A STUDY OF CANINE AND EQUINE SKELETAL MUSCLE

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ABSTRACT OF THESIS

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Title of Thesis A Study of Canine and Equine Skeletal Muscle.

(1) This study compares Greyhounds and Thoroughbreds - breeds selected for high speed running - with other breeds of their species by gross dissection, histometric and histochemical and biochemical methods, to identify adaptations which would favour their superior athletic capacity. Skeletal muscle has been the primary tissue of interest because of its power-generating nature.

(2) Carcass dissection was carried out on 44 Greyhounds from birthweight to 37 kg, 31 other dogs from birthweight to 47 kg, 30 Thoroughbreds from 0·69 kg to 509 kg and 33 other horses from 2·2 to 547 kg liveweight.

(2a) Measurements on the humerus, radius and ulna, femur and tibia and fibula indicated that their combined lengths were not different in Greyhounds and other dogs, but tended to be longer in adult Thoroughbreds than in adult other horses.

(2b) Within limb variations in bone lengths were not apparent between breeds. However the epipodial segment in dogs and the propodial segment in horses grows faster.

(2c) There is no difference in fresh bone density between the types of dog and horse, but dog bones tend to be more dense than horse bones.

(2d) The proportions of muscle, bone and fat relative to liveweight were compared between athletes and others in adults and during growth. In adults the most functionally significant difference is that muscle occupies a greater proportion of liveweight in athletes. Adult Greyhounds have less fat than other dogs while bone weight forms a remarkably similar proportion of liveweight in all adult dogs and horses. In athletes there is a greater growth rate of muscle which explains the difference in adult proportions. Growth changes in muscle distribution explain the greater propulsive capacity of the Greyhound spinal column and femoral region and of the Thoroughbred hindlimb. It is also compatible with the potentially higher stride frequency of the Greyhound hindlimb.

(2e) Athletes tend to have heavier hearts than non-athletes at adult liveweights, despite the lower growth rate of the heart in athletes.

Use other side if necessary.

(3) In all 33 Greyhounds from birth to 37 kg, 26 other dogs from birth to 47 kg, 34 Thoroughbreds from 11 kg to 598 kg and 34 other horses from 2·3 to 560 kg liveweight were used for histometric and biochemical assay, of samples of their m. semitendinosus, m. diaphragma and m. pectoralis transversus. Mean fibre areas were established in samples of all three muscles, and in m. semitendinosus only the transverse sectional area and total number of fibres in it were also estimated. Histochemical profiles of individual fibres were estimated using myosin adenosine triphosphate (myosin ATPase), succinate dehydrogenase (SDHase), and glycogen phosphorylase (GPase) reactions; capillaries were also demonstrated using a modification of the myosin ATPase reaction.

(3a) Athletes have more larger fibres in m. semitendinosus than non-athletes. The mean fibre area of m. diaphragma is also larger in Greyhounds and Thoroughbreds than in their fellows but the mean fibre area of m. pectoralis transversus is similar in the two types of animal within each species. Although the mean fibre area of corresponding muscles is significantly larger in horses than in dogs the difference is not related to their liveweight difference.

(3b) The major histochemical difference between fibres is their myosin ATPase activity, which differentiates them according to whether they have a high or low activity. In adult dog muscle, all fibres have a high SDHase activity and myosin ATPase low-reacting fibres have a low activity of GPase. In adult horse muscle all fibres have a high activity of GPase. In m. diaphragma and m. pectoralis transversus all fibres also have a high SDHase activity so that only the myosin ATPase reaction differentiates fibres in these muscles, however fibres with a low activity of SDHase are present in samples of m. semitendinosus.

(3c) The myosin ATPase reaction differentiates fibres at the earliest stage of growth observed. The GPase and SDHase activities gradually develop from an amorphous staining pattern in the young to the appropriate adult type. The proportional area of myosin ATPase low-reacting fibres in the three muscles studied is related to liveweight from birth to near adulthood. Thereafter the relationship is less obvious in "athletes" than "non-athletes."

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(3e) It is concluded that the proportions of fibre types in muscles are related to the function of muscles and its parts. Although the proportions of fibre types in different muscles and parts of muscles and in different types of animals resemble those of adults at the earliest stages investigated, histochemical evidence has been obtained which suggests transformation of the physiological properties of fibres as a normal occurrence but to differing extents during growth of normal athletes and non-athletes.

(3f) Capillary density is remarkably similar between muscles of all groups of animals at all except very early stages of growth.

(4) The biochemical estimation of SDHase activity does not show a within species difference in the adult but indicates an increase in activity in both species during growth. It has also been found that there is a greater aerobic activity in m. diaphragma than in the other two muscles and a greater activity in the deep medial than in the superficial lateral region of m. semitendinosus.

(5) M. longissimus is proportionally lighter in Greyhounds taken out of training than in others. Such specimens have a greater myosin ATPase high-reacting fibre area in their m. diaphragma and lesser capillary density in their m. pectoralis transversus than trained Greyhounds.

Use other side if necessary.

(6) The crosses of Thoroughbreds with other horses, show anatomical properties more like Thoroughbreds than non-athletic horses.

(7) The results are discussed in relation to stride length and frequency. It is suggested that in adult athletes enhanced stride length is favoured by longer limbs in horses, and a greater acceleration capacity in both species. A higher natural frequency of the Greyhound hindlimb, and a greater intrinsic speed of sarcomere contraction in the athletes of both species favour enhanced stride frequency. The combination of these endowments aids a greater maximum speed of running in both Greyhounds and Thoroughbreds when compared with their fellows.

Do mho Mhathair agus Alan R. Muir

a thug an mhead dhuim.

With whitening hedges, and uncrumpling fern,
and blue-bells trembling by the forest-ways,
and scent of hay new-mown.
..... we still had Thyrsis then.

Matthew Arnold (1822 - 1888).

It is meet not to imagine or think out, but to find out what nature does or produces.

Francis Bacon (1561 - 1626).

Truth is rarely pure and never simple.

Oscar Wilde (1856 - 1900).

I hereby declare that this thesis has been composed by myself and comprises my own work.

H. Michael Gunn.

During the period in which this study was carried out the following related communications were made conjointly with A.S. Davies:

DAVIES, A.S. & Gunn, H.M. (1971). A comparative histochemical study of the mammalian diaphragm and m. semitendinosus. Journal of Anatomy, 110, 137 - 139P.

DAVIES, A.S. & Gunn, H.M. (1972). Histochemical fibre types in the mammalian diaphragm. Journal of Anatomy, 112, 41 - 60.

GUNN, H.M. & Davies, A.S. (1971). Histochemical characteristics of muscle fibres in the diaphragm. Biochemical Journal, 125, 108 - 109P.

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SUMMARY

(1) This study compares Greyhounds and Thoroughbreds - breeds selected for high speed running - with other breeds of their species by gross dissection, histometric and histochemical and biochemical methods, to identify adaptations which would favour their superior athletic capacity. Skeletal muscle has been the primary tissue of interest because of its power-generating nature.

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(7) The results are discussed in relation to stride length and frequency. It is suggested that in adult athletes enhanced stride length is favoured by longer limbs in horses, and a greater acceleration capacity in both species. A higher natural frequency of the Greyhound hindlimb, and a greater intrinsic speed of sarcomere contraction in the athletes of both species favour enhanced stride frequency. The combination of these endowments aids a greater maximum speed of running in both Greyhounds and Thoroughbreds when compared with their fellows.

1.0 GENERAL INTRODUCTION

The ability of some human beings and animals to propel themselves or objects higher, further, or faster than their fellows has been a source of power, pride and enjoyment to man throughout the ages. The inquiring mind may ask how is it that the athletic performance of one individual regularly excels that of similar members of the species?

Numerous factors influence athletic performance. Many of these can be assessed in the living being, such as the effect of exercise on cardiac, respiratory and circulatory function which have been reviewed by Åstrand (1956). However the morphological and biochemical properties of an athlete's propulsive machine - his musculoskeletal system have not been extensively studied in relation to athletic ability. Therefore it was decided to study muscle in relation to physical performance in two species, strains of which have been selected by man for athletic ability over a long period.

The Thoroughbred horse has been selected for swiftness for approximately 300 years (Willett, 1970), and the Greyhound dog for appropximately 3,000 years (Clarke, 1965). Unlike man, who has not been selected for athletic ability, these animals typify extremes of their species and are therefore more suitable subjects for an investigation into athletic characteristics.

The relationship of different body proportions to physical performance capacity of animals is discussed by Hayes (1904) and Howell (1965). However, although gross morphological attributes are doubtlessly an important

consideration, the intrinsic characteristics of skeletal muscle - the source of locomotory power - must be considered. It is expected that exponents of swiftness might have a greater rate of production of, and a greater capacity for the rapid utilization of energy than would their counterparts. Thus studies comparing Greyhounds and Thoroughbreds with less athletic breeds of their species might reveal an advantageous rate of synthesis or utilization of the major energy mediator in muscle, adenosine triphosphate.

Adenosine triphosphate (ATP) is dephosphorylated in muscle by the enzyme myosin adenosine triphosphatase, and it is regenerated by either an aerobic or an anaerobic process. An indication of the rate of ATP utilization and of the aerobic and anaerobic capacity for ATP production may be obtained by using histochemical methods for myosin ATPase and for indicator enzymes of aerobic and anaerobic metabolism. When these techniques are applied to serial transverse sections of a muscle fibre, it is possible to construct its metabolic profile. The ability of a whole muscle to synthesise and degrade ATP may be elucidated either by studying the metabolic profile of all its fibres, or by sampling an area of muscle, known to be representative. If muscles with a similar function in different animals are chosen entire animals may be compared.

By comparing the musculoskeletal system of Greyhounds and Thoroughbreds with members of their species less specialised for athletic performance, this study attempts to describe the changes induced by selection for this trait and therefore to define athletic characteristics. This

comparison is made by gross morphometric, histological, histochemical and biochemical studies. Gross morphometric comparisons entail the determination of the relationship between muscle weight and body weight, and the changes in muscle distribution during growth. These considerations form Part 1 of this thesis. In Part II differences in the histochemical properties of muscle fibres of representative muscles are investigated in relation to the function of the muscle, and to the athletic capacity of the breed. The adaptation of muscle fibres to changing demands during growth are also examined.

2.0 Part 1: MACROSCOPIC FEATURES RELATED TO LOCOMOTION

2.1 INTRODUCTION

A terrestial animal propels itself by exerting forces against the ground. These forces are created by the contraction of skeletal muscles acting over components of an articulated skeleton. In this way, bones act as levers, passively transmitting the forces exerted by the muscles. This study is primarily concerned with the muscular forces of the locomotive machine but other relevant factors are considered briefly.

2.1.1 The consideration of limbs as pendulums and skeletal levers

If a limb of an animal is likened to a pendulum then the natural oscillatory frequency of the limb will depend on the length of the leg and the distance of the centre of gravity of the leg from the centre of rotation of the limb. Muscular forces are required to cause a limb to move faster or slower than its natural oscillatory frequency. Tricker and Tricker (1967) demonstrate that the distance covered by the end of the limb in a given time when the limb is oscillating at its natural frequency is related to the square root of the length of the limb. However, if the oscillatory frequency is forced to increase by muscular action, the distance covered per unit time is independent of the length of the limb.

By moving the center of gravity of a limb nearer to the pivot the natural frequency of the limb is increased. This happens in limb protraction when flexion of the joints shortens the length of the pendulum and increases the natural frequency of the swing of the limb; a similar result is

achieved by the adaptative development of the cursorial limb in which much of the muscle mass is located near the pivot. Howell (1944) suggests that in general, the hind limbs of terrestial mammals are better endowed for propulsion than are the fore limbs, and that adaptation for swift running may be more highly developed in the hind than in the fore limb. The possibility that animals specialised for running quickly may have a greater propulsive capacity in their hind limbs and a greater proportional weight of their hind limbs nearer the hip joint in comparison to other members of their species will be investigated.

Hayes (1904) shows that animals of great strength have proportionally a long body and short legs, whereas those renowned for their high speed are distinguished by having a short trunk and long limbs. More specifically he shows that among horses those specialised for speed of running - Thoroughbreds - have longer limb length in relation to their trunk. However Tesio (1958) indicates that within the Thoroughbred breed of horse, the shape of the skeleton has no particular influence on speed or "staying power." When discussing the specialisation of animals for running, Howell (1965) suggests that decreasing the length of the femur and humerus in relation to the other bones of the limb seems to facilitate speed of running in various species of animals. Measurements made by Ewart (1894) suggest that irrespective of breed, the lengths of the radius of adult horses varying in height from 96·5 to 157 cm bear a constant relationship to those of the humerus.

However he finds that the fetuses of "long legged" and "well bred" horses over 157 cm have a higher radius to humerus length ratio than fetuses from other types of horses, and suggests that depending on the breed, adult horses over 157 cm may have relatively short humeri. However during the postnatal growth of several breeds of horse including the Thoroughbred, the proximal bones grow more rapidly than do the distal bones of the forelimb (Krüger, 1939; Green, 1970). The disproportionate humeral lengths in "well bred" horse fetuses (Ewart, 1894) may therefore be merely a result of normal growth. This may be investigated by comparing bone lengths at different stages of growth in the Thoroughbred and in horses less specialised for speed. According to Lamb (1935) differences such as thickness of shoulder and breadth of chest in proportion to length of body in horses are related to differences in the size of muscles attached to the body to assist in movement. He also comments that the shapes of the bodies of different types of horses are similar, and that the only real breed differences lies in the relative "thickness of the legs." Nevertheless, a preferential development of those parts of the body best located for a propulsive effort would be a decided advantage to the cursorial animal.

2.1.2. Muscle design to account for strength, range of movement and speed

The effectiveness of a muscle for a propulsive effort depends on the force it can exert and its mechanical advantage. The point of insertion of a muscle on a limb influences the range and speed of movement of the limb

(Howell, 1965). Alexander (1968) shows that the mechanical advantage of similar muscles in different animals depends on the distance from the insertion to the pivoting joint. The further away the attachment of a muscle is from the joint the greater will be its mechanical advantage. Also the force capable of being produced by a given volume of muscle is influenced by its fibre architecture - a pennate muscle is capable of producing a greater force than a similar volume of a parallel fibred muscle acting in a similar position (Alexander, 1968). The speed of movement of the insertion of a muscle will also depend on the arrangement and length of its fibres since there is an increase in velocity of the free end of a fibre with an increase in number of serially arranged sarcomeres (Gans & Bock, 1965). These effects on the extrinsic speed of contraction of a muscle are distinct from those governing the intrinsic speed of contraction of individual fibres.

Although alterations in the relative positions of the origins, insertions and in the fibre architecture of muscles may enhance athletic capacity, as may other factors such as supplementation of hind limb propulsion by movements of the spinal column, these factors are not investigated in depth in this study.

2.1.3. <u>The quantity and distribution of muscle in relation to function</u>

The weight of a muscle may be considered as an estimate of its potential work capacity. Therefore weight measurement of the gross contractile apparatus of horses and dogs

should determine to what extent the total amount of muscle in relation to body weight is greater in the Thoroughbred and Greyhound and hence to what extent the ability to perform propulsive work is different.

Total muscle dissection has been used as a means for carcass evaluation of meat producing animals by Walker (1961), Dumont, Le Guelte and Arnoux (1961), Butterfield (1962), Berg and Mukhoty (1970) on cattle; Lohse, Moss and Butterfield (1971), Fourie, Kirkton and Jourie (1970) on sheep; and by Cuthbertson and Pomeroy (1962), Dumont, Schmitt and Roy (1969), Richmond and Berg (1971) and Davies (1974 a,b) on pigs. Fowler (1968), Lohse, Moss and Butterfield (1971) and Davies (1974 b) suggest that different growth patterns of muscles in meat producing animals are associated with different functional demands. Similarly, in non-domesticated animals Bryden (1973) shows that the growth rates of individual muscles of the elephant seal correspond to the change from a fully terrestial to a mainly aquatic existence.

A distinct feature of the Greyhound breed is the exceptional development of the hind quarters (Clarke, 1965). This may be attributed to the high ratio of upper hind limb muscle mass to total body weight found in the Greyhound, in contrast to the German Shepherd and July Hounds of differing body weights studied by Riser and Shirer (1967). Variation in the distribution of muscle weight in the carcasses of different breeds of cattle is considered to be generally of little economic significance (Butterfield, (1974). However Davies (1974 a,b) finds that the superior muscularity

of the Belgian Pietran pig in comparison to British pigs is due to a greater muscle mass acting over the hip joint, suggesting that in this breed the normal craniocaudal growth gradient is exaggerated. If as suggested by Davies (1974b), this growth pattern is an adaptation of the animal to maintain a constant propulsive acceleration during growth, then it may also be a feature of animals selected for speed of running. This study examines whether selection for swiftness in cursorial animals is associated with changes either in proportion of total muscle in relation to body weight, or changes in muscle distribution.

Watson (1973) shows that when a group of 91 young adult humans are subjected to a 26 day period of moderate exercise the majority of the participants increase in weight although their skinfold measurements - an index of body fatness - actually decreases. The author concludes that a small decrease in adipose tissue occurs concurrent with a large increase in the mass of total body tissue, probably accounted for by an increase in skeletal muscle. However, the attempt to demonstrate an increase in muscular strength was unsuccessful. The possibility that prolonged periods of moderate exercise may alter body composition will be considered in this thesis.

Although it is generally accepted that after 5 years of age racing horses are not as fleet of foot as when younger, there is no scientific evidence that there is an optimal age for swiftness in horses (Lees, 1974). Since the author had no control over the environment of the adult animals and foals, this study compares the growth

of body components relative to each other rather than relative to time.

2.2 MATERIALS AND METHODS

2.2.1 Sources of material

Seventy-five dogs, comprising forty-four Greyhounds and thirty-one other dogs; and sixty-three horses, composed of thirty Thoroughbreds and thirty-three horses of other breeds were used for partial or total body dissection. The animals were of different sexes and were obtained between 1970 and 1973. All animals except the pups were randomly selected.

The forty-six adult dogs were obtained from two sources; those given to the author to be put down and used for this study, and dogs from the post-mortem room at the Royal (Dick) School of Veterinary Studies. The Greyhound pups were obtained from two litters which were bred and reared for the purpose of this study, at the Centre for Laboratory Animals, Easter Bush, Midlothian. The other pups used were also obtained from the Centre for Laboratory Animals. Both types of pups were reared under similar conditions. They were weaned at approximately 5 weeks of age. Their diet was gradually changed from a predominantly milk one, to one which contained 22% protein, 69% carbohydrate, 4% fat and 5% ash on a dry matter basis.

The adult Thoroughbreds used were obtained from the post-mortem rooms at the Field Station of the Royal (Dick) School of Veterinary Studies; the Equine Research Station, Newmarket, Suffolk; and the Irish Veterinary College, Dublin. Adult horses of other breeds were acquired from the abbatoirs at Motherwell, Lanarkshire and Cupar, Fifeshire; and the post-mortem rooms at the Field Station of the

Royal (Dick) School of Veterinary Studies and the Animal Diseases Research Institute, Moredun, Edinburgh. The post-mortem rooms at the Equine Research Station, Newmarket, Suffolk and the Irish Veterinary College, Dublin provided the Thoroughbred fetuses and young horses. The other fetuses and young horses were obtained from the post-mortem rooms at the Field Station of the Royal (Dick) School of Veterinary Studies, and the Animal Diseases Research Institute, Moredun, Edinburgh. Dogs over 15 months of age and horses two years old and over are considered to be adults for the purpose of this study.

2.2.2 Dissection procedure

2.2.2.1 Preliminary preparation of material

Total body weight was recorded before death, except in the case of the fetal horses when it was recorded immediately they were received. Most of the animals used for dissection provided samples for the histological and histochemical study of Part 2 of this thesis. These samples were removed as soon as possible after death.

The method of dissection was similar for both dogs and horses, but the extent varied between individuals. Where applicable evisceration was effected via a midline incision from the angle of the mandible to the pubis. In each case the sternum was separated from the costal cartilages on the left side by an incision through the sterno costal junction. The weights of the viscera and m. diaphragma were recorded. The weight of gut and bladder contents was included with the weight of the digestive system. The head was removed and weighed. The carcass

was then dissected or stored for up to 24 hours at a temperature between 0 and 4°C. Meat knives were used on the larger carcasses and scalpels and forceps on the smaller ones. Only the right half of the carcasses were dissected. Some muscles were weighed individually, others were assigned to groups and their weights recorded with that of the group. Appendices 1 and 2 list the constituents of the groups and give an example of the data recorded, from one horse and one dog respectively. The nomenclature follows the recommendations of the Nomina Anatomica Veterinaria (1968). During dissection the portions of the carcass not being used and those parts being collected to be weighed in groups were covered by damp paper towels.

2.2.2.2 Dissection of the hindlimb

The skin and subcutaneous fat where present were removed and weighed separately. Individual muscles were dissected from the carcass, cleared of intermuscular fat, fasci and tendon and the weights were recorded. Intermuscular and subcutaneus fat were weighed together. The weights of fascia, ligaments, blood vessels, nerves and lymph nodes were recorded as one group and subsequently added to the weights of these structures from other parts of the carcass. The third phalanx was not removed from the hoof or claw and so contributed to the weight of the integument. Extensor and flexor tendons were weighed together. The bones were cleaned and weighed. The volumes of the femur, tibia and fibula were assessed by the displacement of water. The length of a bone was taken

as its greatest linear dimension and measurements were made as follows:-

Femur: Horse - Apex of trochanter major to the distal articular surface of medial condyle.

Dog - Proximal part of the head to the distal articular surface of the lateral condyle.

Tibia: Horse - Spine to the medial part of the distal articular surface of the intermediate ridge.

Dog - Proximal part of the tibial tuberosity to the medial malleolus.

2.2.2.3 Removal and dissection of the forelimb

The manner of dissection was similar to that of the hindlimb. The skin, subcutaneous fat and m. cutaneus were removed and their weights recorded. The extrinsic muscles of the limb were severed at their insertions on the trunk, and after removal from the limb their weight as a group was recorded. The tendon weights were recorded as for the hindlimb. All the muscles on the limb with insertions above the elbow joint were removed and weighed together. Muscles below the elbow joint were treated similarly. Bone lengths were measured as follows:-

Humerus: Horse - Apex of the medial tuberosity to the distal articular surface on the lateral condyle.

Dog - Apex of tuberculum majus pars cranialis to the distal articular surface of the medial condyle.

Radius and Ulna: Horse - Tuber olecrani to the distal articular surface of the radius.

Dog - Tuber olecrani to the distal articular surface of the radius.

2.2.2.4 Dissection of the trunk

After removal of the skin and where present subcutaneus fat and m. cutaneus, the abdominal and costal muscles were removed and weighed as groups. The ribs and sternum were cleaned and weighed. M. longissimus and the iliopsoas group were separated from the other spinal muscles. Cervical and spinal muscles were incorporated as one group. Fat was included in total body fat. The weight of ligaments, blood vessels, nerves and lymph nodes from half the trunk was added to those from the limbs. The vertebrae and pelvis were weighed together after cleaning and disarticulation from the ribs on the left side.

2.2.3 Analysis of data

The number of samples of 13 body components obtained by dissection are listed in Table 1. The amount, type and values of all the data collected from each animal are given in Appendices 3&4. The data were analysed to test for differences in body components. The parameters of the regression lines, the difference between slopes of two regression lines, and the determination of whether there was a significant difference between adjusted group means of the dependent variable in similar comparisons of carcass components in the two types of animals in both species were estimated using computer facilities and the methods

outlined by Dixon (1971). The term covariate relationship is used in the text in reference to the relationship of adjusted group means of samples. The regression coefficient b is the differential growth ratio of a carcass component y in relation to a carcass component x. The significance of the difference between b and 1 and the comparisons of the value of y, for the two groups of animals at nominal values of x,(i.e. comparing the 95% confidence limits of y at a given value of x) were calculated by methods outlined by Diem and Lentner (1970).

2.3 RESULTS

2.3.1 Measurements on limb bones

2.3.1.1 Length of limb bones

The lengths of the femur, tibia, humerus and radius and ulna in cm. are listed on Table 2. The femurs of three athletic and non-athletic dogs and horses spread over the range of liveweights studied are shown on Figs. 1 and 2 for dogs and horses respectively. Both types of animals within each species are compared by:

(a) determining the relationship of both the sum of the lengths, and the individual lengths of both the propodial (humerus or femur) and epipodial (radius and ulna or tibia) skeletal segments in either fore- or hindlimb with liveweight in each type of animal;

(b) comparing the propodial : epipodial skeletal length ratio within each limb of each type of animal.

(A) The relationship of individual and combined bone lengths with liveweight during growth.

It is assumed that the combined lengths of the propodial and epipodial skeletal segments is an index of limb length.

Dogs

The mean individual and combined lengths of the bones of the adult Greyhounds calculated from Table 2 (22·3 cm for the femur, 23·4 cm for the tibia and 45·7 cm for both these bones combined; 21·1 cm for the humerus, 25·1 cm for the radius and ulna and 46·2 cm for the combined length of both these bones) and the adult other dogs (femur: 23·4 cm, tibia: 24·4 cm and 42·8 cm for the mean combined length of these bones; humerus: 25·4 cm, radius and ulna 21·8 cm and

the mean combined length of these bones being 42·2 cm) are not significantly different between the two types of dog although the combined length of the bones in both the fore and hindlimb are longer in the Greyhounds.

The comparison of absolute limb lengths between groups of adult dogs is of limited merit as it is obvious that a Greyhound has longer legs than a Dachshound but shorter legs than a Great Dane, whereas these results are of more value when related to liveweight. The double logarithmic regression equations describing the relation of the combined lengths of the femur, tibia and fibula, and of the humerus and radius and ulna to liveweight indicate that the limb length increases at a rate greater than that for a proportionate relationship (i.e. $b > 0{\cdot}333$ since weight $\propto$ volume $\propto l^3$), for both limbs of the Greyhounds ($P < 0{\cdot}001$), and for the hindlimb ($P < 0{\cdot}05$) and forelimb of the other dogs ($P < 0{\cdot}02$) (Table 3 and Fig. 3). Similar relationships also hold when the lengths of the individual bones are related to liveweight ($b > 0{\cdot}333$; $P < 0{\cdot}001$ for all the Greyhound bones considered individually; $P < 0{\cdot}05$ and $P < 0{\cdot}025$ for the femur and humerus of the other dogs respectively, and $P < 0{\cdot}02$ for the tibia and fibula; radius and ulna of the other dogs). No other significant results have been found relating bone lengths to liveweight in the dog (Tables 3 & 4). The liveweights of 0·5 and 30 kg for which computations are made on Table 4 are considered to represent neonatal and adult liveweights in large dogs.

Horses

The regression equations for horses (Table 3; Fig. 4) computed from the data in Table 2 less the smallest fetus of each type (both of which are much smaller than the other specimens of their type and may apparently have a different relationship of bone length to liveweight than the other members of their group (see Fig. 4) indicate that the increase in the combined lengths of the humerus, radius and ulna of the other horses remain proportional to liveweight (i.e. b is not significantly different from 0·333). However the combined lengths of the humerus, radius and ulna of the Thoroughbreds and the femur and tibia of both types of horse increase at a lower rate than does the body weight (Table 3).

Although there is no significant difference in the rate of increase in the combined lengths of the forelimb and hindlimb bones either within or between the two types of horse, the Thoroughbreds at 500 kg liveweight (adult weight for Thoroughbreds) have greater ($P < 0.05$) combined lengths of their femora and tibiae (98·4 cm) than the other horses (83·3 cm) at a similar liveweight (Table 5).

When the smallest fetus of each type are included the values at 50 kg liveweight (perinatal weight in Thoroughbreds) for both the forelimb and hindlimb (51 cm and 51·9 cm respectively) are significantly greater in Thoroughbreds than in other horses (42·4 cm and 40·2 cm respectively), but there is no significant difference between the values for the forelimb and hindlimb bones of the two types of horse at 500 kg liveweight. (The weights

of 50 and 500 kg are considered to represent neonatal and adult liveweights of large horses).

Although the rate of increase in the individual bone lengths relative to liveweight does not differ between the two types of horse the length of the Thoroughbred tibia corresponding to 500 kg liveweight is greater than that of the other horses at the similar liveweight. There are no other significant differences in individual bone lengths at either 50 or 500 kg liveweight. The rate of increase in length of the femur ($P < 0.01$) and tibia ($P < 0.001$) of the Thoroughbreds and of the tibia ($P < 0.01$) of the other horses are less than the rate of increase in liveweight of these animals; the same is true for the humerus ($P < 0.02$); and radius and ulna ($P < 0.025$) of the Thoroughbred. However the humerus, radius and ulna, and femur of the other horses increase in length proportional to the increase in liveweight.

It would appear, therefore, that the limbs grow at a disproportionately low rate relative to liveweight in all horses but that Thoroughbreds have longer limbs relative to liveweight when adult (at 500 kg liveweight) and perhaps neonatally (at 50 kg liveweight) compared with other horses. Both the horse and dog change their shape during growth since one or both limb lengths in the two species do not remain proportional to liveweight. There is no difference in limb growth relative to liveweight between athletic and non-athletic dogs and horses.

(B) The propodial : epipodial skeletal length ratio within each limb.

Dogs

The rate of growth in length of the femur is significantly less than that of the tibia ($P < 0.01$) and the rate of growth in length of the humerus is significantly less than that of the radius and ulna ($P < 0.01$) in the Greyhound (Table 6, Fig. 5). There are similar but not significant trends in the other dogs. The relative rate of increase in length of the propodial forelimb segment when compared with that of the propodial hindlimb segment is not significantly different between the two types of dog or within either type of dog.

Horses

There are no statistical differences in either limb of the horse when the relative growth of the epipodial and propodial segments are considered for all the horses shown on Table 2.

Although the femur is significantly shorter ($P < 0.001$) than the tibia in 9 of the 11 Thoroughbreds and significantly longer ($P < 0.001$) than the tibia in the 14 other horses (Table 2) this comparison is made between animals of different ages and so the difference may be age-related.

When the two species are compared, the growth of the femur relative to the tibia in all the dogs ($\log y = 0.927 \log x + 0.012$) is less than the growth of the femur relative to the tibia in all the horses ($\log y = 1.055 \log x - 0.054$; $P < 0.001$). Similarly the rate of growth of the humerus relative to the radius and ulna in all the dogs

(log y = 0·943 log x -0·002) is less than that in all the horses (log y = 1·012 log x -0·176; $P < 0.02$).

Summing up, the results obtained from the two methods used to study bone lengths, it is shown that:

(1) limb bones grow at a disproportionately high rate relative to liveweight (i.e. $b > 0.333$) in dogs but a similar or even a disproportionately lower rate in horses;

(2) at the same liveweight, (either neonatal or adult) athletic and non-athletic dogs do not differ significantly in length of limb bones. Adult Thoroughbreds, however, have longer limbs relative to their liveweight than do the other horses, they also possibly have longer limbs relative to their liveweight perinatally;

(3) the epipodial skeleton grows at a greater rate than the propodial skeleton in dogs, but at a lower rate in horses;

(4) there is no significant difference between the two types of dog in the propodial to epipodial skeletal length ratio of either fore- or hindlimbs but in the horse hindlimb only, the ratio is lower in the Thoroughbred, i.e. tibia is longer;

(5) compared between species, the length of the radius and ulna relative to the humerus is longer in the horse than in the dog (Fig. 5).

2.3.1.2 Density of limb bones

Allometric equations describing growth in weight relative to the growth in volume of bones are given in Table 8,

calculated from the weights and volumes of the femur, tibia and fibula, humerus and radius and ulna of dogs and horses shown in Table 7. If the growth ratio equals 1, density remains constant during growth, hence values of b greater or less than 1 indicate an increasing or decreasing density of bone respectively.

Dogs

The mean densities of the bones of the adult Greyhounds (femur:1·46 g/cm ^{3}, s.d. 0·17; tibia:1·41 g/cm ^{3}, s.d. 0·20; humerus:1·44 g/cm ^{3}, s.d. 0·11; radius and ulna:1·53 g/cm ^{3}, s.d. 0·04) and of the adult other dogs (femur:1·36 g/cm ^{3}, s.d. 0·14; tibia:1·45 g/cm ^{3}, s.d. 0·06, humerus:1·33 g/cm ^{3}, s.d. 0·15; radius and ulna:1·58 g/cm ^{3}, s.d. 0·06) are not significantly different. There is no significant differences between the different limb bones of adult Greyhounds, but in adult other dogs the density of the radius and ulna is significantly greater ($P < 0·025$) than that of the other measured limb bones.

There is no significant difference in the growth ratio (change in density) of corresponding bones between the two types of dog. Since the growth ratio of the femur of the Greyhound is significantly greater than 1 ($P < 0·02$); its density increases during growth. Changes in density of the other bones are not significant. In the other dogs the density increases in the femur, tibia and fibula, and radius and ulna, but decreases in the humerus. However these changes are only significant for the tibia and fibula ($P < 0·05$).

The density of the hindlimb bones of both types of

dog combined increases ($P < 0.001$) during growth but the increase in the forelimb bones is not significant. However the density of all the dog bones combined increases during growth ($P < 0.001$). The density of the Greyhound femur at a femur volume corresponding to 30 kg liveweight (1·43 g/cm ^{3}) is greater ($P < 0.05$) than the density at a femur volume corresponding to 0·5 kg liveweight (1·20 g/cm ^{3}). There are no other significant differences between the density of the bones of the Greyhounds and the other dogs.

