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Differences in the histochemical properties of skeletal muscles of different breeds of horses and dogs

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INTRODUCTION

One would expect exponents of swiftness in a species to have the capacity for a greater rate of energy production and utilization than their fellows. Studies comparing thoroughbred horses and greyhounds – breeds selected for racing – with other members of their species should therefore 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 is regenerated by either an aerobic or 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 enzymes representative of aerobic and anaerobic metabolism.

This study investigates such histochemical properties of muscle fibres in appropriate muscles of athletic and non-athletic horses and dogs. A preliminary report has already been communicated (Gunn, 1973). Other attributes of athletically superior animals have been reported elsewhere (Gunn, 1975*a*).

MATERIALS AND METHODS

The animals from which samples of three muscles (semitendinosus, diaphragm and pectoralis transversus) were obtained are listed in Table 1. All the horses were over 2 years old and all the dogs over 15 months old. Four greyhounds had recently completed a one year detraining period, while the other greyhounds were still in training for racing at the time of death. Four thoroughbreds were also out of training. Samples of muscle were removed from the left side and they contracted fully on removal. In both horses and dogs blocks were taken from the costal part of the diaphragm, and the superficial part of the pectoralis transversus lateral to the manubrium sterni. In horses, blocks were taken from the caudal superficial region of the transverse section of semitendinosus where it crosses the tuber ischii; in dogs, complete transverse sections were taken at the mid-belly.

The canine semitendinosus sections were trimmed to a thickness of 0·5 cm with a brain knife, and all other samples reduced to transverse blocks of $2 \times 2 \times 0{\cdot}5$ cm. Each sample was mounted on cork 0·5 cm thick frozen to a cryostat chuck. Samples were then frozen rapidly by plunging into dichlorodifluoromethane (Arcton 12, I.C.I.) cooled to its melting point of -158 °C by liquid nitrogen. The samples were serially sectioned at 10 μm in a cryostat at -20 °C. About 15 adjacent sections

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Table 1. *The numbers of each type of animal from which samples of each muscle were obtained for histochemical analysis*

Type of animal	Muscle: Semitendinosus	Diaphragm	Pectoralis transversus
Thoroughbreds	9	6	10
Other horses	15	11	16
Thoroughbred crosses	5	3	6
Greyhounds	21	10	9
Other dogs	10	7	8

were mounted directly onto coverslips and allowed to thaw and dry rapidly at room temperature. Fibre outlines were demonstrated by fixing sections in 4 % formaldehyde for 10 minutes, then washing and staining for 20 minutes in Ehrlich's haematoxylin. Enzyme activities were demonstrated by modifications of the methods described by Nachlas *et al.* (1957) for succinate dehydrogenase (SDHase); Takeuchi & Kuriaki (1955) for glycogen phosphorylase (GPase) and Padykula & Herman (1955), as modified by Davies & Gunn (1972), for myosin adenosine triphosphatase (ATPase).

