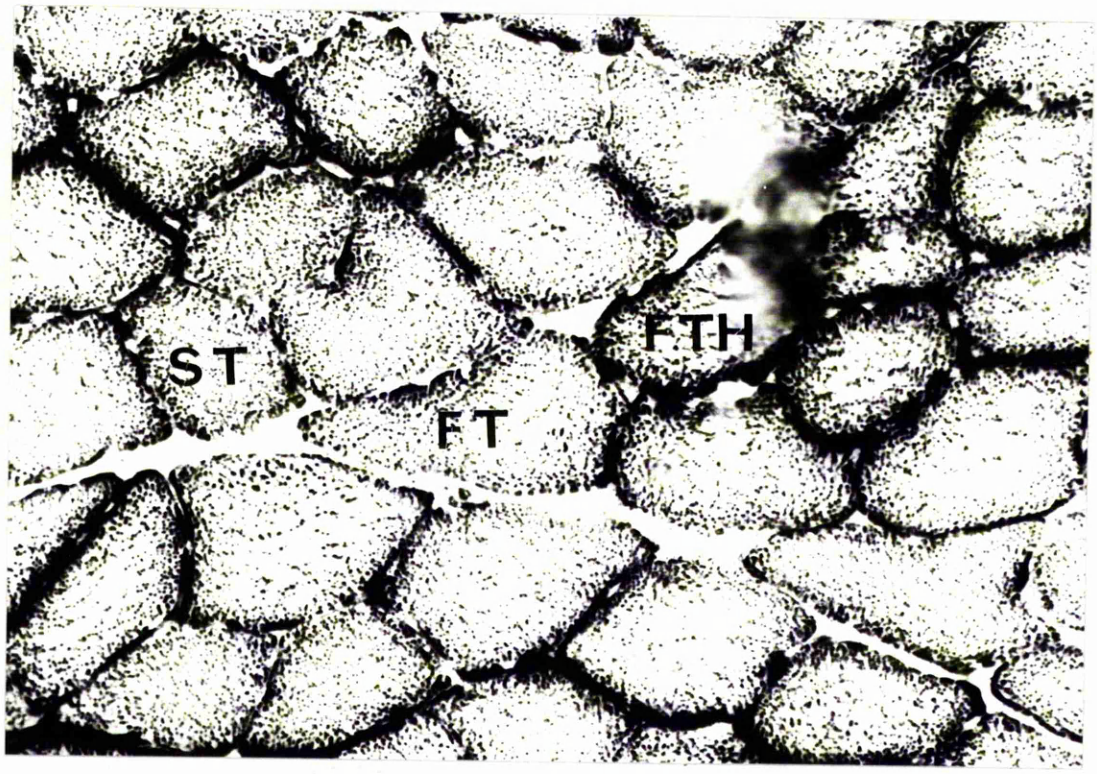

ST
FT
FTH

Fig 4.6: Serial sections of horse gluteus medius muscle stained for (a) myosin ATPase (pre-incubation pH 4.3), (b) SDH. Serial section of Fig 4.4. FTH fibres stain lightest. X 250.


ST
FTH
FT

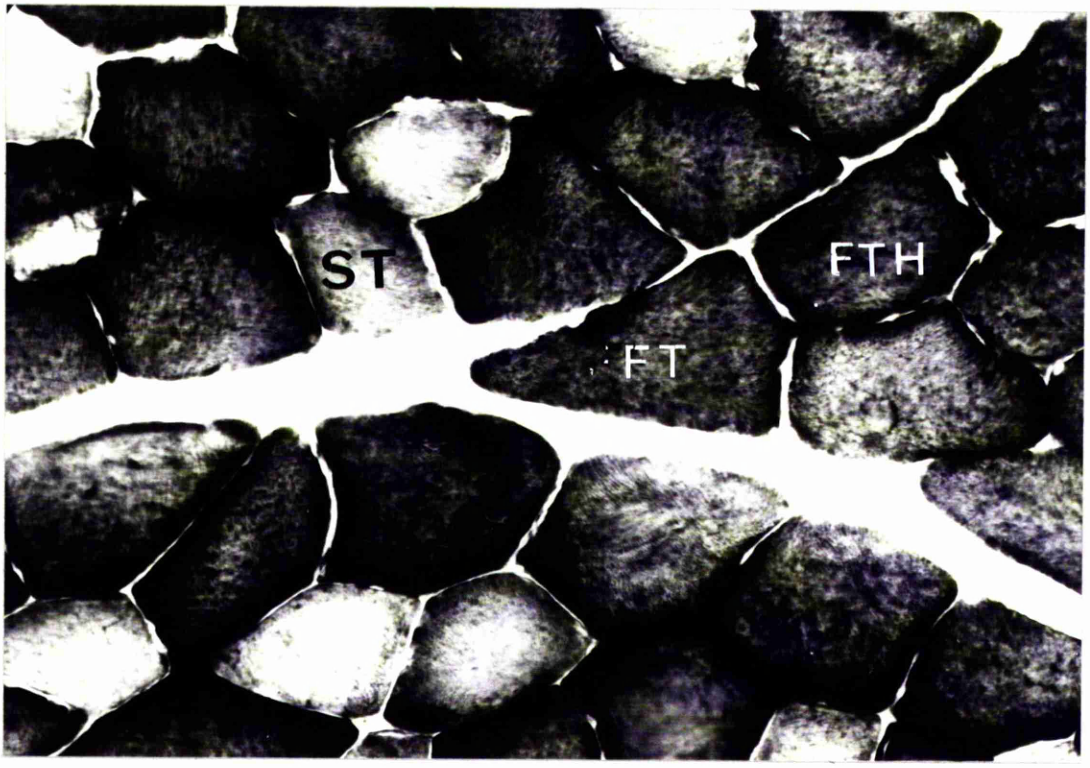

ST
FTH
FT

a dark and intermediate staining fibre. The intensity of the low activity fibre at this pH remained unchanged. The intermediate fibre corresponded to the FTH fibre with the dark and light fibres being respectively the FT and ST fibres. At pH 4.3, three fibre types were again distinguished and again there were dark, intermediate and light staining fibres. The darkest staining fibre was now the ST fibre whilst the FT fibre stained up intermediately. The FTH fibre did not exhibit much activity and stained up very lightly (Fig 4.6).

Phosphorylase a: The staining intensity of phosphorylase in the fibres varied from an intense blue through to a pale pink depending on the glycolytic capacity. The darker the stain, the greater the capacity. The dark fibres corresponded with the high myosin ATPase activity at pH 9.4 fibres, ie the fast twitch fibres (Fig 4.7).

Dog: Differentiation of the fibre types in the dog was more difficult than in the horse. Although two populations of fibres could be distinguished by the myosin ATPase activity at pH 9.4, it proved impossible to further sub-divide the fibres by using pre-incubations at other pH's. Using serial sections stained for SDH and myosin ATPase activity three basic fibre types could be distinguished in all of the greyhounds and most of the mongrel dogs (Figs 4.8; 4.9) but because of the distribution of the diformazan deposits, separation was difficult. Some of the mongrels had an unusual distribution of SDH in their muscle fibres with the low myosin ATPase fibres having a much lower staining intensity than the high myosin ATPase fibres. None of these latter fibres had a lower oxidative potential than the low myosin ATPase activity fibres (Fig 4.10).

Fig 4.7: Serial sections of horse gluteus medius muscle stained for (a) phosphorylase (b) myosin ATPase (pH 9.4). Serial section of Fig 4.4. High phosphorylase activity and high myosin ATPase activity correspond. X 250.


ST
FTH
FT

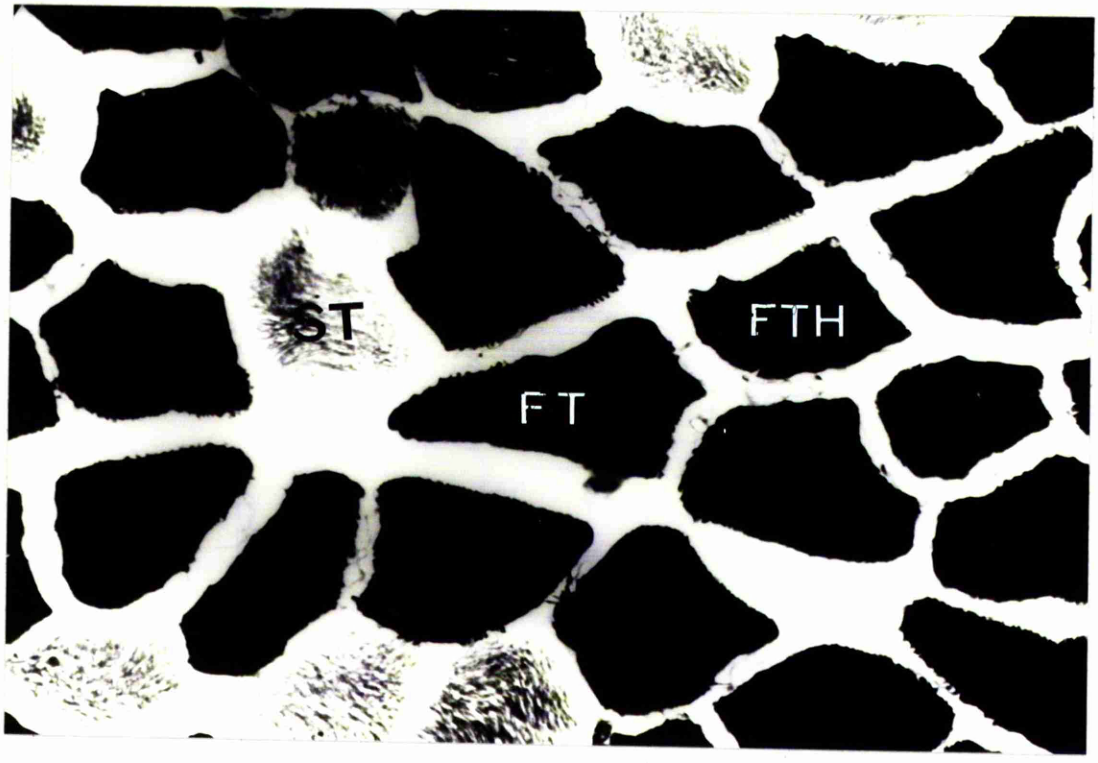

ST
FTH
FT

Fig 4.8: Greyhound biceps femoris muscle stained for (a) myosin ATPase (pH 9.4) and (b) SDH. Three main fibre types are evident, FTH, FT and ST. X 250.

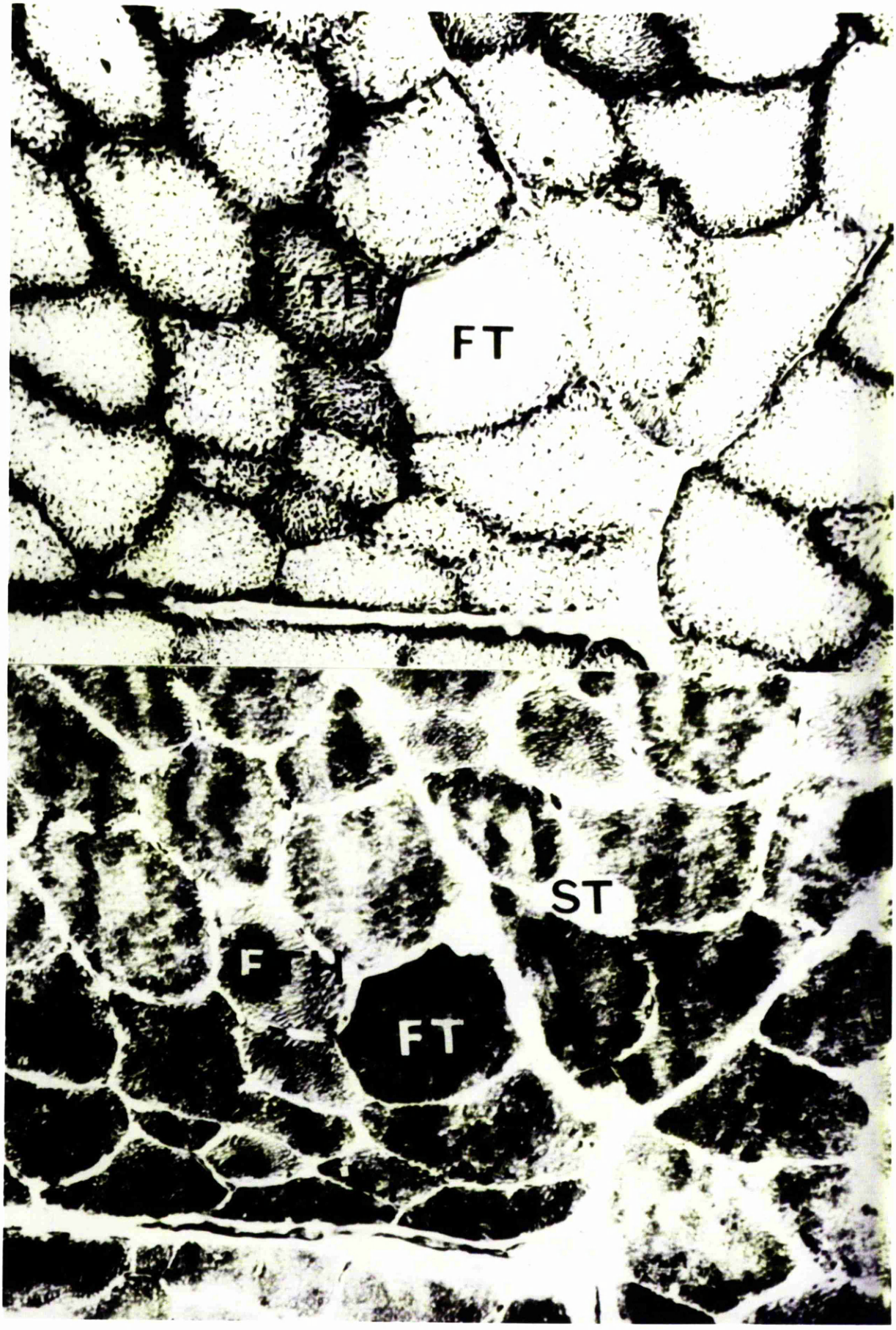

FT
ST
F
FT

Fig 4.9: Mongrel gluteus medius muscle stained for (a) myosin ATPase (pH 9.4) and (b) SDH. Three fibre types evident, FTH, FT and ST.
X 110.

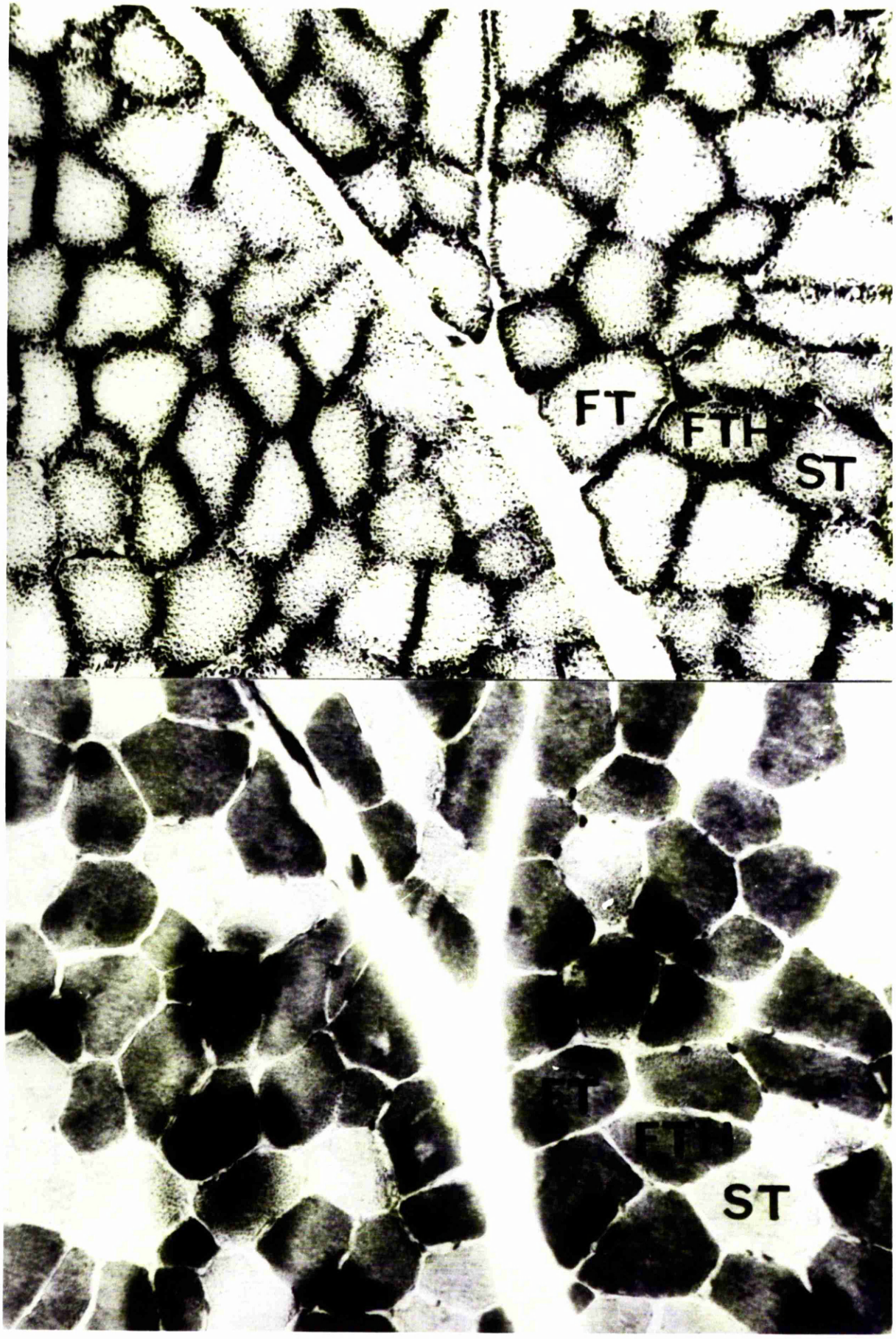
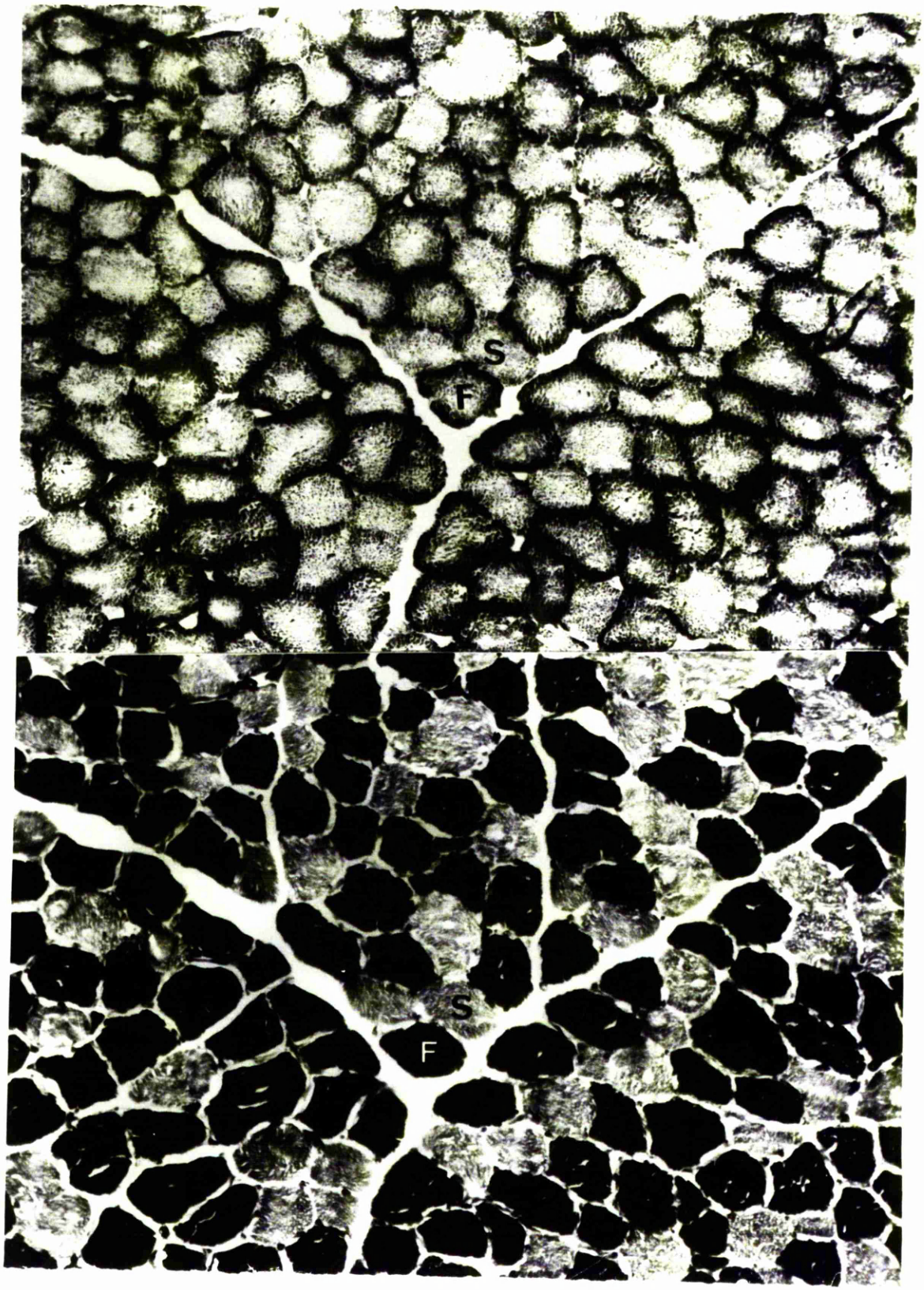

FT
FTH
ST
FT
ST

Fig 4.10: Mongrel gluteus medius muscle stained for (a) myosin ATPase (pH 9.4) and (b) SDH. Only two fibre types observed,

F -- high myosin ATPase (pH 9.4), high oxidative, and

S -- low myosin ATPase (pH 9.4), low oxidative.

X 110.


S
F
S
F

As with the horse, the Sudan Black B stain corresponded to the SDH stain activity and the phosphorylase activity was highest in the fast twitch fibres.

Fig 4.11: Histogram of the percentage fibre types (mean $\pm$ S.E.M.) in the deltoideus, long head of the triceps brachii and vastus lateralis in several breeds of horse.
Key. Q = Quarterhorse, T = Thoroughbred, A = Arab, S = Shetland pony, P = pony, D = donkey and H = heavy hunter.

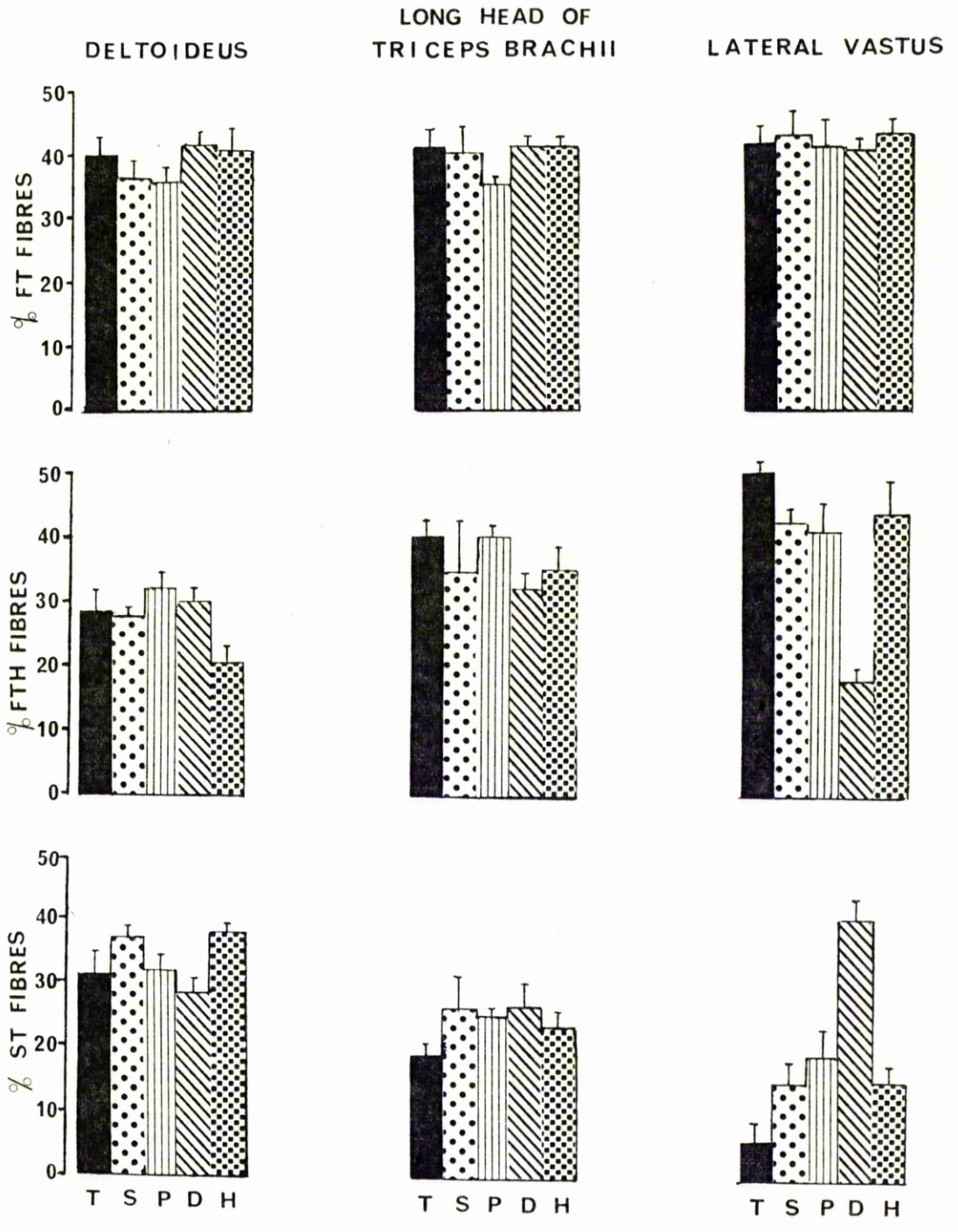
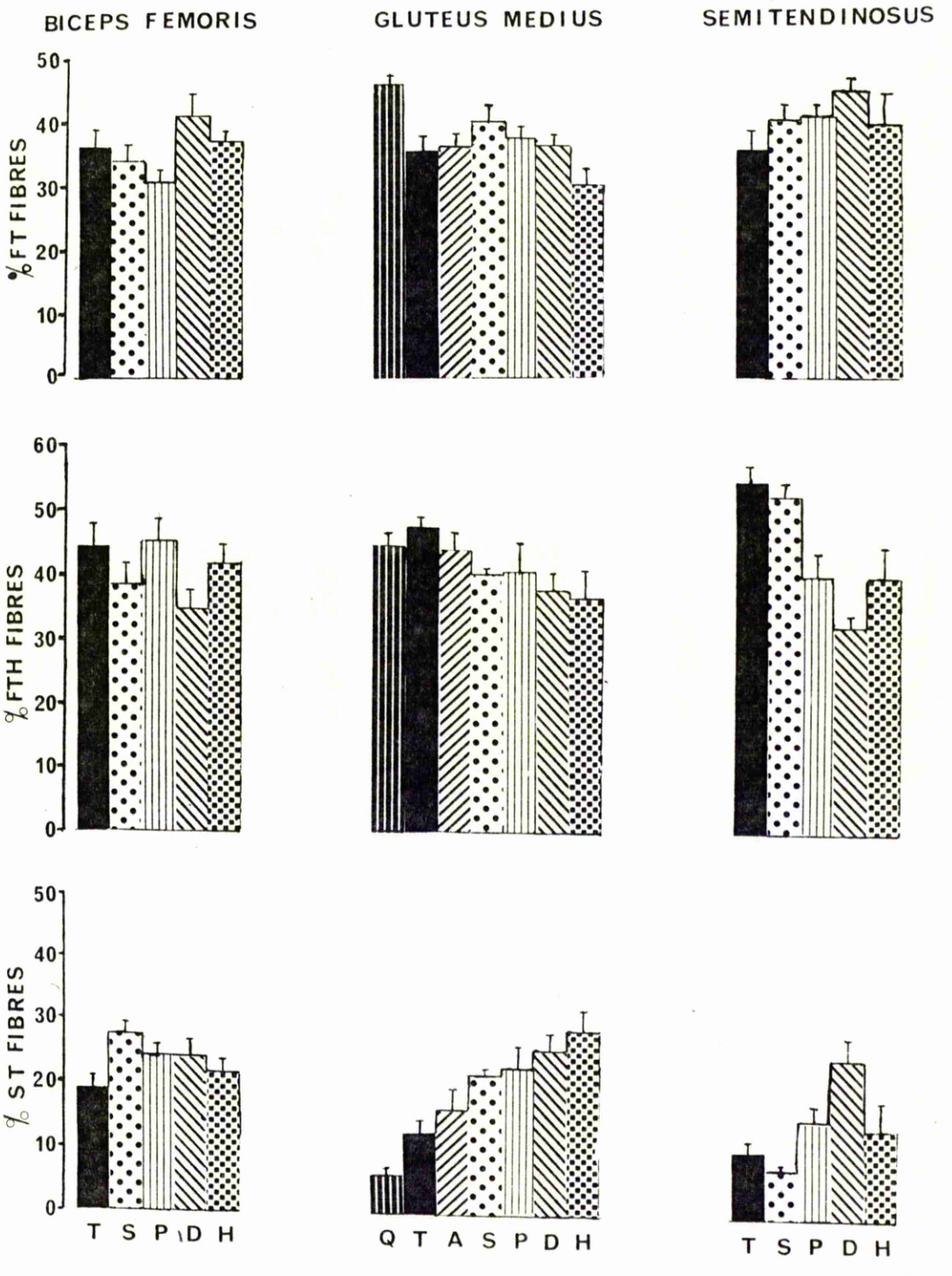

DELTOIDEUS
LONG HEAD OF TRICEPS BRACHII
LATERAL VASTUS
% FT FIBRES
% FTH FIBRES
% ST FIBRES
0
10
20
30
40
50
T S P D H

Fig 4.12: Histogram of the percentage fibre types (mean $\pm$ S.E.M.) in the biceps femoris, gluteus medius and semitendinosus in several breeds of horse. For explanation see Fig 4.11.


BICEPS FEMORIS
GLUTEUS MEDIUS
SEMITENDINOSUS
% FT FIBRES
% FTH FIBRES
% ST FIBRES
0
10
20
30
40
50
60
T S P D H
Q T A S P D H
T S P D H

Fibre Type Distribution in the Muscles of the Various Breeds

Horse: The results of the percentage fibre types for the various breeds of horse are shown in Figs 4.11 and 4.12. The percentage of high oxidative fibres was very constant between the various muscles and between the various breeds. The one exception found was the gluteus medius muscle of the Quarterhorse which had a significantly greater percentage of low oxidative fibres than the other breeds.

The muscle with the highest percentage of slow twitch fibres in all breeds, except the donkey, was the deltoideus. In the donkey, the vastus lateralis was the muscle with the highest percentage of slow twitch fibres. In most breeds the muscle with the next highest percentage of slow twitch fibres was the long head of the triceps brachii. The two exceptions to this were the heavy hunter where the gluteus medius came next and the donkey where it was the deltoideus. The muscles with the lowest percentage of slow twitch fibres were for all breeds, except the donkey, the semitendinosus and vastus lateralis. The other two muscles examined, the gluteus medius and biceps femoris usually had intermediate values between the two extremes.

The muscle for which the greatest number of different breeds were obtained was the gluteus medius. The breed with the smallest percentage of slow twitch fibres in this muscle was the Quarterhorse (6.6% ST). This was a significantly lower amount than all other breeds. The Thoroughbred was next with 12.3% of the fibres slow twitch and this was significantly lower than the remaining breeds. The order of these breeds were in increasing magnitude of percentage slow twitch fibres,

Arab < Shetland pony < pony < donkey < heavy hunter.

The Arab was significantly lower than the donkey and heavy hunter and this latter breed was significantly higher in its percentage of slow twitch fibres than all breeds except the donkey. This pattern was not repeated in the other muscles, although generally, the Thoroughbred had the lowest percentage of slow twitch fibres. In most cases, no correlation was found between the percentage of slow twitch fibres in these muscles (Table 4.3).

Dog: Due to the difficulties in classifying the fibres on the basis of their oxidative stain, only the percentage of high and low myosin ATPase activity at pH 9.4 fibres will be considered. The percentage fast twitch fibres in the various muscles of the dog are in Table 4.4. The greyhound was significantly higher in the percentage of fast twitch fibres in all of the muscles examined, when compared to the foxhound and cross-bred mongrel. The foxhound was significantly lower in the percentage of fast twitch fibres in the deltoideus, long head of the triceps brachii and the semitendinosus when compared to the cross-bred mongrel. In the greyhound, the percentage of fast twitch fibres was almost 100% in most muscles.

	Deltoideus	Long head of Triceps Brachii	Vastus Lateralis	Gluteus Medius	Biceps Femoris	Semitendinosus
Deltoideus	1.0000 (0) S=0.001	0.0949 (31) S=0.306	-0.2546 (29) S=0.091	0.0411 (30) S=0.415	-0.0276 (31) S=0.441	-0.3192 (31) S=0.040
Long head of Triceps Brachii		1.0000 (0) S=0.001	0.3220 (29) S=0.044	0.1886 (30) S=0.159	0.2624 (31) S=0.077	-0.1442 (31) S=0.219
Vastus Lateralis			1.0000 (0) S=0.001	0.2145 (29) S=0.132	0.2013 (30) S=0.143	0.5104 (29) S=0.002
Gluteus Medius				1.0000 (0) S=0.001	0.2071 (31) S=0.132	0.2748 (31) S=0.067
Biceps Femoris					1.0000 (0) S=0.001	0.2278 (31) S=0.109
Semitendinosus						1.0000 (0) S=0.001

(Coefficient /(no. of animals)/ significance)

Table 4.3: Correlation coefficients between the various muscles for the % ST fibres.

	Greyhound	Cross-Bred Mongrel	Foxhound
Deltoideus	99.8±0.2	74.4±3.0*	56.6±6.8*ϕ
Long Head of the Triceps Brachii	94.2±5.4	77.2±2.7*	64.9±4.4*ϕ
Lateral Vastus	96.6±1.7	61.4±10.2*	80.7±8.2*
Middle Gluteal	97.4±0.7	68.6±5.6*	65.3±4.9*
Biceps Femoris	88.6±2.2	67.2±1.3*	63.0±1.5*
Semitendinosus	98.9±0.8	85.3±2.6*	69.6±6.8*ϕ
N	6	5	4

*P < 0.05 when Mongrel and Foxhound compared to Greyhound

ϕP < 0.05 when Mongrel compared to Foxhound

Table 4.4: The percentage of skeletal muscle high myosin ATPase activity fibres in various muscles of the Greyhound, Foxhound and Cross-Bred Mongrel (Mean $\pm$ S.E.M.).

Discussion (1)

Histochemistry

Fibre Types

Horse: As with most other species, horse skeletal muscle was found to consist of three distinct fibre types, distinguished by their contractile and metabolic properties and designated ST, FTH and FT as previously described. The results from this study are in agreement with those of Shubber (1971/72), Lindholm and Piehl (1974) and Aberle et al. (1976) but at variance with those of Gunn (1975). This latter author described only two fibre types, one having high activity of myosin ATPase at pH 9.4, succinate dehydrogenase and glycogen phosphorylase and the other having a low activity of myosin ATPase at pH 9.4 but high activities of succinate dehydrogenase and phosphorylase. Why Gunn's study is different from the present study and other reports is not known, but as histochemical methods are dependent to a great extent on the opinion of the investigator it is understandable why this could occur. His definition of high and low may be different from that in the present study and that of other workers.

Pre-incubation of the sections after fixation in buffered formaldehyde (pH 7.0) at pH 10.2 produced two fast contractile fibres and one slow contractile fibre. This was a similar situation to that found by Aberle et al. (1976) who observed, using only the fixation in buffered formaldehyde (pH 7.0), that in the semitendinosus of the pony three distinct fibre types were produced. These corresponded to the fibre types found in the present study. Man and rat also exhibit this staining pattern with

the high oxidative fast twitch fibre having an intermediate staining intensity with pre-incubation at pH 10.2 (Guth and Samaha, 1969; Prince et al., 1976).

Fast twitch fibres can also be separated into two after pre-incubation at an acid pH of 4.35 in man, rat and rabbit (Brooke and Kaiser, 1970a). At this pH, a reversal of staining properties occurs, to that at alkaline pH, with the slow twitch, low myosin ATPase fibre at pH 10.2 staining darkly, the fast twitch, high oxidative fibre staining light and the fast twitch low oxidative fibre staining intermediate. This was similar to the results obtained in the horse in this study. A fourth type of fibre, usually designated as type IIC, which stains dark at all pH's down to pH 3.9 has been found in some muscles of various species but usually only constitutes a few percent of the total number of fibres. This fibre type was not found in the present study but its presence in the horse cannot be ruled out, as this fibre type, it is suggested, only occurs in growing muscles (Riley, 1973).

In the past, an oxidative stain, usually SDH or NADH diaphorase has been used to classify fibre composition in skeletal muscle (Padykula and Gauthier, 1966) and the classification used indicated the oxidative capacity of the fibre, either high, intermediate or low and designated by these authors as A, B or C. It has since been discovered, that this system is inaccurate when the oxidative and myosin ATPase properties of muscle fibres are examined over a large range of species. In small laboratory animals such as the rat and rabbit, the intermediate SDH fibre is the low myosin ATPase activity at pH 9.4 fibre (Brooke and

Kaiser, 1970a) whilst in larger species such as man (Brooke and Kaiser, 1970a), cat (Schmalbuch and Kamieniecka, 1975) and pig (Fitts et al., 1975) the intermediate SDH staining fibre is a high myosin ATPase activity at pH 9.4 fibre. The horse is similar to those larger species with the high myosin ATPase activity at pH 9.4 fibre usually staining intermediate. It is slightly more confusing in the horse, as the FTH fibre can have as high an oxidative stain as the slow twitch fibre. This difference may be size dependent although against this theory is the finding that in cattle, the staining pattern is similar to the rat (Ashmore and Doerr, 1971).

The Sudan black B stain for lipids has also been used as the basis of classifying the fibre composition of muscle fibres (Gauthier and Padykula, 1966). The fact that this stain follows the staining pattern for SDH is to be expected as lipid is utilised in the mitochondrion, the site of SDH activity and electron micrographs of horse skeletal muscle (Fig 2.4) indicated that lipid droplets are found in close association with the mitochondrion.

The various responses of the myosin ATPase activities to different pH's are due to a variable susceptibility to inhibition of the myosin molecules. Thus, the slow contracting fibres are inhibited at an alkaline pH of 10.2 whilst the fast contracting fibres are inhibited only slightly (FTH fibres) or not at all (FT fibres). Similarly, with an acid pH it is the FTH fibres which are most inhibited in their myosin ATPase activity. Brooke and Kaiser (1970b) using the sulphydryl blocking agent PHMB inhibited the activity of all fibres but restored the activity in the type I and type IIB fibres by following the PHMB incubation with cysteine

incubation for fifteen minutes. These authors suggest that the susceptibility of the myosin ATPase molecules to the various pH's are therefore dependent on the sensitivity of some of the amino acids in the myosin molecules to changes in pH. They also noted that the pK of cysteine is 10.3 although in the protein, this value will be altered to some extent.

High phosphorylase activity is correlated with high myosin ATPase activity at pH 9.4. This is because of the nature of these fibres which are required to contract rapidly utilising large amounts of glycogen (Karlsson and Komi, 1976). This is also the case in the horse although, again Gunn (1975) disagrees with this classification and he claims that in the horse all fibres have high phosphorylase activity.

Dog: Typically, only two fibre types are described in the dog, one type (slow twitch) being characterised by low myosin ATPase activity at pH 9.4, low phosphorylase activity and high SDH activity. The other type exhibits the opposite staining pattern (Cardinet et al., 1969; Gunn, 1975). In the present study, the staining for SDH activity was less clearly divided into high and low activities. A random examination of serial sections revealed that the three fibre types found in the horse were also present in the dog. The incidence of the FTH fibre was less than in the horse. The results were further confused by the findings with some of the dogs that the low myosin ATPase fibre at pH 9.4 had a much lower SDH intensity than the high myosin ATPase fibres at pH 9.4. It was therefore decided to separate dog muscle fibre types only on the basis of their myosin ATPase properties.

Unlike the horse, the skeletal muscle fibres of the dogs in

this study, could not be sub-divided into three types by the use of acid and alkali pH's. At pH 4.5 and less, only two types exhibiting reciprocal staining activities to that at pH 9.4 were found. The amino acid composition of the myosin molecule may be different in the dog thus preventing only partial inhibition of one of the fibres at a given pH.

Breed Muscle Composition

Horse: The skeletal muscles of the horse displayed some differences in their fibre composition with in general the front limb muscles (deltoideus and long head of the triceps brachii) being lower in their percentage of fast twitch fibres. A difference in fibre composition between muscles has previously been reported in other species (Ariano et al., 1973; Johnson et al., 1973). At one time, it was thought that muscles involved mainly in the maintenance of posture were composed of a high percentage of slow twitch, high oxidative fibres whilst muscles used during movement consisted mainly of fast twitch fibres. This does not now appear to be the case in view of the range of fibre composition found in any given muscle of the human (Johnson et al., 1973). The reason that the front limb muscles have a lower percentage of fast twitch fibres may be related to the style of running of the horse. Most of the power used during running is derived from the hind quarters whilst the front limbs are mainly used to absorb the shock of landing*.

Low variability has been reported between different muscles of the same subject (Johnson et al., 1973) and a correlation has been found between different muscles of the same subject (Komi et al., 1977b). This correlation between muscles was not generally found in the present study (Table 4.3).

The muscle for which the greatest amount of data was available was the gluteus medius. This was due to several reasons, including the fact that it was relatively easy to obtain biopsies from this muscle, there

*"The Thoroughbred in Action" filmed by the New York Racing Association

were no large blood vessels to cause much bleeding and the previous work of Lindholm and coworkers (1973, 1974a,b) was performed on this muscle. It was of interest that in this muscle, the breed with the highest percentage of fast twitch fibres was the Quarterhorse. This breed has been specifically bred over the years for extremely fast sprinting over a very short distance, after which it is exhausted. The next highest breed, the Thoroughbred, was developed for fast sprinting over longer distances than the Quarterhorse. This difference in the type of running for which these two breeds have been bred is further highlighted when it is considered that the Quarterhorse has a much greater percentage of low oxidative FT fibres than the Thoroughbred, and because of the short distances which it is required to run, the Quarterhorse has less need for high oxidative fibres. The Arab, a breed capable of great endurance, had the next highest percentage of fast twitch fibres followed by the ponies, hunters and donkeys which are not as fast as these three previous breeds. In general, the donkey had the lowest percentage of fast twitch fibres although it must be remembered that the donkey is not actually a breed of horse but a member of the equine species. A similar effect has been reported by Gunn (1975) who examined the transverse pectoral muscle of the Thoroughbred and found that the percentage of fast twitch fibres was significantly higher ($P < 0.01$) than other breeds.