Horses

The density of the limb bones of the largest Thoroughbred in which these values were assessed (femur:1·26 g/cm ^{3}; tibia:1·23 g/cm ^{3}; humerus:1·12 g/cm ^{3}; radius and ulna 1·30 g/cm ^{3}) are less than the densities of similar bones in the adult other horses (femur: 1·18 g/cm ^{3}, s.d. 0·13; tibia: 1·28 g/cm ^{3}, s.d. 1·11; humerus:1·27 g/cm ^{3}, s.d. 0·11; radius and ulna 1·34 g/cm ^{3}, s.d. 0·16).

The increase in density of the Thoroughbred femur is significantly greater than the increase in density of the femur of the other horses ($P < 0.001$; Table 8), but there is no significant difference between the rate of change in density of the other bones of the two types of horse. The femur of the Thoroughbreds is the only bone of both types of horse to show a significant increase in density during the periods of growth studied, i.e. $b < 1$, ($P < 0.001$).

The hind limb bones of both types of horse grouped together show an increase in density with growth ($P < 0.005$), but there is no significant increase in density in the

forelimb bones. Neither is there a significant change in density in all the bones of all the horses as a group.

The density of the femur of the Thoroughbreds at a femur volume corresponding to 500 kg liveweight (1·76 g/cm ^{3}) is significantly greater ($P < 0·05$) than the density at 50 kg liveweight (1·17 g/cm ^{3}). There is no significant difference in femur density between the two types of horse at femur volumes corresponding to 50 kg liveweight (i.e. 1·17 g/cm ^{3} for the Thoroughbreds and 1·14 g/cm ^{3} for the other horses). However the density of the femur of the Thoroughbreds at 500 kg liveweight (1·76 g/cm ^{3}) is greater ($P < 0·05$) than that of the other horses (1·17 g/cm ^{3}). There are no other significant differences in the density.

In general therefore the femur of the Greyhounds and of the Thoroughbreds; and the tibia and fibula of the other dogs are the only individual bones which increase in density significantly during growth. The mean density of all dog bones (irrespective of age of type) is 1·38 g/cm ^{3}, and the mean density of all the horse bones (irrespective of age or type) is 1·16 g/cm ^{3}.

2.3.2 <u>The relative position of the centre of gravity of the hindlimb</u>

An index of the position of the centre of gravity of the hindlimb (a determining factor for the natural frequency of the hindlimb) may be obtained by comparing the weight of the proximal hindlimb muscles plus femur with the weight of the distal hindlimb muscles plus other bones of the hindlimb. The higher the proximal : distal ratio the nearer will be the centre of gravity of the

limb to the pivot i.e. the hip joint and caudal part of the vertebral column in dogs and hip joint in horses.

Allometric equations describing the change in this ratio are given in Table 9 and plotted on Figs 6 & 7 for dogs and horses respectively.

Dogs

The mean ratio of proximal to distal limb component weights in the 7 adult Greyhounds (4·38, s.d. 0·21) is significantly greater ($P < 0.005$) than that in the 5 adult other dogs (3·45, s.d. 0·61). Similarly the covariate relationship of the proximal to the distal limb components is greater ($P < 0.01$) in the Greyhound adults than the similar relationship in the adult other dogs. The proximal component of the Greyhounds enlarges at a significantly greater rate ($P < 0.001$) than the distal component (i.e. $b > 1$); although it increases at a greater rate in the other dogs also, the difference in growth rate of the two components is not significant. There is no significant difference between the rate of growth of the proximal component relative to the distal component between the two types of dog.

Horses

Only three adult other horses and no adult Thoroughbreds were available for this comparison. The mean ratio of the proximal to distal components of the three adult other horses is 4·66, s.d. 0·83 (4·19 for a Shetland pony, 5·62 for Thoroughbred cross and 4·16 for a Clydesdale). This is less than that for the largest Thoroughbred sampled - 4·70.

No significant changes were found in the proximal: distal ratio of either type of horse, either when the values for the smallest Thoroughbred is omitted (because this single prenatal Thoroughbred appears to have a relationship of proximal : distal limb components extraneous to the rest of the data on Thoroughbreds, Fig. 7) or included in calculations. The difference between the two types of horse at the lower distal limb component weights (Fig. 7) is probably because the Thoroughbreds having these distal limb component weights have a lesser development of their musculoskeletal system than the older ponies which have similar distal hind limb component weights.

Therefore, adult Greyhounds have a greater weight of their limb nearer the pivot than adult other dogs. Although young Thoroughbreds have a smaller proximal to distal limb component weight ratio than the other small horses of this study (more mature ponies, Fig. 7), they have a tendency to a greater increase in this ratio. Therefore, the adult Thoroughbreds should have a similar or equal ratio to the adult other horses. However as previously mentioned, no adult Thoroughbreds were investigated.

2.3.3 Proportions in adults and growth of muscle, bone and fat

2.3.3.1 Total muscle and total bone

Allometric equations comparing the growth of either total muscle or total bone relative to liveweight or to combined total muscle plus bone weight in 18 Greyhounds and 9 other dogs, 6 Thoroughbreds and 5 other horses are shown in Table 12 and Figs. 8 and 9. These equations

are calculated from the data in Tables 10 and 11. The regressions for horses in Table 12 and Fig. 9 are calculated without the data for the smallest horse of each type. The relationship of muscle and bone to liveweight and combined muscle plus bone weight was apparently different in these animals than in the others of their type (Fig. 9), but as both are much smaller than the other specimens of their type, no data from nearby was available to substantiate real differences.

Dogs

Total muscle weight has a mean of 57·1% (s.d. 1·9) of liveweight in all the adult Greyhounds (Table 14) which is significantly greater ($P < 0.001$; $t = 5.74$) than that of all the adult other dogs (mean 43·5, s.d. 6·0). There is no significant difference between the total bone weights of the two types of dog expressed as a percentage of their liveweights, nor in the weight of total muscle as a percentage of liveweight between the detrained and trained Greyhounds. However, the detrained adult Greyhounds still have significantly more muscle as a percentage of liveweight (56·2, s.d. 1·2) than the adult other dogs (43·5, s.d. 6·0; $P < 0.005$, $t = 4.11$).

The mean of the muscle to bone ratios of the seven adult Greyhounds (4·71, s.d. 0·35) is significantly greater than that of the other dogs (3·63, s.d. 0·60; $t = 3.967$, $P < 0.005$).

The growth of total muscle is significantly greater than the increase in liveweight in both types of dog ($P < 0.001$ for the Greyhound and $P < 0.02$ for the others)

but there is no significant difference in the growth rate of muscle relative to liveweight between the two types of dog. The increase of bone weight is significantly less than that of liveweight in the Greyhounds ($P < 0{\cdot}05$), but not in the other dogs. The growth of total bone relative to liveweight is actually greater in the other dogs than in the Greyhounds ($P < 0{\cdot}05$). The covariate relationship of total muscle relative to liveweight is greater in the 7 adult Greyhounds than in the 5 other adult dogs ($P < 0{\cdot}01$) and is similarly greater in all the Greyhounds (i.e. both adults and pups) than in all the other dogs ($P < 0{\cdot}01$).

At 0·5 kg liveweight the weight of total muscle in the Greyhound (0·112 kg) is not significantly different from that of the other dogs (0·121 kg) at this liveweight, but at 30 kg liveweight there is more muscle in the Greyhound (16·7 kg) than in the other dogs (13·1 kg; $P < 0{\cdot}05$). However the weight of bone at 0·5 kg (0·087 kg for the Greyhound and 0·054 kg for the other dogs) or at 30 kg liveweight (3·61 kg for the Greyhound and 3·67 kg for the other dogs) is not significantly different between the two types of dog.

The growth of muscle is significantly greater than that of total muscle plus bone in the Greyhounds ($P < 0{\cdot}001$) but not significantly greater in the other dogs. The growth rate of total muscle relative to total muscle plus bone is greater in the Greyhound ($P < 0{\cdot}025$) than in the other dogs. The growth of bone is significantly less than that of muscle plus bone weight combined ($P < 0{\cdot}001$) in the Greyhounds; but this is not significantly different

in the other dogs. When the two types of dog are compared the rate of growth of total bone relative to that of total muscle plus bone is significantly greater in the other dogs ($P < 0.02$). The covariate relationship of total muscle to total muscle plus bone is greater in the 7 adult Greyhounds than in the 5 adult other dogs ($P < 0.01$), but that of total bone relative to total muscle plus bone is greater in the adult other dogs ($P < 0.01$).

The total muscle weight of the Greyhound either at 0.2 kg or 20 kg total muscle plus bone weight is not significantly different from the other dogs at corresponding total muscle plus bone weights. Similarly there is no significant difference in the weight of bone between the two types of dog at either 0.2 kg or 20 kg total muscle plus bone weight, although the bone weight of the other dogs at 20 kg is greater than that of the Greyhounds (while the reverse is true at 0.2 kg).

Horses

The muscle to bone ratio of the largest Thoroughbred is 3.56, while that for the largest other horse (a Clydesdale female) is 3.18 and the Shetland pony gelding is 3.10 - both less than that of the Thoroughbred (Table 11). However the muscle to bone ratio of the Thoroughbred cross is 4.94; this may be due to the Thoroughbred influence on its morphology (the heaviest Thoroughbred was under two years of age and it is likely that the adults have a higher muscle to bone ratio due to the growth rates of muscle and bone). There is no significant difference between the rate of muscle or bone growth relative to

liveweight within or between the two types of horse. There is no significant difference in the total muscle weight or total bone weight between the two types of horse at 50 kg or 500 kg.

The growth of muscle is significantly greater than that of total muscle plus bone in the Thoroughbreds ($P < 0.005$). The growth rate of muscle relative to total muscle plus bone is significantly greater in the Thoroughbreds than in the other horses ($P < 0.005$) (Table 12). The growth of bone is significantly less ($P < 0.005$) than that of total muscle plus bone in the Thoroughbreds but there is no significant difference in the rate of bone growth relative to total muscle plus bone weight between the two types of horse (Table 12). Differences referred to above are not significant when the data for all the horses are used for computations.

There is no significant difference between the total muscle weights of the two types of horse at 300 kg combined total muscle plus bone weight but at 20 kg total muscle plus bone weight the Thoroughbreds have less ($P < 0.05$) total muscle (11.6 kg) than the other horses (14.8 kg). There is no significant difference in the total bone weight between the two types of horse at 20 kg or 300 kg total muscle plus bone weight.

Total muscle represents 57% of liveweight in Greyhounds, 44% in adult other dogs and 42% in adult other horses. Total bone weight is 12% of liveweight in these three types of animal. In adult Thoroughbreds it is probable that muscle will be more than 42% of liveweight; but it is

unlikely that there will be such a disparity between the two types of horse as between the two types of dog. Whether the figure for bone is similar to that in the other three types of animal remains to be investigated.

2.3.3.2 The growth of total fat

Both the absolute weights of fat and fat weights expressed as percentages of liveweight are given on Table 10 for dogs and Table 11 for horses. Some Greyhounds and Thoroughbreds did not have dissectable fat on their carcasses. It is apparent from the tables that the growth of fat is more variable than the growth of total muscle and total bone.

Dogs

The mean percentage of liveweight as fat in all the adult Greyhounds is 0·29 (s.d. 0·35) which is significantly less ($P < 0·025$, $t = 2·67$) than in all the adult other dogs (0·94, s.d. 0·50). Table 10 shows that the detrained Greyhounds accumulate fat during the detraining period. The mean percentage of liveweight as fat in the detrained Greyhounds is not significantly different from that in all the adult other dogs.

Horses

The majority of Thoroughbreds did not have any dissectable fat on their carcasses, whereas all the other horses had. However, the mean percentage of liveweight as fat in the adult other horses 1·04 (s.d. 1·27) is not very different from that of the largest Thoroughbred 1·0.

2.3.4 Growth and development of muscle groups relative to liveweight

The weights of the six muscle groups and the proportions formed of liveweight in adult dogs and horses are shown in Tables 13 and 14 respectively. Where present, significant differences between similar groups as proportions of liveweight are indicated. The percentages of liveweight formed by 5 of these groups are illustrated topographically in Fig. 10. The values for all the muscle groups of the Thoroughbreds except the femoral group are those for the largest (young) Thoroughbred, i.e. one specimen only in which these values were assessed. Muscles are grouped according to their skeletal attachments and the weights refer to one side of the animal only.

The allometric equations comparing growths of the six muscle groups relative to liveweight are listed in Table 15. Where present, significant differences between the growth rate of the muscle group and that of liveweight and in the growth ratios between the two types of animal within each species are indicated. The adjusted group means of each type of animal within a species were compared by analysis of covariance and where present, differences are also shown in Table 15. The topographical distribution of 5 of the 6 muscle groups and the differences between their regression coefficients or adjusted group means are shown in Fig. 11 for both dogs and horses. Data for the pectoral girdle group is supplied in tabular form only (Table 15). The weights of the muscle groups of dogs at 0·5 kg and 30 kg liveweight are given in Table 16 and those of horses at 50 kg and 500 kg liveweight are shown in Table 17.

Dogs

The brachial, pectoral, femoral muscle groups and m. longissimus occupy a significantly ($P < 0.02$) heavier proportion of liveweight in the adult Greyhounds than in the adult other dogs (Table 13). M. longissimus forms a smaller percentage (2·85% ± 0.06) of liveweight in detrained than in the trained Greyhounds (4·00% s.d. 0·76).

All groups in the Greyhounds and the brachial and pectoral groups in the other dogs grow at a faster rate than liveweight ($P < 0.05$). There are significant differences in the adjusted group means of the brachial, pectoral, longissimus and femoral groups between the two types of dog when compared by analysis of covariance either when animals of all ages, or adults only are compared, those for the Greyhounds are larger (Table 15). The 95% confidence limits of values of the muscle groups corresponding to 0·5 kg liveweight are not significantly different for any of the groups (Table 16), but m. longissimus and the femoral group are significantly greater in the Greyhounds at 30 kg liveweight.

Horses

The femoral group forms a significantly greater ($P < 0.05$) percentage of liveweight in the adult Thoroughbreds (8%) and largest young Thoroughbred than in the other adult horses (6·41%) (Table 14). It is not possible to compare the other muscle groups since there is no data for Thoroughbred adults.

The growth of the femoral group is significantly greater ($P < 0.05$) than the increase in liveweight in the

Thoroughbreds. The growth of m. longissimus in the other horses is significantly less ($P < 0.05$) than their increase in liveweight. The growth of the femoral group is significantly greater in Thoroughbreds ($P < 0.02$) than in other horses (Table 15). There are no significant differences between the weights of the six muscle groups of the Thoroughbreds and other horses when compared at 50 kg and 500 kg liveweight (Table 17).

The unusually low growth ratio of m. longissimus relative to liveweight of the other horses (0·690, Table 15) may be partially attributed to a very high value for m. longissimus (138 g) in a foetus of 2·98 kg liveweight. If this specimen is omitted the correlation coefficient becomes higher (0·9967 from 0·9964) and the growth ratio also increases (though not significantly) to 1·187. Nor is this modified ratio significantly higher than that of m. longissimus of the Thoroughbreds. Omission of the data for the femoral group of the smallest Thoroughbred results in an increase of the growth ratio for this group from 1·150 ($r = 0.9935$) to 1·220 ($r = 0.9944$), statistically, the difference from the other horses is more significant. If the value for the distal hindlimb group of the smallest Thoroughbred is not used in the calculation of the regression equation, the growth ratio of this group is reduced from 1·108 ($r = 0.9920$) to 1·025 ($r = 0.9762$); this is still not significantly different from the growth ratio of the same muscle group in other horses (0·966).

2.3.5 Muscle groups relative to total muscle weight

The weights of the six muscle groups and the percentages of total muscle weight in adult dogs and horses are shown on Tables 13 and 14 respectively. Where present, differences between muscle groups expressed as percentages of total muscle weight are indicated. The percentages of total muscle weight formed by 5 of these groups are illustrated topographically in Fig. 12. The values for all muscle groups in the Thoroughbreds are those for the largest (young) Thoroughbred, i.e. one specimen only in which these values are assessed.

The growth of 6 muscle groups relative to total muscle weight is described by allometric equations given in Table 18. Where present, significant differences between the growth rates of muscle groups and those of total muscle weight and the growth ratios for the two types of animal within each species have been indicated. Also the adjusted group means for all the animals within a species, tested by analysis of covariance, are indicated in Table 18. The topographical distribution of 5 of the 6 muscle groups and the differences between their regression coefficients or adjusted group means relative to total muscle weight are shown on Fig. 13. Data for the pectoral girdle is supplied in tabular form only. Allometric equations describing the growth of brachial and femoral groups and m. longissimus of dogs and the brachial, femoral and distal forelimb groups of the horses relative to total muscle weight are given on Figs. 14 and 15 respectively.

Dogs

The weights of the distal forelimb and of the distal hindlimb as percentages of total muscle weight are less ($P < 0.02$) in adult Greyhounds than in adult other dogs (Table 13). In Greyhounds, the weight of m. longissimus as a percentage of total muscle weight is less ($P < 0.025$) in detrained (mean 5.08% s.d. 0.10) than in trained animals (mean 6.77 s.d. 1.08).

In the Greyhounds, the growth of the distal forelimb, brachial and pectoral girdle groups is less (P 0.05) while that of the longissimus and femoral groups is greater (P 0.05) than that of total muscle. However in the other dogs there is no significant difference between the growth rate of any of the muscle groups and that of total muscle (Table 18). The growth of the femoral group relative to total muscle weight is greater in the Greyhounds than in the other dogs ($P < 0.05$), while that of the pectoral group is greater in the other dogs ($P < 0.05$). The covariate relationship of the distal forelimb group to total muscle weight in all the other dogs is greater ($P < 0.05$) than that in Greyhounds, but that of m. longissimus is significantly greater in the Greyhounds ($P < 0.05$). When adults alone are considered, the covariate relationship of the distal forelimb ($P < 0.01$), brachial ($P < 0.05$) and distal hindlimb group ($P < 0.01$) are all greater than in the other dogs.

Greyhound pups may attain the same total muscle weight as in adults of some other breeds, therefore patterns of growth ratios as in Fig. 13 could be attributed to differing

relationships to total muscle weight with chronological age in the two types of dog. Therefore regression equations relating the growth of muscle groups to total muscle weight in dogs less than 15 months of postnatal age (i.e. puppies) were calculated and these are shown in Table 19. The muscle groups (longissimus, femoral and distal hindlimb) of Greyhounds under one year of age have a higher (though not significantly so) growth rate relative to that of total muscle weight than in the other dogs of the same age. A similar result is obtained when the data for all the Greyhounds and all the other dogs are computed (Table 18). Thus although the two types of dog have different total muscle weights at similar chronological ages, the grading of the muscle groups in each type of dog by their growth rates is not markedly different if calculated for pups alone or for all specimens. Therefore the greater weight of the muscle groups of the Greyhounds at 30 kg liveweight (Table 16) may be attributed to greater relative growth rates in this breed. It does not necessarily mean a significantly greater relative weight of these groups at all stages of growth.

Horses

The femoral group of the largest young Thoroughbred forms a higher proportion of total muscle weight (17·9%) than in the three adult other horses (15·7%) (Table 14).

The growth of the femoral group in both types of horse is greater ($P < 0{\cdot}05$) than the increase in total muscle weight while the growth of longissimus in the other horses is less ($P < 0{\cdot}05$) than that of total muscle (Table 18).

There is no significant difference in the growth rates of any of the muscle groups relative to total muscle weight between the two types of horse (Table 18). The covariate relationship of brachial muscle relative to total muscle weight is greater in all the Thoroughbreds than in all the other horses ($P < 0{\cdot}05$; Table 18 and Fig. 13).

As the regression equations listed in Table 18 are calculated for young Thoroughbreds and both young and adult other horses they are recalculated for young horses only of both types in Table 19 to enable the patterns of growth ratios to be established over an equivalent age range. However the growth ratios of all the muscle groups relative to total muscle weight (except the femoral group) are higher, though not significantly so in the young Thoroughbreds than in the young other horses (Table 19). The femoral group has a lower (though not significantly) growth coefficient relative to total muscle weight in young Thoroughbreds than in the young other horses, although relative to liveweight the growth coefficient is higher - but not significantly so - in the young Thoroughbreds ($b = 1{\cdot}135$) than in the other young horses ($b = 1{\cdot}047$). Whether the lower growth rate of the femoral group relative to total muscle of the young Thoroughbreds when compared with the young other horses is a breed difference (as all the other foals happen to be of the one breed - Welsh Mountain ponies -) or due to the different development of the muscular system at similar chronological ages cannot be determined from the data.

Thus the main difference between the muscle distribution of the Thoroughbreds and that of the other horses studied would appear to be due to the greater relative growth of the hindlimb muscles. Due to the nature of the animals sampled, differences between types, due to the comparisons of animals at differing chronological ages are more likely to occur in horses than in dogs. However the differences between the two types of horse do not appear to be solely associated with age.

2.3.6 Distribution of muscle in the hindlimb

The growth of the proximal (femoral) relative to the distal hindlimb muscle group in both types of dogs and horses is depicted in Fig. 16 calculated from the allometric equations of Table 20. The data for the smallest Thoroughbred is omitted from the equations for the Thoroughbreds because this early prenatal animal when compared with much older Thoroughbreds appears to have an extraneous relationship of femoral to distal hindlimb muscle (Fig. 16).

Dogs

The growth of the proximal hindlimb muscle group is greater than that of the distal hindlimb group in the Greyhounds ($P < 0.05$) but is not significantly greater in the other dogs. However there is no significant difference in the growth of the proximal relative to the distal muscle groups between the two types of dog. The covariate relationship of proximal to distal hindlimb group is higher in Greyhounds than in other dogs, whether all dogs ($P < 0.01$), adults only ($P < 0.01$) or pups only ($P < 0.05$) are considered.

Horses

The muscles of the proximal hindlimb grow at a faster rate ($P < 0{\cdot}05$) than those of the distal hindlimb in the other horses, but a similar trend in Thoroughbreds is not significant (Table 20). The growth of the proximal hindlimb muscles relative to the distal hindlimb muscles is greater (though not significantly so) in the Thoroughbreds than in the other horses.

When the data for all the Thoroughbreds is computed the correlation coefficient is reduced to 0·9916; and the regression coefficient (0·9832) is lower (though not significantly so), than both, the value for the Thoroughbreds when the smallest Thoroughbred is omitted from the calculations; and the regression coefficient for all the other horses.

Thus it appears that Greyhounds differ from other dogs in having a higher proportion of their hindlimb muscle mass in the proximal part of the limb. This is due to a slightly greater growth rate of the proximal relative to the distal limb groups in the Greyhound. On the other hand, the Thoroughbreds do not differ significantly from the other horses in this respect.

2.3.7 Growth in weight of limb bones

The growth of total forelimb bone and of total hindlimb bone relative to total bone, of humerus relative to total forelimb bone and of femur relative to total hindlimb bone are compared for the two groups of dog and horse in Table 23. The weight of total forelimb bone, total hindlimb bone, humerus and femur and their proportion

of liveweight and total bone weight in adult dogs and horses are given in Tables 21 and 22 respectively. The values for the bones of Thoroughbreds except those for the femur are those of the largest (young) Thoroughbred in which these were assessed.

Dogs

There is no significant difference between the two types of adult dog in the weight of total forelimb or total hindlimb bone either when expressed as a percentage of liveweight or of total bone weight (Table 21).

The growth of total forelimb bone is less ($P < 0{\cdot}05$) and the growth of hindlimb bone greater ($P < 0{\cdot}05$) than that of total bone in Greyhounds (Table 23). There is no significant difference between the two types of dog in the rate of forelimb bone growth relative to the growth of total bone, but the rate of hindlimb bone growth relative to total bone growth is greater ($P < 0{\cdot}01$) in the Greyhounds. The absolute rate of growth of hindlimb bone is faster ($P < 0{\cdot}001$) than that of forelimb bone in the Greyhounds but there is no significant difference in the other dogs.

The femur forms a higher ($P < 0{\cdot}05$) percentage of liveweight and of total bone weight (0·49% and 4·12% respectively) in the adult Greyhound than in adult other dogs (0·43% and 3·84% respectively) (Table 21). There are no significant differences between the two types of dog concerning the growth rates of the humerus or of the femur relative to the fore or hindlimb bones.

Thus in the dog in general, the forelimb bones are heavier than the hindlimb bones; in Greyhounds the femur

is heavier than the humerus although in some representatives of other breeds of dog the reverse is true (Table 21). Also the femur of the Greyhound is heavier than that of other dogs, but otherwise there is little difference in the bone weights of the two types of dog.

Horses

There are few significant differences between the two types of horse concerning bone weights and rates of growth (Tables 22 and 23). However, the covariate relationship of forelimb bone relative to total bone and of hindlimb bone relative to total bone is significantly greater ($P < 0.05$) in Thoroughbreds than in other horses. This may reflect merely the greater proportion of bone relative to liveweight found in the young Thoroughbreds (Table 11 section 2.3.3.1).

3.2.8 Ratio and growth of muscle relative to bone in the brachial and femoral regions

The absolute ratios of brachial muscle weight to humerus weight and of femoral muscle to the femur are shown in Tables 24 and 25 for dogs and horses respectively. The allometric equations describing the growth (in weight) of the brachial muscles relative to the humerus and femoral muscles relative to the femur are listed in Table 26.

Dogs

In the adult Greyhounds and other dogs the ratio of the absolute weight of femoral muscles to the femur are greater ($P < 0.001$ for the Greyhounds and $P < 0.005$ for the other dogs) than the ratio of brachial muscles to the humerus (Table 24). The ratio of the femoral muscles

to the femur and brachial muscles to the humerus are significantly greater in the Greyhounds ($P < 0{\cdot}01$ and $P < 0{\cdot}02$ respectively) than in the other dogs. Lack of training produces rather an unusual effect: the ratio of the brachial muscles to the humerus in the detrained Greyhounds (8·11, s.d. 0·31) is actually higher ($P < 0{\cdot}02$) that that in the trained Greyhounds (7·17, s.d. 0·31). This suggests that detraining actually makes Greyhounds even more unlike other dogs! On the other hand it may be just a feature of these particular Greyhounds.

The brachial and femoral muscles both grow at a greater rate ($P < 0{\cdot}05$) than the humerus and femur respectively in both types of dog. The growth of the brachial muscles relative to the humerus and femoral muscles relative to the femur are greater in the Greyhound ($P < 0{\cdot}02$ and $P < 0{\cdot}05$ respectively) than in the other dogs. The growth of the femoral muscles relative to that of the femur is not significantly different from the growth of the brachial muscles relative to that of the humerus within either type of dog, although in both Greyhounds and other dogs the values for the hindlimb relationship are greater (though not significantly) than those for the forelimb relationship (Table 26).

Horses

In the adult horses the ratio of the femoral muscles to the femur is greater ($P < 0{\cdot}005$) than the ratio of the brachial muscles to the humerus (Table 25). Although the mean of the ratios of the femoral muscles to the femur in adult Thoroughbreds is greater than in adult other horses,

it is not significantly so. The value for the eldest young Thoroughbred (Table 25) indicates that the ratio of the femoral muscles to the femur in this animal is less in adult Thoroughbreds and should therefore increase with growth.

The growth of the femoral muscles is greater than that of the femur ($P < 0.05$) in the Thoroughbreds only (Table 26). The growth of the femoral muscles relative to the femur tends to be greater in Thoroughbreds than in other horses and the growth of the brachial muscles relative to the humerus less in the Thoroughbreds than in the other horses (Table 26); however, although these differences are not significant, the greater femoral muscle to femur ratio in the adult Thoroughbreds is probably related to these results. Although the ratios describing the growth of the femoral muscles relative to the femur are greater than those describing the growth of the brachial muscles relative to the humerus within both type of horse, the differences are not significant.

2.3.9 Growth of cardiac muscle

The growth of the heart relative to liveweight and to total muscle in dogs and horses is indicated in Figs. 17 and 18 and by the regression equations shown on Table 29. The weight of the heart of adult dogs and horses and the percentage which it forms of liveweight and of total muscle weight are indicated in Tables 27 for dogs and 28 for horses, the Thoroughbreds are represented by the largest (young) Thoroughbred in which these values were assessed.

Dogs

Heart weight forms a greater percentage of liveweight ($P < 0.001$) and of total muscle weight ($P < 0.02$) in adult Greyhounds than in the adult other dogs (Table 27). Detraining reduces but does not significantly alter the proportion of liveweight and total muscle weight occupied by the heart in adult Greyhounds. Although the largest Greyhound pup was not in training its heart weight (1·3% of its liveweight and 2·4% of its total muscle weight) is larger than that of the other dogs.

The growth rate of the heart is less ($P < 0.05$) than that of total muscle weight in both types of dog. Although there is no significant difference in the rate of increase in heart weight between the two types of dog, either when compared with that of liveweight or total muscle (Table 29), the covariate relationships of heart weight to both liveweight and total muscle weight are greater ($P < 0.01$ and $P < 0.05$ respectively) in all the Greyhounds than in all the other dogs. A similar result is obtained if adults only are compared ($P < 0.01$). The predicted heart weight of the Greyhounds at 30 kg liveweight (379 g) is greater ($P < 0.05$) than that predicted for the other dogs at 30 kg liveweight (277 g). However there is no significant difference between the heart weights of the Greyhounds (6·62 g) at 0·5 kg liveweight or of the other dogs (4·70 g) at the same liveweight.

Horses

The heart of the largest young Thoroughbred forms a higher percentage of liveweight than in the adult other

horses, with the exception of a Thoroughbred cross male.

There is no significant difference in the rate of growth of the heart relative to the increase in liveweight or total muscle weight between the two types of horse (Table 29). Although the growth rate of the heart relative to total muscle weight is even lower in Thoroughbreds than in the other horses (Table 30) it is significantly ($P < 0.05$) lower only in the other horses. The covariate relationship of heart weight relative to liveweight is greater ($P < 0.05$) in all the Thoroughbreds than in all the other horses. The weight of the heart of the Thoroughbreds (0.55 kg) at 50 kg liveweight is greater ($P < 0.05$) than that of the other horses (0.37 kg) at the same liveweight, but there is no significant difference between the predicted heart weights of Thoroughbreds and other horses at 500 kg liveweight.

Thus over the range of liveweight investigated the "athletes" have a greater relationship of heart weight to liveweight than "non-athletes". However the growth rate of heart weight relative to total muscle weight tends to be less in "athletes" than in "non-athletes".

2.3.10 Growth of m. semitendinosus

The regression equations describing the growth of m. semitendinosus relative to the femoral muscle group (a rapidly growing muscle group in both types of dog and horse) are shown in Table 30.

Dogs

The growth of m. semitendinosus is greater than the increase in liveweight (P 0.05) in Greyhounds but is not

significantly different from that of liveweight in other dogs. Also, the growth rate of m. semitendinosus relative to femoral muscle is greater ($P < 0.01$) in Greyhounds than in other dogs (Table 30). The correlations between the growth of m. semitendinosus and that of the femoral muscles are highly significant ($P < 0.001$) in both types of dogs.

Horses

The growth of m. semitendinosus is greater ($P < 0.05$) than that of the femoral muscles in Thoroughbreds but not significantly different from that of the femoral muscles in other horses. The rate of growth of m. semitendinosus relative to femoral muscle is also greater in the Thoroughbreds ($P < 0.05$) than in other horses. The correlations between the growth of m. semitendinosus and that of the femoral muscles are highly significant ($P < 0.001$) in both types of horse.

In general it appears that the growth of m. semitendinosus reflects the high growth impetus of the femoral muscle group in both types of dog and horse, and is therefore a suitable muscle on which to base a histometric comparison of muscles of athletic and non-athletic dogs and horses.

2.4 DISCUSSION

2.4.1 Analysis of Growth

In this study the musculo-skeletal system is considered primarily as the support and propulsive mechanism of the animal. It is obvious that there is a greater weight of muscle acting around the hip in a Greyhound than in a Chihuahua or in a heavy Drafthorse than in a Thoroughbred. However as each animal has to support its own liveweight, comparisons of the absolute weight of the musculoskeletal components between different types of dog and horse are not as relevant as comparisons of tissue proportions or tissue distribution.

In adult animals measurements expressed as ratios of one body component to another or to total body weight enable comparisons of these proportions to be made between adults of different types. However Huxley (1932) suggests that the use of percentages to compare differences in body proportions between animals is unsatisfactory as they generally change with absolute size. While this may be true the study of the development with growth of adult proportions - and inquiry which may explain differences between adults or highlight adult differences in growth patterns between two types of animals may be more informative although requiring more elaborate analysis.

Such studies may be influenced by external factors e.g. disease processes. Therefore if it is desired to compare the growth pattern of a similar body component in different types of normal animal it is best done by comparing the relationship of one body component,y,with that of another

body component, x, which should both be similarly influenced by external factors. Error can be further reduced if the dependent variable y (e.g. weight of a muscle group) is part of the independent variable x (e.g. liveweight)

The investigation of the relative growth of body components in this study uses the logarithmic values of the variables. The method of analysis is based on the concept of allometry, embodied in the allometric equation $y = ax^b$ (Huxley, 1932). For the purpose of this study growth is considered to be multiplicative and exponential. During exponential growth at any instant, the rate of change in the dimension of a body component - x - is proportional to its value at that time, i.e. $\frac{dx}{dt} = k.x$ - where k is a constant. Brody (1945) defines k as the instantaneous relative growth rate and indicates that it is constant over limited ranges of growth in bacteria, rats and humans.

The ratio of the values of the instantaneous relative growth rates of two body components - y and x - may be considered as b,

$$\text{i.e. } b = \frac{Ky}{Kx} = \frac{\frac{dy}{dt} \cdot \frac{1}{y}}{\frac{dx}{dt} \cdot \frac{1}{x}}$$

Huxley (1924) calls b the 'differential growth ratio'. If b does not alter in value then the ratio between the instantaneous relative growth rates in y and x is constant in the interval being measures as shown in data presented by Huxley (1932) and Brody (1945).

