A STUDY OF CAPILLARIES IN EQUINE SKELETAL MUSCLE

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ABSTRACT

Four horses of varying ages, breeds and sex were used to evaluate the variation in capillary supply and fibre type proportions in a range of equine skeletal muscles. A total of 100 muscles were sampled from the four horses. These samples were stained for myosin ATPase activity from which fibres were classified as ATPase low or ATPase high. Visualisation of capillaries was also achieved by the use of this stain. The 100 muscle samples were assessed for capillary/fibre ratio and for capillary density. From these muscles, 22 were chosen for more detailed analysis on the number of capillaries surrounding each fibre and the fibre area for ATPase low and ATPase high fibres individually.

Fibre type distribution was found to vary from 0% to 100% ATPase low fibres and therefore a complete range of muscle fibre type proportions were obtained. An extensive range was found within a muscle or between adjacent muscles.

There was no significant difference in mean fibre area between the two fibre types in the muscles examined.

The percentage of ATPase low fibres and capillary/fibre ratio showed a significant regression (regression coefficient = 0.36, $p < 0.05$)

For both the ATPase low fibres and the ATPase high fibres, larger fibres were surrounded by more capillaries.

It was concluded that, although fibre size is the primary determinant for capillary supply, the contractile properties of the muscle in which a fibre is found will also determine the number of capillaries it should have at its disposal.

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CHAPTER ONE : INTRODUCTION

This introduction represents an uncritical discussion of the role of exercise physiology in the horse. It outlines the areas which are to be covered in this thesis and the reasons why these areas are relevant to the study of the physiology of equine athletes.

The effect of exercise on the structural and metabolic characteristics of skeletal muscle has become a subject of interest. Studies on a range of species including humans have shown that there are a number of physiological changes associated with the improvement of performance as the result of training.

Horses compete in a wide range of disciplines from endurance rides through to races over short distances as well as performing the more precise movements required for disciplines such as jumping and dressage. The way in which energy is produced for these activities differs depending on the intensity and duration of the exercise. The changes which occur in response to training also differ depending upon the type of exercise that is performed and therefore the training program an individual undertakes is of vital importance. Exercise of submaximal intensity that is maintained over a relatively long period is fuelled by aerobic respiration during which ATP is formed from the breakdown of glycogen in the presence of oxygen. Event horses use aerobic metabolism during the lengthy 'roads and tracks' section of the cross-country phase during a competition while it is also the major form of energy production for endurance horses. In contrast, during sprinting and jumping which involve short bursts of maximal power, the muscles require energy much more rapidly than the aerobic pathway can manage. In this case the individual must utilise anaerobic respiration which also involves the breakdown of glycogen to ATP but this breakdown is not complete and results in the production of lactate which builds up and finally results in fatigue. Training

programs which involve a large amount of endurance type work will result in an increase in the individuals aerobic capacity while those involving sprinting over short distances will increase the horses anaerobic capabilities. The design of a training program must therefore take into account the needs of each individual horse.

The rate of diffusion of oxygen into working muscles is one factor that affects performance. An increase in oxygen diffusion is facilitated by an increase in capillary supply and it has been shown that an increase of aerobic capacity in an individual is associated with an increase in capillary supply during training. Capillary supply differs between muscles depending on the function of the muscle. For example, muscles which contract slowly and constantly such as the heart and diaphragm require large amounts of oxygen and therefore have a more extensive capillary supply than those muscles which contract very quickly but only occasionally and therefore rely mostly on anaerobic metabolism and consequently do not have a large requirement for oxygen.

In this study the variation in capillary supply from muscle to muscle in the horse is investigated in order to obtain a range of muscles varying from those with an extremely low capillary supply to those which have a high capillary density.

Not all muscles within an animal are the same colour, some being redder and others whiter with many variations between these two extremes. Through the use of histochemical methods, it has been shown that most mammalian skeletal muscle consists of a mosaic of fibres with distinct variations in metabolic and functional characteristics. Fibres may be differentiated into two distinct types on the basis of contractility through staining for myosin adenosine triphosphatase (ATPase) activity. ATPase low or 'slow twitch' fibres have relatively slower contraction and relaxation times and are considerably more fatigue resistant than ATPase high or 'fast twitch' fibres.

The colour of various muscles has been related to capillary supply with the general belief being that the red (slow contracting) muscles are better supplied with blood vessels than the white (fast contracting) muscles. Studies have also extended into the capillary supply of individual fibres and the difference in supply between ATPase low and ATPase high fibres. It would be expected that because ATPase low fibres are slower contracting and have a high oxidative (aerobic) capacity they would require a greater oxygen supply and would therefore be surrounded by more capillaries than the ATPase high fibres which have more anaerobic capabilites and require less oxygen.

In the following chapter the literature available on capillary supply to various skeletal muscles in all species will be reviewed. The objectives of the present study will be given at the conclusion of this chapter.

CHAPTER TWO : REVIEW OF LITERATURE

2-1 FUEL UTILISATION

2-1-1 ENERGY SUPPLY

In order for muscles to operate, a suitable form of energy is required. The production of adenosine triphosphate (ATP) as a form of energy is an important metabolic process for muscle cells and is produced by the degradation of fats (lipids) and carbohydrates.

During exercise, energy derived from carbohydrates is of prime importance in the first few minutes while lipids become the most important fuel as exercise progresses (Goodman, 1986). Carbohydrate oxidation does, however, continue throughout exercise and is probably important in preventing exhaustion. The use of lipid as a fuel during exercise spares the use of carbohydrate and therefore glycogen stores are not so rapidly depleted.

Although horses have a variety of energy sources, fat constitutes the largest reserve (Rose, 1983). The relative energy sources for the horse are given in table 2-1-1. M^{c}Miken (1983) suggested that it was apparent that 'fast' energy sources are limited despite equine muscle having a high capacity for glycogen storage.

Glycogen is the largest intramuscular fuel source and is rapidly utilised during the first few minutes of exercise at submaximal intensities (speeds of less than 600m/min in the horse). The rate of glycogen utilisation decreases during exercise as other sources such as intramuscular triglycerides and free fatty acids are preferentially utilised. Muscle glycogen stores have been shown to decrease dramatically in horses competing in endurance events

(Hodgson et al, 1983 ; Snow et al, 1981). Snow et al (1981) showed a 56% decrease in muscle glycogen content following an 80km endurance ride.

Table 2-1-1 : Total energy stores available in the horse (From M^{c}Miken, 1983)

These values were calculated by M^{c}Miken (1983) on the basis of a 500kg horse. Tissue mass was derived from allometric equations which yielded a muscle mass of 206kg, adipose tissue of 25kg and liver of 6.5kg. Values were then derived from energy densities and substrate concentration.

Energy Sources	**Energy (KJ)**
ATP	38
Creatine Phosphate	188
Glycogen	75000
Fat	640000

2-1-2 FACTORS REGULATING FUEL UTILISATION

The type of fuel which is utilised during exercise is dependant upon the composition of the muscles utilised during exercise, the type of exercise performed (speed, distance) and also on the level of fitness of the individual. All three of these factors are discussed below.

2-1-2-1 Muscle Fibre Types

Most mammalian muscles consist of a mixture of fibres with distinct metabolic and functional characteristics (Snow, 1983). These fibres differ in their ability to obtain materials from the blood (Rose, 1983) and in the rate at which they use various metabolites (i.e. rate at which ATP is split). The mechanisms they use for metabolising various substrates also differs.

Generally three different fibre types are recognised. The classification systems used for these vary and more than one term can be used for each fibre type. The main systems used are shown in Table 2-1-2.

Table 2-1-2 : Summary of muscle fibre type classification systems shown as they correspond to the classification system described by Peter et al (1972).

REFERENCE	**FIBRE TYPE**		
Peter et al (1972)	**F-T-O-G**	**F-T-G**	**S-T-O**
Brooke and Kaiser (1970)	IIA	IIB	I
Ashmore and Doerr (1971)	α-red	α-white	ß-red
Davies and Gunn (1972)	Fast,aerobic, anaerobic	Fast, anaerobic	Slow, aerobic

When fibres are stained with oil red O, it is observed that S-T-O (slow-twitch-oxidative) fibres contain larger amounts of lipid than the F-T-O-G (fast-twitch-oxidative-glycolytic) fibres which in turn contain more than the F-T-G (fast-twitch-glycolytic) fibres (Essen, 1977).

Gollnick et al (1973) found that in humans working at an intensity of 30-85% VO_2 max, the S-T fibres were the first to become depleted of glycogen but as exercise continued, there was a progressive depletion of glycogen in the F-T fibres. The fibres with more glycolytic activity are therefore activated when the duration of the exercise exceeds the capacity of the oxidative fibres as the F-T-O-G fibres were more rapidly depleted than the F-T-G fibres.

2-1-2-2 Intensity and Duration of Exercise

The pattern of fuel utilisation differs for exercise of high intensity and short duration e.g sprinting and for exercise of a lower intensity but of a longer duration e.g marathon running.

Human sprinters have a relatively high proportion of F-T fibres (Newsholme and Leech, 1983) which are characterised by low capillary density, low myoglobin, few mitochondria, low activity of the enzymes involved in aerobic metabolism and high activity of those enzymes involved in anaerobic metabolism. Because of these characteristics almost all the energy for this type of exercise must be obtained from fuels which produce ATP under anaerobic conditions such as phosphocreatine and glycogen. Phosphocreatine is the major source of energy during the first four seconds of a 100m sprint and is utilised via the transfer of a phosphoryl group from phosphocreatine to ADP to form ATP as shown in the following reaction:

$$\text{phosphocreatine} + \text{ADP} + H^+ \rightleftharpoons \text{ATP} + \text{creatine}$$

Following the first four seconds of a sprint, anaerobic glycolysis of glycogen provides most of the energy.

The initiation of sprinting results in decreases in the concentrations of ATP and phosphocreatine and increases in the concentrations of AMP, phosphate and possibly fructose-1,6-diphosphate. This leads to a large increase in the rate of fructose-6-phosphate phosphorylation. Further on in the sprint when the maximum power output is achieved, the activity of fructose-1,6-diphosphate may be inhibited so that the cycling rate is reduced.

In comparison to sprinters, marathon runners have a high percentage of S-T-O fibres which have a good blood supply, high content of myoglobin, have many mitochondria and show high activities of the enzymes of the citric acid cycle and B-oxidation of fatty acids. Therefore at work intensites of about 30% VO_2 max lipids are the most important substrate. At this intensity a decline in glycogen concentration of only 50-60% is observed after 3 hours of exercise (Essen et al, 1977) as oppossed to almost total depletion of glycogen following 1-2 hours exercise at an intensity of 70-80% VO_2 max (Hermanson et al, 1967).

It has been found in human muscle that at moderate work intensities, blood-bourne free fatty acids account for only about 50% of the total lipid oxidation while the rest is derived from local lipid stores (Havel et al, 1967).

The oxidation of fatty acids during moderate intensity exercise has a regulatory role to ensure that blood glucose can be conserved for tissues that have a high requirement for it as a fuel such as the brain. The regulation of glucose utilisation by fatty acid oxidation is achieved by the inhibitory effect of citrate on the enzyme phosphofructokinase (PFK) (Newsholme, 1977). If, however, there is an increase in the concentration of any of the deinhibitors of PFK (e.g AMP, NH_4^+, inorganic phosphate and fructose diphosphate) the citrate inhibition of PFK is reduced and therefore there is an increase in the rate of glycolysis and glucose utilisation (Newsholme and Randle, 1964). If there is an increased energy demand by the muscle, therefore, that cannot be met by increased fat mobilisation, there is

an increase in the rate of glycogen utilisation . Towards the end of a marathon, when the levels of muscle and liver glycogen are low, blood glucose is utilised decreasing its level which results in muscular fatigue. The type of fuel used in relation to period of exercise is shown in Table 2-1-3.

Table 2-1-3 : Contribution of glucose, glycogen and fatty acids to oxygen consumption of leg muscle of man (From Goodman, 1986)

Period of exercise (Mins)	Percentage Contribution to Oxygen Uptake		
	Muscle Glycogen	Plasma Glucose	Plasma Fatty acids
40	36	27	37
90	22	41	37
180	14	36	50
240	8	30	62

2-1-2-3 Physical Training

Glycogen utilisation during exercise is decreased by training and the maximum rate of oxygen uptake (VO_2 max) is increased (Newsholme and Leech, 1983 ; Karlsson et al, 1974). This indicates a greater contribution from lipids to the total energy metabolism.

A glycogen-saving effect is also observed during training with the muscle increasing its mitochondrial content in order to increase its oxidative capacity (Morgan et al, 1971 ; cited in Essen, 1977). There is also an increased enzymatic activity for oxidative metabolism (Henriksson and Reitman, 1977) which possibly accounts for an enhanced oxidation of lipids.

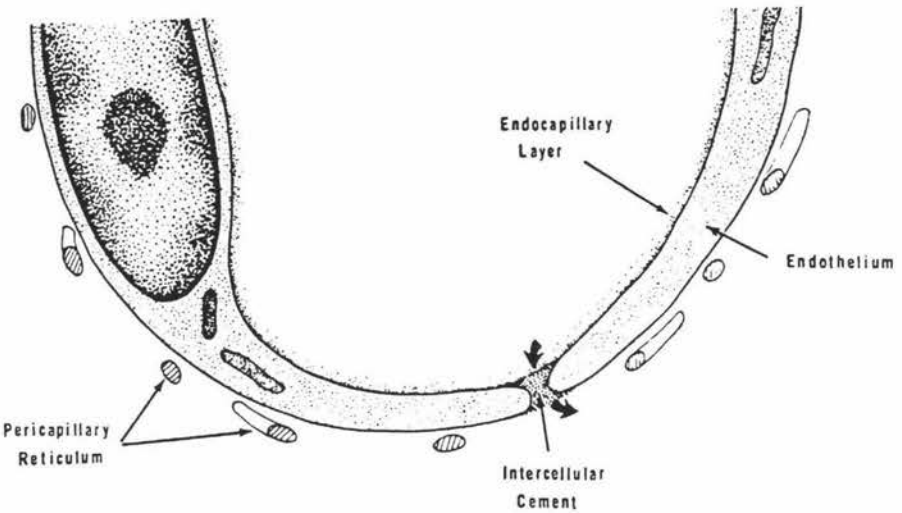
2-2 CAPILLARIES

2-2-1 CAPILLARY STRUCTURE

Capillaries have been identified as the thin-walled exchange vessels of the microcirculation that form anastomosing networks within the tissues (Cliff, 1976). They are just large enough to allow the passage of red and white blood cells and connect the smallest arteries with the smallest veins (Bone, 1982).

The capillary was traditionally described by Mayerson (1962) as being made up of three layers (Fig. 2-2-1). The layer closest to the lumen was known as the endocapillary layer which was believed be an adsorbed layer of some protein constituent of blood plasma. The middle layer was the endothelium which was composed mostly of endothelial cells held together by intercellular cement. The outer layer was described as the pericapillary sheath and consisted of a closely woven investing layer of silver-staining connective tissue. At this time, the exchange of substances through the capillary wall was considered to occur via channels or pores which penetrated the capillary wall through which the blood communicates directly with the extravascular fluid (Pappenheimer, 1953). The pores were thought in general to be too small to allow the passage of large protein molecules but were of sufficient size and number to account for observed rates of passage of water and other lipid insoluble molecules.

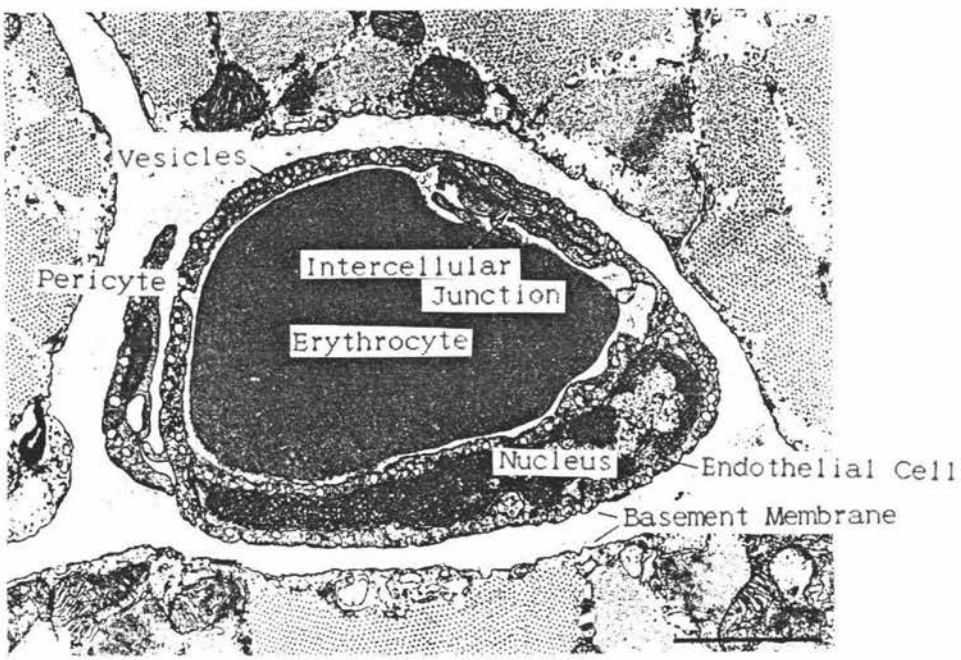
Fig. 2-2-1 : Traditional concept of the structure of a capillary (From Mayerson, 1962).



More recently, through the use of the electron microscope, the capillary wall has been described in only two layers, an endothelial cell and a basement membrane (Weibel, 1984) (Fig. 2-2-2). The endocapillary layer described by Mayerson (1962) is not observed under these conditions. It was thought that either it did not exist or was not preserved by the methods used.

The endothelial cell is a thin, flat cell of approximately 3μm thickness in the nuclear region and flattening to about 0.2μm or less towards the periphery (Bruns and Palade, 1968a). They have a usually flattened nucleus. Most of the cell is made up of greatly attenuated cytoplasmic extensions which are no more than 0.1μm thick and are built essentially of two plasma membranes with some cytoplasmic substance in between.

Fig. 2-2-2 : An electron micrograph showing the current concept of the structure of a capillary. (From Weibel, 1984).



The extensions reach out until they meet and become closely apposed to form an intercellular junction in which the two cells are joined by a complex of molecular bridges. These junctions are also known as zona occludens. The pores described by Pappenheimer (1953) are not observed. Bruns and Palade (1968a) reported that the endothelial cells come together bluntly or they may overlap or interdigitate. The junctions described by Bruns and Palade (1968a) were said to measure approximately 15nm across and were fused at the level of the central layer (Fig. 2-2-3). They also described regions of more advanced fusion which formed an S-shaped intercellular space and which were similar to those described by Weibel (1984) (Fig. 2-2-4).

Fig. 2-2-3 : Cell junctions in the endothelium of rat tongue capillaries (From Bruns and Palade, 1968a).