As previously mentioned, high myosin ATPase activity at pH 9.4 fibres is generally assumed to be fast contracting and this is based on the work of Barany (1967) who found that the activity of myosin ATPase is directly proportional to the intrinsic speed of sarcomere shortening in normal muscle. Peter et al. (1972) have reported that the contraction

time of a muscle is inversely proportional to the percentage of high myosin ATPase activity at pH 9.4 fibres. This implies that the greater the proportion of fast twitch fibres, the greater the frequency of contraction and the potential for the animal to run fast. Other factors are also indicated which may influence the speed of running of an animal and these are summarized in Fig 4.13 after Gunn (1975). As can be seen, the force produced by the muscle during acceleration is also important and again a high percentage of fast contractile fibres would be an advantage. It has been reported in human studies that not only does the maximal velocity increase with an increasing percentage of high myosin ATPase fibres but also that the peak torque (force) produced at high velocities is related to the proportion of high myosin ATPase fibres (Thorstensson et al., 1976b).

The relationship between speed and a high percentage of fast twitch fibres is also found in humans. In general, athletes that are good in power events such as sprinting and weight-lifting have a higher proportion of fast twitch fibres than other athletic types or sedentary control subjects. The average percentage of fast twitch fibres in the gastrocnemius or vastus lateralis in man is about 54% (Komi et al., 1977a) whereas elite sprinters examined by Gollnick et al. (1972) and Costill et al. (1976a) possessed a much higher percentage of those fibres, ranging from 73% to 79%. These values are at the lower end of the range in the horse but have been suggested to be near optimum in man (Thorstensson et al., 1977). This is because in man, marked fatiguability occurs in muscles with a large percentage of those fibres within a few maximal contractions (Thorstensson and Karlsson, 1976). The picture is probably different in the horse,

SPEED OF RUNNING

CO-ORDINATED GAIT

STRIDE LENGTH	X	STRIDE FREQUENCY
LENGTH OF LIMBS		NATURAL FREQUENCY OF LIMBS
RANGE OF MOVEMENT OF JOINTS		MECHANICAL ADVANTAGE OF MUSCLES
ACCELERATION CAPACITY (Force produced by muscle relative to body weight)		SPEED OF SARCOMERE CONTRACTION
INTERNAL MUSCLE ARCHITECTURE		REPETITIVITY OF LIMB MOVEMENTS (Anaerobic and aerobic energy supply mechanisms)

Fig 4.13: Factors influencing running speed in animals (Gunn, 1975).

however, due to the large percentage of FTH fibres. These fibres will enable not only fast contractions but allow those contractions to be continued for a relatively longer period of time when compared to man. In this respect, it is interesting that the Quarterhorse, the breed which is most easily fatigued, had the highest percentage of low oxidative FT fibres.

In man, it has been found that a prerequisite for good endurance running ability was a high percentage of low myosin ATPase activity at pH 9.4 fibres (Gollnick et al., 1972). This has been confirmed from work in several studies sampling both good (Edstrom and Ekblom, 1972) and elite endurance event athletes (Costill et al., 1976a,b). Some of the athletes examined in these latter studies had a value for their percentage slow twitch fibres of as high as 98%. Little difference was found when the percentage of slow twitch fibres or the percentage area occupied by these fibres was considered. A large number of slow twitch, high oxidative fibres would be thought to be advantageous during distance running as these fibres produce most of the tension required during this type of exercise (Burke and Edgerton, 1975). These fibres are also extremely resistant to fatigue (Burke et al., 1973). Costill et al. (1976b) concluded, however, that although a high percentage of low myosin ATPase activity at pH 9.4 fibres distinguished elite long distance runners from middle distance runners, within the group of elite distance runners, this parameter was of limited value in guaging the success an athlete would enjoy. Other factors would have to be taken into account.

In the present study, most of the muscles examined had a high percentage of high myosin ATPase activity at pH 9.4 fibres with only

the occasional muscle having values below 50%. The horse as a species is noted for endurance and strength, some being able to travel 160 kilometres in a day (Carlsson , 1975). This poses the question, do these horses have a high percentage of low myosin ATPase activity at pH 9.4 fibres, like human endurance athletes or are their fibres different from those in human subjects. As previously stated, the horse possesses a high percentage of FTH fibres as well as some ST fibres and both of these types are resistant to fatigue. Well trained humans also have FTH fibres in their muscles to the exclusion of FT fibres (Jansson, 1975) and so the difference may be in the relative efficiency of utilising fuels and preventing fatigue in the FTH fibres in the horse compared to man.

The results with the greyhound compared to the foxhound and mongrel also corroborate the idea of fibre type composition determining to some extent the ability of an animal to run fast. The greyhound excels at short bursts of sprinting and so it is not surprising that it has almost 100% fast twitch fibres in all of the muscles examined. It is also of interest that the front limb muscles have as high a percentage of these fibres as the hind limbs. This may be an indication of the different style of running in the dog where propulsion is obtained from muscles acting on the spinal column and hip as compared to the horse where propulsion is derived mainly from hind limb muscles (Gunn, 1975).

Results (2)

Biochemistry

Horse

The mean activities of the enzymes assayed and glycogen content for the various breeds of horse in the gluteus medius and semitendinosus muscles are shown in Table 4.5 and 4.6. The relative magnitudes between the various breeds are shown in Table 4.7.

LDH: In the gluteus medius, the Quarterhorse had a significantly higher LDH activity than all the other breeds, being 50% greater than the Arabs and over 100% greater than the rest. The donkey had the lowest activity in both muscles examined.

CPK: The activity of CPK was similar in all breeds except the donkey which was significantly lower in activity.

ALD: The pattern of activity for ALD was similar to that of LDH in both muscles.

AST: The breed with the highest AST activity in the gluteus medius was the Arab which was significantly higher than the Quarterhorse, pony and donkey. As with the three previous enzymes, the donkey had the lowest activity. A similar pattern existed in the semitendinosus.

ALT: The activity of ALT was similar in both muscles for all the breeds. The donkey was, however, half the activity of all the other breeds.

CS: In the gluteus medius, the donkey had the lowest activity of CS.

	Heavy Hunter (9)	Pony (8)	Thoroughbred (8)	Arab (5)	Quarterhorse (8)	Donkey (4)
LDH	2885 ± 366^{ab}	2947 ± 575^{ab}	2613 ± 458^{ab}	4106 ± 555^{ab}	6213 ± 526^{a}	1548 ± 255
CPK*	16930 ± 984^{a}	17578 ± 838^{a}	20220 ± 1984^{a}	19912 ± 1352^{a}	19940 ± 1071^{a}	10346 ± 1237
ALD*	391 ± 18^{abc}	402 ± 45^{ab}	348 ± 39^{abc}	470 ± 36^{ab}	632 ± 37^{a}	203 ± 25
AST*	553 ± 55^{a}	448 ± 70^{c}	618 ± 55^{a}	810 ± 160^{b}	486 ± 40	340 ± 60
ALT*	49.3 ± 81^{a}	41.1 ± 8.1^{a}	44.8 ± 7.1^{a}	55.1 ± 6.2^{a}	53.7 ± 5.4^{a}	17.5 ± 2.6
CS*	12.7 ± 1.3^{ab}	10.7 ± 0.8^{abd}	15.1 ± 1.2^{ab}	15.1 ± 2.4^{ab}	8.2 ± 0.8^{a}	2.8 ± 0.3
HK*	7.6 ± 0.9^{d}	7.0 ± 0.2^{ad}	11.1 ± 1.3^{a}	7.6 ± 0.5	8.7 ± 0.4^{a}	5.9 ± 0.6
HAD*	187 ± 17^{b}	160 ± 32	159 ± 20^{b}	172 ± 21^{b}	112 ± 8	148 ± 32
GDH*	157 ± 17^{d}	149 ± 34	142 ± 19^{a}	156 ± 23^{a}	181 ± 20^{a}	53 ± 6
Glycogen ‡	321 ± 18^{a}	300 ± 15^{a}	329 ± 12^{ab}	264 ± 40^{a}	252 ± 49^{a}	234 ± 6

a = $P < 0.05$ compared to donkey; b = $P < 0.05$ compared to Quarterhorse; c = $P < 0.05$ compared to Arab;

d = $P < 0.05$ compared to Thoroughbred

*µmoles. $(\text{min.gm dry weight of tissue})^{-1}$ ‡ µmoles . $(\text{gm dry weight of tissue})^{-1}$

Table 4.5: Enzyme activities and glycogen concentration in the gluteus medius of various breeds of horse. Number in brackets is number of subjects.

	Heavy Hunter (9)	Pony (7)	Thoroughbred (7)	Donkey (5)
LDH*	2749 ± 358[a]	3075 ± 470[a]	2290 ± 257[a]	1367 ± 166
CPK*	16976 ± 1100[a]	18562 ± 534[a]	16573 ± 1157[a]	9974 ± 840
ALD*	418 ± 37[a]	387 ± 39[a]	349 ± 53[a]	172 ± 25
AST*	529 ± 78[a]	465 ± 58[a]	595 ± 83[a]	272 ± 31
ALT*	39 ± 5[a]	42 ± 6[a]	50 ± 8[a]	22 ± 2
CS*	11.4 ± 1.8[a]	10.0 ± 0.7[a]	13.4 ± 1.9[a]	2.2 ± 0.3
HK*	10.0 ± 1.0[ac]	6.8 ± 0.5[b]	11.0 ± 1.0[a]	6.8 ± 1.0
HAD*	143 ± 27	126 ± 15	122 ± 17	91 ± 15
GDH*	130 ± 13[a]	164 ± 23[a]	122 ± 25[a]	38 ± 5
Glycogen ‡	298 ± 30[a]	312 ± 28[a]	340 ± 18[a]	155 ± 15

a = $P < 0.05$ compared to donkey; b = $P < 0.05$ compared to pony.

* μmoles.(min.gm dry weight of tissue)$^{-1}$ ‡ μmoles.(gm dry weight of tissue)$^{-1}$

Table 4.6: Enzyme activities and glycogen concentration in the semitendinosus of various breeds of horse. Number in brackets is number of subjects.

Enzyme	
CPK	Arab > Thoroughbred > Quarterhorse > pony > heavy hunter > donkey
LDH	Quarterhorse > Arab > pony > heavy hunter > Thoroughbred > donkey
ALD	Quarterhorse > Arab > pony > heavy hunter > Thoroughbred > donkey
AST	Arab > Thoroughbred > heavy hunter > Quarterhorse > pony > donkey
CS	Arab > Thoroughbred > heavy hunter > Quarterhorse > pony > donkey
ALT	Arab > Quarterhorse > heavy hunter > Thoroughbred > pony > donkey
HK	Thoroughbred > Quarterhorse > Arab = heavy hunter > pony > donkey
HAD	heavy hunter > Arab > pony > Thoroughbred > donkey > Quarterhorse
GDH	Quarterhorse > heavy hunter > Arab > pony > Thoroughbred > donkey

Table 4.7: The relative mganitudes of the various enzymes in gluteus medius muscle of six breeds of horse.

This was followed by the Quarterhorse and both of these breeds were significantly lower than the rest. The Thoroughbred and Arab had the highest activity, but there was no significant difference between these breeds and the heavy hunter. The Thoroughbred was significantly higher than the pony. A similar pattern existed in the semitendinosus.

HK: The breed with largest HK activity was the Thoroughbred being significantly higher than the Arab, pony, heavy hunter and donkey in the gluteus medius and higher than the pony in the semitendinosus. In both muscles, the donkey had the lowest HK activity.

HAD: The activity of HAD in the gluteus medius was similar in all of the breeds except the Quarterhorse. The activity in this breed was significantly lower than the heavy hunter, Arab and Thoroughbred. The breed with the highest HAD levels in both muscles was the heavy hunter but the difference was not significant except when compared to the Quarterhorse.

GDH: The donkey had the lowest GDH activity in both muscles, being significantly different from all horse breeds except the gluteus medius of the pony. The activity in the other breeds were similar.

Glycogen: Only the Quarterhorse and donkey were significantly lower in glycogen concentration.

Enzyme Ratios

Table 4.8 displays several important enzyme ratios in the gluteus medius of the various breeds of horse. In general, the heavy hunter, pony, Thoroughbred and Arab had similar values for a given ratio

	Heavy Hunter (9)	Pony (8)	Thoroughbred (8)	Arab (5)	Quarterhorse (8)	Donkey (4)
$\frac{LDH}{ALD}$	7.4 ± 0.9^{b}	7.4 ± 1.1	7.8 ± 0.9^{b}	8.6 ± 0.8	9.8 ± 0.5^{a}	7.4 ± 0.4
$\frac{LDH \times 10^{-2}}{CS}$	2.5 ± 0.5^{ab}	2.8 ± 0.6^{ab}	1.8 ± 0.3^{ab}	3.0 ± 0.6^{ab}	7.9 ± 0.8	5.7 ± 1.0
$\frac{LDH \times 10^{-1}}{HAD}$	1.7 ± 0.3^{b}	2.2 ± 0.4^{b}	1.7 ± 0.3^{b}	2.8 ± 0.4^{ab}	5.8 ± 0.7^{a}	1.2 ± 0.3
$\frac{ALD \times 10^{-1}}{CS}$	3.5 ± 0.5^{ab}	3.8 ± 0.4^{abd}	2.4 ± 0.3^{ab}	3.4 ± 0.4^{ab}	8.2 ± 0.8	7.4 ± 1.0
$\frac{ALD}{HAD}$	2.3 ± 0.2^{b}	3.3 ± 0.7^{b}	2.3 ± 0.4^{b}	2.9 ± 0.4^{ab}	6.0 ± 0.7^{a}	1.5 ± 0.3
$\frac{CS}{HAD}$	7.5 ± 0.6^{a}	8.4 ± 1.4^{a}	10.1 ± 1.4^{a}	9.2 ± 1.8^{a}	7.0 ± 0.6^{a}	2.1 ± 0.5

a = $P < 0.05$ compared to donkey; b = $P < 0.05$ compared to Quarterhorse; d = $P < 0.05$ compared to Thoroughbred.

Table 4.8: Several important enzyme ratios in the gluteus medius muscle of various breeds of horse. Number in brackets is number of subjects.

for all of the enzyme ratios examined. The Quarterhorse and donkey had significantly different values for several of the ratios.

Correlations Between the Various Enzymes

Pearson correlation coefficients between the various enzymes were determined with the aid of a computer (Table 4.9). A high correlation was found between the enzymes connected with the anaerobic production of energy, ie ALD, LDH and CPK. These enzymes were also highly correlated with ALT and GDH. Of the aerobic enzymes examined, a high correlation was found between CS and AST and HAD and AST.

Correlations Between Enzyme Activity and Muscle Fibre Composition

The scattergrams illustrating the enzymes that were significantly correlated with either the percentage ST fibres or the percentage FT fibres are shown in Figs 4.14 to 4.24. Although significant, the R value for the correlations was never very large. A negative relationship was found with the anaerobic energy producing enzymes LDH, ALD and CPK and the percentage of ST fibres. These fibres were also significantly correlated with ALT and GDH. A negative correlation was found between the "aerobic" enzymes CS and HK and the percentage of FT fibres.

	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
LDH	1.0000 (0) S=0.001	0.5468 (96) S=0.001	0.8118 (96) S=0.001	0.3882 (96) S=0.001	0.6138 (96) S=0.001	0.0632 (96) S=0.270	-0.0387 (66) S=0.379	0.1602 (65) S=0.101	0.7078 (64) S=0.001
CPK		1.0000 (0) S=0.001	0.6348 (96) S=0.001	0.5563 (96) S=0.001	0.5387 (96) S=0.001	0.4435 (96) S=0.001	0.0597 (66) S=0.317	0.2646 (65) S=0.017	0.6810 (64) S=0.001
ALD			1.0000 (0) S=0.001	0.3762 (96) S=0.001	0.4967 (96) S=0.001	0.1839 (96) S=0.036	0.0298 (66) S=0.406	0.0999 (65) S=0.214	0.6291 (64) S=0.001
AST				1.0000 (0) S=0.001	0.6133 (96) S=0.001	0.6397 (96) S=0.001	-0.0431 (66) S=0.366	0.5534 (65) S=0.001	0.6549 (64) S=0.001
ALT					1.0000 (0) S=0.001	0.3882 (96) S=0.001	-0.1294 (66) S=0.150	0.5702 (65) S=0.001	0.8016 (64) S=0.001
CS						1.0000 (0) S=0.001	0.2809 (66) S=0.011	0.4676 (65) S=0.001	0.4199 (64) S=0.001
HK							1.0000 (0) S=0.001	-0.2324 (65) S=0.031	-0.1378 (64) S=0.139
HAD								1.0000 (0) S=0.001	0.5703 (63) S=0.001
GDH									1.0000 (0) S=0.001

(Coefficient / (no. of animals) / significance)

Table 4.9: Correlation coefficients between the different enzymes in horse skeletal muscle

Fig 4.14: Scattergram of % ST fibres against LDH activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).

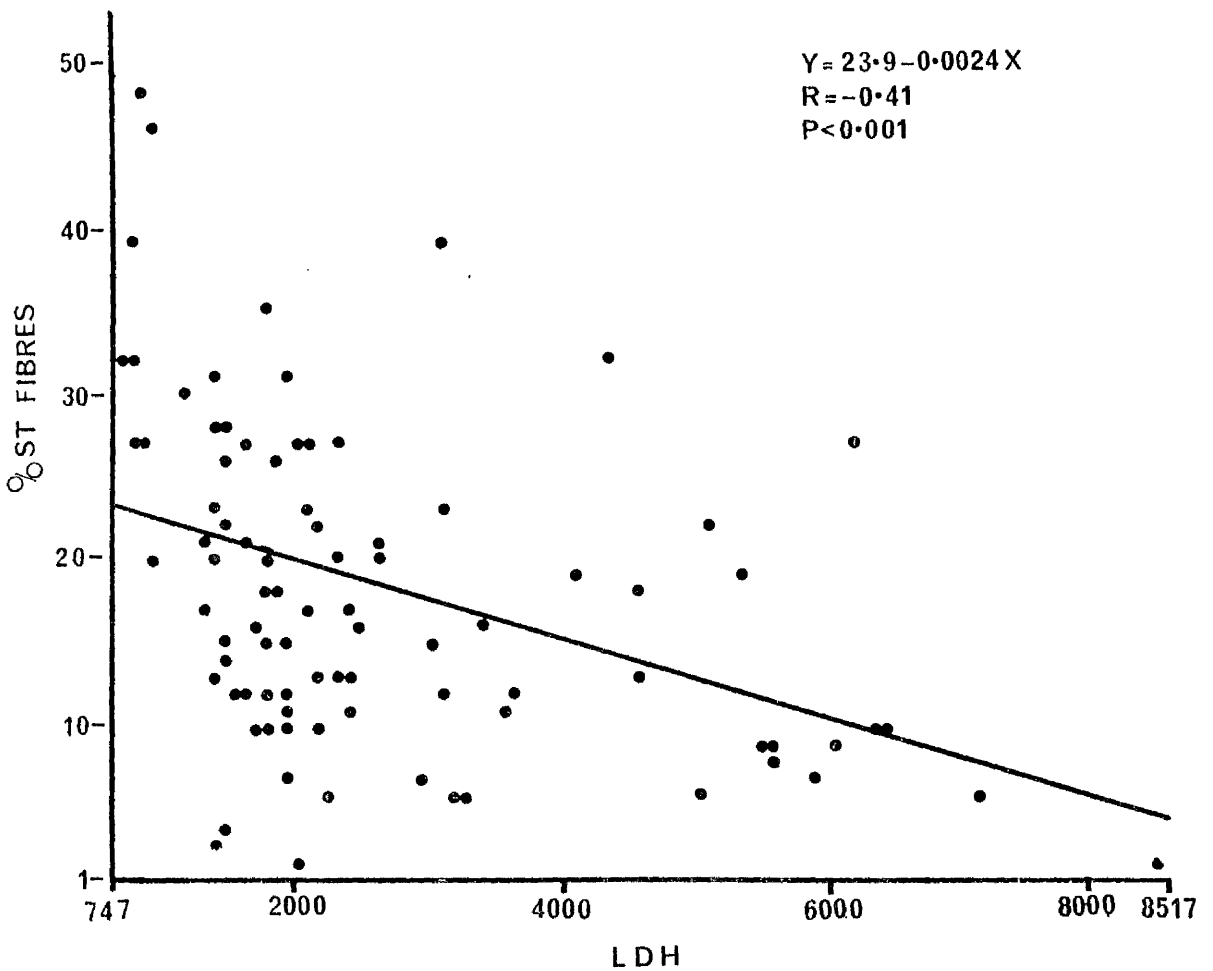

Y = 23·9 – 0·0024 X
R = –0·41
P < 0·001
% ST FIBRES
50
40
30
20
10
1
747
2000
4000
6000
8000
8517
LDH

Fig 4.15: Scattergram of % ST fibres against ALD activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).

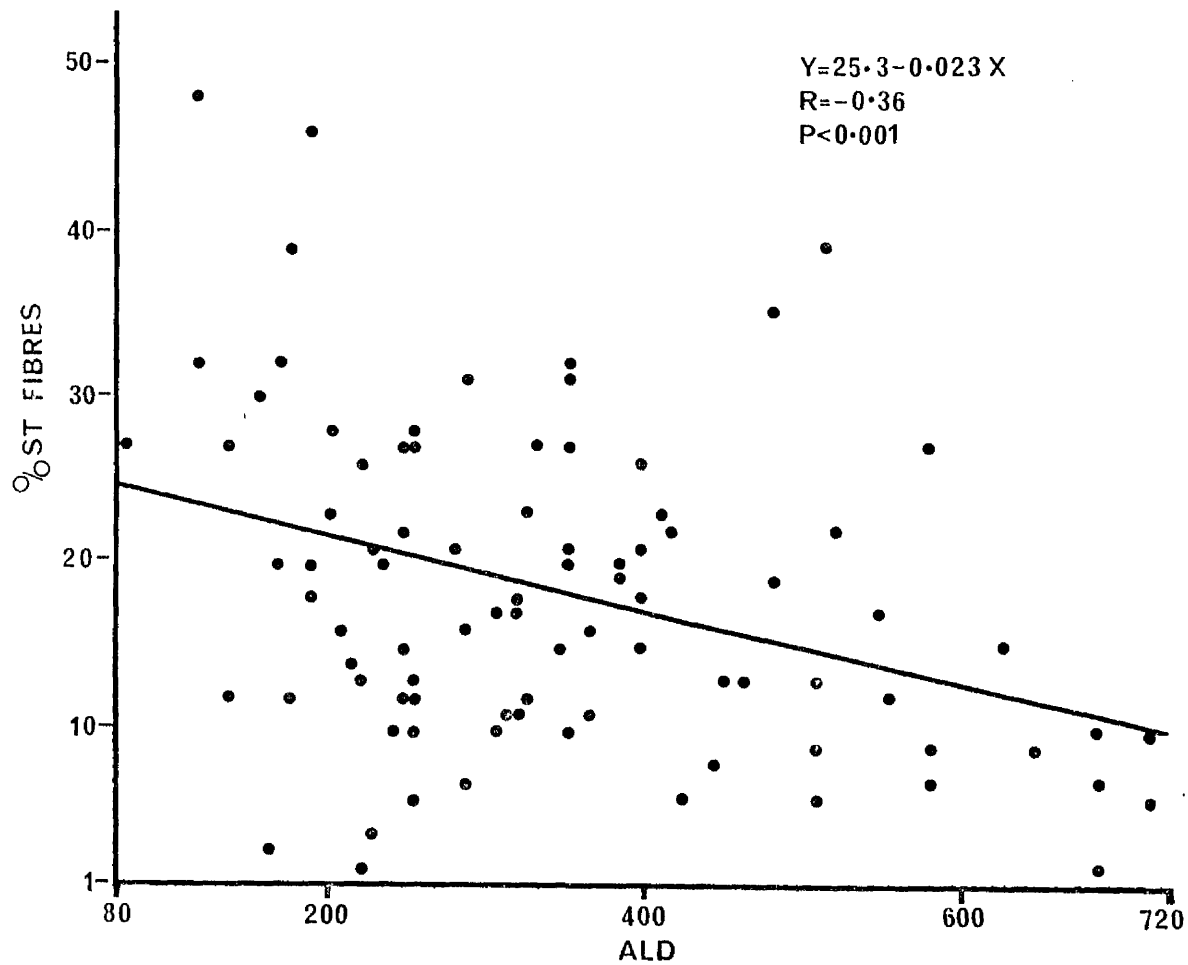

Y=25·3-0·023 X
R=-0·36
P<0·001
%ST FIBRES
50
40
30
20
10
1
80
200
400
600
720
ALD

Fig 4.16: Scattergram of % ST fibres against ALT activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).

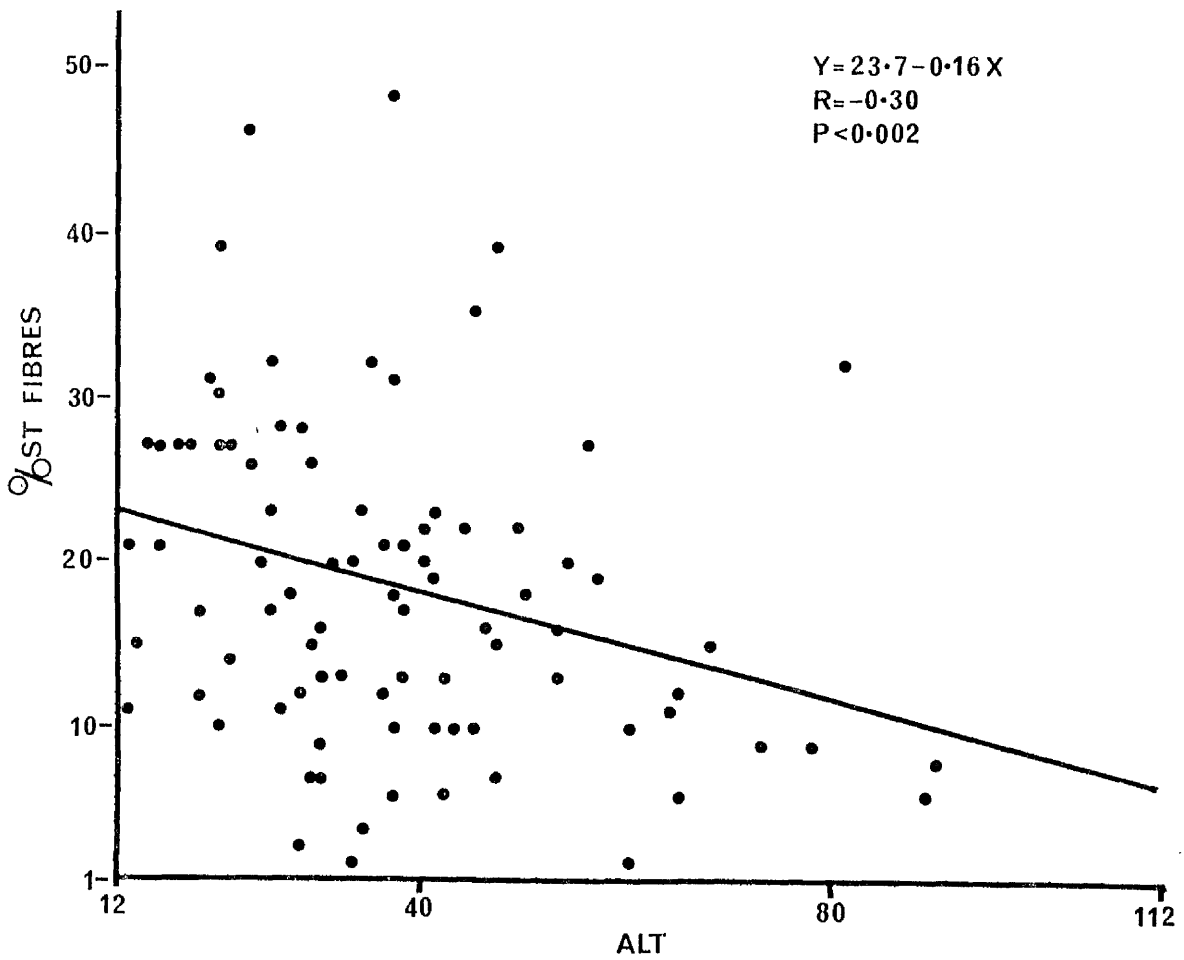

Y=23·7−0·16X
R=−0·30
P<0·002
%ST FIBRES
50
40
30
20
10
1
12
40
80
112
ALT

Fig 4.17: Scattergram of % ST fibres against CPK activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).

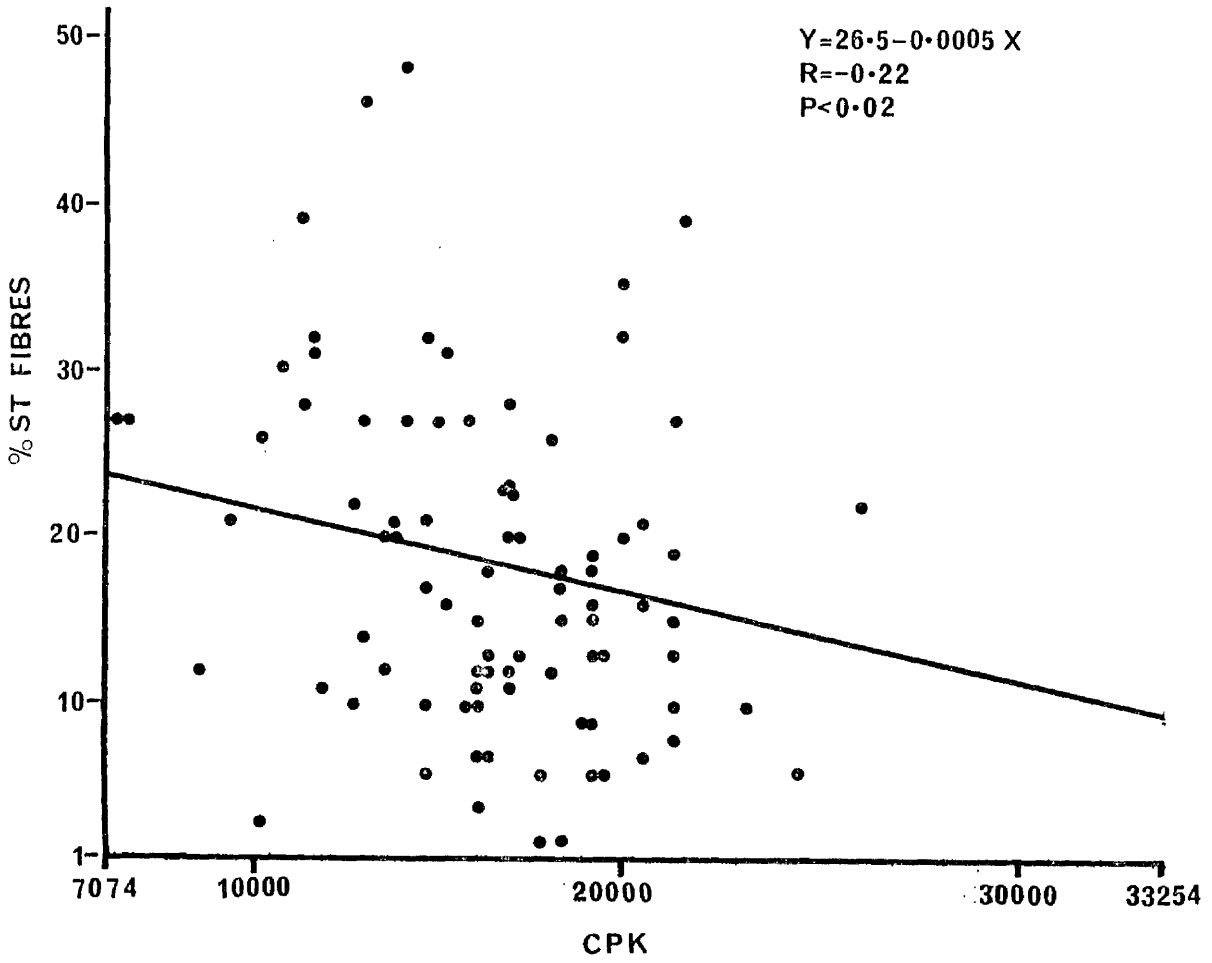

Y=26·5−0·0005 X
R=−0·22
P<0·02
% ST FIBRES
50
40
30
20
10
1
7074
10000
20000
30000
33254
CPK

Fig 4.18: Scattergram of % ST fibres against HAD activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).

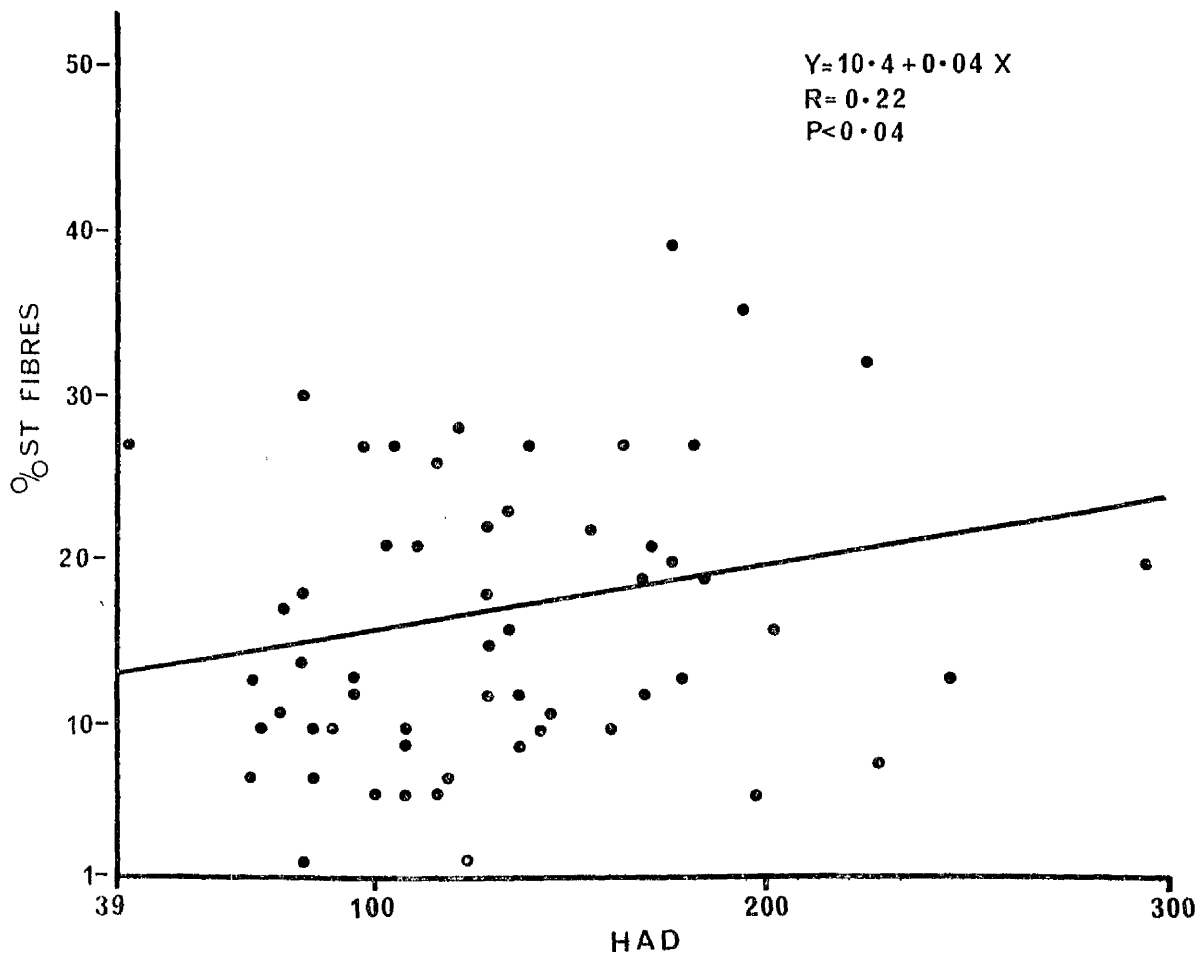

Y=10·4+0·04 X
R=0·22
P<0·04
50
40
30
20
10
1
%ST FIBRES
39
100
200
300
HAD

Fig 4.19: Scattergram of % ST fibres against GDH activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).

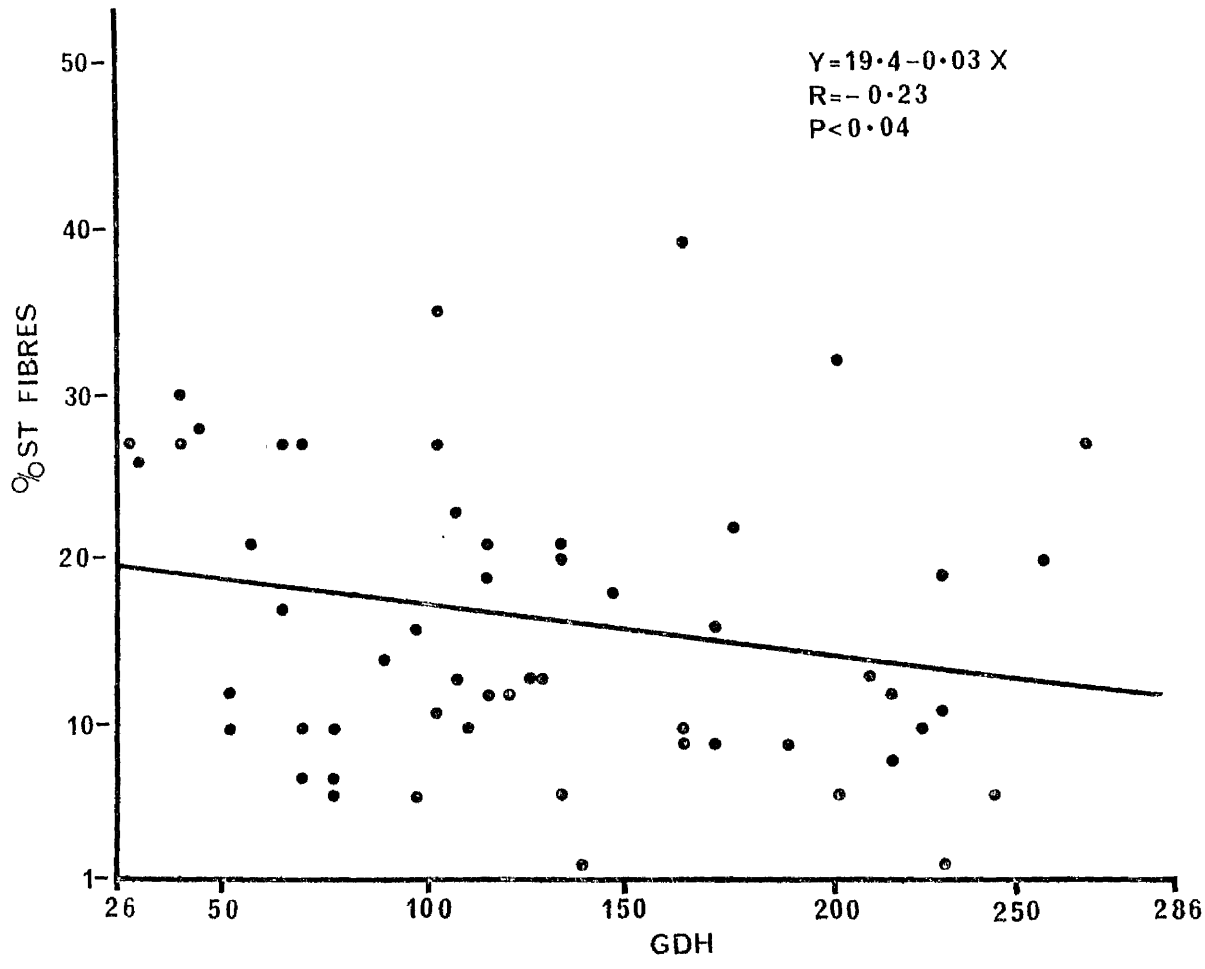

Y=19·4−0·03 X
R=− 0·23
P<0·04
50
40
30
20
10
1
% ST FIBRES
26
50
100
150
200
250
286
GDH

Fig 4.20: Scattergram of % ST fibres against HK activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).