Thus when the natural logarithm of x is plotted against the natural logarithm of y the presence of a straight line with a gradient equal to b demonstrates an allometric

relationship between x and y i.e. log y = b log x + log a. Therefore although the rates of multiplicative growth of two body components need not be constant themselves, if these growth rates bear a constant relationship to one another then their relative growth is said to be allometric, and the constant b may be considered as a value which represents the relative growth rates of parts y and x.

White and Gould (1965) intimate that the interpretation of a (in the equation log y = b log x + log a) is not as clear as that of b since a represents the value of y when x = 0. Thus its value will depend on the units of measurements used and whether the calculations are based on the natural or common logarithms of the data (the latter being used in this study). Alterations in values of b are not necessarily associated with alterations in value of a e.g. when the equations produce parallel lines on the graph. Frequently in this study when the values of b for two regression lines are not significantly different comparisons of the adjusted group means of the relationship between y and x are made. The data for the allometric equations in this study are assumed to be represented graphically by straight lines in the intervals over which measurements are taken. However it is recognised that breaks or jumps in allometric plots may occur so that data cannot be fitted by a single regression line. Examples of such discontinuities in regression lines may be seen in the growth of the testes of the mouse (Bertalanffy, 1951) or of m. splenius cervicis of rams (Lohse, 1973). The influence of sex hormones indicated by these authors are not apparent

in the present study. However the investigation of possible breaks in allometric regression lines of similar data could form the basis for future study.

Analysis based on the use of allometric equations carries one further advantage. It is a matter of common experience that if a small and a large animal carry an equal extra weight, the relative effort required is greater for the smaller animal as the weight forms a greater proportion of the liveweight than in the larger animal. Therefore a system of comparison which maximizes differences in small values and minimizes differences in large values - as an allometric relationship does - is particularly useful in the study of cursorial animals.

The allometric equation has been rarely used to study dogs and horses but it has been employed to describe the relationship of heart weight to liveweight in dogs and horses by Brody (1945) using the data of others (Stewart, 1921; Crile & Quiring, 1940) and the evolutionary changes that have occurred in equine skulls by Reeve & Murray (1942). Double logarithmic regression equations allow the comparison of y values of two types of animals at nominal values of x, as used by Elsley, McDonald & Fowler (1964) in pigs and sheep, Fourie, Kirkton & Jury (1970) on sheep, Mukhoty & Berg (1971) on cattle, Fowler & Livingstone (1972) and Davies (1974) on pigs.

2.4.2 Problems of sampling

2.4.2.1 Problems of breeds used

Prior to its introduction into Western Europe the Greyhound breed was probably closely related to other breeds of hound such as the Saluki or Afghan (Clark, 1965); it may

therefore have morphological or biochemical similarities to these breeds. However the Greyhound is the oldest of all pure breeds of dog (Clarke, 1965) and therefore its similarity to other breeds of dog is unlikely to be as close as that between Thoroughbreds and other breeds of horse. The Thoroughbreds originated from the crossing of Arabian ponies with native horses of Britain in the 17th and 18th centuries (Willett, 1970). The relation of these native breeds of British horse to present-day breeds of England, Scotland and Ireland is unclear, although Jones & Bogart (1971) suggest that the Connemara was a prime contributor to the Thoroughbred breed. Wymalen (1950) says that many native breeds of British pony "have received from time to time some infusion of Oriental or Arab blood", and that some larger breeds such as the Cleveland Bay, have had genetic contribution from the Thoroughbred breed. The Thoroughbred breed is therefore closely related genetically to some of the other horses with which it is compared in this study. Thus if differences are established in this study between Thoroughbreds and the other horses (a classification which includes first and second cross Thoroughbreds), even greater differences between the Thoroughbred breed and breeds totally uninfluenced by the Thoroughbred breed may exist. Also the grouping of dogs or horses of different breeds together and comparing then en bloc with Greyhounds or Thoroughbreds respectively has the disadvantage that the variability within the other animals may be so large as to preclude statistical significance between them and "athletic" animals.

2.4.2.2. Mathematical analysis relative to samples

Tulloh (1964) has shown that within the bovine, ovine and porcine species, variation in muscle and bone weights (but not fat weights) is explained almost entirely by the effect of body size, and that comparisons of animals on the basis of body composition may be invalid unless compared at the same body sizes. Ideally comparisons should be made only between animals having representatives at similar values of an independent variable. However Tulloh (1964) indicates that this condition may be met by designing experiments which can be analysed using regression techniques.

Few animals of equivalent body size were available for comparison with adult Greyhounds and Thoroughbreds; similarly, because of the large body size of young athletic animals they frequently have independent variable values which are similar to those of adults in other breeds. As it was not possible to collect specimens in this investigation to provide an ideal population for allometric analysis in some instances, particularly in the horses, data is not always evenly distributed along the x axis. Also due to lack of a complete set of data for each animal, different comparisons frequently utilize different animals.

Despite the shortcomings of drawing conclusions from measurements made on animals of different sizes (Tulloh, 1964), comparisons of tissue proportions and distributions in mature animals of different sizes have been investigated as well as the development of these proportions and distribution.

2.4.3 Measurements on live athletes

Human physiques (based on measurements of shape in live humans) may be classified into three broad somatotypes - ectomorphs, mesomorphs and endomorphs, Sheldon, Stevens & Tucker (1940). Tanner (1964) indicates that Olympic athletes are more mesomorphic than a sample population of students in America or England and suggests that a "proper" physique may be necessary for an individual to reach Olympic qualifying standards.

Tanner (1964) also demonstrates differences in relative limb and trunk skeletal measurements in Olympic athletes and relates these to the events in which these athletes excel e.g. 100 m sprinters and marathon runners have relatively shorter legs (the sprinters are however more "heavily muscled" than the long distance runners) than middle distance (400 m and 1,500 m) runners. He believes that differences in bone proportions emerge by a process of natural selection rather than as a result of training.

As is the case with human athletes, individual Greyhounds and Thoroughbreds may perform optimally over a specific distance on the track although it is recognised that some animals perform well over a greater range of absolute distances (i.e. recognised short and long distances for their type) than human athletes (Clarke, 1965; Tesio, 1958). This may indicate that certain athletic animals are more "multipurpose" than are human runners; or alternatively of course, differences between short distance or long distances in human track events may be relatively greater for man than are the conventional flat race distances for dogs and horses.

All Greyhounds and Thoroughbreds may - due to their commitment to perform well in track events, be considered in the same context as human Olympic athletes. However somatotyping these breeds by measurements on live specimens has not been attempted in the present study due to the difficulty of obtaining repeatable accurate measurements on live animals (Walton & Hammond, 1938). In any case it is interesting in view of Tanner's (1964) finding of differences between various human athletes that Thoroughbreds which are likely to show speed (of running) "have size and substance, depth of chest, prominent muscles and good girth of cannon bone below the knee whereas the typical stayer is smaller and more often of light build, lean and with less prominent muscles" (Tesio, 1958). A similar description of Greyhound "sprinters" and "stayers" is offered by Clarke (1965). This study does not attempt to correlate the observed differences of the measured parameters between individuals with their track performances (or that of their progenitors).

2.4.4 Factors controlling effective limb length

The effectiveness of forces created by an animal in order to propel itself from or along a surface will depend on the efficiency with which these forces are applied to the surface. If two animals of the same liveweight but different limb length move their limbs at similar frequencies, then the animal having the longer limb length has longer strides and moves faster over the ground.

2.4.4.1 Angles of articulation of limb bones

The length of the limb is a function of the angles with which the limb bones articulate as well as of the lengths of the bones. Adams (1962) supports the idea that there are variations in the angles of articulation of the bones of the forelimb between different breeds of horse. The angle of inclination of the scapula from the horizontal probably offers the greatest scope for variation, as it is suggested that sloping shoulders favour fast running in dogs (Clarke, 1965) and horses (Hayes, 1904). The measurements of Goubaux and Barrier (1892) indicate that the mean inclination of the scapula in fast horses is 55° measured from the horizontal at the shoulder joint, whereas it is between 65° and 70° in those used for slow work. Although variations in the angles of other joints of the forelimb and the joints of the hindlimb are supposed to exist between fast running and other types of horse (Goubaux & Barrier, 1892; Hayes, 1904; De Vine, 1946; Wymalen, 1950) there are no recorded measurements to support this concept. Moreover, Krüger's (1939) data comparing Trakehenen (racing-type) and Mecklenburgh (heavy-type) horses indicate that there is no variation in limb joint angles (including slope of the scapula) between these breeds and that no variation in joint angles occur within the breeds during growth.

Thus differences in the angles of inclination of joints of standing horses appear slight, if they are present, involve mainly the shoulder (probably for dogs also). Differences in effective limb length are more

likely to be due to differences in the sum of bone lengths. In the present study therefore the relationship of limb length, angle and inclination of joints and range of movement of joints has not been investigated.

2.4.4.2 Limb bone lengths

The general view that animals noted for their high speed of running are characterised by having long legs in relation to other parts of their body (Hildebrand, 1968) is given support within the equine species by Hayes (1893), and Hammond (1940). These authors show that the limb length below the trunk relative to trunk length is greater in Thoroughbreds than in heavy (draft) horses. Although Jones & Bogart (1971) indicate that there are variations in the numbers of vertebrae between members of different breeds of equidae, it is unlikely that this small and infrequent variation constitutes a significant factor in the limb length: trunk length ratio.

Maximum bone lengths were used in this study rather than distances between effective articulations because measurement of bone length is more accurate on dead animals. The combined propodial and epipodial segment lengths were taken to represent limb lengths although it is recognised that these segments in the horse are relatively longer in the adult than in the fetus, (Ewart, 1894). The data presented in section 2.3.1.1 indicate that the growth rate of the combined length of the propodial plus epipodial bones of the forelimb (humerus and radius/ulna) and hindlimb (femur and tibia/fibula) is greater relative to body weight in the dog, but less or dimensionally proportional to body

weight in the horse. Although there is no significant difference between the two types of dog, the bone lengths in the adult Thoroughbreds are greater than in controls and a similar tendency exists between the young. This would support the concept that the Thoroughbred has relatively long legs. Hayes (1893) found more obvious differences, perhaps because he used a more homogenous population of controls (i.e. Drafthorses).

2.4.4.3 Relative bone lengths within limbs

Although the total length of the combined propodial and epipodial segments of the limbs may not vary between the two types of dog and horse over all their growth stages it is possible that their length relative to each other may. Thus Howell (1965) and Hildebrand (1968) find in various species that the length of the epipodial segments relative to the propodial segments increases with relative adaptation for high speed running. Within the equine species, Goubaux and Barrier (1892) conclude that an elongated epipodial segment relative to the propodial segment in both fore and hind limbs facilitates fast running.

Evidence to the contrary is presented by Krüger (1939) who finds that there is no significant difference in this ratio between the fast running Trakehnen breed and the heavy Meklenburgh breed. Similar observations were made by Ewart (1894) on the lengths of the radius and ulna relative to the humerus in two Thoroughbreds (Hermit and Eclipse) and a Shetland pony. Ewart (1894) also noted that the fetuses of "well bred" (fast-running) horses have higher epipodial : propodial ratios than other fetuses.

This comparison was made at different stages of development, however, and there is evidence of disproportionate growth of limb components (Ewart, 1894; Lebre, 1897; Krüger, 1939, Hammond, 1940; Green, 1970). In cattle also, Berg & Butterfield (1975) were unable to show breed differences in limb segment length above the carpus and tarsus.

This study confirms that there is no significant difference in relative bone lengths between athletic and non-athletic animals. However the propodial segment grows at a similar or greater rate relative to the epipodial segment in horses but at a lesser rate in dogs.

No significant sex-dependent bone length correlation has been found in the present study. Although Brannäng (1971) found that either ovariectomy or castration in cattle causes reduction of growth in length of the proximal limb bones and an increase in length of the distal limb bones these effects may be attributable to a general effect of the loss of steroid hormones.

In the present study the metapodial segment lengths have not been measured since a large number of bones would have been involved and also because the epipodial segment length bears a close correlation to metacarpal length in adult horses (Ewart, 1894; Hayes, 1904; Krüger, 1939). Hence comparison of propodial with metapodial segment length should give similar results to a comparison of propodial with epipodial segment length in the forelimbs, and similarly in the hindlimbs (Krüger, 1939). It appears that there is no significant difference in the epipodial : propodial ratio between the two types

of horse and dog.

It is concluded that the athletic performance of Thoroughbreds is enhanced by their possessing relatively longer legs although composed of bones of similar proportional lengths than their fellows. The limbs of Greyhounds are not relatively longer than those of their fellows, and are also composed of bones of similar proportional lengths.

2.4.5 Bone quality

Hayes (1904) states (without giving data) that "the race-horse, like all quadripeds of which speed is a chief characteristic, has comparatively slender bones of extremely dense texture". No difference in specific gravity of the bones of "athletes" and "non-athletes" was found in this study. There appears to be only one study concerning the specific gravity of dog or horse bones and that is where Hedhammer, Krook, Kallfelz, Schryver & Hintz (1974) show that variations in nutrition do not alter the specific gravity of limb bones of growing Great Dane dogs.

The increase in bone density with growth in the dogs and the similar trend in most of the horse bones is obviously related to the increase in ash and calcium with advancing age, occurring in vertebrae, ribs and femur of chickens, turkeys, sheep, pigs and cattle (Field, Riley, Mello, Corbridge & Kotula, 1974).

2.4.6 Natural frequency of hind limbs

On the lines of the pendulum theory if two limbs of unequal length but with their mass similarly distributed, are allowed to swing freely under gravity without being acted upon by forces, the foot of the longer limb will cover a greater distance in a cycle resulting in a longer stride length than that of the shorter limb. It will however have a lower frequency of oscillation although this could be increased if its mass were brought nearer the pivot. If the two limbs are required to swing at a rate higher than their natural frequencies of oscillation then the limb with the higher natural frequency will require the smaller additional force.

This study indicates that the hindlimb - which in terrestial animals is supposed to be better adapted for running than the forelimb (Howell, 1965) - of the adult Greyhound has a greater proportion of its mass nearer its pivot, i.e. the hip joint, than in the adult other dogs (Section 2.3.2). However some dogs have proximal hindlimb component weights greater than those of young Greyhounds even though the distal hindlimb components are similar in the two groups (Fig. 4). This may be explained by the greater "maturity" of the musculoskeletal system in the other dogs sampled since in both types of dog the femoral muscle group (the larger part of the proximal limb component) grows at a greater rate than the distal hindlimb muscle group (Section 2.3.6).

Disparity in musculoskeletal maturity is even more significant in the comparison made in this study between

Thoroughbreds and the more mature other horses; this factor is probably responsible for the greater proximal limb component weights in the other horses at the lesser distal limb component weights. However the growth potential of the proximal component in the still immature Thoroughbreds might well alter the situation so that the adult Thoroughbreds rather than the other horses would have a greater proximal hindlimb component weight. The greater proportional mass of the femoral muscles relative to liveweight in the Thoroughbred adults than in the adult other horses and also the greater femoral muscle : femur ratio in the adult Thoroughbreds (section 2.3.8) supports this contention as the femoral muscles are the main constituents of the proximal hindlimb component.

Despite the difficulties of comparing dogs and horses of different sizes and maturity, it is concluded that in adult Greyhounds and, to a lesser extent, in adult Thoroughbreds, there is an anatomical basis for a higher stride frequency than in members of their species of similar body size, due to the location of the greater mass of the hindlimb near the pivot of the limb.

2.4.7 The effect of selection for running ability on the proportions of muscle, bone and fat in dogs and horses

The ideal animal for the meat producer is one which produces the maximum amount of meat (muscle altered by post-mortem processes) in the shortest time using the cheapest fodder. Because of their mechanical function in the body, muscle and bone are associated closely anatomically. However, the reason for the proximity of fat to these tissues is not clear. Because of the commercial importance of the relative proportions of fat, muscle and bone in various "cuts" of meat, the carcasses of meat-producing animals have been the subject of numerous and variously analysed studies (see reviews by Tulloh, 1964; Butterfield, 1974). The proportion of muscle, bone and fat in an animal are related to the functions of these tissues and to the habitus of the whole animal. In this context muscle and bone may be considered to have primary support and locomotory functions, their metabolic functions being secondary, whereas except in certain anatomical sites, fat may be considered to have only a metabolic function. Therefore the investigators of locomotory and of meat-producing capacity in animals have a common interest in skeletal muscle but for different reasons, either because of its work potential or because of its food value.

The economic significance of muscle as meat has led to most of the investigations concerning the proportions

of muscle, bone and fat in animals to be carried out on sheep, pigs and cattle. Within these three species the proportions of muscle and bone bear a direct relationship to body weight and as carcass weight increases the proportion of carcass muscle remains roughly constant while that of carcass bone falls (Tulloh, 1964). However nutritional extremes that produce quantitatively variable fat deposition can obviously alter this relationship (Boccard, Le Guelte and Arnoux, 1964; Boccard and Dumont, 1970). Elsley, McDonald & Fowler (1964) conclude, by re-analysing the data of McMeekan (1940a,b,c) on Large White pigs and that of Pálsson & Vergés (1952) on cross-bred lambs, that nutritional extremes have little effect on either of muscle or bone provided the animals are compared at the same total muscle plus bone weight.

Muscle and Bone

Davies (1974a) by analysis of his own data on Large White and Pietrain pigs and that of Tulloh (1964) based on cattle, sheep and pigs concludes that between species maturity has a greater effect than absolute body size on the relationship of muscle to bone in a carcass. This conclusion is based on the similarity of muscle to bone ratios in the newborn sheep, pig and ox, although the ratios increase with growth in the three species - they are similar in adults. Since at similar body sizes differences exist between species in the proportion of muscle and bone so also do breed differences within a species exist as shown in cattle (Berg & Butterfield, 1966; Dumont & Boccard, 1967; Mukhoty & Berg, 1971), sheep (Fourie, Kirton &

Jury, 1970) and pigs (Dumont, Schmitt & Roy, 1969; Davies, 1974a).

The disparity of maturity and body size is apparent in the comparison of the growth of muscle and bone between the two types of horse. Mature ponies have higher muscle to bone ratios than young Thoroughbreds compared at similar liveweights or at muscle plus bone combined weights.

Lawrie (1974) quoting the data presented by Callow (1948 & 1961) states that the three domestic species used for meat production carry 30 - 40% of their liveweight as muscle when slaughtered. The present study shows that adults of both types of dog and horse have a greater proportion (57% in Greyhounds, 44% in adult other dogs, 44% in the largest young Thoroughbred and 42% in the adult other horses) of muscle than this figure. The very high proportion of muscle in the adult Greyhounds (over half of their liveweight) appears to be unique for terrestial mammals. Nevertheless the muscle to bone ratio in 800 kg cattle (4·6) 60 kg sheep (4·8) and 60 kg pigs (3·5) as calculated by Davies (1974a) from the data of Tulloh (1964) compares favourably with the mean ratios of the adult Greyhounds (4·7), other dogs (3·6), adult other horses (3·5) and the largest Thoroughbred (3·6). However, it must be remembered that total bone occupies a smaller proportion of liveweight in meat-producing animals - 7% in both sheep and cattle (Lawrie, 1974); compared to 12% in the horses and dogs in this study. Therefore although the more cursorially adapted animals - dogs and horses- have a high proportion of muscle relative to their liveweights, this

is not associated with a higher muscle to bone ratio than meat animals.

Bone forms a greater proportion of liveweight in young sheep, pigs and cattle than in older members of their species, whereas the reverse is true for muscle (Pálsson, 1955). The present investigation demonstrates this trend in dogs and horses also. Thus when the growth of muscle and bone in the two types of dog is assessed in relation to liveweight, there is a greater rate of development of muscle in the Greyhound and of bone in other dogs. But when muscle or bone weight are considered separately in relation to combined weight, it is found that the difference between the two types of dog is due to a slower rate of development of bone in the Greyhound. The muscle weights in both types of dog are remarkably similar at the same muscle plus bone combined weight (Fig. 8), but since small alterations in the larger component - total muscle weight - could alter the relationship of total bone compared with combined muscle plus bone weight the apparent difference in the rates of bone growth during development may not be meaningful. However since the proportion of muscle increases and bone decreases with development the Greyhounds may be considered to have a greater "maturity" of their musculoskeletal system. It is apparent from the growth rate of muscle and bone in both types of horse (Fig. 9) that the difference in the two types of horses in the growth of these tissues resembles that between the two types of dog.

Although no previous study has been carried out relating the carcass composition of dogs and horses to their athletic ability, the proportion of muscle and bone in the carcasses of weanling foals of the Belgian draft (Butaye, 1966) and mixed breeds of "working horse" (Deskur & Doroszewski, 1966) has been investigated for meat production purposes. The foals in both studies have 71% muscle on the carcass equivalent to 44% of liveweight) with a mean muscle to bone ratio of 3·56 (a similar figure to the largest horses in the present study).

This study indicates that selection for speed of running a criterion apparently unrelated to meat production is associated with elevated proportions of muscle and bone in athletic animals compared with other members of their species. It is a matter for debate whether selection of meat producing animals based on visual appraisal is meritorious or not (Butterfield, 1974), Davies (1973) comments that 30 years of genetic selection on the Large White pig (the best breed of British pig, which because of its reproductive rate, should offer considerable scope for genetic improvement) has failed to improve their muscle to bone ratio. It is possible therefore that breeds which have a high ratio of meat to bone on their carcasses such as the Pietrain pig or European draft breeds of cattle have arisen without having undergone "selection" by man for this trait.

Fat

Although the distribution of fat is an important economic consideration in meat-producing animals

(Butterfield, 1974) total body fat only has been measured in the present investigation and its location in the body has not been studied. It is apparent from this study and indeed to the layman that although Greyhounds may lay down fat when not in training they are not able to store as much fat as do other breeds of dog although fed on similar diets. It is similarly equally apparent to the horseman, though it has not been so obvious in this study, that Thoroughbreds lay down less fat than ponies even if both are fed on similar diets. Tulloh (1964) finds that variations in carcass composition of sheep, pigs and cattle are due primarily to variations in the quantity of fat in the carcass; he suggests that efforts to change body composition should be devoted to altering the rate of fat deposition. In children, Brook, Huntley and Slack (1975) find, by using skinfold thickness as an index of the amount of fat, that there are appreciable differences between limb and trunk fat and in the amount of fat between sexes at different ages. They state that environmental factors contribute more to fat quantity in younger children while over ten years of age, hereditary factors are more important. The present study also suggests that despite variations in diet and exercise regimes, some genotypes lay down less fat than others of the same species. The mechanisms controlling fat deposition in athletic and non-athletic animals is unknown but may be related to their capacity to metabolise fat during exercise.

2.4.8 The effect of selection on the distribution of muscle and bone

2.4.8.1 The distribution of muscle in relation to athletic ability

It is generally recognised that in the galloping canine, propulsion is derived from its back and hindlimbs, while the galloping horse is propelled predominantly by its hindlimbs and very little by the supportive movement of its back (Hildebrand, 1959). It has been shown (sections 2.3.4 and 2.3.5) that relative to liveweight and total muscle weight, the weight of the m. longissimus and femoral muscles is greater in the adult Greyhound than in other dogs and that of the hindlimb muscle groups in Thoroughbreds greater than in other horses. Thus this finding indicates a greater propulsive capacity in the musculoskeletal parts of athletic animals most relevant to propulsive effort.

Pálsson (1955) states that the developmental changes demonstrated by McMeekan (1940 a,b,c and 1941) in the pig and Vergés (1939 a,b), Pálsson and Vergés (1952) and Wallace (1948) in the sheep may be summarised in terms of growth waves of cranio-caudal and disto-proximal orientation. Davies (1974b) agrees with this statement, demonstrating cranio-caudal and disto-proximal growth gradients of increasing growth in pigs, but also shows that in the Pietrain bréed there is a preferential development of the muscles anatomically best located for propulsion - m. longissimus and the femoral group.

The present investigation also demonstrated cranio-

caudal and disto-proximal gradient of growth rate in muscle in the dog but with exaggeration of these gradients in the Greyhound. Cranio-caudal and disto-proximal gradients are not so apparent in the horse as in the dog in this study although the femoral muscle group is best developed in both types of horse. These gradients are apparent in the change in shape of the Greyhound and Thoroughbred during growth as depicted by the Cartesian transformations shown on Figs 19 and 20 respectively. The reasons for this disparity in growth pattern between the dog and horse may be related to the different locomotory patterns of the dog and horse, the smaller range of liveweights studied in the horse (only a 10 fold increase in liveweight) than in the dog (a 60 fold increase in liveweight), or the sampling problems of this study; the relative significance of these factors cannot be determined.

Therefore this study shows that the animals selected for speed of running have an enhancement of the normal growth gradients of the non-athletic members of their species with a preferential development of those parts of their bodies which would be desireable in meat-producing animals. Thus the difference between the muscle distribution of Pietrain and of Large White pigs (Davies, 1974b) parallels the difference between Greyhounds and other dogs, while the difference between Culard cattle and normal cattle (Vissac, Menissur and Perrean, 1971) parallels that between Thoroughbreds and other horses.

The similarities in altered body composition in athletic and meat-producing animals merits further investigation into the determination of the stimuli operating in the latter. The findings also suggest that the selection of meat producing animals might be based on functional criteria.

A further point concerns the incidence of distal limb injuries in Greyhounds and of flexor tendons in fast working horses. These injuries might well be related to the lesser development of the distal limb muscles of the fore and hindlimb of the Greyhound and forelimb of the Thoroughbreds. The mean pelvic muscle mass (corresponding to twice the percentage of liveweight of the femoral muscle group in this study) of Greyhounds used by Riser & Shirer (1967) 14·2 is similar to that in the present investigation - 15·2. The present study also indicates that Greyhounds have a higher proportion of femoral muscle to liveweight than other dogs whether these are of different, or similar adult sizes. Whether this is related to the development of hip dysplasia in other large breeds of dog, as suggested by Riser & Shirer (1967), or is due to the selection of the Greyhound for functional reasons requires further investigation.

Athletic animals have more fibres in their m. semitendinosus, a member of the femoral group, than their fellows (section 3.3.1.1.3). It is likely therefore that the greater mass of this muscle group in the athletic animals is due to a greater number of fibres in these muscles of the athletic animals.

2.4.8.2 The distribution of bone in relation to athletic ability

The greater weight of the limb bones of perinatal Thoroughbreds (i.e. 50 kg liveweight, section 2.3.7) may be related to the disparity in "maturity" of the musculoskeletal system of the two types of horse, i.e. the adult relationship of muscle to bone occurring at a lower liveweight in members of the other horses (ponies) than in the Thoroughbreds, as well as to the greater length of limb of the Thoroughbreds at this age. Schumacher (1972) shows that muscular actions have a formative influence on bones in the skull. The femur of the Greyhound is heavier than that of the other dogs and there is also a non-significant tendency for the Thoroughbred femur to be heavier than that of the other horses. In view of Schumacher's (1972) results, this may be associated with the greater development of the femoral muscle groups in these animals.

2.4.9 Circulatory potential

Although the various parts of the heart function differently and may, at least in the dog, grow at different rates (Kirk, Smith, Hutcheson & Kirkby, 1975), for the purposes of this study the heart is considered as a circulatory pump with a pumping capacity related to its weight (Grande & Taylor, 1965). In the Greyhound the heart forms a greater proportion of liveweight than in other dogs (Herrmann, 1925), and similarly in Thoroughbreds compared with other horses (Herrmann, 1929; Quiring & Baker, 1953). The present study confirms these findings and also indicates

that athletic animals have a greater heart weight relative to total muscle weight.

Kubo, Senta & Sugimoto (1974) demonstrate that increase in heart weight in the Thoroughbred is associated with the time (in months) spent "in training". A similar finding in rats is reported by van Liere & Northup (1957). The largest young Thoroughbred in this study (although not in training) had a higher ratio of heart weight to liveweight than all the other horses, with the exception of a Thoroughbred cross.

By analysing the data of Crile & Quiring (1940) from horses and dogs and Stewart (1922) from dogs, Brody (1945) shows that heart weight increases at slower rate than liveweight in these species. In accordance with this analysis Northup, van Liere & Stickney (1957) show that young dogs have a higher ratio of heart weight to liveweight than adults. In the present study immature "athletic" animals had heavier hearts (at similar liveweights or total muscle weights) than their "non-athletic" fellows. Since the rate of increase in heart weight with increasing liveweight (or total muscle weight) is smaller in athletic animals than in their fellows, but that this difference is reduced with increasing size. Even so, it is apparent from Figs 17 and 18 that within the ranges of liveweight observed in this study the difference persists.

Steel (1963) finds that the size of a racehorse's heart may be estimated by electro cardiography and suggests that this measurement may be an indicator of racing potential. It would appear that if such measurements are to

be meaningful they should be related to liveweight or preferably total muscle weight. Accepting that measurements of heart size are remote from those of stride length and stride frequency, (the dictators of running speed) an indication of the potential blood pumping capacity may be useful in certain athletes, if it is considered a limiting factor to athletic performance. Although Quiring & Baker (1953) find that the "anatomy of the Thoroughbred heart does not differ greatly from that of other breeds", investigation of the mechanisms of growth and normal hypertrophy of athletic animal hearts may prove a fruitful basis for the examination of pathological hypertrophy of the heart.

2.4.10 The effect of detraining

In this investigation only one year could be devoted to detraining Greyhounds. Such specimens were used to represent untrained adult Greyhounds, which are very rare and difficult to obtain.

As regards total muscle weight although this constituted a smaller percentage of liveweight in detrained than in trained Greyhounds, the difference between the two groups is not significant. M. longissimus is the only muscle in detrained Greyhounds to have a significantly lower ratio to liveweight or total muscle weight than in the trained Greyhounds. This result may be due to a greater disuse atrophy in this muscle than in all other muscles, possibly indicating its importance in propulsion. However the reverse argument that training produced hypertrophy of the muscle (by increasing fibre size) is not supported by Fig. 14 which shows that the values for the weight

of m. longissimus in young untrained Greyhounds lie above the regression line relating the weight of m. longissimus with total muscle weight in all the Greyhounds.

As detraining had little effect on muscle weight it is not surprising that it had less effect on bone.

The slightly lower heart weight in detrained Greyhounds suggests that training may effect heart size. However although immature (untrained) Greyhounds have in general similar ratios of heart weight to liveweight or to total muscle weight as the immature other dogs, these relationships for the largest Greyhound pup (23·5 kg liveweight) is nearer to that of the Greyhounds than the other dogs (Fig. 17). It is unlikely therefore that, at least as carried out in this study, detraining causes a marked reduction in heart size in the Greyhound. A similar finding is reported by Leon and Bloor (1968) for the rat.

2.4.11 The effect of sex and cross breeding

There is no difference between the growth rates of Thoroughbred colts and fillies up to 12 months of age (Wojciechowski, 1964; Green, 1969; McCarthy & Mitchell, 1974). Nor in the present study could significant sex differences be found in either species in any of the traits assessed. It is of course well recognised that male are larger than female adult horses (Brody, 1945); it is too often a matter for personal regret that female racehorses receive weight allowances over males in handicap races.

Horsemen recognise the potential of the Thoroughbred in transmitting its "conformation" to its progeny, but the small number of specimens obtained in this study precluded

statistical comparisons between Thoroughbred-cross horses only and pure Thoroughbreds or non-Thoroughbreds. However the proportion and distribution of muscle in the Thoroughbred-cross horses investigated in this study is similar to that expected for Thoroughbreds (Figs. 9, 15 and 16).

3.0 Part 2: MICROSCOPIC FEATURES RELATED TO LOCOMOTION

3.1 INTRODUCTION

The first part of this study investigates adaptations in the gross morphology of the skeletal muscle of athletic animals which may favour their cursorial ability. The second part investigates the quality of their musculature.

3.1.1 Structural features of muscle

The acceleration capacity of an animal is related to the propulsive force that it can produce in relation to the weight it has to propel (Gray, 1968). The force produced by a muscle is related to the transverse sectional area of the muscle (Hettinger & Müller, 1953; Bendall, 1969). Excluding connective tissue, the transverse sectional area of a muscle is the product of the number of fibres in the section and their mean area.

Numbers of fibres

The total number of fibres in the cross section of a muscle is defined at birth or very soon afterwards in the pig (Staun, 1963; Davies, 1972), rat (Enesco & Puddy, 1964) and mouse (Rowe & Goldspink, 1969). Such studies may be complicated by the presence of intrafasicular-terminating fibres which may influence the number of fibres seen in a transverse section (Swatland & Cassens, 1972). Within the same species there may be a genetic variation in the number of muscle fibres in a given anatomical muscle, as in "culard" cattle (Ouhayoun & Beaumont, 1968; McKellar, 1968), and in certain breeds of pigs (Staun, 1963) and in mice selected for high body weight at five and ten weeks of age (Byrne, Hooper & McCarthy, 1973). Therefore it is possible

that selection within the equine and canine species may cause variations in the numbers of fibres in similar muscles of different breeds of horse and dog. This possibility will be investigated in this study.

Area of fibres

On the basis of the rate of diffusion of oxygen and other blood borne metabolites, Hill (1956) predicts that the diameter of muscle fibres should vary as the square root of the linear dimension of an animal. However, Julian and Cardinet (1961) find that the area of muscle fibres bears a constant relationship to body weight in different breeds of adult dogs. But Davies and Gunn, (1972) find no relationship between muscle fibre area and body size in the diaphragm of nine different species. Apart from hypertrophy during normal growth, other known sources of variation in fibre transverse sectional area are exercise, nutrition and the action of anabolic steroids.

Exercise results in an increase in the mean area of, but not the number of fibres in a muscle. This was originally demonstrated by exercising dogs a distance of 7 to 50 kilometres daily for 20 days, and increasing to 60 to 80 kilometres for the following 40 days (Morpurgo, 1897). Exercise helps equine muscles to become bigger and firmer (Stewart, 1969), suggesting changes in either fibre number or diameter.