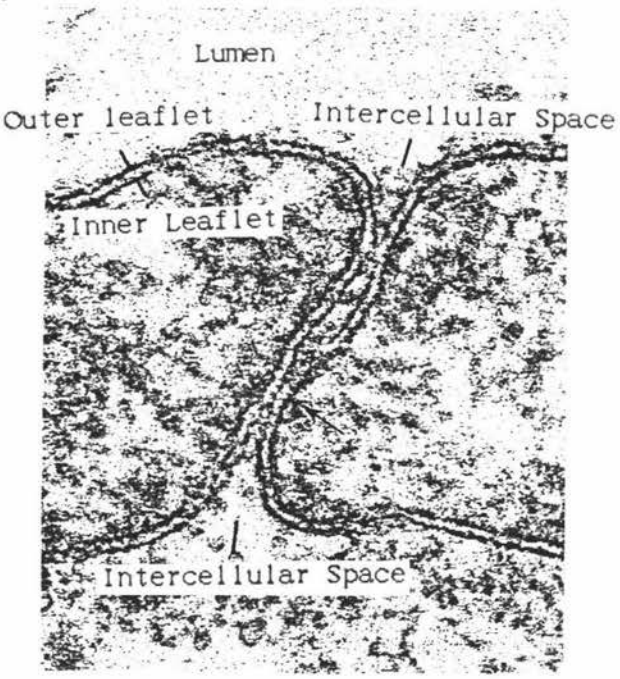
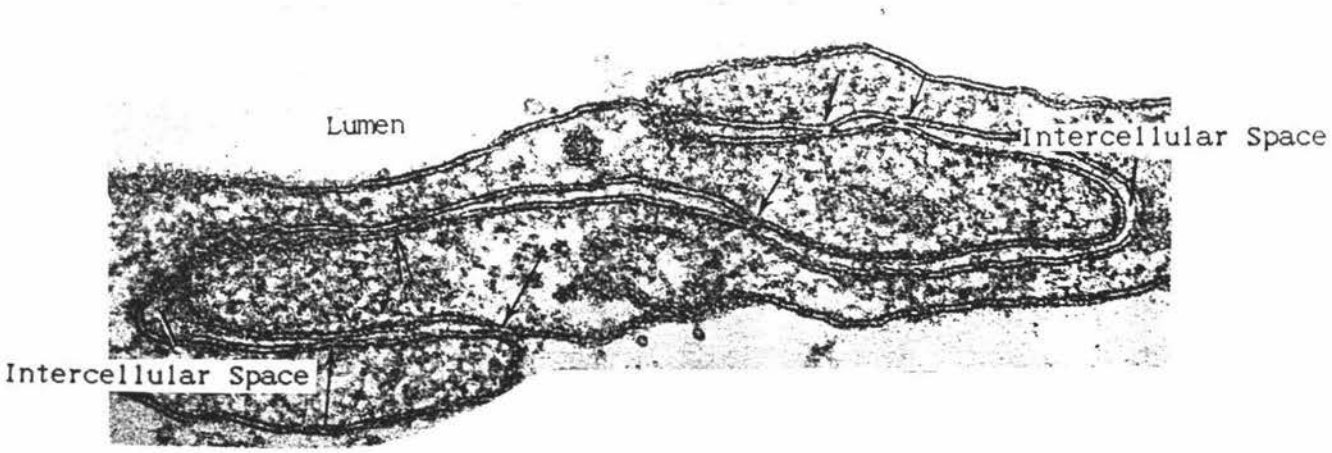


Fig. 2-2-4 : More complex intercellular junction in the endothelium of a rat capillary. (From Bruns and Palade, 1968a)



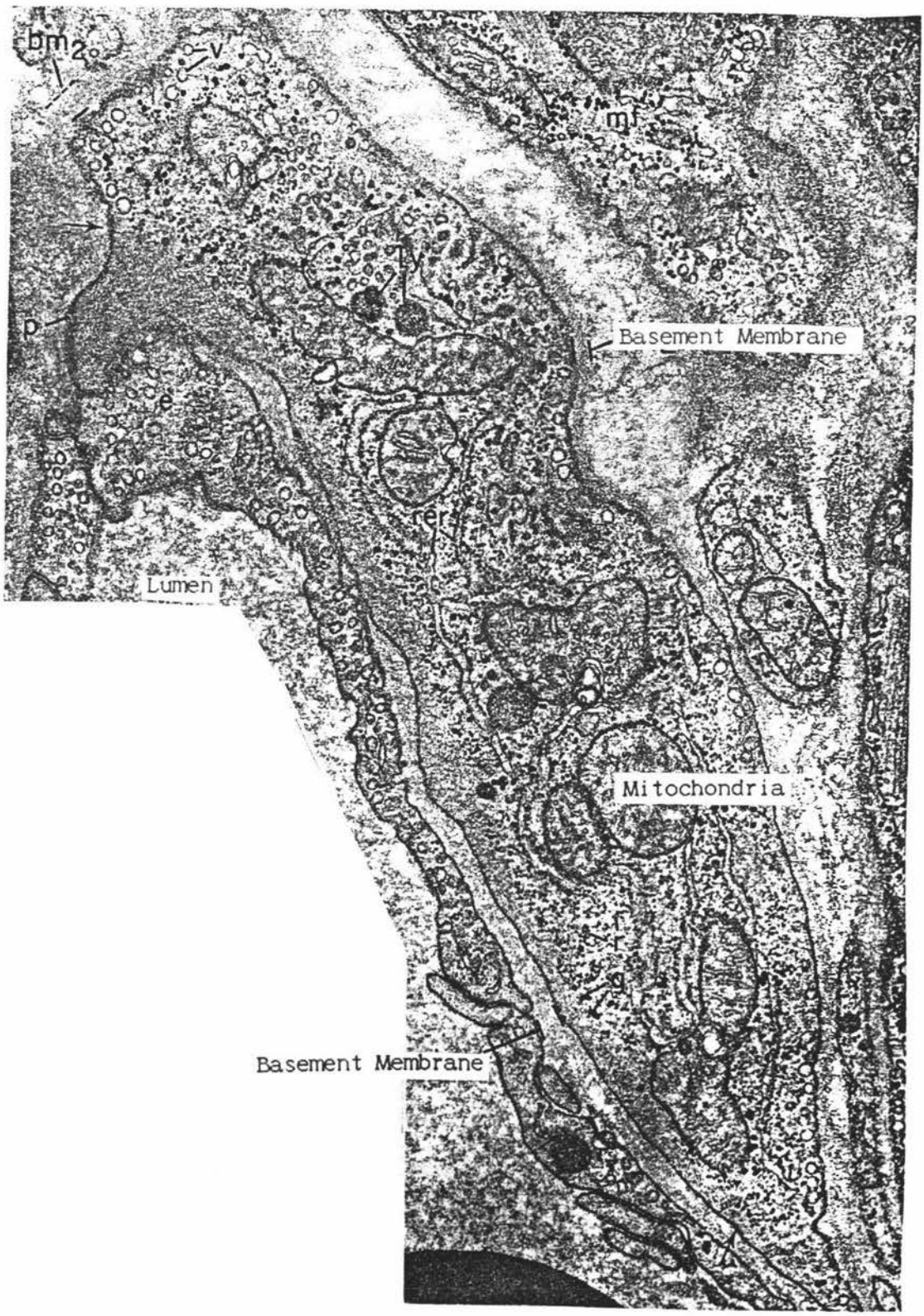
Mayerson (1962) reported that there are numerous inpocketings in the endothelial plasma membranes. Some communicate with the lumen while others form small vesicles which have been associated with the active transport of materials by pinocytosis. These vesicles have been found to be normally concentrated along the two fronts of the cell next to the plasmalemma (Bruns and Palade, 1968b ; Majno, 1965). Bruns and Palade (1968a) reported that the membrane of those vesicles that are opened onto either the blood or tissue side is continuous with the cell membrane. They also reported that when the vesicles open onto the surface of the cell they do so through a stoma which is connected to the main cavity of the vesicle through a narrow neck. In some cases a band which has a small central density spans the stoma of the vesicle. In addition to these vesicles, Bruns and Palade (1968a) describe the occasional occurence of slightly larger and structurally different vesicles. These are reported to be flask-shaped and to appear either free in the cytoplasm or connected with the cell surface by a narrow neck or a solid strand.

The basement membrane forms a continuous lining on the outer surface of the endothelium and adheres tightly to it. It is made up of proteoglycans and a type of collagen which allows solutes and small macromolecules to pass through quite freely (Weibel, 1984). It is approximately 30-50nm in thickness and has an inner margin which is relatively well defined and usually separated from the endothelium by a narrow subendothelial space (Bruns and Palade, 1968a). The outer margin is less distinct. Bruns and Palade (1968a) described the basement membrane as occurring mostly as a single layer but reported that it sometimes splits into two or more leaflets of unequal thickness. The innermost leaflet is frequently interrupted by relatively large gaps while the outer leaflet is generally continuous and therefore the gaps do not lead through the membrane into the pericapillary space but form closed pockets.

Weibel (1984) described an additional set of cells that partially envelop the capillary and are closely associated with the basement membrane. These cells are known as pericytes and are thought to have contractile properties. Pericytes were also described in detail by Bruns and Palade (1968a) as special cellular elements located entirely within the basement membrane. It has been suggested that their topography makes them comparable to the smooth muscle cells of larger vessels such as terminal arterioles (Rodin, 1967). The general form of pericytes is more easily visualised under a light microscope and has been extensively studied by Rouget (1873, cited by Bruns and Palade, 1968a) (Fig. 2-2-5) . Each cell has a perikaryon and from this arises an elaborate sytem of processes. The perikaryon contains a large nucleus which protrudes towards the pericapillary space, an endoplasmic reticulum, a small population of free ribosomes and a large number of mitochondria. The cytoplasm has a relatively high content of dense cytoplasmic particles. Spinous processes extend in large numbers from the perikaryon and measure from 0.05 to 0.4μm in diameter. Their cytoplasm contains a high concentration of fine fibrils which are preferentially oriented parallel to the long axis of the pseudopodium. Many of these processes penetrate the subendothelial space through gaps in the inner leaflet of the basement membrane and come into contact with the endothelial cells.

Bruns and Palade (1968a) reported the presence of a third layer which they named the outer tunic and described as a highly discontinuous layer comprising cellular and extracellular elements. This layer was said to be well outlined by the basement membrane inwardly but outwardly it has no distinct limits. Its cellular and extracellular components were reported to be similar to those which populate the pericapillary spaces.

Fig. 2-2-5 : Pericyte in a capillary of rat myocardium. (From Bruns and Palade, 1968a)



2-2-2 MEASUREMENT OF THE SPATIAL DISTRIBUTION OF CAPILLARIES

The spatial distribution of capillaries in transverse sections of skeletal muscle has traditionally been described in terms of capillary density (i.e. capillaries/mm^2), number of capillaries per muscle fibre or as the number of capillaries around each fibre. Each of these parameters, however, has its problems and these must be taken into account when any results are reported.

The major problem with measurement of capillary distribution in terms of capillaries/mm^2 occurs as the result of tissue shrinkage during histological procedures (Plyley and Groom, 1975). Goldspink (1961) reported that the area of tissue is reduced to approximately 56% by histological procedures including fixation, dehydration and embedding. Capillary density also varies with fibre type with red muscle having a higher density of capillaries than white muscle (Schmidt-Nielsen and Pennycuik, 1961 ; Lee, 1958). Schmidt-Nielsen and Pennycuik (1961) reported that the red fibres in skeletal muscle of the guinea-pig contained 1094 capillaries/mm^2 while white fibres in the same animal contained only 489 capillaries/mm^2. They observed similar differences in other species such as the mouse, rat and rabbit. Much variation in capillary density also occurs because of differences in mean fibre diameter (Myrhage, 1978). Henckel (1983) found that capillary density increased with increasing fibre area in horses grouped by age. The youngest horses (6 months of age) had a mean fibre area of 1957um^2 and a capillary density of 497 capillaries/mm^2 while older horses (more than 10 years of age) had a mean fibre area of 3053um^2 and a capillary density of 692 capillaries/mm^2. They concluded that the lower capillary density of the foals was the result of both smaller mean fibre areas and lower capillary:fibre ratios and that fibre areas as well as capillary:fibre ratios increase with maturation. Anderson and Henriksson (1977) found that mean fibre area increased from 4150um^2 to 5020um^2 during training in men while

capillary density also increased (329 to 395 capillaries/mm^2).

All these factors lead to a large range of values reported by various investigators (Table 2-2-1). For example, values of capillary density in the gastrocnemius muscle in the cat has been reported to be as low as 379 capillaries/mm^2 (Schmidt-Nielsen and Pennycuik, 1961) and as high as 2341 capillaries/mm^2 (Paff, 1930). Plyley and Groom (1975) suggested that, as the discrepancies appear to relate to the period in which the study was undertaken, the differences in results may be due to some inherent error in technique.

Table 2-2-1 : Reported values for some capillary densities in the guinea-pig, cat and mouse. (Modified from Plyley and Groom, 1975)

Author	Year	Animal	Muscle	Capillaries/mm^2
Paff	1930	Guinea-pig	Rectus femoris	3060
		Guinea-pig	Gastrocnemius	2614
		Cat	Rectus femoris	2474
		Cat	Gastrocnemius	2341
Sjostrand	1935	Guinea-pig	Gastrocnemius	1100
		Mouse	Gastrocnemius	2700
		Mouse	Masseter	4500
Schmidt-Nielsen and Pennycuik	1961	Mouse	Masseter	2272
		Mouse	Gastrocnemius	975
		Cat	Gastrocnemius	379
Hammersen	1970	Cat	Rectus femoris	811

Another parameter which is used more extensively to describe the distribution of capillaries is the capillary/fibre ratio. This is not affected by either tissue shrinkage or fibre size but it is affected by the particular arrangement of vessels and fibres in the section (Plyley and Groom, 1975). An increase in the capillary/fibre ratio reflects the formation of new capillaries in the muscle (Andersen and Henriksson, 1977). The capillary/fibre ratio does not

provide any information about the diffusion conditions and because of this, mean fibre area must be taken into account when diffusion is the object of study.

Plyley and Groom (1975) reported that the capillary/fibre ratio is dependent upon how many fibres share each capillary. This is known as the sharing factor and varies depending on the manner in which the fibres are packed together. It can be computed using the quotient, (mean number of capillaries around a fibre) / (capillary:fibre ratio). They suggested that the sharing factor could never be less than 2 and rarely exceeded 4.

The parameter of the number of capillaries in contact with each fibre is another method used to describe capillary supply. This parameter can be used to determine the number of capillaries surrounding fibres of different types and is not affected by tissue shrinkage although it does not take into account the size of different fibre types. In order to compare muscles with different fibre areas, the number of capillaries in contact with each fibre relative to fibre area can be calculated as in a study on humans by Anderson and Henriksson (1977) who found that the number of capillaries surrounding each fibre relative to fibre area for type I fibres was always greater than corresponding values for type IIA and IIB fibres.

A recent method of assessing capillary supply involves the determination of capillary/fibre ratios and capillary densities for individual fibre types. It also provides an index of the heterogeneity in capillary spacing (Degens et al, 1992). Their work was done on rats and involved the use of an algorithm which constructed capillary domains. These domains were defined as the area surrounding a capillary delineated by equidistant boundaries from adjacent ones. This is a Voronoi tessellation. The heterogeneity of capillary spacing could be determined from the logarithmic standard deviation of the domain area. The capillary/fibre ratio for each fibre was described as the local capillary fibre ratio (LCFR) and

was defined as the sum of the fractions of each domain area overlapping the fibre. They suggested that this parameter could take into account remote capillaries and therefore allow an estimate of the capillary supply to a fibre which lacked direct contact with an adjacent capillary. The capillary density for a particular fibre was also determined by dividing the LCFR by the cross-sectional area of the fibre and was described as the capillary fibre density (CFD). These parameters were considered to estimate the capillarisation of separate fibre types.

2-2-3 IDENTIFICATION OF CAPILLARIES

As well as the use of different parameters for estimating spatial distribution of capillaries, variation amongst the values reported has probably also arisen from the use of different techniques for staining capillaries.

In the late 1950's, the common method for studying capillary distribution was to inject the desired muscles with Indian ink at pressures of around 300-400 mm Hg thereby presumably filling all the vessels including the capillaries (Smith and Giovacchini, 1956). The muscles were then removed, fixed in formalin, sectioned in paraffin and lightly stained with eosin. Smith and Giovacchini (1956) found that when using this method some areas of the muscle appeared to be well injected while other areas were devoid of ink. It was therefore concluded that not all capillaries were necessarily filled. Any counts of capillary density in this case would therefore be lower than the actual capillary density. Carrow et al (1967) also used the perfusion of Indian ink to study capillaries in the gastrocnemius muscle of rats and also commented that while some areas of their preparations were well injected others were devoid of ink and therefore counting of capillaries in these areas was impossible.

Perfusion has also been performed using an opaque, silicone elastomer of very low

viscosity (Microfil) (Plyley and Groom, 1975). This was injected manually from a syringe into the popliteal artery and vein of cats and dogs and into the abdominal aorta in rats and guinea-pigs. Once sectioned the samples were stained with a modified Gomori trichrome stain which stained red cells a brilliant red and nuclei a very pale purple. They commented that although they frequently suceeded in completely filling the vascular bed there were often poorly filled areas as well.

Another method for staining capillaries in the 1970's was the use of a modified form of the periodic-acid-Schiff (PAS) reaction (Hermansen and Wachtlova, 1971 ; Parizkova et al, 1971 ; Anderson, 1975). This method allowed a more accurate measurment of capillary distribution because it stained a mucopolysaccharide in the capillary basement membrane (Anderson, 1975). Capillaries were therefore stained whether they were empty or full which meant all capillaries present could be counted.

The capillary wall itself is labeled by the unspecific alkaline phosphatase reaction which labels the capillary endothelium (Myrhage, 1978) because it's alkaline phosphatase concentration is high. Since ATP can be hydrolysed by alkaline phosphatase as well as specific ATPases, the methods described by Gomori (1939 ; cited in Myrhage, 1978) and by Guth and Samaha (1970) are quite comparable and all capillaries should be stained by either of these two methods (Myrhage, 1978 ; Reis and Wooten, 1970).

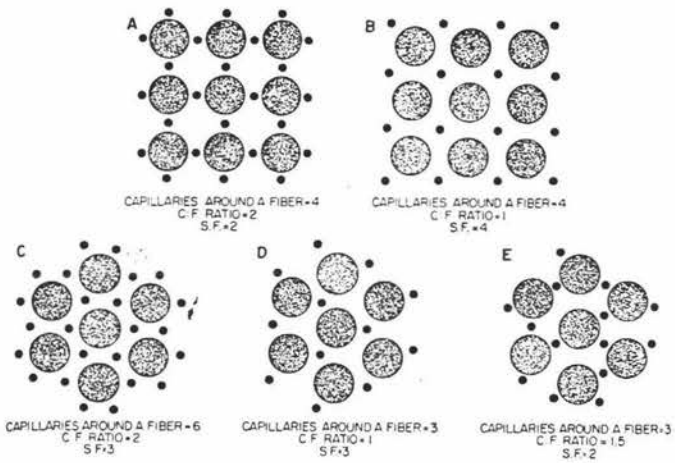
Electron microscopy has also been employed to study capillary distribution in muscle (Brodal et al, 1977). The identification of capillaries was based on the following criteria : diameter no less than 6μm, continuous basal lamina and no continuous cellular layer external to the endothelium. In this way lymphatic capillaries were not included because they are larger than 6μm and lack a continuous basal lamina. It was therefore claimed that all capillaries were positively identified. Although electron microscopy may be useful for

checking that capillaries are being positively identified, it can only be performed on a small scale and is therefore not viable for studies involving large counts of capillaries.

2-2-4 ARRANGEMENT OF CAPILLARIES AND FIBRES IN TRANSVERSE SECTIONS OF MUSCLE

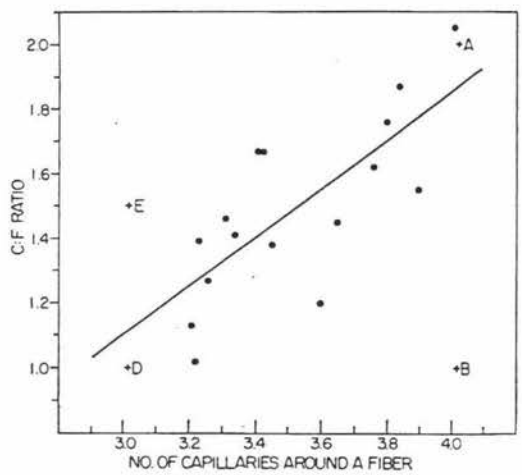
The value of the parameter capillary/fibre ratio depends upon the way in which the vessels and fibres are arranged in a transverse section. Different models for such arrangements have been proposed and are based either on a square array of fibres (Hammersen, 1968 ; cited in Plyley and Groom, 1975) or a hexagonal array (Schmidt-Nielsen and Pennycuik, 1961). In a square array each vessel may either be between two adjacent fibres (Fig. 2-2-6A) or at the junction of 4 fibres (Fig. 2-2-6B). In a hexagonal array, the vessels generally appear at the corners of each hexagon (Schmidt-Nielsen and Pennycuik, 1961). It is suggested that in red muscle all 6 corners of the hexagon are occupied (Fig. 2-2-6C) while in white muscle only 3 of the corners are occupied (Fig. 2-2-6D). A further model was suggested by Plyley and Groom (1975) which said that there might be a total of 3 capillaries per fibre but instead of being at the corners they may be located between two adjacent fibres (Fig. 2-2-6E). If the sharing factors for each of these models is considered it can be seen that the sharing factor for model B is 4 which means that this model may be ruled out as in Plyley and Groom's study the sharing factor never exceeded 3.2. On account of the mean number of capillaries around a fibre, Plyley and Groom (1975) suggested that the model proposed in Figure 2-2-6C could also be ruled out as it requires 6 capillaries around a fibre while the results in their study showed the maximum number of capillaries around a fibre to be 4 (Rat soleus = 4.01). In other studies the maximum number of capillaries around a fibre have been 3.5 (Henkel, 1983), 9 (Myrhage, 1978) and 5.5 (Anderson and Henriksson, 1977).

Fig. 2-2-6 : Possible models for the description of capillary : fibre geometry in striated muscle (From Plyley and Groom, 1975). SF = sharing factor.



Plyley and Groom (1975) studied the relationship between capillary/fibre ratio and the mean number of capillaries around a fibre and obtained a graph as shown in Figure 2-2-7. The models A, B, D and E were placed on the graph as shown. It was observed that the points lay scattered about a line roughly joining A and D. They therefore suggested that a muscle such as rat soleus (Capillaries around a fibre = 4.01, C:F = 2.05) may be represented by model A while a muscle such as dog tongue (Capillaries around a fibre = 3.22, C:F = 1.02) may correspond to model D. Other muscles may then correspond to a series of transitional models between these two extremes.

Fig. 2-2-7 : Relationship between capillary/fibre ratio and mean number of capillaries surrounding a fibre. (From Plyley and Groom, 1975).



2-2-5 CAPILLARY PERMEABILITY

2-2-5-1 Models for Capillary Permeability

The degree to which a capillary is permeable to various substances affects the supply of oxygen and other nutrients to the muscle fibre. This will in turn affect the number of capillaries required to sustain the needs of each fibre.

Various models to describe capillary permeability have been proposed over time. In 1951 Pappenheimer, Renkin and Borrero proposed a model that required the existence of a system of water-filled pores which occupied about 0.2% of the capillary surface area. These pores were described as being either uniform pores of 3nm radius or rectangular slits with widths of 1.85nm or pores with a Gaussian distribution of radii of 2.4 ± 1.2nm. They suggested that it was likely that capillary permeability to gases and other lipid-soluble molecules involves the whole surface of the endothelial cell. Permeability to water and lipid-

insoluble solutes, however, only involved a very small fraction of the capillary wall. They postulated that this small area may correspond to the intercellular junctions although they did not have any direct evidence to support this. With respect to oxygen, they reported that the small capillary area available for passage of water and lipid-insoluble molecules is insufficient to account for the observed rate of transfer of oxygen by diffusion and they therefore hypothesized that oxygen and other lipid-insoluble molecules could penetrate the plasma membrane of the capillary endothelial cells and were not restricted to aqueous intercellular spaces.