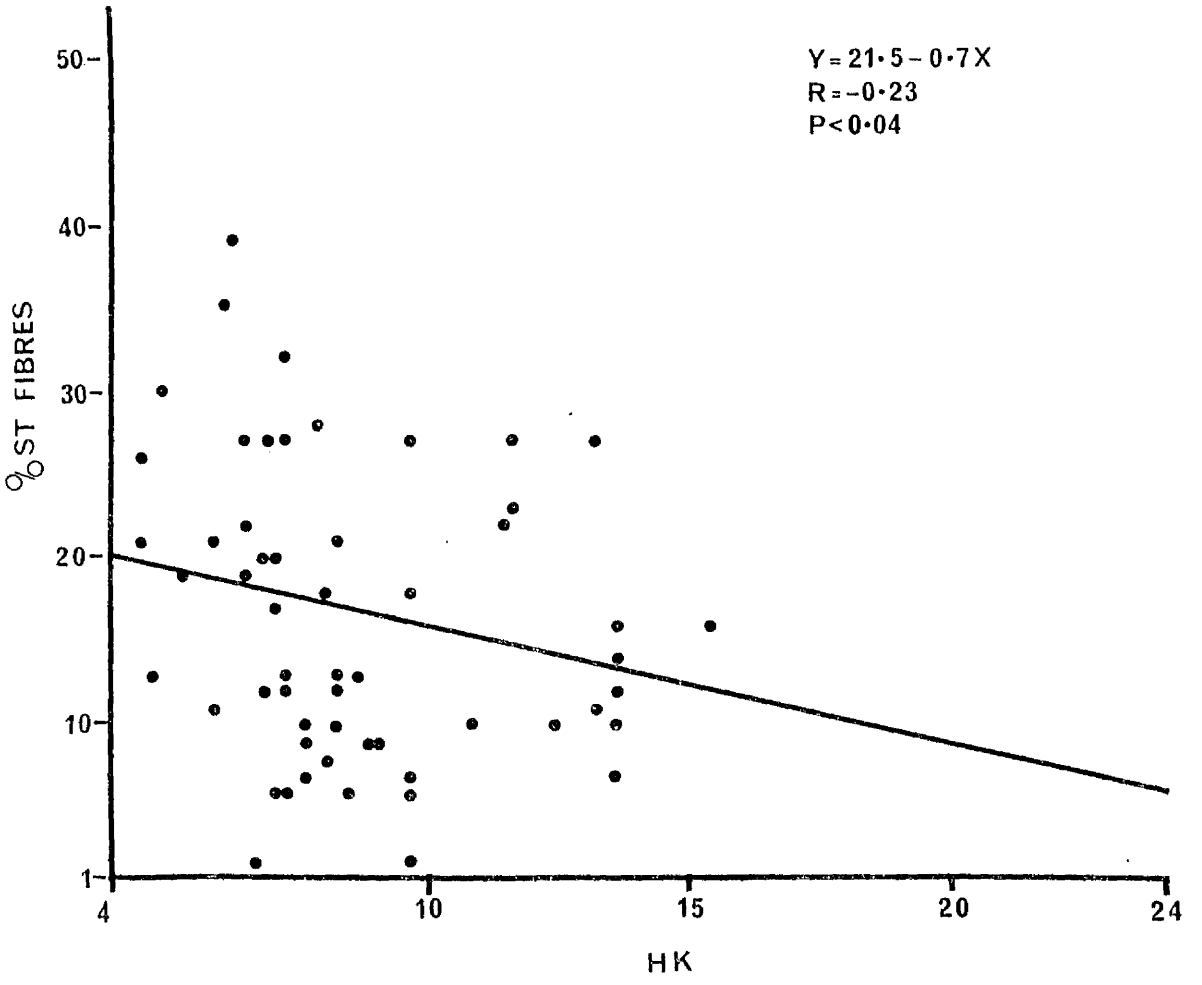

Y = 21·5 – 0·7X
R = –0·23
P < 0·04
% ST FIBRES
50
40
30
20
10
1
4
10
15
20
24
HK

Fig 4.21: Scattergram of % FT fibres against CS activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).

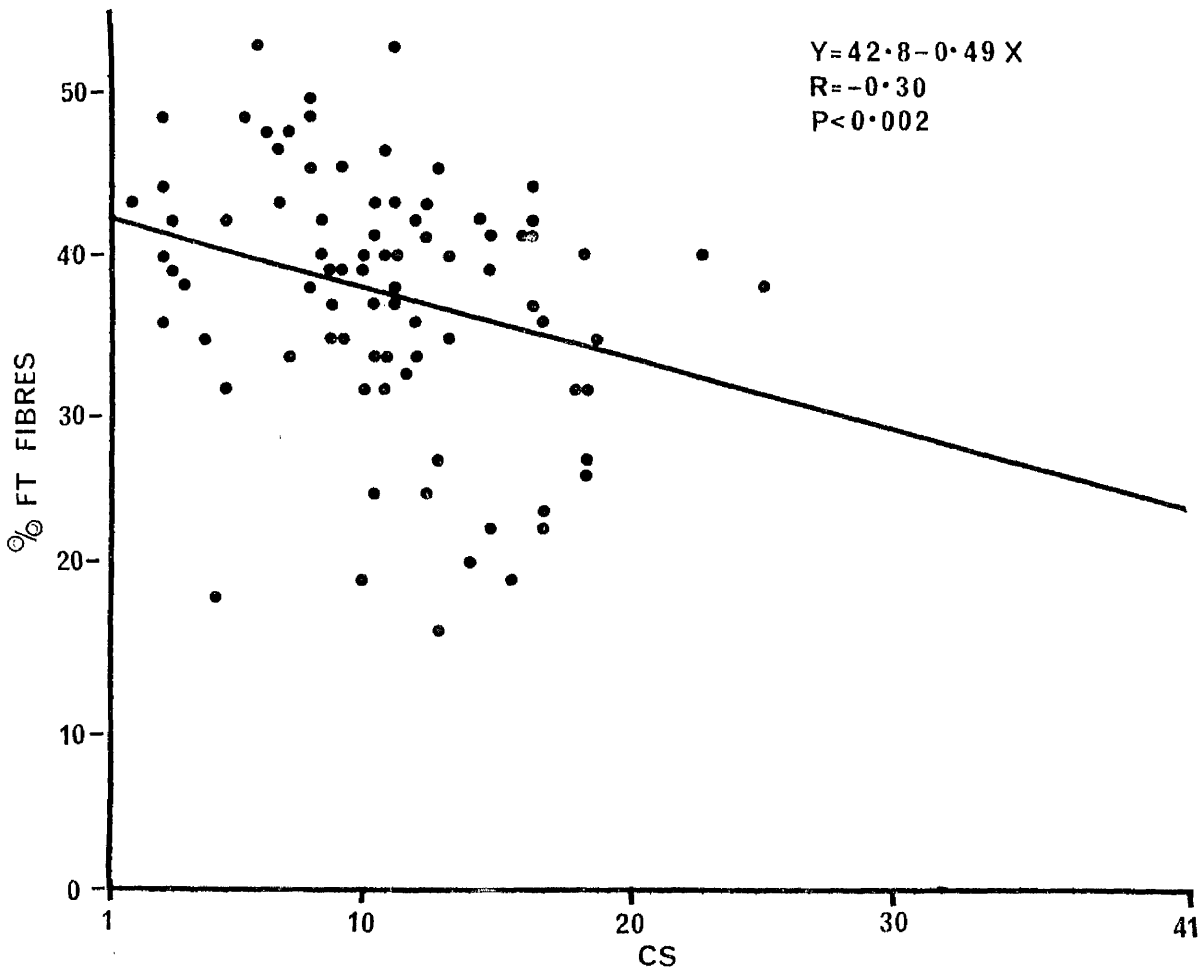

Y=42·8-0·49 X
R=-0·30
P<0·002
% FT FIBRES
50
40
30
20
10
0
1
10
20
30
41
CS

Fig 4.22: Scattergram of % FT fibres against ALT activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).

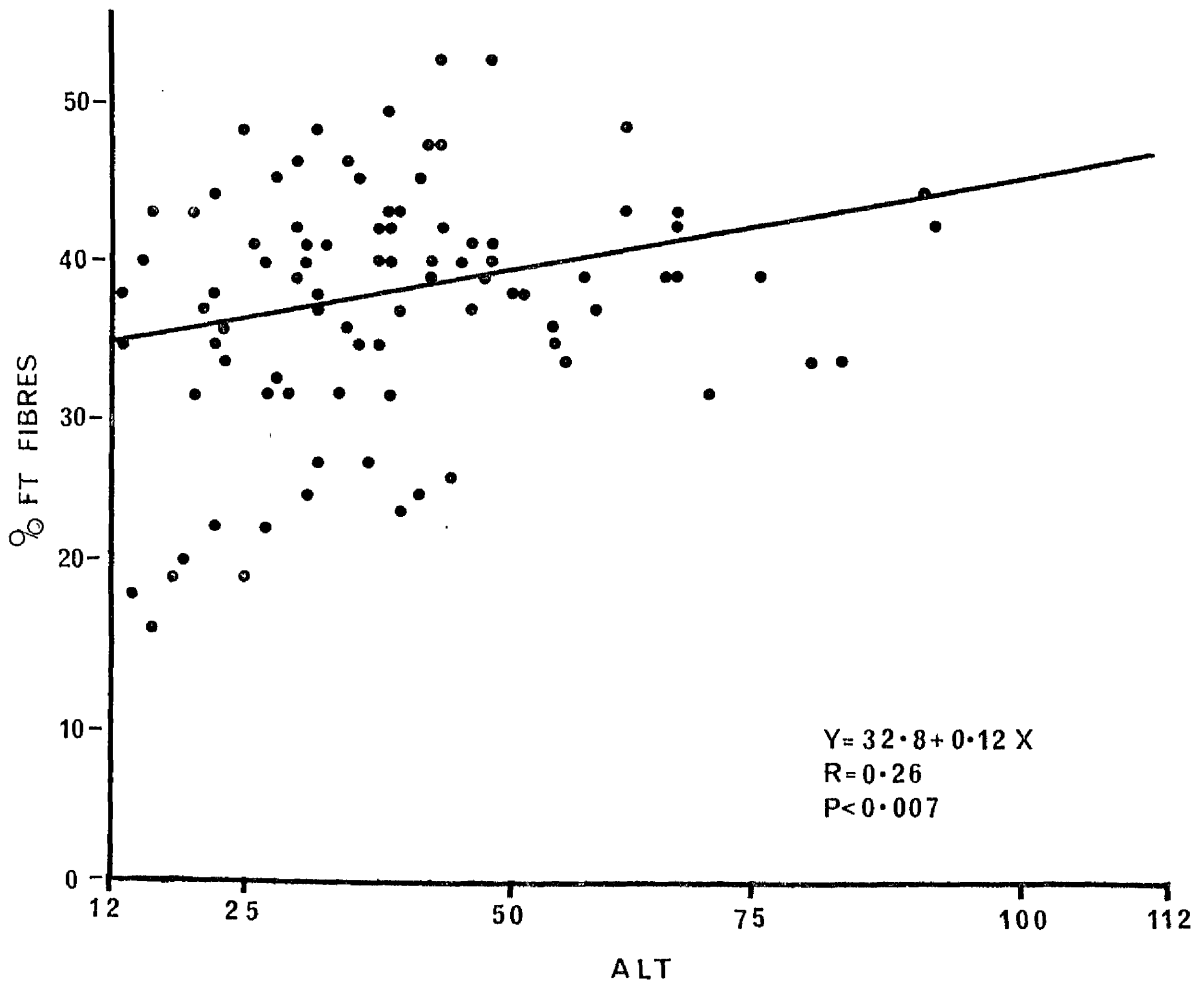

50
40
30
20
10
0
% FT FIBRES
12
25
50
75
100
112
ALT
Y= 32·8+ 0·12 X
R= 0·26
P< 0·007

Fig 4.23: Scattergram of % FT fibres against LDH activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).

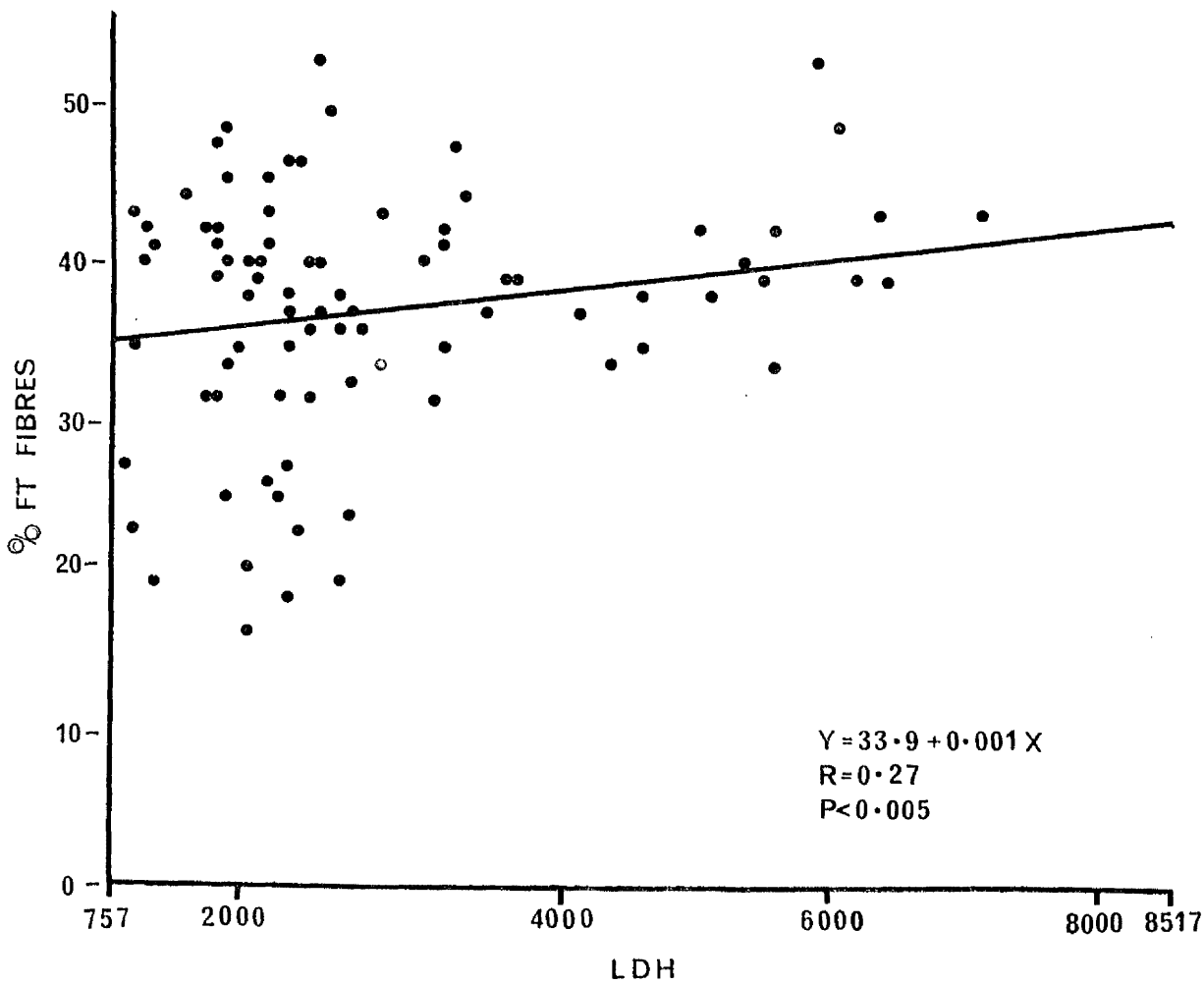
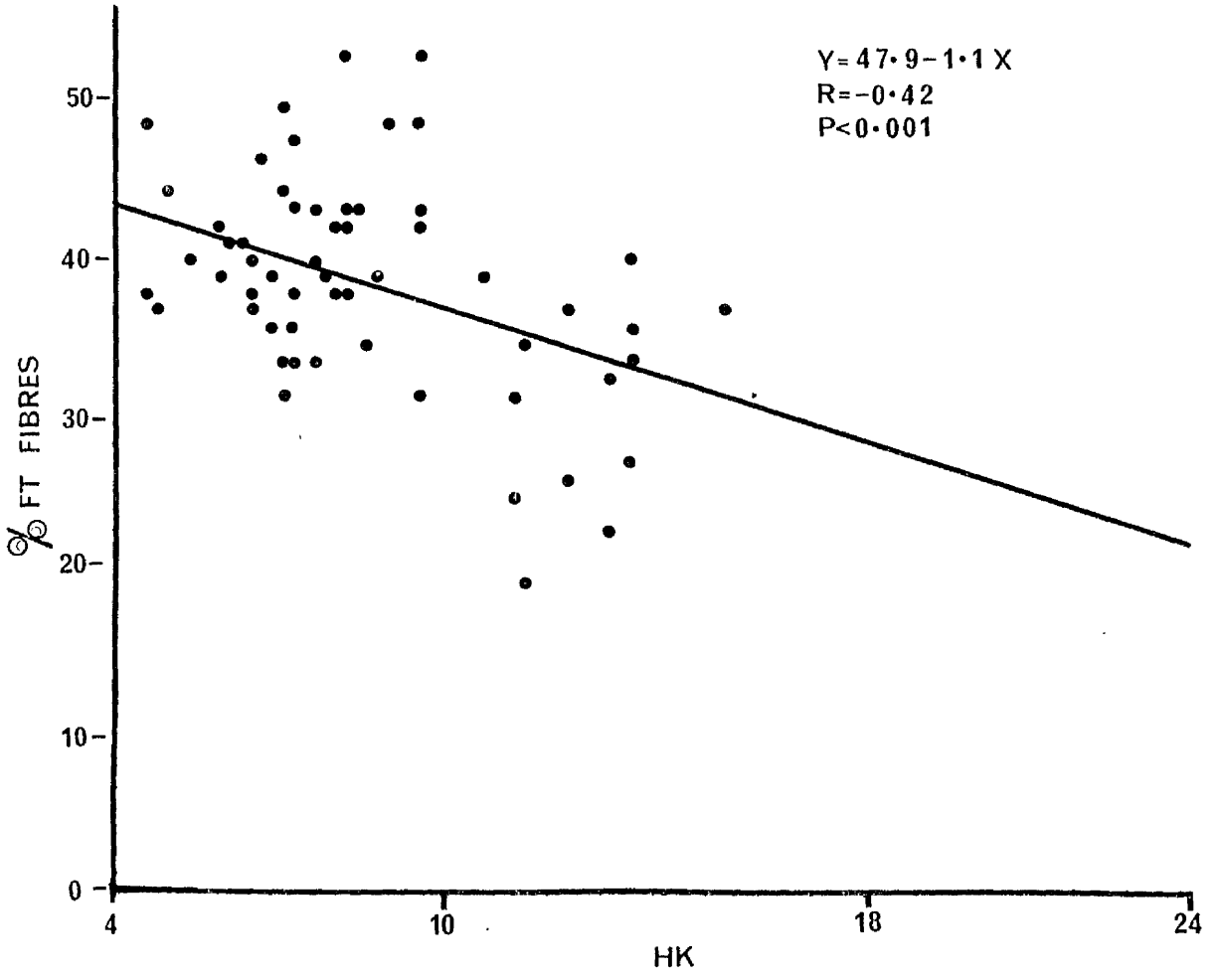

50
40
30
20
10
0
% FT FIBRES
757
2000
4000
6000
8000
8517
LDH
Y = 33·9 + 0·001 X
R = 0·27
P < 0·005

Fig 4.24: Scattergram of % FT fibres against HK activity in the horse (μmoles.(min.g.dry weight of tissue)$^{-1}$).


Y=47·9−1·1X
R=−0·42
P<0·001
50
40
30
20
10
0
% FT FIBRES
4
10
18
24
HK

Dog

The mean activities for the various enzyme activities and glycogen in the cross-bred mongrel and the greyhound are shown in Table 4.10. The greyhound had significantly higher levels of CPK, ALD, ALT and CS and significantly lower activities of HAD and HK in the gluteus medius muscle. A similar pattern existed in the semitendinosus. Glycogen was similar in the two breeds for both muscles.

Enzyme Ratios

The mean values for several important enzyme ratios in the gluteus medius are in Table 4.11. In most cases, there was a significant difference between the greyhound and the cross-bred mongrel.

Correlations Between the Various Enzymes

In the dog, a high correlation was found between the anaerobic energy producing enzymes, ALD and CPK. These enzymes also correlated highly with the aerobic enzymes CS and AST as well as with ALT. The highest correlation was observed between AST and CS (Table 4.12).

Correlations Between Enzyme Activity and Muscle Fibre Composition

Figs 4.25 to 4.29 illustrate the scattergrams for the various enzymes which exhibited a significant correlation with the percentage of ST fibres.

	Gluteus Medius		Semitendinosus	
	Greyhound	Mongrel	Greyhound	Mongrel
No. of animals	6	5	6	5
LDH	2201 ± 205	2330 ± 281	2122 ± 189	2241 ± 172
CPK	34250 ± 3129	22849 ± 629*	30418 ± 2281	20302 ± 1094*
ALD	442 ± 16	245 ± 18*	466 ± 26	265 ± 13*
AST	1038 ± 61	915 ± 42	806 ± 80	626 ± 70
ALT	76 ± 5	48 ± 5*	56 ± 6	27 ± 4*
CS	23 ± 2	8 ± 1*	19 ± 2	6 ± 1*
HK	5.2 ± 0.4	7.0 ± 0.4*	4.9 ± 0.6	7.0 ± 0.4*
HAD	115 ± 14	291 ± 16*	83 ± 9	106 ± 12
GDH	143 ± 7	156 ± 9	126 ± 13	94 ± 8
Glycogen	149 ± 15	192 ± 26	130 ± 11	164 ± 18

* $P < 0.05$

Table 4.10: Enzyme activities (μmoles.(min.gm dry weight tissue)$^{-1}$) in the gluteus medius and semitendinosus muscles of the greyhound and the cross-bred mongrel. (Mean ± S.E.M.).

	Greyhound (6)	Mongrel (5)
$\frac{LDH}{ALD}$	5.0 ± 0.5	9.4 ± 0.8*
$\frac{LDH \times 10^{-1}}{CS}$	9.9 ± 1.0	30.9 ± 5.9*
$\frac{LDH}{HAD}$	20.0 ± 2.4	8.1 ± 1.0*
$\frac{ALD \times 10^{-1}}{CS}$	2.0 ± 0.2	3.2 ± 0.5*
$\frac{ALD}{HAD}$	4.0 ± 0.4	0.85 ± 0.06*
$\frac{CS \times 10^{-1}}{HAD}$	2.1 ± 0.3	0.3 ± 0.04*

* = $P < 0.05$ compared to greyhound.

Table 4.11: Several important enzyme ratios in the gluteus medius of the greyhound and cross-bred mongrel. Number in brackets is number of subjects.

	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
LDH	1.0000 (0) S=0.001	0.1297 (22) S=0.283	-0.0490 (22) S=0.414	0.1799 (22) S=0.211	0.1102 (22) S=0.313	-0.0182 (22) S=0.468	0.0986 (22) S=0.331	0.2220 (22) S=0.160	0.1255 (22) S=0.289
CPK		1.0000 (0) S=0.001	0.7653 (22) S=0.001	0.6024 (22) S=0.002	0.8003 (22) S=0.001	0.8362 (22) S=0.001	-0.3031 (22) S=0.085	-0.3338 (22) S=0.064	0.3847 (22) S=0.039
ALD			1.0000 (0) S=0.001	0.4408 (22) S=0.020	0.7123 (22) S=0.001	0.8575 (22) S=0.001	-0.5308 (22) S=0.006	-0.5464 (22) S=0.004	0.2463 (22) S=0.135
AST				1.0000 (0) S=0.001	0.8429 (22) S=0.001	0.6648 (22) S=0.001	-0.0871 (22) S=0.350	0.2091 (22) S=0.175	0.5937 (22) S=0.002
ALT					1.0000 (0) S=0.001	0.9020 (22) S=0.001	-0.2935 (22) S=0.092	-0.0795 (22) S=0.363	0.5685 (22) S=0.003
CS						1.0000 (0) S=0.001	-0.4801 (22) S=0.012	-0.3608 (22) S=0.050	0.3305 (22) S=0.067
HK							1.0000 (0) S=0.001	0.4388 (22) S=0.021	0.1832 (22) S=0.207
HAD								1.0000 (0) S=0.001	0.5333 (22) S=0.005
GDH									1.0000 (0) S=0.001

(Coefficient / (no. of animals) / significance)

Table 4.12: Correlation coefficients between the different enzymes in dog skeletal muscle.

Fig 4.25: Scattergram of % ST fibres against CPK activity in the dog (μmoles.(min.g.dry weight of tissue)$^{-1}$). Analysed by discriminant analysis.

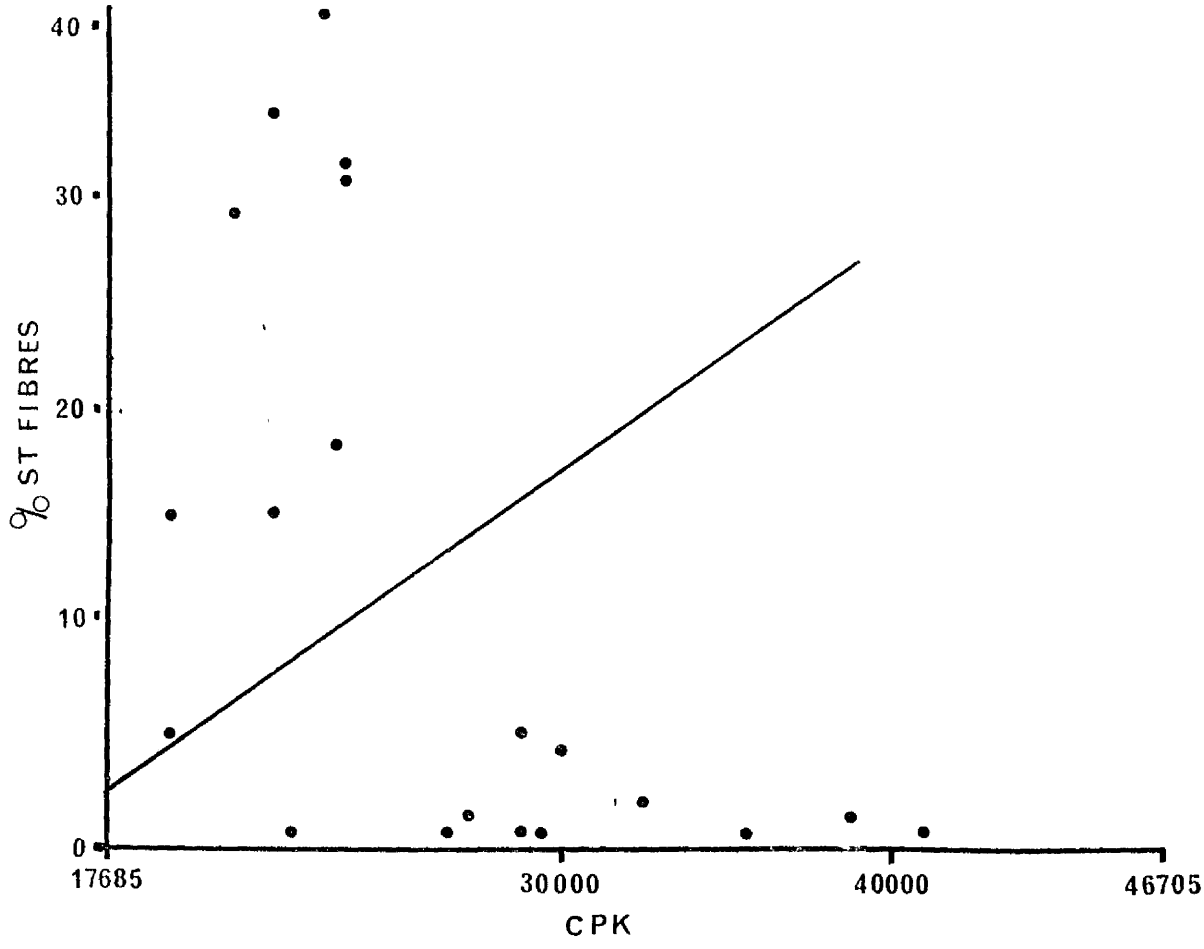

40
30
20
10
0
% ST FIBRES
17685
30000
40000
46705
CPK

Fig 4.26: Scattergram of % ST fibres against ALD activity in the dog (μmoles.(min.g.dry weight of tissue)$^{-1}$). Analysed by discriminant analysis.

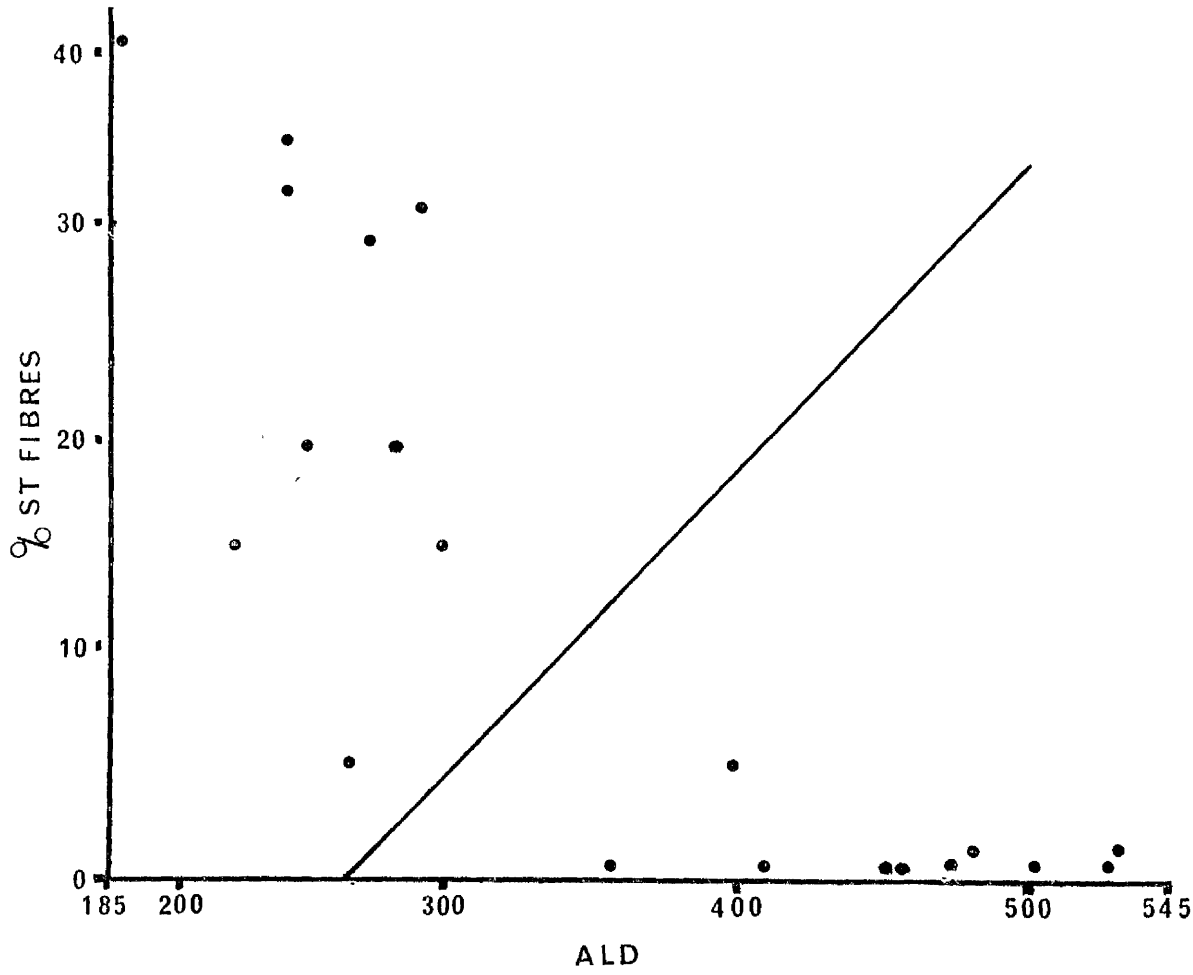

40
30
20
10
0
% ST FIBRES
185
200
300
400
500
545
ALD

Fig 4.27: Scattergram of % ST fibres against ALT activity in the dog (μmoles.(min.g.dry weight of tissue)$^{-1}$). Analysed by discriminant analysis.

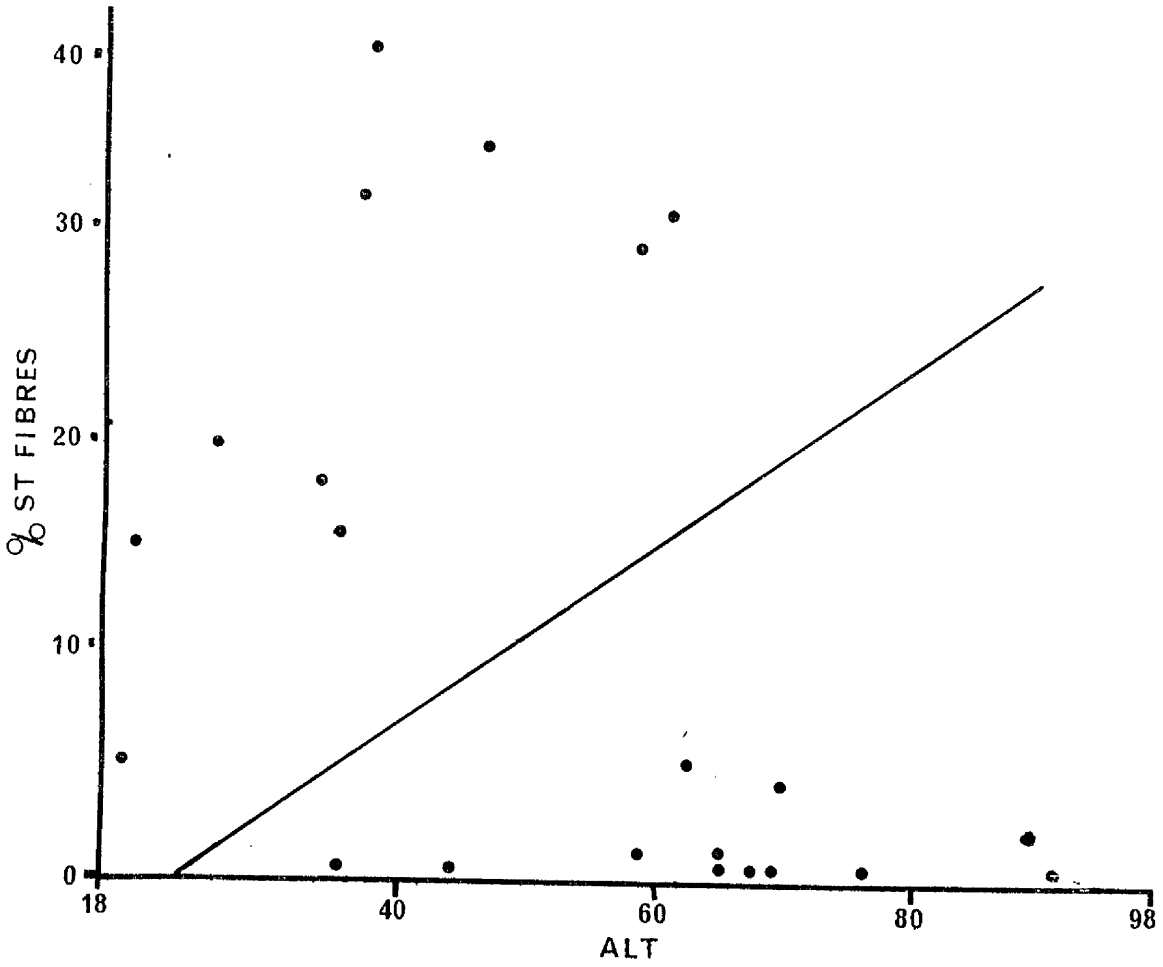

40
30
20
10
0
% ST FIBRES
18
40
60
80
98
ALT

Fig 4.28: Scattergram of % ST fibres against CS activity in the dog (μmoles.(min.g.dry weight of tissue)$^{-1}$). Analysed by discriminant analysis.

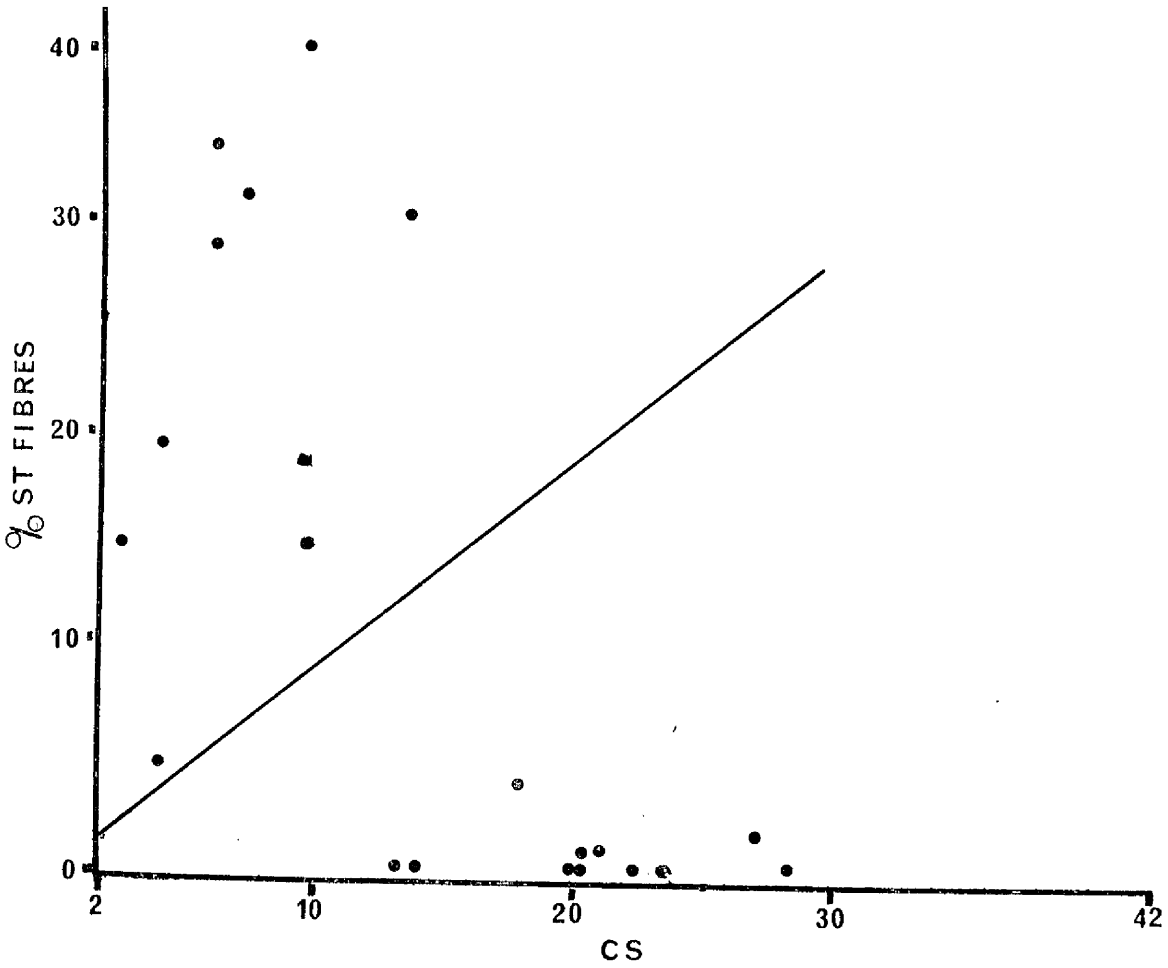
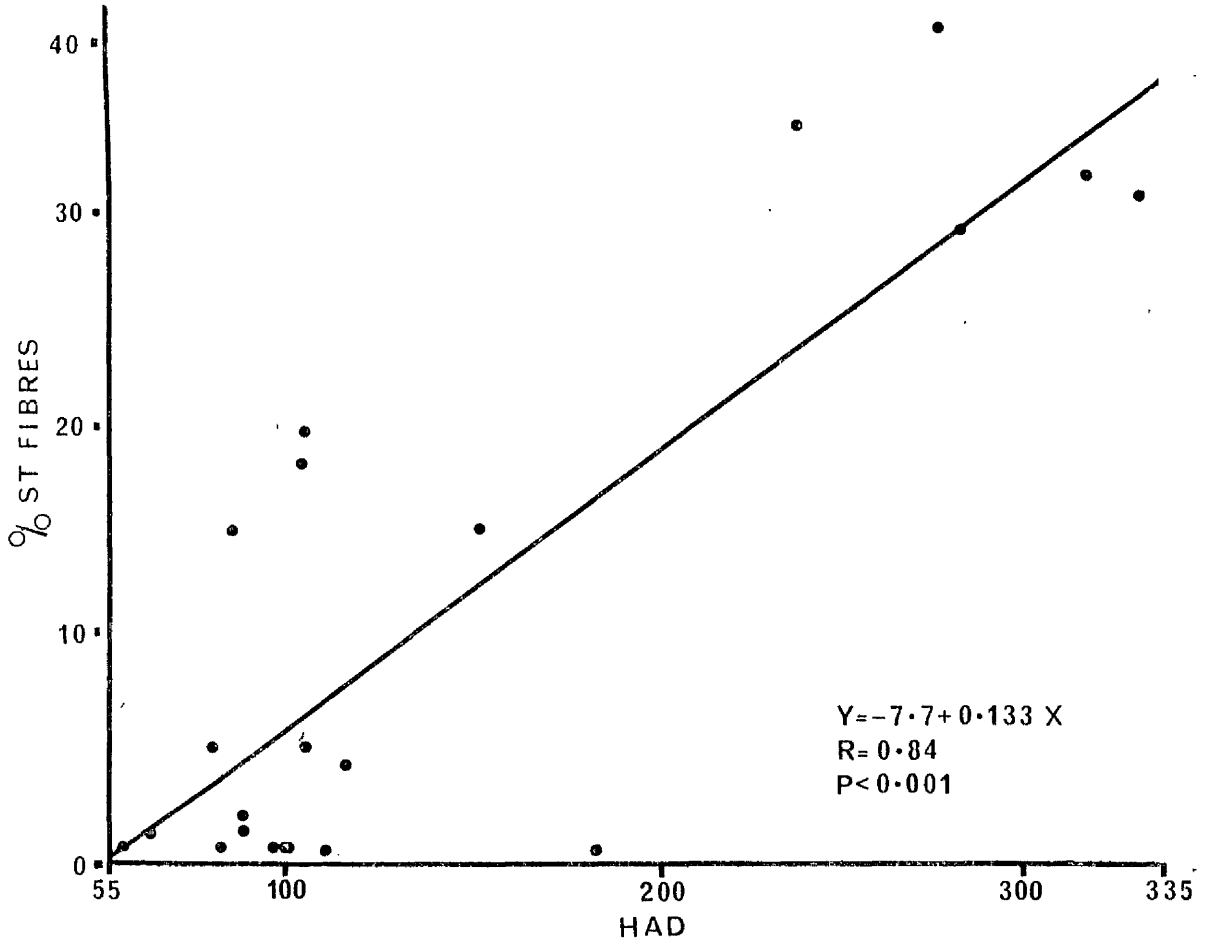

40
30
20
10
0
% ST FIBRES
2
10
20
30
42
CS

Fig 4.29: Scattergram of % ST fibres against HAD activity in the dog (μmoles.(min.g.dry weight of tissue)$^{-1}$).


40
30
20
10
0
% ST FIBRES
55
100
200
300
335
HAD
Y=-7·7+0·133 X
R=0·84
P<0·001

Discussion (2)

The enzyme pathways of skeletal muscle contain certain key enzymes which are found in more or less constant proportion to each other. (Pette and Bücher, 1963). This means that to obtain an indication of the capacity of a pathway, it is necessary only to measure the activity of one of those key enzymes. The ratios of these enzymes will therefore reflect the relationship between the various enzyme systems. There are two types of relationship to be found between these systems, constant systems relations which are said to be independent of the muscle fibre type and variable system relations which are dependent on several factors including fibre type and degree of activity (Pette and Bücher, 1963; Pette and Staudte, 1973; Pette and Dölken, 1975). The results in this section were therefore viewed in the above context, assaying only one enzyme from a given metabolic pathway.