Starvation causes a reduction in fibre diameter (Goldspink, 1965). Although administration of large amounts of androgens early in post-natal life to neonatally

castrated rats results in an increase in the number of fibres in the levator-ani muscle (Homna, Saito, Tsunenari & Maekawa, 1972), Venable (1966) finds that testosterone given to castrated mature rats has an effect on fibre area only, producing hypertrophy. Castration of adult male rats without testosterone administration results in an atrophy of the fibres (Venable, 1966). The growth of the head and neck muscles of the male guinea-pig (Kochakian, Tillotson & Austin, 1957), and the neck muscles of bulls (Berg & Mukhoty, 1970) and rams (Lohse, 1973) is also dependent on anabolic hormones.

Thus although the number of fibres in the muscles of an adult animal appears to be predominantly genetically controlled, the mean transverse sectional area of the fibres, is strongly influenced by environmental conditions. Therefore a better indication of the potential athletic ability of a breed may be obtained by comparing fibre number rather than mean fibre area.

3.1.2. Metabolic features of muscle

The ability of skeletal muscle to split and resynthesise ATP determines the speed and repetitivity of limb movement. However intramuscularly ATP may be synthesised from a number of sources of chemical energy. Exercise studies on humans have established that the two intramuscular anaerobic energy sources for ATP resynthesis- creatine phosphate and glycogen are used during short term high intensity exercise: glycogen is more important quantitatively (Margaria, Aghemo & Sassi, 1971). If the duration of exercise is extended and at the same time

its intensity is recorded, energy sources needing oxygen for their combustion are used to varying degrees depending on the maximal oxygen consumption of the participant (Margaria, 1972). Glucose (Keul & Doll, 1973) and free fatty acids (Keul, Haralambie & Tritten, 1974) are brought to working muscle via the blood stream during intermittent exercise. However intramuscular triglycerides are used in animals during exhaustive exercise (Reitman, Baldwin & Holloszy, 1973) although the intramuscular glycogen store determines the duration of long term heavy exercise (Bergström, Hermansen, Hultman & Saltin, 1967).

Thus the particular energy store used for either anaerobic or aerobic metabolism in the musculature of an individual of a given species, depends on the intensity and duration of the exercise, as well as the capacity of its skeletal muscle to deal with different types of energy sources. However in order to compare this intrinsic quality of muscle, the relative ability of the fibres to synthesise ATP either by an anaerobic or an aerobic method and their capacity for rapid splitting of ATP, must be established.

3.1.2.1 Types of fibre

Ranvier (1873) noted that the fibres of rabbit and rat red muscles were more granular than those of the white muscles; he concluded from his physiological experiments on whole muscles that the red or granular fibres contract more slowly than the white ones. Schaefer (1913) states that there are two types of fibres in the horse muscle, and that the muscles contain different proportions of the two types of fibre. On the evidence of staining for

"muscle granules" he shows that the proportion of clear fibres is higher in light weight than in heavy horses. Schaefer considered that the difference between these fibre proportions in the horse was not due to different levels of nutrition, because although the circus horses used in his study had more fibres of the dark type than did the hunter or racehorse, they were not fed any better. Tesio (1958) advances the hypothesis that in Thoroughbreds different types of muscle fibre may be associated with the ability to run quickly over different distances, 'stayers' having "dark red" fibres, and 'sprinters' "pink" fibres.

3.1.2.2 Aerobic capacity

The ability of muscle to sustain prolonged activity is well correlated with its content of respiratory enzymes (Lawrie, 1953). Training by means of submaximal exercise produces an adaptive increase in exercise capacity. Holloszy (1967), exercising rats on a treadmill, at first for 10 minutes per day at a speed of 22 meters per minute but progressing to a level of 120 minutes per day at 31 meters per minute, finds an adaptive increase in exercise capacity in the gastrocnemius muscle is associated with a twofold rise in respiratory enzyme activity. In biopsies of the human vastus lateralis muscle physically fit individuals show higher aerobic capacities than those who are less fit (Bjornthorp, Fahlen, Holm, Schersten & Szostak, 1970). Horse muscle appears to have a relatively higher capacity for oxygen transport than other species: Drabkin (1950) finds that the ratio of total muscle cytochrome C to the 0·7 power of body weight is seven times

greater in the horse than in the rat, dog, man and ox. Adams, Denny-Brown and Pearson (1953) state that highly trained horses have muscles containing more myoglobin, than untrained horses. Since there is a direct correlation between the content of myoglobin and oxidative enzyme activity in the muscles of the horse (Lawrie, 1953), it might be assumed that the horse has a greater capacity for prolonged muscular work than other species.

The proportion of predominantly oxidative-type fibres in a muscle can be determined by the histochemical demonstration of enzymes of aerobic metabolism. The proportion of such fibres increase in the plantaris and gastrocnemius of adult guinea-pigs (Barnard, Edgerton & Peter, 1970a), after 18 weeks of treadmill running, by which time they are able to run for 30 minutes per day at speeds of up to 49 metres per minute, for five days a week. Young guinea pigs, trained to run on motor-driven treadmills, at 30 metres per minute for 60 minutes per day, had a higher proportion of aerobic fibres in their plantaris muscle compared to age matched controls. A detraining period of 4 to 16 weeks causes the proportion of aerobic fibres to return to that found in age-matched controls (Faulkner, Maxwell & Lieberman, 1972). Using electron microscopy, Gollnick and King (1969) have shown that exercise causes both an increase in mitochondrial numbers and a change in their conformation in the gastrocnemius of rats trained for 10 weeks. A similar result is found by Kraus, Kirsten and Wolff (1969). Schaefer's (1913) observation that the psoas muscle of a "much worked"

horse contained a greater proportion of "muscle granules", suggests that the oxidative capacity of equine muscle is also increased with exercise, since the "granules" might well have been mitochondria or lipid droplets, both associated with oxidative metabolism.

3.1.2.3 Anaerobic capacity

Several studies (Saltin & Hermansen, 1967; Edgerton, Barnard, Peter, Simpson & Gillespie, 1970; Edgerton, Simpson, Barnard & Peter, 1970 and Margaria, 1972) suggest that regular exercise increases the oxidative ability of muscle and so spares anaerobic energy production. Nevertheless it appears that a considerable period of intermittent exercise is necessary for muscle to adapt in this manner.

As with oxidative capacity, histochemical studies of phosphorylase activity and glycogen content have been used to supplement the estimates of anaerobic capacity made on homogenates. In man, Saltin and Hermansen (1967) show that enhanced glycogen synthesis occurs after and as a result of exercise; after exercising one leg only the glycogen content is higher in the muscles of the exercised than of the non-exercised leg. Similar biopsy studies in the dog may prove difficult, since Chapler and Moore (1970) find that the distribution of glycogen in the canine gastrocnemius is uneven. In sodium pentobarbital anaesthetised guinea-pigs, Edgerton, Barnard et al. (1970) show that electrical stimulation of the motor nerve to the gastrocnemius for one hour at five pulses per second selectively reduces total phosphorylase activity in the

fibres as determined histochemically; this was associated with glycogen depletion immediately after electrical stimulation. In the muscle as a whole, loss of glycogen and phosphorylase activity is less pronounced in animals previously trained by treadmill running. The programme of treadmill running was one of increasing training schedule for nine weeks. At the ninth week, the guinea-pigs were exercised for 50 minutes a day and during this time they were subject to 20 minutes of sprints as well as endurance running - 40 metres per minute uphill on a 1 in 50 gradient. Forty eight hours were allowed to elapse after the animals last bout of exercise before the animals were subjected to electrical stimulation studies. Since it was found that the proportions of oxidative type fibres had increased with exercise Edgerton Barnard et al (1970) conclude that glycogen is preferentially spared in the "red" fibres as a result of their capacity for producing energy from oxidative phosphorylation. However the amount and form of glycogen depletion can vary with the type of usage: for example, treadmill running in untrained guinea-pigs at 26 metres per minute (this speed is well below that at which guinea-pigs can run in the experiment quoted above) for five minutes, 10 minutes, or to exhaustion, preferentially depletes glycogen in the aerobic fibres of plantaris at the higher levels of exercise (Edgerton et al. 1970b). This may be due either to a higher initial glycogen content in the white than in the red fibres or to the preferential use of red fibres during treadmill running by guinea-pigs running at low speeds.

In the cat, motoneurons innervating oxidative-type fibres are more easily stimulated than those controlling fibres with a low aerobic capacity (Henneman & Olson, 1965); this also may account for a preferential use of aerobic fibres during treadmill running.

Similar differences in the effects of varying schedules of exercise are seen in other species. In trotting horses the glycogen depletion pattern in the muscle fibres of the gluteus medius varies with the intensity and duration of trotting (Lindholm, Bjerneld & Saltin, 1974). After four hours of trotting at low speed - 18 kilometres per hour, glycogen is reduced in the slow-twitch oxidative fibres. Lindholm et al. (1974) suggest that these fibres are heavily employed in performing this type of exercise. However during short term maximal trotting speed, consisting of 6 runs of 400 metres at a speed of 46 kilometres per hour with ten minutes rest between runs, the glycogen content of the fast-twitch fibres is markedly reduced - suggesting a greater usage of these types of fibres in fast trotting. In human subjects performing short term high intensity exercise, requiring 150% of their aerobic power, on a bicycle ergometer Gollnick, Armstrong, Sembrowich, Shepard and Saltin (1973) find that the fast-twitch anaerobic fibres become glycogen-depleted first. However Gollnick et al. (1973) find that the aerobic slow-twitch fibres are the first to become depleted of glycogen as a result of prolonged exercise of lower intensity on a bicycle ergometer. Likewise by using glycogen depletion in fibres as an indication of fibre utilization

Gillespie, Simpson and Edgerton (1974) find that while bushbabies run on a treadmill at a speed of 1·75 metres per minute the slow-twitch oxidative fibres become glycogen depleted first, then the fast-twitch oxidative-glycolytic fibres and finally the fast-twitch glycolytic fibres. However when these animals are made to jump by increasing the treadmill speed to 2·4 or 2·9 metres per minute the order of usage of fibre types is reversed, in that fast-twitch glycolytic fibres are first to be depleted followed by the fast-twitch oxidative-glycolytic fibres and finally the slow-twitch oxidative fibres.

Such findings may demonstrate preferential utilization of different fibre types at different intensities of trotting in the horse, or of cycling in man and during running and jumping in the bushbaby. However the mode of stimulation of various types of work leading to usage of different types of fibres, remain to be elucidated.

3.1.2.4 Capillary density in muscle

Oxygen is brought to muscle fibres through capillaries, therefore capillary density may be used as an index of the capacity of muscle for aerobic metabolism. Carrow, Brown and van Huss (1967), using India ink injection studies, find an increase in the number of capillaries after forced and voluntary exercise associated with both red and white fibres. Krogh (1919), also using India ink, finds 400 capillaries per mm^2 in the gastrocnemius of the horse, and 2,500 capillaries per mm^2 in the semimembranosus of the dog. Capillary density in dog and horse muscle may also vary with athletic capacity.

3.1.2.5 Intrinsic speed of contraction

It is generally believed that the force generated by skeletal muscle results from the cyclic attachment and detachment of cross-bridges projecting from the myosin filaments and interacting with the actin filaments in such a way as to draw them towards the centre of the sarcomere. Cross bridge activity is energetically coupled to the splitting of ATP catalyzed by a part of the myosin molecule - the heavy meromyosin segment, which forms the movable cross-bridge (Fuchs, 1974).

Homogenates from fast-twitch muscle have a higher myosin ATPase activity than those from slow-twitch muscles (Bárány, 1967). It is possible to show this biochemical difference histochemically (Guth & Samaha, 1969). A close correlation between the cross sectional area of fibres with high myosin ATPase activity and the speed of contraction in the pectineus muscle of the dog is shown by Cardinet, Fedde and Tunell (1972). This has been demonstrated in a variety of mammalian muscles (Peter, Barnard, Edgerton, Gillespie & Stempel, 1972; Teig & Dahl, 1972 and Edström & Lindquist, 1973). Using intraneuron stimulation of the cat gastrocnemius Burke, Levine, Tsairis and Zajac (1973) find a correlation between myosin ATPase activity as shown histochemically and the speed of contraction of individual motor units.

Several studies have been carried out concerning the effect of exercise on the myosin ATPase activity in muscle, as demonstrated histochemically in tissue sections and biochemically in homogenates. Barnard, Edgerton and Peters (1970 a,b) show that, although the proportion of aerobic

fibres in crural muscles of the adult guinea-pig increases with exercise the ratio of fast to that of slow-twitch fibres is not altered, nor is there a difference in the time taken to reach peak tension, half relaxation time, tetanic fusion frequency, twitch to tetanus ratio or in the rate of tension development. Other histochemical (Maxwell, Faulkner & Lieberman, 1973) and physiological (Walker, 1968) investigations on adult animals confirm that the proportion of fibres, as differentiated by the myosin ATPase reaction, does not change, and that the contractile properties of the whole muscle does not alter significantly due to exercise. Bagby, Sembrowich and Gollnick (1972) support these findings by a combined biochemical and histochemical study on adult rats. Although Syrový, Gutmann and Melichna (1972) show in 14 day old rats that a swimming regime causes an increase in the proportion of fibres with a high activity of myosin ATPase in the soleus, this result may be attributed to the immaturity of the animals or to the muscles examined: such changes do not occur in the extensor digitorum longus of these animals nor in the soleus of mature (105 day old) rats subjected to the same regime. However in adult pigs Campbell, Onan, Thomas, Weirlich, Will, Cassens and Briskey (1971) demonstrate that the proportion of total fibre area composed of fibres with alkali-stable myosin ATPase increases with treadmill running. They exercised the animals for a period of one hour per day, at a speed of 2·4 kilometres per hour for two weeks. Also Gollnick, Armstrong, Saltin, Saubert, Sembrowich and Sheperd (1973) find in the human adult that a five month training period

of one hour per day for four days per week on a bicycle ergometer at a work load requiring 75 to 90% of the subjects maximal aerobic power, causes an increase in the relative area occupied by slow-twitch fibres in biopsies from vastus lateralis. These contrasting results may be due to the different species and exercise regimes studied.

In general myosin ATPase activity of homogenates has been shown to remain constant after exercise (Rawlinson and Gould, 1959; Hearn and Gollnick, 1961), although Wilkerson and Evonuk (1971) have shown that exhaustive exercise may increase the myosin ATPase levels. Thus a group of adult rats exercised by swimming unweighted for 30 minutes every other day for 10 weeks exhibited no change in myosin ATPase activity of homogenates of m. gastrocnemius, but a group weighted by 5% of their total weight and which swam to exhaustion every other day for six or 10 weeks showed a significant increase in enzyme activity. However Bagby, Sembrowich and Gollnick (1972) subjecting adult rats to a similar exercise regime to the one used by Wilkerson and Evonuk (1971) are unable to detect a change either in the proportion of slow and fast twitch fibres in m. gastrocnemius or in the myosin ATPase activity of homogenates from that muscle.

Thus there is not complete agreement from the earlier work as to the effects of exercise on muscle: different types and intensities of exercise may effect some fibres more than others. While it may be true that training does not increase the absolute number of one type of fibre in the adult, nevertheless, it may do so in the young animal:

even in the adult in two species at least the area occupied by a particular type of fibre may be changed by exercise. Can, therefore, the superior athletic ability of certain breeds of animals be associated with changes in their musculature, either in absolute number, or proportional areas of fibres with different mechanical properties?

3.1.3 Present investigation

The aim of this study has been to establish if selection for speed of running has caused an adaptive change in the mechanical or metabolic properties of skeletal muscle. This may be shown by changes in fibre number and diameter, histochemical patterns, capillary supply and biochemical aerobic capacity of the musculature of Greyhounds and Thoroughbreds in comparison to other members of their species. The semitendinosus muscle has been chosen as the principal muscle for this study because it is easily identified, has parallel fibres, and is one of the main propulsive muscles in both species. Samples were also taken from m. pectoralis transversus and m. diaphragma because these muscles have a simple fibre architecture and function differently during locomotion both from each other and from m. semitendinosus.

It was not considered possible to perform physiological studies on the musculature of the animals used due to sociological implications. The known difficulties of extracting myosin from muscle prohibited the biochemical estimation of its activity in this study (Bárány & Close, 1971). However an index of aerobic capacity may be obtained by histochemical and biochemical methods for

succinate dehydrogenase activity: this system is an indicator of aerobic metabolism and is of significance in the present investigation because oxidative metabolism takes place during submaximal exercise in the dog (Paul, 1970), and horse (Lindholm, Bjerneld & Saltin, 1973). The biochemical estimation of glycogen content and phosphorylase activity was not carried out but the anaerobic ability of muscle fibres has been demonstrated by the histochemical method for phosphorylase activity. The histochemical reaction for myosin ATPase has been used to distinguish fibres of fast and slow intrinsic speed of contraction. By using serial frozen sections and these enzyme techniques, histochemical profiles of the muscle fibres may be determined, permitting a comparison of the metabolic patterns of muscle fibres in the different breeds.

3.2 MATERIALS AND METHODS

3.2.1 Sources of material

The samples used for microscopic examination were taken from animals used in Part 1 of this thesis, and from six other animals. All the samples were from the left side. Three muscles were sampled - m. semitendinosus, m. pectoralis transversus and m. diaphragma. Samples were obtained pre-rigor and therefore were contracted. Appendices 5 and 6 list the samples taken, the types of animals from which they were taken and the data recorded from each sample.

3.2.2 Sampling and initial preparation of material

In both horses and dogs samples were taken from the costal diaphragm and superficial part of the m. pectoralis transversus lateral to the manubrium sterni. In dogs a complete transverse section of m. semitendinosus was taken from the mid-belly of the muscle. The transverse section of m. semitendinosus of horses was taken from where the muscle passes over the tuber ischii. The area of the fresh sections of m. semitendinosus in both species was measured by drawing the outline of the section when placed on ruled paper. The complete transverse section of this muscle in dogs and some young horses was subjected to histological and histochemical procedures. In the remainder of the horses samples from the most caudal part of the superficial region of the muscle were used.

Specimens for biochemical estimation of succinate dehydrogenase activity were removed from sites adjacent to the sampling sites.

Samples from horses were chilled (0 - 4°C) before being brought to the Royal (Dick) School of Veterinary Studies or the Physiological Laboratories, Cambridge, for microscopic preparation. Biochemical assays on samples obtained at the Equine Research Station, Newmarket, were carried out at the Station. Samples from Ireland taken prior to the day that they were flown to Edinburgh were frozen to -30°C and maintained at that temperature. On arrival at Edinburgh they were allowed to thaw before being prepared for microscopic examination. Samples from Ireland obtained on the same day as they were to be processed were chilled. Biochemical examination was not carried out on any of the samples from Ireland.

3.2.3 Biochemical estimation of succinate dehydrogenase activity

The method of Bocek (1964) as modified by Beecher, Cassens, Hoekstra and Briskey (1965) was used. Samples were cleaned of connective tissue and fat and 0·1 g of each was homogenised with 10 ml of 0·2M phosphate buffer (pH 7·5) in a "Quickfit" all glass homogeniser cooled by ice and powered by a 30 watt motor (Griffin & George). One ml of the homogenate was added to an incubation medium consisting of:

0·1 M Sodium succinate	1 ml
0·2 M Phosphate buffer (pH 7·5)	1 ml
0·002 M 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT)	1 ml

The reaction mixture was incubated for 15 min at 37°C and stopped by adding 3 ml of 0·6M trichloro acetic acid (TCA). The formazan produced was extracted into 7 ml ethyl acetate

by vigorous hand shaking followed by centrifugation at 1,000 r.p.m. for 2 minutes. The optical density of the ethyl acetate layer was measured at 490 μm, in a Unicam SP.600 spectrophotometer. The results were estimated from a standard graph prepared by determining the optical density of a range of concentrations from 5 to 50 μg of a commercial preparation of INT formazan in 7 ml of ethyl acetate. They are reported as mg of formazan produced per 15 min per g of fresh tissue. The mean of duplicate one ml aliquots of the homogenate from each sample was taken to represent the sample.

3.2.4 Procedure for microscopic examination

The complete transverse sections of m. semitendinosus were cut to about ½ cm thick with a brain knife. The other samples were trimmed into transverse blocks of about 2 x 2 x 0·5 cm. The samples were mounted on a piece of cork 5 mm thick which was frozen to a cryostat chuck. The samples were then frozen rapidly by plunging the chuck and tissue into dichlorodifluoromethane (Arcton 12, I.C.I.) cooled to its melting point of -158°C by liquid nitrogen. About 15 adjacent transverse serial sections were cut 10 μm thick in a cryostat at -20°C. The sections were mounted directly on to coverslips, and allowed to thaw and dry rapidly at room temperature. Fibre outlines, the activity of the enzymes succinate dehydrogenase, glycogen phosphorylase and myosin ATPase, and the presence of capillaries were demonstrated by the following methods.

Fibre outlines

Sections were fixed for 10 minutes in 4% formaldehyde, washed, and stained for 20 minutes in Ehrlich's haematoxylin.

Succinate dehydrogenase (E.C. 1.3.99.1, SDH)

Sections were incubated for 20 minutes at 37°C in a medium composed of:

0·2 M Sodium succinate	10 ml
0·2 M Phosphate buffer (pH 7·6)	10 ml
1 mg/ml nitro blue tetrazolium	20 ml

after Nachlas, Tsou, deSouza, Cheng and Seligman (1957). Gas bubbles frequently formed between the section and the coverslip; these were often eliminated by drying the section between washing and fixation in 4% formaldehyde.

Glycogen phosporylase (E.C. 2.4.1.1, GP)

Takeuchi's (1956) modification of the method of Takeuchi and Kuriaki (1955) was used. Sections were incubated for 3 hours at 37°C in a medium consisting of:

Glucose-1-phosphoric acid	75 mg
Adenosine-5-monophosphoric acid	15 mg
Glycogen	3 mg
Distilled water	22·5 ml
0·1 M Acetate buffer (pH 5·9)	15 ml
Insulin	1 i.u.
Ethanol	7·5 ml

1·5 gm of Dextran, MW 275000 was added to this medium after the method of Meijer (1968). The sections were subsequently washed, dried, fixed in absolute ethanol, dried, and stained with dilute Lugol's iodine for three minutes. Because the colour faded, iodine staining was repeated

immediately before subsequent use of the section.

Myosin ATPase

The calcium-cobalt method of Padykula and Herman (1955) was modified by substituting tris for the barbitone buffer to improve the buffering capacity of the medium. Sections were fixed for exactly two minutes in cacodylate buffered 4% formaldehyde at pH 7·0. Without fixation, the sections floated off the coverslip, and prolonged fixation affected the characteristics of the enzyme (Stein & Padykula, 1962; Guth & Samaha, 1969). Sections were incubated for 20 minutes at 37°C in a freshly made medium consisting of:

1·0 M Tris-(hydroxymethyl)-aminomethane (M.W. = 121·14)	8 cm^3
0·18 M $CaCl_2$ $6H_2O$ (2g/100 cm^3)	4 cm^3
ATP Disodium salt	60 mg
Distilled water	to 30 cm^3

this medium was then adjusted to a pH of 9·5 with 0·1 N HCl and made up to a final volume of 40 ml. The final concentration of ATP was therefore 2·4 mM. With two washes in distilled water between treatments, the sections were immersed in 2% cobalt chloride for three minutes and developed in dilute ammonium sulphide for one minute.

Capillaries

These were demonstrated immediately following incubation of the sections for myosin ATPase, by lowering the pH of the medium (Freiman & Kaplan, 1960; Padykula & Gauthier, 1963).

3.2.5 Histometric measurements and calculations

In dogs and small horses, mean fibre area was determined by sampling nine areas of the whole transverse section of m. semitendinosus - three superficial, three middle and three deep. In the dogs other determinations were carried out on the whole transverse section; in horses these were done on an area of the section corresponding to the sample site in the large horses. Histometric assessments were carried out on 800 to 1,000 fibres from smaller samples. Two squares of approximately 400 fibres each, about 20 fibre-breadths deep to the surface of the muscle and about 200 fibre-breadths apart were examined. The mean of the data from the two areas was taken to represent the sample.

3.2.5.1 Determination of mean fibre area and area of frozen section of m. semitendinosus

Mean fibre areas of the samples were estimated by back-projecting a haemotoxylin stained section on to a glass screen and counting the numbers of fibres within an area of known magnification. The areas of 3,000 to 3,500 fibres were assessed in complete transverse section of m. semitendinosus; and in samples, 800 to 1,000 fibres were assessed. The area of small frozen sections of m. semitendinosus was measured by drawing their outline of the section on transparent paper, their area was calculated and comparing the weight of the outlined paper with the weight of a known area of paper. The area of larger sections was measured on transparent ruled paper.

The total numbers of fibres in m. semitendinosus of

dogs and small horses was calculated from the mean fibre area of the muscle, and the area of the whole frozen section. In larger horses the total numbers of fibres was calculated from the mean fibre area of the part of the transverse section sampled, and the total unfrozen sectional area.

3.2.5.2. Method of establishing histochemical profiles of muscle fibres

Profiles of from 800 to 1000 individual fibres from the samples were established by back-projection on to a glass screen. First tracings of the fibre outlines from a haemotoxylin stained section were made on transparent paper. Each serial section was then projected in turn. The histochemical reaction of each fibre was indicated on the tracing; Figs.23-26 illustrate the type of material used. Where there was a continuous spectrum of enzyme activity between fibres, a simple division into "high" and "low" was made for each fibre, relative to the overall activity of fibres in each section. It was not possible to compare one sample with another, because of difficulties in standardisation of the preparation and processing of the material. This source of variation between samples in the quantitative data precludes the possibility of a comparison between breeds and species based on overall enzyme activity.

3.2.5.3 Estimation of the proportions and proportional areas of fibre types

The total numbers of fibres showing a low activity of myosin ATPase in the sections of m. semitendinosis of

dogs were counted by sampling the muscle at 0·6 mm to 2 mm intervals across its entire transverse section. The mean area of 50 to 100 of these fibres, sampled from the entire transverse section, was established by the paper weighing method. From these data and the total numbers of fibres in the section and the total sectional area; the total area, proportion and proportional area of both types of fibre were calculated.

Similarly, in other muscles of the dog and in horse muscles the numbers of the various types of fibre were recorded and their proportions calculated while their histochemical profiles were being determined. The areas of paper representing each fibre type were weighed to measure both the proportion of the transverse sectional area occupied by each fibre type, and the mean area of each fibre type.

In some samples for all species the area of fibre types as shown by the myosin ATPase reaction was determined using a Quantimet 720 (Imanco), which determined areas of a microscopic field occupied by fibres of a given light transmittance. The mean area of 50 to 100 fibres with either a high or low reaction was assessed. The mean area of the other fibre type i.e. the one not directly measured by Quantimet, was derived by calculation from this data, i.e. the area of the fibre type assessed by Quantimet, the proportions of each fibre type, and the mean fibre area of the section (obtained by back-projection). In the m.semitendinosus of dogs only, a similar procedure permitted calculation of the total numbers, the total proportion, and the total proportional area of the other

type of fibre.

3.2.5.4 Capillary density

The ratio of capillary numbers to fibre numbers, and the number of capillaries observed per cm^2 in a transverse section of the muscle were studied by counting the number of capillaries and number of fibres projected within an area of known magnification on the screen of the back-projection apparatus.

3.2.6 Analysis of data

The number of samples of m. semitendinosus, m. diaphragma and m. pectoralis transversus used for histometric and histochemical analysis are given in Table 31. The amount of data collected from the muscle samples may be seen in Appendices 5 and 6, where they are arranged in order of increasing body weight. The data were analysed to test for differences between the two types of animal within each species using, where applicable, Students' 't' test, the parameters of the regression lines, the difference between slopes of two regression lines, comparison of the value of y for two groups of animals at a nominal value of x, and comparison of the adjusted means of groups of samples from each type of animal within each species using computer facilities and methods outlined by Diem and Lentner (1970) and Dixon (1971).

3.3 RESULTS

3.3.1 Histometric results

3.3.1.1 Measurements on m. semitendinosus

3.3.1.1.1 Comparison of whole muscle transverse sectional area (TSA) of m. semitendinosus of athletic and non-athletic dogs and horses

(a) The effect of freezing on the TSA of the muscle.

The areas of fresh and frozen cross sections of m. semitendinosus of young horses are given on Table 32. These data were compared using Student's 't' test for paired samples and found not to be significantly different. Therefore freezing does not have a significant effect on the total transverse sectional area of m. semitendinosus of the young Thoroughbred. It appears valid to use, where necessary, the area of the frozen complete transverse sectional area of m. semitendinosus in the horse instead of the fresh transverse sectional area.

(b) Effect of sampling on mean fibre TSA.

The mean fibre TSA of the complete cross section of m. semitendinosus from the same animals and samples from the caudal superficial part of the muscle are also given on Table 32. Although the mean fibre area of the superficial sample of the muscle tends to be less than that of the whole muscle this difference is not significant when compared by Student's 't' test for paired samples at the 5% level. The mean fibre area of a superficial sample of the muscle may therefore be used to represent the mean fibre area of the complete transverse section of the whole muscle.

3.3.1.1.2 Growth of the TSA of m. semitendinosus relative to liveweight

The allometric equations describing the change in TSA of m. semitendinosus relative to liveweight are given on Table 33 and plotted on Fig. 21 for dogs and Fig. 22 for horses. The predicted areas of the TSA for dogs at 0·5 kg and 30 kg liveweight, and horses at 50 kg and 500 kg liveweight are given on Tables 37 and 38 respectively.

Dogs

The rate of increase of the TSA is greater ($P < 0·05$) than 0·67 (i.e. proportionate growth of muscle area) in Greyhounds but not significantly different from 0·67 in the other dogs. This suggests that the TSA of m. semitendinosus of the other dogs grows at a slightly greater rate (though not significantly so) than the two-thirds power of liveweight, while the TSA of the Greyhound m. semitendinosus grows at a much greater rate than the two-thirds power liveweight (i.e. is disproportionate). The covariate relationship of TSA of m. semitendinosus relative to liveweight is significantly greater ($P < 0·01$) in the 21 adult Greyhounds than in the 10 adult other dogs; this same relationship is significantly greater ($P < 0·01$) in all the 33 Greyhounds (both adults and pups) than in the 26 other dogs (both adults and pups). The TSA of the Greyhound m. semitendinosus at 30 kg liveweight (1,008 mm^2) is significantly greater ($P < 0·05$) than that of the other dogs at the same liveweight (620 mm^2), however, there is no significant difference between the TSA of m. semitendinosus of the two types of dog at 0·5 kg liveweight (Table 37).

Horses

The growth ratio of the TSA of m. semitendinosus relative to liveweight in the Thoroughbred is significantly greater ($P < 0.05$) than 0·67 (i.e. it grows disproportionately) but not significantly different from 1, while that of the other horses is not significantly different from 0·67. Therefore the growth in TSA of the Thoroughbred m. semitendinosus is disproportionate to liveweight, while that of the other horses is proportionate to liveweight. The rate of increase of the TSA of m. semitendinosus relative to liveweight is significantly greater ($P < 0.01$) in the Thoroughbred than in the other horses (Table 33) which is apparent from Fig. 22. There is no significant difference between the TSA of the muscle in the two types of horse at 50 kg liveweight, but the TSA is significantly greater ($P < 0.05$) in the Thoroughbreds at 500 kg liveweight (6,124 mm^2) than in the other horses (3,595 mm^2) at the same liveweight (Table 38).

3.3.1.1.3 Total fibre numbers in cross sections of m. semitendinosus

Dogs

The mean of the actual numbers of fibres in the adult Greyhounds (319,000 s.d. 67,000) is significantly greater (P 0·001) than the mean of the other dogs (177,000 s.d. 37,000; Table 34). The regression coefficients describing the change in total fibre numbers in the cross section of m. semitendinosus relative to liveweight is significantly greater ($P < 0.05$) than zero for both types of dog (Table 36). This indicates that the total number

of fibres in a cross section of m. semitendinosus increases during growth. There is no significant difference in the rate of increase of fibre numbers during growth between the two types of dog. The covariate relationship of total fibre numbers relative to liveweight is significantly greater ($P < 0.01$) in all the Greyhounds (both adults and pups) than in all the other dogs (both adults and pups). The numbers of fibres in the Greyhound m. semitendinosus at 0·5 kg (228,000) and 30 kg (314,000) liveweight are significantly greater ($P < 0.05$) than the number in the other dogs at 0·5 kg (122,000) and 30 kg (184,000) liveweight (Table 37). Also the number of fibres in each type of dog at 30 kg is greater ($P < 0.05$) than those for the same type of dog at 0·5 kg (Table 37).

The regression coefficients describing the growth of the TSA relative to the growth of its mean fibre area although not significantly different between both types of dog are significantly ($P < 0.05$) greater than 1 for both (Table 36). This confirms the finding that the number of fibres counted in the transverse section of m. semitendinosus actually increases during growth in both types of dog, and are greater in the Greyhound (as the covariate relationships are greater in the Greyhound) (Table 36).