Mean fibre areas

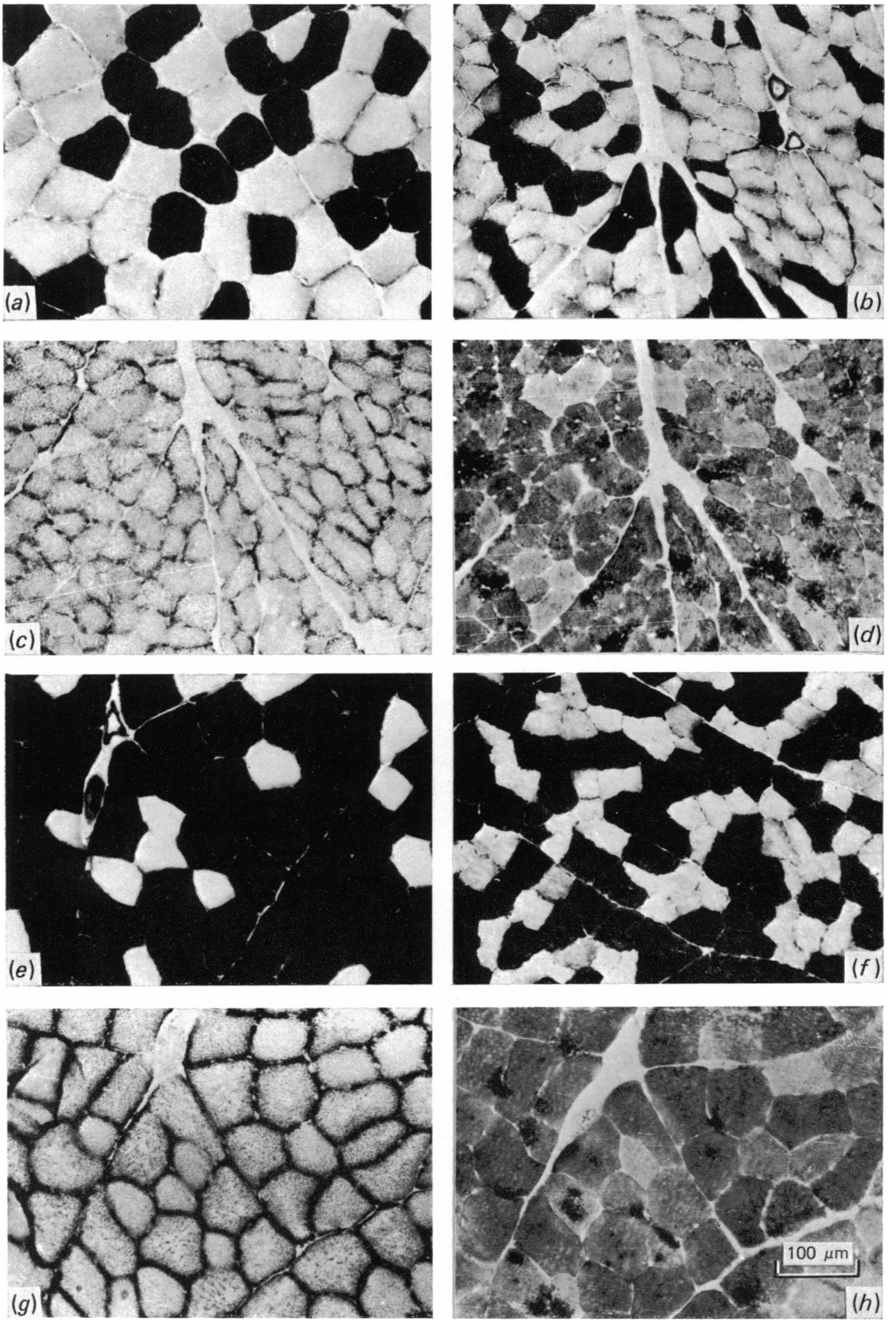
The areas of transverse sections of semitendinosus were measured on ruled paper. The mean fibre areas of the samples were estimated from this value by back projecting haematoxylin-stained sections on to a glass screen and counting the numbers of fibres within a known area. In horses the total number of fibres in the semitendinosus was calculated from the mean fibre areas of the part of the muscle sampled and the total unfrozen sectional areas. In dogs the total number of fibres in the semitendinosus was calculated from the mean fibre area of the muscle and the area of the whole frozen section; nine areas of the section were sampled – three superficial, three middle and three deep – together comprising 3000–3500 fibres.

Histochemical profiles

In small samples, profiles of the fibres in two squares, each containing approximately 400 fibres, and lying about 20 fibre-breadths deep to the surface of the muscle and about 200 fibre-breadths apart, were established by back projection. First, the fibre outlines in a haematoxylin-stained section were traced on transparent paper. Subsequently, each serial section was projected in turn and the histochemical reaction for each fibre entered on the tracing. The type of material used is illustrated in Figures 1–4. Where the fibres exhibited a continuous spectrum of staining intensity, a simple division was made into 'high' and 'low' relative to the overall activity of fibres in the section. Difficulties in standardization and processing precluded qualitative comparisons between samples.