A high oxidative capacity and a high level of mitochondrial enzyme activity is assumed necessary for good endurance running ability, and indeed, training increases the levels of these enzymes in many species whilst increasing the time to exhaustion (see section 5). The habitual level of activity has been stated to be the single most important factor in determining the level of oxidative enzymes in a muscle, more important even than the fibre composition of the muscle (Jansson and Kaijser, 1977). Endurance athletes have been shown to have a much higher SDH activity than control subjects (Gollnick et al., 1972; Costill et al., 1976a,b) even when the fibre composition of the two groups were similar (Jansson and Kaijser, 1977).

Although the horses in the present study were in an untrained condition, the two mitochondrial oxidative enzymes CS and HAD were significantly lower in the Quarterhorse, the breed with the highest percentage of FT fibres in the gluteus medius muscle. This suggests, that in the horse at least, the level of oxidative enzyme activity is determined to some extent by fibre composition. The two breeds with the highest CS were the Thoroughbred and Arab. Both of these breeds can travel large distances with the Arab especially being noted for its endurance ability. It was of interest that the Arab with the highest CS activity (25.5 μmoles.(min.gm dry weight of tissue)$^{-1}$) was extremely good during endurance events. This animal also had the highest percentage of slow twitch fibres (21.6%) in its gluteus medius muscle although this value was not significantly different from the other Arabs which were examined. The donkey had a very low CS activity but a similar HAD activity to the breeds of horse. The donkey probably depends on fat oxidation for energy, and in support of this, the donkey also was found to have a much lower glycogen content than the horses. The greyhound exhibited a much higher CS activity than the mongrel but a much lower HAD activity. This may be due to the absence of fat depots, in the greyhound, both in muscle and in the amount of adipose tissue present (Bogan, J.A. pers. comm.).

The levels of activity of AST tended to follow the same pattern as CS in both horse and dog. Thus the highest AST activities were found in the Arab and Thoroughbred in the horse and the greyhound in the dog. AST is part of a shuttle that is used by the cell to transfer NADH from the cytoplasm to the mitochondrial matrix for oxidation by the electron

transport chain. The cell employs this indirect method of transfer as the mitochondrial membrane is impervious to NADH (Van Dam and Meyer, 1971). The other known NADH shuttle involves the enzyme GDH. Little difference is found between the various breeds of horse although, the donkey is significantly lower than the horses. Again this may be because the donkey is not actually a breed of horse, but part of the equidae family. GDH activity is said to follow glycolytic activity and indeed is highest in the Quarterhorse, the breed with the highest ALD activity. The difference, however, was not significant.

As a prerequisite for an ability to run at speed, the muscle cells of an animal must be able to contract quickly and deliver energy at a rate that is not limiting. This implies that a high glycolytic/ anaerobic capacity would be an advantage. The Quarterhorse fulfils both of these criteria with very high LDH and ALD activity compared to the other breeds. This breed, when compared to the other breeds, will be able to break glycogen down at a faster rate and regenerate NAD^+ faster for the continuation of glycolysis during high output anaerobic exercise. Although the greyhound has a higher ALD activity than the mongrel, it does not possess a higher LDH activity. This may be due to the high CS activity in the greyhound reducing the need for the anaerobic metabolism of glycogen. A similar finding occurs in man with sprinters and power event athletes having a higher activity of LDH and phosphorylase than control subjects and endurance athletes (Costill et al., 1976a; Komi et al., 1977a). There is no difference between control subjects and endurance athletes (Costill et al., 1976b). It may be that the difference between the power event athletes and the others is due to both a training

effect and the fibre composition of the muscles. These athletes have in general a high percentage of fast twitch fibres when compared to other athletes (Gollnick et al., 1972) and these fibres are known to have the highest glycolytic/anaerobic potential (Essen et al., 1975). A training effect is also likely, however, as Komi et al. (1977a) comparing power event athletes and other athletes with a similar muscle fibre composition found that the former group of athletes had a higher LDH activity. A high CPK activity does not appear to be important for fast sprinting in the horse as levels were similar in the various breeds. When the dogs were examined, the greyhound, the fastest breed, had the highest CPK activity. This may be an indication of a different type of adaptation for fast sprinting in the two species with the greyhound being dependent on "phosphagen" derived energy whilst the horse derives sufficient energy from other sources. This adaptive response with CPK also occurs in man with "power" athletes having a higher CPK activity than control subjects or other athletes (Komi et al., 1977a). CPK activity is also determined to some extent by the fibre composition of a muscle, fast twitch fibres having a higher activity than slow twitch fibres (Pette and Staudte, 1973) and so the greyhound with its greater percentage of fast twitch fibres would be expected to have a higher CPK activity than the mongrel. Possibly this relationship is not as great in the horse because the CPK activity in the fast and slow twitch fibres are similar, a situation found in man (Thorstensson et al., 1977).

When the ratios of some of the enzymes in the various metabolic pathways are considered, one can obtain an indication of the relative importance of these pathways in the various breed. Thus, the

Quarterhorse had a high dependence on anaerobic energy metabolism during exercise as indicated by the high LDH/CS ratio and ALD/CS ratio when compared to the other breeds. Similarly the Thoroughbred had the lowest values for these ratios, indicating the relatively greater importance of aerobic metabolism in this breed. Most of the other enzyme ratios examined were similar in the various breeds. The donkey had an unusual enzyme ratio pattern, however, with an apparently high dependence for energy on fat oxidation rather than carbohydrate oxidation as indicated by the low CS/HAD ratio when compared to other breeds. When the enzyme ratios are examined in the two breeds of dog the high dependence of the greyhound on carbohydrate metabolism as opposed to fat oxidation is highlighted by the greater ALD/HAD, LDH/HAD and CS/HAD ratios when compared to the mongrel.

Metabolic Differentiation in Horse Skeletal Muscle Fibres

An attempt was made to classify the enzyme composition of the various fibre types in horse skeletal muscle by plotting scattergrams of the various enzyme activities against the percentage of ST fibres or FT fibres. None of the correlations obtained were very large, but this was to be expected as muscles of mixed fibre composition were being used, as opposed to the relatively homogeneous samples which can be obtained in several other species (Ariano et al., 1973). A negative correlation was found between the percentage of slow twitch fibres and the anaerobic/glycolytic enzymes. This was similar to the work of Pette and Staudte (1973) who found that the level of LDH, CPK, GDH and triose phosphate-dehydrogenase (in the same pathway as ALD) was greater in the fast twitch vastus lateralis than the slow twitch soleus of the guinea pig. This negative correlation was also found between the percentage of slow twitch fibres and ALT and HK. The conversion of pyruvate to alanine is catalysed by ALT and as pyruvate is the end product of a glycolysis, it is not surprising if this enzyme is higher in the fast twitch fibres. This is different from the rabbit, where the amino acid metabolism enzymes ALT and AST are both lower in the fast twitch fibres (Pette and Dölken, 1975).

The correlation between CS and the percentage of high oxidative fibres is to be expected as CS is found only in the mitochondria and the stain for oxidative capacity stains only the mitochondria. HK is unique among the glycolytic enzymes as its activity is directly proportional to the respiratory capacity of a fibre (Crabtree and Newsholme, 1972). Thus the negative correlation with the percentage of low oxidative fibres was

to be expected. The positive relationship between LDH and the percentage of low oxidative fibres arises because these fibres are also fast twitch fibres and will have a high anaerobic capacity.

If enzymes are correlated with the percentage of a given fibre type, it is not surprising that a relationship is found between the different enzyme activities. Thus, LDH, GDH, ALD and CPK, enzymes which are connected with glycolysis or anaerobic metabolism, all had a high correlation with each other. Similarly, the correlations between AST, HAD and CS, enzymes connected with aerobic metabolism, were to be expected. Surprisingly, in this study, the correlation between HK and CS and HAD was extremely low. As previously stated, normally a high relationship between these enzyme activities is found (Crabtree and Newsholme, 1972) but no explanation can be offered for this discrepancy.

Metabolic Differentiation in Dog Skeletal Muscle Fibres

As with the horse a significant correlation was found with the percentage of fast twitch fibres in dog skeletal muscle and the enzymes ALD and CPK. No correlation was, however, found with LDH and the percentage of fast twitch fibres which was unusual when compared to other species (Pette and Staudte, 1973). This was because of the similar LDH activities found in both breeds examined.

There was also a significant correlation with CS and AST and the percentage of fast twitch fibres caused mainly by the high dependence of the greyhound on carbohydrate metabolism. Similarly the significant correlation between the percentage of slow twitch fibres and HAD was because of the high fat dependence in the mongrel. The HK and slow twitch fibre correlation was as expected from other studies where higher HK activities are found in slow contractile fibres (Pette and Staudte, 1973).

As the greyhound and mongrel had unusual enzyme patterns in their muscles several unexpected correlations occurred between the various enzymes. CPK and ALD were not only correlated with GDH and ALT as in the horse, but also with CS and AST.

The major disadvantage in the present study has been that the muscles examined consisted of different fibre types of distinct metabolic and contractile properties. This has led to difficulties in interpreting the meaning of the values obtained for the various enzymes. It would therefore be of interest to examine the muscle fibres of these two species using the "single fibre technique."

SECTION 5

THE EFFECT OF TRAINING AND DETRAINING ON MUSCLE FIBRE COMPOSITION IN THE HORSE

Introduction

An adaptive response to training occurs in many of the body's systems, e.g. the muscular system, the central nervous system, the autonomic system and the cardiovascular system (Holloszy, 1976). These adaptations improve co-ordination and the transport of oxygen, fuels and metabolites during exercise. Training adaptations in muscle can be brought about by two main types of exercise. The first is exercise of an explosive nature involving relatively few very forceful muscle contractions and is exemplified by the exercise done by weight-lifters and sprinters. This type of exercise results in hypertrophy of the muscle cells with an increase in strength (Edstrom and Ekblom, 1972; Gollnick et al., 1972; Thorstensson et al., 1975). The increase in muscle cell proteins responsible for this hypertrophy appears to be due to changes in both the rate of synthesis and degradation (Goldberg et al., 1975). The second type of exercise is endurance, exemplified by activities such as those involved in long distance running or swimming. This results in increased endurance and more efficient oxygen utilisation due to adaptations in the skeletal muscle as well as in the cardiovascular and autonomic nervous systems. An increase in the capacity for aerobic exercise can occur without an increase in strength or hypertrophy of the muscle (Holloszy, 1976).

Over the past decade, studies in several species have shown that, with training, there are alterations in the activities of several enzymes in skeletal muscle and these findings have been reviewed by Holloszy (1973) and Holloszy and Booth (1976). The most extensive

investigations have been carried out on rats by Holloszy and his co-workers who found that the capacity of muscle homogenates to oxidise fats and carbohydrates approximately doubled following a 13-18 week programme of treadmill running (Holloszy et al., 1973; Holloszy and Booth, 1976). This increase was found to occur in all three fibre types (Baldwin et al., 1972). A high level of respiratory control and tightly coupled oxidative phosphorylation accompanied this increased oxidative capacity, indicating an increased capacity to regenerate ATP via oxidative phosphorylation (Holloszy, 1967; Mole et al., 1971).

It was found that underlying this increase in respiratory capacity was an increase in the activities of enzymes in the T.C.A. cycle (Holloszy et al., 1970), the activation, transport and β-oxidation of long-chain fatty acid enzymes (Mole et al., 1971) and several components of the respiratory chain (Holloszy et al., 1970; Baldwin et al., 1972). Not all of the constituent parts of the mitochondria changed to the same extent and indeed some, notably GDH (Holloszy and Oscai, 1969), CPK and MK (Oscai and Holloszy, 1971), did not alter at all. These latter three enzymes in fact exhibited a significant decrease in specific activity when the results were expressed per milligram of mitochondrial protein. These findings have essentially been confirmed by other workers in rats and other species. Studies using either longitudinal (i.e. serial samples collected from the same individuals during a progressive training programme) or cross-sectional (i.e. comparing groups of trained and untrained subjects) sampling techniques in the guinea pig (Barnard et al., 1970), lesser bush baby (Edgerton et al., 1972), man (Gollnick et al., 1973), horse (Lindholm and Piehl, 1974) and pig (Fogd Jorgensen and Hyldgaard-Jensen, 1975), have all shown that training produces an

increase in the oxidative capacity and/or enzymes connected with oxidative metabolism.

Reports on the effect of training on glycolytic and glycolytic related enzymes have proved more confusing with a variety of differing results being obtained. Depending on the study, glycolytic enzymes have been reported to increase (Taylor et al., 1972a; Gollnick et al., 1973; Lindholm and Piehl, 1974), decrease (Baldwin et al., 1972; Edgerton et al., 1972; Hickson et al., 1975) or remain unchanged (Fogd Jorgensen and Hyldgaard-Jensen, 1975; Bylund et al., 1977).

The responses of other enzyme systems to training have been less well documented and have been studied mainly in rats and man. They include the following enzymes or enzyme systems.

1) The Lowenstein cycle (Fig. 5.1) which is concerned with ammonia production (Lowenstein, 1972). With training, AMP deaminase parallels changes in PFK whilst adenylosuccinase is unchanged (Winder et al., 1974b).
2) Enzymes involved in ketone utilisation which increase in all three fibre types (Winder et al., 1974a).
3) Enzymes involved in the immediate production of ATP, i.e. CPK and MK which vary in their response to training (Oscai and Holloszy, 1971; Fogd Jorgensen and Hyldgaard-Jensen, 1975; Thorstensson et al., 1975, 1976a).
4) Actomyosin ATPase which also varies in its response to training (Wilkerson and Evonuk, 1971; Edgerton et al., 1972; Baldwin et al., 1975).

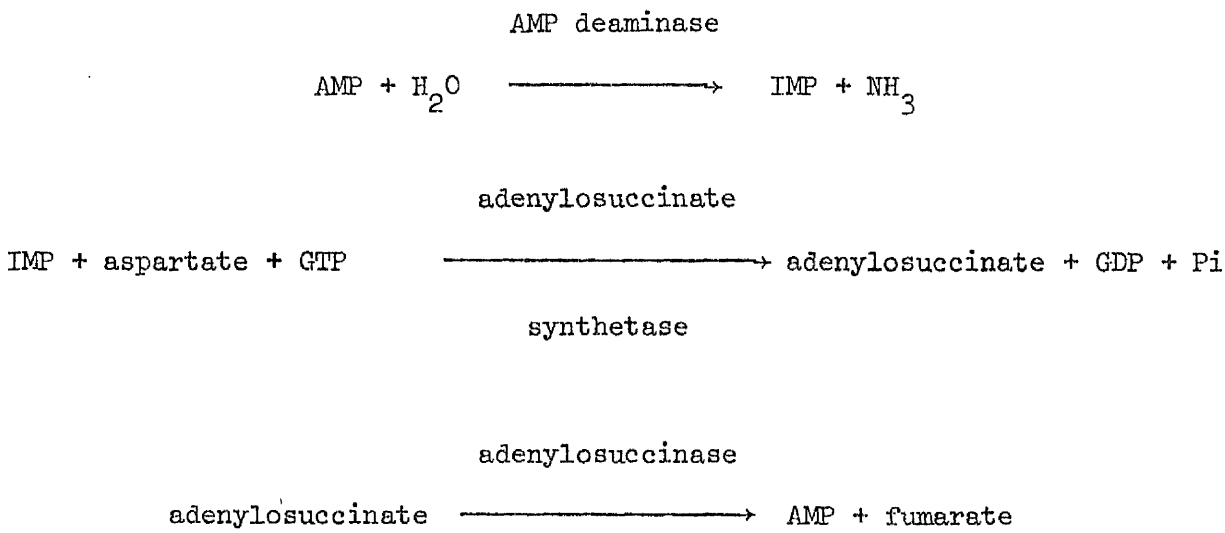


Fig 5.1: The Lowenstein Cycle.

5) Enzymes involved in the NAD shuttles, i.e. GDH, AST and MDH. GDH is unchanged by training (Holloszy and Oscai, 1969) whereas both AST and MDH are increased (Raimondi et al., 1975; Holloszy, 1976).

6) Enzymes connected with the fate of pyruvate, such as LDH which decreases slightly in rat (Mole et al., 1973) and is unchanged in man (Sjodin et al., 1976), ALT which increases with training and malate dehydrogenase which increases (Mole et al., 1973).

7) Enzymes involved in the control of glycogen synthesis, i.e. GS (Jeffress et al., 1968; Taylor et al., 1972b; Piehl et al., 1974), HK (Peter et al., 1968; Baldwin et al., 1973; Piehl et al., 1974) and the glycogen debranching enzyme (Taylor et al., 1973), which in general increase.

8) Adenyl cyclase which increases with training in rats (Dohm et al., 1976).

9) Isoenzymes of LDH which in general have a tendency to have an increased H subunit percentage with training (York et al., 1974; Fogd Jorgensen and Hyldgaard-Jensen, 1975; Sjodin et al., 1976).

Most work has been done on rats and other small laboratory animals as it is possible to dissect out portions of muscle which are relatively homogeneous with respect to their fibre types and thus changes in the three fibre types could be detected readily. In animals with a mixed fibre composition such as man (Johnson et al., 1973) and horse (Lindholm and Piehl, 1974), however, biochemical techniques have been of more limited value as indicators of changes in a specific type. Thus, until the advent of the "single fibre" technique of Essen et al. (1975), the only method of determining the metabolic characteristics of a specific

fibre type in mixed muscle was by the use of histochemical techniques. Reports on the effect of training on fibre types have been contradictory and depend largely on whether oxidative capacity or myosin ATPase activity was used to define the fibre type. Morgan et al. (1971) staining only for SDH an oxidative enzyme, concluded that there was a change in fibre type with training. In studies where both oxidative and myosin ATPase properties were examined, it is generally agreed that whilst an increase in the staining for oxidative enzymes may occur, no change in the proportion of high to low myosin ATPase (pH 9.4) activity fibres occurs (Barnard et al., 1970; Gollnick et al., 1973; Lindholm and Piehl, 1974).

Much less is known about the effects of detraining after a period of intense training. Several early studies reported on the effect of detraining on heart-weight:body-weight ratio (Secher, 1921; Steinhaus et al., 1932) and more recently, Gollnick and Simmons (1967) found a regression of adrenal and heart weight and cholesterol concentration after training had ceased. Biochemically and histochemically, it has generally been assumed that a decrease in enzyme activity occurs, similar to that which occurs following immobilisation of limbs by plaster casts or pinning following orthopaedic surgery (Booth and Kelso, 1973). Three studies which support this assumption have been carried out in guinea pigs (Faulkner et al., 1972), where histochemical analysis revealed a regression of red compared to white fibres, indicative of a reduced oxidative capacity, and in rats (Terjung, 1975) and man (Henricksson and Reitman, 1977) where biochemical analysis of some mitochondrial components has revealed a decreased oxidative capacity. Apart from these studies there are few studies which have examined the effect of detraining on skeletal muscle.

Although there is considerable information on the effects of training, surprisingly few investigations have been carried out on the horse, a species which has been bred specifically for speed or heavy endurance work. As mentioned previously, it has been reported that racing Standardbred horses of different ages have different muscle enzyme activities and this has been attributed to a training effect (Lindholm and Piehl, 1974). Similarly, a very limited study in Thoroughbreds has shown a difference in the activities of some mitochondrial enzymes when two trained and two untrained animals were examined (Straub et al., 1975). Virtually nothing is known about the changes with detraining in the horse and so the purpose of this section was to make a detailed examination of the effects of training and detraining on enzymes representative of several metabolic pathways in horse skeletal muscle.

Methods

The analytical methods used were as described in section 2. Muscle samples were obtained by the technique of percutaneous needle muscle biopsy (Bergström, 1962) as described previously.

Animals

Six clinically healthy horses (4 Thoroughbreds and 2 heavy hunters - horses 1 to 6) were used.

Training Programme

For the three months prior to the training programme, the horses underwent only maintenance exercise consisting of walking and slow cantering. The programme each week consisted of four days submaximal exercise, the distance being gradually increased until the horses were trotting and cantering approximately 10-15 kilometres per day and two days of maximal sprinting, galloping a 600 metre course three times with a few minutes rest between each gallop. The training programme lasted ten weeks. A further period of five weeks of sprinting over various distances was also included for the four Thoroughbreds. At the end of the training programme, the horses were slowly detrained by walking and slow cantering over two weeks in order to prevent fluid accumulation in lower limbs, followed by a period of walking exercise only (being led as opposed to being ridden) for one kilometre per day during the rest of the detraining period.

Sampling Procedure

Biopsy samples were taken from six limb muscles - *deltoideus*,

long head of the triceps brachii, vastus lateralis, gluteus medius biceps femoris and semitendinosus. In order to allow specimens to be obtained from all six sites, it was found necessary to use a tranquilliser in most cases. This was administered thirty minutes before sampling and one of three drugs was used.

Drugs

Acepromazine : Acetylpromazine 10 mg/ml. C-Vet Limited.

Azaperone : Suicalm 4% w/v. Crown Chemical Company Limited.

Xylazine : Rompun 50 mg/ml. Bayer U.K. Limited.

The drugs were administered at the following dose rates;

i) Acepromazine : 0.5 $mg.kg^{-1}$ intramuscularly

ii) Azaperone : 0.7 $mg.kg^{-1}$ intramuscularly

iii) Xylazine : 2.0 $mg.kg^{-1}$ intramuscularly

as described by MacKenzie and Snow (1977). Biopsies were taken from the six sites at weeks 0, 5, 10 and in the case of the four Thoroughbreds week 15. Samples were also obtained at weeks 5 and 10 during the detraining programme. For seventy two hours before a biopsy sample, the horses were given walking exercise only, in order to minimise any effects of acute exercise. Samples were all obtained from within a 10 cm square and at a similar depth, in order to reduce any possible variation in the muscle. The muscle was divided into two sections at weeks 0 and 10, one section being used for histochemistry, the other for biochemistry. At other times, only a sample for biochemistry was collected.

Preparation of Samples for Analysis

Histochemistry: Samples were prepared and stained as in section 2. Fibres were then typed.

Biochemistry: Samples were prepared and analysed as described in section 2. Assays were performed on all six muscles for LDH, CPK, ALD, AST, ALT, CS, glycogen and protein and on the gluteus medius and semitendinosus for LDH isoenzymes, HAD, GDH and HK.

Results

Training

Histochemistry: Mean values for the percentage of each fibre type before and after training are given in Table 5.1. From the limited number of subjects available, it appears that the oxidative capacity of all the fibres in a given muscle increase with training (Fig 5.2). Only the deltoideus had a significant increase in the percentage of high oxidative fibres. Similarly, when the myosin ATPase activity at pH 9.4 was considered, only the fibre composition of the deltoideus was significantly changed, with a decrease in the percentage of low myosin ATPase activity fibres at pH 9.4. For several reasons, some of the values for the fibre percentages are not available after training (usually due to the section obtained not being in a transverse orientation and less commonly, the horse proving too difficult to allow a sample to be taken) and so the resulting small number of samples may be the reason that more muscles did not exhibit a significant alteration in fibre composition. When all of the muscles were considered together, a significant decrease in the number of low myosin ATPase activity at pH 9.4 fibres and a significant increase in the number of high oxidative fibres were found with training. This had the effect of increasing the percentage of FTH fibres at the expense of the ST and FT fibres.

Biochemistry: Enzyme activities and glycogen concentrations for the Thoroughbreds and heavy hunters were considered together as levels and changes with training were similar in the two breeds. Tables 5.2 to 5.14 show the mean values for the various enzymes at the different sampling

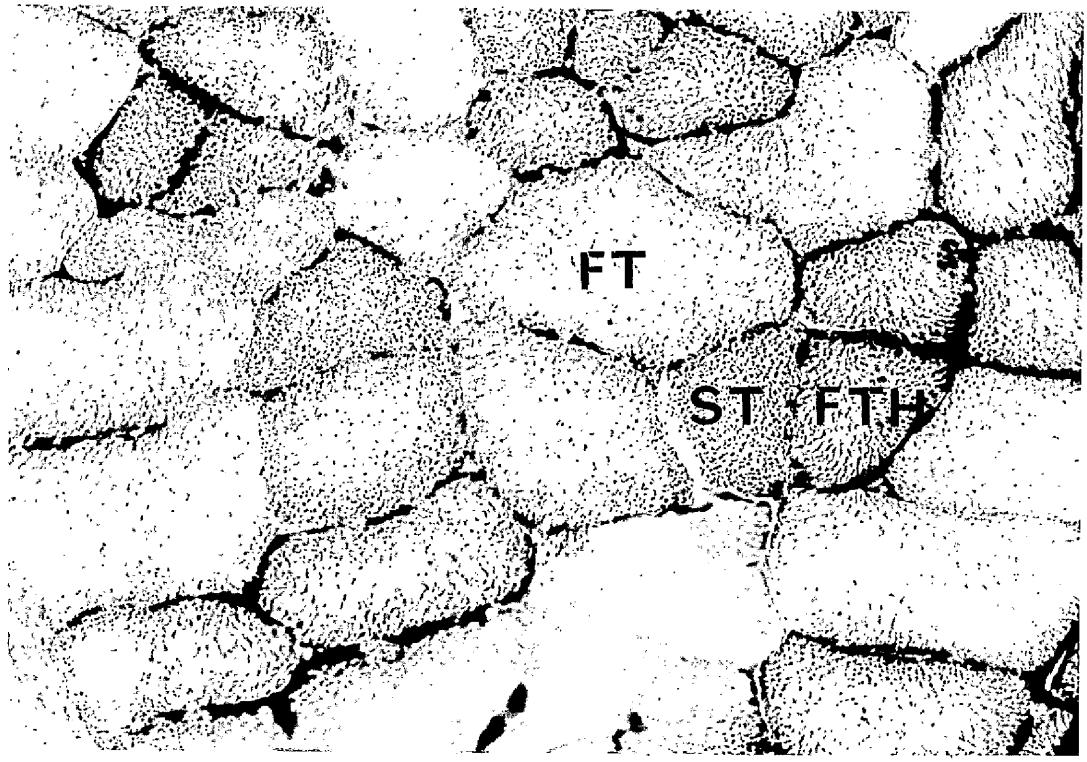
Table 5.1 Percentage Fibre Types (Mean ± S.E.M.) Before and After 10 weeks Training for 6 Limb Muscles (n = numbers in brackets)

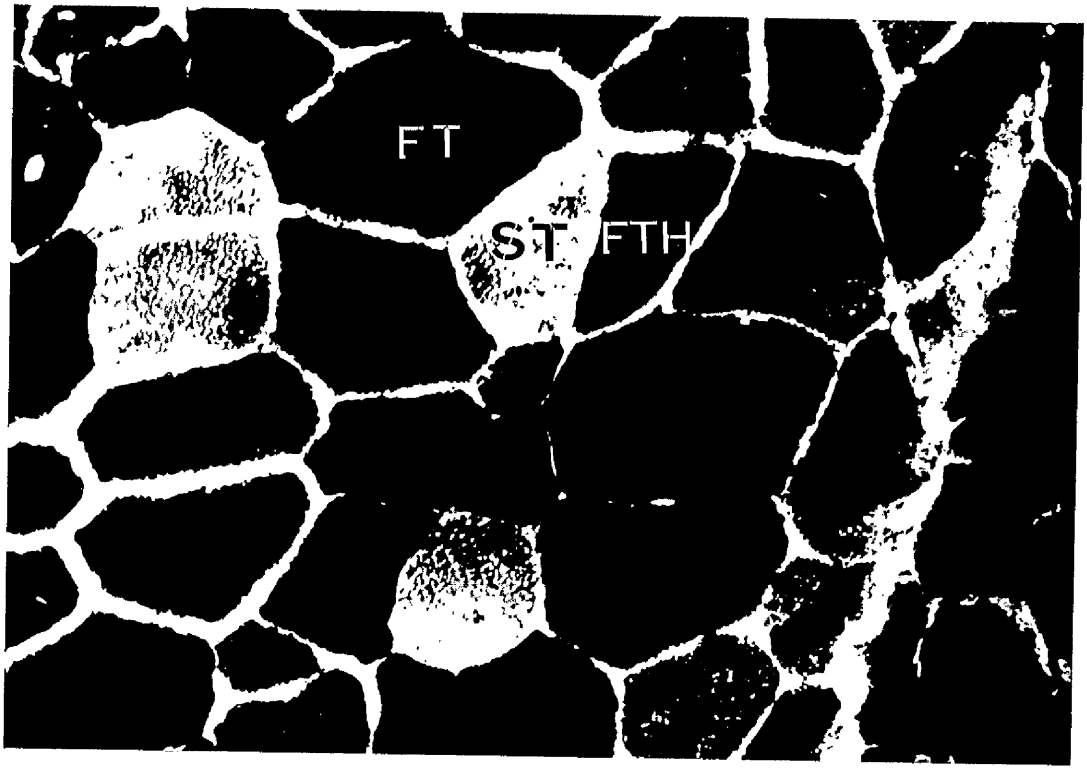
	ST		FTH		FT	
	Before	After	Before	After	Before	After
Deltoideus	35.3±4.1 (6)	28.4±3.9* (5)	27.7±4.1 (6)	41.9±5.0* (5)	37.0±3.9 (6)	29.6±3.4 (5)
Long Head Triceps	20.4±2.4 (6)	19.3±2.0 (5)	40.9±1.7 (6)	46.3±4.4 (5)	38.7±2.1 (6)	34.3±3.2 (5)
Lateral Vastus	11.5±1.9 (6)	6.9±2.5 (4)	46.5±3.2 (6)	50.6±3.6 (4)	42.0±2.5 (6)	38.8±1.2 (4)
Gluteus Medius	15.5±3.1 (6)	17.5±1.8 (6)	50.0±2.2 (6)	48.8±1.4 (6)	34.5±2.0 (6)	33.7±1.3 (6)
Biceps Femoris	19.6±2.0 (6)	16.9±2.4 (4)	43.0±2.9 (6)	49.7±4.5 (4)	37.4±1.7 (6)	33.4±1.2 (4)
Semitend-inosus	14.0±2.7 (6)	12.6±3.3 (5)	55.0±2.7 (6)	58.1±5.7 (5)	31.0±2.5 (6)	29.2±3.8 (5)
Mean	20.0±1.9 (36)	17.3±1.6* (29)	44.9±2.1 (36)	49.4±1.8* (29)	35.1±1.3 (36)	33.3±1.2* (29)

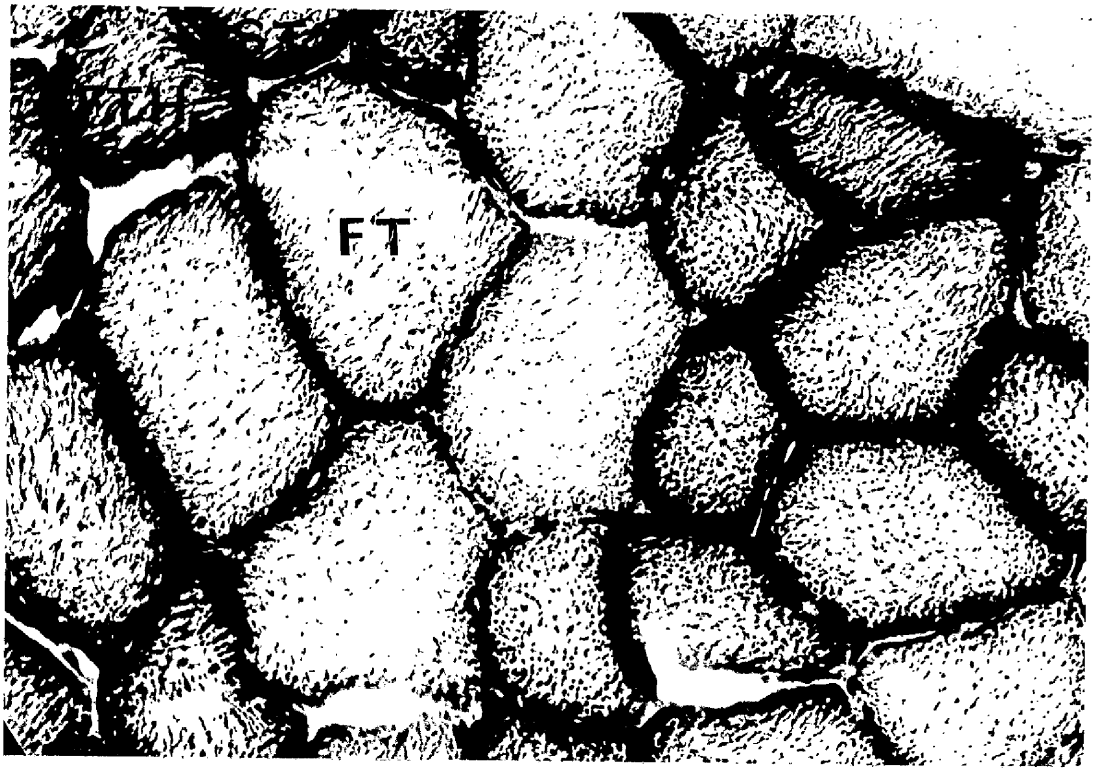
* = $P < 0.05$

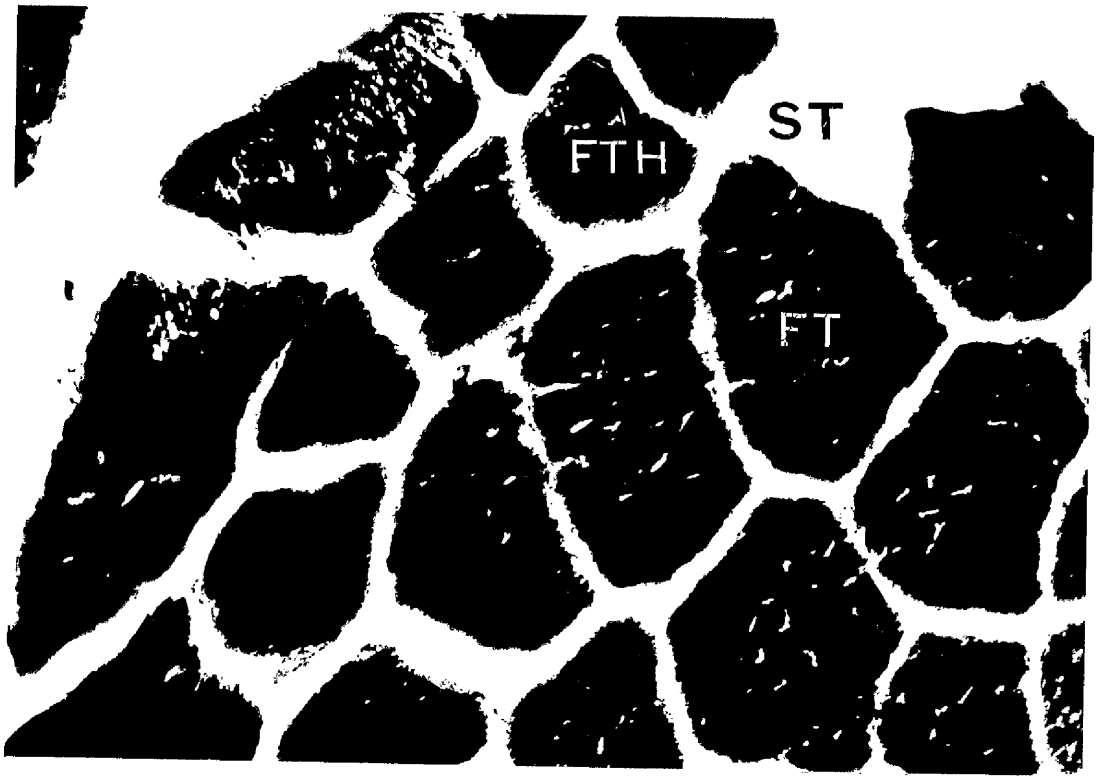
Fig 5.2: SDH activity in horse skeletal muscle (a) before and (b) after 10 weeks of training.
X 250.

a






FT


FTH
ST
FT

times during the training and detraining programme. Preliminary results showed that changes in enzyme activity were similar in the six muscles originally biopsied and so some of the enzymes were examined in only two muscle sites, the gluteus medius and the semitendinosus. As the number of subjects available was small, the mean activity of each enzyme for all the muscles examined at each period of sampling was also calculated to determine whether there was an overall change in activity for each enzyme with training and detraining. As the changes found with training were much larger than the errors in the methods, they have been considered as a real effect due to training.

CS activity was found to have almost doubled by the end of the training programme. The activity increased by the same amount, about 25%, in both the first five weeks and second five weeks training period. With the four Thoroughbreds, the extra five weeks of sprint training increased the activity further until the final increase as compared to week 0 was about 100% (Table 5.2: Fig 5.3). The activity changes in HAD, the other enzyme examined, which is situated only in the mitochondria were remarkably similar to those of CS (Fig 5.3). An approximately 50% increase was found after ten weeks training with a further 50% increase in the four Thoroughbreds after the additional five weeks of training (Table 5.3).

The glycolytic enzyme ALD, did not increase as much as the oxidative enzymes with training. The greatest increase occurred in the first five weeks of training (Table 5.4: Fig 5.4) with the activity increasing by 35% as compared to only 55% in the second five weeks when

Table 5.2 Effect of Training and Detraining on Citrate Synthase Activity ($\mu moles.min^{-1}.g$ dry weight $tissue^{-1}$) for 6 Limb Muscles. (Mean $\pm$ S.E.M. n = 6 (week 15, n = 4))

	Training				Detraining	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Deltoideus	9.62 ± 0.76	12.58 ± 1.03	15.54 ± 1.77*	19.83 ± 1.48*	16.83 ± 2.56	21.83 ± 2.95
Long Head Triceps Brachii	13.82 ± 0.97	20.33 ± 1.01*	21.30 ± 1.46*	24.95 ± 1.25*	21.00 ± 2.49	22.75 ± 2.48
Vastus Lateralis	8.02 ± 1.17	9.00 ± 1.20	10.74 ± 0.94*	18.00 ± 3.43*	11.25 ± 2.46	13.50 ± 2.29
Gluteus Medius	15.84 ± 1.12	17.11 ± 2.45	21.88 ± 2.34*	28.62 ± 1.52*	16.78 ± 3.56+	25.25 ± 3.72
Biceps Femoris	16.36 ± 1.64	19.55 ± 2.55	22.29 ± 2.86*	30.88 ± 4.25*	24.42 ± 2.88	26.20 ± 3.80
Semitendinosus	12.92 ± 1.82	18.30 ± 2.45	18.95 ± 1.77*	27.00 ± 1.68*	19.91 ± 2.78+	17.08 ± 1.67
Mean	12.76 ± 0.71	16.15 ± 1.18*	19.42 ± 1.02*	25.08 ± 1.36*	18.64 ± 1.22+	20.95 ± 1.32

* = $P < 0.05$ compared to week 0

+ = $P < 0.05$ 5 weeks detrained are compared to last week of training

Table 5.3 Effect of Training and Detraining on 3-Hydroxyacyl CoA Dehydrogenase Activity ($\mu moles.10^{-1}.min^{-1}.g$ dry weight of $tissue^{-1}$) for 2 Limb Muscles. (Mean $\pm$ S.E.M., n = 6 (week 15, n = 4))

	Training			Detraining	
Muscle	0 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Gluteus Medius	14.8 ± 1.8	22.1 ± 3.0*	23.4 ± 3.0*	14.9 ± 2.3+	19.3 ± 1.6
Semitendinosus	10.9 ± 1.3	16.3 ± 1.2*	25.4 ± 2.4*	13.1 ± 2.2+	14.4 ± 1.9
Mean	12.8 ± 1.2	19.0 ± 1.8*	24.4 ± 1.8*	14.1 ± 1.6+	16.9 ± 1.4

* = $P < 0.05$ compared to week 0

+ = $P < 0.05$ 5 weeks detrained are compared to last week of training

Table 5.4 Effect of Training and Detraining on Aldolase Activity ($\mu moles.10^{-1}.min^{-1}.g$ dry weight tissue^{-1}) for 6 Limb Muscles (Mean $\pm$ S.E.M., n = 6 (week 15, n = 4))

	Training				Detraining	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Deltoideus	18.38 ± 2.12	25.43 ± 1.83*	30.52 ± 4.43*	27.83 ± 1.92	28.88 ± 3.63	38.05 ± 3.50
Long Head Triceps Brachii	24.00 ± 2.25	34.43 ± 3.23*	40.00 ± 2.25*	38.40 ± 5.33*	33.90 ± 2.35+	40.00 ± 2.15
Vastus Lateralis	24.86 ± 3.14	33.24 ± 3.09	40.48 ± 2.16*	42.78 ± 4.35*	35.37 ± 2.23+	43.65 ± 2.57
Gluteus Medius	31.82 ± 2.25	41.02 ± 2.33*	46.18 ± 2.28*	47.50 ± 3.00*	41.14 ± 1.88+	49.70 ± 2.20
Biceps Femoris	24.92 ± 1.90	33.60 ± 2.15*	38.97 ± 2.82*	40.10 ± 2.05*	34.93 ± 1.43	41.80 ± 4.49
Semitendinosus	31.05 ± 2.57	40.87 ± 2.62	45.17 ± 3.08*	49.30 ± 2.52*	40.78 ± 2.27	50.40 ± 1.65
Mean	25.50 ± 1.20	34.70 ± 13.25*	40.20 ± 14.06*	41.50 ± 12.01*	35.80 ± 1.15+	44.00 ± 1.35

* = $P < 0.05$ compared to week 0

+ = $P < 0.05$ 5 weeks detrained are compared to last week of training

Fig 5.3: Time course of the percentage change in CS, HAD and AST activity with training and detraining.