Horses

Although the mean of the actual numbers of fibres in the 5 Thoroughbred crosses studied is not significantly different from the mean of the 5 adult Thoroughbreds, the mean of all the other horses - both Thoroughbred crosses and others is significantly less ($P < 0.001$) than in the

Thoroughbreds (Table 35). The regression coefficients describing the relationship of total fibre numbers with liveweight are significantly greater than 0 ($P < 0{\cdot}05$) for both types of horse indicating that the total number of fibres in the transverse section increases during growth. However, there is no significant difference between the rate of increase in fibre numbers during growth between the two types of horse. The covariate relationship of total fibre numbers relative to liveweight is significantly greater ($P < 0{\cdot}01$) in all the Thoroughbreds than in all the other horses (i.e. when both adult and young of each type are grouped together and compared). The number of fibres in the Thoroughbred m. semitendinosus at 50 kg and 500 kg liveweight are significantly greater ($P < 0{\cdot}05$) than the number in the other horses at 50 kg and 500 kg liveweight (Table 38). As with the dogs the numbers of fibres at the heavier liveweight is greater ($P < 0{\cdot}05$) than the number in the same type of horse at the lighter liveweight (Table 38). Also the regression equations describing the increase in muscle TSA relative to total fibre numbers indicates that the number of fibres actually increases during growth in both types of horse and that they are greater in the Thoroughbreds (Table 36).

3.3.1.2 Mean fibre areas of m. semitendinosus, m. diaphragma and m. pectoralis transversus

3.3.1.2.1 Comparison of fibre areas between the two types of dog

The mean fibre areas of the three muscles of the detrained Greyhounds, although larger, are not significantly

different from those of the trained Greyhounds (Table 39) and so they are included as a group with the trained Greyhounds. The mean fibre areas of m. semitendinosus and m. diaphragma of the Greyhound adults are significantly greater ($P < 0{\cdot}02$ and $P < 0{\cdot}05$ respectively) than the same muscles in the adult other dogs, however, there is no significant difference between the mean fibre areas of m. pectoralis transversus of the two types of adult dogs (Table 39).

The rate of increase of mean fibre area relative to liveweight is significantly greater in the Greyhound m. semitendinosus ($P < 0{\cdot}05$) and m. diaphragma ($P < 0{\cdot}01$) than in the same muscles of the other dogs, but there is no significant difference in the growth rates of mean fibre area in m. pectoralis transversus between the two types of dog (Table 40), but the covariate relationship of mean fibre area relative to liveweight is greater ($P < 0{\cdot}01$) in m. pectoralis transversus of all the other dogs than in the Greyhounds. The mean fibre areas of m. semitendinosus and m. diaphragma of the other dogs at 0·5 kg liveweight are greater ($P < 0{\cdot}05$) than those of the Greyhounds at the same liveweight, but there is no significant difference between the fibre areas of m. pectoralis transversus at 0·5 kg liveweight. When the two types of dog are compared at 30 kg liveweight there is no significant difference between the two types of dog in the mean fibre areas of any of the three muscles (as shown on Table 41).

3.3.1.2.2 <u>Comparison of fibre areas between the three muscles within each type of dog</u>

There is no significant difference between the fibre

areas of males and females (of either type) within any of the muscles sampled.

Athletic dogs

In the adult Greyhounds the mean fibre area of both m. semitendinosus and m. pectoralis transversus are greater than in m. diaphragma ($P < 0{\cdot}001$ and $P < 0{\cdot}01$ respectively). There is no significant difference between the mean fibre area of m. semitendinosus and m. pectoralis transversus in the adult Greyhounds. Although the mean fibre area of m. diaphragma of the newborn pups is greater than in either m. semitendinosus or m. pectoralis transversus when adult this difference is reversed (Table 39); this feature may be explained by the growth impetus of both the mean fibre area of m. semitendinosus and m. pectoralis transversus being greater ($P < 0{\cdot}01$) than that for the mean fibre area of m. diaphragma (Table 40). There is no significant difference between the growth rate of the fibres of m. semitendinosus and m. pectoralis transversus relative to liveweight.

Non-athletic dogs

The mean fibre area of the diaphragm of the adult other dogs is less ($P < 0{\cdot}01$) than that of both m. semitendinosus and m. pectoralis transversus in these animals, however there is no significant difference between the mean fibre areas of m. semitendinosus and m. pectoralis transversus in these animals. Although the mean fibre area of m. diaphragma in newborn pups tends to be greater than in m. pectoralis transversus and in m. semitendinosus (Table 39) - like in the Greyhounds, this difference disappears with growth. This may be explained by the

growth ratios of the mean fibre areas being, relative to liveweight, highest in m. semitendinosus, intermediate in m. pectoralis transversus and lowest in m. diaphragma (Table 40), although only the growth rate of the mean fibre area of m. diaphragma relative to liveweight is significantly smaller ($P < 0{\cdot}01$) than that of the other two muscles.

3.3.1.2.3 Comparison of fibre areas between each type of horse

The mean fibre areas of the three muscles of the Thoroughbred cross adults are not significantly different from those in the adult other horses, and so they are included with the other horses for computations.

The mean fibre areas of m. semitendinosus and m. diaphragma of the adult Thoroughbreds are greater than those of the adult other horses ($P < 0{\cdot}005$ and $0{\cdot}01$ respectively), but there is no significant difference between the mean fibre area of m. pectoralis transversus of the Thoroughbreds and the adult other horses (Table 42).

The growth rate of the mean fibre areas of the three muscles is greater ($P < 0{\cdot}01$) in the Thoroughbreds than in the other horses (Table 40). The mean fibre areas of the three muscles at 50 kg liveweight in the Thoroughbreds are significantly less ($P < 0{\cdot}05$) than those for the other horses at the same liveweight, but there is no significant difference between the predicted mean fibre areas of the two types of horse at 500 kg liveweight (Table 43).

3.3.1.2.4 Comparison of mean fibre areas between the three muscles within each type of horse

There is no significant difference between the mean fibre areas of castrated and entire males and between either type of male and females within any of the muscles sampled in both types of horse or between Thoroughbreds in training or out of training and so all the adult horses irrespective of their sex or training status are grouped together when comparisons are made between muscles within each type of horse. Unlike in both types of dog the mean fibre area of m. diaphragma is not greater than that of the other muscles in the smallest horses of each type (Table 42).

Athletic horses

There is no significant difference between the mean fibre areas of the three muscles in the Thoroughbred adults. Although the growth ratios of the fibres in m. diaphragma is less than that for m. semitendinosus, which is less than that for m. pectoralis transversus, there is no significant difference between the growth ratios of the fibres of the three muscles (Table 40).

Non-athletic horses

The mean fibre area of m. pectoralis transversus of the other adult horses is significantly greater than that of m. semitendinosus ($P < 0.02$) and m. diaphragma ($P < 0.01$) but there is no significant difference between the mean fibre areas of m. semitendinosus and m. diaphragma in the adult other horses. Although the growth ratio of the mean fibre areas of m. semitendinosus is less than that of

m. diaphragma which is less than that of m. pectoralis transversus, there is no significant difference between the growth rates of the three muscles (Table 40).

3.3.1.2.5 Comparison of fibre areas between dogs and horses

An obvious interspecies difference between the fetal horses and neonatal dogs is not apparent (Tables 39 and 42). However the mean fibre area of m. semitendinosus of 31 adult dogs of both types (2,993, s.d. 604 μm^2) is significantly less (P 0·005) than that of all the adult horses (3,748, s.d. 1,086 μm^2). Similarly the mean fibre area of m. diaphragmaof 17 adult dogs (1,647, s.d. 352 μm^2) is significantly less ($P < 0·001$) than that of 20 adult horses (3,305, s.d. 1,086 μm^2) and also the mean fibre area of m. pectoralis transversus of 17 adult dogs (3,300 s.d. 1,028 μm^2) is less ($P < 0·01$) than that of 32 adult horses (4,501,s.d. 1,586 μm^2). This indicates that although the muscle fibres of the adult horses are larger than those of the dogs the difference is not related to their liveweight difference.

3.3.2 Histochemical results

3.3.2.1 Qualitative histochemistry

Succinate dehydrogenase, E.C. 1.3.99.1, SDH (Figs. 23, 27 and 30).

The diformazan deposits occur as blue dots or irregular areas that appear to form a network around the myofibrils (Fig. 30). In fibres with a high level of activity diformazan deposition is highest in the subsarcolemmal region. Frequently fibres shown in serial

sections to have a low activity for the myosin adenosine triphosphatase (myosin ATPase) reaction, have an evenly distributed, moderately dense pattern of greenish blue punctate dots, whereas the colour of the fibres having a high activity for myosin ATPase is purplish. This difference although more obvious in freshly stained sections is not sufficiently consistent to use in identifying fibre types, nor is it apparent in black and white microphotographs.

Glycogen Phosphorylase E.C. 2.4.1.1, GP (Figs. 24, 28, 31)

Fibres vary in reaction from an intense blue network to a paler blue, to a diffuse pink, to fibres coloured only by iodine.

Myosin Adenosine Triphosphatase (Myosin ATPase) (Figs. 25, 29, 32).

Myosin ATPase-high reacting (AH) fibres show a dense brown reaction in which a brown network can usually be seen. These fibres are distinct from the much lighter coloured "low reacting" fibres in which only the brown network may be seen. At certain stages of growth, fibres with an intermediate intensity of reaction are evident (see section 3.3.2.5). Myosin ATPase-low reacting (AL) fibres frequently have an activity of SDHase equal to or greater than adjacent myosin ATPase high reacting (AH) fibres (Figs. 23, 25, 27, 29). Therefore it is not possible to grade the activity of SDHase from the myosin ATPase reaction.

Capillaries (Figs. 33, 34)

When sections were incubated in a modified medium - which had been used to demonstrate myosin ATPase activity in fibres - the capillaries appeared as dark brown dots between fibres. However, capillaries may also though inconsistently be stained with the myosin ATPase reaction.

3.3.2.1.1 Fibre types in adults

The types of fibre in the muscles of the dog and horse were identified by establishing profiles with three histochemical reactions.

Dogs

All the fibres in m. semitendinosus, m. diaphragma and m. pectoralis transversus in both types of dog have a high activity of succinate dehydrogenase (SDHase) and glycogen phosphorylase (GPase). Thus it is not possible to distinguish between different types of fibres by means of the histochemical reaction for SDHase or GPase, although as mentioned in paragraph 3.3.2.1 the myosin ATPase low reacting (AL) fibres frequently have a different pattern of diformazan deposits from that in the myosin ATPase high reacting (AH) fibres. However AL fibres also have a low intensity of glycogen phorphorylase activity. For these reasons the myosin ATPase reaction is used to distinguish between fibre types in the dog.

Horses

It is not possible to distinguish accurately between fibre types in m. diaphragma and m. pectoralis transversus of the horse by means of the histochemical reaction for SDHase and GPase because all the fibres tend to have a

high activity of these enzymes. AL fibres may show variations in the pattern of diformazan distribution and in some animals a slightly lower GPase activity. However in general the myosin ATPase reaction differentiates fibres in these two muscles into

(i) those with a high activity of this enzyme and which also have a high activity for both SDHase and GPase, and

(ii) those with a low activity of myosin ATPase but having a high activity of SDHase and a medium to high (interpreted as high) activity of GPase.

In m. semitendinosus the situation is more complex. The AL fibres in m. semitendinosus have a high activity for SDHase. However, the AH fibres may be differentiated by the SDHase reaction - although a continuous spectrum of activity of this enzyme occurs between fibres each fibre was categorized by a simple division into having a high or low activity. Usually all fibres have an intense blue reaction for GPase activity, but sometimes the AL fibres have a paler blue reaction; however no differentiation could be achieved using this reaction due to the small disparity in colour between the two types of fibre. Using the aforementioned characteristics three types of fibre may be differentiated in m. semitentinosus

(i) those having a high activity for myosin ATPase, GPase and SDHase,

(ii) those with a high activity for myosin ATPase and GPase and a low activity for SDHase;

(iii) those having a low activity for myosin ATPase and a high activity for SDHase and GPase.

3.3.2.2 Quantitative histochemistry of adult muscle

3.3.2.2.1 Quantitative histochemistry of canine muscle

(a) Comparison of fibre type proportions between regions of the same muscle of the dog.

There is no apparent variation in the proportion of fibre types in the vicinity of the sampling site of m. diaphragma. Samples of m. pectoralis transversus were assessed at a constant distance - 20 fibre breadths deep from the surface of the muscle - because the proportion of AL fibres increased towards the deep regions of the muscle. This variation was not investigated further. In m. semitendinosus there is a marked gradation in the proportion of fibre types across the transverse section of the muscle in both types of dog. The relative incidence of AL fibres in the transverse sections of m. semitendinosus of a Greyhound and a Collie which were assessed by sampling the muscle at 2 mm intervals across its entire transverse section is indicated in Fig. 35. It may be seen that the gradation of AL fibres starts off at a much lower figure in the deeper part of the Greyhound muscle than it does in the Collie muscle and progressively gets less towards the lateral part. The Greyhound has no AL fibres at the exterior of m. semitendinosus but some are present near the periphery (Fig. 36) while the Collie has AL fibres at the exterior (Fig. 37). The higher proportion of AL fibres at the inside of

m. semitendinosus in both types of dog is shown in Figs. 40 and 41, the mean of the differing proportions of fibre types across the whole muscle or part of the muscle sampled was taken to represent the muscle. The method of sampling overcomes the variation in distribution in fibre types in m. pectoralis transversus and m. semitendinosus.

(b) <u>The effect of detraining and sex on the proportions of fibre types in m. semitendinosus, m. diaphragma and m. pectoralis transversus</u>.

The percentage numbers of fibres and the percentage areas of the transverse section of m. semitendinosus, and of samples of m. diaphragma and m. pectoralis transversus occupied by AL fibres were assessed in various groups of dogs. No sex difference were found within either the Greyhounds or the other dogs and hence males and females have been grouped together in subsequent calculations. However detraining has a small effect on the results. There is no significant difference in the percentage of or in the percentage areas occupied by AL fibres in m. semitendinosus and m. pectoralis transversus between the trained and detrained adult Greyhounds. However in the m. diaphragma the percentage numbers of AL fibres is not significantly different between the two types of Greyhound but the percentage area of AL fibres is greater ($P < 0{\cdot}005$) in the trained Greyhounds.

(c) Comparison of the incidence of fibre types in similar muscles between the two types of dog.

The percentage AL numbers and the percentage AL area of the complete transverse section of m. semitendinosus in the Greyhounds - irrespective of training status or sex - is significantly less ($P < 0.001$) than that of the other dogs (Table 44). Similarly, the percentage numbers of AL fibres in m. diaphragma of the adult Greyhound is significantly less ($P < 0.001$) than that of the other dogs. Either the trained Greyhounds alone or the trained and detrained Greyhounds grouped together have a significantly lower ($P < 0.001$) area of m. diaphragma occupied by AL fibres. In m. pectoralis transversus also the percentage numbers of AL fibres as well as the percentage area occupied by AL fibres are significantly less ($P < 0.001$) in the Greyhound than in the other dogs.

Therefore the three muscles in the adult Greyhound have fewer AL and a smaller area occupied by AL fibres than the corresponding muscles in the adult other dogs (Figs. 36, 37; 40 - 45).

(d) Comparison of the incidence of fibre types between muscles within each type of dog.

In the Greyhounds the percentage numbers of, and the percentage area occupied by, AL fibres in the complete transverse section of m. semitendinosus is significantly less ($P < 0.001$) than in samples from m. diaphragma and m. pectoralis transversus. There is no significant difference between the percentage numbers of AL fibres in samples of m. diaphragma and m. pectoralis transversus although there tend to be fewer in m. pectoralis transversus.

However the percentage area occupied by AL fibres in m. pectoralis transversus is significantly smaller ($P < 0{\cdot}005$) than in m. diaphragma.

In the other dogs the percentage number of, and area of, AL fibres in the complete transverse section of m. semitendinosus is less ($P < 0{\cdot}001$) than in m. diaphragma and m. pectoralis transversus. However although there tends to be a greater number and area of AL fibres in m. diaphragma than in m. pectoralis transversus there is no significant difference between the two muscles.

Therefore in both types of dog m. semitendinosus has a lower proportional number and area of AL fibres than in m. diaphragma and m. pectoralis transversus; also there tend to be fewer AL fibres and a smaller area occupied by AL fibres in samples of m. pectoralis transversus than in samples of m. diaphragma.

3.3.2.2.2 Quantitative histochemistry of equine muscle

(a) Comparison of muscle fibre type proportions as differentiated by the myosin ATPase and SDHase reactions between different regions of the same muscle of the horse.

The proportion of AL and SDHase high reacting (SH) fibres increases towards the deep region of m. semitendinosus (Figs. 48 - 55). The proportion of AL fibres also increases towards the deep region of m. pectoralis transversus, but there is no apparent difference in the proportions of fibre types in the vicinity of the sampling site of m. diaphragma.

(b) The effect of sex and training status on fibre types in adult horse muscle.

There is no significant difference in the percentage numbers and percentage areas occupied by AL fibres or fibres reacting highly for SDHase (SH fibres) between corresponding samples of m. semitendinosus, m. diaphragma and m. pectoralis transversus of trained and untrained adult Thoroughbreds; and female, castrated and entire male adult Thoroughbreds. So the proportional numbers and proportional areas of fibre types of all the adult Thoroughbreds irrespective of training or sex are compared as a group with all the other horses, as a group.

(c) Comparison of muscle fibre type proportions in similar muscles between the two types of horse.

The percentage number and area occupied by AL fibres in the samples of m. semitendinosus and m. pectoralis transversus is less ($P < 0{\cdot}001$) in the Thoroughbreds than in the other horses. There is no significant difference in the percentage numbers of, or percentage areas of, AL fibres in m. diaphragma between the two types of horse,(see Table 45; Figs. 48, 50, 52, 54, 56, 57, 60, 61).

Although there are significant differences between the values of means of corresponding muscles between the Thoroughbred crosses and the Thoroughbreds and with other horses in general, the Thoroughbred cross horses have values which lie between those of the Thoroughbreds and the other horses (Table 46). When the percentage AL numbers and the percentage AL areas of m. diaphragma of Thoroughbreds are compared with those in the other horses - without the Thoroughbred crosses - there is still no significant

difference between the percentage AL numbers of their m. diaphragma but the percentage area of AL fibres is significantly less ($P < 0.01$) in the Thoroughbred m. diaphragma than in m. diaphragma of the other horses.

Although there tends to be more SDHase high reacting (SH) fibres in the Thoroughbreds there is no significant difference between the proportions of fibres reacting highly to SDHase between the two types of horse (Table 47). However, the proportion of AH.SH fibres in the sample is significantly higher ($P < 0.05$) in the Thoroughbred than in the other horses.

Therefore Thoroughbreds have fewer AL fibres and a lesser area occupied by AL fibres in samples of m. semitendinosus and m. pectoralis transversus, but there is no significant difference in the percentage numbers or percentage area of AL fibres in m. diaphragma of the two types of horse - unless the Thoroughbred cross horses are not included in the other horse group. Thoroughbreds have more AH.SH fibres in their m. semitendinosus than other horses.

(d) <u>Comparison of muscle fibre type proportions between muscles within each type of horse</u>

In both types of horse there are significant differences between the three muscles in the percentage numbers and areas of AL fibres. There are fewer ($P < 0.001$) AL fibres and a smaller ($P < 0.001$) area of AL fibres

(i) in m. semitendinosus than in either m. diaphragma or m. pectoralis transversus, and

(ii) in m. pectoralis transversus than in m. diaphragma.

M. semitendinosus is the only muscle in the two types of horse to have SDHase low reacting (SL) fibres.

3.3.2.2.3 Comparison of muscle fibre type proportions between the dog and horse

All the adult dogs and all the adult horses irrespective of type were compared with one another. The proportional numbers and area occupied by AL fibres in samples of m. diaphragma is greater in the horses ($P < 0.001$) than in the dogs. There is no significant difference between the proportional numbers and areas of AL fibres in m. pectoralis transversus between the two species.

3.3.2.3 Growth changes in the proportion of fibre types in canine muscle

3.3.2.3.1 Fibre type differentiation in postnatal pups

The myosin ATPase reaction differentiates muscle fibres at all postnatal ages in the dog (Figs. 64 - 69).

At birth the overall density of reaction products from the succinate dehydrogenase reaction is generally higher in m. diaphragma than in m. pectoralis transversus, which in turn is slightly higher than in m. semitendinosus. The reaction in all three muscles is less in the immature than in adults. It is not possible to differentiate fibre types in the 3 muscles of the pup using the SDHase reaction similar to in the adults, although the fibres shown histochemically to have a low myosin ATPase activity frequently develop a punctate distribution of diformazan deposits like that of the adults within 2 - 3 days of birth in m. diaphragma, and at about 3 weeks of age in m. pectoralis transversus, but not until about 5 - 6 weeks in m. semitendinosus.

At birth all fibres in the three muscles are stained a purplish blue by the glycogen phosphorylase reaction. AL fibres may be distinguished by a lower phosphorylase activity from 2 days of age in m. diaphragma, from 4 - 5 weeks in m. pectoralis transversus, and 5 - 6 weeks in m. semitendinosus. The AH fibres become darker staining during these intervals.

After seven weeks of age therefore fibre type differentiation either with the SDHase or GPase reactions is similar to that in adults. There is no significant difference between the classification of fibre types by the SDHase and GPase reactions in the two types of dog.

3.3.2.3.2 Changes in the numbers and area occupied by fibre types in m. semitendinosus, m. diaphragma and m. pectoralis transversus.

The percentage numbers and area occupied by AL fibres in samples of the three muscles of the young dogs are given on Table 48. The logarithmic regression equations describing the change in percentage area of the samples occupied by AL fibres with liveweight are given in Tables 49 and 50. The distribution of AL fibres across the transverse section of m. semitendinosus of the two types of dog at 0·5 and 6 kg liveweight are shown on Figs. 70 and 88 and the increase in AL fibre number and proportion of the samples occupied by AL fibres in the three muscles is also shown in Figs. 71 - 87; 89, 90.

M. semitendinosus

The percentage numbers and areas of AL fibres in the complete transverse section of m. semitendinosus does not

vary very much with increasing age in the six youngest Greyhounds of the series i.e. up to 21 days of age, or in the six youngest other pups sampled i.e. up to 23 days of age Table 48. The variation in distribution of AL fibres across the muscle in the two types of pups or between the two types of pup (Fig. 70) is not as great as in their respective adults (Fig. 35). When the pups of each type less than 1 kg liveweight and not older than two weeks of age (4 Greyhounds and 3 other pups) are compared the percentage AL numbers in the Greyhounds (mean 2·17, s.d. 0·56) is less ($P < 0·005$) than in the other pups (mean 3·87, s.d. 0·23). There is no significant difference between the percentage area of AL fibres in the Greyhounds (mean 3·60, s.d. 0·95) and in the other pups (mean 4·30, s.d. 0·46).

The percentage numbers of AL fibres in the complete cross section of m. semitendinosus of the 4 Greyhound pups (mean 2·18, s.d. 0·56) is significantly less ($P < 0·025$) than in m. diaphragma of the same animals (mean 5·88, s.d. 2·33); and although less than that in m. pectoralis transversus (mean 3·65, s.d. 1·90) not significantly so. Similarly the percentage area of AL fibres in the transverse section m. semitendinosus of the Greyhounds (mean 3·60, s.d. 0·95) is significantly less ($P < 0·005$) than in m. diaphragma of the same animals (mean 7·93, s.d. 1·49) and although the mean area is less than in m. pectoralis transversus (mean 3·85, s.d. 1·38) it is not significantly so. The percentage numbers of AL fibres in the complete cross section of m. semitendinosus of the three other pups

(mean 3·87, s.d. 0·23) is significantly less (P 0·02) than the percentage numbers in m. diaphragma of the same animals (mean 8·07, s.d. 1·64); similarly the percentage area of AL fibres in m. semitendinosus (mean 4·30, s.d. 0·46) is significantly less (P 0·025) than in m. diaphragma of the same animals (mean 9·07, s.d. 2·18).

At about 2 kg liveweight in the Greyhound (2 - 4 weeks of age) and 1 kg liveweight in the other dogs (1·5 - 3 weeks) the total number (Figs. 85 and 86) and percentage numbers (Fig. 87) of AL fibres increases so that from 4 - 6 weeks of age onward there is a marked increase in the number of AL fibres in the transverse section of the muscle in both types of dog. At about 4 to 6 weeks of age pups use their hind limbs for support, before this stage the hind limbs may aid crawling but not quadripedal walking. At this stage of growth the differential density of AL fibres both across the muscle and between the two types of dog becomes apparent (Fig. 88). The increase in AL fibres in the transverse section of m. semitendinosus is only transitory in the Greyhound and with increasing age and liveweight the proportional numbers and area occupied by AL fibres drops to those of the adults, however the proportion in the other dogs is more permanent (Tables 44 and 48; Figs. 85 - 87,89,90).

The logarithmic regression equations comparing the growth of the total sectional area and the total area of AL fibres in m. semitendinosus with liveweight in 9 Greyhound pups less than 12 kg liveweight and in all the other pups (16) less than 12 kg liveweight are given on Table 49.

The regression equations comparing total AL area with liveweight over the whole range of liveweight studied are not significant for both types of dogs ($F = 4{\cdot}037$, degrees of freedom = 31 for the Greyhound and $F = 3{\cdot}308$ degrees of freedom = 24 for the others). The total area of m. semitendinosus in these Greyhounds increases at the same rate as liveweight (i.e. b is not significantly different from 1), while the total sectional area of muscle increases at the same rate as the 2/3 power of the liveweight of these pups (b not significantly different from 0·666, i.e. proportional growth). Fig. 89 indicates that the total AL area of the muscle may not alter appreciably or may actually decrease with increasing liveweight in the 3 Greyhound pups over 12 kg and in the Greyhound adults, while the transverse sectional area of the muscle in these animals actually increases. Although the rate of growth of total AL area in m. semitendinosus is greater than the growth of total sectional area this difference is not significant. In all the other pups the rate of increase in total sectional area and total AL area of the section are greater ($P < 0{\cdot}02$) than 0·666 and 1 respectively. The rate of growth of AL area relative to liveweight is greater ($P < 0{\cdot}002$) than the rate of increase of total sectional area in these pups.

The rate of increase in the transverse sectional area of m. semitendinosus is less though not significantly so in all the other dogs than in all the Greyhounds (Section 3.3.1.1.2) and is concurrent with a greater increase in total AL area of the muscle in the other dogs than in the Greyhounds during growth.

M. diaphragma

The proportions of fibre types as differentiated by the myosin ATPase reaction did not vary in the region of the sampling site of m. diaphragma, the percentage number and area of AL fibres is higher at birth in samples of m. diaphragma than in the transverse section of m. semitendinosus, and the proportion and proportional areas of AL fibres increases with increasing liveweight until the adult figures are reached (Table 48).

There is no significant difference in the percentage numbers and areas of AL fibres in both types of pups less than two weeks of age and 1 kg liveweight (4 Greyhounds and 3 other pups) although the mean percentage numbers and areas for the Greyhounds (5·9, s.d. 2·3 and 7·9 s.d. 1·5 respectively) are less than in the other pups (8·1, s.d. 1·6 and 9·1, s.d. 2·2 respectively). The percentage AL area of m. diaphragma increases with liveweight in both types of dog but there is no significant difference between the rate of increase in AL area between the two types of dog (Table 50, Fig. 90). The predicted percentage of AL fibres in m. diaphragma of the other dogs at 30 kg liveweight (70%) is significantly greater ($P < 0.05$) than that for the Greyhounds at the same liveweight (24%). There is no significant difference between the predicted percentage area of AL fibres in the two types of dog at 0·5 kg liveweight.

M. pectoralis transversus

Although the proportion of AL fibres tends to increase towards the deeper region of m. pectoralis transversus - but to a lesser extent than in the adults - there is no apparent variation in the vicinity of the sampling site. The percentage number of AL fibres (5·7) and percentage area of AL fibres (6·1) in the solitary other pup sampled is greater than the mean percentage number (3·7) and mean percentage area (3·9) in the 4 Greyhound pups under 1 kg and 2 weeks of age. Similarly the percentage numbers (3·0, s.d. 1·7) and percentage area (4·0, s.d. 1·26) of the 6 Greyhounds less than 6 weeks of age are less ($P < 0.005$ and $P < 0.02$ respectively) than the percentage numbers (16·3, s.d. 8·3) and percentage areas (18·5, s.d. 11·4) in the 4 other pups less than 6 weeks of age. Although the percentage numbers of AL fibres in m. pectoralis transversus (5·9, s.d. 2·3) is not significantly different from that in m. diaphragma (3·7, s.d. 1·9) in the 4 Greyhounds less than 1 kg liveweight, the percentage area of AL fibres in m. pectoralis transversus of these animals (3·9, s.d. 1·4) is significantly less ($P < 0.01$) than the percentage area of AL fibres in m. pectoralis transversus of these animals (7·9, s.d. 1·5). There is no significant difference in the percentage numbers or areas of AL fibres in m. semitendinosus and m. pectoralis transversus in these pups. As only one other pup less than 1 kg and 2 weeks of age was sampled (Table 48) the muscles within the other pups less than 6 weeks of age were compared. In these 4 pups

the percentage numbers of (16, s.d. 8·3) and areas of (19, s.d. 11) AL fibres in m. pectoralis transversus are greater ($P < 0{\cdot}05$ and $P < 0{\cdot}025$ respectively) than the percentage numbers (4·7, s.d. 1·5) and percentage area (5·3, s.d. 1·8) in m. semitendinosus of these animals. There is no significant difference between the percentage numbers or area occupied by AL fibres in m. pectoralis transversus or m. diaphragma of these pups.

There is no significant difference in the rate of increase in AL area of m. pectoralis transversus relative to liveweight between the two types of dog (Table 50; Fig. 90). However the percentage area of AL fibres at 0·5 kg (4·9 in the Greyhounds and 18 in the other dogs) and 30 kg liveweight (16 in the Greyhounds and 56 in the other dogs) are significantly greater ($P < 0{\cdot}05$) in the other dogs.

It appears therefore that from the earliest postnatal ages in dogs the percentage numbers and areas of AL fibres is least in m. semitendinosus, intermediate in m. pectoralis transversus and greatest in m. diaphragma. Differences occur between the two types of pups but they are not as significant as in the respective adults. Within m. semitendinosus and m. pectoralis transversus an increase in the percentage numbers and areas of AL fibres may be associated with the increasing functional load borne by the muscles. The total area of AL fibres increases with liveweight up to a point in m. semitendinosus in both types of dog and then drops off markedly at about 10 to 15 kg liveweight in the Greyhounds.

This causes the large difference between Greyhounds and other adult dogs. The percentage area of AL fibres increases with liveweight in m. diaphragma and m. pectoralis transversus in both types of dog.

3.3.2.4 Changes in the proportion of fibre types in m. semitendinosus, m. diaphragma and m. pectoralis transversus in the growing horse

3.3.2.4.1 Fibre type differentiation in young horses

The myosin ATPase reaction differentiates fibres at all stages of growth studied, i.e. from 158 days in utero onward. The percentage of fibres in m. semitendinosus, m. diaphragma and m. pectoralis transversus having a low reaction for succinate dehydrogenase (SL), glycogen phosphorylase (PL) and myosin ATPase (AL) are listed in Table 51.

In the three muscles in the 158 day Welsh Mountain fetus and the 160 day Connemara X fetus all the fibres irrespective of their reaction for myosin ATPase tend to have a uniform pinkish reaction for succinate dehydrogenase (SDHase) activity, and a pinkish purplish reaction for glycogen phosphorylase (GPase) activity (Figs. 91 - 97). From about 300 days in utero onwards it is possible to differentiate fibres in m. semitendinosus with the SDHase reaction (Fig. 99), the overall density of the reaction products due to the SDHase reaction tends to be higher in m. diaphragma than in m. pectoralis transversus which in turn is slightly higher than in m. semitendinosus. It is not possible to differentiate fibre types with the

SDHase reaction in m. pectoralis transversus of the young horses with the exception of two Thoroughbred foeti and one Welsh Mountain yearling (Table 51).

The overall colour of the reaction products due to the GPase reaction tends to get darker with increasing liveweight. In the youngest horse the reaction product is a light purple-blue colour in the three muscles. With increasing age the colours tend to get darker so that at birth the reaction product in m. semitendinosus and m. pectoralis transversus is blue while that in m. diaphragma tends to be purplish. As with the SDHase reaction it is not possible to distinguish fibre types in m. semitendinosus or m. pectoralis transversus of young horses by means of GPase since in the vast majority of cases all the fibres have a high activity of the enzyme. Although occasionally some AL fibres have a low activity of the enzyme. However AL fibres in m. diaphragma frequently have a low activity for GPase (Table 51). With increasing body size fewer fibres with low GPase activity are seen in m. diaphragma, although the overall density of the reaction products tend to increase in all three muscles, but frequently AL fibres have a lower GPase activity than AH fibres.

So with increasing age and bodyweight the adult pattern of fibre type differentiation in the three muscles is gradually assumed i.e. AH, SH, PH; AH, SL, PH and AL, SH, PH fibres in m. semitendinosus; AH, SH, PH and AL, SH, PL in m.diaphragma and AH.SH.PH and AL.SH.PH in m. pectoralis transversus.

3.3.2.4.2 Changes in the number of fibre types and area occupied by myosin ATPase low-reacting (AL) fibres in m. semitendinosus, m. diaphragma and m. pectoralis transversus during growth in the horse

Although the data collected from both types of horse are not evenly distributed over the weight range of animals studied, some comparisons may be made between the two types of horse. There is no significant difference between the percentage numbers or areas occupied by AL fibres in the three muscles between the 3 young Thoroughbred cross horses and the 9 other young horses so both Thoroughbred crosses and the other young horses are grouped together when computations are made. Data for the three muscles are given on Tables 45 and 51. The change in proportions of fibre types with increasing liveweight are depicted on Figs. 91 to 111; and the increasing proportion of AL fibre area with increasing liveweight in the three muscles is plotted in Fig. 112.