Grotte (1956, cited in Cliff, 1976) also reported the existence of pores with radii of 3.5-4.5nm. He postulated the presence of a large number of "large pores " or "leaks" with radii ranging from 12 to 34nm. These leaks were proposed to allow bulk flow of water to occur as opposed to diffusion.

A similar model to those proposed by Pappenheimer et al (1951) and Grotte (1956, cited in Cliff, 1976) was suggested by Landis and Pappenheimer (1963) who reported that the area occupied by pores was less than 0.1% of the capillary surface area and their radii were 4-4.5nm. They also postulated the existence of "leaks" which they suggested were probably located in the venules. They suggested that the small pores they observed may correspond to the endothelial cell junctions although they later discovered that the blood vessel endothelial junctions had regions of membrane fusion. Majno (1965) reported that the regions of the endothelial junctions could be considered as filters rather than seals.

In the early 1970's, by the use of the freeze-fracture technique for examination by electron microscopy, Leeson (1971) confirmed the existence of endothelial vesicles which had been previously described by Bennett et al (1959), Majno (1965) and Bruns and Palade (1968a). Bruns and Palade (1968a) observed that some of these vesicles lie free in the

cytoplasm while others open onto the blood or tissue front of the cell. They reported that they could be arranged as 1) single spherical vesicles, 2) chains of vesicles or 3) tortuous channels of circular cross-section and that each configuration could be isolated in the cytoplasm, open on one front or open on both fronts of the cell. They found, however, that plasmalemmal vesicles do not establish continuous channels from one cell front to the other although they do frequently form chains of two or three vesicles and they concluded that transport by vesicles appears to be a discontinuous process.

Firth et al (1983) in their work on pores in guinea-pig placental capillaries, suggested that routes for protein transport across the endothelium could either be transcellular i.e. via the vesicles ar paracellular i.e. via the lateral intercellular spaces. They found that the role of vesicles in the transport of a haempeptide tracer is very slight while transfer of the tracer across the endothelium was correlated to tracer filling of the lateral intercellular spaces.

Mayerson et al (1960) reported that the permeability of capillaries to macromolecules varied in different parts of the body of the dog. Liver capillaries were found to be much more permeable than capillaries in the leg and lung areas. They postulated the presence of two discrete sets of pores. One of these was a uniform set of small pores which allow the passage of molecules with a molecular weight of no larger than 250,000. The other set includes large pores which permit the passage of molecules with a molecular weight of at least 412,000. The exact size of these large pores was not determined but they were shown to allow the passage of the dextran molecules of about 14nm. They suggested that small molecules probably cross the capillary membrane by diffusion across through the pores while for larger colloid particles, filtration is most likely the primary method of exchange.

Later, Weibel (1984) reported that the two extensions of the endothelial cell meet and become closely apposed. They are joined by a band-like complex of molecular bridges

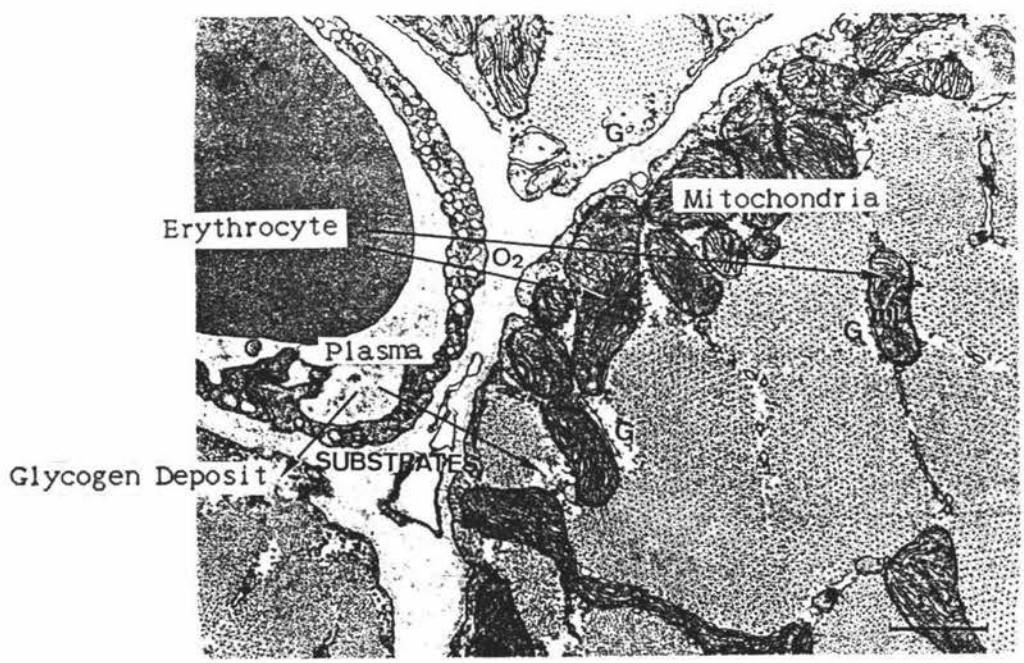
forming a tight junction or zona occludens. It was suggested that this complex forms a leaky seal so as to form passageways allowing ions and small macromolecules to "leak" out from the plasma.

2-2-5-2 Oxygen Flow From Blood to Cells

Weibel (1984) provided an explanation of the transfer of oxygen from the blood in the capillary to the muscle cell. He also addressed the question of how far oxygen can penetrate a cell.

The transfer of oxygen from capillary to cell is shown diagramatically in Figure 2-2-8. The oxygen diffuses across the tissue spaces to the cell where it meets the mitochondria which may absorb some of it in the process of oxidative phosphorylation.

Fig. 2-2-8 : Oxygen flow from the capillary to the muscle cell (From Weibel, 1984).



One factor that determines the flow rate of oxygen into the tissue via diffusion is the pressure or PO_2 in the capillary. This is generally higher at the arterial end of the capillary and lower at the venous end (Schmidt-Nielsen, 1987). Weibel (1984) reported that the PO_2 at the arterial end is approximately 95mmHg while at the venous end it falls to approximately 40mmHg or less. When the pressure inside the capillary is greater than the pressure outside (i.e. colloidal osmotic pressure), the fluid is forced out through the capillary wall and when the pressure inside the capillary is lower than that outside the fluid moves into the capillary (Schmidt-Nielsen, 1987). The decrease in PO_2 along the capillary from arteriole to to venule is not a linear function due to the non-linear shape of the O_2Hb dissociation curve which maintains the PO_2 at a fairly high level in spite of O_2 discharge (Weibel, 1984).

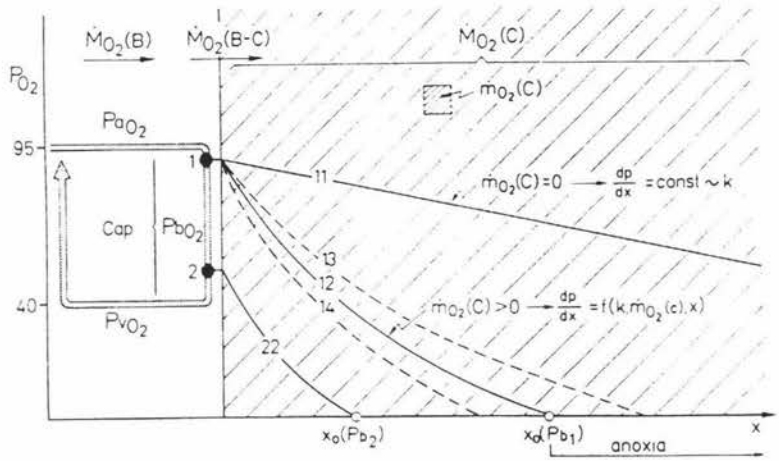
Another factor which affects the rate of O_2 flow to the tissues is the rate at which the cell consumes oxygen which in turn depends upon the density of mitochondria in the cell.

A model for oxygen consumption was described by Weibel (1984) through the use of the diagram shown in Figure 2-2-9. Two assumptions were made as follows :

1. Assume that the mitochondria are finely and homogeneously distributed throughout the cell ; can expect that the unit cell volume, say $1\mu m^3$, will consume oxygen at a constant rate $mo_2(c)$.

2. Assume the cell to be exposed to a capillary on one side but to extend indefinately in direction, x.

Fig. 2-2-9 : Simple model of a slab-like cell supplied by a capillary to show the profile of PO_2 as a function of distance into the cell (From Weibel, 1984).



First consider diffusion of oxygen that occurs at the arterial end (point 1) where the PO_2 is approximately 90mmHg and where no oxygen consumption is occurring i.e. $mo_2(c) = 0$. A linear PO_2 gradient occurs (curve 11) which depends exclusively on the Kroghs diffusion coefficient, K. This diffusion coefficient was described by Krogh (1959) as the number of cc of oxygen which will in 1 minute diffuse through an area of $1cm^2$ when the pressure gradient is 1 atmosphere of oxygen per μm.

If, however, oxygen is consumed at a constant rate $mo_2(c) > 0$ then the PO_2 gradient falls more rapidly and follows a parabolic curve (curve 12). When $mo_2(c)$ is small the curve is less steep (curve 13) and when it is large the curve is steeper (curve 14).

At some distance, x_o, the PO_2 falls to zero and the tissue beyond this point will be anoxic. The location of this point depends on K, the rate of oxygen consumption and on the oxygen head pressure, PbO_2 according to the following equation :

$$x_o = \sqrt{2K^* PbO_2/mo_2(c)}$$

At the venous end of the capillary, point 2, the PbO_2 is low and x_o is shorter i.e. the point at which the tissue becomes anoxic is closer to the capillary.

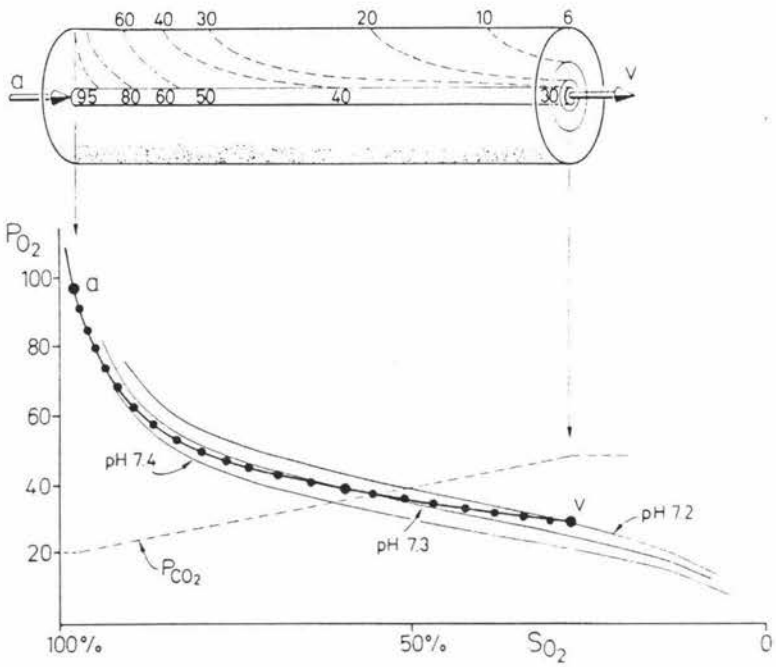
A more complex model for oxygen diffusion through a capillary is also described by Weibel (1984). This involves the Krogh cylinder model where it is assumed that oxygen diffuses radially into the tissue that surrounds an axial capillary. In this model the PO_2 is still highest near the capillary and falls off at a radial distance, r. At an equal distance, r, the PO_2 will decrease along the cylinder from the arterial end to the venous end. Also therefore, the anoxic region will become closer to the capillary.

Figure 2-2-10 shows the model for oxygen delivery into the Krogh cylinder and represents one capillary path from arteriole to venule. The plot of the relationship between PO_2 and O_2 saturation shown in Figure 2-2-10 depends on the O_2Hb equilibrium curve and makes two assumptions as follows :

1. The quantity of oxygen delivery in each unit length of the path is constant so that saturation decreases linearly from approximately 95% in the arteriole to 28% in the venous blood.

2. CO_2 uptake along the path is also constant so the Pco_2 increases linearly and pH decreases linearly from 7.4 to 7.2.

Fig. 2-2-10 : Model for oxygen delivery into the Krogh cylinder and a plot of the relationship between PO_2 and oxygen saturation (From Weibel, 1984).



The solid line in Figure 2-2-10 represents the physiological O_2Hb equilibrium curve. The PO_2 remains relatively high towards the venous end and its profile depends on the diffusion constant, K, the rate of oxygen consumption ($mo_2(c)$) and on the capillary PO_2. These factors determine the radial distance R_o to which oxygen can diffuse according to the following equation :

$$PO_2\ (cap) = mo_2(c)/2k * [R_o^2 * \ln(R_o/r_c) - (R_o^2 - r_c^2)/2]$$

where r_c = capillary radius

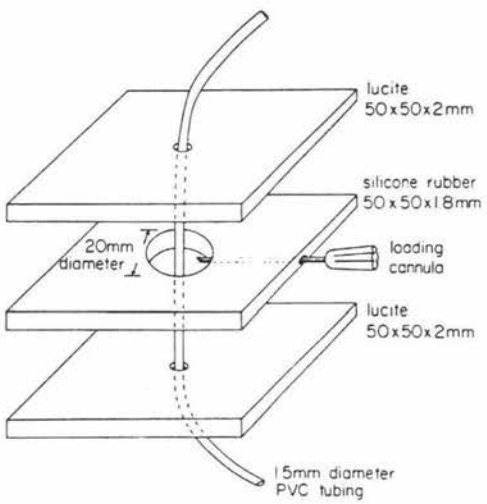
It can be calculated that $mo_2(c)$ should be about 0.05 to 0.1 ml O_2/min/ml and the capillary radius can be taken as 3μm. If K = 2.10^{-18} ml/min/cm/mmHg, then R_o is about 50 to 25μm for PO_2 values of 40mmHg or less.

If the radial diffusion of oxygen and the gradual loss of oxygen along the capillary is studied, they show an interesting PO_2 profile in the tissue sleeve around the capillary. If points of equal PO_2 are considered, it is found that they do not lie on cylindrical surfaces but on a "cone" or "funnel" that tapers towards the venule end. This leads to the postulation that the tissue which is near the venule end is less well supplied with oxygen than other parts of muscle. However, it has been observed in skeletal muscle (Weibel, 1984) that the number of capillaries increases towards the venous end so that the capillary density increases as PO_2 falls and the distance R into the cells that must be furnished with oxygen from one capillary must decrease. This increase in capillary density towards the venous end was not, however, quantified. Because of this it is suggested that the Krogh cylinder must taper toward the venule and should therefore be replaced by a model called the "Krogh cone" as it still obeys the same laws (Weibel, 1984).

Quistorff et al (1977) developed an experimental model of the Krogh tissue cylinder through the use of a bacterium (Photobacterium Phosphoreum) as an oxygen indicator. This bacteria was used because when it is suspended in a hyperosmotic medium it emits a green luminescence when oxygen is present. This luminescence increases in intensity as the oxygen concentration increases. They designed a model of the Krogh cylinder as shown in Figure 2-2-11. A suspension of the Photobacteria containing 4% gelatine was introduced into the chamber via a syringe and allowed to form a gel. Once this gel was stable, the PVC tube was removed leaving a channel in the gel. This channel constituted the capillary in the model through which oxygen was suuplied as a gas. Various substrates and mitochondria could be

added to the gelatine suspension to either facilitate oxygen diffusion or vary oxygen consumption rates. Their results indicated that anoxic zones may develop with only a slight decrease in arterial tension or by occlusion of a single capillary in a tissue with high oxygen consumption for example in the brain.

Fig. 2-2-11 : Schematic diagram of the macro model of *The Krogh tissue Cylinder.* (From Quistorff et al, 1977)



A problem that still exists is that the PO_2 near the capillary is higher than it is deeper in the cell (Weibel, 1984). This means that in the centre of the muscle fibre there is less oxygen available than at the periphery. However, the mitochondria are not evenly distributed throughout the cell as assumed in the original model but they occur at a higher density at the periphery leaving the fibre centre relatively poor in mitochondria (Hoppeler et al, 1981). It is possible to form two hypotheses from the radial distribution gradient of mitochondria:

1. Local oxygen consumption is not constant throughout the cell but decreases as one moves away from the capillary and causes the PO_2 profile to become flatter. This allows the PO_2, the driving force for oxygen diffusion, to remain relatively higher in the deeper parts of the cell.

2. There is more ATP generated at the periphery of the cell which means that the concentration of ATP decreases towards the centre of the cell. This may cause high-energy phosphates to move inward using the creatine phosphate transfer system. This would improve energy supply to the centre of the cell because it would be affected by the parallel diffusion of both oxygen and energy-rich phosphates.

Another factor which helps to bring oxygen into the deeper parts of the cell is the presence of myoglobin which can bind oxygen reversibly and serves as a cellular oxygen store. It also facilitates the diffusion of oxygen which is an important function because the facilitation is greater the lower the PO_2 (Scholander, 1960).

Therefore as oxygen molecules diffuse radially into the cell, the PO_2 is kept relatively high due to :

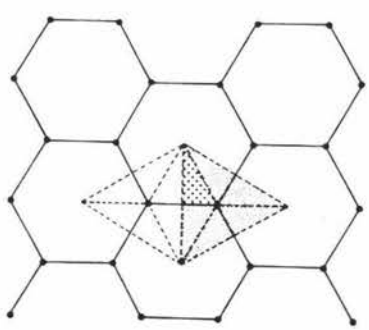
1. The uneven distribution of the mitochondria
2. Facilitated diffusion due to the presence of mitochondria
3. The increase in capillary density as PO_2 falls.

Another model to describe oxygen transport to skeletal muscle was proposed by Akmal et al (1977). This model is an extension of the Krogh cylinder and is modified to include a three dimensional arrangement of capillaries around muscle fibres. They postulated that six capillaries are arranged at the apices of a hexagonal muscle fibre with a cross-sectional area

of about $3000\mu m^2$ and it is assumed that the capillaries are of identical length and are arranged such that all arterial ends are in the same plane. Also the blood flow through the capillaries is assumed to be identical as far as PO_2 is concerned.

On internalising a capillary with no-flux boundaries through each of the hexagons, an equilateral triangle is obtained in cross-section which is completely supplied by one capillary (Fig. 2-2-12).

Fig. 2-2-12 : A hexagonal fibre area is completely supplied by 6 capillaries. Each capillary participates in the supply of 3 adjacent fibres. The equilateral triangle (fine stippled area) is divided by bisectors into 6 triangular micro-units (coarse stippled area). (From Akmal et al, 1977)



Akmal et al (1977) suggested that there can be no efficient perfusion of tissue if Krogh cylinders are circumscribed around the capillaries and they postulated that the most economical and efficient solution is to assume no-flux boundaries between adjacent equilateral triangles which results in an enhanced PO_2 at any point.

2-2-6 CAPILLARY SUPPLY OF SKELETAL MUSCLE

The capillary supply of skeletal muscle has been studied in a wide range of species and muscles yeilding a range of results. The major methods of assessing capillary supply in transverse sections of muscle have traditionally been (1) number of capillaries per muscle fibre i.e. capillary/fibre ratio, (2) number of capillaries per mm^2 i.e. capillary density and (3) number of capillaries surrounding each fibre.

2-2-6-1 Capillary/fibre Ratio

Capillary/fibre ratio has been shown to vary according to species (Plyley and Groom, 1975), muscle (Gray and Renkin, 1978), fibre type (Schmidt-Nielsen and Pennycuik, 1961), level of fitness (Hermansen and Wachtlova, 1971) and age (Henkel, 1983).

Henkel (1983) found that, in horses, the capillary/fibre ratio increased during maturation with horses of six months of age having a mean capillary/fibre ratio of 0.97 while horses over 10 years of age had a capillary/fibre ratio of 2.05. In contrast Parizkova et al (1971) who also studied the effect of age on capillary/fibre ratio and observed that, in humans, the capillary/fibre ratio decreased with increasing age. They reported that the mean capillary/fibre ratio of men with an average age of 20.76 was 0.81 ± 0.22 while in older men, those which had been physically active (mean age : 73.90) had a capillary/fibre ratio of 0.65 ± 0.3 and those which were relatively sedentary (mean age : 72.49) had a ratio of 0.59 ± 0.08.

Schmidt-Nielsen and Pennycuik (1961) studied the variation in capillary/fibre ratio in the masseter muscle of 10 species of mammal. They found that as body size increases, the number of capillaries/fibre generally increases. The smallest species included in their study was the bat which had a capillary/fibre ratio of 1.6 while the largest was cattle which showed a ratio of 2.5.

A number of species was also included in the study of Plyley and Groom (1975) who investigated the gastrocnemius and soleus muscles of cats, guinea pigs, rats and rabbits. They found that the capillary/fibre ratios for these animals ranged from 1.27 (guinea pig soleus) to 2.05 (rat soleus) with a mean of 1.63. Other studies have yielded a wide range of results for various species and muscles with considerable variation even for the same muscle (Table 2-2-2). Values for rabbit gastrocnemius have ranged from 0.5 (Smith and Giovacchini, 1956) to 1.10 (Schmidt-Nielsen and Pennycuik, 1961) and those for rabbit semitendinosus from 1.18 (Hammersen, 1970 ; cited by Plyley and Groom, 1975) to 1.7 (Smith and Giovacchini, 1956).

The effect of exercise on capillary/fibre ratio has been studied mostly in the human (Hermansen and Wachtlova, 1971 ; Brodal et al, 1977 ; Andersen and Henriksson, 1977).