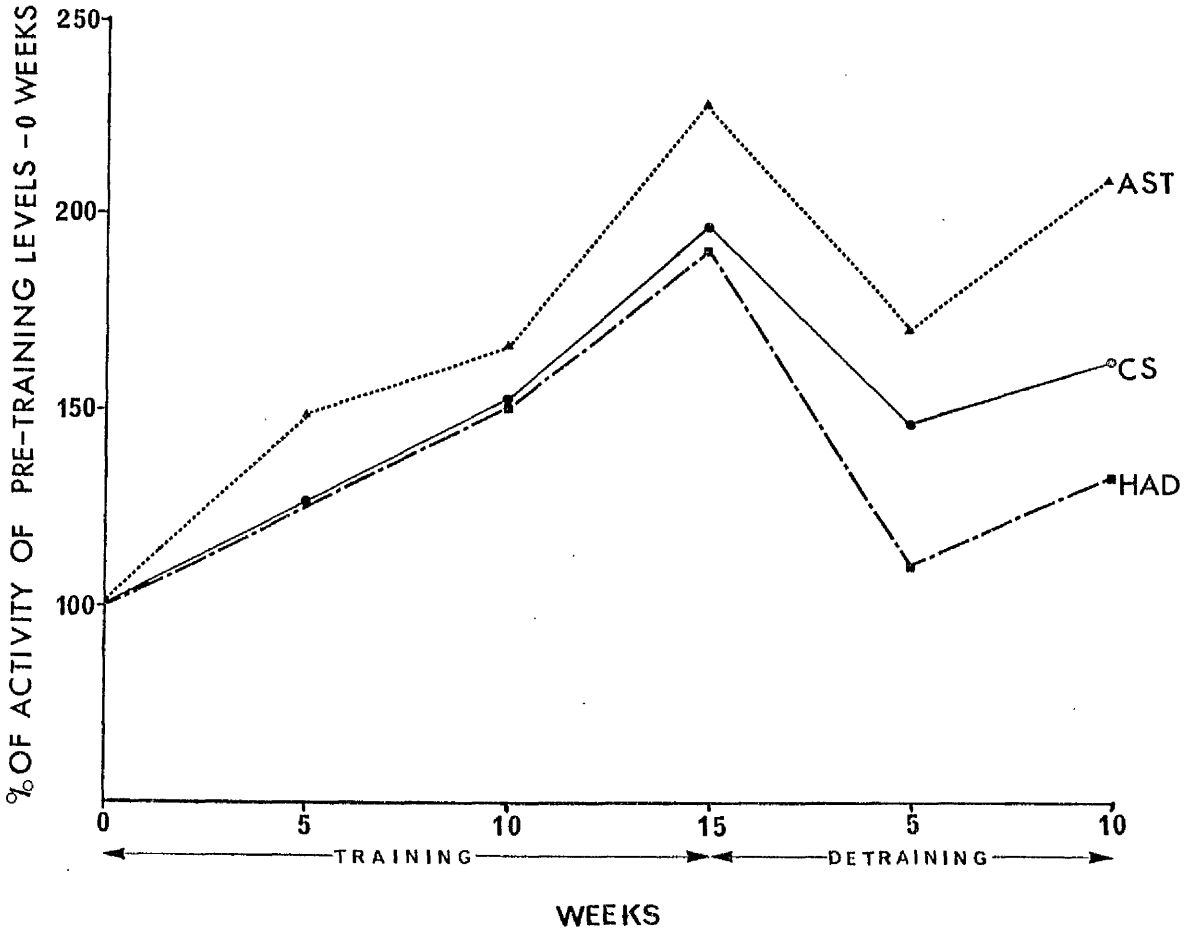
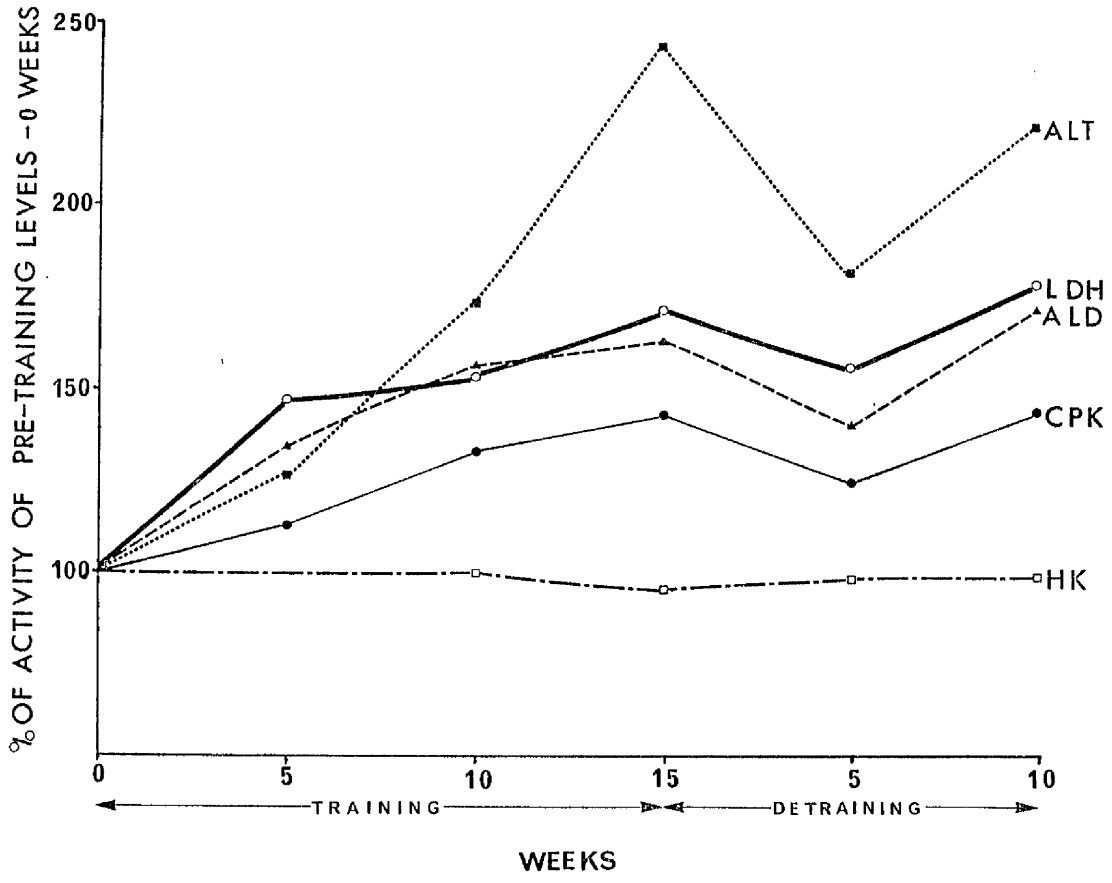

% OF ACTIVITY OF PRE-TRAINING LEVELS - 0 WEEKS
250
200
150
100
AST
CS
HAD
0
5
10
15
5
10
TRAINING
DETRAINING
WEEKS

Fig 5.4: Time course of the percentage change in ALD, HK, LDH, ALT and CPK activity with training and detraining.


% OF ACTIVITY OF PRE-TRAINING LEVELS - 0 WEEKS
250
200
150
100
ALT
LDH
ALD
CPK
HK
0
5
10
15
5
10
TRAINING
DETRAINING
WEEKS

both periods are compared to week 0. Perhaps surprisingly the five weeks of sprint training had little effect on ALD. The other glycolytic enzyme examined was HK which was little changed by training. The activity of this enzyme remained at about 13.0 μmoles g.dry weight of tissue^{-1}.minute^{-1} (Table 5.5: Fig 5.4).

Apart from CS, the two other enzymes which are involved with the immediate fate of pyruvate were LDH and ALT. Both of these enzymes increased with training. As with ALD, the greatest increase in LDH occurred in the first five weeks (over 45%) with a much smaller increase in the second (52%) and third five weeks (71%). In contrast, ALT increased less than 20% in the first five weeks of training but increased markedly during the second five weeks by over 75% when compared to week 0. The increase in the activity of ALT during the sprint period was even greater with the activity eventually reaching 2.5 times resting values at week 0 (Tables 5.6 and 5.7: Fig 5.4).

In order to clarify the changes underlying the overall change in LDH activity, the composition of the various LDH isoenzymes in the muscle were examined during the training and detraining programme. Skeletal muscle LDH in this study was found to consist of five separate isoenzymes with by far the greatest activity being found in LDH_5, the most muscle specific isoenzyme (Fig 5.5). It was not always possible to detect the activity of LDH_1 by densitometric methods. Training increased the percentage of $LDH_{1,2,3}$ and $_4$ and decreased that of LDH_5 with a consequent increase in the percentage of H subunits (Tables 5.8 and 5.9).

When the activities of the subunits were examined, it was found that the activity of both subunits increased, with the percentage increase

Table 5.5 Effect of Training and Detraining on Hexokinase Activity ($\mu moles.min^{-1}.g$ dry weight of $tissue^{-1}$) for 2 Limb Muscles (Mean $\pm$ S.E.M., n = 6 (week 15, n = 4))

	Training			Detraining	
Muscle	0 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Gluteus Medius	12.8 ± 0.4	13.0 ± 0.6	12.4 ± 1.1	12.3 ± 0.4	12.7 ± 0.8
Semitendinosus	13.4 ± 0.5	13.1 ± 0.4	12.5 ± 0.4	13.6 ± 0.4	12.8 ± 0.4
Mean	13.1 ± 0.3	13.1 ± 0.3	12.5 ± 0.5	12.9 ± 0.4	12.7 ± 0.4

Table 5.6 Effect of Training and Detraining on Lactic Dehydrogenase Activity ($\mu moles.10^{-2}.min^{-1}$. g dry weight $tissue^{-1}$) for 6 Limb Muscles (Mean $\pm$ S.E.M., n = 6 (week 15, n = 4)

	Training				Detraining	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Deltoideus	10.93 ± 1.39	17.38 ± 1.47*	19.57 ± 2.15*	20.39 ± 2.17*	19.56 ± 2.42	24.15 ± 2.54
Long Head Triceps Brachii	15.04 ± 2.00	23.13 ± 3.04*	25.06 ± 2.13*	22.53 ± 2.23*	23.24 ± 2.69	27.95 ± 2.51
Vastus Lateralis	17.50 ± 0.81	24.47 ± 2.78	28.54 ± 2.94*	33.47 ± 3.70*	27.27 ± 2.42	30.02 ± 2.38
Gluteus Medius	23.93 ± 2.07	33.73 ± 1.90*	27.55 ± 1.71*	37.84 ± 2.84*	33.82 ± 1.93	39.13 ± 1.71
Biceps Femoris	17.27 ± 1.18	24.98 ± 2.13*	27.52 ± 1.71*	30.74 ± 0.45*	28.30 ± 1.25	29.84 ± 1.59
Semitendinosus	21.48 ± 2.84	32.68 ± 2.91	31.99 ± 1.59*	34.88 ± 1.15*	34.09 ± 1.69	37.80 ± 2.85
Mean	17.69 ± 0.99	26.05 ± 1.32*	26.74 ± 1.48*	30.39 ± 1.57*	27.73 ± 1.19+	31.63 ± 1.24

* = $P < 0.05$ compared to week 0

+ = $P < 0.05$ 5 weeks detrained are compared to last week of training.

Table 5.7 Effect of Training and Detraining on Alanine Aminotransferase Activity ($\mu moles.min^{-1}.g$ dry weight $tissue^{-1}$) for 6 Limb Muscles (Mean ± S.E.M. n = 6 (week 15, n = 4))

	Trained				Detrained	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Deltoideus	31.30 ± 3.45	28.98 ± 3.79	46.28 ± 5.52*	61.00 ± 2.94*	61.15 ± 10.97	73.25 ± 13.17
Long Head Triceps Brachii	30.07 ± 4.94	47.13 ± 8.04*	61.15 ± 4.60*	64.90 ± 3.93*	69.00 ± 9.98	78.20 ± 15.30
Vastus Lateralis	27.30 ± 4.87	28.96 ± 4.06	38.88 ± 6.93	62.55 ± 7.91*	38.06 ± 6.04	51.53 ± 10.89
Gluteus Medius	33.48 ± 4.99	37.78 ± 7.15	65.70 ± 8.10*	92.37 ± 11.15*	56.11 ± 9.36	76.18 ± 15.63
Biceps Femoris	33.45 ± 4.50	44.40 ± 8.00	63.88 ± 9.84*	90.67 ± 8.08*	70.18 ± 10.73	77.05 ± 19.73
Semitendinosus	33.90 ± 4.45	40.00 ± 5.00	53.95 ± 4.51*	86.90 ± 8.76*	62.33 ± 11.97	65.40 ± 8.21
Mean	31.50 ± 1.70	37.80 ± 2.60*	54.90 ± 3.00*	77.00 ± 4.00*	57.40 ± 4.10+	70.20 ± 5.60

* = P < 0.05 compared to week 0

+ = P < 0.05 5 weeks detrained are compared to last week of training

Table 5.8 Effect of Training and Detraining on the % of LDH Isoenzymes in Horse Gluteus Medius Muscle

(Mean ± S.E.M.; 6 horses (4 horses week 15))

	I	II	III	IV	V	M Subunits
0 weeks	0.90 ± 0.14	1.83 ± 0.37	4.20 ± 0.88	7.70 ± 2.00	85.37 ± 2.12	93.68 ± 0.96
10 weeks	1.76 ± 0.43	3.13 ± 0.91	6.82 ± 1.62	9.27 ± 1.18	79.02 ± 3.50	90.33 ± 1.99
15 weeks	2.51 ± 0.71*	3.06 ± 0.85	6.05 ± 1.53	13.43 ± 3.10	74.95 ± 2.41	88.80 ± 1.17*
5 weeks detraining	1.80 ± 0.84	2.88 ± 0.41	5.38 ± 0.91	7.57 ± 1.44	82.37 ± 1.61	91.58 ± 0.73
10 weeks detraining	0.92 ± 0.19	2.10 ± 0.47	5.30 ± 0.46	8.90 ± 2.66	82.78 ± 2.48	92.65 ± 0.71

* $P < 0.05$ vs 0 weeks

Table 5.9 Effect of Training and Detraining on the % of LDH Isoenzymes in Horse Semitendinosus Muscle (Mean ± S.E.M.; 6 horses (4 horses week 15))

	I	II	III	IV	V	M subunits
0 weeks	0.17 ± 0.32	1.42 ± 0.55	2.88 ± 0.77	3.23 ± 0.80	92.30 ± 1.85	96.33 ± 0.81
10 weeks	1.03 ± 0.23*	2.75 ± 0.77	6.68 ± 1.43	9.08 ± 1.42*	80.46 ± 2.69*	91.30 ± 1.11*
15 weeks	2.97 ± 1.20*	4.58 ± 1.10*	9.58 ± 0.90*	11.85 ± 1.35	73.02 ± 2.62*	86.35 ± 2.32*
5 weeks detraining	0.90 ± 0.34	2.52 ± 0.45	6.17 ± 1.00*	8.51 ± 1.43*	81.90 ± 1.79*	92.00 ± 0.91
10 weeks detraining	0.30 ± 0.89	0.90 ± 0.34	3.63 ± 0.60	7.18 ± 1.67	87.99 ± 2.38	92.25 ± 0.85

* $P < 0.05$ vs 0 weeks

being greater in the H subunits. As the changes in the activities of the H and M subunits and total LDH were similar in the 2 muscles examined the results have been combined as shown in Fig 5.6.

The other enzyme connected with anaerobic energy production, CPK, increased by just over 30% by the end of the training programme. This enzyme increased by about 12% in the first five weeks and by 30% in the second five weeks as compared to week 0 (Table 5.10 : Fig 5.4). Again, as with LDH and ALD, very little alteration in the activity of CPK occurred during the last five weeks of training in the four Thoroughbreds.

Enzymes from the two extramitochondrial NADH "shuttles" known to exist in skeletal muscle were also examined. These were GDH from the "Glycerol-3-phosphate shuttle" and AST from the "Malate-Aspartate shuttle." Perhaps surprisingly, different effects were observed in the two shuttles with GDH being unaffected by training (Table 5.11 : Fig 5.7) whereas AST increased markedly, eventually attaining a value approximately 2.5 times that of the pre-training value. The greatest increase in AST occurred during the sprint training with an 80% change being found when compared to week 0 (Table 5.12 : Fig 5.7).

All of the enzymes previously mentioned have been concerned mainly with catabolism and the production of energy. GS, on the other hand, is one of the major controlling enzymes in the formation of glycogen. As with other studies, GS activity was found to be considerably enhanced by the addition of glucose-6-phosphate to the incubation medium (Table 5.13). The percentage of GS in the a-form was about 10% and this percentage did not vary greatly with training. Similarly, the activity of the a-form and

Table 5.10 Effect of Training and Detraining on Creatine Phosphokinase Activity ($\mu moles.10^{-3}.min^{-1}.g$ dry weight $tissue^{-1}$) for 6 Limb Muscles (Mean $\pm$ S.E.M. n = 6 (week 15, n = 4))

	Training				Detraining	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Deltoideus	14.39 ± 0.96	16.66 ± 0.82	19.53 ± 0.95*	20.63 ± 0.31	19.48 ± 1.66	23.64 ± 1.61
Long Head Triceps Brachii	16.21 ± 1.04	18.75 ± 1.48	21.57 ± 1.13*	21.20 ± 0.24	18.97 ± 1.35	22.75 ± 1.49
Vastus Lateralis	15.68 ± 0.90	17.11 ± 1.07	20.66 ± 1.55*	22.58 ± 1.08*	18.98 ± 0.85	21.99 ± 1.84
Gluteus Medius	16.95 ± 0.75	17.90 ± 1.34	21.34 ± 1.36*	23.17 ± 0.88*	19.88 ± 0.81	23.82 ± 1.73
Biceps Femoris	16.77 ± 0.87	18.73 ± 0.83	21.28 ± 2.06	23.60 ± 1.03*	20.97 ± 0.88	23.28 ± 2.12
Semitendinosus	14.86 ± 0.54	16.58 ± 1.23	20.26 ± 0.70*	22.99 ± 0.84*	20.34 ± 1.16	24.81 ± 0.99
Mean	15.79 ± 0.36	17.63 ± 0.46*	20.78 ± 0.53*	22.43 ± 0.37*	19.77 ± 0.45+	22.78 ± 0.65

* = $P < 0.05$ compared to week 0

+ = $P < 0.05$ 5 weeks detrained are compared to last week of training.

Table 5.11 Effect of Training and Detraining on Glycerol-3-Phosphate Dehydrogenase Activity (μmoles $10^{-1}.min^{-1}.g$ dry weight of $tissue^{-1}$) for 2 Limb Muscles (Mean $\pm$ S.E.M., n = 6 (week 15, n = 4))

	Training			Detraining	
	0 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Gluteus Medius	11.3 ± 1.4	10.1 ± 0.8	9.5 ± 0.5	10.5 ± 0.4	9.6 ± 0.7
Semitendinosus	8.34± 0.9	9.1 ± 1.2	8.2 ± 0.4	8.2 ± 0.5	8.1 ± 0.7
Mean	9.32 ± 0.6	9.6 ± 0.7	8.9 ± 0.5	9.3 ± 0.4	8.8 ± 0.5

Table 5.12 Effect of Training and Detraining on Aspartate Aminotransferase Activity (μmoles $10^{-1}.min^{-1}.g$ dry weight of $tissue^{-1}$) for 6 Limb Muscles (Mean ± S.E.M. n = 6 (week 15, n = 4))

	Trained				Detrained	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Deltoideus	36.35 ± 5.00	52.03 ± 7.66*	70.15 ± 5.02*	87.27 ± 5.34*	71.77 ± 11.30	92.60 ± 12.1
Long Head Triceps Brachii	44.23 ± 5.77	75.58 ± 12.82*	98.48 ± 7.86*	104.63 ± 5.31*	76.62 ± 11.44	96.47 ± 12.(
Vastus Lateralis	35.12 ± 6.07	45.28 ± 7.57	55.03 ± 8.53*	78.17 ± 6.30*	53.13 ± 11.79	62.90 ± 12.1
Gluteus Medius	51.73 ± 6.97	67.03 ± 1.52	78.52 ± 6.18	122.15 ± 9.35*	77.18 ± 11.87+	103.72 ± 15.(
Biceps Femoris	52.98 ± 9.79	76.10 ± 12.62	69.28 ± 7.89	114.15 ± 12.19*	96.08 ± 10.74	104.95 ± 17.3
Semitendinosus	43.66 ± 5.66	74.10 ± 10.52*	68.45 ± 5.12	119.32 ± 9.34*	91.71 ± 15.52	88.15 ± 9.69
Mean	44.00 ± 2.70	65.00 ± 4.70*	73.30 ± 3.40*	105.00 ± 4.60*	74.90 ± 5.30+	91.40 ± 5.60

* = P < 0.05 compared to week 0

+ = P < 0.05 5 weeks detrained are compared to last week of training.

Table 5.13 Effect of Training and Detraining on Glycogen Synthase Activity ($\mu moles.min^{-1}.g$ dry weight of $tissue^{-1}$) for 2 Limb Muscles (Mean ± S.E.M., n = 6 (week 15, n = 4))

		Training			Detraining	
	Muscle	0 weeks	10 weeks	15 weeks	5 weeks	10 weeks
a - form	Gluteus Medius	2.0 ± 0.3	2.2 ± 0.3	2.2 ± 0.3	1.4 ± 0.2	2.2 ± 0.3
	Semitendinosus	1.6 ± 0.3	2.0 ± 0.3	1.5 ± 0.3	1.8 ± 0.3	1.8 ± 0.2
	Mean	1.8 ± 0.2	2.1 ± 0.2	1.8 ± 0.2	1.6 ± 0.2	2.0 ± 0.2
a+b-form	Gluteus Medius	19.2 ± 2.4	22.4 ± 4.4	15.3 ± 1.8	17.5 ± 1.5	21.7 ± 3.4
	Semitendinosus	17.0 ± 1.7	21.3 ± 3.4	18.8 ± 2.2	17.9 ± 2.5	13.8 ± 1.5
	Mean	18.1 ± 1.5	21.8 ± 2.7	17.1 ± 1.5	17.7 ± 1.4	17.7 ± 2.3
%a - form	Gluteus Medius	10.5 ± 0.6	10.1 ± 0.9	15.0 ± 3.0	8.0 ± 0.5	10.4 ± 1.1
	Semitendinosus	9.3 ± 1.5	10.0 ± 1.3	7.6 ± 0.6	10.9 ± 1.9	14.0 ± 2.0
	Mean	9.9 ± 0.8	10.0 ± 0.8	11.3 ± 1.9	9.4 ± 1.0	12.2 ± 1.2

Fig 5.5: The effect of training and detraining on poly-acrylamide gel separations of LDH isoenzymes. (bl = blank; 0, 10 and 15 = weeks of training; 5d and 10d = weeks of detraining).

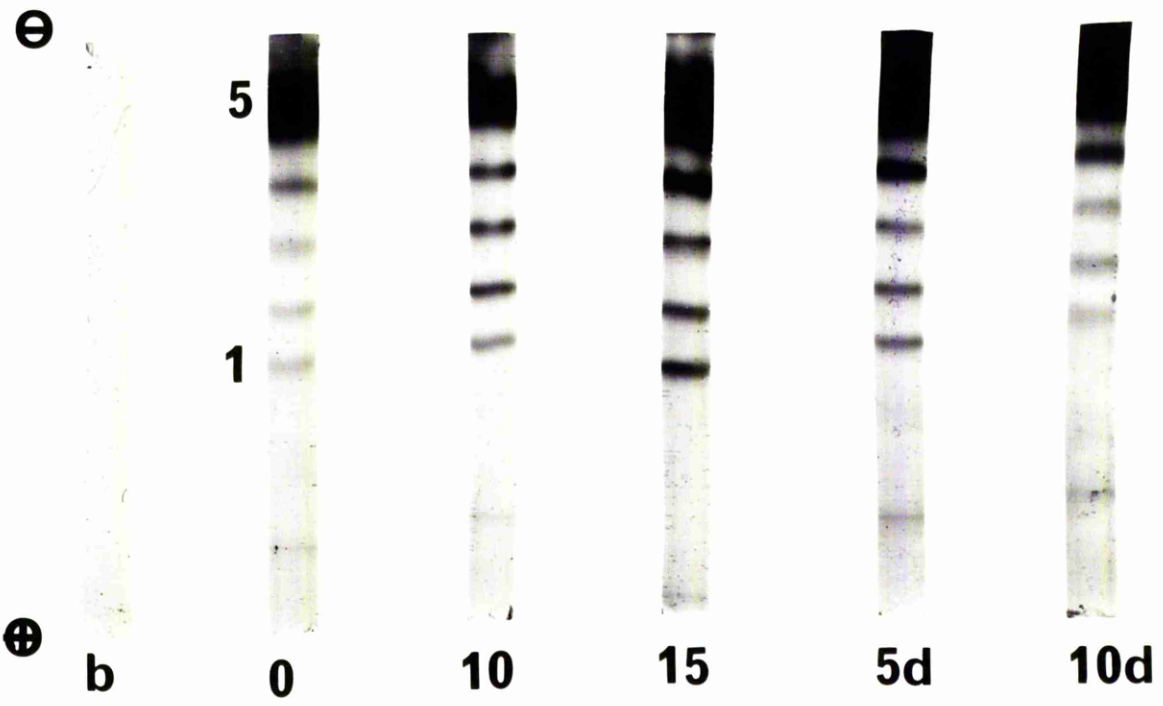

⊖
5
1
⊕
b
0
10
15
5d
10d

Fig 5.6: The effect of training and detraining on total LDH activity and the activity of the H and M subunits.

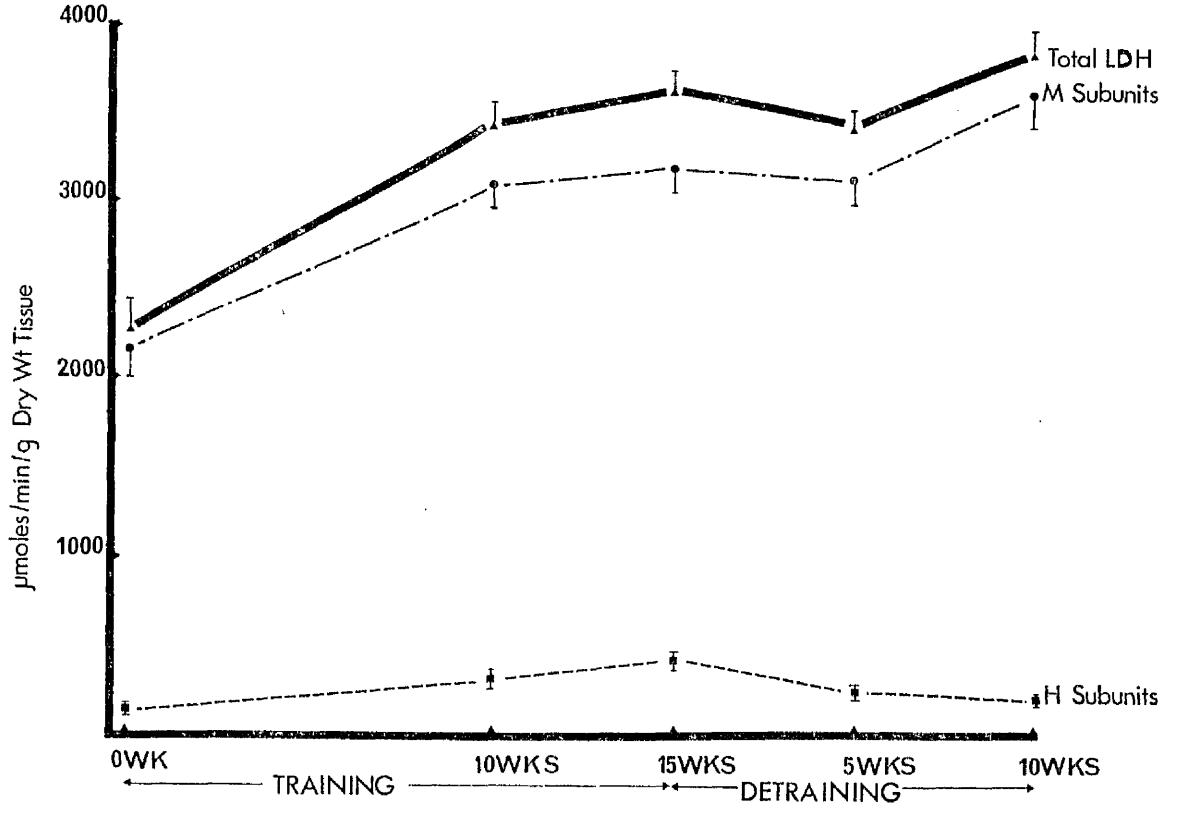
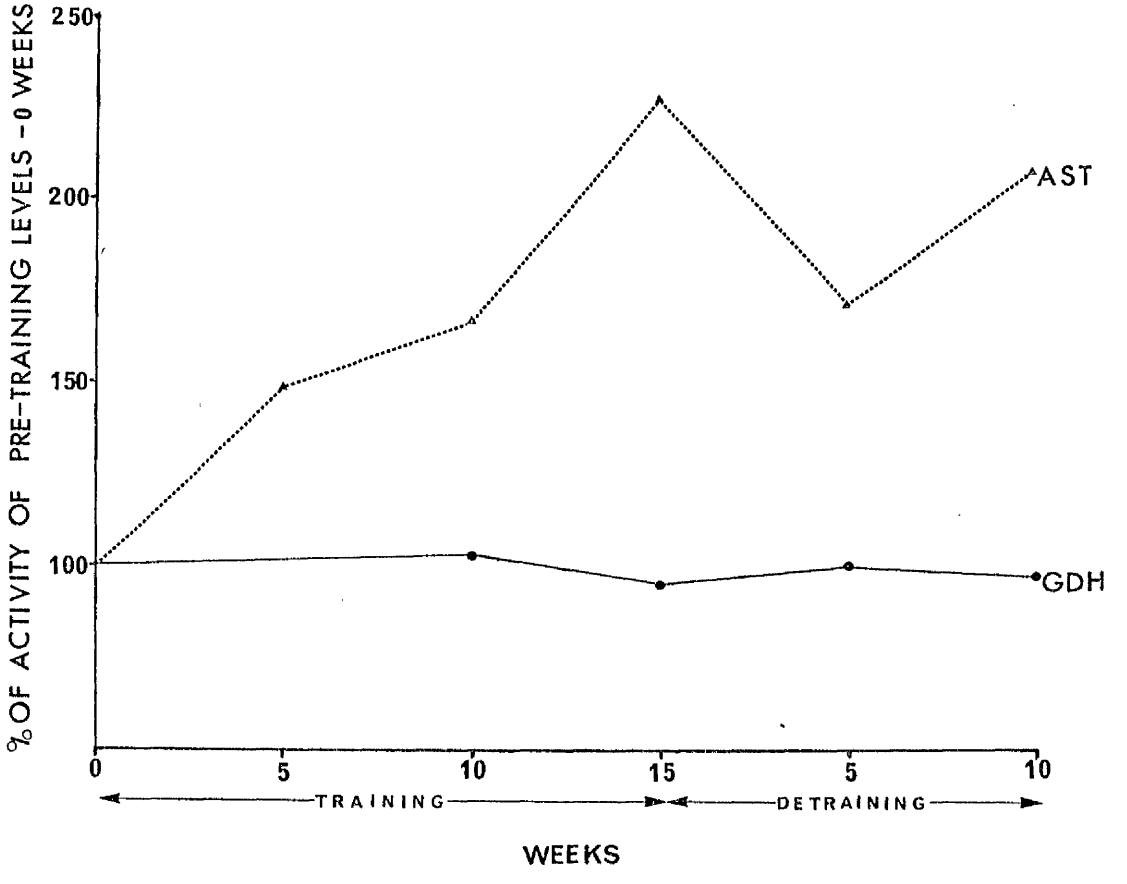

4000
3000
2000
1000
µmoles/min/g Dry Wt Tissue
Total LDH
M Subunits
H Subunits
0WK
10WKS
15WKS
5WKS
10WKS
TRAINING
DETRAINING

Fig 5.7: Time course of the percentage change in the activity of the "NAD-shuttle" enzymes, GDH and AST with training and detraining.


% OF ACTIVITY OF PRE-TRAINING LEVELS - 0 WEEKS
250
200
150
100
AST
GDH
0
5
10
15
5
10
TRAINING
DETRAINING
WEEKS

the a-b forms were unaffected with training (Table 5.13 : Fig 5.8).

The concentration of glycogen itself increased, although this increase was not significant in the various muscles until week 15 (Table 5.14). If all of the muscles were considered together, the increase in glycogen concentration was significant by week 5.

Total protein content was also assayed during training, but no significant change was found (Table 5.15).

Detraining

After five weeks of detraining, all of the mean enzyme activities of those enzymes which had increased with training had decreased significantly over the corresponding levels at the end of the training programme (week 10 for the heavy hunters and week 15 for the Thoroughbreds), but in most cases were still significantly higher than week 0 levels. The exception was HAD which had decreased in activity to a similar activity to week 0 levels. Enzymes which had been unaffected by training were similarly unaffected by detraining.

Surprisingly, however, following ten weeks detraining, an opposite effect to that seen at week 5 detraining occurred in the enzymes which had increased in activity with training. This increase was found to be significant in ALD, LDH, ALT and AST but not significant in CS and HAD.

Glycogen levels remained elevated over those at week 0 during the detaining period and protein was unaffected by detraining.

Table 5.14 Effect of Training and Detraining on Glycogen concentration (μmoles.g dry weight tissue^{-1}) for 6 Limb Muscles (Mean $\pm$ S.E.M., n = 6 (week 15, n = 4))

	Training				Detraining	
Muscle	0 weeks	5 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Deltoideus	265 ± 19.8	326 ± 18.8	271 ± 27.7	350 ± 5.8	297 ± 32.9	320 ± 22.7
Long Head Triceps Brachii	297 ± 15.4	376 ± 12.7	360 ± 30.3	444 ± 23.2*	408 ± 31.4	347 ± 15.4
Vastus Lateralis	280 ± 18.4	294 ± 17.2	278 ± 17.2	322 ± 9.9	289 ± 21.1	276 ± 27.5
Gluteus Medius	348 ± 27.8	367 ± 40.6	374 ± 27.7	459 ± 6.6	399 ± 27.7	429 ± 16.4
Biceps Femoris	299 ± 13.4	328 ± 22.3	374 ± 23.2	448 ± 28.9*	397 ± 22.7	436 ± 16.4
Semitendinosus	323 ± 25.4	340 ± 23.0	376 ± 25.5	479 ± 5.5*	406 ± 26.2	351 ± 20.1
Mean	302 ± 9.0	338 ± 11.3*	339 ± 12.4*	418 ± 15.0*	366 ± 13.5	361 ± 12.1

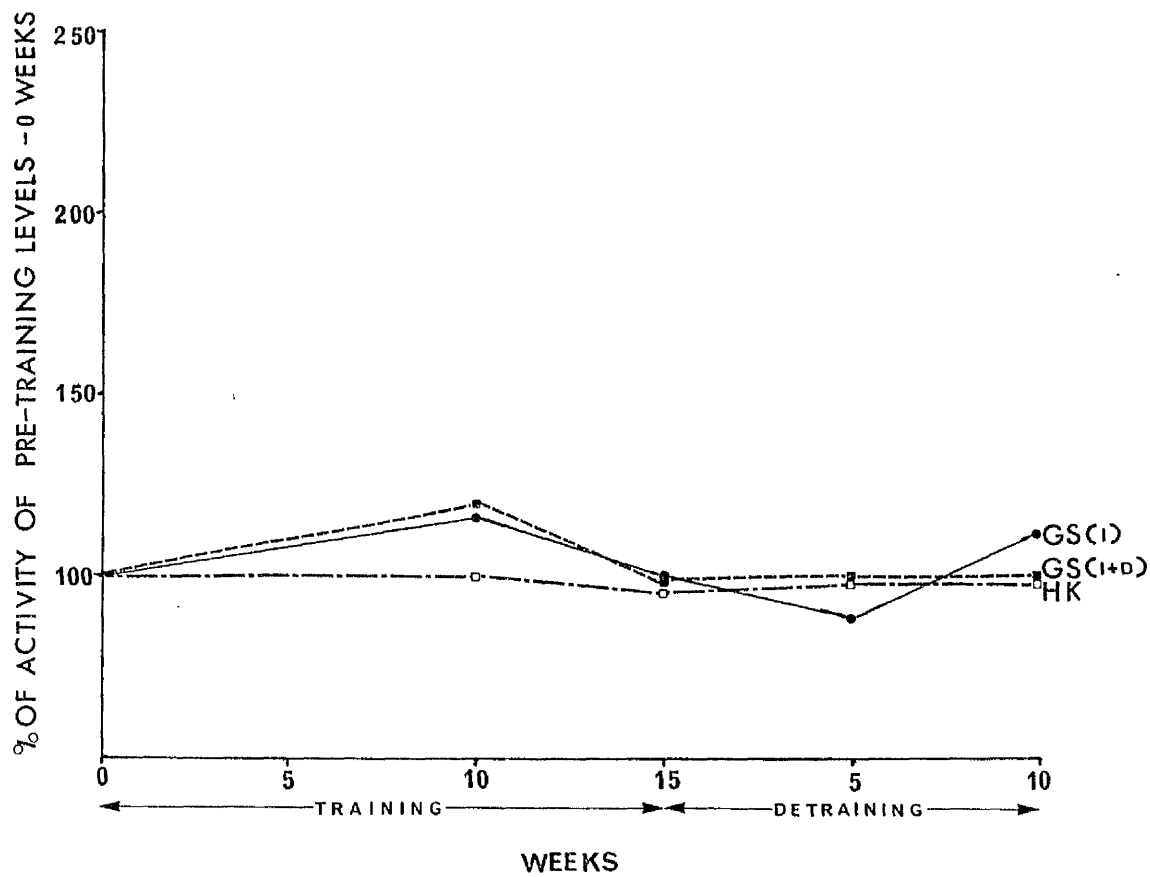
* $P < 0.05$ compared to week 0

+ $P < 0.05$ 5 weeks detrained are compared to last week of training

	Training			Detraining	
Muscle	0 weeks	10 weeks	15 weeks	5 weeks	10 weeks
Gluteus Medius	550±41	568±48	545±26	549±24	586±48
Semitendinosus	520±25	579±31	598±45	619±45	597±54
Mean	535±23	573±35	571±26	584±26	591±35

Table 5.15: Effect of Training and Detraining on Protein Concentration (μmoles.(gm dry weight of tissue)$^{-1}$) for 2 Limb Muscles (Mean ± S.E.M. n = 6 (week 15 n = 4)).

Fig 5.8: Time course of the percentage change in GS and HK activity with training and detraining.


% OF ACTIVITY OF PRE-TRAINING LEVELS - 0 WEEKS
250
200
150
100
GS(I)
GS(I+D)
HK
0
5
10
15
5
10
TRAINING
DETRAINING
WEEKS

Discussion

Histochemistry

It appears from this study, that although there is a significant increase in the percentage of FTH fibres at the expense of ST and FT fibres, training has only a small influence on the fibre composition of the muscles examined, with the possible exception of the deltoideus. When the intensity of the oxidative stain is examined, however, there appears to be an increase in the intensity of staining in all of the fibres rather than in one particular fibre type. This indicates that the difference between fibre types remains constant. A similar result was found in the miniature pig (Fitts et al., 1973) and Lindholm and Piehl (1974) observed only a slight increase between trained and untrained Standardbred horses. These findings are, however, in disagreement with those of Edgerton et al. (1969), Barnard et al. (1970), Morgan et al. (1971), Faulkner et al. (1972) and Prince et al. (1976) who reported an increase in high oxidative fibres with training. An explanation for these different findings may be that enzyme histochemistry is a qualitative technique from which one can estimate the oxidative capacity of a fibre in a given muscle. It is inadequate for quantitating changes due to training, however, as many factors, including the time which elapsed between cutting and staining, can influence the staining intensity (Spamer and Pette, 1977). Further proof of this inadequacy comes from the work of Baldwin et al. (1972) who found that a two-fold increase in mitochondrial marker enzyme levels and oxidative capacity occurred in all three fibre types. When the muscles were examined histochemically, they found an apparent increase in "red" appearing fibres and a decrease in "white" appearing fibres. They attributed this discrepancy to insensitivity in the histochemical staining techniques.

The finding of a conversion of myosin ATPase fibres is interesting in that it is generally agreed that training has no effect on the high and low myosin ATPase activity at pH 9.4 (Edgerton et al., 1969 (rat); Barnard et al., 1970 (guinea pig); Edgerton et al., 1972 (lesser bush baby); Fitts et al., 1973 (miniature pig); Gollnick et al., 1973 (man)). The only studies where training has induced a change in the high/low myosin ATPase ratio have been by Syrovy et al. (1972) who reported a conversion from low to high myosin ATPase at pH 9.4 fibres in the soleus of rats in response to swimming and Muller (1974) who reported the reverse happening in rat soleus in response to treadmill running. Both of these studies were with young animals and as Syrovy et al. (1972) did not find the same conversion with adult rats, it appears that at least in these studies age has an effect.

A change in the contractile properties of a muscle has been achieved by cross-innervation (Buller et al., 1960b), long term electrical stimulation (Salmons and Vbrova, 1969) and removal of synergistic action (Vbrova, 1963). It has been found by cross-innervation of the slow contracting soleus muscle with a fast motor nerve (designated X-SOL) that the physiological, histochemical and biochemical data all indicate that the X-SOL has become fast contractile. The reverse effect was found when the fast flexor hallucis longus was innervated with a slow innervating nerve (designated X-FHL) but the effect took much longer to manifest itself. It is also possible to implant a stimulator which subjects a fast muscle to low frequency pulses similar to those of a slow nerve impulse pattern and produce a slowing of the muscle as indicated by histochemical, biochemical and physiological measurements. Due to the

nature of the slow motorneurone impulses, it is not possible to impose a fast motorneurone impulse pattern (Salmons and Vbrova, 1969).

If the spinal column of a few days old animal is isolated to prevent the motorneurons from firing at all, muscles which would normally have become slow contracting, develop as fast contracting (Buller et al., 1960,a). A similar finding has been reported in humans who due to a lesion of the spinal cord are paretic in their lower extremities and have developed spasticity, have close to 100% fast contracting fibres in their vastus lateralis, soleus and gastrocnemius muscles, whereas their deltoideus has the usual mixed fibre pattern (Grimby, cited in Essen et al., 1975).

A change in the myosin ATPase properties of muscle fibres with training has been found in adult human subjects. By using different pH's, it is possible to divide the fast contracting fibres into two populations, called in human studies, type IIA and type IIB. This latter fibre usually has a lower oxidative capacity than the type IIA. Recently, it has been observed that endurance athletes have almost no type IIB fibres (Jansson, 1975; Nygaard-Jensen, 1976) and similarly, an eight week period of endurance training was shown to increase the percentage of type IIA fibres at the expense of the type IIB fibres (Andersen and Henricksson, 1977). According to Brooke and Kaiser (1970a) the difference in pH sensitivity of the myosin ATPase, which is the basis of the different staining patterns, is related to the reactivity of the sulphydryl groups in the myosin. It may be that the change in staining pattern in response to training is indicative of a change in the structure or composition of the myosin molecule.

Further evidence that training can alter the percentage of high to low myosin ATPase fibres comes from a study in cats exercised by making them lift weights (Gonyea et al., 1977). These authors found a significant increase in the total number of fibres in the trained flexor carpi radialis (FCR) when compared to the untrained FCR. There was also a 10% increase in the percentage of FTH fibres at the expense of both ST and FT fibres. This increase was not significant, but it was suggested that this was possibly due to the small sample size (only three cats) with a large variation. Fibre splitting in both slow twitch and fast twitch fibres was found, and Gonyea et al. (1977) suggested that the increase in the total number of fibres is due to a parallel increase in both types of fibres due to fibre splitting. Whether their data actually shows a parallel increase in view of the decrease in ST fibres (6.2%) may be open to question.