M. semitendinosus

The graph of the relationship of percentage AL area of the sample with liveweight shows a considerable scatter of data points (Fig. 112), with the result that the regression equation $\log y = 0{\cdot}101 \log x - 0{\cdot}192$, ($r = 0{\cdot}190$ $F = 1{\cdot}157$ degrees of freedom = 31) does not significantly describe the relationship, but it indicates a tendancy for an increase in AL area with liveweight as occurs in the other horses (Table 50).

When the prenatal horses of each type (Table 51) are compared as groups with one another by Student's 't'

test there is no significant difference in the percentage numbers or percentage area occupied by AL fibres in the two types of horse. The percentage numbers of AL fibres in samples of m. semitendinosus of all the Thoroughbreds as a group (mean 2·98, s.d. 2·03) is less ($P < 0.001$) than the percentage numbers in samples of m. diaphragma (mean 43·8, s.d. 14·9), and is also less ($P < 0.001$) than in m. pectoralis transversus (mean 21·1, s.d. 10·2) in the same animals (Table 51). Similarly the percentage AL area of samples of m. semitendinosus (mean 2·4, s.d. 1·87) is less ($P < 0.001$) than the percentage AL areas of m. diaphragma (mean 49·6, s.d. 15·6) and of m. pectoralis transversus (mean 18·1, s.d. 8·51).

Despite 2 of the 3 young Thoroughbred cross horses having values nearer those of the young Thoroughbreds than the young other horses (Fig. 112), there is no significant difference in the percentage numbers of, or areas occupied by AL fibres in the three muscles between the Thoroughbred crosses and the other horses, so the Thoroughbred crosses and the other horses are treated as a group. The percentage numbers of AL fibres in samples of m. semitendinosus of all the other young horses as a group (mean 8·90, s.d. 6·69) is less ($P < 0.001$) than the percentage numbers in samples of m. diaphragma (mean 48·9, s.d. 23·7) and is also less ($P < 0.01$) than the percentage numbers of AL fibres in samples of m. pectoralis transversus (mean 33·4, s.d. 14·9). Similarly the percentage areas occupied by AL fibres (mean 4·45, s.d. 2·65) is less ($P < 0.001$) than in m. diaphragma (mean 50·8, s.d. 25·0) and than in m. pectoralis transversus (mean 25·0, s.d. 11·5).

The number of SL fibres tended to decrease with increasing liveweight in both types of horse. One yearling Thoroughbred had 4% SL fibres in a sample from its m. semitendinosus.

It appears that the difference in the proportion of both AL fibres and AL areas between m. semitendinosus and the other two muscles has been established early in life in both types of horse, and that the differences between the two types of horse are not as obvious early in life as they are later.

M. diaphragma

The logarithmic regression lines calculated from the data on Tables 45 and 51, comparing the growth of percentage area of m. diaphragma relative to liveweight are shown on Table 50 and plotted on Fig. 112. The growth ratio is significantly greater ($P < 0.05$) than zero in both types of horse (indicating an increase in AL area with liveweight) and the growth of percentage AL area is greater ($P < 0.01$) in the other horses.

The percentage numbers of AL fibres is greater ($P < 0.02$) in the 17 prenatal Thoroughbreds than in the 3 prenatal other horses (mean 20·3, s.d. 18·8); and the percentage AL area of samples of m. diaphragma is greater ($P < 0.005$) in the prenatal Thoroughbreds (mean 43·4, s.d. 10·5) than in the prenatal other horses (mean 18·7, s.d. 12·5).

There are PL fibres in samples of m. diaphragma in 4 out of the 16 Thoroughbreds and in 5 of the 9 other horses (Table 51). The values for the Thoroughbreds are

all below 5%. Although these fibres obviously occur irregularly it may suggest that PL fibres are more likely to occur in young other horses than in young Thoroughbreds.

Therefore the percentage AL area in m. diaphragma increases with liveweight in both types of horse and particularly so in the other horses, and the prenatal Thoroughbreds have a greater area and numbers of AL fibres in samples of m. diaphragma than in the other horses.

M. pectoralis transversus

The graph describing the relationship of percentage AL area in m. pectoralis transversus in Thoroughbreds (Fig. 112) shows a considerable scatter of the data, but the non-significant regression, $\log y = 0{\cdot}063 \log x + 0{\cdot}920$ ($r = 0{\cdot}185$, $F = 0{\cdot}992$, degrees of freedom = 28) suggests that the rate of increase in AL area is less in the Thoroughbreds than in the other horses.

There is no significant difference in the percentage numbers or areas of AL fibres in m. pectoralis transversus between both types of prenatal horse.

When all the young Thoroughbreds are grouped together and the percentage numbers and areas of AL fibres compared in these animals using Student's 't' test the percentage numbers of AL fibres in m. pectoralis transversus (mean 21·1, s.d. 10·2) is less ($P < 0{\cdot}001$) than in m. diaphragma (mean 43·8, s.d. 14·9) of the same animals; and also the percentage areas of AL fibres is also less ($P < 0{\cdot}001$) in m. pectoralis transversus (mean 18·1, s.d. 8·5) than in m. diaphragma (49·5, s.d. 15·6). However in the young other horses the intermuscular

differences between m. diaphragma and m. pectoralis transversus is not so marked as there is no significant difference between the percentage numbers of AL fibres between the two muscles, but the percentage areas of samples of m. diaphragma (mean 50·7, s.d. 25·0) is greater (P 0·01) than that of m. pectoralis transversus (mean 25·0, s.d. 11·5).

Fibres with a low activity of SDHase (SL fibres) were present in samples of m. pectoralis transversus of 2 Thoroughbreds and 1 other young horse, and 2 other horses had fibres with a low activity of GPase (PL fibres) (Table 51).

There is an increase in AL area of m. pectoralis transversus during growth in the other horses, but it is not possible to demonstrate differences in numbers or areas of fibre types in young horses as are seen in adult horses.

In general therefore, it appears that the differences between m. diaphragma and m. pectoralis transversus of the Greyhound and other dogs are roughly paralleled by the Thoroughbred when compared with the other horses although more thorough comparisons within horses could be aided by more suitable data. The remarkable reduction in AL area relative to liveweight seen in m. semitendinosus of the Greyhound may even occur in Thoroughbreds as indicated by samples from the muscle (Fig. 112). Fibres with a low activity of SDHase (SL fibres) are seen in m. semitendinosus and occasionally in m. pectoralis transversus in the young of both types of horse. Fibres

with a low activity of GPase (PL fibres) are seen in the three muscles of the young Thoroughbreds and in m. diaphragma and m. pectoralis transversus of the young other horses.

3.3.2.5 Fibres with an intermediate reaction for myosin ATPase in growing animals

Fibres having an intermediate reaction for myosin ATPase frequently have a distribution of diformazan granules similar to that seen in AL fibres (Figs. 113 - 118).

(a) Canine m. semitendinosus

Fibres with an intermediate reaction for myosin ATPase are seen when a rapid increase in AL fibres occurs in this muscle in both types of dog and also when there is a decrease in the muscle of AL fibres in the Greyhounds. Table 52 and Figs. 89 and 119 indicate that the rate of increase in AL area and AL numbers is greater than the increase in transverse sectional area and total fibre numbers in the muscle. As indicated in section 3.3.2.3.2 the growth of m. semitendinosus in pups less than 12 kg liveweight is less than the growth of AL area in these animals. In animals over 12 kg the total AL area of the muscle may not alter or actually decrease with increasing liveweight (Fig. 89). Similarly the total number of fibres in m. semitendinosus increases with increasing liveweight in both types of dog (section 3.3.1.1.2 and Fig. 112) while the total number of AL fibres in the muscle increases with liveweight from about 1 kg to 12 kg in both types of dog and then decreases in the Greyhound.

This alteration in AL fibre numbers and therefore

total AL area without concomitant alterations in total sectional area or total fibre numbers of the muscle can only be explained by one type of fibre decreasing and the other increasing in number at the relevant times. This is best explained by a conversion of a fibre of one type into another due to lack of evidence of structural alterations (such as splitting) of fibres. The case for conversion of one type of fibre into another is supported by the occurrence of fibres with an intermediate reaction for myosin ATPase at the time when a large increase in AL numbers occurs in young animals.

It is easier to see 'transitional' fibres when AH fibres are changing to AL fibres (Figs. 113 - 118) than when AL fibres are changing to AH fibres as only 3 young Greyhounds were studied in which this may have been occurring (Table 48). All the other dogs over 12 kg liveweight were adults and the change may have taken place in these before they were submitted for histochemical study.

By studying the complete cross section of the muscle the possibility of rearrangement of types within the muscle is accounted for (Figs. 35, 70 and 88). So it appears that fibres with an intermediate reaction for myosin ATPase belong to a population of muscle fibres which are changing from one type to another.

(b) <u>M. diaphragma and m. pectoralis transversus of the dog, m. semitendinosus, m. diaphragma and m. pectoralis transversus of horses</u>

Fibres with an intermediate reaction for myosin ATPase activity are also observed in samples from muscles other than

the canine semitendinosus when the proportions of AL fibres in them is increasing.

3.3.2.6 The relative growth of, and the transverse sectional area of, fibre types as differentiated by the myosin ATPase reaction

The transverse sectional area of AH and AL fibres in each of the three muscles of the two types of animals in both species is shown in Tables 53 and 55 and the growth of AH relative to AL fibres as indicated by the allometric equations on Table 54 is shown in Fig. 120.

Dogs

In the 3 smallest dogs of each type the mean AL area of the three muscles is greater than the mean AH area (Table 53), but only significantly so ($P < 0.02$) in m. diaphragma of the Greyhounds. With the exception of m. diaphragma of the other dogs, the AH fibres grow at a greater rate than the AL fibres in the three muscles but only significantly so in m. semitendinosus of both types of dog ($P < 0.001$ for the Greyhound and $P < 0.025$ for the other dogs). When the rate of increase of AH area relative to AL area is compared in the same muscles between each type of dog there is no significant difference between the two types of dog, but when a comparison is made between muscles within each type of dog the only significant difference is that the relationship in the Greyhound m. semitendinosus is greater ($P < 0.05$) than in m. pectoralis transversus. But there is a general trend for a decreasing relative growth of AH fibres from m. semitendinosus to m. pectoralis transversus to m. diaphragma,

but the Greyhound m. diaphragma is an exception to this trend. This results in the mean AH fibre area of the three muscles of the adult being greater than the mean AL area of the same muscles - but only significantly so in m. semitendinosus and m. pectoralis transversus of the Greyhound ($P < 0{\cdot}001$) and m. semitendinosus of the other dogs ($P < 0{\cdot}02$).

When the fibre areas of the adults of the two types of dog are compared both the AH ($P < 0{\cdot}05$) and AL fibres ($P < 0{\cdot}005$) of the Greyhound m. semitendinosus are larger than the corresponding fibre types in the other dogs, and the AL fibres of the Greyhound m. diaphragma are larger than the AL fibres in m. diaphragma of the other dogs.

The only significant difference between the sexes is that the Greyhound males have larger ($P < 0{\cdot}05$) AH fibres in m. pectoralis transversus than the Greyhound females have in the same muscle. Detraining also has a small effect - detrained Greyhounds actually have larger ($P < 0{\cdot}05$) AL fibres in m. diaphragma than the trained Greyhounds.

The absolute size of AH and AL fibres varies between each muscle in the adults. Table 53 indicates that the mean AH area in all the adults of each type of dog are greatest in m. pectoralis transversus least in m. diaphragma with the mean areas for m. semitendinosus between these two. The mean AL areas for all the adult Greyhounds is greatest in m. semitendinosus followed by m. pectoralis transversus and m. diaphragma having the smallest AL fibre

areas. In the adult other dogs however the situation is a little different in that the AL fibres of m. pectoralis transversus are larger than those of m. semitendinosus which in turn are larger than those in m. diaphragma.

Therefore the greater area of AH fibres relative to AL fibres in the adults of both types of dog is due to the greater relative growth rate of these fibres during postnatal life.

Horses

The mean area of AL fibres is greater than that of the AH fibres in the three muscles of 2 of the 3 smallest Thoroughbreds (Table 55). However in adults the reverse is true in m. semitendinosus and m. pectoralis transversus - (but only significantly so in m. pectoralis transversus - $P < 0{\cdot}001$) but in m. diaphragma the mean AL area is larger than the mean AH area (Table 55, Fig. 120). In the other horses the mean AH area of m. semitendinosus and m. pectoralis transversus is greater at all stages of growth - in adults these differences are significant ($P < 0{\cdot}001$ for m. semitendinosus and $P < 0{\cdot}02$ for m. pectoralis transversus). However the mean AL area of m. diaphragma is larger at all stages of life (Table 55).

The growth of mean AH fibre area is greater than that of mean AL fibre area in m. semitendinosus of both types of horse and in m. pectoralis transversus of the Thoroughbred but is only significantly so ($P < 0{\cdot}001$) in the latter muscle, while the growth of mean AH fibre area is less (though not significantly so) than that of mean AL

area in m. diaphragma of both types of horse and in m. pectoralis transversus of the other horses (Table 54). The growth of AH fibre area relative to AL fibre area is greater in the three muscles of the Thoroughbreds than in the corresponding muscles of the other horses - but is only significantly so in m. pectoralis transversus ($P < 0{\cdot}001$).

When the mean AH and AL areas are compared in all the adults of each type - the values for the Thoroughbred crosses not being significantly different from the other horses, or the detrained Thoroughbreds not being significantly different from the trained Thoroughbreds and there being no significant difference between entire, or castrated males or females within each type of horse - the mean area of AH and AL fibres in m. semitendinosus are greater ($P < 0{\cdot}005$ and $0{\cdot}01$ respectively) in the Thoroughbreds than in the other horses; similarly the mean AL area of m. diaphragma is greater ($P < 0{\cdot}02$) in the Thoroughbreds than in the other horses. The mean AH areas of m. diaphragma and m. pectoralis transversus are larger in the Thoroughbreds but the mean AL area is larger in the other horses, however none of these differences are significant.

It appears therefore that as in the dog the rate of increase in area of AH relative to AL fibres tends to be greater in the athletically selected breed of the species, but this difference in growth of fibres is exemplified in m. pectoralis transversus of the horses studied rather than as in the dog in m. semitendinosus.

3.3.2.7 The potential blood supply of dog and horse muscle

The ratio of the number of capillaries to the number of fibres in a sample, and the area of muscle fibres (in μm^2) supplied by a capillary - and index of the diffusion distance involved in the nutrition of the muscle - in the three muscles of the dog and horse are listed in Tables 56 and 57. The logarithmic regression equations comparing the growth of capillary-fibre ratios with live-weight in both dogs and horses are given in Table 59. The type of material used is shown on Figs. 33, 34, 121 to 126.

3.3.2.7.1 Capillary density in m. semitendinosus, m. diaphragma and m. pectoralis transversus of dogs

(a) The effect of training and sex in adult dogs.

There is no significant difference between the male and female adult Greyhounds, or between the male and female adult other dogs when the capillary-fibre ratios and area per capillary of the three muscles are compared using Student's 't' test. Although there is no significant difference in the capillary fibre ratios of the three muscles or the mean area per capillary of m. semitendinosus and m. diaphragma, in m. pectoralis transversus the mean area per capillary is greater ($P < 0{\cdot}025$) in the detrained than in the trained Greyhounds (Table 56).

(b) Differences in similar muscles between the adults of the two types of dog.

The capillary-fibre ratio of the Greyhound m. semitendinosus is significantly higher ($P < 0{\cdot}02$) than the ratio in m. semitendinosus of the other adult dogs.

However there is no significant difference between the two types of dog in the area per capillary of that muscle or in the capillary-fibre ratios and area per capillary of the other muscles. Although the area per capillary of m. pectoralis transversus in the detrained is greater than in the trained Greyhounds, there is no significant difference in the area per capillary between the adult other dogs and either all adult Greyhounds irrespective of their training status or trained adult Greyhounds only.

(c) Comparison of different muscles within each type of adult dog.

In the adult Greyhounds the capillary-fibre ratio of m. diaphragma is less than the capillary-fibre ratio either of m. semitendinosus or m. pectoralis transversus ($P < 0{\cdot}001$ and $P < 0{\cdot}02$ respectively; Table 56). But the mean area per capillary of m. diaphragma is less than the mean area per capillary either in m. semitendinosus or in m. pectoralis transversus ($P < 0{\cdot}005$ and $P < 0{\cdot}001$ respectively; Table 56). In the young Greyhounds there is no significant difference in the capillary-fibre ratios or mean area per capillary between the three muscles.

In the adult other dogs the capillary-fibre ratio of m. diaphragma is less ($P < 0{\cdot}02$) than that of m. semitendinosus, but there is no significant difference in the capillary-fibre ratio either between m. semitendinosus and m. pectoralis transversus or between m. pectoralis transversus and m. diaphragma. There is no significant difference in the area per capillary between the three muscles. Within the other pups as a group there is no

significant difference either in the capillary-fibre ratios or area per capillary between the three muscles.

(d) Growth changes in capillary density.

In general although the capillary fibre-ratio of the three muscles increases with increasing liveweight, the area of muscle fibres per capillary - a better indicator of circulatory system potential - remains reasonably constant, so growth changes mostly occur in capillary-fibre ratios (Tables 56 and 58). The rate of increase in capillary-fibre ratios in m. semitendinosus and m. diaphragma with liveweight is not significantly different in the two types of dog. However the growth rate of capillary-fibre ratios in m. pectoralis transversus is greater ($P < 0{\cdot}01$) in the Greyhounds. The covariate relationship of capillary-fibre ratios relative to liveweight is greater in m. diaphragma of all the Greyhounds ($P < 0{\cdot}05$) when compared with all the other dogs, but there is no significant difference in the case of m. semitendinosus.

In general therefore it appears that there is little between type difference in the capillary density in adult dog muscles although the capillary-fibre ratio is greater in m. semitendinosus of adult Greyhounds than in the other adults, although the capillary-fibre ratio increases with growth the area per capillary does not alter markedly. Detraining increases the area per capillary in m. pectoralis transversus of the Greyhounds. Capillary-fibre ratios in m. diaphragma are less than those in both m. semitendinosus and m. pectoralis transversus in adult

Greyhounds; and in the adult other dogs are also less than in m. semitendinosus, but however in the adult Greyhounds this is compensated for by a smaller area per capillary in m. diaphragma than in the other two muscles.

3.3.2.7.2 Capillary density in m. semitendinosus, m. diaphragma and m. pectoralis transversus of horses

(a) The effect of training, sex and cross breeding.

Although some of the Thoroughbreds were "out of training" as indicated in Table 57, there is no significant difference between the trained and detrained animals. There is no significant difference between the entire male, castrated male and female adult other horses. The Thoroughbred cross adults have a greater ($P < 0{\cdot}02$) area per capillary in m. semitendinosus than the adult other horses. However there is no significant difference between Thoroughbred crosses and adult other horses in the area per capillary of the other two muscles, or in the capillary-fibre ratios of all three muscles. So trained and detrained, male and female, Thoroughbred crosses and other horses where no significant differences between them have been found are grouped together into appropriate groups as shown on Table 57 for computations.

(b) Comparison between similar muscles in the adults of the two types of horse.

In m. semitendinosus the capillary-fibre ratio of the adult Thoroughbreds is significantly greater ($P < 0{\cdot}005$) than that of the adult other horses. But there is no

significant differences in the area per capillary of the Thoroughbred adults compared with all the other adult horses. However when the Thoroughbred crosses - which have a greater area per capillary in m. semitendinosus than the others - are omitted from the group of other adults the area per capillary of the other adults is significantly less ($P < 0.05$) than that of the Thoroughbred adults. In m. diaphragma there is no significant difference between the capillary-fibre ratio or area per capillary between the two types of adult horse, although both are greater for Thoroughbreds (Table 57). The capillary-fibre ratio of m. pectoralis transversus is greater in the Thoroughbreds ($P < 0.001$) than in the other adult horses, but there is no significant difference in the area per capillary of m. pectoralis transversus between the two types of adult horse.

(c) Comparison of muscles within each type of horse.

There is no significant difference in the capillary-fibre ratio or area per capillary between the three muscles within the adult Thoroughbreds or within the young Thoroughbreds. Similarly within the other horses there is no significant difference in the capillary-fibre ratio or area per capillary between the three muscles within the adult other horses. Within all the other foals as a group the only significant difference in potential blood supply between muscles is that the capillary-fibre ratio of m. semitendinosus is less than ($P < 0.05$) that of m.pectoralis transversus (Table 57).

(d) Growth changes in capillary density.

Although the area per capillary increases during growth in the three muscles of the young horses of each type most of the growth changes occur in the capillary-fibre ratios. There is no significant difference in the rate of increase of capillary-fibre ratios with liveweight in m. semitendinosus and m. diaphragma of the two types of horse. But the rate of increase of capillary-fibre ratios relative to liveweight in m. pectoralis transversus of the Thoroughbreds is greater ($P < 0{\cdot}01$) than in the other horses (Table 58).

In general therefore, Thoroughbred adults have a greater capillary-fibre ratio in m. semitendinosus and in m. pectoralis transversus than the adult other horses. Intermuscular differences in the capillary-fibre ratios and area per capillary within the adults and young of each type of horse is negligible with the exception that the capillary-fibre ratio in m.pectoralis transversus of the other foals is less than the capillary-fibre ratio in m. semitendinosus of the same group.

3.3.3 Biochemical results

The absolute values for the biochemical estimation of SDHase activity (E.C. 1.3.99.1) in m. semitendinosus, m. diaphragma and m. pectoralis transversus of dogs and horses are shown in Table 59. The absolute levels are expressed as mg of INT formazan produced per g of muscle per 15 minutes.

(a) SDHase activity of dog muscles

There is no significant training or sex differences in SDHase levels of the muscles of adult Greyhounds. The activity of the enzyme in the three muscles tends to increase with increasing body weight. There is no significant breed difference in any of the muscles between the young of both types of dog (there is no data for adult other dogs for comparison with adult Greyhounds). There is no significant difference between the values for m. semitendinosus and m. diaphragma of the adult Greyhounds. The activity of the deep region of m. semitendinosus of three Greyhounds (mean 2·67, s.d. 0·145) is greater ($P < 0·02$) than that of the lateral superficial region of the same muscle (mean 2·25, s.d. 0·15). There is no significant difference between the values in m. semitendinosus and m. diaphragma of the young Greyhounds but the values for m. diaphragma of these dogs (mean 1·66, s.d. 0·58) are greater than in m. pectoralis transversus of the same group (mean 1·02, s.d. 0·289; $P < 0·02$). Although the values for m. diaphragma (mean 1·53, s.d. 0·25) are greater than those for m. semitendinosus (mean 1·25, s.d. 0·21) and m. pectoralis transversus (0·95, s.d. 0·16) there is no significant difference between the values for the three muscles within the other pups.

(b) SDHase activity of horse muscles

The activity of SDHase tends to increase with increasing liveweight in the three muscles of both types of horse. Although the three Thoroughbred adults were in training

there is no significant difference between the SDHase values for m. semitendinosus and m. pectoralis transversus either within the adults or within the young Thoroughbreds. However the values for m. diaphragma of the young Thoroughbreds (mean 2·55, s.d. 0·86) are greater than those for m. semitendinosus (mean 0·91, s.d. 0·11; $P < 0{\cdot}005$). There are no significant intermuscular differences in the young other horses.

In general therefore, adults of both types of animal have higher SDHase levels in their muscles than the younger animals. M. diaphragma has a higher activity than the other muscles within the same type of animal.

3.4 DISCUSSION

3.4.1 Sample dimensions after tissue preservation

Apart from their chemical effects on cellular metabolism (Culling, 1974), fixatives for light microscopy - formalin - (and electron microscopy - osmium tetroxide) - cause alterations in volume of muscle slices (Bahr, Bloom & Friberg, 1957). It is recognised that freezing tissues to -160°C has an advantage over chemical fixation in that it causes reversible and not necessarily permanent stoppage of numerous cellular processes. An additional advantage is the absence of change in area of large sections of skeletal muscle, when the areas of fresh sections are compared with sections mounted on glass after freezing and cutting. It is assumed that as the complete cross sections of muscles are not altered by freezing the individual fibre areas are not altered either. Whether the absence of area changes due to freezing occurs in other organs needs further investigation.

3.4.2 Acceleration capacity

The acceleration capacity of the animal is directly related to its inherent propulsive forces. Hettinger & Müller (1953) demonstrate that in man the force capable of being produced by the arm muscles is directly proportional to the cross sectional area of the muscles. Similar results have been obtained by Ikai (1973) who finds, while investigating human arm muscles during a training period of 100 days, an increase in strength without an increase in transverse sectional area of the muscles up to twenty days, but thereafter the cross sectional

area of the muscles increases progressively with increasing strength. He postulates that neuronal factors may cause the discrepancy. However the pattern of glycogen depletion in muscle fibres during exercise suggests that, within the same muscle, different exercise stimuli may cause preferential usage of different types of fibre in the bushbaby (Gillespie, Simpson & Edgerton, 1974), man (Gollnick, Armstrong, Sembrowich, Shepherd & Saltin, 1973; Costill, Jansson, Gollnick & Saltin, 1974) and horse (Lindholm, Bjerneld & Saltin, 1974). Therefore the complete cross-section of the muscle may be considered only as an indicator of the potential force producing capacity of the muscle i.e. when all the fibres in the cross-section are being utilized. This study shows that at similar adult liveweights the athletic animals should have a greater acceleration capacity due to the greater cross-sectional area of their m. semitendinosus which is representative of the femoral muscle group (section 2.3.10).

Although the athletic animals have larger cross-sectional areas of m. semitendinosus relative to their liveweights, it is apparent from Figs. 21 and 22 that at lower liveweights Greyhounds and Thoroughbreds may have smaller cross-sectional areas of m. semitendinosus than the non-athletic animals. Possibly the non-athletic animals are more "mature" at lower body weights than the athletic animals chosen.

3.4.3 Factors affecting the estimated numbers of fibres in a muscle

3.4.3.1 Problems of sampling

It has been shown that the total numbers of fibres in the complete cross-section of m. pectineus in dogs may be over estimated when calculated from sampled areas (Jimenez, Cardinet, Smith & Fedde, 1975) expecially where the number of fibres present is large (i.e. 2% error or 5% error for every 1,000 fibres per mm^2 or 100,000 fibres per section respectively). However in the present study the method employed to assess total fibre numbers (e.g. in m. semitendinosus of dogs and horses, calculations were based on 9 sampled areas of 300 to 400 fibres each) was similar for both athletic and non-athletic animals. Hence it is unlikely that a sampling error of this kind would cause a major discrepancy in comparisons between the two types of animals. Another source of error lies in alterations in orientation of the internal architecture of a muscle during growth, as may happen in a pennate muscle (Maxwell, Faulkner & Hyatt, 1974). This also may produce wrong estimations of the total numbers of fibres in the cross section of the muscle due to changes in obliquity of the section relative to its fibres. Since there is no apparent change in the shape of either the canine or equine m. semitendinosus during growth, the number of fibres in the cross-section of the muscle should be directly correlated with the total numbers of fibres in the muscle. The fibres in m. semitendinosus of both dog and horse run in parallel from origin towards insertion. So a cross-

section of m. semitendinosus, either at the mid-belly of the muscle in the dog or where the muscle plays over the tuber ischii in the horse, cuts the fibres transversely. However in both species it should be noted that such sections do not pass through all the fibres in the muscle. In the dog m. semitendinosus is divided at the junction of its proximal and middle thirds by a tendinous intersection; in the horse, m. semitendinosus has two heads, the first arising from the transverse processes of the first and second coccygeal vertebrae, the coccygeal fascia and the proximal end of m. biceps femoris and a second head arising from the ventral surface of the tuber ischii. In the present study samples were taken only from the first part. Therefore the total number of fibres in the muscle could not be estimated in either species.

3.4.3.2 Growth, body size and genetics

A number of studies have been made which estimate the total number of fibres in the transverse sections of muscles. These include the investigation of the tibialis anterior, biceps brachii, extensor digitorum longus, soleus and sternomastoid of the mouse (Rowe & Goldspink, 1969), m. radialis, m. biceps brachii, m. extensor carpi radialis, m. gastrocnemius, m. tibialis anterior, m. soleus and m. plantaris of the rat (Morpurgo, 1898; Enesco & Puddy, 1964; Chiakulas & Pauly, 1965), m. longissimus in the pig (Staun, 1963; Davies, 1972), and m. sartorius in man (MacCallum, 1898; Montgomery, 1962). These studies have shown that - depending on the muscle and "maturity" of the

species at birth - fibre numbers may increase for a short period after birth but that subsequent postnatal growth of the muscle is due to hypertrophy of the fibres present in the neonatal period. A dissenting view is held by Rayne & Crawford (1975) who find that the number of fibres in the lateral pterygoid muscle of the rat (whose fibres run from origin to insertion) increases progressively from birth to adulthood, the precursors of muscle fibres seen at birth having disappeared by 4 weeks of age. They surmise that satellite cells may be the source of new fibres in the adult. However in the present study no undifferentiated fibres were observed in the m. semitendinosus of the dog or horse at any of the stages of growth studied.

In mice selected for high body weight at 5 and 10 weeks of age, Byrne, Hooper & McCarthy (1973) show that an increase is observed in the numbers of fibres in m. tibialis anterior, m. pectoralis major, m. brachio-radialis, m. rectus femoris and m. psoas major. In the present investigation it appears that in animals whose total fibre numbers in the transverse section of m. semitendinosus was assessed, the greater numbers of fibres in m. semitendinosus of the Greyhound and Thoroughbred compared with the other members of their species may be the effect of enhanced body size. However the smallest adult Greyhound (19 kg liveweight) has more fibres (280,000) in m. semitendinosus than the largest other dog a Great Dane (46·5 kg liveweight; 248,000) similarly the smallest adult Thoroughbred has more fibres than any of the other non-Thoroughbred-cross adults with the exception of an adult Clydesdale mare

(Tables 34 and 35). The covariate relationship between fibre numbers and liveweight is greater in the athletic animals over the entire weight range studied. Secondly the total numbers of fibres at weights corresponding to neonatal and adult animals are greater for the athletic animals. Hence it is unlikely that body size is the sole determining factor controlling the numbers of fibres in a muscle. Therefore the greater number of fibres in m. semitendinosus of athletic animals may be a genetically controlled attribute. This possibility certainly merits further investigation in relation to meat production.

3.4.4 Factors affecting mean fibre areas

The effect of environmental conditions (and inherited factors) determine the eventual size of the growing animal. Similarly it is probable that the maximum size of a cell is dictated by genetic and environmental conditions, with limits being imposed by morphological and physiological constraints.

Using his own data, (Young, 1970) and that of others (Enesco & Puddy, 1964; Chiakulas & Pauly, 1965), Young (1974) concludes that although the DNA content of the rat m. extensor carpi radialis increases somewhat during growth, the weight of the muscle increases to a much greater extent. Loewy & Siekevitz (1969) suggest that the nucleo-cytoplasmic ratio may be one of the limiting factors of absolute cell size, as well as intracellular diffusion capacities and transport across the plasma membrane. As muscle fibres are multinucleated cells the constraints of cytoplasmic to nuclear size may be less

rigid than in mononuclear cells. In the case of membrane depolarization a special mechanism - the T-tubules - facilitates conduction within muscle fibres. As regards metabolic requirements, it would appear that the major constraint on muscle fibre size is the surface to volume ratio of the fibre in relation to diffusion into and out of the fibre.

3.4.4.1 Contraction of fibres on removal of samples from the animal

The muscle samples used in this study were all sampled pre-rigor and therefore contracted fully on removal. As the transverse sectional areas were estimated only in regions in which fibres were cut transversely and where the amount of endomysium and perimysium was minimum, it is considered that the areas are comparable between samples and between species. However this assumes that all the muscles used contracted to a similar extent when the samples were excised.

3.4.4.2 Sites of sampling

It may be noted that the mean fibre area of the superficial sample of m. semitendinosus in young Thoroughbred horses appears to underestimate the mean fibre area of the whole section (Table 30), although the difference is not satistically significant. In any case myosin ATPase high-reacting (AH) fibres predominate towards the superficial caudal region of the muscle (the source of the samples in both types of horse) and this type of fibre increases in area at a slightly greater rate than the myosin ATPase low-reacting (AL) fibres during growth. Hence during development the preferential growth of AH fibres should

compensate for any under-estimation (section 3.3.2.6). Therefore it is probable that the mean fibre area of the samples from m. semitendinosus (predominately AH fibres in both types of horse) is a good indicator of the mean fibre area of the transverse section at all stages of growth.

Johnson & Beattie (1973) stress the need for accurate specification of sampling sites in muscles as they find variations in fibre diameter between teased fixed samples from closely associated sections along the long axis of the ox m. biceps brachii, m. longissimus or m. semitendinosus. There are also variations in the mean area of fibres within samples of dog and horse muscles due to the variation in the size of myosin ATPase high and low reacting fibres: in general AL fibres are larger than AH fibres when young but the reverse is true in adults (section 3.3.2.6). It is for these reasons that the mean fibre area of samples from well-defined regions of muscles are used in this study.

3.4.4.3 Body size

A direct relationship of fibre diameter with body size would be inconsistent with tissue perfusion at least in larger animals. Hill (1956) predicts that the diameter of a muscle fibre should vary as the square root of the body size. However the available evidence does not support his prediction. Thus although Gauthier & Padykula (1966) claim a direct relationship between fibre diameter and body size in m. diaphragma of 13 species (ranging in size from a harvest mouse to an ox), Schmidt-Nielsen &

Pennycuik (1961) find from their studies on m. masseter and m. gastrocnemius that except in very small mammals there is little difference in diameter of muscle fibres over a wide range of body size (bat to ox). Similarly Davies & Gunn (1972) find no correlation between body size and mean fibre area in m. diaphragma of nine mammalian species ranging in size from mice to horses. The present study has also shown that the disparity in fibre size in similar muscles between adult horses and dogs is not directly related to the difference in their body sizes.