Subjects in Hermansen and Wachtlova's study consisted of 15 young males, 8 of which were untrained (average maximal oxygen uptake : 50.2ml/kg x min) and the other 7 well trained (average maximal oxygen uptake : 71.4ml/kg x min). The capillary/fibre ratios from the lateral portion of the quadriceps femoris muscle of the two groups were found to be lower (1.08 capillaries/fibre) for the untrained subjects than for the well trained individuals (1.49 capillaries/fibre).

Table 2-2-2 : Reported values for capillary/fibre ratios in striated muscle of the rabbit, rat, mouse, guinea pig, cat and dog. (Modified from Plyley and Groom, 1975)

Author	Year	Animal	Muscle	C/F
Smith and Giovacchini	1956	Rabbit Rabbit	Semitendinosus Gastrocnemius	1.7 0.5
Heroux and St. Pierre	1957	Rat Rat	Gastrocnemius Soleus	1.6 1.6
Schmidt-Nielsen and Pennycuik	1961	Mouse Rat Guinea Pig Cat Rabbit Dog Rat Cat Rabbit	Masseter Masseter Masseter Masseter Masseter Masseter Gastrocnemius Gastrocnemius Gastrocnemius	1.78 1.71 2.67 0.90 2.12 2.19 1.72 0.74 1.10
Nishiyama	1965	Cat Cat	Gastrocnemius Soleus	1.40 0.60
Carrow et al	1967	Rat Rat	Gastroc (red) Gastroc (white)	2.53 1.22
Hammersen	1970	Rabbit Rabbit Rabbit Cat Cat	Sartorius Gracilis Semitendinosus Rectus femoris Sartorius	0.58 0.68 1.18 1.04 1.87
Henckel	1983	Horse	Gluteus medius	2.05

A higher capillary/fibre ratio in trained subjects was also found by Brodal et al (1977) who also studied the lateral part of the quadriceps muscle in 12 trained and 11 untrained young males. Their values were higher than those of Hermansen and Wachtlova with the trained subjects having an average capillary/fibre ratio of 2.49 and the untrained subjects an average of 1.77 capillaries per muscle fibre.

Andersen and Henriksson (1977) observed values which were between those of Hermansen and Wachtlova (1971) and Brodal et al (1977) in their investigation of the quadriceps femoris muscles of 5 men who participated in 8 weeks of training. Before training the average capillary/fibre ratio of the men was 1.36. This increased to 2.0 capillaries per fibre by the end of the three week training program.

The 7 young females that participated in 24 weeks of intensive endurance training in a study by Ingjer (1979) showed very similar capillary/fibre ratios to the subjects in Andersen and Henriksson's study. Samples were also taken from the lateral part of the quadriceps muscle and showed an average capillary/fibre ratio of 1.39 before training which increased significantly to 1.79 following training.

Apart from humans, the effect of exercise on capillary/fibre ratios has also been examined in the rat (Carrow et al, 1967). 30 male albino rats were divided into 3 groups (1) sedentary, (2) voluntary exercise and (3) forced exercise. The capillary/fibre ratios for the predominantly red areas of the gastrocnemius muscle were 2.53, 2.65 and 2.63 for the three groups respectively and were 1.22, 1.42 and 1.35 respectively for the predominantly white areas.

As seen in the study of Carrow et al (1967), the parameter capillary/fibre ratio also shows variability from one type of muscle fibre to another (Schmidt-Nielsen and Pennycuik, 1961 ; Gray and Renkin, 1978). Schmidt-Nielsen and Pennycuik (1961) in their study of the gastrocnemius muscle of 10 species of mammal separated their capillary counts into two groups (1) those from the red (slow contracting) portion and (2) those from the white (fast contracting) portion of the gastrocnemius muscle. They found that the capillary/fibre ratios of the red portion of the muscle were higher than those in the white portion in all species that they studied, for example, in the bat the capillary/fibre ratio in the red portion was 2.0 while

in the white portion it was 0.9 and in the rabbit the values were 1.7 and 1.0 for the red and white portions respectively.

Gray and Renkin (1978) classified the muscle fibres in the gastrocnemius (lateral and medial portions), soleus and cranial tibial muscles of the rabbit as αW (fast contracting, low oxidative), αR (fast contracting, high oxidative) and ß (slow contracting, high oxidative) through the use of the staining techniques for succinic dehydrogenase and myosin ATPase. They observed that the capillary/fibre ratios of individual fibre types showed different patterns of variation in different muscles. There was no significant differences among the fibre types in the medial gastrocnemius but in the lateral gastrocnemius, ratios for αR (1.30 capillaries/fibre) and ß (1.30 capillaries/fibre) were significantly greater than the average for the muscle (1.18 capillaries/fibre) while for αW fibres (1.07 capillaries/fibre) the ratio was significantly smaller. For the cranial tibial muscle, all the capillary/fibre ratios were significantly different from the average (1.61 capillaries/fibre) with values of 1.42, 1.76 and 2.38 capillaries/fibre for the αW, αR and ß fibres respectively.

2-2-6-2 Capillary Density

The measurement of capillary density i.e. number of capillaries per mm^2 is another common method for assessing capillary supply in particular muscles.

Changes in capillary density can be seen with increasing age (Henkel, 1983 ; Parizkova et al, 1971). Henkel (1983) divided 8 Thoroughbred horses into 3 groups according to age. These groups were comprised of 3 six month old foals (Group I), 2 two year olds and 1 three year old (Group II) and 3 horses over 10 years (Group III). The capillary density values obtained from the middle gluteal muscles of these horses were 497, 627 and 692

capillaries/mm_2 for Group I, II and III respectively showing an increase in capillary density with increasing age.

In humans, capillary density has also been shown to increase with increasing age (Parizkova et al, 1971). In the study of Parizkova et al (1971), men with an average age of 74 years who had been physically active during their lives had a mean capillary density of 362.4 capillaries/mm^2 while young men with an average age of 21 years had a mean capillary density of only 270 capillaries/mm^2.

Training has generally been found to result in an increase in the capillary density of skeletal muscle (Hermansen and Wachtlova, 1971 ; Brodal et al, 1977). Hermansen and Wachtlova (1971) found that the capillary density in the lateral part of the quadriceps muscle of the 15 young male subjects in their study was slightly lower in a group of untrained subjects (600 ± 23.8 capillaries/mm^2) than in a group of well trained subjects (640 ± 54.5 capillaries/mm^2) although this difference was not statistically significant.

Brodal et al (1977) working on the same muscle in young males did, however, find a significant difference between the untrained group (585 ± 40 capillaries/mm^2) and the trained group (821 ± 28 capillaries/mm^2) in their study. Andersen and Henriksson (1977) also found a significant difference in the capillary density of the lateral part of the quadriceps muscle in 5 young men before 8 weeks of training (329 capillaries/mm^2) and after (395 capillaries/mm^2).

In young females, capillary density has also been found to increase with training (Ingjer, 1979). Ingjer (1979) found that capillary density counts from the lateral part of the quadriceps muscle increased significantly from 348 ± 29 capillaries/mm^2 before to 438 ± 31 capillaries/mm^2 after 24 weeks of training.

Capillary density can vary significantly between muscles with the general trend being

that the more aerobic the muscle (i.e. the higher the percentage of ATPase low fibres) the higher the capillary density. Myrhage (1978), in a study on 5 muscles from the hindlimb of the cat, found that the capillary density ranged from 570 ± 34 capillaries/mm^2 in the lateral gastrocnemius to 948 ± 47 capillaries/mm^2 in the soleus muscle. The other muscles studied were the biceps femoris, the medial gastrocnemius and the caudal crural abductor muscles which showed values of 617 ± 66, 695 ± 49 and 657 ± 54 capillaries/mm^2 respectively.

Plyley and Groom (1975) reported that in the four muscles, gastrocnemius, soleus, masseter and tongue of the cat, the capillary density ranged from 369 capillaries/mm^2 in the gastrocnemius to 1440 capillaries/mm^2 in the tongue. They found a similar range in the dog which showed capillary densities of 706 capillaries/mm^2 in the gastrocnemius to 1008 capillaries/mm^2 in the tongue.

As well as varying between muscles, capillary density also shows variation between different muscle fibre types. Schmidt-Nielsen and Pennycuik (1961), in their study of the red and white portions of the gastrocnemius muscle in 10 species of mammal, found differences between these portions in terms of capillary density in most species. For example, in the mouse, the capillary density in the red portion of the gastrocnemius was found to be 1848 capillaries/mm^2 while in the white portion it was only 635 capillaries/mm^2. Corresponding values for the rat were 1252 and 374 capillaries/mm^2 for the red and white portions respectively and for the guinea pig 1094 and 489 capillaries/mm^2 respectively.

Gray and Renkin (1978) found that, in the white rabbit, there was a greater overall capillary density in muscles with a larger proportion of red fibres : soleus had the highest capillary density (643 ± 43 capillaries/mm^2) and the lateral gastrocnemius the lowest (349 ± 53 capillaries/mm^2). The individual fibre types showed capillary densities of 258 (αW), 514 (αR) and 642 (ß) for the lateral gastrocnemius and since the soleus contained only ß fibres,

the capillary density for ß fibres in the soleus was 642 ± 43 capillaries/mm^2. Capillary densities for the individual fibre types in the cranial tibial muscle were 430, 866 and 829 capillaries/mm^2 for the αW, αR and ß fibres respectively.

2-2-6-3 Number of Capillaries Surrounding Each Fibre

A somewhat less common method of assessing capillary supply is the use of the number of capillaries surrounding each fibre (CAF). This parameter has been used to describe capillary supply without distinguishing between different fibre types (Brodal et al, 1977 ; Plyley and Groom, 1975) as well as in the context of the number of capillaries surrounding the fibres of a particular type (Andersen and Henriksson, 1977 ; Myrhage, 1978).

Brodal et al (1977), when they did not separate the fibres depending on type, found in their study on the effect of endurance training on capillary supply to the lateral part of the quadriceps muscle in human males that the CAF was higher in trained (5.87 ± 0.18) than untrained (4.43 ± 0.19) subjects. When they classified the fibres on the basis of the number of subsarcolemmal mitochondrial aggregates (M1, no subsarcolemmal aggregates ; M2, one or two subsarcolemmal aggregates ; M3, three or more subsarcolemmal aggregates) they found that the number of capillaries around each fibre increased with the number of mitochondria in both trained and untrained subjects.

Andersen and Henriksson (1977) also studied the effect of training on the capillary supply to the lateral part of the quadriceps muscle of young men and classified the fibres into three groups : Type I (slow twitch, high oxidative), Type IIA (fast twitch, high oxidative) and Type IIB (fast twitch, low oxidative) using the ATPase stain. They found that the number of capillaries surrounding each fibre significantly increased for all fibre types over the 8 week training period. The number of capillaries surrounding the Type I fibres increased from 3.9

to 5.4, the number surrounding the Type IIA fibres from 4.2 to 5.5 and the number surrounding the Type IIB fibres from 3.0 to 4.2. They also found that, before training, the number of capillaries surrounding the Type IIB fibres was 0.77 times that of the Type I and 0.71 times that of the Type IIA, while following training the corresponding values were 0.78 and 0.76 respectively.

Ingjer (1979) also classified the fibres in the lateral part of the quadriceps of females as Type I, Type IIA and Type IIB. He found that over a 24 week training program, the CAF for the Type I fibres increased from 4.11 to 5.04, for the Type IIA fibres an increase from 3.40 to 4.15 was observed while the CAF for the Type IIB fibres increased from 2.33 to 2.68.

The number of capillaries surrounding fibres of different types was also studied by Myrhage (1978) for the caudal crural abductor, biceps femoris and gastrocnemius (lateral and medial portions) muscles of the cat. The fibres in this study were described as FG (fast twitch, glycolytic), FOG (fast twitch, oxidative glycolytic) and SO (slow twitch, oxidative). It was reported that the absolute values for CAF varied from 0-5 in the caudal crural abductor, biceps femoris and lateral gastrocnemius and from 0-7 in the medial gastrocnemius. The FG fibres in all four muscles had a smaller number of surrounding capillaries (range: 1.5-3.3) than the FOG fibres (range: 2.0-3.8). The SO fibres in these muscles had CAF values (range: 2.0-3.4) which were slightly lower or similar to the corresponding values for the FOG fibres.

The variation of CAF between muscles and species has also been studied (Plyley and Groom, 1975). From a selection of species (cat, dog, rat, rabbit and guinea pig) and muscles (gastrocnemius, soleus, gracilis, cranial tibial and masseter) the values for the mean number of vessels surrounding a fibre ranged from 3.21 (cat masseter) to 4.01 (rat soleus) with an overall mean value for 16 different muscles taken from 5 species, of 3.53 ± 0.07. From all

the muscles the number of vessels surrounding each fibre ranged from 0 to 9.

2-2-6-4 Relationship Between Oxidative Capacity and Capillary Supply

It has been reported that a greater capillary network surrounds fibres that have a higher activity of oxidative enzymes than those which have a lower activity for such enzymes (Gray and Renkin, 1978 ; Hoppeler et al, 1981).

Gray and Renkin (1978) classifed fibres in the medial gastrocnemius, lateral gastrocnemius, cranial tibial and soleus muscles of New Zealand white rabbits as αW (fast contracting, low oxidative capacity), αR (fast contracting, high oxidative capacity) and ß (slow contracting, high oxidative capacity). Capillaries were identified using alkaline phosphatase. They found that the capillary/fibre ratios for αR and ß fibres in the lateral gastrocnemius and tibialis anterior muscles were greater than for αW fibres in the same muscles. They also observed that while capillary densities for red fibres were twice those for white fibres in the same muscle, estimated maximal oxygen uptakes for red and white fibres are in the ratio of approximately five to one. It was concluded that capillary supply to white fibres is excessive, or the supply to red fibres is deficient, in relation to their capacities for oxidative metabolism. This conclusion ignores the fact that, occasionally, white muscles are required to perform at maximum capacity and therefore needs enough capillaries to quickly remove metabolites such as lactate.

Hoppeler et al (1981) also commented that a relationship between oxidative capacity and capillary supply is apparent on the level of single fibres but they observed that this relationship becomes much less obvious on the level of individual muscles. In their study on

twenty muscles from wildebeest and dik-dik, they found that only in the diaphragm were higher mitochondrial volume densities consistently combined with higher capillary counts.

The capillarity and oxidative capacity of the heart, diaphragm, vastus medialis and semitendinosus muscles of dogs, goats, ponies and calves was assessed by Conley et al (1987). These species were choosen because of results of Taylor et al (1987) which suggested that 'athletic' animals such as dogs and ponies are capable of reaching higher limits of aerobic metabolism than 'less athletic' species of the same size such as goats and calves. They addressed the question of whether the structure supporting oxygen delivery from the blood, the capillary network, meets the demands imposed by the mitochondria. Capillary density, mean fibre area and mitochondrial volume density was calculated for each muscle. Capillary density was found to be 1.2 to 1.4 times higher in skeletal muscles of the "more aerobic" dogs and ponies than in the goats and calves. The statistical significance of these differences was, however, weak in general. It was also found that the parameters of capillary density and mitochondrial volume density are linearly correlated ($r^2 = 0.89$).

A study by Enad et al (1989) examined the relationship between fibre capillarity and oxidative capacity at the level of single motor units which are the basic functional units of motor control. Motor units in the cat diaphragm were isolated in situ and were classified on their isometric contractile force responses and fatigue indexes. Muscles fibres which belonged to the individual units were identified using the glycogen-depletion method and were classified as type I or type II based on myosin ATPase activity. The capillarity of muscle unit fibres was determined using the parameters, number of capillaries surrounding each fibre and number of capillaries per fibre area. A significant positive correlation was observed between motor unit fatigue resistance and the mean capillary/fibre ratio of unit fibres. Also, when the number of capillaries surrounding a fibre was normalised for fibre cross-sectional

area, a significant positive correlation was found between unit fatigue resistance and fibre capillary density. They concluded that diaphragm motor unit fatigue resistance depends, at least in part, on the oxidative capacity and capillary density of muscle unit fibres.

In contrast to these studies, Maxwell et al (1980) reported that the results of their study did not support the hypothesis that capillarity correlates with the oxidative capacity of skeletal muscles. A range of muscles (soleus, plantaris, medial gastrocnemius, extensor digitorum longus, flexor carpi radialis, semitendinosus, psoas, diaphragm, masseter and temporalis) and species (cats, rhesus monkeys, rabbits, guinea pigs and rats) were included in the study. Fibres were classified as type I, type IIA (fatigue resistant) and type IIB (fatigable) from their succinic dehydrogenase and myosin ATPase activity. It was concluded that there was no consistent pattern relating the number of capillaries adjacent to individual fibres to the histochemical enzymatic characteristics of the fibres.

2-3 ADAPTATION TO EXERCISE IN HORSES

The morphological and biochemical changes that occur during training in the equine athlete have become a subject of interest in recent years. The changes which occur can depend on the type of exercise performed (Lindholm and Saltin, 1974) while it is also known that horses differ individually with respect to the composition of muscle fibres, muscle fibre area and the enzymes involved in aerobic and anaerobic metabolism (Lindholm et al, 1983 ; Essen-Gustavsson et al, 1983).

2-3-1 FIBRE TYPE PROFILES

The skeletal muscle of most animals is composed of a mixture of fibre types which differ from each other with respect to metabolic and contractile characteristics (Barnard et al, 1971 ; Essen et al, 1975). Fibres can be separated histochemically on the basis of contractility by staining for myofibrillar ATPase (Stein and Padykula, 1962) into two groups. Type I or slow twitch fibres have slow contraction times and Type II or fast twitch fibres have fast contraction times. The Type II fibres can then be subclassified into 2 subpopulations through the use of the histochemical method for the oxidative enzyme succinic dehydrogenase (Nachlas et al, 1957). Through this method, the muscle fibres of the horse have been identified as slow twitch oxidative (ST), fast twitch oxidative (FTH) and fast twitch glycolytic (FT) by Lindholm and Piehl (1974) and by Snow and Guy (1976). Alternatively they have been described as Type I, Type IIA and Type IIB respectively (Essen-Gustavsson et al, 1983 ; Lindholm et al, 1983).

Currently these two classification systems are used interchangeably although it has been suggested that they are not completely identical (Valberg et al, 1988; Snow and Guy,

1980). Valberg et al (1988) evaluated the oxidative capacity of skeletal muscle fibre types in racehorses histochemically using the nicotinamide dinucleotide diaphorase (NADH-D) staining technique. Fibres were rated as high (H), medium (M) or low (L) according to staining intensity. Oxidative capacity was also assessed biochemically by measuring the activity of citrate synthase (CS). Fibres were also classified on the basis of ATPase activity following acid preincubation (pH 4.6) as type I, IIA or IIB. It was observed that while most type I fibres were rated as H, type IIA fibres could be rated as either H or M. It was only type IIB fibres that rated L. It was concluded that differences exist between fibre type distribution determined histologically and that determined from ATPase staining.

Lopez-Rivero et al (1990a) studied the degree of correspondance between contractile and oxidative capacities in horse muscle fibres. Samples from the middle gluteal muscle of 95 untrained adult horses were assessed on myosin ATPase activity and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) activity. Fibres were classified as type I, IIA or IIB according to ATPase activity. The percentage fast twitch glycolytic (FT) fibres and the proportion of IIB fibres with high and low oxidative capacity were determined from NADH-TR staining. It was observed that, generally, type I and IIA fibres showed some degree of oxidative capacity while most IIB fibres showed low oxidative capacity and were therefore classified as FT. The oxidative capacity of IIB fibres, however, was not found to be constant as a considerable proportion of them showed some reaction to NADH-TR staining. It was concluded that the contractile capacity of a muscle fibre does not determine its oxidative profile and that oxidative capacity should be assessed separately. They also suggested that oxidative capacity should not be used as a criterion for fibre classification in horses.

The distribution of fibre types has been shown to differ between muscles and between

different parts of the same muscle (van den Hoven et al, 1985; Hermanson and Hurley, 1990). Large differences in fibre type distribution were found to exist in different parts of four muscles in the horse by van den Hoven et al (1985). Nine different parts of the triceps brachii, longissimus dorsi, gluteus medius and biceps femoris muscles were studied. These nine parts were selected so that samples from three cranial, three intermediate and three caudal areas were obtained from locations at 1cm, 5cm and 8cm below the superficial muscle surface. It was found that the distribution of type I fibres in the four muscles was not homogeneous with the percentage of these fibres increasing with increasing depth. It was suggested that the deeper parts of the muscles have a more aerobic character which is related to their postural functions. They reported that, even at a depth of 8cm, differences of up to 30% exist in the percentage of type I fibres between individual samples.

Bruce and Turek (1985) also studied the muscle fibre variation at different depths in the gluteus medius of the horse. An increase in the mean percentage of slow twitch fibres occurred as depth increased. Significant differences in the percentage of slow twitch fibres were also found along the length of the muscle with a general increase in slow twitch fibres from cranial to caudal. The percentage slow twitch fibres in the whole muscle ranged from 2.4% to 64.5% depending on the location of the biopsy. It was concluded that small biopsy samples of fibres are not necessarily representative of the whole muscle.

Hermanson and Hurley (1990) observed that the medial head of the biceps brachii muscle in horses contains fewer type I fibres than the lateral head. They suggested that the lateral head contributes to the postural role of the muscle while the medial head is more important during dynamic activity.

In contrast to these studies, Snow and Guy (1980) found no significant differences in the percentage of FT, FTH and ST fibres between 36 different muscles in five different

breeds of horse. In only three of the 36 muscles examined was there a significant difference in the proportion of high myosin ATPase fibres between the deep and superficial regions of the muscle.