How does the change in fibre composition occur in this study? The increase in high myosin ATPase activity fibres may be due to the alterations in the level of contractile activity inducing a change in the myosin isoenzyme pattern. Baldwin et al. (1975) in fact provide evidence that this can happen with endurance training and Müller (1974) postulates that this change also occurs in the young rats in his study. This then prompts the further questions, does the motorneuron transform as suggested by Gerchman (1968) and if so, is it the neuron or the fibre which changes first? If it is the neuron, other fibres innervated by the neuron would be expected to undergo transformation simultaneously.

Preferential fibre splitting in the high myosin ATPase activity fibres leading to an increase in the number of these fibres would also

alter the percentage of high to low myosin ATPase activity fibres. Like the first theory, however, there is no evidence that this happens in the horse.

Biochemistry

The field of biochemical adaptations to exercise is extremely complex with great variation being reported in different studies, even within the one species. The major problem has been that several different types of exercise have been used to train the subject under examination. For example, as a result of the work of Hearn and Waino (1956) and of Gould and Rawlinson (1959) it was generally believed for several years that no major enzymatic adaptations occurred in skeletal muscle in response to endurance exercise. The training programme used by those investigators was thirty minutes of swimming per day for five to eight weeks. These findings have since been confirmed by Holloszy (1967) who found, however, with other workers (Holloszy et al., 1973) that an adaptive change occurred with more prolonged swimming. The discrepancy arose because thirty minutes of swimming per day is well within the capabilities of an untrained rat and it is generally true that a stimulus must be sufficiently intense to tax an organism's capacity to maintain homeostasis if it is to bring about an adaptive response. Differences may also arise according to whether aerobic and/or anaerobic metabolism is primarily involved. The magnitude of these changes during the training programme is proportional to the intensity of exercise, at least for some mitochondrial marker enzymes (Fitts et al., 1975; Benzi et al., 1976). As the magnitude of the effect of training on a given enzyme was similar in all of the muscles

biopsied in the horses in this study, it appears that all of the limb muscles examined for a given enzyme were being worked to a similar degree of activity.

The approximately two-fold increase in citrate synthase activity following training confirms the greater oxidative capacity seen with histochemical staining. This change occurs due to both the sprint and endurance segments of the training programme. A number of studies have shown that endurance exercise produces varying increases in TCA cycle enzymes (Barnard et al., 1970 (guinea pig); Holloszy et al., 1970 (rat); Edgerton et al., 1973 (lesser bush baby); Gollnick et al., 1973a(man); Fogd Jorgensen and Hyldgaard-Jensen, 1975 (pig)). As aerobic metabolism is reputed to contribute very little to the energy required for muscle contraction during sprinting (Hickson et al., 1975) this form of exercise might be expected to have little effect on TCA cycle enzymes. This is not so, however, as sprinting in rats has been shown to induce an increase in fumarase (Hickson et al., 1975) in agreement with the present work where an increase in CS was found in the last five weeks of training. Also in agreement are the results of Exner et al. (1973) and Grimby et al. (1973) who found that isometric exercise in which much of the energy for muscle contraction is also derived from anaerobic sources, also increases the levels of mitochondrial enzymes in muscle.

Why is this adaptation advantageous to the cell during training? Holloszy (1973) gives an elegant theoretical description as to why this occurs during endurance exercise training, and this is given below.

In association with this increase in TCA cycle enzymes, there

is also an increase in the size and number of skeletal muscle mitochondria (Gollnick et al., 1971) as well as a change in the composition of the mitochondria (Holloszy et al., 1973). Although this increase in respiratory capacity of the myofibres occurs with training no increase in oxygen consumption occurs for submaximal exercise of a given intensity. Therefore to attain a given submaximal level of oxygen consumption, the concentration of ADP and Pi must increase less in trained subjects when compared to untrained subjects, since more mitochondria per gram of tissue are present and therefore each mitochondrion has to produce less ATP to balance that being hydrolysed by the myofibrils. In other words, the more mitochondria per gram of muscle, the lower the oxygen uptake per mitochondrion for a given submaximal level of oxygen consumption. In attaining this steady state, the levels of CP and ATP must therefore fall less in trained individuals. This should also result in a lower steady state concentration of AMP, ADP, Pi and possibly also ammonia per mitochondrion.

As the intracellular concentrations of ATP, CP, Pi, ADP, AMP and ammonia control the rate of glycolysis in the muscle, this should result in the alteration of the rate of glycogen consumption during exercise. ATP and CP are allosteric inhibitors of PFK and Pi, ADP, AMP and ammonia are stimulators of PFK (Hoffman, 1976) and so the changes in the concentrations of those metabolites during training should result in a decreased consumption of glycogen and lactate formation. This is important, as glycogen depletion has been implicated as a causitive factor in the onset of muscle fatigue (Ahlborg et al., 1967). Thus, there is an increase with submaximal exercise, in the duration to exhaustion following training. The effect

was apparent in the horses in this study, as they all appeared less fatigued following a standard endurance run after training than before.

In addition to the above mechanism causing a decreased utilisation of glycogen with training, another contributing factor to glycogen sparing is the increased ability of trained muscle to metabolise fat as an energy source (Issekutz et al., 1966). Fat oxidation is regulated by three factors, the metabolic rate, the concentration of free fatty acids (Bremer, 1967; Paul, 1970) and the capacity of the tissue to oxidise free fatty acids, i.e. the activity of the free fatty acid metabolising enzymes. Trained subjects have a greater release of free fatty acids during submaximal exercise (Havel et al., 1964; Issekutz et al., 1965). Also, the increase in HAD in this study and the increases with training in free fatty acid metabolising enzymes found in other investigations (Mole et al., 1971; Moesch and Howald, 1973) will lead to an increased oxidation of free fatty acids with a corresponding increase in the concentration of acetyl CoA. The increase in acetyl CoA in turn leads to an inhibition of pyruvate dehydrogenase (Randle et al., 1966) and the increased levels of citrate which occur will further inhibit PFK (Hoffman, 1976). These effects result in a shift in the carbon source for energy from carbohydrate to fat.

As well as the above adaptations in aerobic metabolism occurring to increase the time to exhaustion, adaptations in anaerobic metabolism by changes in the glycolytic capacity of a muscle may also occur. The large increase in the activity of ALD found in this work which is in agreement with several other reports in man (Taylor et al., 1972a; Gollnick et al., 1973a; Ericksson et al., 1972) indicates that

this is the case. Since the work load in the present investigation required a large consumption of glycogen (Section 6), an increased glycolytic capacity would aid in the degradation of glycogen to supply energy for muscular contractions by both the aerobic and anaerobic pathways. This may at first appear to be a contradiction of the statements made earlier about aerobic enzymes increasing to decrease glycogen consumption but this is not the case. It is likely that the increased ALD levels will be important only during anaerobic exercise when glycolysis is working maximally, rather than during submaximal exercise when glycolysis is proceeding at a slower rate.

Not all studies have shown an increase in glycolytic enzymes and in general those which do have involved a training programme requiring high anaerobic requirements (Gollnick et al., 1973a; Staudte et al., 1973; Lindholm and Piehl, 1974) whilst those using endurance training have generally demonstrated little or no change in the enzyme activities (Baldwin et al., 1973; Fogd Jorgensen and Hyldgaard-Jensen, 1975; Bylund et al., 1977). The study by Baldwin et al. (1973) found a small but significant decrease in several glycolytic enzymes in the FTH muscle fibres of the rat and a small but equally significant increase in the ST fibres of the soleus. The low oxidative FT fibres were generally unchanged and reflects the fact that those muscle fibres are little used during endurance treadmill running. In the investigation in humans by Gollnick et al. (1973a), the increase in glycolytic enzymes appeared to take place in the fast twitch fibres and prompts the question, which muscle fibres are representative of their type? Is it the fibres in the mixed muscles of man and many other species or is it the fibres in small

laboratory rodents. It has also to be taken into consideration that the soleus muscle, which is made up of mainly slow contracting fibres in most species is a specialised muscle and may not be representative of slow contracting fibres in other muscles of mixed composition. It may just be, however, that variation between species causes the differences.

The length of time of the exercise sessions may also have some effect on the glycolytic enzyme activities, as a study using a training session of exercising for twenty seven minutes at 101% VO_2 max failed to produce an increase in PFK (Henricksson and Reitman, 1976) whereas one using sixty minutes at 75% VO_2 max produced a two-fold increase in this enzyme (Gollnick et al., 1973a).

The effect of training on glycolytic enzyme activities is further complicated, if the PFK values of various athletes including cyclists, canoeists, swimmers, runners - both sprint and endurance, weightlifters and orienteerers are examined as in the report of Gollnick et al.(1972). They found that there was no difference in the PFK activities of these athletes when compared to untrained individuals. When all factors are taken into account, it may be that the increase in glycolytic enzymes found with training are only transitory and return to pre-training levels following long term training.

The large elevation in LDH activity with training seen in this study is at variance with the changes reported in most other species, where little effect (Fogd Jorgensen and Hyldgaard-Jensen, 1975; Thorstensson et al., 1975; Bylund et al., 1977) or even a decrease (York et al., 1974; Suiminen and Heikkinen, 1975) has been reported. LDH

levels in rat skeletal muscle do in fact follow a similar trend as the glycolytic enzymes with a decrease in FTH and FT and an increase in ST fibres (Baldwin et al., 1973). In view of the small number of ST fibres in these horses, an increase in these fibres alone is unlikely to be the reason for the findings in the horse. A decrease in the activity of LDH with endurance training might be expected as, already described, this results in a decreased breakdown of glycogen and consequently a decreased pyruvate production. This results in a decrease in the requirement for anaerobic degradation of pyruvate. It has also been reported in man (Hermansen et al., 1967), that following training the same relative work load results in a decreased production of lactate which again indicates a decreased requirement for LDH. On the other hand, in these horses an increase in lactate production was found after training for a given sprint exercise involving riding at maximum speed for 600 metres (Snow and MacKenzie, 1977). Although no direct evidence was available to show that the horses were being exercised at the same relative work load, it is thought that the increase in lactate production and LDH activity are linked.

It might be thought that sprint training would produce an increase in LDH to enable a more rapid turnover of pyruvate but this does not appear to be the case. Several studies in rat (Staudte et al., 1973; Hickson et al., 1975) and man (Thorstenssen et al., 1975) have failed to show an increase and indeed the Hickson et al. (1975) study actually found a decrease, similar to that found with endurance exercise. Why this adaptive difference between the horse and other species occurs is not known. It may be that during high intensity exercise in the horse, the

requirements for glycolysis are greater due to a greater energy demand. This demand cannot be met from other sources such as fatty acid oxidation. As already described, training results in an increased glycolytic capacity and if increased glycolysis occurs, increased levels of pyruvate will occur. The pyruvate will be unable to enter the TCA cycle due to the increased metabolism of fat producing acetyl CoA which inhibits the pyruvate dehydrogenase complex (Randle et al., 1966). Therefore increased LDH activity will result in more rapid conversion of pyruvate to lactate and the replenishment of NAD^+ to permit further glycolysis.

Supporting the idea of an increased LDH activity occurring with anaerobic training in the horse is a study of the anaerobic performance capacity of various types of athletes (Komi et al., 1977a). LDH activities in alpine skiers who could be classified as anaerobic type athletes were significantly higher than those of endurance type athletes such as cross-country and nordic skiers. This difference is not due to a difference in the fibre composition of the muscles as all three groups had a similar spread and the same means for the percentage of ST fibres (Table 5.16). The other studies which have shown different results may have been using the wrong type of stimulus for increasing LDH activity.

LDH is a tetrameric molecule made up of four subunits from two parent molecules designated M (muscle specific) and H(heart specific) which are under separate genetic control. The subunits combine to form five isoenzymes arranged M_4, M_3H, M_2H_2, MH_3, H_4 with the M form being maximally active with high concentrations of pyruvate whilst the H form is most active at low concentrations (Dawson et al., 1964). It has been

Event (Energy source)	% fast twitch fibres in vastus lateralis	Enzyme activities $\mu mole.gm.10^{-3}.min^{-1}$	
		LDH	CPK
Ski jumping (Phosphogen)	45.0	1.54	7.96
Alpine skiing (Anaerobic)	37.0	1.75	10.02
Nordic Combination (Aerobic)	37.0	1.30	6.97
Cross Country (men) (Aerobic)	37.0	1.10	7.96
Cross Country (women) (Aerobic)	40.0	1.07	7.38

Table 5.16: Fibre composition and enzyme activities in the vastus lateralis muscle of several different athletic groups. (Komi et al., 1977a).

suggested that this is a method by which the cell regulates the production of lactate (Karlsson et al., 1974b). Evidence is available to show that a definite relationship exists between the specific activity of LDH and the amount of M subunit in several muscle types (Peter et al., 1971; Karlsson et al., 1974b) and so in any discussion of the changes in LDH activity with training, account has also to be made of the relative and quantitative changes in the H and M subunits.

Previous reports have indicated that total LDH activity decreases with training and that the proportion of H subunits increase (York et al., 1974; Fogd Jorgensen and Hyldgaard-Jensen, 1975; Sjodin et al., 1976). This is in contrast with this study where both the percentage of H subunits and total LDH increased markedly. Alterations in the percentage of LDH isoenzymes can be brought about by increases or decreases in both the H and M subunit activity and so the effect of training in terms of the total H and M subunit activity was also examined (Fig 5.6), and calculated from the data in other studies (Table 5.17). Due to the great variation in these activities between species, due as much to differing assay methods as to possible species variation, changes in total subunit activity have been expressed as a percentage of the pre-training levels. In all cases except the horse, a decrease in M subunit activity occurred with generally an increase in the H subunit activity. It has been suggested that the decrease in total LDH activity is due to this decrease in the amount of M subunits (York et al., 1974).

The increase in H subunits with submaximal type training has been ascribed to a change in skeletal muscle metabolism towards that of

Table 5.17 The Effect of Training on the Activity of the LDH M and H Subunits in Various Species (% of the activity of the Untrained Value)

	Man [+]		Pig [*]		Rat [†]		Horse	
	H	M	H	M	H	M	H	M
Untrained	100	100	100	100	100	100	100	100
Trained	155	55	133	71	88	56	216	148

[+] Sjodin et al. (1976)

[*] Fogd Jorgensen and Hyldgaard Jensen (1975)

[†] York et al. (1974).

cardiac muscle, i.e. a greatly increased oxidative capacity which favours synthesis of the H subunit (York et al., 1974). As the H subunit is situated on the inside of the mitochondrial membrane (Baba and Sharma, 1971) it is not surprising that this increases as, the number of mitochondria and mitochondrial cristae increase with training (Gollnick and King, 1969).

The difference between the horse and other species for the change in M subunit activities is not readily explicable but again may be related to the intensity of exercise used in the various studies. Reductions in the M subunit activity have generally been associated with endurance exercise programmes (York et al., 1974; Fogd Jorgensen and Hyldgaard-Jensen, 1975; Sjodin et al., 1976) which lead to an increased oxidative capacity and a subsequent decrease in the requirement for LDH_5. In contrast to our findings, anaerobic exercise in man has been reported to have no effect on the total LDH activity or the isoenzyme pattern (Sjodin et al., 1976). It may be that the type of programme utilised in our study was a greater stimulus for the synthesis of both forms. In this respect, it would be of interest to know the isoenzyme composition of the alpine skiers in the study by Komi et al. (1977a). Pette et al., (1973) also found achange from M to H with electrical stimulation

A third pathway for pyruvate metabolism is the conversion to alanine by ALT and increased levels of this metabolite have been reported following exercise in man (Felig and Wahren, 1971; Bloom et al., 1976) and the horse (Snow and Chalmers, personal communication). With training, increases in this enzyme have been demonstrated in rats (Mole et al., 1973) and the present study. An increase in ALT would be advantageous especially during anaerobic exercise as it would permit greater amounts of pyruvate to be metabolised to alanine. This would aid in maintaining

intracellular homeostasis by reducing the amount of lactate produced and hence the decrease in pH. High lactate and low pH have been implicated as a cause of fatigue with strenuous exercise (Karlsson and Saltin, 1970) by causing inhibition of glycolysis at the PFK level (Hoffman, 1976) and also by interfering with muscular contractions due to the H^+ ions competing with Ca^{2+} for the binding sites that control actin-myosin interactions (Katz, 1970). The increase in ALT takes place after that of LDH and it may be speculated that the increase in ALT occurs to counteract the increase in lactate that may arise from this earlier adaptation.

CPK is another enzyme which is connected with sprinting, as high intensity exercise makes large demands on the mechanisms which supply immediate energy to the cell. These mechanisms are, in the main, replenishment of ATP from creatine phosphate by CPK and ADP by myokinase. Therefore, an increase in CPK activity with training would increase ATP resynthesis for high intensity sprinting. The 30% increase in CPK activity was similar to that in man (Thorstensson et al., 1975) and rat (Staudte et al., 1973) using sprint training. Other studies using endurance exercise have found no difference in man (Souminen and Heikkinen, 1975), pig (Fogd Jorgensen and Hyldgaard-Jensen, 1975) and rat (Oscai and Holloszy, 1971). Further evidence that the increase in CPK activity is due to anaerobic exercise is the finding that alpine skiers have, as with LDH, a higher activity of CPK when compared to endurance sportsmen (Komi et al., 1977a). Not all anaerobic exercise induces this increase in enzyme activity, as weight training which also involves mainly anaerobic exercise has no effect on enzyme levels. It is likely that higher demands are made on the muscle to synthesise ATP during sprinting when

many maximal contractions are repeated than during weightlifting.

CPK, like the other anaerobic energy producing enzymes, LDH and ALD, increased least during the five weeks of sprinting, contrary to expectations. One reason may be that each fibre has a certain potential that it can reach with training and the sprint exercise during the first ten weeks was enough to allow the anaerobic enzymes to approach this maximum. Benzi et al. (1975) have found in rats, that in response to a constant training stimulus, enzyme activities tend to level off and only alter if the level of activity alters. It may be, therefore, that the anaerobic enzymes levelled off because the amount of exercise being given in the last five weeks was not sufficiently different from the first ten weeks to warrant a change in the anaerobic enzyme activities. As CPK is only of importance during the first few seconds of exercise, it is unlikely to be affected more by the different distances of sprinting imposed in the last five weeks than in the shorter sprint distances in the first ten weeks.

Extramitochondrial NADH produced during glycolysis is unable to penetrate normal mitochondria for oxidation. A number of mechanisms have been proposed therefore to explain how this NADH is oxidised. The best documented of these are, the "malate-aspartate shuttle" and the "glycerol-3-phosphate shuttle" (Van Dam and Meyer, 1975). As it is known that trained individuals produce less lactate during submaximal exercise than untrained individuals, even at comparable rates of glycolysis, it was thought of interest to determine whether an adaptive response increasing the capacity to transfer reducing equivalents to the respiratory chain from cytoplasmic NADH occurs. The results from this

study are similar to those in the rat where an increase was found in the "malate-aspartate shuttle" in the form of an increase in AST activity (Holloszy et al., 1975) whilst little change occurred in the activity of GDH. The reason why one shuttle adapts whereas the other does not is unknown, but may be related to the fact that the "malate-aspartate shuttle" is used mainly by aerobic fibres whereas the "glycerol-3-phosphate shuttle" is associated with anaerobic fibres. These fibres (the FT fibres) are only stimulated at or near maximum exercise and it may be that the stimulus was not enough to bring about an adaptation in this enzyme system. This latter shuttle has been reported to be closely related to the activity of glycolytic enzymes but this does not appear to be the case with training in this study.

The high values of glycogen concentrations reported in this work are similar to those of Lindholm and Piehl (1974) and are greater than those of most other species (Table 5.18). The increase in glycogen concentration, with training, is in agreement with that observed in man (Gollnick et al., 1973a; Taylor et al., 1972b), rat (Fitts et al., 1975) and pig (Rulcker, 1968) although the changes are of a smaller magnitude than in most other species. In order to elucidate a reason for this, two enzymes GS and HK connected with the synthesis of glycogen in muscle were examined during the training programme.

GS, the rate limiting enzyme in glycogen synthesis, consists of two interconvertible forms, the less active b-form being dependent on glucose-6-phosphate for activity, whereas the a-form is independent of this cofactor for activity (Friedman and Larner, 1963). The proportions of the two forms in the muscle vary with the glycogen content of the muscle,

with the percentage in the a-form being inversely related to the amount of glycogen (Danforth, 1965). The increase in glycogen with training is attributed to an increase in total GS activity and not to any dietary adjustments (Piehl et al., 1974). Although an increase in total GS activity is usually found, reports on changes in the percentage a-form with training are contradictory, with an increase being reported in guinea pigs (Jeffress et al., 1968) and a decrease in man (Piehl et al., 1974). The unaltered GS activity in the horse may be due to the high glycogen levels found before training and the relatively small increase during training. Similarly, no increase in GS activity was reported in a study in man where training caused only a slight increase in glycogen levels (Bylund et al., 1977). (Table 5.18).

HK converts glucose to glucose-6-phosphate, a substrate for glycogen synthesis and an activator of GS. Therefore, HK may promote glycogen synthesis by increasing the intracellular glucose-6-phosphate pool (Hultman et al., 1971). An increase in HK has been found in several studies (Peter et al., 1968; Baldwin et al., 1973; Piehl et al., 1974) and this tends to indicate that an adaptation takes place that is beneficial to the cell. However, in contrast to these findings but in agreement with the present findings, is a report in man that training had no effect on HK activity (Moesch and Howald, 1973). HK consists of two forms and insulin deprivation results in a marked decrease in total HK activity, thought to be mediated by the decreased entry of glucose affecting the type II activity. The reverse effect is also true, with an increase in HK activity if insulin is administered (Katzen et al., 1970). Repeated muscle contraction markedly increases muscle cell permeability to glucose

Authors	Species	Muscle	Glycogen (μmoles glucose units.(gm wet tissue)$^{-1}$)
Wilber (1963)	Mouse	unspecified	9
Hultman (1967)	Man	Vastus Lateralis	61 - 131
Chapter and Stainsby (1968)	Dog	Gastrocnemius	21 - 85
Gillespie et al. (1970)	Guinea Pig	"red" Vastus Lateralis	59
		"white" Vastus Lateralis	45
Baldwin et al. (1973)	Rat	"red" Vastus Lateralis	58
		"white" Vastus Lateralis	49
		Soleus	39
Gollnick et al. (1973)	Man (untrained)	Vastus Lateralis	72
	Man (trained)	Vastus Lateralis	182
Lindholm and Piehl (1974)	Horse	Gluteus Medius	126
Fitts et al. (1975)	Rat (untrained)	Gastrocnemius	44
	Rat (trained)	Gastrocnemius	58

Table 5.18: Muscle glycogen levels in several species.

(Holloszy and Narahara, 1965, 1967) and this tends to support the idea of training increasing HK activity. It may be that the increase in glucose uptake did not occur in the present study to the same extent.

One of the problems when dealing with the effect of training on HK activity is the fact that acute exercise also has an effect. It has been shown that a single bout of exercise results in an increase which lasts for up to seventy two hours (Bostrom et al., 1974). This means that the significance of at least some of the findings with HK can be questioned (Peter et al., 1968; Baldwin et al., 1973). This effect occurs with several other enzymes, but the changes are not as marked.

The decrease with detraining in the enzyme activities of those enzymes which increased with training is to be expected from various studies where the parameters measured regressed to levels similar to those before training (Secher, 1921; Steinhaus et al., 1932; Gollnick and Simmons, 1967; Faulkner et al., 1972). Few biochemical studies have been carried out on the effects of detraining, but the findings of Terjung (1975) and Henricksson and Reitman (1977) indicate that the mitochondrial components regress to levels similar to those found before training. This phenomenon is similar in many respects to the effect of disuse produced by casting or bone pinning of hind limbs, where a loss of mitochondrial function occurs (Max, 1972) with a decrease in oxidative capacity (Booth and Kelso, 1973; Haggmark, Jansson and Eriksson, cited by Henricksson, 1976). With this type of disuse, there is a rise in lysozomal enzyme activity (Max et al., 1971) which may be related to the decrease in energy supplying enzyme activities. The atrophy which follows may also be connected as mitochondrial dysfunction may contribute to a loss of muscle

mass through a decreased amount of energy being available for the synthesis of muscle proteins (Max, 1972). Whether in fact the effects of immobilisation disuse are similar in all respects to detraining remains to be discovered.

The "rebound" effect occurring after ten weeks of detraining is surprising, as it would have been expected that the levels of enzyme activity would have decreased further towards the levels of activity at week 0. No satisfactory explanation of this effect can be given and no comparable studies have been found in the literature. The finding of an unchanged total protein content during detraining indicates that it is not due to a marked loss of myofibrillar protein making the activity of the enzymes relative to the weight of the tissue greater. It may be, that a relative hypoxia in the tissues is stimulating the synthesis of new enzyme, as trained subjects have a higher pO_2 content in the blood than sedentary subjects (Schroeder et al., 1976). Supporting this idea is the finding that the M form of the LDH isoenzyme increases whilst the H form decreases during detraining, a response which has been previously reported to occur in conjunction with hypoxic conditions (Dawson et al., 1964). Further support comes from the work of Tappan et al. (1957) who found that hypoxia increases the levels of oxidative enzymes in rats. On the other hand, there are reports that the levels of mitochondrial enzymes are positively correlated to the pO_2 (Kellerman, 1969) and supporting this, is the report that there is a decreased cytochrome content in rat heart in response to one to two weeks of hypoxia (Kinnula, 1976).

All of this work raises the question, what is responsible for the changes in enzyme activities? An increase in enzyme activity is

generally equated with an increase in the amount of enzyme present. This can be the result of an increased synthesis and/or decreased degradation. Reports as to which mechanism is important, vary with all three possible mechanisms being implicated (Terjung et al., 1973; Schott and Terjung, 1975; Booth and Holloszy, 1976). Increased synthesis can be caused by action at several points, such as on transcription by activation of RNA polymerase, by inhibition of the degradation of m-RNA or on translation of polysomes (Gehlerter, 1976) and in rats nuclear DNA-dependent RNA polymerase increases in response to swimming (Rogozkin, 1976). Decreased degradation can be due to a change in the hydrolytic system which is thought to bring about degradation and/or an altered susceptibility for proteolysis. This latter method has been suggested as responsible by Schott and Terjung (1975) and occurs because the elevated energy output due to exercise keeps the mitochondrial cristae components of working muscle in a more stable conformation.

The stimuli triggering the adaptive changes in skeletal muscle are unknown. As these responses take place only in muscles or muscle fibres which have been activated during exercise, this suggests that whatever it is, it is unlikely to be blood borne, i.e. a hormone, but is present in the working cell. Henricksson (1976) reported that the presence of the motor nerve is not necessary for these adaptive changes but that the triggering factor is related to the activation and contraction of the muscle fibre, even if it cannot completely be excluded that a trophic influence of the nerve is of importance for the enzyme adaptation in the intact organism (Gutmann, 1976).

Another idea put forward, is that specific activation of β-receptors on the muscle fibres triggers the enzyme adaptation (Hari and Valtola, 1975) and Yakovlev (1975) claims that chemical and surgical desympathization markedly limits the extent of the adaptation.

As mentioned before, hypoxia has also been suggested as a possible candidate for the stimulus. This has been based on investigations on patients with arterial insufficiency and subjecting rats to hypoxia and obtaining an increased oxidative potential (Holm et al., 1972). The problem with this theory is that there is doubt as to whether muscle becomes hypoxic during submaximal exercise (Chance et al., 1964; Jobsis and Stainsby, 1968). Another problem is that, as mentioned before, hypoxia has been shown to decrease the oxidative potential (Simon and Robin, 1970; Kinnula, 1976). These findings suggest that for the oxidative enzymes at least, the trigger for adaptation is the flux of oxygen through the muscle cells. In agreement with this is that the increase in the number of capillaries comes before the increase in oxidative enzymes in electronically stimulated muscle (Cotter et al., 1973).

The increase in other enzyme systems is not explained by this theory, however, since if oxygen was the only trigger for adaptation, those systems would be expected to change with submaximal exercise also, and, in fact they do not. What is more probable is that the stimulus for adaptation is the exercise induced increases in the concentrations of various metabolites such as ADP, AMP, creatine and lactate (Rogozkin, 1976; Yakovlev, 1976).

SECTION 6

FACTORS INFLUENCING GLYCOGEN LEVELS DURING EXERCISE

Introduction

Energy consumption during exercise has been studied by many authors during the last century and it was shown that the energy required for muscular work is derived from body fats and carbohydrates (Krogh and Lindhard, 1920; Christensen and Hansen, 1939). No energy is derived from the breakdown of protein in the muscle (Poortmans, 1975). Using the respiratory quotient (R.Q.), it was demonstrated that the amount of carbohydrate utilised was in proportion to the intensity of work being undertaken (Christensen and Hansen, 1939). Although this method permitted the estimation of the total amount of carbohydrate being used, information concerning local changes in the muscle were lacking, until the advent of the percutaneous needle muscle biopsy (Bergström, 1962). Using this technique, it was possible to obtain serial samples within a short time on the same subject and follow the changes in muscle constituents at rest or during exercise.

Several studies have shown that glycogen depletion occurs in the muscle during exercise (Ahlborg *et al.*, 1967; Bergström and Hultman, 1967; Costill *et al.*, 1973) and that glycogen depletion may be indicated in the onset of fatigue. Total carbohydrate utilisation during a work period, calculated from the R.Q. exhibited a high correlation with the decrease in the local muscle glycogen content. The time to exhaustion for an individual was proportional to the initial glycogen concentration in the muscle (Bergström *et al.*, 1967). Several factors, including whether the subject is on a high carbohydrate or high fat diet (Bergström *et al.*, 1967), undergone previous exercise (Hultman, 1967) and training (Gollnick *et al.*, 1973a) influenced this initial concentration of glycogen.

Training also decreased the rate at which the glycogen was utilised (Karlsson et al., 1974c).

Glycogen depletion during exercise has been shown to be selective in the different fibre types, depending on the intensity and duration of the exercise. In man, ST fibres are the first to be depleted during submaximal exercise with the FT fibres only becoming depleted after 2-3 hours of exercise (Gollnick et al., 1973b,c). With high intensity exercise, however, it is the FT fibres which are depleted first (Gollnick et al., 1974a). The depletion pattern in the horse (Lindholm et al., 1974a) and rat (Armstrong et al., 1973) is somewhat different from that found in man, with both ST and FTH fibres being depleted during submaximal exercise, although again FT fibres are the last to be depleted.

The other carbohydrate fuel utilised in exercise in mammals is blood glucose. Its role as a fuel in exercise is controversial with varied effects being reported in both man and horse, largely depending on the intensity and duration of exercise (Krzywanek, 1973; Engelhardt et al., 1973; Lindholm and Saltin, 1974; Rennie et al., 1974; Snow and MacKenzie, 1977a,b). The levels of circulating blood glucose during exercise are dependent on the ratio of uptake by working muscle and the rate of hepatic glycogenolysis and gluconeogenesis. It has been suggested that glucose is only of minor importance as a fuel whilst sufficient glycogen stores exist (Skinner et al., 1971).

Less is known about the utilisation of the other main fuel in muscle, triglycerides, but in man, the immediate oxidation of plasma free

fatty acids accounted for less than 50% of the carbon dioxide produced during exercise at an R.Q. of 0.75 (Havel et al., 1964). This led to the assumption that fatty acids derived from muscle triglycerides were also important during exercise, however, direct studies on the concentration of triglycerides in the muscle gave varying results (George and Jyoti, 1955; Masoro et al., 1966; Valyathan et al., 1970). From the work of Carlson et al. (1971) and Fröberg and Mossfeldt (1971) in man and Reitman et al. (1973) in rats, it is now clear that intramuscular triglycerides participate in energy supply during exercise.

Catecholamines play an important role in exercise metabolism, stimulating free fatty acid mobilization as well as hepatic gluconeogenesis and glycogenolysis. Over the last decade or so, attempts have been made to elucidate the importance of catecholamines in the control of energy substrate utilisation during exercise and the relationship between the activity of the adrenergic system and exercise metabolism (Häggendal et al., 1970; Kotchen et al., 1971). Some of those studies have involved the use of adrenergic β blockers and examining their effects on various muscle constituents. Conflicting results have been reported (Harris et al., 1971; Nazar and Kozlowski, 1975).

This section is therefore concerned with the effect of exercise on intramuscular glycogen and triglyceride levels during both maximal and submaximal exercise. As part of a more comprehensive study on the metabolic effects of adrenoreceptor β-blockers during maximal and submaximal exercise (Snow, D.H. and Summers, R.J. unpublished) the effect of these drugs on glycogen levels during exercise was also determined.

Methods (1)

The analytical methods used were as described in section 2. Muscle samples were obtained by the technique of percutaneous needle muscle biopsy (Bergström, 1962). The incisions in the muscle were made prior to exercise as previously described. In order to minimise the effect of local damage, biopsies after each period of exercise were taken from different incisions. The biopsies were taken in the order from the distal to proximal incision.

Animals: Horses 1 to 6 were used in this section.

Exercise Tests: The exercise tests used in this section were as follows.

Maximal Exercise: Gallop tests were carried out in the morning after overnight fasting. The 4 x 500 metre gallops were over a distance which was essentially flat with a short incline in the last 50 metres. Biopsies were taken before the exercise commenced and when possible, after each gallop. The test was performed twice for each animal, the biopsies being obtained from the left *gluteus medius* on the first occasion and from the right *gluteus medius* on the second test.

Sub-Maximal Exercise: The test involved cantering at a steady pace around a large field and was carried out in the morning after overnight fasting. The animals were exercised over a total distance of 22.4 kilometres, biopsies being taken prior to exercise, at 11.2 kilometres and at the end of exercise. Samples were obtained from the *gluteus medius*, long head of the *triceps brachii* and the *deltoideus* muscles.

Results (1)

Although it was hoped to determine the concentration of triglycerides in the muscle, meaningful results proved impossible (Table 6.1). As previously mentioned, the variability of triglycerides in the muscle was extremely large, due to non-homogeneous deposition in the muscle. Only glycogen could therefore be measured.

Maximal Exercise (Horses 1, 2, 3 and 5)

The effect of galloping over the 4 x 500 metre course is shown in Fig 6.1. The greatest depletion of glycogen occurred in the first gallop, averaging 80 μmoles.(g dry weight of tissue)$^{-1}$ with only 20 μmoles.(g dry weight of tissue)$^{-1}$ being utilised during gallops 2 and 3. During gallop 4, the rate increased slightly to 31 μmoles.(g dry weight of tissue)$^{-1}$. The mean time for a 500 metre gallop was 42.2 $\pm$ 3.2 seconds giving a mean velocity of 12.2 $\pm$ 0.8 metres.second^{-1}. The rate of glycogen utilisation was therefore for the four gallops, approximately 55 μmoles.(g dry weight of tissue.minute)$^{-1}$.

Sub-Maximal Exercise (Horses 1 to 6)

The effect of a 22.4 kilometre bout of exercise on the *gluteus medius*, *deltoideus* and long head of the *triceps brachii* are shown in Figs 6.2 to 6.4. In all three muscles, the largest utilisation of glycogen occurred in the first half of the exercise. The largest depletion of glycogen occurred in the long head of the *triceps brachii* with 65% of the available glycogen being utilised by the end of the exercise. The *deltoideus* was next with 47% being utilised whilst the *gluteus medius* muscle used up only 33% of its glycogen by the end of the 22.4 kilometres.

	Horse 1		Horse 2		Horse 4		Horse 5	
	L	R	L	R	L	R	L	R
Pre Exercise	562	230	314	225	339	436	1785	1712
Gallop 4	267	240	212	308	206	219	1613	2737

Table 6.1: The effect of 4 x 500 metre gallop on triglyceride levels (μmoles . (gm dry weight of tissue)$^{-1}$) in the gluteus medius muscles (L = left, R = right) of four horses.

Fig 6.1: Glycogen concentration (μmoles.(gm dry weight of tissue)$^{-1}$) (mean $\pm$ S.E.M.) in the gluteus medius during 4 x 500 m gallops.

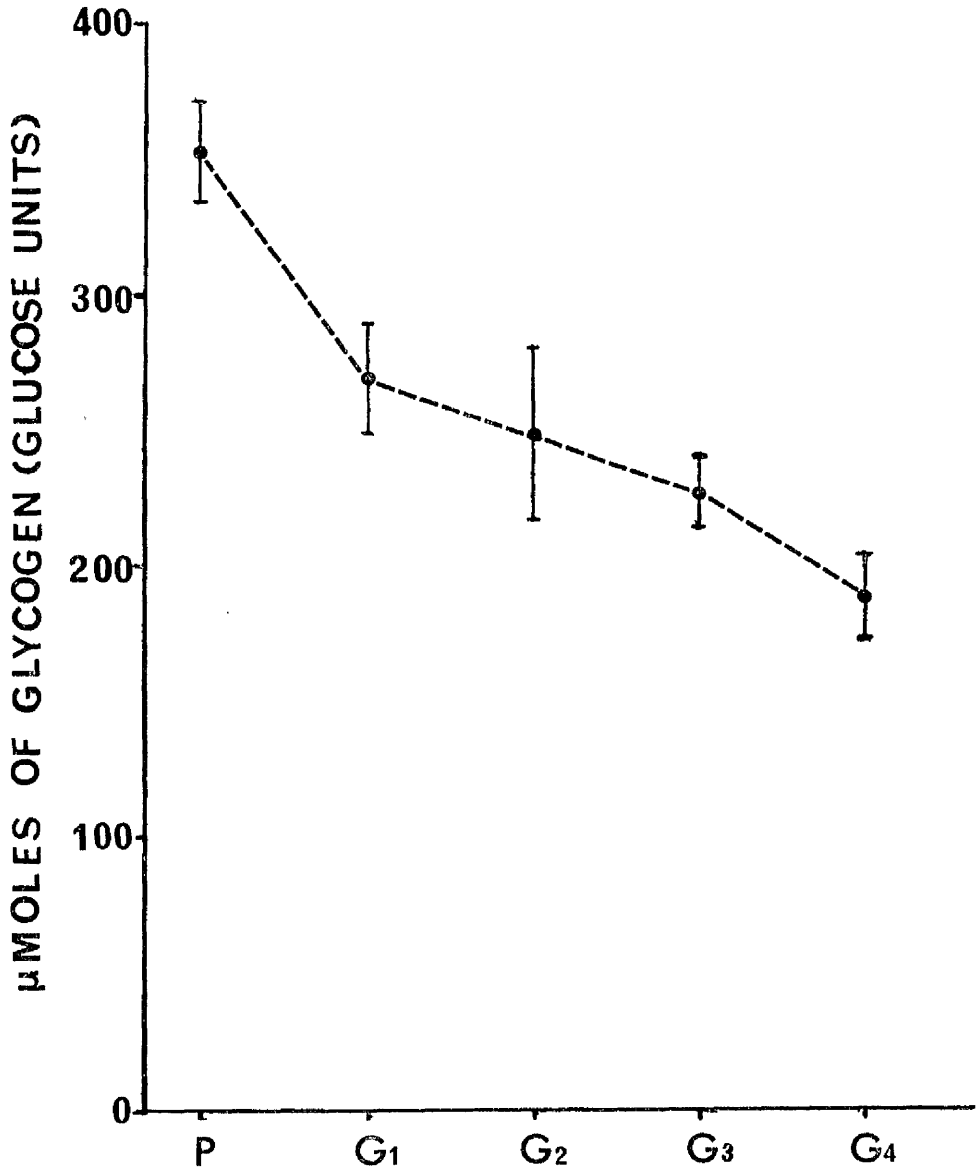

400
300
200
100
0
µMOLES OF GLYCOGEN (GLUCOSE UNITS)
P
G1
G2
G3
G4

Fig 6.2: Glycogen concentration (μmoles.(gm dry weight of tissue)$^{-1}$) (mean $\pm$ S.E.M.) in the deltoideus muscle during a 22.4 kilometre endurance canter.

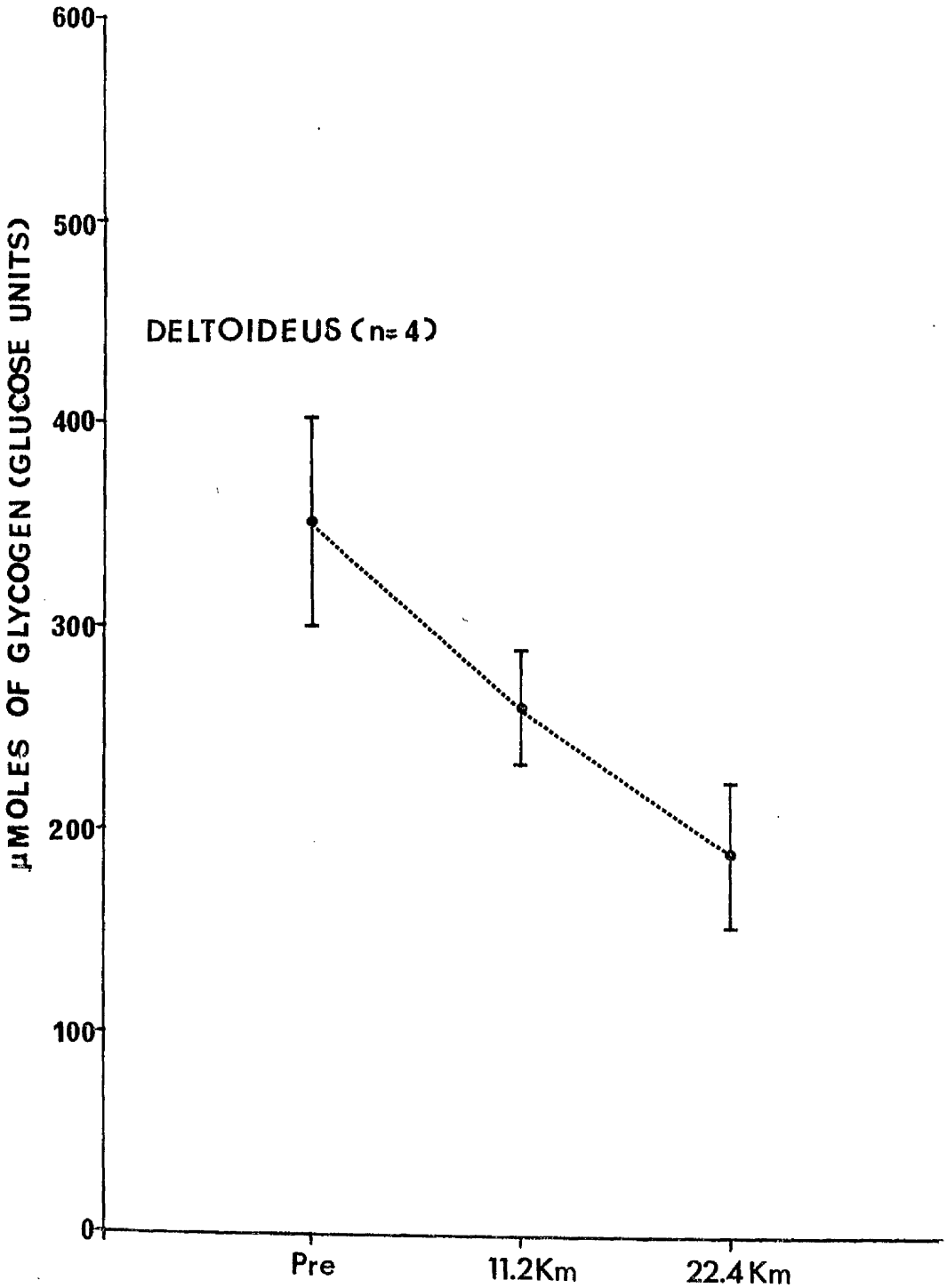

600
500
400
300
200
100
0
µMOLES OF GLYCOGEN (GLUCOSE UNITS)
DELTOIDEUS (n= 4)
Pre
11.2Km
22.4 Km

Fig 6.3: Glycogen concentration (μmoles.(gm dry weight of tissue)$^{-1}$) (mean $\pm$ S.E.M.) in the long head of the triceps brachii muscle during a 22.4 kilometre endurance canter.