When considering the effect of body size within the same species the same principle apparently holds true. Although in adult dogs of body sizes ranging from 1·4 to 56·4 kg, Julian & Cardinet (1961) find that the mean fibre area is larger in the heavier dogs, in the present study the mean fibre area was not always larger in the larger dogs, over a range of adult liveweights from 7·7 to 46·5 kg.

It is probable therefore that when a muscle in an adult horse is larger than a similar muscle in an adult dog it is because it is composed of a greater number of fibres. The difference in muscle size between foetal horses and neonatal pups would appear to be due almost entirely to the greater number of fibres in the foetal horse (section 3.3.1.1.3).

3.4.4.4 Age when sampled

It is recognised that respiratory movements may occur in foetal lambs in utero from the last third of gestation onwards or from the first third onwards when exteriorized (Dawes, Fox, Ludec, Liggins & Richards, 1972).

Although respiratory movements in utero have not been detected in the dog or horse it is also possible that the muscles of the respiratory apparatus should develop earlier than the limb muscles. In dogs this could explain why the mean fibre area of m. diaphragma is larger at birth than those of the limb muscles. However in the most immature foetal horses studied, the mean fibre area of m. diaphragma is not greater than in the other muscles. Possibly the limb muscles in the horse may develop earlier than in the dog, perhaps associated with the greater locomotory capacity of the foal in comparison to the pup at birth.

3.4.4.5 Growth and exercise

The increase in fibre area with growth which occurs to a greater extent in athletic animals of both species may reflect the greater growth potential of their muscle. However immature athletic animals can have smaller mean fibre areas than non-athletes of similar liveweights. This finding is reminiscent of Joubert's (1956) suggestion that muscle growth is a function of physiological rather than chronological age.

Contrary to Morpurgo's (1897) findings of an increase in whole muscle and fibre areas in the dog sartorius after a training regime where the dogs ran 28 to 53 kilometers per day, Thorner (1935) finds in the same species that a running regime of 8 to 45 kilometers per day for 5 months does not alter the mean fibre area of vastus externus and gastrocnemius. The detraining regime of this study is associated with slight increase in fibre area: this may

indicate that the training regime of Greyhounds (mostly walking 3 to 10 kilometers per day) causes a reduction in fibre area - conducive to improved blood perfusion. Alternatively it may be that the muscles sampled are unsuitable for investigating training effects.

3.4.4.6 Fibre metabolism

The smaller mean fibre area of m. diaphragma in comparison to m. semitendinosus and m. pectoralis transversus within both types of horse and dog may be related to the greater density of capillaries in m. diaphragma at least in the dog if not in the horse (section 3.3.2.7). The time needed for oxygen to diffuse into the centre of a fibre is proportional to its transverse sectional area (Hill, 1965). Hence the small fibres of m. diaphragma facilitate a greater degree of vascularity consistent with the higher level of succinate dehydrogenase activity in the muscle than in the other two muscles (section 3.3.3) this would be associated with the need for continuous usage of this muscle. The smaller fibre area of m. diaphragma may also be associated with the higher proportion of AL fibres in this muscle in both types of dog and horse: such fibres always have a high aerobic capacity. However the proportion of AL fibres in a muscle is not always related to its mean fibre area (sections 3.3.1.2 and 3.3.2.2).

3.4.4.7 Genetic factors

In m. longissimus of the Danish Landrace pig Staun (1972) finds that the hereditability of total fibre number is greater than that of fibre diameter and that the hereditability of both is greater in young females than in

castrated males. What hereditability actually means in terms of genetic control over fibre size or numbers is not clear. Byrne, Hooper & McCarthy (1973) find a significant maternal effect on fibre diameter in murine muscles, but no evidence of maternal influences affecting fibre number. This may suggest differing sire and dam effects on the two major parameters governing muscle bulk.

3.4.4.8 Relation to athletic ability

The mean fibre area of m. semitendinosus and m. diaphragma are greater in adult athletic animals than in their fellows - but this is not so in m. pectoralis transversus - indeed in dogs over the range of liveweight studied, the areas are smaller. This suggests that differences in mean fibre areas in different genotypes do not necessarily follow a similar trend in all the muscles. Similarly Byrne, Hooper, & McCarthy (1973) show that selection for increased or decreased liveweight in mice at 5 or 10 weeks of age relative to controls may be accompanied by an increase or decrease in fibre diameter. However their data indicate that the effect may be greater in some muscles than in others. The greater growth rate of fibre area relative to liveweight appears to be the main contributing factor to the greater fibre area of the athletic animals.

The findings of Ethmadi & Hosseini (1968) based on one human 'athlete' (a lumberjack) only, suggest that the human 'athlete' may be similar to the 'athletic' animal in possessing larger and more fibres than the 'non-athlete'.

3.4.4.9 Other factors

The androgen associated larger muscles in mice and rats are attributable to a greater fibre diameter in these muscles (Goldspink, 1972). The same author also presents evidence that starvation or semistarvation is associated with reduced fibre diameter in laboratory and farm animals (Goldspink, 1972). Differing diatetic intake or sex hormone status cannot be responsible for the difference in fibre areas between the 'athletes' and 'non-athletes' of this study, since their diets were similar and no sex difference was apparent.

There is evidence therefore from various sources that the size and numbers of fibres detectable in a muscle of a normal animal are influenced by : the maturity of the animal when sampled, the function of the muscle, the body size and (expected) adult body size of the animal, the animal's exercise, nutritional and anabolic steroid status and the selective processes undergone by the strain of animals to which the individual belongs. These factors which determine the numbers and areas of fibres in muscles and hence gross muscle size are of great importance to both the meat and athletic animal producer. A comparison of athletic and non-athletic members of the same species may elucidate how the number and size of fibres in a muscle are controlled.

3.4.5 Significance of histochemical reactions used in this study to establish fibre type profiles

3.4.5.1 Succinate dehydrogenase E.C. 1.3.99.1

Nitro blue tetrazolium acts as a hydrogen acceptor when the sodium succinate of the incubation medium is dehydrogenated. The tetrazolium is reduced to a monoformazan or diformazan depending on whether it reacts with 2 or 4 hydrogen ions (Altman & Butcher, 1973). Diformazan deposition caused by SDHase activity follows patterns of mitochondria, demonstrated by light microscopy (Nachlas et al. 1957; Scarpelli & Pearse, 1958; Novikoff, Shin & Drucker, 1961) or by electron microscopy in skeletal muscle (Padykula & Gauthier, 1963; Ogata, 1964; Pieper, Fuestel & Hubner, 1969) and kidney (Novikoff et al.1961), Brooke & Engel (1966) provide evidence that nitro blue tetrazolium is selectively absorbed on to mitochondria and sarcoplasmic reticulum of striated muscle fibres. Since, however, SDHase is believed to be entirely intramitochondrial (Roodyn, 1967), non-specific adsorption should enhance the histochemical localisation of the enzyme. A limited amount of non-specific diformazan deposition remote from sites of SDHase activity, such as on lipid droplets (Hitzeman, 1963), should have little effect on the comparison between individual fibres. The report of a heterogeneous all-or-none deposition at random in individual mitochondria (Seligman, Ueno, Morizono, Wasserkrug, Katzoff & Hanker, 1967) could have more serious implications, but the numbers of mitochondria in the large numbers of fibres sampled in this study should overcome this irregularity. It is

possible that SDHase activity of mitochondria from different muscle fibres may vary (Blanchaer, 1964), but the density of diformazan deposited histochemically in a particular fibre after incubation for as long as 20 minutes should depend primarily on the level of SDHase activity itself related to mitochondrial density.

Paul & Sperling (1952) demonstrate a direct relationship between estimates of mitochondrial density, determined by phase microscopy of homogenised tissue, and the oxidative capacity of a variety of muscles from different species. This relationship is supported by observations on the effect of severe exercise on limb muscles: this can produce a twofold increase in their capacity to oxidize pyruvate (Holloszy, 1967), accompanied by an increase in mitochondrial density as seen electron-microscopically (Gollnick & King, 1969). Similar findings are reported by Kraus, Kirsten & Wolff (1969).

Although it lacks direct proof, the assumption that the histochemical succinate dehydrogenase reaction indicates the capacity of an individual fibre for aerobic metabolism appears responsible.

3.3.5.2 Glycogen phosphorylase E.C. 2.4.1.1

Takeuchi & Kuriaki (1955) have shown their method to be specific for glycogen phosphorylase which in vivo catalyses the successive phosphorylation of the terminal glucose units of the glycogen chain, with the production of glucose-1- phosphate. The histochemical method utilises the reversibility of this reaction to synthesise

a polyglucose which stains blue with iodine and is distinct from native glycogen, judged either by iodine staining or by electronmicroscopic appearance (Takeuchi & Sasaki, 1968). Differences in the colour of iodine staining have been attributed to the progressive increase in chain length during synthesis of the glucose polymer: blue indicating chains of over 30 glucose units, and red indicating chains of 7 - 13 glucose units (Swanson, 1948). Iodine colours have been used in this study to indicate different levels of phosphorylase activity in individual fibres.

It is accepted that glycogen is quantitatively the major store of energy for muscular contraction in the absence of oxygen, and that phosphorylation is the first step in glycolysis. The phosphorylase activity of an individual fibre is therefore indicative of the rate at which it can derive energy for contraction anaerobically.

3.4.5.3 Myosin adenosine triphosphatase (Myosin ATPase)

The specificity of the histochemical technique for myosin ATPase (Padykula & Herman, 1955; Padykula & Gauthier, 1963) is supported by the work of Guth & Samaha (1969) They compared the effects of pre-incubation at pH values of 10·4 and 4·35 on the ATPase activity of both actomyosin extracted from fast and slow muscles of the cat, and individual fibres of these muscles examined histochemically. Their study also provides evidence that fibres shown histochemically to be ATPase high-reacting (AH) are fast contracting, and that ATPase low-reacting (AL) fibres are slow contracting. This concept is supported by work showing that the activity of myosin ATPase is directly proportional to the intrinsic speed of shortening in a range of normal muscles with widely varying speeds of contraction (Barany, 1967), and in muscles in which the speeds of contraction have been altered by cross-reinnervation (Barany & Close, 1971). Taylor, Essen & Saltin (1974) have correlated the proportions of myosin ATPase high-reacting fibres with the biochemical activity of the enzyme in human lower limb muscles.

The intermyofibrillar reaction products due to the myosin ATPase reaction (Fig. 32) is attributable to this reaction (Schiaffino & Bermioli, 1973). It is known that muscle mitochondria possess a calcium-activated ATPase still showing activity at a pH as high as 9 to 9·5 (Samaha & Yunis, 1973) and that liberated phosphate may bind to myofibrils suggesting that the histochemical procedure for myosin ATPase does not exclusively demonstrate

the activity of the latter enzyme (Guth, 1973). However, the activity of mitochondrial ATPase falls off rapidly between pH 7·5 and 10. Since in the present study the myosin ATPase reaction is carried out at pH 9·5 - the optimum for the high reacting fibres being between 9·4 and 9·6 (Guth & Samaha, 1969) - the activity of mitochondrial ATPase would be much less at this pH than at pH 9·4. It may be noted that the method of Padykula & Herman (1955) is carried out at pH 9·4 and uses a barbitone buffer, which in practice may allow the pH to fall during incubation (Davies & Gunn, unpublished observations). Also, AL fibres at pH 9·4 (Engel, 1962; Edgerton & Simpson, 1969) or pH 9·5 (Davies & Gunn, 1972) have relatively high activities of the mitochondrial associated enzymes and hence should have a higher level of deposition of "non-specific" phosphate than AH fibres. The clear differentiation of fibres into AH or AL reactions suggests that mitochondrial produced phosphate does not obscure that produced from catalysis by myosin.

The differentiation of populations of AH fibres at pH 9·4 using their sensitivity either to formalin fixation or to pre-incubation of sections at acid of alkaline pH has not been undertaken in this study. There is biochemical evidence for the existence of two types of myosin differing with respect to the pH lability of their ATPase and closely associated proteins (Locker & Hagyard, 1968; Samaha, Guth & Albers, 1970a). However, Samaha, Guth & Albers (1970b) suggest that there are 3 types of fibre, as demonstrated by alkaline lability of their myosin ATPases

at pH 9·4 and sensitivity of the alkali-stable fibres to formalin or acid. This may be due to basic differences in the combinations of the different sub-units of the myosin macromolecule. Whether the responses of the myosin ATPase high-reacting fibres at pH 9·4 (alkali-stable fibres) to formalin fixation or to acid or alkali pre-incubation are due to properties of the myosin molecule or to other properties of the fibres is not certain. In rat crural muscles within the population of fibres that are alkali-stable, the fibres resistant to formalin fixation and sensitive to acid pre-incubation at pH 4·35 are smaller (Samaha, Guth & Albers, 1970b) and have a higher activity of mitochondrial - associated enzymes (Stein & Padykula, 1962) than the fibres with formalin sensitive acid-resistant myosin ATPase.

Yellin (1972) demonstrates that even within the same rats differences in response to acid pre-incubation (at pH 4·4) may occur between fibres of m. diaphragma and m. tibialis anterior. Similarly, Guth & Samaha (1970) and Khan, Papadimitriou& Kakulas (1974) indicate that there may be a species variability in the pH lability of the myosin ATPase high reacting fibres (at pH 9·4). Likewise, it might be suggested further than an increase in lactic acid in a fibre just after or prior to sampling may alter its pH and therefore its staining intensity for myosin ATPase at pH 9·4.

Khan et al (1974) show that the temperature at which pre-incubation (whether acid or alkali) is carried out is as critical a factor as pH for differentiating types of alkali-stable myosin ATPase fibres. They suggest that

the differing staining intensities caused by pre-incubation do not reflect an inherent characteristic of the muscle fibre but instead are associated with denaturation of the myosin molecule and its ATPase activity.

The proportion of myosin ATPase high-reacting fibres in a whole muscle bear a reciprocal relationship to its contraction time (Peter, Bernard, Edgerton, Gillespie & Stempel, 1972; Cardinet , Fedde & Tunell, 1972). Several investigators have shown that three types of fibres may be identified in rat and cat crural muscles based on speed of contraction and fatigability (Close, 1967; Burke, Levine, Zajac, Tsairis & Engel, 1971; Burke, Levine, Tsairis & Zajac, 1973). However it is apparent that two populations of fibre may be differentiated by the myosin ATPase reaction at pH 9·4 in the soleus and gastrocnemius of the cat (Burke et al. 1971; Burke et al. 1973; and Burke, Levine, Salcman & Tsairis, 1974). Their twitch contraction times do not overlap although there is a range of contraction times within each population. Similarly Stephens & Stuart (1974) investigating the medial gastrocnemius of the cat suggest that a bipartite classification of motor units based on speed of contraction alone is more applicable to their data than one based on both speed of contraction and fatigability; they consider that a scheme based on a fast-slow framework is the most desirable way of describing motor unit properties as it may be suggested that resistance to fatique may be dependent on the rate of ATP supply to the muscle.

In man, Johnston, Polgar, Weightman & Appleton (1973)

indicate that despite individual variation, muscles with predominantly postural functions have more slow-twitch fibres than those used for rapid contractions. Similarly Sulemana & Suchenwirth (1972) show that human upper limb muscles have fewer slow-twitch fibres than leg muscles. Using open biopsy electromyography in patients with long-term low-grade motor neuropathies, Warmolts & Engel (1972) show that AL fibres are associated with prolonged sustained discharges of potentials of low frequency AH fibres are associated with irregular bursts of potentials of higher frequency. In hamsters Awan & Goldspink (1972) demonstrate that muscles largely composed of slow-twitch fibres can develop and maintain more tension per μmole of creatine phosphate used than those composed of fast-twitch fibres, concluding that slow-twitch fibres are ergometrically more efficient for isometric function. These studies suggest that slow-twitch fibres are more suited for repetitive isometric or postural function than myosin ATPase high-reacting fibres.

Clearly more correlative work on the physiology, biochemistry and histochemistry of individual muscles (and if possible, muscle fibres) of individuals in different species is necessary. However the evidence summarised above appears to justify the designation of ATPase high-reacting mammalian extrafusal fibres as fast-twitch and ATPase low reacting fibres as slow-twitch.

The distinct difference between the histochemical reactions of these fibres is probably related to the molecular differences between the myosin of fast and of slow

muscle demonstrated chemically by Samaha et al. (1970a) and associated with the "light chains" of the myosin molecule (Sarker, Sreter & Gergely, 1971; Lowey & Risby, 1971). Although chemical procedures may alter the histochemical reaction of muscle fibres, (for example the various types which are differentiated by pre-incubation associated with their unknown physiological properties) it is considered that a classification based on the myosin ATPase reaction differentiates two populations of fibres, one of which is faster contracting than the other. This is at present the most desireable baseline for differentiating fibres in the dog and horse.

3.4.5.3.1 Relationship of myosin ATPase activity to electrical stimulation and the function of muscle

It appears that appropriate patterns of electrical stimulation alter both the histochemical properties and contraction characteristics of a muscle. Continuous electrical stimulation (pulses of 0·5 msec at a frequency of 10 pulses per second for up to four weeks) of the rabbit m. tibialis anterior and m. extensor digitorum longus (both fast contracting muscles) is associated with the synthesis by these muscles of myosin light chains similar to those synthesised by the slow contracting soleus (Sréter, Gergely, Salmons & Romanul, 1973). This alteration is reversible on cessation of stimulation (Sréter, 1975). Following denervation of the rat soleus, Lømo, Westgaard & Dahl (1974) brief periods of electrical stimulation intended to resemble phasic activity of a fast muscle (tetanic stimulation lasting 0·5 seconds and repeated every

25 seconds) and longer periods resembling the tonic activity of a slow muscle (tetanic stimuli lasting 10 seconds and repeated every 50 seconds) cause the muscle to adopt contractile and histochemical properties of a fast and slow contracting muscle respectively. Electrical stimulation (10 pulses per second each of 0·5 msec duration) may also prolong the contraction times of the rabbit tibialis anterior and extensor digitorum longus and the cat flexor hallucis longus - which are all fast contracting muscles (Salmons & Vrbová, 1969). Findings to the contrary (Riley & Allin, 1973) may be associated with too short a period of artificial electrical stimulation.

Altering the nerve supply to muscles has been shown to exert similar changes to those of patterns of electrical stimulation. It has been shown that cross re-innervation of fast and slow twitch muscles:

(i) reverses intrinsic speeds of contraction, as in the cat (Buller, Eccles & Eccles, 1960), guinea-pig (Robbins, Karpati & Engel, 1969) and rat (Close, 1969),

(ii) alters the myosin ATPase activity as demonstrated biochemically in the cat (Buller, Mommaerts & Seraydarian, 1969; Samaha, Guth & Albers, 1970c) and rat (Bárány & Close, 1971; Buller et al. 1971),

(iii) is associated with an increase in the proportion of myosin ATPase high reacting fibres in a slow-twitch muscle (soleus) as in rabbit (Dubowitz, 1967), guinea-pig (Karpati & Engel, 1967; Robbins et al. 1969), cat and rat (Guth, Samaha & Albers, 1970)

and a decrease in the proportion of myosin ATPase high-reacting fibres in fast twitch muscles as in flexor hallucis longus of the cat (Dubowitz, 1967; Guth et al. 1970) and flexor digitorum longus of the rabbit (Dubowitz, 1967), and

(iv) causes reciprocal changes both in contraction times and in light chain patterns of myosin as shown electrophoretically on SDS polycrylamide gels in the cat soleus and flexor hallucis longus (Weeds, Trentham, Kean & Buller, 1974) and the rat soleus and flexor digitorum longus (Sréter, Gergely & Luff, 1974).

Thus the type of electrical stimulation whether natural or artificial has a direct influence on the contractile properties of the muscle fibre. Conversely any change in the contractile properties of a muscle and the proportion of its fibre types (as differentiated by the myosin ATPase reaction) should be associated with altered patterns of electrical stimulation.

Notwithstanding the importance of the type of electrical stimulation the contractile properties of a muscle may be altered by mechanical factors which do not interfere directly with its electrical stimulation pattern. Thus imposition of extra isometric work on limb muscles of laboratory animals causes slowing in their speeds of contraction (Vrbová, 1963; Lesch, Parmley, Hamosh, Kaufman & Sonnenblick, 1968; Olsen & Swett, 1969; Gutmann, Schiaffino & Hanzlikova, 1971), a decrease in myosin ATPase activity (Gutmann & Hajek, 1971) and an increase in the proportion

of myosin ATPase low-reacting fibres (Guth & Yellin, 1971). Davies (1972) demonstrates an increase in proportion of myosin ATPase low-reacting fibres in m. longissimus and m. diaphragma of the pig with growth and increasing liveweight; the proposes that this is an adaptation of these muscles to meet the greater isometric demands of increased liveweight. There is evidence that denervation of antagonists also causes an increase in the speed of contraction and in the proportion of myosin ATPase high-reacting fibres in m. soleus of the rat (Guth & Wells, 1972). However the gait of the experimental animals is not commented on by the authors. It is possible therefore that the effects may be associated with disuse atrophy of the muscle: peroneal denervation is associated with flexion of the stifle and extension of the hock (O'Connor, 1965) and immobilization of the stifle and hock causes shortening of contraction time of the soleus of the rat (Fischbach & Robbins, 1969; 1970).

In summary, it is apparent that although the type of electrical stimulation of a muscle (or muscle fibres) is an important factor in governing physiological, biochemical and histochemical parameters of the muscle (and fibre) its effects are integrated with those of the functional stresses imposed on the muscle.

3.4.6 Classification of fibre types

The interpretation of histochemical investigations of muscle fibre types should relate the reaction results directly to the physiological and metabolic characteristics of each fibre. The evidence given above suggests that the

profile obtained by determining the SDHase, GPase and myosin ATPase reactions will classify an individual fibre according to its capacity for aerobic and anaerobic metabolism and its intrinsic speed of contraction.

Metabolic profiles

It is accepted that a fibre classified as either aerobic or anaerobic may also have a low level of the other type of metabolism. Complete reciprocality in the mode of ATP production is claimed for fibres of the rat, man (Dubowitz, 1960a,b) and cat (Jinnai, 1960) muscles and supported by Engel (1962, 1965, 1970) and Suchenwirth & Bundschu (1970) in human muscles; Nishiyama (1966) in the respiratory muscles of the rat and cat; Kugelberg & Edström (1968) in rat crural muscles; Moody & Cassens (1968) in longissimus and trapezius muscles of the pig; and Jasmin, Bokdawala & Desrosiers (1971) in crural muscles of the hampster. However it is feasible to believe that the aerobic and anaerobic methods of ATP production as outlined in Fig.127 may both contribute significantly to the energy supply of a muscle fibre adapted for repetitive and rapid contractions. Therefore for either fast - or slow - twitch fibres there are three theoretical possibilities for their metabolism; aerobic, combined anaerobic and anaerobic, and anaerobic. Five of the six possibilities are found in significant proportions in m. diaphragma in nine species of mammal (Davies & Gunn, 1972). The present study shows that two of these possibilities occur in the dog muscles and three in the horse muscles studied. In particular samples from all three muscles of the adult

dogs and from m. pectoralis transversus and m. diaphragma of the adult horses all fibres that are AH are also SH and PH. In samples of the other muscle, m. semitendinosus, only 65% of AH fibres in the adult Thoroughbred and 42% in the adult other horses are AH, SH and PH. Although histochemical methods are qualitative and therefore can not indicate the absolute levels of activity of SDHase and GPase, the presence of large numbers of fibres with high enzyme activities for both aerobic and anaerobic metabolism suggests that there need not be an inverse relationship between the two methods of ATP production. The present histochemical evidence for this is supported by other studies. Thus fibres having both a high activity for SDHase and a moderate to high phosphorylase activity have been demonstrated in rat crural muscles (Romanul, 1964) in rat femoral muscles and m. soleus of the monkey and rat (Bocek & Beatty, 1966) in the thyroarytenoid and cricothyroid muscles of the rabbit (Hall-Craggs, 1968), in guinea-pig crural muscles (Edgerton & Simpson, 1969), in cat crural muscles Prewitt & Salafsky (1970), in the gastrocnemius and soleus muscles of the mouse and triceps brachii and rectus abdominis muscles of the pig and ox (Ashmore & Doerr, 1971), in m. diaphragma of eight mammalian species (Davies & Gunn, 1972) and in the longissimus of the pig (Davies, 1972). Further biochemical studies (Gillespie, Simpson & Edgerton, 1970) demonstrate greater stores of glycogen in the 'red' (aerobic) region of m. vastus lateralis of the guinea-pig, composed of 77% SDHase high fibres, than in the 'white' region (anaerobic)

containing only 29% SDHase high fibres.

Fibre type nomenclature

Engel (1962, 1965, 1970) proposed a simple division of muscle fibres into two histochemical types for human muscles and also for crural muscles of the guinea-pig (Karpati & Engel, 1967, 1968). In this scheme "Type 1" fibres have high activities for the enzymes of aerobic metabolism and show little phosphorylase o myosin adenosine triphosphatase (myosin ATPase) activity, whereas their "Type II" fibres have a low activity for aerobic enzymes and a high activity for phosphorylase and myosin ATPase. Stein & Padykula (1962) have established histochemical "profiles" for fibres in rat crural muscles using methods for SDHase, glycogen and myosin ATPase. These authors show that some fibres have high myosin ATPase and SDHase activity. Thus the existence of this type of fibre confounds Engel's over-simplified system.

Edgerton & Simpson (1969), reviewing the classifications that have been used since 1962, favour the descriptive terms "red", "intermediate" and "white" in preference to letters or numbers (this nomenclature is for the most part based on the activity of aerobic enzymes). Fibres low in myosin ATPase activity were described as "intermediate" in SDHase activity by Stein & Padykula (1962), Edgerton & Simpson (1969) and Jasmin et al (1971) in their studies of crural muscles of rat, guinea-pig and rat and hamster respectively. But even this more complex classification is insufficient to accurately describe the observed histochemical properties of all mammalian muscle as the term "intermediate" has no

general significance (Table 60). Also from the results of Ashmore & Doerr (1971) for limb muscles of the pig and ox, Burke et al (1971) for the cat gastrocnemius, Davies & Gunn (1972) on m. diaphragma of nine mammalian species, Davies (1972) on the longissimus of the pig, Gunn (1972, 1973a) on the laryngeal muscles of the horse show that this ("intermediate") type of fibre frequently has SDHase activity equal to or even greater than surrounding myosin ATPase high fibres.

More recently a description of muscles fibres based on their capacity to synthesise and degradate ATP has been used (Gunn & Davies, 1971; Davies & Gunn, 1972). Peter, Bernard, Edgerton, Gillespie & Stempel (1972) have since adopted a similar classification with more specific biochemical connotations, based on histochemical methods and supported by biochemical and physiological observations. The classification of Gunn & Davies (1971), Davies & Gunn (1972) is that used in this study: Table 60 indicates how it complies with other nomenclature systems.

3.4.6.1 Histochemical fibre types in canine muscle

Trevino, Demaree, Saunders & O'Donnell (1973) differentiate three types of fibres, 'red', 'white' and 'intermediate', in the triceps muscle of German Shepherd dogs in agreement with reports on other species, but their photomicrographs do not distinguish 'white' and intermediate' fibres by their reaction for DPNH dehydrogenase. Three groups of investigators have used the "Type I/Type II" of Engel (1962) to describe both normal and pathological dog muscle (Cardinet , Wallace, Fedde, Guffy & Bardens, 1969;

Cardinet , Fedde & Tunell, 1972; Griffiths, Duncan, McQueen, Quirke & Miller, 1973). The "Type I" and "Type II" fibres described by these groups correspond to the slow-twitch aerobic and the fast-twitch combined aerobic and anaerobic fibres of the present study, and similarly therefore the myosin ATPase reaction is used in this study to differentiate fibre types in the dog. But it cannot be assumed that the methods of ATP production holds a similar relationship to the rate of ATP utilization in pathological as in normal muscle.

3.4.6.2 Histochemical fibre types in equine muscle

Using the histochemical method for SDHase, Shubber (1972) distinguishes three types of fibres in forelimb muscles of three two year old horses. As in m. semitendinosus in the present study, it may be possible to distinguish three types of fibres using SDHase alone in agreement with investigations on other animals. This method only differentiates fibres by their aerobic capacity and hence does not describe their metabolic profile. Lindholm & Piehl (1974) use histochemical methods for myosin ATPase to differentiate fibres into fast or slow contracting, and nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase), to estimate the oxidative capacity of a fibre. They describe fibres of the gluteus medius as slow-twitch high oxidative; fast-twitch, high oxidative; and fast-twitch low oxidative. Although they also show that all the fibres in samples from this muscle have large amounts of the anaerobic energy source, glycogen, this is ignored in their terminology of fibre types. In any case,

classifications of fibres based on their differing capacities for aerobic metabolism cannot be used in all horse muscles as, for example, all fibres in m. diaphragma (Davies & Gunn, 1972) and in the laryngeal muscles (Gunn, 1972) have a high activity of succinate dehydrogenase in the normal. The present study indicates also that all the fibres in m. pectoralis transversus of adult horses have a high activity for SDHase. In m. pectoralis transversus and m. diaphragma of adults the myosin ATPase reaction may be used only to differentiate slow-twitch fibres with combined aerobic and anaerobic metabolism and fast-twitch fibres with combined aerobic and anaerobic metabolism; however, in m. semitendinosus the reaction differentiates fibres into slow- and fast-twitch, the fast-twitch fibres being subdivided further by their aerobic capacity.

Drabkin (1950) and Lawrie (1953) find that homogenates of horse muscle have an unusually high oxidative capacity in comparison to those of other species. Lindholm & Saltin (1974) indicate that homogenates of horse muscle are also rich in glycogen in comparison to other species. Although these studies on aerobic and anaerobic capacity were carried out on different muscles, they substantiate the present histochemical evidence for the high aerobic and anaerobic capacity in some horse muscles.

3.4.7 Quantitative differences in fibre type between individual animals

The increase in the proportion of slow-twitch fibres in samples of m. diaphragma and in complete cross-sections

of m. semitendinosus which occurs with increasing live-weight in mammals pari passu with decreasing respiratory rate and speed of limb movement (Davies & Gunn, 1971) receives confirmation in this study from comparisons of m. diaphragma in the horse and dog. However this change is not evident in m. semitendinosus or m. pectoralis transversus. In the case of m. semitendinosus this may be due to the comparison of the cross section of the whole muscle in the dog with only superficial portions of the muscle in the horse; and in the case of m. pectoralis transversus, the muscle may function differently in the two species.

Within either species factors associated with breed apparently over ride the effect of body size. The athletic animals, although frequently heavier than the non-athletes, have fewer slow-twitch fibres in their limb muscles and (in dogs only) in m. diaphragma. It is concluded therefore that the limb muscles both of Greyhounds and Thoroughbreds have a greater speed of sarcomere contraction than their fellows. This indicates a greater rate of work per gramme of muscle in athletic animals, and suggests subtle differences in their nervous system.

There is some evidence that "double muscled" cattle have a lower proportion of slow-twitch fibres in their muscles than normal cattle (Holmes & Ashmore, 1972; Hendricks, Aberle, Jones & Martin, 1973; West, 1974). However the two types of cattle could still have the same absolute proportions of slow-twitch fibres in the same muscle but the larger numbers of muscle fibres in

"double muscled" cattle (Ouhayoun & Beaumont, 1968) reduces the proportion. Although this feature was not investigated in the studies of "double muscled" cattle or in horses in the present study, it is apparent from Fig. 89 that m. semitendinosus of the adult Greyhound has a smaller total area of slow-twitch fibres than in other dogs of similar weight.

Using biopsy studies Saltin (1974) suggests that prime human sprinters have fewer slow-twitch fibres in m. vastus lateralis than others and on the basis of rather limited evidence suggests that middle distance runners who are capable of a fast finishing spurt have fewer slow-twitch fibres than their less fortunate competitors. Although variations have been found in the proportions of slow-twitch fibres within the Greyhound and Thoroughbred populations of this study, a correlation with the performance of individuals has not been attempted. In different muscles in different individuals it is possible that similar intensities and durations of (short-term) exercise may be limited by muscular ATP utilization in some individuals but by ATP supply to muscles in others. Certainly the rate of ATP utilization i.e. the speed of sarcomere contraction, is only one factor dictating stride frequency and hence speed of running. Although it is recognised that differences between athletes and non-athletes in the proportion of fast-twitch and slow-twitch fibres in similar muscles should be kept in perspective, a high proportion of fast-twitch fibres undoubtedly enhances stride frequency and in this study appears to be the most significant attribute of fleet runners.

3.4.8 Functional relationship of fibre type proportions within adult animals

The location and fibre architecture of the various anatomical muscles suggests different functions for whole muscles, and the difference in the proportions of fibre types between muscles supports this belief. The distribution of fibres suitable for isometric work within a muscle (Guth & Samaha, 1969; Davies & Gunn, 1971; Gunn, 1973b; Johnson et al., 1973) confirmed by this study suggests that different parts of muscle function differently. Inspection of Figs. 128 & 129 suggests that the deep medial region of the m. semitendinosus is adapted for a postural function while the superficial lateral region of the muscle is suited for a predominantly propulsive function. So different parts of the same muscle may function differently and may even be considered separately. Gross muscles may be considered to be groups of smaller muscles distinguishable by different histological properties, i.e. fibres, and it is their disposition which dictates the function of the whole muscle.

As the distribution of fibre types in a given muscle is probably not random in any species (James, 1971a,b, 1972: rabbit and rat; Jennekins, Tomlinson & Walton, 1971: man) the appreciation of the function of a muscle may be aided by analysis of its complete cross section.