Fibre type distributions are also known to differ between breeds of horse (Lopez-Rivero et al, 1989; Snow and Guy, 1980). Snow and Guy (1980) examined muscles from the following breeds of horse : American racing Quarterhorse, Thoroughbred, Arab, Shetland pony, pony and heavy hunter. They also examined muscles from donkeys. All the horses sampled were undergoing only maintenance exercise. In the gluteus medius muscle, a difference between the breeds with respect to percentage of high myosin ATPase fibres was found. The Quarterhorse had a significantly greater proportion of these fibres than any other breed with the Thoroughbred having the next highest proportion. It was suggested that these variations were related to the sprinting speed of the breed. In the vastus lateralis, mean values of myosin ATPase fibres ranged from 91.8% in the Thoroughbred to 58.2% in the donkey (samples of this muscle were not taken from the Quarterhorse or the Arab). In the semitendinosus muscle, the Shetland pony had the greatest proportion of high ATPase fibres.

Galisteo et al (1992) compared the gluteus medius fibre composition in young Andalusian and Arabian horses. It was found that Arabian yearlings have a higher IIA/IIB ratio than Andalusians. In general it was reported that in any given age group (foals, weanlings and yearlings), Arabian horses had a significantly higher proportion of type I and IIA fibres and a lower proportion of IIB fibres than Andalusians.

The fibre type composition of Andalusians, Thoroughbreds and Arabians has been studied by Lopez-Rivero et al (1989). They found highly significant differences in the proportion of type I and type II fibres between breeds. The percentage of type I fibres was highest in the Andalusian (35.2%) and lowest in the Arabian (23.8%). The Thoroughbred had

29.5% type I fibres. Thoroughbreds showed a higher proportion of type IIA fibres than the other two breeds. This contradiction between the studies of Galisteo et al (1992) and Lopez-Rivero et al (1989) may have occurred due to the differences in the age of the horses used in their respective studies. In the formers study the horses were all aged between 10 days and 18 months while in the latters study the age of the horses was much higher (5 to 12 years). Age has been shown to affect fibre type proportions by Henckel (1983).

The proportions in which fibres exist has been shown to change following training (Lindholm et al, 1983 ; Essen-Gustavsson et al, 1983) although due to a large variation between individuals, fibre type composition is thought to be to some extent genetically determined (Essen-Gustavsson et al, 1983).

Guy and Snow (1977) studied the effect of training on the muscle fibre composition of 4 Thoroughbreds and 2 heavy hunters. The training program lasted 10 weeks and consisted of 4 days submaximal endurance work and 2 days maximal sprinting (galloping over 3 x 600m) per week. The fibres were classified as ST, FTH and FT. With training, the deltoideus muscle showed a significant change in fibre type proportions, with a decrease in the percentage of ST fibres and an increase in the percentage of FTH fibres. When all the muscles studied (deltoideus, long head of triceps brachii, vastus lateralis, gluteus medialis, biceps femoris and semitendinosus) were considered together, a significant decrease in ST fibres and a significant increase in FTH fibres was also found with training.

Lindholm et al (1983) also found fibre type changes in the middle gluteal muscles of 14 Thoroughbred yearlings during the sprint training and racing of their season as 2 year olds. They found that by the end of this racing season, the proportion of Type IIA fibres had increased from 31% to 38% while the proportion of Type IIB fibres had decreased from 56% to 49%. They also found that there was no change in the ratio of Type I to Type II fibres

during racing and training. They commented that all fibre types displayed a large individual differences both before and after training.

Similar differences in fibre type populations has been observed in Standardbred horses (Essén-Gustavsson and Lindholm, 1985). The 36 horses were placed into 3 groups as follows:

Group 1 : 12 horses neither in training nor had been raced during the past two years.

Group 2 : 12 horses which were moderately good performers that had been actively trained during the past year and were still racing.

Group 3 : 12 horses which were excellant performers and that were being actively trained and raced.

They observed that the percentage of Type I fibres was similar in all three groups while there was a marked difference among the subgroups of the Type II fibres. The 2 groups of well trained horses had a higher proportion of Type IIA fibres and a lower proportion of Type IIB fibres (58% IIA and 15% IIB in excellant performers ; 49% IIA and 26% IIB fibres in moderate performers) than the inactive horses (41% IIA and 35% IIB). In the well trained horses the best performers were found to have a higher proportion of Type IIA fibres and a lower proportion of IIB fibres than the moderate performers.

A slight increase in the IIA/IIB ratio of young Standardbreds during training was also observed by Essén-Gustavsson et al (1983). 10 Standardbred colts aged between 6 and 8 months were divided into 2 groups, one of which was subjected to regular controlled exercise while the other group only participated in the amount of activity normally occurring in a group of yearlings. Muscle biopsies were taken at the beginning of the study and then 4 and 10 months later. An increase in the IIA/IIB ratio was also seen in the control group and therefore growth was considered to be a significant factor in this change.

Muscle fibre composition has also been studied in untrained and endurance trained Andalusian and Arabian horses (Lopez-Rivero et al, 1991). Fibres from the middle gluteal muscle of these horses were classified as type I, IIA and IIB following staining for myosin ATPase and nicotinamide adenine dinucleotide-tetrazolium reductase activity. The group of horses with the hardest training were found to have a higher proportion of type IIA fibres and a lower proportion of type IIB fibres than the untrained horses. The trained horses also had a higher percentage of type IIB oxidative fibres and a lower percentage of type IIB non-oxidative fibres than the untrained horses. It was suggested that while fibre type proportions are stable within a given breed, the stimulus of training facilitates changes in both contractile and oxidative properties of the type II subgroups.

As seen in the study of Essén-Gustavsson and Lindholm (1985), the proportion of various fibre types has also been related to performance. Snow et al (1981) studied the muscle fibre composition in horses competing in an endurance ride. They found that although the proportion of ST fibres in the horses varied between 7 and 38%, the horses with the highest proportion of these fibres usually had the best performance record. Elite horses were also found to have a higher proportion of Type I fibres than average horses in a study on endurance horses by Hodgson et al (1983).

2-3-2 FIBRE SIZE

The size of individual fibres in horses has been shown to differ between breeds (Lopez-Rivero et al, 1990b) and between different fibre types (Andrews and Spurgeon, 1986). The size of fibres in the middle gluteal muscle of 25 Andalusians, 20 Thoroughbreds and 10 Arabians was analysed in a study by Lopez-Rivero et al (1990b). No statistical difference in the size of type I fibres between breeds was found (Andalusian, 3250μm^2; Thoroughbred,

2880μm^2; Arabian, 3010μm^2). A significant difference was found, however, in the size of the type IIA and IIB fibres between breeds. The area of type IIA fibres was significantly higher in the Andalusian (4170μm^2) than in the Thoroughbred (3380μm^2) and the Arabian (3520μm^2). The size of type IIB fibres was also found to be higher in the Andalusian horses than the other two breeds.

Fibres in Andrews and Spurgeon's (1986) study on five breeds of horse were classified as type I, type IIA and type IIB. Type IIB fibres had the greatest minimal fibre diameter, type IIA fibres intermediate minimal fibre diameter and type I fibres had the smallest minimal fibre diameter. Type IIB fibres have also been found to be the largest of the three fibre types in both Spanish (Lopez-Rivero et al, 1990c) and Arabian horses (Lopez-Rivero et al, 1990d). In both cases type IIA fibres were the next largest with type I fibres being the smallest.

Fibre size has been shown to change during training. Essén-Gustavsson et al (1983) studied fibre ares in all fibre types in 2 groups of colts, one of which underwent controlled regular exercise for 1 year between the ages of 6 months to 1.5 years while the other group had only the amount of physical exercise that normally occurs in a group of yearlings. They found that mean fibre area increased significantly over the whole period in both the middle gluteal (control group by 29% ; exercised group by 50%) and semitendinosus muscles (control group by 24% ; exercise group by 35%).

Inactive Standardbred horses in Essén-Gustavsson and Lindholm's study (1985) were found to have significantly larger Type IIA and IIB fibres (IIA 3714 μm^2 ; IIB 5935 μm^2) than active horses (excellant performers : IIA 3075 μm^2, IIB 3075 μm^2 ; moderate performers : IIA 3185 μm^2, IIB 4252 μm^2)

Lindholm et al (1983), however, found that there was no significant change in fibre areas during the training and racing of 14 Thoroughbred yearlings through the two year old

season although there was a tendancy towards an increase in Type I and IIA fibre areas and a decrease in Type IIB fibre area.

Lopez-Rivero et al (1992) also found no significant difference in fibre areas, neither between untrained and endurance trained Arabians nor between untrained and endurance trained Andalusian horses.

2-3-3 ENZYME LEVELS

The major energy sources for the rephosphorylation of ADP to ATP during prolonged exercise are fats and carbohydrates. Fats which are stored in the muscle are mobilised through mitochondrial membranes into the mitochondria where they combine with coenzyme A and are oxidised to form acetyl CoA which can then enter the citric acid cycle. Carbohydrates are broken down through the process of glycolysis and, in the presence of oxygen, also form acetyl CoA (Rose, 1986).

With training, the levels of the enzymes responsible for oxidation of fats (e.g citrate synthase (CS), 3-OH-acyl CoA dehydrogenase (HAD)) and glycolysis (e.g. triose phosphate dehydrogenase (TPDH), lactate dehydrogenase (LDH)) can alter significantly.

The oxidative enzyme citrate synthase showed a significant increase in a group of Standardbred colts which were given regular controlled exercise over a period of 1 year (Essén-Gustavsson et al, 1983). Another oxidative enzyme, HAD was shown to be unchanged in this group. Two glycolytic enzymes were also studied and showed a significant decrease (20-40%) in both the regularly exercised group and a group of colts which had had no regular controlled exercise.

A study by Lindholm et al (1983) also showed an increase in the level of citrate synthase (28 to 60 μmol/g/min) during the two year old racing seasons of 14 Thoroughbreds.

They also showed an increase in the level of HAD (14 to 23 μmol/g/min) and therefore reported an overall increase in oxidative capacity. They also studied glycolytic capacity and found that although the level of lactate dehydrogenase did not change over the racing period, triose phosphate dehydrogenase decreased in activity.

Guy and Snow (1977) studied 6 different enzymes (Lactate dehydrogenase, creatine phosphokinase, aldolase, citrate synthase, aspartate aminotransferase and alanine aminotransferase) in the muscles of 4 Thoroughbreds and 2 heavy hunters during a 10 week training period. They reported that training resulted in an increase of approximately 70-100% for all the enzymes except creatine phosphokinase (CPK) which increased approximately 30%. These increases in enzyme activity were noticed to occur at different rates. The greatest increase in the anaerobic (glycolytic) enzymes, lactate dehydrogenase and aldolase was in the first 5 weeks whereas that of CPK and alanine aminotransferase occurred during the second 5 weeks. The percentage change, however, in aspartate aminotransferase and citrate synthase was similar for both periods of time.

The levels of the oxidative enzymes citrate synthase and HAD have been shown by Essén-Gustavsson and Lindholm (1985) to be higher in active (CS 91.6 μmol/g/min ; HAD 58.8 μmol/g/min) than inactive horses (CS 54.4 μmol/g/min ; HAD 43.2 μmol/g/min).

The depletion of glycogen during exercise has been observed for both endurance exercise and during maximal exercise over shorter distances.

Nimmo and Snow (1983) galloped 6 Thoroughbred horses over distances of 506, 1025, 1600 and 3620m to investigate the effect of maximal exercise on muscle glycogen. They found that in the 506m trial the horses used significantly less glycogen than in the 1025 and 1600m trials and in the 3620m trial they used significantly less than in the 1025m trial. They also investigated the levels of lactate in the horses and found that lactate accumulation over

the 3620 trial was significantly lower than over all other distances.

Snow et al (1981) found that in horses which completed an 80km endurance ride there was a decrease in glycogen content of 56 ± 7.2%. They also reported that in all horses examined before the ride there was a high glycogen content in the FT and FTH muscle fibres with a slightly lower amount in the ST fibres. Following the ride there was an apparent complete depletion of glycogen in the ST fibres in all cases. In the FTH fibres there was a marked to moderate depletion while only a small to moderate depletion was observed in the FT fibres.

Patterns of glycogen depletion in endurance horses has also been studied by Hodgson et al (1983) who collected biopsy samples from the middle gluteal muscles of horses competing over three distances (Ride 1, 40km ; Ride 2, 110km and Ride 3, 160km). Their results also showed that in all rides, Type I fibres showed almost complete glycogen depletion with the most extensive depletion occurring in the horses competing in the 160km ride. As the ride distance was increased a progressive recruitment of the Type IIA and Type IIB fibres became apparent.

2-4 OBJECTIVES OF THIS STUDY

In the present study the relationship between contractility, as measured by the percentage of ATPase low fibres, and capillary supply is examined in a wide variety of muscles from horses of differing breeds, ages and sex.

The objectives of this study are :

1. To examine the variation in capillary supply of a range of muscles in the horse.

2. To determine the relationship between contractility, as measured by percentage of ATPase low fibres, and capillary supply.

3. To examine and compare the problems associated with the measurement of capillary supply using the parameters, capillary/fibre ratio, capillaries/mm^2 and number of capillaries surrounding each fibre.

4. For selected muscles, to determine the difference in capillary supply for ATPase low and ATPase high fibres.

CHAPTER THREE : MATERIALS AND METHODS

3-1 SAMPLING

Muscle samples were obtained from 4 horses which were of differing breeds, ages and sex (Appendix 1) within 2 hours of death. All the horses were healthy except for Horse 3 which had a stifle joint problem on its near hind leg. In this case samples were taken only from the off hind leg. The muscles taken from each horse are listed in Appendix 2. The sampling site in each muscle was randomly chosen and was not necessarily consistant between horses.

3-2 METHOD OF FREEZING AND STORING

Once all the samples had been taken, a cube of muscle with a side of 5mm was removed from each sample. This was immediately frozen in 100ml of isopentane cooled in 50 ml of liquid nitrogen for at least 90 seconds. The frozen cube of muscle was then wrapped in tin foil and stored in a -70°C freezer until analysis.

3-3 CUTTING OF SECTIONS

The samples, still wrapped in tin foil, were placed in the cryostat (LIPSHAW CRYOTOME Model 1500) for at least 2 hours before cutting in order to equilibrate to -20°C. Each sample was then mounted on a disk of cork (approximately 15mm diameter and 3mm thick) using a few drops of mountant (Tissue-Tek 11 O.C.T compound) so that the muscle fibres ran perpendicular to the cork and therefore the cutting blade. The sample was then mounted, via the cork, onto a cryostat chuck, which had been allowed to chill in the cryostat, using a few drops of Tissue-Tek 11 O.C.T compound. The chuck was then placed on the cryostat stage for 10-15 minutes to allow the mountant to set.

Transverse sections, 10μm thick, were cut and mounted on clean glass slides (5-6 sections/ slide).

3-4 STAINING OF SECTIONS

The unstained mounted sections were left in a refrigerator for at least 30 minutes prior to staining to avoid separation of the sections from the slides during staining. The sections were stained for myosin ATPase activity at a pH of 7.2 (Appendix 3).

3-5 CLASSIFICATION OF FIBRE TYPES

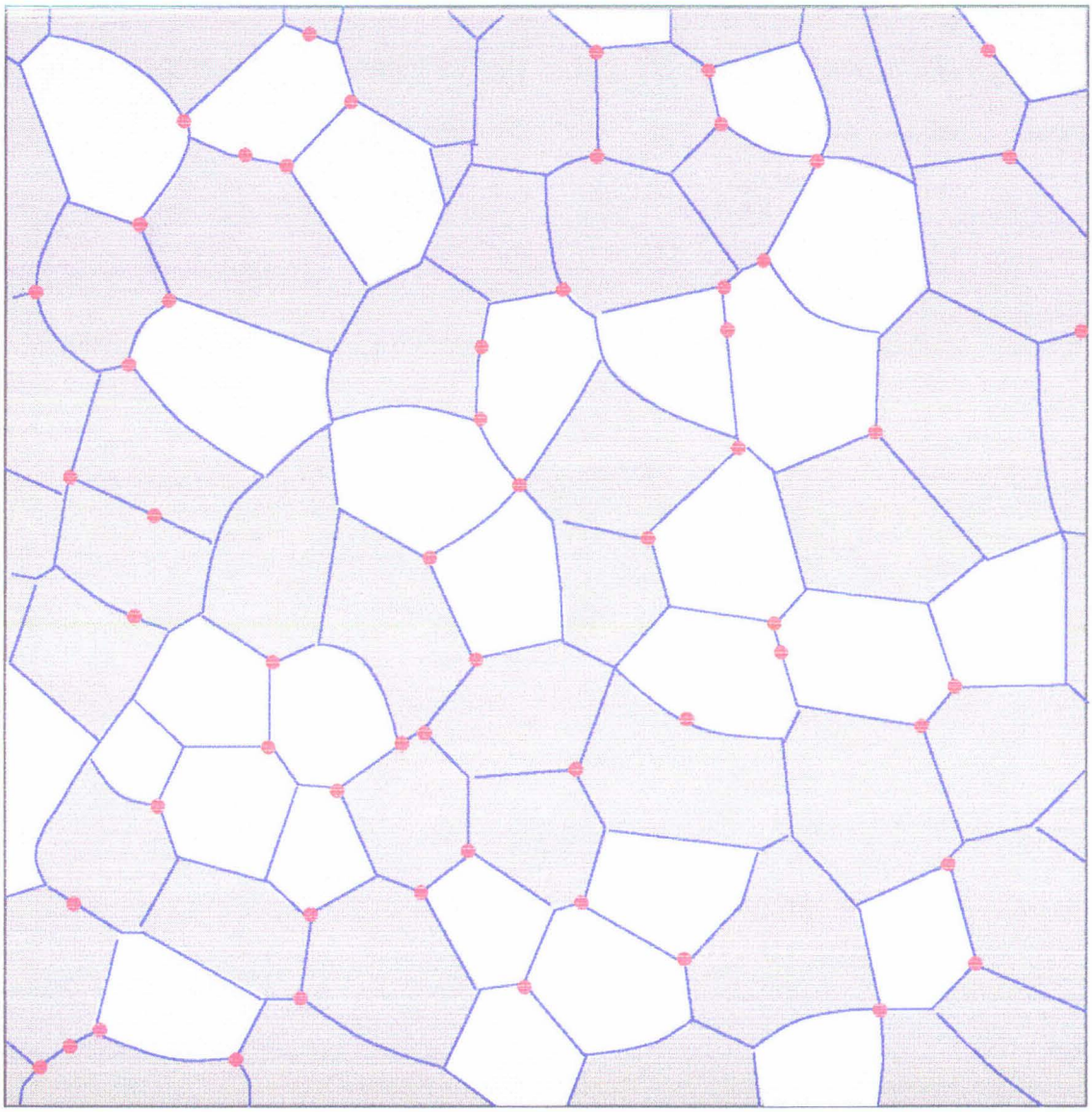
On the basis of myosin ATPase activity, fibres were classified as ATPase low or ATPase high on the basis of staining intensity.

3-6 MEASUREMENT OF CAPILLARY SUPPLY

The sections were examined at 315 X magnification using a projection microscope (Ernst Leitz GMBH Wetzlar). The section was projected onto a rectangle (150 x 150mm) which was considered as 1 field. For each sample, 3 fields were examined, in 2 of these each fibre was traced and identifed by type and the capillaries surrounding it drawn in (Fig 3-1) while in the third field the fibres were identified by type but the fibres were not traced and the capillaries were not drawn in. All fibres completely inside the field, as well as those which were half or more than half way inside were counted. The total number of ATPase low and ATPase high fibres was then counted from the 3 fields and the percentage of ATPase low fibres/total number of fibres was obtained.

From each of the two fields which were traced, the parameters, number of capillaries/fibre, number of capillaries per mm^2 and mean fibre size were calculated.

Fig. 3-1: An example of the type of illustration used to calculate the parameters of capillary / fibre ratio, capillary density and number of capillaries surrounding each fibre.



ATPase high fibre

ATPase low fibre

capillary

Parameters were calculated as:

$$\text{Capillary/ fibre ratio} = \frac{\text{Total number of capillaries}}{\text{total number of fibres}}$$

$$\text{Capillary density (number of capillaries / mm}^2\text{)} = \frac{\text{Total number of capillaries}}{\text{total area (mm}^2\text{)}}$$

3-7 SELECTION OF MUSCLES FOR DETAILED ANALYSIS

The percentage of ATPase low fibres was plotted against capillary/fibre ratio and those muscles which were considered outliers on the graph were examined in greater detail. These muscles were choosen in order to maximise the variation in results while having less material to analyse. A total of 22 muscles were selected (four from Horse 1, five from Horse 2, seven from Horse 3 and six from Horse 4). The traced diagrams of these selected muscles were examined using a digitizer (Genitizer GT-1212B) in conjunction with the Sigma-Scan program to determine the area of each fibre and the number of capillaries surrounding it. The following 5 parameters were calculated:

1. Average number of capillaries surrounding each fibre
2. Average number of capillaries surrounding each ATPase low fibre
3. Average number of capillaries surrounding each ATPase high fibre
4. Average size of ATPase low fibres
5. Average size of ATPase high fibres

3-8 STATISTICAL ANALYSIS

As it was thought that the variables of percentage ATPase low fibres and capillary/fibre ratio may be interdependant, regression analysis was performed on these variables using the Minitab statistical package (Ryan et al, 1986). The significance of the resulting regression coefficient was determined by the Minitab program at the levels of 5% and 1%.

Regression analysis was also used to determine the relationship between the number of capillaries surrounding each fibre and fibre area for the two fibre types separately for the

22 selected muscles.