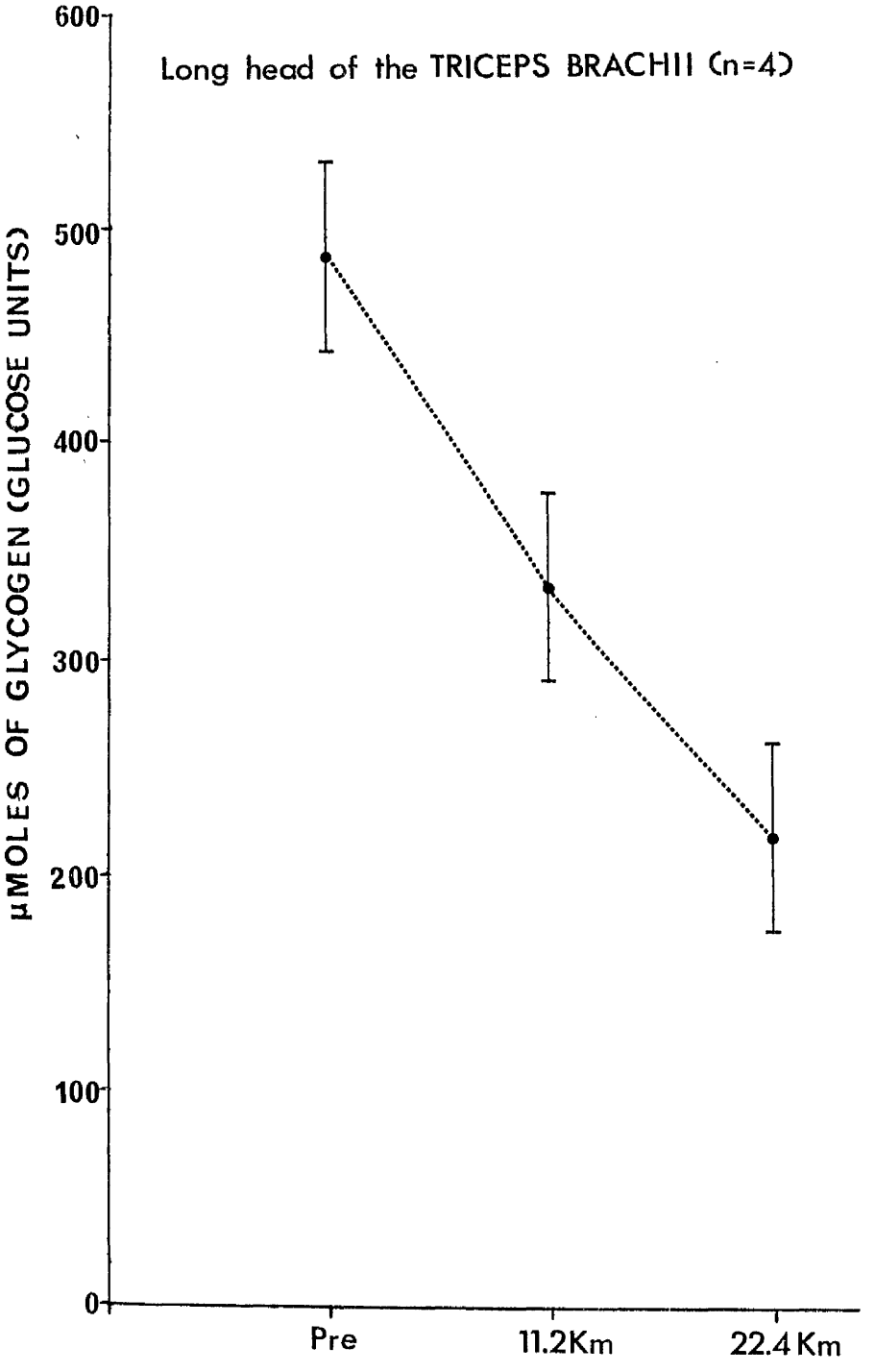
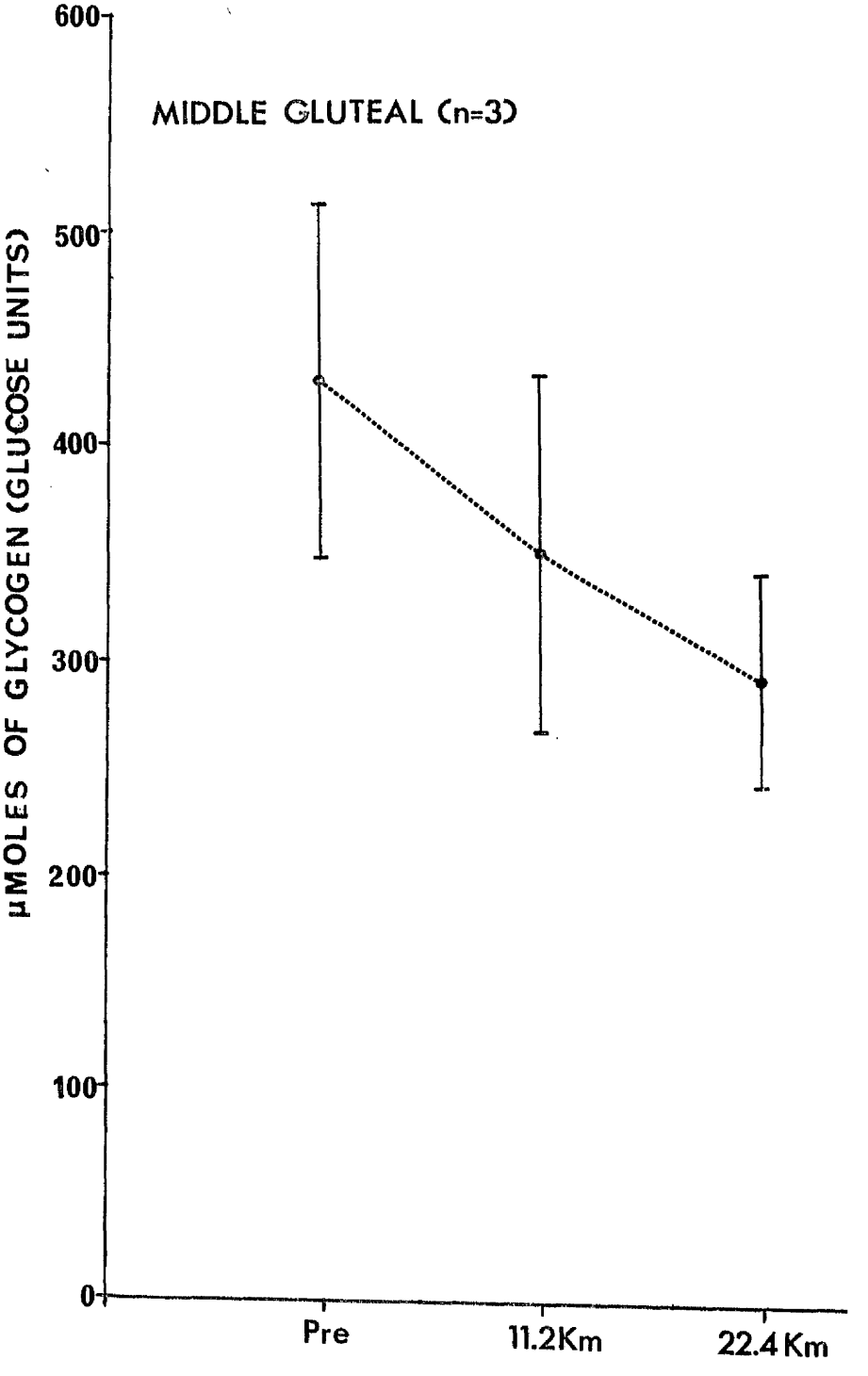

Long head of the TRICEPS BRACHII (n=4)
µMOLES OF GLYCOGEN (GLUCOSE UNITS)
600
500
400
300
200
100
0
Pre
11.2Km
22.4 Km

Fig 6.4: Glycogen concentration (μmoles.(gm dry weight of tissue)$^{-1}$) (mean $\pm$ S.E.M.) in the gluteus medius muscle during a 22.4 kilometre endurance canter.


MIDDLE GLUTEAL (n=3)
µMOLES OF GLYCOGEN (GLUCOSE UNITS)
600
500
400
300
200
100
0
Pre
11.2Km
22.4 Km

Discussion (1)

As found in many other studies, exercise depletes the muscle of glycogen (Bergström and Hultman, 1967; Reitman et al., 1973; Lindholm et al., 1974a; Lindholm and Saltin, 1974). During maximal exercise, this depletion appears to be extremely constant at approximately 33.0 μmoles.(g dry weight of tissue.minute)$^{-1}$ from gallops 2 to 4. This is much higher than in the trotting standardbred where the rate of depletion at maximal trotting speed was 14 μmoles.(g dry weight of tissue.minute)$^{-1}$ (Lindholm, 1974). Why this difference should occur in the two studies is unknown as the speeds being used were similar - 80 seconds.kilometre^{-1} for the standardbreds of Lindholm (1974) and 84 seconds.kilometre^{-1} in the present study. It may be that the gluteus medius muscle is more important for propulsion during galloping than trotting.

There was a much larger depletion of glycogen between taking the pre-exercise sample and the sample taken after gallop 1. This may be due in part to the fact that the field in which the exercise was performed was about 1.5 kilometres from the room where the pre-exercise sample was taken. The amount of glycogen utilisation was probably minimal, however, as the animals were walked to the field. Increased glycogen consumption occurs with an increase in speed, but although the speed in gallop 1 was slightly faster than in any of the other gallops (Table 6.2) it is unlikely that the faster speed would cause such a large difference in the amount of glycogen being used. This suggests that there is a greater consumption during gallop 1 and a similar effect occurred in man at near maximal work loads (Bergström and Hultman, 1967).

	Time for 500 metre gallop (secs)	Speed m . sec^{-1}
Gallop 1	40.2 ± 3.4	13.0 ± 0.6
Gallop 2	41.5 ± 2.8*	12.4 ± 0.7*
Gallop 3	42.8 ± 3.2*	12.1 ± 0.8*
Gallop 4	43.9 ± 3.4*	11.8 ± 0.8*

Table 6.2: The times (mean ± S.E.M.) and speed (mean ± S.E.M.) for four 500 metre gallops by four horses. (Three Thoroughbreds and one heavy hunter).

*$P \leq 0.05$ compared to Gallop 1.

Lactate increases greatly with exercise (Lindholm and Saltin, 1974) and lactate accumulation brings about a pronounced increase in the hydrogen ion concentration in the muscle. This leads to a decreased muscle pH, causing inhibition of phosphofructokinase and phosphorylase b to phosphorylase a conversion (Hoffman, 1976). Phosphorylase b is also inhibited by glucose-6-phosphate (Morgan and Parmeggiani, 1964) which also increases with exercise (Lindholm and Saltin, 1974). As these metabolites are only present in low concentrations before exercise commences, this may be one reason why a greater glycogen utilisation occurs during gallop 1. It is also possible that blood borne fuels such as glucose and free fatty acids are only important after exercise has been taking place for a few minutes.

As with maximal exercise, sub-maximal exercise produced a steady fall in glycogen levels. Although the animals were extremely tired and reluctant to continue, there was still much glycogen left in the muscles. Lactate accumulation has been implicated in the onset of fatigue by enzyme inhibition as mentioned above but this is not the reason in this study as the type of exercise given does not lead to an increase in lactate levels in the blood (Snow and MacKenzie, 1977a)and muscle (Table 6.3). A similar result was found in man (Gollnick et al., 1974a) and these authors also ruled out lactate as a possible factor in the onset of fatigue. They suggested that reduced glycogen stores were the cause of exhaustion but that measurement of the total glycogen content in the muscle was unreliable. During exercise, some fibres become completely depleted of glycogen whilst others still retain most of their glycogen. In the horse, (Lindholm et al., 1974a) at low intensity exercise ST fibres were

Horse No.	1	2	4	5	6
Pre-exercise	21.4	23.0	3.6	20.2	21.4
11.2 k.	16.5	20.4	12.6	24.4	24.2
22.4 k.	15.8	21.4	14.8	-	-

Table 6.3: Effect of endurance on lactate levels (μmoles.(gm dry weight of tissue)$^{-1}$) in the long head of the triceps brachii muscle.

predominantly depleted. Once a few of the ST fibres were depleted, FTH fibres also began to become deficient in glycogen. At exhaustion, most of the glycogen remaining in the muscle was found in the FT fibres. Glycogen utilisation in these latter fibres occurred mainly during maximal exercise.

The physiological basis behind the depletion of the various fibre types is based on the selective recruitment theory. This in turn is based on the fact that motor neurons have different activation thresholds (Henneman and Olson, 1965). The fibres which have the lowest threshold are generally considered to be the high oxidative fibres (FTH + ST) which derive a lot of their energy requirements from fat oxidation as well as carbohydrate utilisation. A similar pattern of activation was found in rats (Armstrong et al., 1973).

Returning to why the horses were exhausted after the sub-maximal endurance exercise despite the fact that much glycogen remained in the muscles, it is probable that fatigue arose because of changes in the metabolic processes in the ST and possibly the FTH fibres. These fibres might not be able to maintain the required tension due to the absence of glycogen in many of the fibres and because of the high activation threshold of the FT fibres, these will not come into play during submaximal exercise.

It was noticed that in all three muscles examined, the rate of glycogen utilisation was less in the second 11.2 kilometres. A similar finding was reported in man (Bergström and Hultman, 1967) and attributed to the rapid breakdown of muscle glycogen during the first few minutes of exercise. This was due to relative anoxia in the muscle causing an

increase in lactate formation. As the exercise progressed, a stabilisation of the metabolic processes took place with a more constant utilisation of glycogen and lipid. The rates of depletion differ in the three muscles and are probably an indication of the activity of the muscles during the exercise. The anatomical location of the muscles and the importance of their actions during a particular movement probably play a greater role in the amount of depletion than any differences in the fibre composition. Costill et al. (1973) have reported that this also occurs in man with the rate of glycogen utilisation during normal running being less in the vastus lateralis than in the soleus and gastrocnemius.

Methods (2)

The analytical methods were as described in section 2. The precautions taken in section 6(1) when biopsies were taken were also observed in this section.

Animals: Horses 1 to 5 were used in this section.

Drugs: The drugs used in this section were as follows.

dl propranolol (0.2 $mg.kg^{-1}$ I.V.) I.C.I., Macclesfield, England

d propranolol (0.2 $mg.kg^{-1}$ I.V.) I.C.I., Macclesfield, England

dl metoprolol (0.2 $mg.kg^{-1}$ I.V.) Hassle, Goteborg, Sweden.

Drugs were administered thirty minutes prior to exercise.

Exercise Tests

Maximal Exercise: Each horse was galloped by the same rider at the maximum speed of which it was capable over a distance of 500 metres. This was repeated four times with an interval of five minutes between each gallop. The test was performed over the same ground as the maximal exercise test in section 6(1). Samples were obtained from the gluteus medius muscle.

Sub-Maximal Exercise: This test was performed in a riding arena approximately 500 metres from the room in which the pre-exercise biopsy was taken. The horses were walked to the school before the exercise and so glycogen depletion was minimal. The test consisted of 100 laps of the riding arena (total distance 9.0 kilometres) plus the distance from the arena to room. The horses were cantered throughout the exercise. Biopsies were taken from the gluteus medius muscle before and after exercise.

Results (2)

Maximal Exercise: Following the administration of dl propranolol, the performance of both horses was decreased as indicated by the increase in gallop times (Table 6.4). A decrease in glycogen consumption occurred in both horses following dl propranolol. In the control test, there was a decrease of 172 and 134 μmoles.(g dry weight of tissue)$^{-1}$ after the four gallops whilst after dl propranolol the decrease in glycogen was 137 and 114 μmoles.(g dry weight of tissue)$^{-1}$ respectively. This was a percentage decrease of 20% and 15% respectively, compared to the control values.

Sub-Maximal Exercise: As can be seen from Table 6.5 β-adrenoreceptor blockers had little effect on the performance or glycogen depletion during submaximal exercise.

	Horse 1		Horse 2	
	Control	Propranolol	Control	Propranolol
Pre-Exercise	400	358	500	384
Post-Exercise	266	244	328	247
Time	44.1	48.7	37.3	39.4
Speed	11.3	10.2	13.4	12.7

Fig 6.4: The effect of dl propranolol (0.2 $mg.kg^{-1}$) on gluteus medius glycogen depletion ($\mu moles \cdot (gm\ dry\ weight\ of\ tissue)^{-1}$), time (sec) and speed ($m.sec^{-1}$) during maximal exercise. Post exercise samples were taken after 4 x 500 m gallops. The time and speed are the mean of the four gallops.

	Glycogen (μmoles.(gm dry weight of tissue)$^{-1}$)			Speed (m.sec^{-1})
	Pre-Exercise	9 Kilo-metres	Difference	
Control	329±8	26±19	68±25	5.1±0.2
dl-propranolol	379±32	343±22	36±13	4.9±0.1
dl-metoprolol	395±33	342±37	32±8	5.1±0.1
d-propranolol	391±24	336±28	53±20	5.0±0.1

Table 6.5: Effect of dl-propranolol, d-propranolol and dl-metoprolol on glycogen depletion and speed (mean ± S.E.M.) in four Thoroughbred horses during sub-maximal exercise.

Discussion (2)

The decrease in performance of the maximally exercised horses seen with propranolol is similar to that reported in man (Ekblom et al., 1972) and dog (Nazar et al., 1971). Using a much higher dose of propranolol (1-2 mg.kg I.V. as opposed to 0.25 $mg.kg^{-1}$ I.V.), however, Donald et al. (1968) found only a slight decrease in the performance of racing greyhounds. The delitatory effect on performance is probably due to a reduction in both circulatory and metabolic responses to exercise following beta blockade.

The heart rate increase found with exercise was partially prevented in the two horses and others not sampled for glycogen determinations (Snow and Summers, 1977). Again this is similar to man (Ekblom et al., 1972; Johnsson, 1975) but different from the greyhound where dl-Propranolol did not significantly alter the heart rate attained during maximal exercise (Donald et al., 1964). This decrease in heart rate leads to a reduction in cardiac output in maximal and submaximal exercise. This may be the reason for the impaired performance found in this study, but complete (Ekblom et al., 1972) or partial (Epstein et al., 1965) compensation for the reduced cardiac output during maximal exercise may have occurred by an increased oxygen extraction from the tissues as indicated by a greater arteriovenous oxygen difference. This tends to indicate that the reduced maximal performance after dl-propranolol is due mainly to effects on catecholamine mediated metabolic responses.

Catecholamines stimulate the breakdown of glycogen to glucose-1-

phosphate by a mechanism termed an amplification cascade. Each step in the sequence is catalytic and therefore for a small number of hormone molecules becoming bound to a receptor, a large increase in phosphorylase-a activity is obtained. Peak activity is reached within a few seconds. This occurs in both the muscle and liver, but in this latter organ, the glycogen is converted to glucose to augment blood glucose levels, rather than shunted to pyruvate. Catecholamines also inhibit the synthesis of glycogen by stimulating a protein kinase which phosphorylates glycogen synthetase to the inactive b-form. Lipases in fat cells are also stimulated by catecholamines, thus increasing the breakdown of triglycerides to yield free fatty acids bound to serum albumin. Again this is thought to be due to phosphorylation of the lipase by an activated protein kinase (Lehninger, 1975). These effects in the horse are thought to be mediated by the stimulation of β-adrenoreceptors (Snow, D.H. pers. comm.).

Plasma noradrenaline levels provide an indication of sympathetic activity and the plasma levels of this hormone increase in the horse (Snow and Summers, 1977) and man (Häggendal, et al., 1970) at high intensity work loads. This would in turn lead to a marked increase in glycolysis and lipolysis. The decrease in glycogen utilisation seen after β-blockade in the two horses in this study is therefore in agreement with inhibition of the glycogen breakdown cascade. A similar effect has been found in man by Galbo et al. (1976). However, on the other hand, Harris et al. (1971) found that dl propranolol had no effect on glycogen consumption.

As indicated from the glycogen depletion rates and the performance of the horses, sub-maximal exercise was little affected by the β-adrenoreceptor blockers. In the dog, submaximal exercise after administration of propranolol causes a more rapid fall in glycogen levels than submaximal exercise without drugs (Nazar and Stanislaw, 1975). It may be that the sympathetic system is less important during submaximal exercise in the horse than in the dog. d-Propranolol was included as it is the optical isomer of l-propranolol but does not possess any β-blocking effects. As such it was not expected to alter the rate of depletion of glycogen and this was the case.

SECTION 7

GENERAL DISCUSSION

General Discussion

Analysis of the fibre composition in the horse revealed three fibre types, a high myosin ATPase activity at pH 9.4, low oxidative high glycolytic capacity fibre (FT), high myosin ATPase activity at pH 9.4, high oxidative, high glycolytic capacity fibre (FTH) and a low myosin ATPase activity at pH 9.4, high oxidative, low glycolytic capacity fibre (ST). This was in agreement with other studies (Lindholm and Piehl, 1974; Aberle et al., 1976). The differences in the fibre composition of the muscles examined may be related to their function during running. The highest percentages of fast twitch fibres were found in the rear limb muscles, from which the horse derives most of its propulsive power. The front limbs are used mainly to absorb the shock of landing* and would therefore have less need of a high percentage of fast twitch fibres.

Although the same three basic fibre types could be distinguished in the dog, they were harder to classify than in the horse using the oxidative stain. The classification was further complicated by the fact that some of the cross-bred mongrels examined had an unusual oxidative staining pattern with none of the slow twitch fibres having a higher oxidative capacity than the fast twitch fibres. Less difference was found between the various muscles in the dog and this may be related to a different running gait in this species.

It was found when the gluteus medius muscles of the various breeds of horse were examined, that the Quarterhorse, the breed which was fastest over a short distance, had the highest percentage of fast twitch

*"The Thoroughbred in Action" filmed by the "New York Racing Association."

fibres. A similar finding in the greyhound when compared to the cross-bred mongrel and foxhound confirmed that the faster breeds had the higher percentage of fast twitch fibres. This is in agreement with findings in man where power event athletes such as sprinters and weight lifters have in general 70-80% fast twitch fibres in their vastus lateralis muscles. Good endurance event athletes on the other hand have between 80-98% slow twitch, high oxidative fibres (Gollnick et al., 1972; Costill et al., 1976a,b; Komi et al., 1977a). It has been shown that the greater the percentage of fast twitch fibres in a muscle, the faster the muscle will contract (Peter et al., 1972) and the greater the stride frequency. Similarly, for endurance running, muscles which are resistant to fatigue would be required, and this is best achieved if the muscle is composed mainly of high oxidative fibres (in man mainly ST fibres but in the horse both FTH and ST fibres).

The enzyme activities in the muscles between the various breeds of horse and dog were also different. The Quarterhorse had the highest capacity for anaerobic exercise with the highest levels of ALD and LDH whereas the breeds best suited for exercise of a longer duration with speed, the Arab and Thoroughbred had the highest levels of activity of oxidative enzymes. CPK, the enzyme which is most important for synthesis of ATP during the first few seconds of exercise was not significantly different between the various breeds of horse. The donkey was significantly lower than the others in the equine species but this may have been due to the sedentary life which they led or because they are not a member of the horse family. The greyhound had a greater CPK activity than the mongrel dog and this is probably a reflection of the greater percentage of

fast twitch fibres in the greyhound. Oxidative enzymes in the dog exhibited an unusual pattern with the greyhound having a higher CS activity but lower HAD activity than the mongrel. This dependence on carbohydrate for fuel in the greyhound may be because the greyhound as a breed has very little fat deposition compared to the other breeds of dog (Bogan, J.A., pers. comm.).

The type of training, which corresponded to the type of training programme used by race-horse trainers, given to the horses in this study stimulated an adaptation in both aerobic and anaerobic pathways. This was to be expected as the type of exercise given included a large amount of both sprint and endurance training. Sprint training or training involving work at near maximal output stimulates an increase in glycolytic and TCA cycle enzymes in several species including man, (Gollnick et al., 1973a), rat (Staudte et al., 1973) and horse (Lindholm and Piehl, 1974). Endurance training alone on the other hand has been found to stimulate an increase only in aerobic pathways such as the TCA cycle, fat oxidation and ketone body utilisation (Holloszy and Booth, 1976).

The training given to the horses induced an increased synthesis of both the H and M subunits of LDH. This was at variance with other studies in the rat (York et al., 1974) and man (Sjodin et al., 1976) where endurance exercise induced an increase in the H-subunit. The difference may have been due to the mixed type of exercise given to the horses with an increase occurring in the total H subunit activity because of the endurance portion and the increase in the M subunit activity due to the sprint training.

The adaptations due to exercise were selective in nature as

several enzymes, notably those concerned with glycogen synthesis remaining unchanged. Similarly, the response to detraining was selective with only those enzymes which had increased with training, decreasing in activity, towards pre-training in the first five weeks. Likewise the increase in the second five weeks of training was only in those enzymes which had increased with training. The decrease after training was expected from work in other species (Faulkner et al., 1972; Terjung, 1975; Henricksson and Reitman, 1977) but the response with prolonged detraining was unexpected. No comparable studies have been found to corroborate or challenge this finding and no explanation can be given why it should occur.

The finding that histochemically, the percentage of high to low myosin ATPase fibres altered was interesting as it is generally agreed that this did not occur in mature animals (Saltin, 1973). It may be that the horses in this study were exercising at a higher intensity than is possible in other species and that this induced the change. How this alteration occurs is not known, but it may be due to changes in the myosin ATPase isoenzymes in the fibres or due to preferential fibre splitting in the FTH fibres (Gonyea et al., 1977).

Glycogen depletion occurs in the horse during both submaximal and maximal exercise, with the rate of utilisation per minute being much greater during maximal exercise. The performance during maximal exercise could be affected by prior administration of a β-adrenoreceptor blocking drug and this may be due in part to a decreased utilization of glycogen.

In conclusion, several findings arise from this work and may

be of interest to both the economic viability of the racing industry and basic physiology. It may in the future be possible to predict at an early age, the potential of a horse for a given type of exercise by determining the fibre composition of certain muscles. This is already carried out to some extent in man. More work is required into the responses during training to determine if the muscles of horses with the best performance respond differently from horses of a lower performance. The detraining effect was also interesting and further work is required. Detraining is extremely difficult to study in man because of the unwillingness of subjects to submit to a detraining programme after becoming trained. This is especially true if the subject is an athlete who competes regularly. The horse, which is easy to exercise and train is therefore an ideal subject for experiments in exercise physiology and in many cases, may even be superior to the normal laboratory animals by allowing serial sampling as in man.

APPENDIX

Horse 1 : Thoroughbred, Gelding, 9 years

	% ST	%FTH	%FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus	31.0	42.3	26.7	757	12026	125	262	36.0	13.0	-	-	-
Triceps	31.6	37.8	30.6	860	14855	174	396	25.9	17.0	-	-	-
Vastus Lateralis	11.9	54.1	31.0	1634	13794	251	331	37.0	9.5	-	-	-
Gluteus Medius	6.5	55.2	38.3	1978	16129	294	507	31.4	18.5	13.5	119	71
Biceps Femoris	13.0	54.3	32.7	1462	17331	260	490	32.8	18.0	-	-	-
Semitendinosus	9.1	62.8	28.1	1806	16270	309	421	44.4	18.5	12.3	86	78
Horse 2 : Thoroughbred, Mare, 5 years												
Deltoideus	22.1	30.6	47.3	1479	16978	211	550	42.0	7.5	-	-	-
Triceps	19.5	35.0	45.5	1806	19807	241	684	40.7	13.0	-	-	-
Vastus Lateralis	3.4	51.0	45.6	1548	16270	232	490	35.1	8.0	-	-	-
Gluteus Medius	15.4	48.8	35.8	2580	20515	289	817	53.6	17.0	13.5	203	175
Biceps Femoris	19.1	37.3	41.0	1419	14148	193	546	32.4	16.5	-	-	-
Semitendinosus	14.0	52.0	34.0	1557	13441	223	434	22.2	7.5	13.5	84	90
Horse 3 : Thoroughbred, Gelding, 6 years												
Deltoideus	27.6	26.3	46.1	1548	17119	260	456	26.8	9.5	-	-	-
Triceps	19.5	39.1	41.4	998	13794	178	408	25.1	16.0	-	-	-
Vastus Lateralis	11.8	41.4	46.8	1972	16972	141	434	29.0	11.0	-	-	-
Gluteus Medius	11.5	48.6	39.9	1720	16624	256	593	37.0	18.5	13.5	169	116
Biceps Femoris	21.8	37.8	40.4	2236	13087	251	908	44.6	23.0	-	-	-
Semitendinosus	9.6	53.2	37.2	2236	13087	354	705	46.2	16.5	12.3	162	111

Horse 4 : Thoroughbred, Gelding, 5 years

	%ST	%FTH	%FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus	47.3	10.7	42.0	903	14502	125	385	37.9	8.5	-	-	-
Triceps	14.9	45.5	39.6	1548	19100	251	529	48.1	13.5	-	-	-
Vastus Lateralis	17.1	39.3	43.6	1836	18312	198	473	37.9	10.5	-	-	-
Gluteus Medius	10.3	57.8	31.9	2494	16270	318	456	27.2	11.8	13.1	79	103
Biceps Femoris	15.5	45.4	39.1	1741	19100	212	310	47.1	15.2	-	-	-
Semitendinosus	10.7	61.9	27.4	1741	14855	246	398	38.4	8.5	13.5	91	52

Horse 5 : Heavy Hunter, Mare, 12 years

Deltoideus	38.6	27.3	34.1	860	11672	183	240	21.1	9.2	-	-	-
Triceps	16.8	43.0	40.2	2150	14855	309	327	25.8	10.4	-	-	-
Vastus Lateralis	10.1	55.4	34.5	1978	12380	323	155	12.0	4.4	-	-	-
Gluteus Medius	22.4	43.4	34.2	3182	16978	415	378	35.1	13.5	11.5	135	109
Biceps Femoris	21.7	47.0	31.3	2150	16978	328	318	25.8	11.0	-	-	-
Semitendinosus	15.9	47.1	37.0	3483	15563	371	335	31.4	11.5	15.4	135	100

Horse 6 : Heavy Hunter, Gelding, 13 years

Deltoideus	45.0	28.8	26.2	1015	13440	193	288	24.0	10.0	-	-	-
Triceps	20.0	45.0	35.0	1660	14855	287	310	14.8	13.0	-	-	-
Vastus Lateralis	14.6	38.0	47.4	1961	16270	347	194	12.8	4.7	-	-	-
Gluteus Medius	26.7	46.7	26.6	2408	15209	338	353	16.6	15.8	11.5	182	103
Biceps Femoris	26.7	36.1	37.2	1720	14289	251	335	18.0	14.4	-	-	-
Semitendinosus	26.3	51.6	22.1	2064	15916	357	327	20.8	15.0	13.1	98	71

Horse 7 : Thoroughbred, Mare, 17 years	%ST	%FTH	%FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus	37.4	19.7	42.9									
Triceps	15.5	33.2	51.3									
Vastus Lateralis	2.0	47.5	50.5									
Gluteus Medius	12.8	49.2	36.4	2453	19453	426	701	99.9	24.0	11.5	312	148
Biceps Femoris	15.7	44.7	39.6									
Semitendinosus	5.7	44.3	50.0	2301	17685	378	834	67.5	24.0	13.1	206	186
Horse 8 : Shetland Pony, Stallion, 1 year												
Deltoideus	32.1	24.0	43.9									
Triceps	26.4	23.2	50.4									
Vastus Lateralis	8.5	37.3	54.2									
Gluteus Medius	19.3	38.8	41.9									
Biceps Femoris	26.8	33.4	39.8									
Semitendinosus	7.8	50.2	42.0									
Horse 9 : Thoroughbred, Gelding, 5 years												
Deltoideus	31.5	32.7	35.8									
Triceps	15.9	39.6	44.5									
Vastus Lateralis	1.6	57.8	40.6									
Gluteus Medius	8.0	50.2	41.8	5677	21222	442	894	90.6	16.7	8.0	230	218
Biceps Femoris	16.9	47.7	35.4									
Semitendinosus	5.3	50.3	44.4	3339	19453	426	877	89.7	16.5	6.9	197	244

Horse 10: Pony, Gelding, 3 years

	% ST	% FTH	%FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus	31.0	-	-									
Triceps	31.7	36.2	32.1									
Vastus Lateralis	29.4	31.9	38.7									
Gluteus Medius	35.2	24.4	40.4	6236	21222	579	533	57.3	9.3	6.8	164	269
Biceps Femoris	23.0	45.4	31.6									
Semitendinosus	11.6	49.6	38.8	3741	18039	554	592	65.6	9.5	6.8	129	218

Horse 11: Pony, Gelding, 10 years

Deltoideus	27.0	27.1	45.9									
Triceps	23.0	38.8	38.2									
Vastus Lateralis	11.0	42.0	47.0									
Gluteus Medius	31.3	34.8	33.9	4424	19807	354	671	82.3	12.3	7.2	226	205
Biceps Femoris	27.0	36.4	36.6									
Semitendinosus	18.2	41.3	40.5	5376	21222	386	714	41.6	11.5	5.3	170	231

Horse 12: Pony, Gelding, age unknown

Deltoideus	44.2	20.0	35.8
Triceps	23.2	38.2	38.6
Vastus Lateralis	7.0	58.9	34.1
Gluteus Medius	25.4	34.2	40.4
Biceps Femoris	18.4	52.5	29.1
Semitendinosus	15.4	44.4	40.2

	% ST	%FTH	% FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Horse 13 : Heavy Hunter, Gelding, age unknown												
Deltoideus	35.8	26.2	38.0									
Triceps	29.7	32.7	37.6									
Vastus Lateralis	13.5	52.5	34.0									
Gluteus Medius	34.8	34.0	31.2									
Biceps Femoris	19.7	47.0	33.3									
Semitendinosus	2.4	62.0	35.6									
Horse 14 : Thoroughbred, Gelding, 6 years												
Deltoideus	25.0	35.0	39.0									
Triceps	15.9	51.6	32.5									
Vastus Lateralis	9.7	55.7	34.6									
Gluteus Medius	21.5	54.0	24.5	548	33248	418	593	40.7	10.5	11.4	131	180
Biceps Femoris	29.5	46.0	24.5									
Semitendinosus	14.6	54.3	31.1	3053	21222	627	877	68.5	18.5	11.4	131	180
Horse 15 : Heavy Hunter, Mare, 10 years												
Deltoideus	31.9	13.6	54.5									
Triceps	28.7	23.9	47.4									
Vastus Lateralis	27.2	23.4	52.4									
Gluteus Medius	20.5	37.7	41.8	1376	14148	450	774	36.9	14.5	5.8	172	116
Biceps Femoris	22.8	35.5	41.7									
Semitendinosus	12.3	34.6	53.1	2236	16624	418	714	42.5	11.5	8.1	71	131

Horse 16 : Heavy Hunter, Mare, 6 years

	% ST	%FTH	% FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus	41.6	12.2	46.2									
Triceps	27.7	31.2	41.1									
Vastus Lateralis	16.2	37.4	46.4									
Gluteus Medius	30.5	38.1	31.4	1428	12026	354	464	37.9	5.0	-	-	-
Biceps Femoris	15.8	45.4	38.8									
Semitendinosus	2.2	55.8	42.0	1419	10611	169	378	28.7	5.0	-	-	-

Horse 17 : Heavy Hunter, Gelding, age unknown

Deltoideus	37.5	17.6	44.9									
Triceps	19.3	36.7	44.0									
Vastus Lateralis	12.1	41.5	46.4									
Gluteus Medius	38.2	20.2	41.6	3141	21500	514	568	48.1	16.0	6.2	177	167
Biceps Femoris	21.2	38.4	40.4									
Semitendinosus	20.2	35.7	43.7	2668	20500	402	490	38.8	12.5	8.1	111	135

Horse 18 : Pony, Gelding, age unknown

Deltoideus	29.5	41.2	29.3									
Triceps	24.6	38.3	37.1									
Vastus Lateralis	-	-	-									
Gluteus Medius	16.3	52.8	30.9	1398	14800	322	215	18.5	10.0	7.0	78	64
Biceps Femoris	19.9	55.9	24.2									
Semitendinosus	2.0	51.7	46.3	2065	18300	225	267	33.6	7.0	6.5	84	141

Horse 19 : Pony, Gelding, 3 years	% ST	%FTH	% FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus	41.5	31.1	27.4									
Triceps	32.3	32.6	35.1									
Vastus Lateralis	16.9	42.0	41.1									
Gluteus Medius	6.3	53.4	40.3	3010	16623	579	378	29.6	11.0	7.6	85	77
Biceps Femoris	35.3	33.0	31.7									
Semitendinosus	12.9	49.3	37.8	2408	19100	466	430	31.4	11.5	7.3	97	109
Horse 20 : Pony, Gelding, age unknown												
Deltoideus	32.4	38.5	29.1									
Triceps	27.7	39.6	32.7									
Vastus Lateralis	7.8	52.6	39.6									
Gluteus Medius	34.1	25.1	40.8	1850	19800	482	267	46.2	15.0	6.0	194	103
Biceps Femoris	27.4	41.8	30.5									
Semitendinosus	19.1	45.1	35.8	2367	17300	386	430	34.2	12.0	6.8	164	269
Horse 21 : Pony, Gelding, 7 years												
Deltoideus	31.9	31.3	36.8									
Triceps	21.5	50.0	28.5									
Vastus Lateralis	33.9	25.2	40.9									
Gluteus Medius	19.0	47.2	33.8	2698	16978	354	627	54.5	11.0	6.9	297	257
Biceps Femoris	13.4	57.6	29.0									
Semitendinosus	10.4	50.9	38.7	3634	16978	370	507	64.7	9.0	5.8	145	231

Horse 22 : Pony, Gelding age unknown

	% ST	%FTH	% FT
Deltoideus	24.0	36.0	40.0
Triceps	18.9	43.9	37.2
Vastus Lateralis	26.7	30.7	42.6
Gluteus Medius	15.2	46.7	38.1
Biceps Femoris	27.7	37.9	34.5
Semitendinosus	27.4	24.5	48.1

Horse 23 : Shetland Pony, Gelding, 5 years

Deltoideus	41.0	29.3	29.7
Triceps	36.8	26.8	36.4
Vastus Lateralis	24.5	37.0	38.5
Gluteus Medius	24.3	37.9	37.8
Biceps Femoris	26.1	44.7	29.2
Semitendinosus	9.1	58.8	32.1

Horse 24 : Shetland Pony, Gelding, age unknown

Deltoideus	35.3	29.3	35.4
Triceps	29.7	32.1	38.2
Vastus Lateralis	15.8	44.7	39.5
Gluteus Medius	21.9	39.5	38.6
Biceps Femoris	24.6	44.8	30.6
Semitendinosus	8.2	52.0	39.4

Horse 25 : Shetland Pony, Gelding, age unknown

	% ST	%FTH	%FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus	39.8	24.4	35.8									
Triceps	13.4	56.0	30.6									
Vastus Lateralis	11.7	49.0	39.3									
Gluteus Medius	19.4	40.6	40.0									
Biceps Femoris	32.3	33.7	34.0									
Semitendinosus	5.7	50.5	43.8									

Horse 26 : Donkey, Stallion, age unknown

Deltoideus												
Triceps												
Vastus Lateralis												
Gluteus Medius												
Biceps Femoris												
Semitendinosus	27.3	34.4	39.3	1440	11672	209	320	29.1	3.0	7.7	123	45

Horse 27 : Donkey, Mare, age unknown

Deltoideus												
Triceps												
Vastus Lateralis												
Gluteus Medius	-	-	-	1376	10611	179	482	21.7	2.5	5.5	240	51
Biceps Femoris												
Semitendinosus	26.2	30.9	42.9	817	7074	80	151	15.0	1.5	9.6	39	26

Horse 28 : Donkey, Mare, age unknown

	% ST	%FTH	% FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus												
Triceps												
Vastus Lateralis												
Gluteus Medius	26.7	33.7	39.6	946	7428	145	204	13.9	2.5	6.4	107	38
Biceps Femoris												
Semitendinosus	25.7	24.9	49.4	1505	10611	225	301	24.0	2.5	4.4	118	28

Horse 29 : Donkey, Gelding, age unknown

Deltoideus												
Triceps												
Vastus Lateralis												
Gluteus Medius	26.6	37.5	35.9	2150	13440	257	387	22.2	2.5	7.1	141	64
Biceps Femoris												
Semitendinosus	11.6	45.1	43.3	1828	9196	184	297	18.5	1.3	7.2	97	51

Horse 30 : Donkey, Gelding, age unknown

Deltoideus												
Triceps												
Vastus Lateralis												
Gluteus Medius	20.0	42.1	37.9	1720	9904	233	288	12.0	3.5	4.4	103	58
Biceps Femoris												
Semitendinosus	29.2	26.7	44.1	1247	11318	161	292	21.4	2.5	4.9	82	38