The deep region of the porcine m. semitendinosus has a higher aerobic capacity than the superficial region (Beecher, Cassens, Hoekstra & Briskey, 1965; Tarrant,

Hegarty & McLoughlin, 1972). Similarly in this study the proportion of succinate dehydrogenase high-reacting (SH) fibres increases towards the deep medial region of the equine m. semitendinosus. Although in the dog all the fibres in both regions of m. semitendinosus had a high SDHase activity, biochemical studies of the Greyhound (the only group which had sufficient specimens for investigation) showed a higher activity of this enzyme in the deep medial region than in the superficial portion. The enhanced aerobic capacity of the deep region complements the greater density of slow-twitch fibres present there and provides an efficient energy supply. However the enhanced aerobic capacity shown biochemically is probably not confined to slow-twitch fibres but possibly occurs in fast-twitch fibres as well; certainly this would be compatible with the histochemical observation. This suggests that slow-twitch fibres are used predominantly for postural or low intensity isometric functions (Gollnick, Karlsson, Piehl & Saltin, 1974). If propulsion is then needed, the fast-twitch fibres with the highest aerobic capacity may be recouped next and the fast-twitch fibres with low aerobic capacity recouped thereafter, but this requires further investigation.

Although there was no apparent difference in the biochemical activity of SDHase between similar muscles of athletes and non-athletes in samples from the superficial region of m. semitendinosus, the Thoroughbreds have more aerobic fibres (though not significantly so) than the other horses. Histochemical analysis of muscle samples

from "double muscled" cattle indicate that they have a smaller proportion of aerobic fibres than normal (Hendricks et al., 1973; West, 1974). The athletic animals of this study and "double muscled" animals appear to have similar gross characteristics and more fibres in toto than others, but the "athletes" differ in having more aerobic fibres than their fellows. Ashmore (1974) suggests a relationship between fibre type and meat quality in meat producing animals, the lack of aerobic fibres being an undesirable feature. In this context the factors affecting the different proportion of aerobic fibres in athletic animals (i.e. Thoroughbreds) and "double muscled" cattle could be investigated with profit.

3.4.9 Blood supply to muscle

A useful "by-product" of the myosin ATPase reaction was that capillaries were stained when the pH of the medium was lowered to 9·4 by the addition of a few drops of 0·1 N HCl, after it had been used to demonstrate myosin ATPase activity. Frieman & Kaplan (1960) indicate that the small blood vessels in the dog jejunum may be demonstrated by incubating sections in a similar medium at pH 9·4 to that used to demonstrate myosin ATPase activity in this study. In muscle a similar technique but using an acid pH (4·35) has been used by Guth & Yellin, (1971) and by Padykula & Gauthier (1963) using ADP who comment that enzymic activity in the walls of blood vessels may split ADP.

It would appear that lowering the pH of the medium which has been used to demonstrate myosin ATPase in sections

is a convenient way of demonstrating capillaries whether patent or not.

As an indication of muscle perfusion or drainage potential the ratio of the numbers of capillaries to the number of fibres in a sample is not as useful as measurements of the area of muscle supplied by a capillary. This study only considers the potential blood supply to muscle as indicated by its capillary density. However it is recognised that during contraction the number of open capillaries may increase 2 - 3 times (Krogh, 1922; Martin, Wooley & Miller, 1932; Petrén, Sjöstrand & Sylvén, 1937). Other factors such as oxygen content of the blood; blood pressure and flow rate, capillary length and diffusion across capillaries may also regulate circulatory supply to fibres.

It is established that muscle fibres with a high aerobic capacity have more capillaries associated with them than fibres with a low aerobic capacity (Romanul, 1965) and that muscles with a predominantly aerobic capacity - the cat soleus - have a higher blood flow than muscles with a lower aerobic capacity - cat gastrocnemius - (Reis, Wooten & Hollenberg, 1967; Hilton Jeffries & Vrbová, 1970). However external factors such as training (Petren et al 1937; Rakusán, Ošťádal & Wachtlová, 1971; Carrow, Brown & Van Huss, 1967), high altitude associated hypoxia (Valdivia, 1958; Banchero, 1970); cross-innervation of a prediminately anaerobic muscle by a nerve to predominantly aerobic muscle and administration of thyroxin (Romanul & Pollock, 1969) may well increase capillary density.

Despite variations in capillary; fibre ratios the cross sectional area of muscle supplied by a capillary in "athletes" is remarkably similar to that in non-athletes. Similarly in the dog m. diaphragma is more richly supplied with blood vessels than the other two canine muscles (both of which have similar densities) but in the horse there is little difference in the capillary density between the three muscles. Krogh (1919a) postulated from a study of different muscles in different animals that the capillary density in smaller animals is greater than in larger animals. However Schmidt-Neilsen & Pennycuik (1961), comparing similar muscles of 10 mammals, find that although there is a relationship between capillary density and body size in very small mammals it is not apparent among large mammals and that the capillary : fibre ratio is remarkably similar throughout the range of body size studied. The data presented by Hudlická (1973) and in this study confirms that the blood supply of similar muscles as indicated by capillary density is not related to liveweight in larger mammals.

Krogh (1919a,b) contends that the capillary distribution in muscle is adequate for tissue perfusion. In the present study there is little variation between muscles with differing proportions of fast and slow twitch fibres; (albeit with the exception of the equine m. semitendinosus they are composed of fibres with a high aerobic capacity) this may suggest that a similar degree of local perfusion is adequate for a wide range of functions in dog and horse. Whether the delivery of oxygen or the removal of metabolites such as lactic acid is more important is unknown.

That the muscular ATP-producing systems may be as important a limiting factor for various types of exercise as the circulatory system may be inferred from the experiments of Kaijser (1970) on human arm muscles who concludes that "oxygen transport by blood circulation does not under ordinary conditions limit the aerobic work capacity" (of the muscles). Jobsis & Stainsby (1968) also conclude that blood circulation does not limit the respiratory metabolic activity of the electrically stimulated dog gastrocnemius and gracilis indicating instead that the aerobic capacity of these muscles may be the limiting factor. Similarly Thomson, Dempsey, Chosy, Shahidi & Reddan, (1974) conclude from their studies on human lower limb muscles exercised to two-thirds of their maximum oxygen uptake that oxygen delivery to working muscles is adequate. Although arterio-venous differences in oxygen tension increase with increasing work load (Doll, Keull & Maiwald, 1968) in man, the presence of oxygen in venous blood (PO_2 = 22 mmHg) at the heaviest exercise intensities compared with a PO_2 = 45 mmHg at rest may indicate limitations of skeletal muscle to extract oxygen from the blood during exercise.

Although the heart rate of the foal decreases with increasing liveweight (Adolph, 1971), apparently associated with the decrease in metabolic rate of the animal during growth, the capillary density in skeletal muscle after initial differentiation remains reasonably constant although <u>en bloc</u> the young animals of this study have slightly greater capillary densities than adults. Hudlicka,

Pette & Staudte (1973) claim that the difference in aerobic enzyme activities of the gastrocnemius and soleus of growing kittens is differentiated before their blood flow is, suggesting a secondary relationship of blood supply to changes in muscle fibre chemistry during growth.

3.4.10 Fibre type changes during growth

3.4.10.1 Developmental features of fibre type differentiation

Swatland & Cassens (1973) indicate that in (prenatal) developing porcine muscle secondary myofibres derive from primary myofibres developed earlier. These authors define myofibres as multinuclear myofibril-containing structures formed from fusion of mononuclear myoblasts. A similar system of development of muscle in ovine limb (Ashmore, Robinson, Rattray & Doerr, 1972), avian wing (Ashmore, Addis, Doerr & Stokes, 1973) and porcine limb (Ashmore, Addis & Doerr, 1973) suggest that the primary myofibres possess a low reaction for myosin ATPase and a high reaction for aerobic enzymes and are destined to become the slow-twitch fibres of adult muscles. Such precursory slow-twitch fibres may also be identified in developing muscle by their relative large and uniform size and by their central position among fibres stained intensely using the myosin ATPase reaction.

Ommer (1971) demonstrates large darkly staining fibres with SDHase (presumably precursory slow-twitch fibres) in the longissimus of cattle. Similarly 5 to 10 percent of the fibres in the human quadriceps and gastrocnemius at about 26 weeks of gestation (Dubowitz, 1965) correspond to the large 'b' fibres described by Wohlfart (1937).

Wohlfart (1937) found that 'b' fibres occur in a wide variety of human foetal and neonatal muscles, and that they differ from the surrounding fibres by their relatively larger size; they constitute 0·5 to 2·5% of the fibres in the muscles investigated. Fenichel (1963, 1966) demonstrates that the Wohlfart 'b' fibre in human muscles is the precursory of a slow-twitch fibre in adults.

In many species the histochemical reaction of muscle fibres alters during the perinatal period. The time of onset of differentiation into aerobic and anaerobic fibres varies as shown by studies on the mouse, rat, hamster, guinea-pig, rabbit and man (Dubowitz, 1965), rhesus monkey (Beatty, Basinger & Bocek, 1967), sheep, pig and ox (Ashmore, Tompkins & Doerr, 1972) and man (Toop, 1974). Dubowitz (1965) notes a correlation between "maturity" at birth and rate of development of fibre types. Similarly, in the cat, Nyström (1968) shows that differentiation of fibre types occurs in respiratory and forelimb muscles prior to hindlimb muscles, which correlates with the function of these muscles at birth. In day old kittens, Hudlická, Pette & Staudte (1973) find higher activities of oxidative enzymes in the soleus than in the gastrocnemius which corresponds with the difference in metabolic capacities between these muscles in adults. Davies (1972) finds that the proportion of myosin ATPase low-reacting fibres is greater in m. diaphragma than in m. longissimus of neonatal pigs; similarly the proportion of fibres having a high aerobic and a low anaerobic capacity is greater in m. diaphragma. This neonatal difference in fibre type

composition correlates with the difference between the two muscles in the adult.

There is little doubt therefore that a pattern of muscle development exists in man, animals and birds which consists of a group of myosin ATPase high-reacting (fast-twitch) fibres developing around single myosin ATPase low-reacting (slow-twitch) fibres. Morphologically the latter are recognised by their large size which later becomes less evident as the smaller (fast-twitch) fibres increase in size. Such a pattern is demonstrated in the canine pectineus by Cardinet, et al (1969), and is also found in the muscles of the dog and horse in this study.

Davies (1972) proposes that the changes in fibre types occurring postnatally are not solely related to different functioning requirements of muscle associated with changes in maternal dependence but also the adaption of muscle to meet the mechanical and metabolic demands of support and propulsion accompanying increased body size.

3.4.10.2 Mechanical adaptations during growth

"As similar structures increase in dimensions their weights increase in a higher ratio than the loads which the structures are alike suitable to bear" (Barr, 1899). Due to the lower speed of movement of the limbs of large in comparison to small animals necessitated by the constraints of inertia and gravity, Hill (1950) predicts that the intrinsic speed of contraction of muscles of animals of similar dimensional proportions should decrease with increasing body size. An increase in the numbers of slow-twitch fibres during growth has been reported in a

large number of muscles including the soleus of the rat, cat and guinea-pig (Karpati & Engel, 1967b); m. pectineus of the dog (Cardinet et al. 1969); m. diaphragma and m. longissimus of the pig (Davies, 1972); triceps of the ox (Holmes & Ashmore, 1972) soleus of the rat, rabbit and guinea-pig (Gutmann, Melichna & Syrový, 1974); and posterior and anterior latissimus dorsi, flexor perforatus digitorum secundi and plantaris of the chick (Melichna, Gutmann & Syrový, 1974).

In pig m. longissimus Davies (1972) proposes that the increase in slow-twitch fibre area in direct proportion to the increase in liveweight is an adaptive mechanism of muscle to isometrically support an increasing load without a disproportionate increase in muscle mass as slow-twitch fibres are more suitable for isometric function (Awan & Goldspink, 1972). This author cites the evidence of Close (1964) and Mann & Salafsky (1970) which shows that after the initial postnatal reduction of contraction times the soleus of the cat and rat thereafter contracts more slowly with increasing body size. Similarly Melichna, Gutmann & Syrový (1974) indicate that contraction times of both fast and slow contracting muscles of the fowl increase during growth. The same authors also find that after initially decreasing the contraction time of the extensor digitorum longus and soleus of the rabbit, rat and guinea-pig increases form one month to one year post-natally; however this difference is only significant for m. soleus which has a much greater increase in slow-twitch fibres postnatally (Gutmann et al. 1974). The latter

authors also show that the contraction characteristics of fast and slow muscles are differentiated at birth and that during development the soleus of the guinea-pig and rabbit looses low-molecular weight proteins of myosin normally present in adult fast muscle. Melichna et al (1974) show that the myosin ATPase activity per unit weight of tissue of both fast and slow avian muscles at one year is less than that at one month. Gutmann et al (1974) also find that the myosin ATPase activity of m. soleus of the rat, rabbit and guinea-pig decreases during growth although they find that it increases in extensor digitorum longus. Similarly Trayer & Perry (1966) and Guth & Samaha (1972) had earlier indicated that the myosin ATPase activity of foetal rat, guinea-pig and rabbit muscle is less than in adults. The difference between adult and foetal myosin may not be directly associated with its myosin ATPase activity (Dow & Stracher, 1971) but may be related to the lower activity of "fast-muscle ATPase" rather than the presence of slow-muscle ATPase, in early stages (Drachman & Johnston, 1973).

The increase of slow-twitch fibres with increasing liveweight observed in other animals is also found in the dog and horse. However the directly proportional increase in slow-twitch fibre area found by Davies (1972) in m. longissimus of the pig is not apparent in any of the muscles sampled over the entire growth range studied. There is however, a direct relationship between the total slow-twitch fibre area of m. semitendinosus and liveweight in the growing dogs up to 12 kg liveweight but not thereafter.

Similarly the areas of slow-twitch fibres increases in samples of the other muscles studied in both the dog and the horse but whether a direct relationship between the total transverse sectional area of these or any muscle of the dog and horse occupied by slow-twitch fibres exists remains to be seen. The existence of a "stay apparatus" for postural support in the horse may complicate the problem in this species.

Lindholm & Piehl (1974) state that the proportion of slow-twitch fibres in m. gluteus medius of trotting horses remains the same over the period from six months to eight years of age. This finding is at first sight at variance with those of the present study. However Lindholm & Piehl's photomicrographs indicate fibres with intermediate reaction for myosin ATPase. Since it has been shown that there is an increase in slow-twitch fibre area with increasing liveweight, and liveweight increases most rapidly during the first six months of life in the horse it might be predicted that the greatest increase in slow-twitch area would occur during the same period, the intermediate fibres could well be "transitional fibres." These features may well explain the discrepancy between their results and those of the present study.

The possibility that genetic factors may modify the effect of body size on fibre type has not been considered by earlier investigators. However Davies'(1973) data shows that the proportion of slow-twitch fibres may increase directly proportional to liveweight in Large White pigs up to 90 kg liveweight but only to 70 kg in the (more muscular) Pietrain and then drops to lower values at heavier

liveweights. The limited data on the Pietrain prohibits a definite conclusion however, Cooper, Cassens, Kastenschmidt & Briskey (1970) report a decrease in the area of m. longissimus occupied by slow-twitch fibres in Poland China and Yorkshire pigs and their crosses from birth to 90 kg liveweight. It is possible that such findings indicate breed differences in the development of fibre types, a factor which has yet to be investigated.

The initial increase and subsequent decrease in slow-twitch fibre area seen in m. semitendinosus of the Greyhounds also occurs in m. diaphragma and m. pectoralis transversus of the Greyhounds; and in m. semitendinosus and m. pectoralis transversus of the Thoroughbreds, but the evidence for its occurrence in m. diaphragma of the Thoroughbreds is slight. An increase in the proportion of slow-twitch fibre area with liveweight is also less pronounced in the heaviest non-athletes, both in dogs and in horses (Figs. 90 & 112). The eventual reduction of slow-twitch fibre area at the heavier liveweights is greatest in m. semitendinosus of the athletes of both species. Since in dogs m. longissimus may be even more important in propulsion a histochemical investigation of m. longissimus may be expected to show large breed differences.

The dog and horse seem to be similar to other animals in that the slow-twitch fibres are larger than fast-twitch fibres during early development. During growth the fast-twitch fibres grow at a greater rate; and in adults are larger than the slow-twitch fibres in propulsive muscles of both

species. The rate of growth of the two types of fibres is probably related to the development and functioning of the muscle during the growth range studied. The general trend, of lower growth rates in horses than in dogs (section 3.3.2.6) of fast-twitch relative to slow-twitch fibres may be associated with the greater functional "maturity" of the horse at birth.

3.4.10.3 Metabolic adaptions of dog and horse muscle during growth

During growth the total surface area of the lung alveoli in man (Dunhill, 1962) and morphological pulmonary diffusion capacity in rats from 21 to 131 days (Burri, Dbaly & Weibel, 1974) increase at a rate proportional to the 0·67 and 0·73 power of body weight in humans and rats respectively; thus there are morphological constraints on the ability of the respiratory system to keep pace with increasing body size as regards gaseous exchange. Similar observations have been made on other mammals (Tenney & Remmers, 1963; Kleiber, 1947). Furthermore the ability of the circulatory system to supply oxygenated blood to tissues, with increasing body size, is constrained by the lower rate of increase of diameter of the aorta (Hill, 1950).

It might be predicted from a consideration of these facts that the ability of the muscular system to produce ATP aerobically would be gradually curtailed in the growing animal. Certainly there is evidence that the activity of enzymes associated with anaerobic metabolism increases with increasing body size, as in samples of m. semimembranosus

and m. trapezius of pigs (Dalrymple, Kastenschmidt & Cassens, 1973; Dalrymple, Cassens & Kastenschmidt, 1974) and soleus and gastrocnemius of the cat (Hudlická, Pette & Staudte, 1973); in human leg muscle glycogen utilization increases during growth (Eriksson & Saltin, 1974). Similarly in trotting horses the glycogen concentration in biopsies from m. gluteus medius is greater in horses of 5 to 8 years than in horses under one year (Lindholm & Piehl, 1974). Again, Ashmore (1974) reports that during the postnatal development of relatively "quiescent muscles" (presumably propulsive muscles, since these are not constantly in use) in the chicken, sheep, pig and ox, the majority of fast-twitch fibres develop a reduced aerobic but enhanced anaerobic capacity; fibres that maintain a high aerobic capacity also develop a moderate anaerobic capacity. An interspecies adaptation of skeletal muscle to produce ATP anaerobically with increasing body size is also reported by Davies & Gunn (1972).

In spite of an increase in anaerobic capacity during growth aerobic capacity does not decrease. Davies (1972) finds that in m. diaphragma and m. longissimus of pigs after initial neonatal differentiation the proportion of SDHase high-reacting fibres do not change from birth to 60 kg liveweight. The fast-twitch anaerobic fibres are the largest and form the highest proportion of fibres in m. longissimus of 90 kg pigs, in m. diaphragma they are also the largest type of fibre but are the most infrequent.

Lawrie (1953) finds that the aerobic capacity of homogenates of horse muscle increases up to two years of age.

Moreover, the biochemical assays of the present study indicate that the SDHase activity of dog and horse muscle is greater in adults than in young animals After initial differentiation, the proportion of aerobic fibres increases in m. semitendinosus of the horse and the intensity of SDHase and GPase staining increases during growth in the other two muscles of horses and three muscles of dogs, but quantitative extrapolations cannot be made. However it is probable that both the aerobic and anaerobic capacities of dog and horse muscle increase during growth, in spite of any apparent inadequacies in the respiratory and circulatory systems.

3.4.10.4 Implications of fibre type changes during growth

Alterations in the proportions of fibre types seen during growth (sections 3.3.2.3. and 3.3.2.4) imply changes in the type of nerve supply to fibres during normal growth. It is generally accepted that a motor unit is composed of similar fibres (Edström & Kugelberg, 1968; Burke, Levine, Zajac, Tsairis & Engel, 1971) so alterations in fibre types necessitate changes in entire motor units during growth.

Muir (1974) proposed two mechanisms of motor unit adjustment to explain fibre type changes. The first involves the conversion of one entire motor unit type to another, and the second entails the localised denervation of one type of fibre followed by a colonizing reinnervation by the nerve to an adjacent different type of fibre. The "local denervation" hypothesis may be extrapolated to the death of the entire motor nerve rather than the

death of just that part near the muscle fibre. In the rat Fraher (1974) finds that the ventral root axons decreases in numbers during the first week of postnatal life although they may branch transiently. Hence during this period neurons may be dying and the muscle fibres they supplied could be reinnervated by a surviving neuron, this has yet to be investigated.

There are studies that indicate the plasticity of the nervous system during growth and in its response to changes in usage associated stimuli. Thus during the rearing of rat litters Greenough, Volkman & Juraska (1973) find that environmental complexity correlates with the density of dendritic branching in specific areas of the cerebral cortex. Also exercise causes an increase in diameter of peripheral motor nerves in the mouse (Samorajski & Rolsten, 1975) and rat (Sammeck, 1975). The results of the present study indicate that at the earliest stages of development studied, there are differences in the fibre composition of muscles between species, breeds, muscles (within an individual) and parts of muscles. Also it appears that the mechanical strain on a muscle may determine its fibre type and hence innervation, rather than that the primary control is exerted by motor nerves.

Neither the mechanism nor the sequential events of motor unit alteration are known. Bagust, Lewis & Westerman (1974) suggest that variations in motor unit tension in m. flexor digitorum longus of 2 week and 6 week old kittens may be associated with different motor unit sizes at these ages. In extensor digitorum longus and

tibialis anterior of the rabbit, changes in the type of myosin associated with fast muscle to that associated with slow muscles, induced by electrical stimulation, are preceded by changes in aerobic and anaerobic metabolism - changes in the myosin taking 8 to 10 weeks, the changes in metabolic capacity taking 3 weeks - (Sreter, Romanul, Salmons & Gergely, 1973). This could explain certain observations in the present study. In "transitional fibres" diformazan deposition due to succinate dehydrogenase activity changes from maximal in the subsarcolemmal region to the pattern of intense blue punctate dots associated with slow-twitch fibres: this phenomenon precedes the change in activity of myosin ATPase (Figs. 113 - 118).

These findings indicate both metabolic and myosin ATPase adaptations of growth. On the other hand it has been shown that training effects the aerobic and anaerobic capacity of muscle but not its myosin ATPase activity (Barnard, Edgerton & Peter, 1970a; Staudte, Exner & Pette, 1973; Saubert, Armstrong, Shepherd & Gollnick, 1973). Such possible differing effects of growth and training remain to be investigated. Whether ultrastructural changes in fibres apparently associated with differences in their speeds of contraction (Gauthier, 1971; MacNaughtan, 1974) arise prior to or later than metabolic adaptations also needs consideration.

Bagust, Lewis & Westerman (1973) and Toop (1974) find polyneuronal innervation in developing kitten and human muscle respectively. Jansen, Lømo, Nicolaysen & Westgaard (1973) show that if a muscle fibre does not receive a

stimulus from its own nerve, it attracts another nerve fibre which suggests the possibility of neurotrophic influences in developing muscle fibres. Such trophic influences during growth could involve altered muscle fibre metabolites resulting from the differences in energy supply mechanisms necessary to cope with differing work loads. Such a hypothesis has yet to be investigated.

The suggestion of altering productions of neurogenic growth stimulating factors during growth by muscle of normal animals advises further morphological biochemical and physiological investigation.

3.4.11 The effect of sex, training and cross-breeding on microscopic features of horse and dog muscle

Malsburg (1911) finds that fibres in the stallion gastrocnemius and abdominal muscle are larger than in the mare, but it is not clear whether this finding is related to body weight differences between the sexes or more directly to hormonal effects. Differences in fibre numbers or area between sexes are not significant in this study.

Vaughan, Aziz-Ullah, Goldspink & Nowell (1975) suggest that in m. soleus of the mouse (believed not to be an androgen-sensitive muscle) the male has fewer slow-twitch fibres than the female. There were no sex differences found in the present study hence this factor cannot explain the differences in proportions of fibre types between "athletes" and "non-athletes."

Gordon (1967) shows that training may or may not alter fibre area, depending on the species, muscle and training regime; the detraining programme of the present

study does not significantly alter fibre area, however although not statistically significant the increase in fibre area in all three muscles of the detrained animals may indicate decreasing fibre area due to training, itself facilitating better perfusion of the muscle. The reduced capillary density in all the muscles, but only significantly so in m. pectoralis transversus, may indicate a reduction of blood supply in these muscles possibly due to inactivity. Whether the smaller area of m. diaphragma occupied by slow-twitch fibres in detrained Greyhounds is associated with a higher respiratory rate in these animals was not investigated.

The common observation that the crosses of Thoroughbreds and non-athletic horses are faster than "non-athletes" but slower runners than Thoroughbreds over short distances is given indirect support by this study. Thus the proportions of slow-twitch fibres in the muscles of Thoroughbred crosses lie between those of Thoroughbreds and non-Thoroughbreds. Other cross breeding effects have not been so obvious, or significant.

4.0 GENERAL DISCUSSION

Gross dissection and microscopic analysis of superior athletes and others emphasise the morphological associations of enhanced running capacity. In using these methods the present study has also attempted to investigate problems of propulsion and support associated with increasing mass.

Despite individual variations intensive selection over the generations has produced the Greyhound and Thoroughbred breeds which are unequivocally distinguishable from the other members of their species by their ability to propel themselves at a greater maximum speed (Clarke, 1965; Wenthworth, 1957). Thus the opportunity has arisen to study the anatomically distinctive features which permit swift running - the criterion of athletic ability of interest in this study. However, this necessitates acceptance of large variations in maximal running speed occurring among the other or "non-athletic" group.

The reasons for the choice of skeletal muscle as the major tissue or system investigated in this study are apparent when the factors dictating the speed of co-ordinated running of an animal (Fig. 130) are considered. The muscular system is obviously involved due to features such as its acceleration capacity and internal architecture which dictate stride length, and its intrinsic speed of sarcomere contraction and repetitivity of limb movement which dictate stride frequency. The range of movement of the joints and the natural frequency of the limbs are other features affecting running speed which are indirectly associated with the muscular system. However the integrated

action for all the systems of the body is a prerequisite for allowing the muscles to exercise their full potential.

In both species, the results indicate running speed is enhanced by a greater acceleration potential (section 3.3.1.1) and a greater intrinsic speed of sarcomere contraction (section 3.3.2.2). In the dog a third factor, the higher natural frequency of their hind limbs (section 2.3.2), is also important but in the adult horse running speed is enhanced rather by their longer legs (section 2.3.1.1). The other factors - internal muscle architecture, range of movements of joints and mechanical advantage of muscles - were not considered to offer sufficient variation to warrant their study in normal animals. All these features are considered to have maximum influence at top running speed.

The running speed at which the transition between different gaits occurs is body weight related (Hegland, Taylor & McMahon, 1974). The traits assessed in this study were also related to body weight. But different attributes favouring different gaits were not considered although it is recognised that breeds of dog and horse may be selected for gaits other than galloping.

The comparisons undertaken in this study could be improved by comparing adult athletes with only one other breed, preferably having the same adult liveweight. Similarly, better data on growth could best be obtained by comparing athletes with an individual breed. However the mixed breeds of this study used for comparison with the athletes allow identification of overriding features which favour superior running performance.

Numerous analyses of this study have related data with liveweight since each athlete has to propel its own liveweight (and that of a human as well, in the case of the Thoroughbred) at its maximum speed. Better parameters may become apparent in the future. Notwithstanding such possible inadequacies, the significant findings and trends resulting from this study which can be correlated with athletic performance highlight many topics which require further investigation.

The appraisal of the gross anatomy which has been undertaken emphasises the relevance of knowledge on athletic animals to meat production. Although altering growth rates and sex influences within the two types of animal have been considered, there is insufficient evidence to warrant more elaborate analysis. Further studies, possibly with specific reference to alterations in growth rates and sex effects, may be more revealing although conformational differences between dogs and bitches and between stallions and mares are not apparent on casual observation as those in other domestic animals such as between male and female oxen, sheep, cats or as in man.

As most of the differences between athletes and non-athletes appear to be due to enhanced development of the musculoskeletal system, the inherent growth capacity of muscle is of primary interest. This appears to result from earlier developmental control over the number of fibres in a muscle. The number of fibres in a muscle is genetically controlled (see section 3.4.3) but whether the control is related to a greater mitotic capacity of myoblasts or

other more indirect mechanisms might be investigated using similarly contrasting breeds to those of this study. Thus although carniverous, Greyhounds can be considered as ideal "laboratory animals" for investigation into this important aspect of meat production.

The obvious morphological differences between Olympic standard exponents of human track and field events are delineated by Tanner (1964), who also associated morphological differences between track athletes with their performance. In dogs and horses also, especially Thoroughbreds, it is likely that there may be morphological differences associated with optimum running distance. This is apparent when the average long distance steeplechaser is compared with the average short distance sprinter or flat racer by visual appraisal. It is not known whether such differences are directly related to the aerobic capacity of muscle in the former and anaerobic capacity in the latter or to differences in their growth pattern. The eludication of such factors is of importance for training and breeding policies of both animals and man.

Walton & Hammond (1938) indicate that the ultimate size of the offspring of Shire and Shetland pony crosses is dictated by the dam. As regards individual muscle fibres, the evidence (Byrne, Hooper & McCarthy, 1974) indicates that in mice maternal factors may affect fibre area more than fibre numbers. On the functional side, selection enhances exercise capacity in both male and female birds (Morse & Smith, 1972) but in males has a greater effect on endurance than on power performance.

Irrespective of whether these results are directly applicable to dogs and horses, they suggest that differing male and female factors could affect enhanced athletic ability. Investigation of the reciprocal crosses of donkeys and horses (as the gross features of the mule and hinny are strongly influenced by the dam) or of "athletic" with "non-athletic" dogs and horses may shed light on the differing transmissions of factors favouring athletic ability by males and females.

Genetic improvement based on assessment of running capacity is unlikely to be a simple problem, which indeed is rather common knowledge. Thus it is possible that factors which predispose functionally to athletic ability may be genetically quite unrelated. For example, genetic control over limb length may be different to that controlling the intrinsic speed of sarcomere contraction, although other factors such as acceleration potential and natural frequency of the limb (both influenced by fibre number and area) may be genetically closely related.

The principle criterion for a potential breeder of athletically successful offspring is winning. Frequently however the distance seperating a winner and not is much less than might be supposed from the relative prestige and stakes extracted from the event, which is used as an indicator for selection. Also, such selection ignores the possibility of insidious pathological processes being genetically determined. Thus there may be a relative lack of pathological occurrences in the more successful athletes. But obviously enhancement of one factor predisposing to

excellence in athletic performance may compliment for a deficiency in another or even mask abnormalities.

The considerably greater improvements in human athletic performance compared with racing times for top class races (particularly for horses) may be due to several factors. These include better performances in animals ab initio, the different objectives of racing between animals (where placings are of prime importance) and man; and the better training programmes, equipment and overall improvement in living standards for human athletes. Even a higher incidence of pathological conditions in animals than in human athletes may cause the discrepancy.

The remodelling of the neurogenically controlled prototype of muscle at the neuromuscular level which occurs as normal part of growth, suggests that similar phenomena may occur in the spinal chord and perhaps in the cerebral cortex. Growing animals could be used to investigate this suggestion which, if found to be true, would be of far reaching consequence to both man and animals.

The change in mechanical properties of fibre types seen during growth may be a result of a direct (such as a proprioceptive impulse) or an indirect (such as enhanced muscle mass) stimulus. However muscle spindles were not investigated. Although the athletic animals have a high proportion of muscle and a low proportion of slow-twitch fibres relative to liveweight, others for example the Great Dane have both a low proportion of muscle and of slow-twitch fibres relative to their liveweights (sections 2.3.3 & 3.3.2.2.). Thus the horse and dog are highly suitable species

for such comparisons. Results from animals grown in gravity-free environments could throw more light on the effect of body weight in relation to the proportions of the different types of fibres. Further differences in metabolism and untrastructure of fast and slow-twitch fibres in these two species could be studied by biochemical and electronmicroscopic investigation of uni-fibre preparations from m. diaphragma and the superficial caudal regions of m. semitendinosus.

An understanding of the mechanism of malignant growth might be enhanced by a greater knowledge of energy production and utilization mechanisms in cells. During normal growth it has been shown that alterations in these mechanisms occur in skeletal muscle. The histochemical and biochemical comparison of rhabdomyosarcomas and growing muscle might prove fruitful.

In striving for better physical performance, man frequently resorts to extraneous chemical substances. Although substances such as anabolic steroids enhance physical performance potentials (Exner, Staudte & Pette, 1973), nevertheless, they require morphological and biochemical potential in the individual for their expression. For the best results, it is still necessary that the training methods involved should be optimal. However better appreciation of the factors limiting physical performance in individuals and the temporal changes in these factors in the non-exercising may dispense with any need for artificial approaches to sporting satisfaction. Extrapolation of the properties of skeletal muscle from athletic animals to superior human athletes is already possible at the

histometric (Etmadi & Hosseini, 1968) and histochemical (Saltin, 1973) level. With more knowledge of both the superior human athletes and his animal counterpart, more similarities should appear.

Outstanding physical performances by man and animals are a source of intense interest and inspiration in such diverse places as regal courts and public drinking houses. Although some people believe that the understanding of the phenomenon of excellence in skill and physical ability, whether in a golfer, virtuoso violinist or Derby winner, should stand as a monument to mythology to be as closely guarded as their lives, the basic biological implications of such prodigies and genius always warrant scientific investigation. In many instances a much greater degree of multifactorial analysis is required than for the relatively simple actions used in running. The investigator should not be daunted by complexity in his attempt to explore natural physical ability but believe that it is better "not to imagine or think out, but to find out what nature does or produces" realising however, that "truth is rarely pure and never simple."

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