In order to determine if the number of capillaries surrounding the ATPase low fibres was significantly different from the number surrounding the ATPase high fibres, a two-sample t test (Larsen and Marx, 1986) was performed on the mean values of these variables for each of the 22 selected muscles. This test was also performed on the overall means of the 22 muscles combined. The significance of any differences found between the means was determined using the student t distribution at both the 5% and 1% significance levels. The difference in mean fibre area between the two fibre types was also analysed using the two-sample t test ($p < 0.05$ and $p < 0.001$).

The relationships between mean fibre area and capillary density and between mean fibre area and capillary/fibre ratio for the 22 selected muscles were determined by regression analysis using the Minitab program. The regression coefficients obtained were tested for significance at both the 5% and 1% level.

CHAPTER FOUR : RESULTS

The percentage of ATPase low fibres, the capillary/fibre ratio and capillary density of each of the muscles studied is given for Horses 1, 2, 3 and 4 in Tables 4-1, 4-2, 4-3 and 4-4 respectively. A total of 100 muscles were examined.

4-1 IDENTIFICATION OF CAPILLARIES

In general, the myosin ATPase staining technique was found to be a satisfactory method for the identification of capillaries. In some cases, residue from the staining could be confused with the capillaries but with correct washing following the cobalt chloride stage of the staining, this problem was resolved. Figure 4-1 A, B and C illustrates the staining quality achieved through the use of this method in muscles which have low (cutaneous), medium (tensor fasciae latae) and high (anconeus) proportions of ATPase low fibres respectively.

4-2 SELECTED MUSCLES

Table 4-5 is a summary of the percentage ATPase low fibres, the capillary/fibre ratios and capillary densities of the 22 selected muscles from the four horses.

4-3 FIBRE TYPE DISTRIBUTION

The muscles sampled from the four horses provided a complete range of fibre type distributions from 0 to 100% ATPase low fibres (Horse 1, range 0 to 64.4% ; Horse 2, range 0 to 100% ; Horse 3, range 0 to 100% ; Horse 4, range 0 to 93.2%). In all horses, the

cutaneous muscle contained ATPase low fibres at neither the abdominal, shoulder nor thoracic sampling sites. In both Horse 3 and Horse 4, the gluteus profundus muscle was completely, or nearly so, composed of ATPase low fibres (100% and 93.2% respectively).

4-4 MEAN FIBRE AREA

Mean fibre areas ranged from 135μm^2 to 9842μm^2 for ATPase low fibres and from 107μm^2 to 9326μm^2 for ATPase high fibres.

Table 4-6 shows the mean fibre areas of the ATPase low and ATPase high fibres from the selected muscles. The significance of any difference between the areas of the two types is also given. Overall there was no significant difference in mean fibre area between the two fibre types ($p > 0.05$).

4-5 CAPILLARY/FIBRE RATIO

The capillary/fibre ratios of all muscles from all horses ranged from 0.67 to 3.43 (mean = 1.81). There was no significant regression between mean fibre size and capillary/fibre ratio for the 22 selected muscles (regression coefficient = 0.109, $p > 0.05$).

4-6 CAPILLARY DENSITY

Capillary density for all four horses and all muscles sampled ranged from 180 capillaries/mm^2 to 1543 capillaries/mm^2 (mean = 553 capillaries/mm^2). Capillary density and mean fibre area showed a significantly negative regression (Fig. 4-2, regression coefficient = -0.49, $p < 0.05$) for the 22 selected muscles.

4-7 NUMBER OF CAPILLARIES SURROUNDING EACH FIBRE

The mean number of capillaries surrounding each fibre is given for the two fibre types from the selected muscles in Table 4-7. Any significant differences between the two fibre types with respect to the number of capillaries surrounding each fibre are also shown. Overall the number of capillaries surrounding the ATPase low fibres and the number surrounding the ATPase high fibres were not significantly different ($p > 0.05$).

4-8 RELATIONSHIP BETWEEN %ATPase LOW FIBRES AND CAPILLARY/FIBRE RATIO

The percentage of ATPase low fibres and capillary/fibre ratio were significantly regressed (Fig 4-3) when all muscles from all the horses were considered (regression coefficient = 0.36, $p < 0.05$).

4-9 RELATIONSHIP BETWEEN NUMBER OF CAPILLARIES SURROUNDING EACH FIBRE AND FIBRE AREA

Table 4-8 shows the regression coefficients for the relationship between the number of capillaries surrounding each fibre of each type, and fibre area for the selected muscles. Generally, the regressions were significant. Overall the number of capillaries surrounding each ATPase low fibre and fibre area showed a significant regression (regression coefficient = 0.59, $p < 0.001$). The regression between the number of fibres surrounding each ATPase high fibre and fibre area was also significant (regression coefficient = 0.43, $p < 0.05$).

Table 4-1 : % ATPase low fibres, capillary/fibre ratios (C/F) and capillary densities (CD) of the muscles from Horse 1.

MUSCLE	%ATPase LOW FIBRES	C/F	CD (MM^{-2})
TENSOR Fasciae Latae	48.5	2.43	647
SERRATUS VENTRALIS	39.5	1.97	670
LONGISSIMUS THORACIS	29.9	1.93	514
EXTERNAL INTERCOSTAL	62.9	1.56	450
DIAPHRAGM	64.4	1.88	583
PSOAS MAJOR	25.2	1.63	555
PECTINEUS	21.9	2.10	579
SEMIMEMBRANOSUS	20.1	2.40	1097
SUPERFICIAL SEMITENDINOSUS	55.8	3.04	1185
MEDIAL GASTROCNEMIUS	25.4	1.93	1097
RECTUS FEMORIS	42.3	2.13	675
BICEPS FEMORIS	24.7	1.65	611
SUBSCAPULARIS	24.6	1.48	721
BICEPS FEMORIS	48.0	1.79	455
CUTANEOUS (SHOULDER)	0.0	1.43	831
INFRASPINATUS	36.7	2.14	725
TENSOR FASCIAE ANTEBRACHII	34.4	1.81	771
TRICEPS (LONG HEAD)	25.1	2.34	1387
TRICEPS (MEDIAL HEAD)	46.0	2.05	1543
TRICEPS (LATERAL HEAD)	41.9	1.60	799

Table 4-2 : %ATPase low fibres, capillary/fibre ratios (C/F) and capillary densities (CD) for the muscles from Horse 2.

MUSCLE	% ATPase LOW FIBRES	C/F	CD (MM^{-2})
PECTINEUS	42.6	2.06	413
DIAPHRAGM	38.0	2.80	849
TENSOR FASCIAE LATAE	26.5	2.04	432
VASTUS MEDIALIS	11.7	2.69	629
RECTUS FEMORIS	8.6	2.34	658
SEMIMEMBRANOSUS	16.3	2.71	707
SUPERFICIAL SEMITENDINOSUS	32.6	1.76	533
DEEP SEMITENDINOSUS	100	2.89	600
MEDIAL GASTROCNEMIUS	33.2	1.56	560
POPLITEUS	12.4	1.83	445
LONGISSIMUS THORACIS	22.6	2.17	537
EXTERNAL INTERCOSTAL	71.9	3.33	473
CUTANEOUS (SHOULDER)	0.0	1.94	1165
BRACHIOCEPHALICUS	37.3	1.96	1056
INFRASPINATUS	41.7	1.97	638
SUPRASPINATUS	21.3	1.41	354
BICEPS BRACHII	46.4	2.30	441
SUPERFICIAL PECTORAL	18.5	1.20	181
DEEP PECTORAL	40.4	1.99	400
TENSOR FASCIAE ANTEBRACHII	38.5	3.34	1152
TRICEPS (LONG HEAD)	20.5	2.57	769
TRICEPS (MEDIAL HEAD)	35.9	3.07	592
CORACOBRACHIALIS	22.4	2.62	537

Table 4-3 : % ATPase low fibres, capillary/fibre ratios (C/F) and capillary densities (CD) for the muscles from Horse 3.

MUSCLE	%ATPase LOW FIBRES	C/F	CD (MM^{-2})
BRACHIOCEPHALICUS	30.0	0.77	375
CUTANEOUS (SHOULDER)	0.0	1.17	404
EXTERNAL ABDOMINAL OBLIQUE	44.7	1.26	617
INTERNAL ABDOMINAL OBLIQUE	67.7	1.60	486
TRANSVERSE ABDOMINIS	37.4	1.24	565
RECTUS ABDOMINIS	41.1	1.01	313
SUPERFICIAL SEMITENDINOSUS	3.7	1.14	323
DEEP SEMITENDINOSUS	40.2	1.57	488
SEMIMEMBRANOSUS	11.7	0.97	373
SUPERFICIAL GLUTEAL	34.4	1.45	392
TENSOR FASCIAE LATAE	41.4	0.76	240
BICEPS FEMORIS	15.8	1.30	463
VASTUS LATERALIS	17.7	1.32	515
RECTUS FEMORIS	18.3	1.21	338
LATERAL GASTROCNEMIUS	16.6	1.99	352
GLUTEUS MEDIUS	9.1	0.81	404
GLUTEUS PROFUNDUS	100	2.32	523
ILIACUS	66.9	1.51	523
PECTINEUS	29.7	1.65	483
ADDUCTOR	13.8	1.47	517
OMOTRANSVERSARIUS	30.6	1.15	448
CUTANEOUS (THORAX)	0.0	0.67	250
DEEP PECTORAL	24.6	1.66	581
SUPRASPINATUS	11.3	0.99	450
TENSOR FASCIAE ANTEBRACHII	18.6	1.36	511
TRICEPS (LONG HEAD)	14.2	0.97	484
ANCONEUS	100	2.07	438
BRACILIS	59.9	1.55	550
LONGISSIMUS	31.2	1.29	550

Table 4-4 : % ATPase low fibres, capillary/fibre ratios (C/F) and capillary densities (CD) for the muscles from Horse 4.

MUSCLE	%ATPase LOW FIBRES	C/F	CD (MM^{-2})
LEVATOR OF UPPER LIP	39.7	1.55	427
BRACHIOCEPHALICUS	19.8	1.50	409
TRICEPS (LONG HEAD)	21.1	1.79	454
DELTOIDEUS	34.4	1.81	417
EXTENSOR CARPI RADIALIS	14.8	1.48	433
SUPERFICIAL PECTORAL	38.9	1.62	423
SERRATUS VENTRALIS	49.1	1.85	521
CUTANEOUS (ABDOMEN)	0.0	1.21	306
TRAPEZIUS THORACIS	30.7	1.71	454
EXTERNAL OBLIQUE	45.1	1.99	446
RECTUS ABDOMINIS	39.8	2.17	429
EXTERNAL INTERCOSTAL	29.9	1.89	381
GRACILIS	27.0	2.67	509
PECTINEUS	43.3	1.72	415
ADDUCTOR	23.7	2.33	429
DEEP SEMIMEMBRANOSUS	14.8	2.26	450
SUPERFICIAL SEMIMEMBRANOSUS	12.8	1.95	448
DEEP SEMITENDINOSUS	44.2	1.71	458
SUPERFICIAL SEMITENDINOSUS	12.6	1.25	381
DEEP GLUTEAL	93.3	2.57	557
LATERAL GASTROCNEMIUS	14.9	2.05	421
SUPERFICIAL FLEXOR	36.6	2.58	609
POPLITEUS	12.4	1.92	340
BICEPS FEMORIS	21.5	1.65	452
VASTUS LATERALIS	6.6	1.58	384
RECTUS FEMORIS	35.3	1.91	604
LONG EXTENSOR	22.3	1.30	317
SHORT EXTENSOR	78.2	1.99	481

Table 4-5 : % ATPase low fibres, capillary/fibre ratios (C/F) and capillary densities (CD) of 22 muscles from 4 horses.

HORSE	MUSCLE	% ATPase LOW FIBRES	C/F	CD (MM^{-2})
1	EXTERNAL INTERCOSTAL	62.8	1.56	450
1	DIAPHRAGM	64.4	1.88	583
1	SEMITENDINOSUS (SUPERFICIAL)	55.8	3.04	1185
1	CUTANEOUS (SHOULDER)	0.0	1.43	868
2	SEMITENDINOSUS (DEEP)	100	2.89	600
2	EXTERNAL INTERCOSTAL	71.9	3.32	486
2	CUTANEOUS (SHOULDER)	0.0	1.94	1165
2	PECTORAL (SUPERFICIAL)	18.5	1.20	181
2	TENSOR FASCIAE ANTEBRACHII	38.5	3.43	1152
3	CUTANEOUS (ABDOMINAL)	0.0	1.16	404
3	INTERNAL ABDOMINAL OBLIQUE	67.7	1.59	486
3	TENSOR FASCIAE LATAE	41.4	0.75	240
3	GLUTEUS PROFUNDUS	100	2.32	523
3	ILIACUS	66.9	1.50	523
3	CUTANEOUS (THORAX)	0.0	0.67	250
3	ANCONEUS	100	2.07	438
4	CUTANEOUS (ABDOMEN)	0.0	1.20	306
4	GRACILIS	27.0	2.66	509
4	SEMITENDINOSUS (SUPERFICIAL)	12.6	1.25	381
4	GLUTEUS PROFUNDUS	93.3	2.56	557
4	SUPERFICIAL FLEXOR	36.6	2.57	609
4	SHORT EXTENSOR	78.2	1.98	481
	Overall Mean	47.1	1.95	562
	Standard Deviation	35.2	0.78	280

Table 4-6 : Mean fibre areas for ATPase low (AL) and ATPase high (AH) fibres of 22 muscles from 4 horses.

HORSE	MUSCLE	MEAN FIBRE AREA (μm^2) AL	MEAN FIBRE AREA (μm^2) AH	SIGNIFICANCE
1	EXTERNAL INTERCOSTAL	3756	3477	NS
1	DIAPHRAGM	2615	3734	S^{**}
1	SEMITENDINOSUS (SUPERFICIAL)	2753	4065	S^{**}
1	CUTANEOUS (SHOULDER)	none	1699	---
2	SEMITENDINOSUS (DEEP)	4738	none	---
2	EXTERNAL INTERCOSTAL	5921	5031	NS
2	CUTANEOUS (SHOULDER)	none	1543	---
2	PECTORAL (SUPERFICIAL)	3639	5153	S^{*}
2	TENSOR FASCIAE ANTEBRACHII	2320	2189	NS
3	CUTANEOUS (ABDOMEN)	none	2992	---
3	INTERNAL ABDOMINAL OBLIQUE	3732	1810	S^{**}
3	TENSOR FASCIAE LATAE	3287	3967	S^{*}
3	GLUTEUS PROFUNDUS	4542	none	---
3	ILIACUS	2906	3403	NS
3	CUTANEOUS (THORAX)	none	2796	---
3	ANCONEUS	5048	none	---
4	CUTANEOUS (ABDOMEN)	none	3804	---
4	GRACILIS	3681	6568	S^{**}
4	SEMITENDINOSUS (SUPERFICIAL)	939	3602	S^{**}
4	GLUTEUS PROFUNDUS	4456	2362	S^{**}
4	SUPERFICIAL FLEXOR	3923	4199	NS
4	SHORT EXTENSOR	4028	3784	NS
	Overall Mean	3664	3483	NS
	Standard Deviation	1129	1245	

NS = Not significant
S^{*} = Significant ($p < 0.05$)
S^{**} = Significant ($p < 0.001$)

Table 4-7 : Mean number of capillaries surrounding each ATPase low (AL) and ATPase high (AH) fibre in 22 muscles from 4 horses.

HORSE	MUSCLE	MEAN NUMBER OF CAPILLARIES AROUND EACH AL FIBRE	MEAN NUMBER OF CAPILLARIES AROUND EACH AH FIBRE	SIGNIFICANCE
1	EXTERNAL INTERCOSTAL	4.3	4.58	NS
1	DIAPHRAGM	4.32	5.1	NS
1	SEMITENDINOSUS (SUPERFICIAL)	6.57	6.32	NS
1	CUTANEOUS (SHOULDER)	¶	3.69	----
2	SEMITENDINOSUS (DEEP)	6.34	¶	----
2	EXTERNAL INTERCOSTAL	6.88	4.79	S^{**}
2	CUTANEOUS (SHOULDER)	¶	3.88	----
2	PECTORAL (SUPERFICIAL)	1.78	1.51	NS
2	TENSOR FASCIAE ANTEBRACHII	6.05	6.71	NS
3	CUTANEOUS (ABDOMINAL)	¶	3.06	----
3	INTERNAL ABDOMINAL OBLIQUE	4.26	3.00	S^{*}
3	TENSOR FASCIAE LATAE	2.55	2.31	NS
3	GLUTEUS PROFUNDUS	5.86	¶	----
3	ILIACUS	4.51	4.0	NS
3	CUTANEOUS (THORAX)	¶	2.24	----
3	ANCONEUS	5.03	¶	----
4	CUTANEOUS (ABDOMEN)	¶	3.12	----
4	GRACILIS	4.85	7.69	S^{**}
4	SEMITENDINOSUS (SUPERFICIAL)	2.25	3.19	NS
4	GLUTEUS PROFUNDUS	5.37	3.6	S^{*}
4	SUPERFICIAL FLEXOR	5.04	6.13	NS
4	SHORT EXTENSOR	4.32	3.96	NS
	Overall Mean	4.72	4.15	NS
	Standard Deviation	1.42	1.6	

NS = Not significant
S^{*} = Significant ($p < 0.05$)
S^{**} = Significant ($p < 0.001$)
¶ = no fibres of this type present

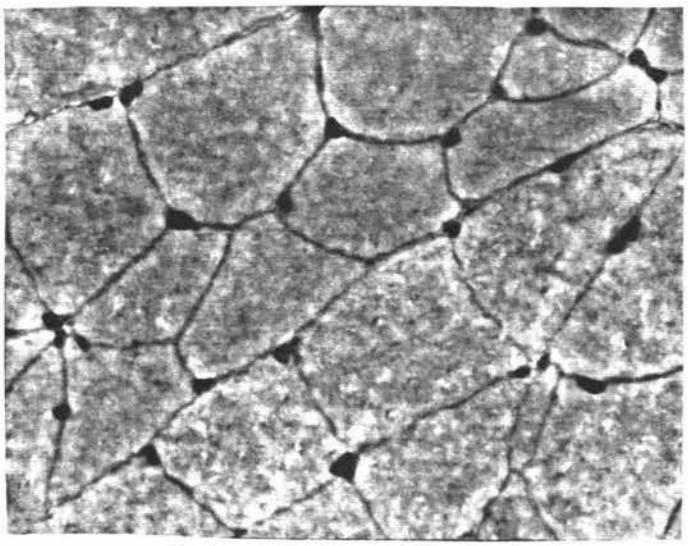
Table 4-8 : Regression coefficients for the relationship between the number of capillaries surrounding each fibre (CAF) and fibre area (FA), for ATPase high (AH) and ATPase low (AL) fibres. N = number of fibres measured

HORSE	MUSCLE	N (AL)	N (AH)	REGRESSION BETWEEN FA AND CAF (AL)	REGRESSION BETWEEN FA AND CAF (AH)
1	EXTERNAL INTERCOSTAL	30	24	0.29	0.76**
1	DIAPHRAGM	37	30	0.68**	0.54**
1	SEMITENDINOSUS (SUPERFICIAL)	47	31	0.62**	0.62**
1	CUTANEOUS (SHOULDER)	none	125	----	0.52**
2	SEMITENDINOSUS (DEEP)	53	none	0.74**	----
2	EXTERNAL INTERCOSTAL	64	24	0.75**	0.64**
2	CUTANEOUS (SHOULDER)	none	42	----	0.51**
2	PECTORAL (SUPERFICIAL)	9	39	0.00	0.30*
2	TENSOR FASCIAE ANTEBRACHII	37	76	0.60**	0.70**
3	CUTANEOUS (ABDOMEN)	none	53	----	0.45**
3	INTERNAL ABDOMINAL OBLIQUE	23	11	0.57*	0.61*
3	TENSOR FASCIAE LATAE	27	36	0.23	0.19
3	GLUTEUS PROFUNDUS	36	none	0.58*	----
3	ILIACUS	42	26	0.52**	0.53**
3	CUTANEOUS (THORAX)	none	50	----	0.17
3	ANCONEUS	40	none	0.24	----
4	CUTANEOUS (ABDOMEN)	none	134	----	0.56**
4	GRACILIS	13	28	0.62*	0.40*
4	SEMITENDINOSUS (SUPERFICIAL)	8	42	0.57	0.41**
4	GLUTEUS PROFUNDUS	102	10	0.64**	0.69*
4	SUPERFICIAL FLEXOR	24	32	0.75**	0.51**
4	SHORT EXTENSOR	92	28	0.38**	0.39*
	Overall correlation	984	841	0.60**	0.43*

* = Significant ($p < 0.05$)

** = Significant ($p < 0.001$)

A.