	% ST	%FTH	% FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Horse 31 : Heavy Hunter, Gelding, 10 years												
Gluteus Medius	17.0	44.8	38.2	4602	16624	402	485	50.8	8.0	7.9	131	148
Semitendinosus	5.4	52.8	41.8	5075	19100	506	439	43.4	3.0	9.5	101	136
Horse 32 : Heavy Hunter, Mare, age unknown												
Gluteus Medius	-	-	-	2365	20515	418	705	42.5	12.0	4.7	183	160
Semitendinosus	-	-	-	3182	21929	482	1066	75.8	21.5	7.2	290	193
Horse 33 : Heavy Hunter, Mare, age unknown												
Gluteus Medius	-	-	-	4064	17685	370	782	98.0	15.0	6.9	254	231
Semitendinosus	-	-	-	1978	16978	540	559	24.0	11.0	8.8	110	108
Horse 34 : Heavy Hunter, Mare, age unknown												
Gluteus Medius	-	-	-	3397	17685	354	464	77.6	14.5	6.0	265	218
Semitendinosus	-	-	-	2636	15563	482	450	42.5	11.5	9.1	225	165
Horse 35 : Thoroughbred, Mare, 3 years												
Gluteus Medius	16.0	60.2	23.8	2453	18300	547	542	38.8	17.0	-	-	-
Horse 36 : Pony, Gelding, age unknown												
Gluteus Medius	30.0	33.4	36.6	1979	15500	289	666	19.6	9.0	-	-	-
Horse 37 : Pony, Gelding, 3 years												
Gluteus Medius	9.9	51.9	38.2	1979	15900	257	232	20.7	8.0	8.1	74	70
Semitendinosus	17.3	51.3	31.4	1936	19000	320	318	27.7	10.0	9.5	83	83

	% ST	%FTH	% FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Horse 38 : Arab, Gelding												
Gluteus Medius	12.7	53.0	34.3	4644	21222	506	1067	53.6	19.0	8.6	248	212
Horse 39 : Arab												
Gluteus Medius	21.9	40.1	38.0	5161	25820	523	1488	49.9	25.4	6.4	156	-
Horse 40 : Arab												
Gluteus Medius	11.1	47.1	41.8	3189	16270	332	633	65.6	12.0	8.1	138	122
Horse 41 : Arab												
Gluteus Medius	8.1	52.5	39.4	5590	19100	579	543	74.0	10.0	8.7	137	174
Horse 42 : Arab												
Gluteus Medius	18.0	45.4	36.6	4171	19100	482	654	58.2	11.5	6.4	185	116
Horse 43 : Arab, Stallion, 9 months												
Gluteus Medius	25.5	49.8	24.5	1936	18000	402	473	29.6	12.5	-	-	-
Semitendinosus	14.4	44.3	41.3	1807	18300	402	550	29.6	12.5	-	-	-
Horse 44 : Quarterhorse												
Gluteus Medius	9.5	51.9	38.6	6535	22991	715	654	41.6	10.0	10.8	143	167
Horse 45 : Quarterhorse												
Gluteus Medius	6.7	39.8	53.5	5935	20515	683	378	48.0	6.0	9.6	70	-

	% ST	%FTH	% FT	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Horse 46 : Quarterhorse												
Gluteus Medius	8.1	57.9	34.0	5676	18746	506	447	78.6	10.5	7.5	-	193
Horse 47 : Quarterhorse												
Gluteus Medius	1.3	49.3	49.4	8514	17685	683	568	60.6	8.3	9.6	124	231
Horse 48 : Quarterhorse												
Gluteus Medius	5.3	51.7	43.0	7225	24405	715	636	65.6	11.3	8.4	118	206
Horse 49 : Quarterhorse												
Gluteus Medius	9.9	47.0	43.1	6450	21222	683	421	61.0	7.0	7.5	110	225
Horse 50 : Quarterhorse												
Gluteus Medius	5.6	46.2	48.2	3268	14855	426	374	43.0	6.5	7.3	110	77
Horse 51 : Quarterhorse												
Gluteus Medius	8.2	42.4	49.4	6107	19100	643	409	31.4	5.8	8.9	108	167

	% ST	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Dog 1 : Greyhound										
Deltoideus	2.0									
Triceps	0.0									
Vastus Lateralis	0.0									
Gluteus Medius	0.0	2409	26881	478	1032	65.8	20.0	3.8	185	141
Biceps Femoris	17.6									
Semitendinosus	0.0	1742	29003	456	705	44.8	14.0	5.1	55	102
Dog 2 : Greyhound										
Deltoideus	0.0									
Triceps	1.5									
Vastus Lateralis	11.3									
Gluteus Medius	4.2	2324	30065	424	895	70.3	18.0	5.2	117	154
Biceps Femoris	12.6									
Semitendinosus	0.0	2212	22637	354	473	35.6	13.0	2.7	83	90
Dog 3 : Greyhound										
Deltoideus	0.0									
Triceps	3.5									
Vastus Lateralis	8.2									
Gluteus Medius	0.0	2151	46689	501	1084	76.7	22.5	6.6	97	167
Biceps Femoris	14.6									
Semitendinosus	0.0	1506	29711	527	912	67.5	23.0	5.4	111	173

Dog 4 : Greyhound

	% ST	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus	0.0									
Triceps	0.0									
Vastus Lateralis	1.9									
Gluteus Medius	0.0	2991	40322	408	1238	90.6	28.5	4.3	99	116
Biceps Femoris	6.0									
Semitendinosus	1.2	2754	38200	530	938	65.2	21.0	4.1	90	128

Dog 5 : Greyhound

Deltoideus	0.0									
Triceps	0.0									
Vastus Lateralis	4.1									
Gluteus Medius	1.8	1592	32540	450	1135	89.2	27.0	5.5	88	141
Biceps Femoris	10.2									
Semitendinosus	1.0	2152	27589	479	1015	58.7	20.5	5.1	63	109

Dog 6 : Greyhound

Deltoideus	0.0									
Triceps	0.0									
Vastus Lateralis	0.0									
Gluteus Medius	4.5	1742	29003	398	843	62.8	20.0	5.8	106	141
Biceps Femoris	6.5									
Semitendinosus	0.0	2637	35370	450	791	69.3	20.0	6.9	99	154

Dog 7 : Mongrel	% ST	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus	27.2									
Triceps	33.6									
Vastus Lateralis	51.4									
Gluteus Medius	39.4	1847	23344	185	869	38.4	9.5	6.4	279	128
Biceps Femoris	36.9									
Semitendinosus	15.2	2645	21929	297	877	36.1	9.5	8.4	152	116
Dog 8 : Mongrel										
Deltoideus	22.0									
Triceps	20.0									
Vastus Lateralis	50.4									
Gluteus Medius	32.3	2623	24052	241	808	37.0	7.0	6.6	318	180
Biceps Femoris	32.4									
Semitendinosus	15.3	2301	19100	225	499	19.4	2.5	6.4	87	96
Dog 9 : Mongrel										
Deltoideus	22.9									
Triceps	20.7									
Vastus Lateralis	16.0									
Gluteus Medius	31.6	2795	24052	289	877	61.0	13.5	8.5	331	154
Biceps Femoris	13.0									
Semitendinosus	18.2	2559	23698	289	626	34.2	8.5	6.6	106	103

Dog 10 : Mongrel	% ST	LDH	CPK	ALD	AST	ALT	CS	HK	HAD	GDH
Deltoideus	19.0									
Triceps	19.7									
Vastus Lateralis	45.5									
Gluteus Medius	34.1	2666	21929	241	972	47.2	6.0	7.4	240	154
Biceps Femoris	12.2									
Semitendinosus	4.9	1935	19100	265	490	18.9	4.0	7.4	81	83
Dog 11 : Mongrel										
Deltoideus	36.8									
Triceps	20.0									
Vastus Lateralis	62.9									
Gluteus Medius	29.3	2322	20868	273	1049	58.7	6.0	6.4	286	167
Biceps Femoris	26.8									
Semitendinosus	19.9	1763	17685	249	628	25.9	4.0	6.1	105	71
Dog 12 : Mongrel										
Deltoideus	33.0									
Triceps	7.3									
Vastus Lateralis	13.1									
Gluteus Medius	34.0									
Biceps-Femoris	23.8									
Semitendinosus	13.2									

	Dog 13 : Foxhound	Dog 14 : Foxhound	Dog 15 : Foxhound	Dog 16 : Foxhound
	% ST	% ST	% ST	% ST
Deltoideus	37.0	54.0	54.6	27.2
Triceps	34.4	24.1	44.2	33.6
Vastus Lateralis	24.4	33.6	0.0	37.6
Gluteus Medius	25.2	46.4	27.7	31.4
Biceps Femoris	33.6	40.7	36.7	36.9
Semitendinosus	34.7	24.7	47.1	15.2

REFERENCES

Aberle, E.D., Judge, M.D., Kirkham, W.W., Page, E.H. and Crawford, B.H. Am. J. vet. Res. 37, 145 (1975)

Abraham, J., Chernian, G., Sathe, B., Bachhawat, B.K. and Chandy, J. Neurol. (Bombay) 15, 152 (1967)

Adolfsson, S. Acta physiol. scand. 87, 465 (1973)

Ahlborg, B., Bergstrom, J., Eklund L.-G. and Hultman, E. Acta physiol. scand. 70, 129 (1967)

Andersen, P. and Henricksson, J. Acta physiol. scand. 99, 123 (1977)

Anderson, M.G. Eq. vet. J. 7, 160 (1975)

Anderson, M.G. Res. vet. sci. 20, 191 (1976)

Ariano, M.A., Armstrong, R.B. and Edgerton, V.R. J. histochem. cytochem. 21, 51 (1973)

Armstrong, R.B., Saubert IV, C.W., Senbrowich, W.L., Shepherd, R.E. and Gollnick, P.D. In: Metabolic adaptations to prolonged physical exercise (H. Howald and J.R. Poortmans eds) pp 397, Birkhavon Verlag, Basel (1975)

Ashmore, C.R. and Doerr, L. Exp. Neurol. 31, 408 (1971)

Asmussen, E. In: Muscle Metabolism During Exercise (B. Pernow and B. Saltin, eds) pp 1 Plenum Press New York (1971)

Baba, N. and Sharma, H.M. J. cell Biol. 51, 621 (1971)

Baldwin, K.M., Klinkerfuss, G.H., Terjung, R.L., Molé, P.A. and Holloszy, J.O. Am. J. Physiol. 222, 373 (1972)

Baldwin, K.M., Winder, W.W., Terjung, R.L. and Holloszy, J.O. Am. J. Physiol. 225, 962 (1973)

Baldwin, K.M., Winder, W.W. and Holloszy, J.O. Am. J. Physiol. 229, 422 (1975)

Bárány, M. J. gen. Physiol. 50, 197 (1967)

Barnard, R.J., Edgerton, V.R., Furukawa, T. and Peter, J.B. Am. J. Physiol. 220, 410 (1970)

Barnard, R.J., Edgerton, V.R. and Peter, J.B. J. appl. Physiol. 28, 762 (1970)

Barnett, H. J. clin. Path. 17, 567 (1964)

Benzi, G., Panceri, P., de Bernardi, M., Villa, R., Arcelli, E., d'Angelo, L., Arrigoni, E. and Berte, F. J. appl. Physiol. 38, 565 (1975)

Bergmeyer, H.U. In: Methoden der Enzymatischen Analyse (H.U. Bergmeyer, ed.), Verlag, Chemie Weinheim (1962)

Bergström, J. Scand. J. clin. Lab. Invest. 14, suppl. 68 (1962)

Bergström, J. and Hultman, E. Scand. J. clin. Lab. Invest. 19, 218 (1967)

Bergström, J., Hermansen, L., Hultman, E. and Saltin, B. Acta physiol. scand. 71, 140 (1967)

Blancher, M.C. Am. J. Physiol. 206, 1015 (1964)

Bloom, S.R., Johnson, R.H., Park, D.M., Rennie, M.J. and Suliman, W.R. J. Physiol. 258, 1 (1976)

Booth, F.W. and Holloszy, J.O. Med. Sci. Sport 8, 60 (1976)

Booth, F.W. and Kelso, J.R. Can. J. Physiol. Pharmacol. 51, 679 (1973)

Bostrom, S., Fahlén, M., Hjalmarson, Å. and Johansson, R. Acta physiol. scand. 90, 544 (1974)

Bremer, J. In: Cellular Compartmentalization and Control of Fatty Acid Metabolism (Bremer, J. and Gran, F.C. eds) pp 65, Academic Press, New York (1967)

Brooke, M.H. and Engel, W.K. Neurol. (Minneapp.) 16, 779 (1966)

Brooke, M.H. and Kaiser, K.K. J. Histochem. Cytochem. 18, 670 (1970,a)

Brooke, M.H. and Kaiser, K.K. Arch. Neurol. (Chic.) 23, 369 (1970,b)

Bücher, Th., Luh, W. and Pette, D. Handbuch der Physiologisch und Pathologisch Chemischem Analyse VIA, pp 292, Springer, Berlin (1964)

Buller, A.J., Eccles, J.C. and Eccles, R.M. J. Physiol. 150, 399 (1960a)

Buller, A.J., Eccles, J.C. and Eccles, R.M. J. Physiol. 150, 417 (1960b)

Burke, R.E. and Edgerton, V.R. Exercise and Sports Sci. Rev. 3, 31 (1975)

Burke, R.E., Levine, D.N., Tsairis, P. and Zajac, F.E. III. J. Physiol. 234, 723 (1973)

Busk, G. and Huxley, T. (tr.) (Kolliker's) Manual of Human Histology, London (1853)

Bylund, A.-C., Björo, T., Ceterblad, G., Holm, J., Lundholm, K., Sjöstrom, M., Angquist, K.A. and Schersten, T. Europ. J. appl. Physiol. 36, 151, (1977)

Cardinet, G.H., Fedde, M.R., Bardens, J.W., Wallace, L.J. and Guffy, M.M. Arch. Neurol. 21, 620 (1969)

Carlsson, G.P. In: Proc. 1st International Symposium on Equine Haematology pp 473 (1975)

Carlsson, L.A., Ekelund, L. and Froberg, S.O. Europ. J. clin. Invest. 1, 248 (1971)

Chance, B., Schoener, B. and Schindler, F. In: Oxygen in the Animal Organism (F. Dickens and E. Neil eds.) pp 122. I.U.B. Symposium Series, Pergamon Press, Oxford (1964)

Chapler, C.K. and Moore, W.G. J. appl. Physiol. 32, 542 (1972)

Chapler, C.K. and Stainsby, W.N. Am. J. Physiol. 215, 995 (1968)

Chernick, S.S. Meth. Enzymol. 14, 627 (1968)

Christensen, E.H. and Hanson, O. Skand. Arch. Physiol. 81, 152 (1939)

Close, R. J. Physiol. 193, 45 (1967)

Costill, D.L., Gollnick, P.D., Jansson, E.D., Saltin, B. and Stein, E.M. Acta physiol. scand. 89, 374 (1973)

Costill, D.L., Daniels, J., Evans, W., Fink, W. and Krahenbuhl, G. J. appl. Physiol. 40, 149 (1976a)

Costill, D.L., Fink, W.J. and Pollock, M.L. Med. Sci. in Sports 8, 96 (1976b)

Cotter, M., Hudlicka, O., Pette, D., Staudte, H. and Vrbova, G.J. Physiol. 230, 34p (1973)

Crabtree, B. and Newsholme, E.A. Biochem. J. 126, 49 (1972)

Danforth, W.H. J. biol. Chem. 240, 588 (1965)

Davies, A.S. and Gunn, H.M. J. Anat. 112, 41 (1972)

Davis, B.J. Ann. N.Y. Acad. Sci. 121, 404 (1964)

Dawson, D.M., Goodfriend, T.L. and Kaplan, N.O. Science 43, 929 (1964)

Dietz, A.A. and Lubrano, T. Analyt. Biochem. 20, 246 (1967)

Dohm, G.L., Pennington, S.N. and Barakat, H. Biochem. Med. 16, 138 (1976)

Donald, D.E., Ferguson, D.A. and Milburn, S.E. Circ. Res. 22, 127 (1968)

Duchennes, G.B. Arch. gen. Med. 11, 179 (1868)

Edgerton, V.R., Barnard, R.J., Peter, J.B., Gillespie, C.A. and Simpson, D.R. Exp. Neurol. 37, 322 (1972)

Edgerton, V.R., Gerchman, L. and Carrow, R. Exp. Neurol. 24, 110 (1969)

Edgerton, V.R. and Simpson, D.R. J. Histochem. Cytochem. 17, 828 (1969)

Edgerton, V.R., Smith, J.L. and Simpson, D.R. Histochem. J. 7, 259 (1975)

Edstrom, L. and Kugelberg, E. J. Neurol., Neurosurg. Psychiat. 31, 424 (1968)

Edstrom, L. and Ekblom, B. Scand. J. clin. Lab. Invest. 30, 175 (1972)

Eggleton, P. and Eggleton, M.E. Nature 119, 194 (1927)

Ekblom, B., Goldberg, A.N., Kolbom, A. and Astrand, P.-O. Scand. J. clin. lab. Invest. 30, 35 (1972)

Engel, W.K. Arch. Neurol. 22, 99 (1970)

Engelhardt, W., Horricke, H., Ehrlein, J.H. and Schmidt, E. Zentbl. Vet. Med. (A) 20, 173 (1973)

Engelhardt, W.A. and Ljubimowa, M.N. Nature 144, 668 (1939)

Eriksson, B.O., Gollnick, P.D. and Saltin, B. Acta physiol. scand. 87, 231 (1972)

Epstein, S.E., Robinson, B.F., Kahler, R.L. and Braunwald, E. J. clin. Invest. 44, 1745 (1965)

Essen, B., Jansson, E., Henricksson, J., Taylor, A.W. and Saltin, B. Acta physiol. scand. 95, 153 (1975)

Exner, G.U., Staudte, H.W. and Pette, D. P fluegers Arch ges Physiol. 345, 15 (1973)

Faulkner, J.A., Maxwell, L.C. and Lieberman, D.A. Am. J. Physiol. 222, 836 (1972)

Felig, P. and Wahren, J. In: Muscle Metabolism During Exercise (B. Pernow and B. Saltin, eds) pp 205, Plenum Press, New York (1971)

Fiske, C.H. and Subbarrow, Y. Science 65, 401 (1927)

Fitts, R.H., Nagle, F.J. and Cassens, R.G. Can. J. physiol. pharmacol. 51, 825 (1973)

Fitts, R.H., Booth, F.W., Winder, W.W. and Holloszy, J.O. Am. J. Physiol. 228, 1029 (1975)

Fletcher, W.M. and Hopkins, F.G. J. Physiol. 35, 247 (1907)

Fogd Jorgensen, P. and Hyldgaard-Jensen, J.F. Acta vet. scand. 16, 368 (1975)

Friedman, D.L. and Larner, J. Biochemistry 2, 669 (1963)

Fröberg, S.O. and Mossfeldt, F. Acta physiol. scand. 82, 167 (1971)

Galbo, H., Holst, J.J., Christensen, N.S. and Hilsted, J. J. appl. Physiol. 40, 855 (1976)

Gauthier, G.F. and Padykula, H.A. J. cell. Biol. 28, 333 (1966)

Gehlerter, T.D. New Engl. J. Med. 294, 10 (1976)

George, J.C. and Jyoti, D. J. anim. morphol. Physiol. 2, 31 (1955)

Gerchman, L.B. Anat. Rec. 160, 352 (1968)

German Society for Clinical Chemistry. Z. klin. Chem. klin. Biochem. 8, 659 (1970)

Ginsberg, V. In: Advances in Enzymology (F.F. Nord ed.) Vol 26 p 35 - Interscience New York (1969)

Goldberg, A.L., Ettlinger, J.D., Goldspink, D.F. and Jablecki, C. Med. sci. Sports 7, 185 (1975)

Gollnick, P.D. and Simmons, S.W. Arbeitsphyiologie 23, 322 (1967)

Gollnick, P.D. and King, D.W. Am. J. Physiol. 216, 1502 (1969)

Gollnick, P.D., Ianuzzo, C.D. and King, D.W. In: Muscle Metabolism During Exercise (B. Pernow and B. Saltin, eds.) pp 69, Plenum Press. New York (1971)

Gollnick, P.D., Armstrong, R.B., Saubert IV, C.W., Piehl, K. and Saltin, B. J. appl. Physiol. 33, 312 (1972)

Gollnick, P.D., Armstrong, R.B., Saltin, B., Saubert IV, C.W., Sembrowich, W.L. and Shepherd, R.E. J. appl. Physiol. 34, 107 (1973a)

Gollnick, P.D., Armstrong, R.B., Saubert IV, C.W., Sembrowich, W.L., Shepherd, R.E. and Saltin, B. Pflüges Arch. ges Physiol. 344, 1 (1973b)

Gollnick, P.D., Armstrong, R.B., Sembrowich, W.L., Shepherd, R.E. and Saltin, B. J. appl. Physiol. 34, 615 (1973c)

Gollnick, P.D., Piehl, K. and Saltin, B. J. Physiol. 241, 45 (1974a)

Gollnick, P.D., Sjödin, B., Karlsson, J., Jansson, E., Saltin, B. Pflüges Arch. ges Physiol. 348, 247 (1974b)

Gould, M.K. and Rawlinson, W.A. Biochem. J. 73, 41 (1959)

Gonyea, W., Ericson, C. and Bonde-Peterson, F. Acta physiol. scand. 99, 105 (1977)

Grunby, G., Bjorntorp, P., Fahlen, M., Hoskins, T.A., Hook, O., Oxhoj, H. and Saltin, B. Scand. J. clin. Lab. Invest. 31, 301 (1973)

Gunn, H.M. New Zeal. vet. J. 23, 249 (1975)

Günther, L. Virchows Arch. path., anat., physiol. 230, 146 (1921)

Guth, L. and Samaha, F.J. Exp. Neurol. 34, 465 (1969)

Guth, L. and Samaha, F.J. Exp. Neurol. 34, 465 (1972)

Guth, L. and Yellin, H. Exp. Neurol. 31, 277 (1971)

Gutmann, E. A. Rev. Physiol. 38, 177 (1976)

Häggendal, J., Hartley, L.H. and Saltin, B. Scand. J. clin. Lab. Invest. 26, 337 (1970)

Harri, M.N.E. and Valtola, J. Acta physiol. scand. 95, 391 (1975)

Harris, R.C., Bergström, J. and Hultman, E. In: Muscle Metabolism During Exercise (B. Pernow and B. Saltin, eds.) pp 301, Plenum Press, New York (1971)

Havel, R.J., Carlson, L.A., Ekelund, L.-G. and Holmgren, A. J. appl. Physiol. 19, 613 (1964)

Hearn, G.R. and Waino, W.W. Am. J. Physiol. 185, 348 (1956)

Henneman, E. and Olsen, C.B. J. Neurophysiol. 28, 581 (1965)

Henricksson, J. Ph.D. Thesis, August Krogh Institute, University of Copenhagen, Denmark (1976)

Henricksson, J. and Reitman, J.S. Acta physiol. scand. 97, 392 (1976)

Henricksson, J. and Reitman, J.S. Acta physiol. scand. 99, 91 (1977)

Hermansen, L., Hultman, E. and Saltin, B. Acta physiol. scand. 71, 129 (1967)

Hermann, D.L. Physiologie des Menschen, Hirschfeld, Berlin (1874)

Hickson, R.C., Heusner, W.W. and Van Huss, W.D. J. appl. Physiol. 40, 868 (1975)

Hitzeman, J.W. J. Histochem. Cytochem. 11, 62 (1963)

Hoffman, E. Rev. Physiol. Biochem. and Pharmacol. 75, 1 (1976)

Hohorst, H.J. In: Methods of Enzymatic Analysis (H.U. Bergmeyer, ed.) Verlag Chemie, Weinheim (1962)

Holloszy, J.O. J. biol. Chem. 242, 2278 (1967)

Holloszy, J.O. Exercise and Sports Sci. Rev. 1, 45 (1973)

Holloszy, J.O. Prog. Cardiovasc. Dis. 18, 445 (1976)

Holloszy, J.O. and Booth, F.W. A. Rev. Physiol. 38, 273 (1976)

Holloszy, J.O., Booth, F.W., Winder, W.W. and Fitts, R.H. In: Metabolic Adaptations to Prolonged Physical Exercise (H. Howald and J.R. Poortmans, eds.) pp 138, Birkhauser Verlag, Basel (1975)

Holloszy, J.O., Mole, P.A., Baldwin, K.M. and Terjung, R.L. In: Limiting Factors in Physical Performance (J. Keul, ed.) pp 66, Thieme, Stuttgart (1973)

Holloszy, J.O. and Narahara, H.T. J. biol. Chem. 240, 3493 (1965)

Holloszy, J.O. and Narahara, H.T. J. gen. Physiol. 50, 551 (1967)

Holloszy, J.O. and Oscai, L.B. Arch. Biochem. Biophys. 130, 653 (1969)

Holloszy, J.O., Oscai, L.B., Don, I.J. and Mole, P.A. Biochem. Biophys. Res. Comm. 40, 1368 (1970)

Holm, J., Bjorntorp, P. and Schersten, T. Europ. J. clin. Invest. 2, 321 (1972)

Huijing, F. Clinica chim. Acta 30, 567 (1970)

Hultman, E. Circ. Res. 20/21, Suppl. 1, 99 (1967)

Hultman, E., Bergström, J. and McLennan Anderson, N. Scand. J. clin. Lab. Invest. 19, 56 (1967)

Hultman, E., Bergström, J. and Roch-Norlund, A.E. In: Muscle Metabolism During Exercise (B. Pernow and B. Saltin, eds.) pp 273, Plenum Press, New York (1971)

Issekutz, B. Jr., Miller, H.I., Paul, P. and Rodahl, K. J. appl. Physiol. 20, 293 (1965)

Issekutz, B. Jr., Miller, H.I. and Rodahl, K. Fedn. Proc. 25, 1415 (1966)

Jansson, E. Acta physiol. scand. 95, 47A (1975)

Jansson, E. and Kaijser, L. Acta physiol. scand. 100, 315 (1977)

Jeffress, R.N., Peter, J.B. and Lamb, D.R. Life Sciences 7, 957 (1968)

Jennekens, F.F., Tomlinson, B. and Walton, J. J. neurol. Sci. 14, 245 (1971)

Jobsis, F.F. and Stainsby, W.N. Resp. Physiol. 4, 292 (1968)

Johnson, M.A., Polgar, J., Weightman, D. and Appleton, D. J. neurol. Sci. 18, 111 (1973)

Johnsson, G., Nyberg, G. and Solvell, L. Acta pharmacol. toxicol. 36, Suppl. V. 59 (1975)

Kalchar, H.M. Chem. Rev. 28, 71 (1941)

Kar, N.C. and Pearson, C.M. Proc. Soc. exp. Biol. (N.Y.) 141, 4 (1972)

Karlsson, J. Acta physiol. scand. 81, Suppl. 358 (1971)

Karlsson, J., Frith, K., Sjodin, B., Gollnick, P.D. and Saltin, B. Scand. J. clin. Lab. Invest. 33, 307 (1974a)

Karlsson, J., Hulten, B. and Sjodin, B. Acta Physiol. Scand. 92, 21 (1974b)

Karlsson, J., Nødesjo, L.O. and Saltin, B. Acta physiol. scand. 90, 210 (1974c)

Karlsson, J. and Komi, P.V. Acta physiol. scand. Suppl. 440, 9A (1976)

Karlsson, J. and Saltin, B. J. appl. Physiol. 29, 598 (1970)

Katz, A.M. Physiol. Rev. 50, 63 (1970)

Katzen, H.M., Soderman, D.D. and Wiley, C.E. J. biol. Chem. 255, 4081 (1970)

Kellerman, A.M., Biggs, D.R. and Linnane, A.W. J. cell. Biol. 42, 378 (1969)

King Engel, W. and Cunningham, G.G. J. Histochem. Cytochem. 18, 55 (1970)

Kinnula, V. Acta physiol. scand. 96, 417 (1976)

Komi, P.V., Rusko, H., Vos, J. and Vihko, V. Acta physiol. scand. 100, 107 (1977a)

Komi, P.V., Vitasalo, J., Hasu, M., Thorstensson, A., Hulten, B. and Karlsson, J. Acta physiol. scand. Suppl. In press (1977b)

Kotchens, T.A., Hartley, L.H., Rice, T.W., Mougey, E.H., Jones, L.G. and Mason, J.W. J. appl. Physiol. 31, 178 (1971)

Krebs, H.A. Adv. in Enzymol. 3, 191 (1943)

Krogh, A. and Lindhard, J. Biochem. J. 14, 290 (1920)

Krzywanek, H. Zentbl. vet. Med. (A) 20, 265 (1973)

Kugelberg, E. and Edstrom, L. J. Neurol. Neurosurg. and Psychiat. 31, 415 (1968)

Kuhne, W. Arch. Path. Anat. Physiol. 33, 79 (1865)

Lehninger, A.L. Biochemistry, 2nd ed., Worth Publishers, New York (1975)

Leloir, L.F. and Goldemberg, S.H. J. biol. Chem. 235, 3054 (1960)

Lindholm, A. Acta physiol. scand. Suppl. 396, 105A, 70 (1973)

Lindholm, A., Bjerneld, H. and Saltin, B. Acta physiol. scand. 90, 475 (1974a)

Lindholm, A., Johansson, H.E. and Kzaersgaard, P. Acta vet. scand. 15, 325 (1974b)

Lindholm, A. and Piehl, K. Acta vet. scand. 15, 287 (1974)

Lindholm, A. and Saltin, B. Acta vet. scand. 15, 310 (1974)

Lipmann, F. Adv. in Enzymol. 1, 99 (1941)

Lohmann, K. Biochem. Z. 233, 460 (1931)

Lohmann, K. Biochem. Z. 271, 264 (1934)

Lohmann, K. Biochem. Z. 282, 120 (1935)

Lowenstein, J.M. Physiol. Rev. 52, 382 (1972)

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. J. biol. Chem. 193, 265 (1951)

Lundsgaard, E. Biochem. Z. 217, 162 (1930a)

Lundsgaard, E. Biochem. Z. 227, 51 (1930b)

Lundsgaard, L. Danske Hospitalstidende 75, 84 (1932)

Margaria, R., Edwards, H.T. and Dill, D.B. Am. J. Physiol. 106, 689 (1933)

Masoro, E.J., Rowell, L.B., McDonald, R.M. and Steiert, B. J. biol. Chem. 241, 2626 (1966)

Max, S.R. Biochem. Biophys. Res. Comm. 46, 1394 (1972)

Max, S.R., Mayer, R.F. and Vogelsang, L. Arch. Biochem. Biophys. 146, 227 (1971)

Meijer, A.E.F.H. Histochemie 22, 51 (1970)

Meijer, A.E.F.H. and Vossenberg, R.P.M. Histochemistry 52, 45 (1977)

MacKenzie, G. and Snow, D.H. Vet. Rec. 101, 30 (1977)

Moesch, H. and Howald, H. In: Metabolic Adaptations to Prolonged Physical Exercise (H. Howald and J.R. Poortmans, eds.) pp 463, Birkhauser Verlag, Basel (1973)

Mole, P.A., Baldwin, K.M., Terjung, R.L. and Holloszy, J.O. Am. J. Physiol. 224, 50 (1973)

Mole, P.A., Oscai, L.B. and Holloszy, J.O. J. clin. Invest. 50, 2323 (1970)

Morgan, T.E., Cobb, L.A., Short, F.A., Ross, R. and Gunn, D.R. In: Muscle Metabolism During Exercise (B. Pernow and B. Saltin, eds.) pp 87, Plenum Press, New York (1971)

Morgan, H.E. and Parmeggiani, A. J. biol. Chem. 239, 2435 (1964)

Morner, K.A.H. Nord. Med. Ark. 30, 1 (1894)

Moses, H.L. and Rosenthal, A.S. J. Histochem. Cytochem. 16, 530 (1968)

Muller, W. Cell. Tiss. Res. 156, 61 (1974)

Nachlow, M.M., Walker, D.G. and Seligman, A.M. J. Histochem. Cytochem. 5, 420 (1957)

Nazar, K., Brzezinska, Z., Lzsyczarz, J. and Danielewicz-Kotowicz, A. Arch. internat. Physiol. Biochem. 79, 873 (1971)

Nazar, K. and Kozlowski, S. In: Proc. Warsaw Convension on Nutrition p 161 (1975)

Needham, D.M. Machina Carnis University Press, Cambridge (1971)

Novikoff, A.B. J. Histochem. Cytochem. 15, 353 (1967)

Nygaard Jensen, E. Acta physiol. scand. Suppl. 440, 291 (1976)

Ogata, T.A. Acta med. Okayama 12, 216 (1958a)

Ogata, T.A. Acta med. Okayama 12, 228 (1958b)

Ogata, T.A. Acta med. Okayama 12, 233 (1958c)

Oliver, I.T. Biochem. J. 61, 116 (1955)

Oscai, L.B. and Holloszy, J.O. J. biol. Chem. 246, 6968 (1971)

Padykula, H.A. and Herman, E. J. Histochem. Cytochem. 3, 170 (1955)

Padykula, H.A. and Gauthier, G. In: Exploratory Concepts in Muscular Distrophy and Related Disorders (A.T. Milhorat, ed.)p 1A, Excerpta Medica, New York.

Paukul, E. Arch. Anat. Physiol. 99, 100 (1904)

Paul, P. J. appl. Physiol. 28, 127 (1970)

Peter, J.B., Barnard, R.J., Edgerton, V.R., Gillespie, C.A. and Stempel, R.E. Biochemistry 11, 2627 (1972)

Peter, J.B., Jeffress, R.N. and Lamb, D.R. Science 160, 200 (1968)

Peter, J.B., Sawaki, S., Barnard, R.J., Edgerton, V.R. and Gillespie, C.A. Arch. Biochem. Biophys. 144, 304 (1971)

Pette, D. Acta Histochem. Suppl. 16, 47 (1975)

Pette, D. and Bücher, T. Hoppe-Seyler's Z. Physiol. Chem. 331, 180 (1963)

Pette, D. and Dolken, G. In: Advances in Enzyme Regulation vol. 13 (G. Weber, eds.) p 355, Pergamon Press, Oxford (1975)

Pette, D., Müller, W., Leisner, E. and Vbrova, G. Pflüges Arch. ges Physiol. 364, 103 (1976)

Pette, D. and Staudte, H.-W. In: Limiting Factors in Physical Performance (J. Keul, ed.) pp 23, Thieme, Stuttgart (1973)

Piehl, K., Adolfsson, S. and Nazar, K. Acta physiol. scand. 90, 779 (1974)

Poortmans, J.R. In: Metabolic adaptations to prolonged physical exercise (H. Howald and J.R. Poortmans, eds.) pp 212, Birkhauser Verlag, Basel (1975)

Prince, F.P., Hikida, R.S. and Hagerman, F.C. Pflügers Arch. ges Physiol. 363, 19 (1976)

Randle, P.J., Garland, P.B., Hales, C.N., Newsholme, E.A., Denton, R.M. and Pogson, C.I. Recent Prog. Horm. Res. 22, 1 (1966)

Ranvier, M.L. Compt. Rend. Acad. Sci. 77, 1030 (1873)

Raimondi, G.A., Puy, R.J.M., Raimondi, A.C., Schwarz, E.R. and Rosenberg, M. Biomed. 22, 496 (1975)

Reitman, J., Baldwin, K.M. and Holloszy, J.O. Proc. Soc. Exp. Biol. Med. 142, 628 (1973)

Reisfeld, R.A., Lewis, U.J. and Williams, D.E. Nature, 195, 281 (1962)

Rennie, M.J., Jennet, S. and Johnson, R.H. J. exp. Physiol. 59, 201 (1974)

Riley, D.A. Exp. Neurol. 41, 690 (1973)

Rogozkin, V.A. Med. Sci. Sports 8, 1 (1976)

Romanul, F.C.A. Arch. Neurol. 11, 355 (1964)

Roodyn, D.B. In: Enzyme Cytology (Roodyn, D.B. ed.) pp 103, Academic Press, London (1967)

Rulcker, C. Acta vet. scand. suppl. 24 (1968)

Rusko, H. Ph.D. Thesis, University of Jyvaskla, Jyvaskla, Finland (Cited by Thorstensson et al. (1977))

Salmons, S. and Vbrova, G.J. Physiol. 210, 535 (1969)

Saltin, B. Med. Sci. Sport 3, 137 (1973)

Saltin, B. and Karlsson, J. In: Muscle Metabolism During Exercise (B. Pernow and B. Saltin, eds.) pp 395, Plenum Press, New York (1971)

Samaha, F.J. and Yunis, E.J. Exp. Neurol. 41, 431 (1973)

Scandinavian Society for Clinical Chemistry and Clinical Physiology, Scand. J. clin. Lab. Invest. 34, 291 (1974)

Schnid, R., Robbins, P.W. and Traut, R.B. Proc. Nat. Acad. Sci. U.S.A. 45, 1236 (1959)

Schott, L.H. and Terjung, R.L. Med. Sci. Sports 7, 64 (1975)

Schroeder, W., Treumann, F., Rathscheck, W. and Muller, R. Europ. J. appl. Physiol. 35, 215 (1976)

Secher, K. Z. ges exp. Med. 14, 113 (1921)

Shubber, A.H. Acta morph. neurl. scand. 9, 229 (1971/72)

Shusheela, A. and Walton, J. J. neurol. Sci. 8, 201 (1969)

Simon, L.M. and Robin, E.D. Comp. Biochem. Physiol. 37, 437 (1970)

Sjodin, B., Thorstensson, A., Frith, K. and Karlsson, J. Acta physiol. scand. 97, 150 (1976)

Skinner, Jr. N.S., Costill, J.C., Saltin, B. and Vastagh, G. In: Muscle Metabolism During Exercise (B. Pernow and B. Saltin, eds.) pp 177, Plenum Press, New York (1971)

Snow, D.H. and MacKenzie, G. Eq. vet. J. 9, 134 (1977a)

Snow, D.H. and MacKenzie, G. eq. vet. J. 9, 226 (1977b)

Snow, D.H. and Summers, R.J. J. Physiol. 271, 31p (1977)

Snedecor, G.W. Statistical Methods 5th ed., Iowa State University Press, Iowa (1956)

Sparapani, A. and Berry, R.E. Am. J. clin. Path. 43, 591 (1965)

Spamer, C. and Pette, D. Histochemistry 52, 201 (1977)

Srere, P.A. Meth. Enzym. 13, 3 (1969)

Staudte, H.-W., Exner, G.U. and Pette, D. Pflugers Arch. ges Physiol. 341, 305 (1973)

Straub, R., Howald, H., Gerber, H., Piehl, M. and Pauli, B. Schweizer Arch. Tierheilk. 117, 453 (1975)

Stein, J.M. and Padykula, H.A. Am. J. Anat. 110, 103 (1962)

Steinhaus, A.H., Kirmiz, J.P. and Launtsen, K. J. Physiol. 99, 487 (1932)

Suominen, H. and Heikkinen, E. Europ. J. appl. Physiol. 34, 249 (1975)

Swanson, M.A. J. biol. Chem. 172, 825 (1948)

Syrovy, I., Gutmann, E. and Melichna, J. Physiol. bohemoslov. 21, 633 (1972)

Takeuchi, T. J. Histochem. Cytochem. 4, 84 (1956)

Tanzer, M.L. and Gilvarg, C. J. biol. Chem. 234, 3201 (1959)

Tappan, D.V., Reynafarje, B.D., Potter, V.R. and Hurtado, A. Am. J. Physiol. 190, 93 (1957)

Taylor, A.W., Booth, M.A. and Rao, S. Can. J. Physiol. Pharmacol. 50, 1038 (1972a)

Taylor, A.W., Thayer, R. and Rao, S. Can. J. Physiol. Pharmacol. (1972b)

Taylor, A.W., Stothart, J., Booth, M.A., Thayer, R. and Rao, S. Can. J. Physiol. Pharmacol. 52, 119 (1973)

Terjung, R.L. Biophys. Biochem. Res. Commun. 66, 173 (1975)

Terjung, R.L., Winder, W.W., Baldwin, K.M. and Holloszy, J.O. J. biol. Chem. 248, 7408 (1973)

Thomas, J.A., Schlender, K.K. and Larner, J. Analyt. Biochem. 25, 486 (1968)

Thorling, E.B. and Jensen, K. Acta path. microbiol. scand. 66, 426 (1966)

Thorstensson, A., Grimby, G. and Karlsson, J. J. appl. physiol. 40, 12 (1976b)

Thorstensson, A., Hulten, B., von Dobeln, W. and Karlsson, J. Acta physiol. scand. 96, 392 (1976a)

Thorstensson, A., Larsson, L., Tesch, P. and Karlsson, J. Med. Sci. Sports 9, 26 (1977)

Thorstensson, A. and Karlsson, J. Acta physiol. scand. 98, 318 (1976)

Thorstensson, A., Sjodin, B. and Karlsson, J. Acta physiol. scand. 94, 313 (1975)

Turner, D.C., Wallimann, T. and Eppenberger, H.M. Proc. Nat. Acad. Sci. 70, 702 (1973)

Uyeda, F. and Racker, E. J. biol. Chem. 240, 4682 (1965)

Vallyathan, N.V., Grinyer, I. and Geroge, J.C. Canad. J. Zoology 48, 377 (1970)

Van Dam, K. and Meijer, A.J. A. Rev. Biochem. 40, 115 (1971)

Van der Helm, H.J. Clin. chim. acta 7, 124 (1962)

Varley, H. Practical clinical Biochemistry (Heinemann, London) (1969)

Varnauskas, E., Bjorntorp, P., Fahlen, M., Prerovsky, I. and Stenberg, J. Cardiovasc. Res. 4, 418 (1970)

Vrbova, G. J. Physiol. 169, 513 (1963)

Wilber, C.G. Life Sciences 8, 564 (1963)

Wilkerson, J.E. and Evonuk, E. J. appl. Physiol. 30, 328 (1971)

Winder, W.W., Baldwin, K.M. and Holloszy, J.O. Europ. J. Physiol. 47, 461 (1974a)

Winder, W.W., Terjung, R.L., Baldwin, K.M. and Holloszy, J.O. Am. J. Physiol. 227, 1411 (1974b)

Wroblewski, F., Ross, C. and Gregory, K.F. New Engl. J. Med. 263, 531 (1960)

Wroblewski, F. and La Due, J.S. Proc. Soc. exp. biol. med. 90, 210 (1955)

Yakovlev, N.N. Ukr. Biokhim. Zh. 48, 388 (1976)

Yakovlev, N.N. In: Metabolic Adaptations to Prolonged Physical Exercise (H. Howald and J.R. Poortmans, eds.) pp 293, Birkhäuser Verlag, Basel (1975)

Yellin, H. and Guth, L. Exp. Neurol. 26, 424 (1970)

York, I.W., Oscai, L.B. and Penney, D.G. Biochem. Biophys. Res. Comm. 61, 1387 (1974)