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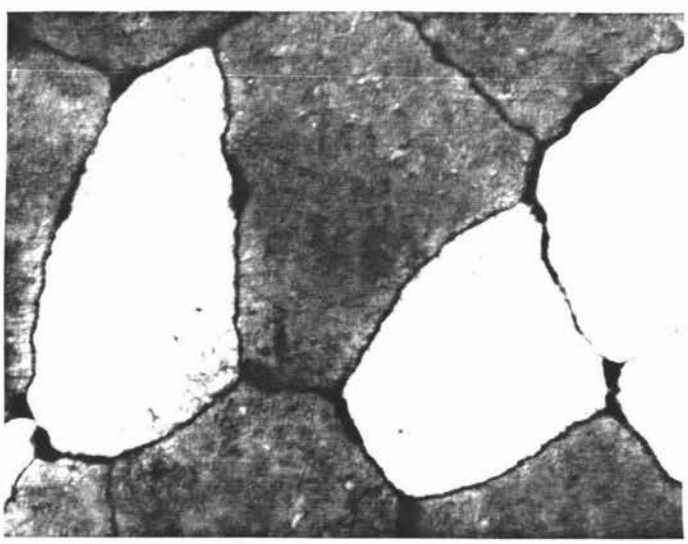

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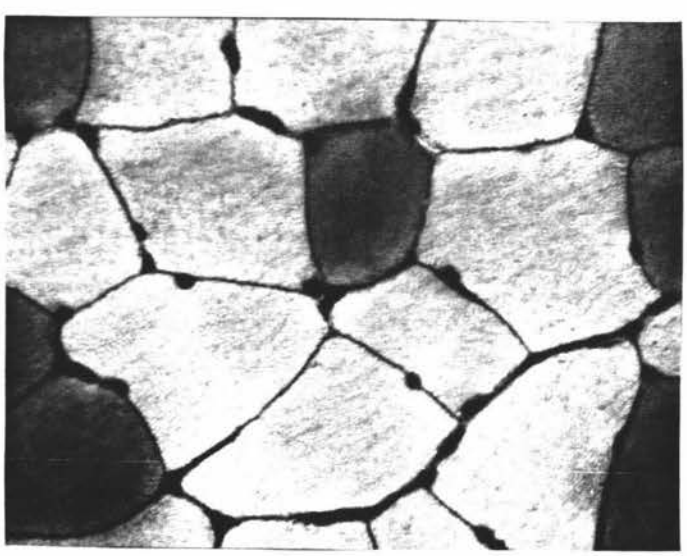

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Fig. 4-1 : Sections of horse muscle stained for myosin ATPase activity at pH 7.2.

A: Shoulder cutaneous: Fibres show a uniformally high level of activity (426X Magnification).

B: Tensor fasciae latae: Approximately equal numbers of fibres are high and low reacting (486X Magnification).

C: Anconeus: Most of the fibres have a low level of myosin ATPase activity (426X Magnification).

Fig. 4-2 : Graph of the relationship between capillary density (capillaries/mm^2) and mean fibre area for the 22 selected muscles

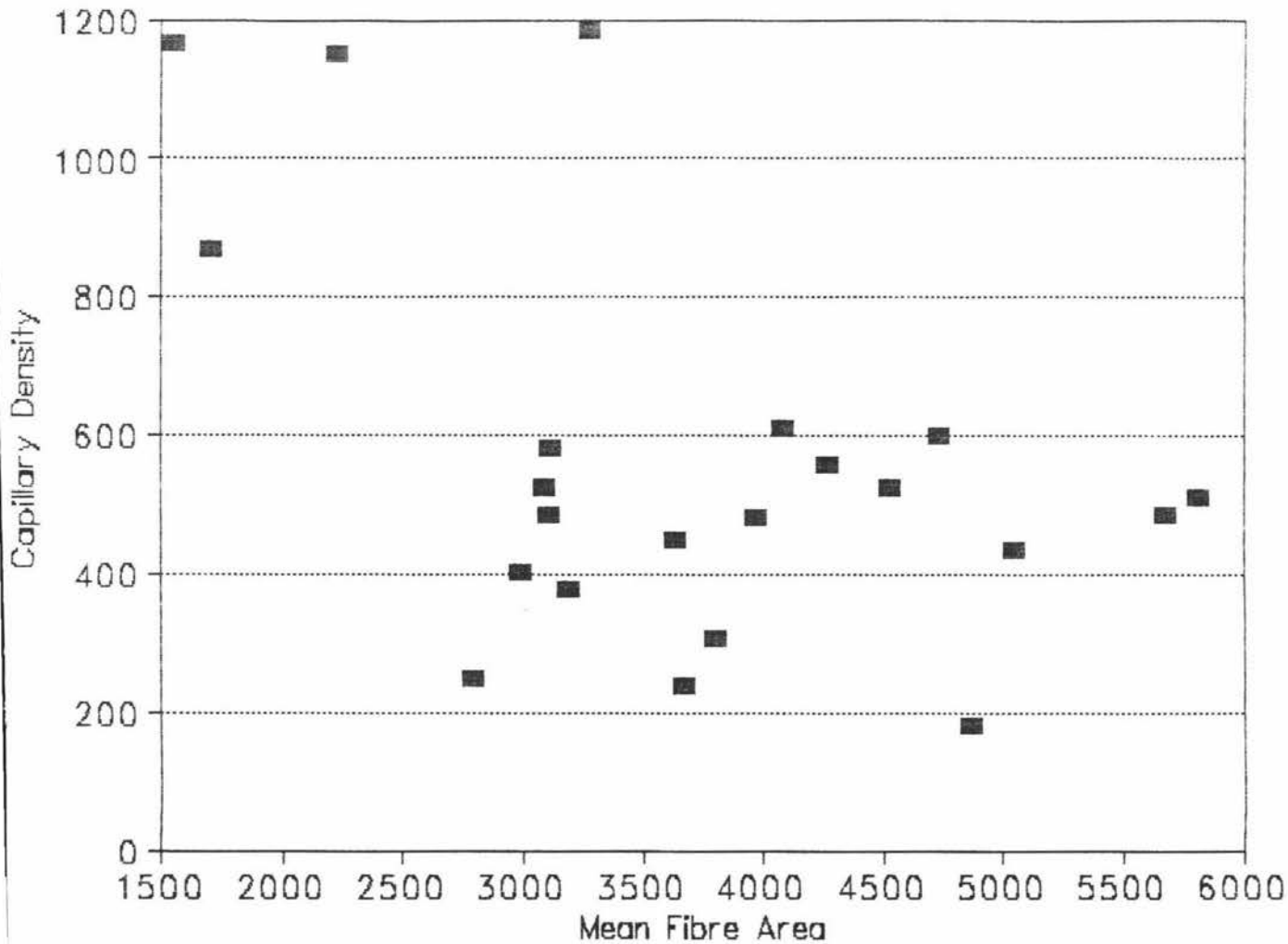
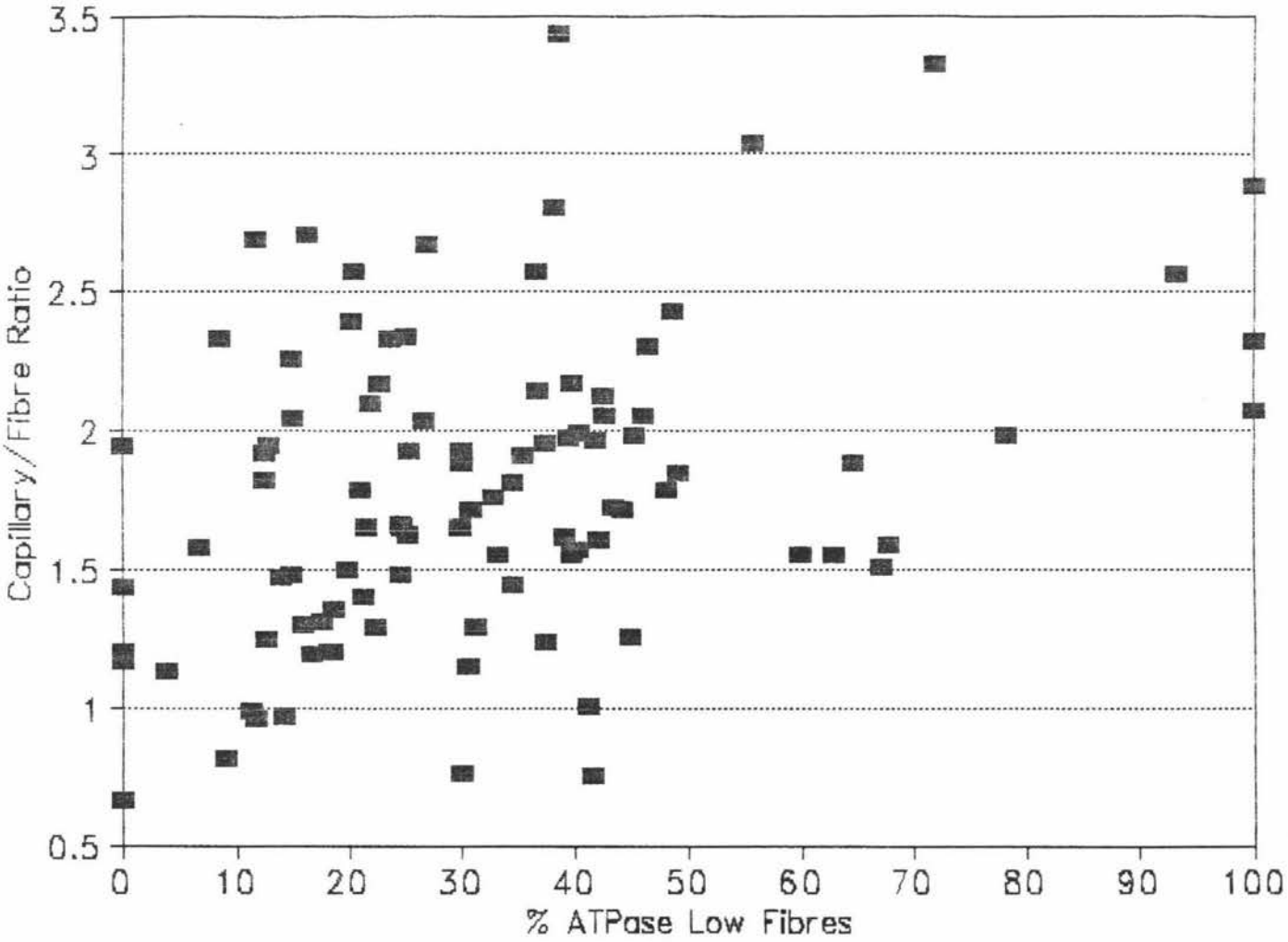


Fig. 4-3 : Graph of the relationship between the percentage of ATPase low fibres and capillary/fibre ratio for 100 muscles from 4 horses



CHAPTER FIVE : DISCUSSION

5-1 METHODS FOR THE IDENTIFICATION OF CAPILLARIES

In the late 1950's, the preferred method of visualising capillaries was by vascular perfusion of Indian ink (Smith and Giovacchini, 1956 ; Carrow et al, 1967). The problem with this method was that while some areas of the muscles were well injected, other areas were devoid of ink and therefore total counts of capillaries were not accurate. Only those capillaries that were open and filled with ink could be counted.

A more accurate method for the positive identification of capillaries was developed in the 1970's and involved the staining of a mucopolysaccharide in the capillary basement membrane (Hermansen and Wachtlova, 1971 ; Parizkova et al, 1971 ; Anderson, 1975). This involved the modified use of the periodic-acid-Schiff (PAS) reaction and allowed capillaries to be visualised irrelevant of their state (i.e. open or closed). However, this method has been criticised as a source of variability in quantitative data as visualisation of capillaries is difficult (Brodal et al, 1977).

Capillary endothelium has been shown to contain a high activity of alkaline phosphatase (Gomori, 1939 ; cited in Myrhage, 1978) which can be histochemically demonstrated using the myofibrillar adenosine triphosphatase (ATPase) reaction (Maxwell et al, 1980). Capillary endothelial phosphatase can be detected whether or not the capillary lumen is open and therefore is considered an accurate method for the visualisation of capillaries.

It has been suggested that the alkaline phosphatase method leaves the venular portion

of the capillary unstained (Mrazkova et al, 1986). If the number of venular portions in any cross-section were constant from one section to another, the results when two sections are compared would not be affected.

A method which reputedly identifies both the arterial and venular portions of a capillary was demonstrated by Degens et al (1992). They used a combined stain of alkaline phosphatase and dipeptidyl peptidase IV (DPP-IV) which does not differentiate fibre types. Therefore serial sections must be prepared in order to calculate the number of capillaries surrounding a fibre of a particular type. Because the method used in the present study demonstrates both capillaries and fibre types, the capillaries surrounding each fibre can be seen from one section. The use of the myosin ATPase stain at pH 7.2 was simple and produced consistent results.

5-2 FIBRE TYPE DISTRIBUTION

Muscle fibres can be separated histochemically on the basis of contractility by staining for myofibrillar ATPase (Stein and Padykula, 1962) into two groups. Through the use of this method, the fibres in this study were classified as ATPase low (Type I) and ATPase high (Type II). In all cases, the fibre types were easily separated because no intermediate staining was evident.

The 100 muscle samples obtained from the four horses in this study provided a wide range of fibre type profiles. The percentage of ATPase low fibres ranged from 0 to 100% and therefore the extremes in muscle type were obtained. No other studies in the horse have involved such a complete range of muscle types and no other studies in other species have involved muscles which contain 0% or 100% ATPase low fibres. Generally fibre type profiles in the horse have been limited to the gluteus medius muscle and therefore

comparisons between the results from this study and others are restricted. The proportion of ATPase low fibres in the gluteus medius muscle of Thoroughbreds has been reported as 15.5% (Guy and Snow, 1977), 13% (Valberg, 1987) and 12.5% (Snow and Guy, 1980). In Standardbreds, the percentage of ATPase low fibres in this muscle has been reported as 13% (Essen-Gustavsson et al, 1983) which is similar to Thoroughbreds and 23% which is slightly higher (Valberg, 1987). Other reported values for Standardbreds are 25% (Essen-Gustavsson and Lindholm, 1985) and 18% (Roneus et al, 1992). Horse 3 in the present study had 9.09% ATPase low fibres in the gluteus medius muscle which is slightly lower than the reported values.

In the semitendinosus muscle, reported values for the percentage ATPase low fibres have been 14% (Guy and Snow, 1977) and 15% (Essen-Gustavsson et al, 1983) while Snow and Guy (1976) reported that the semitendinosus muscle of Thoroughbreds contains 10.9% slow twitch fibres. Horse 4 in this study showed a similar fibre composition to those reported with 12.55% ATPase low fibres in the superficial semitendinosus muscle. The equivalent muscle in Horses 2 and 3 showed values of 32.58% and 3.73% ATPase low fibres respectively which are not consistent with reported values.

The fibre composition of other equine skeletal muscles has been investigated by Snow and Guy (1980) and by Guy and Snow (1977). The results from the present study are in agreement with those of Guy and Snow (1977). They reported that the deltoideus, triceps (long head), vastus lateralis and biceps femoris muscles were composed of 35.3%, 20.4%, 11.5% and 19.6% slow twitch fibres respectively. The corresponding values for the present study were 34.41% (Horse 4), 20.45% (Horse 2), 17.6% (Horse 3) and 21.5% (Horse 4). Snow and Guy (1980) reported proportions of 31.7%, 8.2% and 18.4% slow twitch fibres for the deltoideus, vastus lateralis and biceps femoris muscles respectively which are also in

agreement with the present study.

The differences in fibre composition which exist between muscles is probably related to the specific function of the muscle (Snow and Guy, 1980) and it has been reported that the type of athletic activity an individual is suited to is related to the fibre type profiles of certain muscles (Costill et al, 1976). This theory is supported by the present study. Muscles involved in propulsion in the horse such as the gluteus medius and semitendinosus were found to have a low proportion of ATPase low fibres (9.09% and 12.55% respectively). Those muscles involved in postural functions, however, such as the anconeus and iliacus muscles had a much higher proportion of ATPase low fibres (100% and 66.92% respectively).

Muscles such as the diaphragm and the external intercostal muscles which are involved in slow, constant breathing activity also have high proportions of ATPase low fibres (71.91% and 64.41% respectively).

Fibre type composition in the horse has been shown to differ in different parts of the same muscle with deeper sections of the muscle containing higher proportions of ATPase low fibres (van den Hoven, 1985 ; Hermansen and Hurley, 1990). Samples were taken from superficial and deep parts of the semitendinosus muscle in three out of the four horses in this study and in all cases the percentage of ATPase low fibres was higher in the deep part of the muscle (Horse 2 deep = 100%, superficial = 32.58% ; Horse 3 deep = 40.16%, superficial = 11.65% and Horse 4 deep = 44.16%, superficial = 12.55%). Another example of increasing proportion of ATPase low fibres with increasing depth occurred in the gluteus muscle group; in Horse 3, the gluteus medius muscle had only 9.09% ATPase low fibres while the gluteus profundus muscle was composed completely of ATPase low fibres.

These findings are in contrast to those of Snow and Guy (1980) who reported that in only three out of the 36 muscles they examined in the horse was there a significant difference

in the proportion of high myosin ATPase fibres between the deep and superficial regions of muscle. However, significant differences between the superficial and deep parts of the gluteus medius muscle were found in three out of six horses. They did not examine differences in fibre composition between the gluteus medius and gluteus profundus muscles.

Due to the possibility that differences in sampling depth can effect the fibre composition of the sample, no comparisons could be made between the same muscle from different horses as sampling depth was not constant and could have provided large variation.

Another reason comparisons could not be made between horses is due to reported differences in fibre composition between breeds of horse. These differences have been related to the sprinting speed of the breed (Snow and Guy, 1980). It has been shown that breeds such as the Quarterhorse which can sprint rapidly for short distances have a significantly greater proportion of ATPase high fibres than Thoroughbreds or heavy hunters. Differences may also have occurred due to sex, nutrition and season although there is no information concerning the effects of these factors on fibre type composition.

Galsiteo et al (1992) reported that Arabian horses have a significantly higher proportion of Type I and IIA fibres than Andalusians. Standardbreds have a significantly greater proportion of Type I fibres and significantly lower percentage of Type IIB fibres than Thoroughbreds (Valberg, 1987).

In this study, two of the four horses were Standardbreds, one an Apoloosa and the other a pony of mixed breeding. Due to the large variety in type of these horses and also the different purposes for which they were bred, no conclusions about any differences between the same muscle in different horses have been made.

The fitness level of horses has also been shown to affect the fibre type profiles of particular muscles but mostly seems to affect the proportions of the subgroups of the Type

II fibres. Generally, active or trained horses have a higher proportion of Type IIA fibres and a lower proportion of Type IIB fibres than inactive horses (Guy and Snow, 1977 ; Essen-Gustavsson and Lindholm, 1985 ; Lopez-Rivero et al, 1991 ; Roneus et al, 1992).

Thus the fibre type profile of a muscle is primarily determined by that muscle's particular function, although slight differences in profiles may occur between horses due to differences in breed and state of fitness.

5-3 MEAN FIBRE AREA

The mean areas of the fibres measured in this study are mostly consistent with those in the literature, although, due to shrinkage during histological preparation, absolute comparisons between studies are difficult unless some estimate of shrinkage is provided. Mean fibre area for the ATPase low fibres from the 22 selected muscles ranged from 939μm^2 to 4738μm^2 while areas for the ATPase high fibres ranged from 1543μm^2 to 6568μm^2. Fibres areas for Type I fibres in equine skeletal muscle have been reported as 3145μm^2 for Andalusians and 3194μm^2 for Arabs (Lopez-Rivero et al, 1992), 3285μm^2 (Lopez-Rivero et al, 1990c), 1840μm^2 (Lindholm et al, 1983) and 2577μm^2 for inactive Standardbreds (Essen-Gustavsson and Lindholm, 1985). Type II fibres have been reported as having values for IIB fibres of 5935μm^2 (Essen-Gustavsson and Lindholm, 1985), 4450μm^2 (Lindholm et al, 1983) and 4804μm^2 (Lopez-Rivero et al, 1990d).

Significant differences in area between Type I and Type II fibres have been reported. Type I fibres have been found to have the smallest area (Lopez-Rivero et al, 1990c ; Lopez-Rivero et al, 1990d ; Andrews and Spurgeon, 1986). Type IIA fibres have intermediate areas and Type IIB the largest areas. It has been suggested that the smaller area of the more oxidative Type I fibres means a smaller depth for the diffusion of oxygen and energy

substrates to the fibre centre (Lopez-Rivero et al, 1990d). Fourteen muscles in the present study were examined for significant differences in fibre area between the ATPase low and ATPase high fibres. In most cases where there was a significant difference, the ATPase high fibres were larger than the ATPase low fibres. Overall, however, there was no significant difference in size between the two fibre types.

Differences in fibre area between the horses could not be compared due to reported differences between breeds of horse and also differences due to age. Fibres have been shown to tbe smaller in Arabian horses than in Standardbreds (Lopez-Rivero et al, 1990d) while the areas of Type IIA and IIB fibres were found to be significantly larger in Andalusians than in Thoroughbreds (Lopez-Rivero et al, 1990b).

An increase in age from one to seven months has been related to an increase in the relative area occupied by Type I, Type IIA and Type IIB fibres in Andalusian foals (Galsiteo, 1992). Essen-Gustavsson et al (1983) also reported an increase in fibre area with an increase in age from 1.5 to 2.5 years in Standardbreds. The ages of the horses in this study ranged from nine months to eight years and therefore any differences in fibre size between horses may have been as the result of their age differences. Differences may also have occurred due to the different breeds.

5-4 CAPILLARY SUPPLY

Capillaries provide a means for the delivery of oxygen and energy substrates to cells and also for the removal of carbon dioxide and waste products. The degree to which a muscle fibre is supplied by capillaries gives some indication of the function of that particular fibre. Those fibres which are involved in oxidative metabolism might be expected to be surrounded by a larger number of capillaries than those that are non oxidative in order to

obtain the amount of oxygen required to sustain their needs.

Early in this century, Krogh (1919; cited in Carrow et al, 1967) found that red muscle is more vascular than white muscle and since then further studies have confirmed this (Smith and Giovacchini, 1956 ; Gray and Renkin, 1978 ; Reis and Wooten, 1970). Myrhage (1978) also related muscle type to capillary supply and reported that the number of capillaries in skeletal muscle has a positive correlation first to the oxidative capacity of different muscle fibres and secondly to the dimension of those fibres. Enad et al (1989) also positively related oxidative capacity to muscle type, although Maxwell et al (1980) concluded that they found no support for relations between oxidative capacity and capillarity of whole muscle or individual fibres. The present study involved a wide range of muscle types including the pale cutaneous muscle and the deep red gluteus profundus and anconeus muscles. Any relationship between the percentage of ATPase fibres and capillary supply should be detected through the study of these extreme muscles.

The various methods used for the analysis of capillary supply have made the comparison of results from different studies difficult. Tissue shrinkage during histological procedures causes a problem when the parameter of capillary density is used to estimate capillary supply (Plyley and Groom, 1975). While tissue shrinkage does not affect the capillary/fibre ratio, this parameter does not provide any information about diffusion conditions and therefore mean fibre area needs to be taken into account when capillary/fibre ratios are considered. Capillary/fibre ratio is also thought to depend on the number of fibres which share each capillary (Plyley and Groom, 1975). The number of capillaries surrounding each fibre is also not affected by tissue shrinkage although it does not take into account the size of different fibre types.

All three parameters were used to estimate capillary supply in this study. The degree

of tissue shrinkage was not estimated and therefore the capillary density values may be overestimated. The other two parameters, however, were not affected by tissue shrinkage. The number of capillaries surrounding each fibre was only calculated for the 22 selected muscles and was calculated individually for the two fibre types. By treating the fibre types separately, the size of the different types could be taken into account.

Capillary supply has been shown to be affected by sampling depth (Lopez-Rivero et al, 1993). They found that both capillary/fibre ratio and capillary density increased, by 53% and 57% respectively, between the most superficial and the deepest sampling site of the gluteus medius muscle of horses. Because sampling depth was not consistent between the muscles in the present study, comparisons of the same muscle between different horses were not made.

5-4-1 CAPILLARY/FIBRE RATIO

The assessment of capillary/fibre ratios has mostly been performed on humans and small species such as rabbits and rats with very few studies involving horses. There have been no extensive studies comparing the capillary/fibre ratios of a wide range of muscles from individual horses or any other species. The variety of muscles sampled in this study enabled a comparison of extreme variation in the capillary supply within different muscles in the same horse as well as between different parts of the same muscle.

Plyley and Groom (1975) indicated that the capillary/fibre ratios of muscles should be between one and three. They reported that values of less than one could only occur with incomplete counting of capillaries due to technical problems and that a capillary/fibre ratio of more than three meant that there were more than six capillaries surrounding each fibre which was not observed in their study. Capillary/fibre ratios of less than one only occurred

in Horse 1 in this study and only exceeded three in Horse 2. Contrary to Plyley and Groom's observations, the number of capillaries surrounding a fibre did exceed six in some cases and therefore it may be possible to get a capillary/fibre ratio of greater than three. In most cases, however, capillary/fibre ratios in this study were within the range of one to three.

Generally, capillary/fibre ratio estimations have not accounted for different fibre types. Gray and Renkin (1978) first proposed the idea of calculating capillary/fibre ratios separately for individual fibre types. For each of the rabbit muscles they studied, fibres were classifed according to type and each capillary identified in one field. A table was set up with a column for each fibre type. Each capillary was then studied to see how it was apportioned among the fibre populations. If a capillary was adjacent to four fibres of which one was ATPase low and the other three were ATPase high, the proportion of 0.25 would be entered in the ATPase low column and 0.75 in the ATPase high column. Once all capillaries were apportioned, the columns were added and the sum divided by the number of fibres of that type present in the field to give the capillary/fibre ratio for that fibre type.

This idea was extended by Degens et al (1992) who introduced capillary domains which estimate capillary density and heterogeneity of capillary spacing (see review of literature pg 33). For each fibre, the local capillary fibre ratio was determined. This was defined as the sum of the fractions of each domain area overlapping the fibre. They suggested that this parameter allowed an estimate of the capillary supply to a fibre which may lack direct contact with a capillary and therefore depend on remote capillaries. However, they did not allow for the probability that remote capillaries are separated from a fibre by connective tissue which may have different diffusional capacities than a muscle fibre. The area to which oxygen could diffuse from such a capillary may therefore be different from one which is directly adjacent to a fibre.

A special algorithm required for the determination of these parameters was not available for the present study and therefore these parameters were not calculated. It would be interesting to further the studies by Degens et al, which were on rats, on horse muscle.

Due to the range of methods used to visualise capillaries, it is only valid to compare results from studies which have used the myosin ATPase or PAS staining techniques with the results from the present study. Henckel (1983) visualised capillaries by the use of the PAS stain in the middle gluteal muscle of eight Thoroughbred horses. Capillary/fibre ratios in this muscle were found to be 0.97 for a group of 6 month old foals, 1.86 for two to three year olds and 2.05 for horses above the age of ten. The only gluteus medius sample taken in this study was from horse 3 (9 months old) which showed a capillary/fibre ratio of 0.81 which is consistent with that found by Henckel.

Because the number of capillaries/fibre increases with increasing body size (Schmidt-Nielsen and Pennycuik, 1961), it can be expected that the values obtained in this study will be larger than those reported for smaller mammals. Thus the mean capillary/fibre ratio of the quadriceps femoris muscle in humans which has been found to be 1.67 (Andersen, 1975), 1.49 (Hermansen and Wachtlova, 1971) and 1.77 (Brodal et al, 1977) is lower than that found for horses in this study (2.33).

Plyley and Groom (1975) studied various muscles in the dog which, like the horse, can be considered to be an athletic type of animal. They found the capillary/fibre ratio of the gastrocnemius muscle to be 1.45 which is consistent with horse 2 in this study (1.56) but lower than that for horse 4 (2.05). The cat was also examined by Plyley and Groom (1975) and had a capillary/fibre ratio in the gastrocnemius muscle of 1.62 which is comparable to the dogs in the study and horse 2 in the present study. Conley et al (1987) found that capillary supply in skeletal muscle is consistantly higher in more athletic animals.

Cattle, which could be considered to be of a similar body weight to a pony or small horse, were investigated by Schmidt-Nielsen and Pennycuik (1961). They were found to have a capillary/fibre ratio of 1.0 in the gastrocnemius . This value is lower than those obtained for the horses in this study but this may be due to the fact that Schmidt-Nielsen and Pennycuik used the perfusion of indian ink technique and therefore their capillary counts may have been lower than the actual amount present. In general, over a range of species, capillary/fibre ratios seem to lie between one and three with values rarely lying beyond these limits. Schmidt-Nielsen and Pennycuik (1961) found that over a range of species from the bat to cattle, the capillary/fibre ratio only ranged from 1.6 to 2.5 even though there was a significant increase in ratio with increasing body size. Values from other studies also lie within these limits for all species (Gray and Renkin, 1978 ; Maxwell et al, 1980 ; Carrow et al, 1967). It was concluded by Plyley and Groom (1975) that one capillary is the minimum amount required to meet the needs of a fibre while, regardless of body size, the maximum number of capillaries a muscle fibre requires to maintain itself is three. This does not mean that the number of capillaries around each fibre will not exceed three due to the fact that each capillary may be shared by two or more fibres.

5-4-1-1 Relationship Between %ATPase Low Fibres and Capillary/Fibre Ratio

Because red muscles such as the gluteus profundus are more oxidative than white muscle such as the cutaneous muscle, it can be expected that red muscles should have a higher capillary/fibre ratio in order to deliver the larger amount of oxygen they require. The capillary/fibre ratios in this study ranged from 0.67 to 3.43 and therefore it is concluded that a large range of muscle types were included. The ratio of 3.43 was found in the tensor

fasciae antebrachii muscle and exceeds the maximum number of capillaries considered to be necessary to supply a fibre. The study of Plyley and Groom (1975) which concluded this, however, was not on horses and it may be possible that some muscles in the horse have a higher capillary/fibre ratio than 3 due to their large body size. The most non-oxidative muscle involved in this study was the cutaneous muscle which showed capillary/fibre ratios of 1.43, 1.94. 0.67 and 1.21 in horses 1, 2, 3 and 4 respectively. In three out of four of these cases (exception horse 2), this was the lowest capillary/fibre ratio of all the muscles sampled in that particular horse. The most oxidative muscles from these horses included the gluteus profundus, anconeus and deep semitendinosus muscles. These had higher capillary/fibre ratios of 2.57, 2.07 and 2.89 respectively.

The relationship between oxidative capacity and capillarity has been studied in a few species. In some cases a positive correlation has been found (Myrhage, 1978 ; Enad et al, 1989) while Maxwell et al (1980) concluded that their study did not support the hypothesis that capillarity correlates with oxidative capacity.

The parameter % ATPase low fibres is a measure of contractility which has been shown to be unrelated to oxidative capacity in horse muscle (Lopez-Rivero et al, 1990a). Other studies have also concluded that the classification of muscle fibres according to oxidative capacity does not necessarily correlate to classification on the basis of myosin ATPase activity in horses (Essen et al, 1980) and humans (Sjogaard et al, 1978). Because the present study classified fibres on the basis of ATPase activity, it could only investigate the relationship between contractility and capillary/fibre ratio and no conclusions about oxidative capacity could be drawn.

When all muscles from all horses were considered together the relationship between these two parameters was found to be significant (Regression coefficient = 0.36, $p < 0.05$),

a finding in agreement with Reis and Wooten (1970) who showed a direct and linear relationship between capillarity and contraction time in the skeletal muscle of cats. They suggested that the range of values for contractility results from differences in the relative proportion of red and white fibres. The slower twitch times and greater capillarity of red muscles such as the gluteus profundus muscle (Buller et al, 1960) is better suited to its postural function while the lower capillarity but faster contraction times of white muscle (e.g. cutaneous muscle) is more suited to short sudden bursts of activity. Cutaneous muscle supports posturally no greater weight than that of the skin and therefore does not have any need for ATPase low fibres. Faster contracting muscles have been found to depend more on their own stores of glycogen than on the circulation for energy (Beatty et al, 1963) and therefore require fewer capillaries per fibre.

From the results of the present study it can be concluded that that slower contracting muscles have a higher capillary/fibre ratio. Gunn (1975) concluded that, in the diaphragm and pectoralis transversus muscle of the horse, it was not possible to distinguish between fibre types on the basis of the reaction for the oxidative enzyme succinate dehydrogenase because all fibres tended to have a high activity for this enzyme. If all fibres in the horse have a high oxidative capacity, studies into the relationship between oxidative capacity and capillarity are less relevant in the horse than those involving relationships between contractility and capillary supply.

5-4-2 CAPILLARY DENSITY

The parameter of capillary density (number of capillaries/mm^2) is beset with the most problems of measurement of the three parameters investigated in this study. This is mainly because it is susceptible to the tissue shrinkage that occurs during histological procedures.

The amount of shrinkage involved when using perfusion with microfil and staining by a modified Gomori technique has been said to be 56% (Goldspink, 1961). The amount of shrinkage which occurred in the present study was not determined and it was therefore not possible to adjust capillary density values to account for shrinkage. It is assumed that shrinkage is similar for different muscles in this study, and that capillary density comparisons are valid.

Capillary density also varies with fibre type and is reported to be higher in red areas of muscle than in white areas (Schmidt-Nielsen and Pennycuik, 1961 ; Lee, 1958). In contrast, there was no relationship between the percentage of ATPase low fibres and capillary density in the present study.

Due to the fact that tissue shrinkage was not measured and that each of the muscles sampled had varying proportions of fibre types and fibre sizes, capillary density was considered the least accurate method of assessing capillary supply in this study.

5-4-3 NUMBER OF CAPILLARIES SURROUNDING EACH FIBRE

The parameter of the number of capillaries surrounding each fibre (CAF) has been less commonly used than capillary/fibre ratio and capillary density. It is, however, a useful method of measuring capillary supply as it is not affected by tissue shrinkage and can be measured separately for different fibre types. Plyley and Groom (1975) commented that the CAF is remarkably similar for a range of species. They found that over a range of 5 species (cat, dog, guinea pig, rat and rabbit) and 6 muscles, the mean number of capillaries surrounding each fibre only varied from 3.21 to 4.01 with a mean of 3.53 (SE = 0.07). It was also interesting that the muscles used in their study came from widely varying anatomical sites (hindlimb, jaw and tongue).

In the skeletal muscle of humans, CAF has been estimated as 4.4 for untrained and 5.9 for trained subjects (Brodal et al, 1977) while in the cat it has been shown to vary from 1.5 for fast twitch glycolytic fibres in the biceps femoris to 5.0 in the slow twitch fibres of the soleus muscles (Myrhage, 1978).

In this study, the mean number of capillaries surrounding each fibre was calculated only for the 22 selected muscles and was treated individually for the two fibre types. It was found to range from 1.5 to 7.69 which is a larger range than has been found in other species although the individual values are in agreement with those in the literature. The greatest number of capillaries surrounding any particular fibre was 13 which is larger than the 9 observed by Plyley and Groom (1975).

CAF is similar to capillary/fibre ratio in that it does not take into account the size of individual fibres. Plyley and Groom (1975) stated that the diameter of the fibre is the only factor determining the number of capillaries in muscle tissue. They did not, however, actually measure any fibres in their study. Myrhage (1978) observed that the number of capillaries around each fibre increased with increasing fibre diameter. An increase in local capillary fibre ratio with hypertrophy was also observed by Degens et al (1992). They stated that this indicated that capillary supply to a fibre is influenced by its cross-sectional area. Egginton and Ross (1989, cited in Degens et al, 1992) reported that 'capillarization is primarily determined by fibre size, although the basic relationship may be modulated by the absolute level of oxidative metabolism'. Fibre size was found to signifcantly affect the number of capillaries surrounding each fibre for both fibre types in the present study with the larger fibres requiring more adjacent capillaries. The regression coefficients for the two fibre types were 0.59 ($p < 0.001$) and 0.43 ($p < 0.05$) for the ATPase low and ATPase high fibres respectively.

The number of capillaries surrounding each fibre has been shown to differ between fibre types. Within muscles, Myrhage (1978) found that, in cats, fast twitch glycolytic fibres have a smaller number of adjacent capillaries than fast twitch oxidative glycolytic fibres. They observed, however that in the soleus muscle, the slow twitch oxidative fibres had a similar number of capillaries around each fibre as the fast twitch oxidative glycolytic fibres. They concluded that muscles with a highly oxidative metabolism, such as the soleus require more adjacent capillaries than those that were not so oxidative. Andersen (1975) also observed that the number of capillaries around the non oxidative Type IIB fibres was less than the number around the more oxidative Type I and Type IIA fibres. In the horse, since it has been suggested that it is not possible to distinguish between fibre types on the basis of oxidative capacity (Gunn, 1975), studies on the number of capillaries surrounding a fibre of a particular metabolic type are less relevant. The horse is therefore not a useful model for this purpose.

This study is the first to investigate the number of capillaries which surround each fibre of a particular type in equine muscle. It demonstrates that overall, within particular regions of a muscle, there are no significant differences between the number of capillaries surrounding the ATPase low fibres and the ATPase high fibres. However, muscles with a higher percentage of ATPase low fibres have a higher capillary/fibre ratio. The capillarisation of a fibre, therefore, appears to depend on the usage (postural or propulsive) of the muscle in which it is found, rather than to depend on its own contractile properties.

If a capillary has a limited area to which it can supply oxygen, then each fibre should be surrounded by enough capillaries to provide a supply of oxygen to the whole fibre i.e. the areas supplied by each of the capillaries must at least touch or overlap in order to avoid anoxic regions in the fibre. It follows therefore that larger fibres will require a greater

number of adjacent capillaries. Although fibre size is the primary determinant for capillary supply, the contractile properties of the muscle, or region of a muscle, in which a fibre is found will also determine the number of capillaries required to supply it. Due to the extremes of muscle types they possess, the horse is a particularly useful animal for the study of muscle characteristics in athletic animals. The results shown in the present study lead the way to a more detailed and diverse study of the effect of training on skeletal muscle.

CHAPTER SIX : CONCLUSIONS

The following conclusions may be drawn with regard to capillary supply and fibre type proportions of equine skeletal muscles.

1. The myosin ATPase staining technique enables the visualisation of capillaries together with the differentiation of fibre types on the basis of contractility.

2. The skeletal muscles of the horse provide a wide variety of muscle types for the study of differences in capillarity and contractile speed in different muscles. The horse is therefore a useful animal for the study of the effect of contraction speed on other muscle parameters.

3. The percentage of ATPase low fibres in equine skeletal muscle ranges from 0% (cutaneous muscle) to 100% (gluteus profundus muscle). This is the largest range of muscle type proportions found in any animal to date.

4. There is no significant difference in mean fibre size between the two fibre types in horse muscle, whereas previous work in the horse has shown ATPase high fibres to be larger than ATPase low fibres.

5. There was no significant correlation between mean fibre size and capillary/fibre ratio for the 22 selected muscles.

6. Muscles with a higher mean fibre area have a lower capillary density.

7. Overall, the number of capillaries surrounding each ATPase low fibre was not significantly different from the number surrounding the ATPase high fibres. Within muscles of the horse, the oxidative capacity of both fibre types may therefore be similar.

8. Between muscles of the horse, muscles with a low contraction speed, estimated by the proportion of ATPase low fibres, had a higher capillary/fibre ratio.

9. For both high and low reacting ATPase fibres, the larger fibres had a larger number of capillaries in contact.

10. From the results of the present study, it seems that the horse has a unique advantage over other athletic animals studied, in that its complete range of muscle fibre type proportions could be used to show the particular effects of training on extreme types of muscle.

APPENDIX ONE

DESCRIPTION OF HORSES

Horse 1 - Exmoor type pony

- Mare
- 5 years old
- 7-9 months pregnant
- killed for pet food

Horse 2 - Standardbred

- Stallion
- 8 years old

Horse 3 - Standardbred

- Filly
- 9 months old
- Stifle joint problem on near hind

Horse 4 - Apoloosa

- Gelding
- 2 years old
- killed for pet food

APPENDIX TWO

MUSCLES SAMPLED FROM EACH HORSE

MUSCLE	1	2	3	4	MUSCLE	1	2	3	4
ADDUCTOR			*	*	PECTINEUS	*	*	*	*
ANCONEUS			*		PECTORALIS PROFUNDUS			*	
BICEPS BRACHII	*	*			PECTORALIS SUPERFICIALIS		*		*
BICEPS FEMORIS	*		*	*	POPLITEUS		*		*
BRACILIS			*		PSOAS MAJOR	*			
BRACHIOCEPHALICUS		*	*	*	RECTUS ABDOMINIS			*	*
CORACOBRACHIALIS		*			RECTUS FEMORIS	*	*		*
CUTANEOUS (ABDOMEN)			*	*	SEMIMEMBRANOSUS (DEEP)	*	*	*	*
CUTANEOUS (SHOULDER)	*	*			SEMIMEMBRANOSUS (SUP)				*
CUTANEOUS (THORAX)			*		SEMITENDINOSUS (DEEP)		*	*	*
DELTOIDEUS				*	SEMITENDINOSUS (SUP)	*	*	*	*
DIAPHRAGM	*	*			SERRATUS VENTRALIS	*			*
EXTENSOR CARPI RADIALIS				*	SHORT EXTENSOR				*
EXTERNAL ABDOMINAL OBLIQUE			*		SUBSCAPULARIS	*			
EXTERNAL INTERCOSTAL	*	*		*	SUPERFICIAL FLEXOR				*
EXTERNAL OBLIQUE				*	SUPRASPINATUS		*	*	
GASTROCNEMIUS	*	*	*	*	TENSOR FASCIAE ANTEBRACHII	*	*	*	
GRACILIS				*	TENSOR Fasciae Latae	*	*	*	
GLUTEUS MEDIUS			*		TRANSVERSE ABDOMINIS			*	
GLUTEUS PROFUNDUS			*	*	TRAPEZIUS THORACIS				*
GLUTEUS SUPERFICIALIS			*		TRICEPS (LATERAL HEAD)	*			
ILIACUS			*		TRICEPS (LONG HEAD)	*	*	*	*
INFRASPINATUS	*	*			TRICEPS (MEDIAL HEAD)	*			
INTERNAL ABDOMINAL OBLIQUE			*		VASTUS LATERALIS				*
LEVATOR OF UPPER LIP				*	VASTUS MEDIALIS		*		
LONG EXTENSOR				*					
LONGISSIMUS	*	*	*						
OMOTRANSVERSARIUS			*						

1 = Horse 1, 2 = Horse 2, 3 = Horse 3 and 4 = Horse 4 * = muscle sampled

APPENDIX THREE

Myosin ATPase Staining Technique

Substrate:

1.0 M Tris-(hydroxymethyl)-aminomethane	8 ml
0.18 M Calcium Chloride ($CaCl_2.6H_2O$)	4 ml
Adenosine Triphosphate (disodium dihydrogen salt)	60 mg
Distilled water	up to 30 ml

Adjust pH to 7.2 using 2N HCl (approximately 2 ml). Add distilled water to make up a final volume of 40 ml.

Method:

1. Cut fresh frozen sections at 10-15 um thickness
2. Mount on slides (leave in refrigerator for at least 30 min)
3. Fix in 4% Formaldehyde buffered with cacodylate (see below) pH 6.8 for two minutes exactly
4. Wash in two changes of distilled water
5. Incubate for 20 minutes in the above substrate
6. Wash in distilled water
7. Treat with 2% Cobalt Chloride solution for 2-3 minutes
8. Dip in distilled water 2-3 times
9. Develop in dilute (1%) yellow Ammonium Sulphide solution for 30 seconds
10. Wash, mount in aqueous mountant

Cacodylate buffered formaldehyde:

2.14 g Sodium cacodylate $.3H_2O$ in 50 ml distilled water

50 ml of above solution + 6.3 ml 0.2 M HCl

+ 20 ml 40% W/V formaldehyde

Make up to 200 ml with distilled water. Final pH 6.8

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