Figs. 1–4 inclusive show transverse fresh frozen sections.

Fig. 1. (*a*) Diaphragm of a 458 kg 3 years old thoroughbred mare. Myosin ATPase activity (*b–d*). Diaphragm of a 318 kg 6 years old piebald pony mare. Myosin ATPase (*b*), SDHase (*c*) and GPase (*d*) activities. Some AL fibres have a lower GPase activity than AH fibres. (*e, g, h*) Pectoralis transversus of a 458 kg 3 year old thoroughbred mare. Myosin ATPase (*e*), SDHase (*g*) and GPase (*h*) activities. (These sections are taken from the same animal as Figs. 1*a* and 2*a–d*.) (*f*) Pectoralis transversus of a 318 kg 6 years old piebald mare. Myosin ATPase activity. (This section is taken from the same animal as those for Figs. 1*b–d* and 2*e–h*).


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
100 μm

Quantification procedures

In the sections of the semitendinosus of dogs the total numbers of fibres having a low staining intensity for myosin ATPase were estimated by sampling the muscle (at 2 mm intervals in adults and 0·5 mm intervals in pups) across its entire transverse section. The mean area of 50–100 of these fibres was established by paper weighing methods. The total area, numerical proportion, and proportional area of each type of fibre could then be calculated. Similarly, in other muscles of the dog, and in the horse muscles, the various types of fibre were counted and their proportions calculated while their histochemical profiles were determined. The areas of paper representing each fibre type were weighed to measure both the proportional area occupied by each fibre type and the mean area of each fibre type.

In some samples from both species areas of fibre types (as differentiated by the myosin ATPase reaction) were determined with a Quantimet 720 (Imanco) which measures areas of a microscopic field occupied by fibres of a given light transmittance. The mean area of 50–100 fibres of one type was estimated directly by measurement, while that of the other type was derived by calculation. In the semitendinosus of dogs a similar procedure permitted calculation of the total numbers, the proportion, and the total proportional area, of the other fibre type. The data were analysed to test for differences between athletic and non-athletic animals within each species, using Student's '*t*' test.

RESULTS

Fibre types in adults

Horses

It is not possible to distinguish accurately between fibres in the diaphragm and pectoralis transversus of the horse by means of the histochemical reactions for SDHase and GPase, because most fibres exhibit a high activity of these enzymes (Fig. 1*c*, *d*, *g*, *h*). Fibres having a low activity of myosin ATPase (AL fibres) may show variations in the pattern of diformazan distribution with SDHase and, in some species, a slightly lower GPase activity. However, the myosin ATPase reaction differentiates fibres satisfactorily in these two muscles into: (i) those with a high activity of this enzyme and which also have a high activity for both SHDase and GPase (termed AH, SH, PH fibres) and (ii) those with a low activity of myosin ATPase but with a high activity of SDHase and a medium to high (interpreted as high) activity of GPase (termed AL, SH, PH fibres) (see Fig. 1).

In the semitendinosus the situation is more complex; the AL fibres have a high activity for SDHase; however, the AH fibres may be broadly categorized into those with high and low SDHase activities – although there is in fact a fairly continuous spectrum of activity of this enzyme. AL fibres may sometimes have a paler blue reaction with GPase, but no differentiation could be achieved using this reaction because of the small disparity in staining density between AL and AH fibres. Thus, in the semitendinosus three types of fibre could be differentiated:

(i) those having a high activity for myosin ATPase, GPase and SDHase (termed AH, SH, PH fibres);

(ii) those with a high activity for myosin ATPase and GPase and a low activity for SDHase (termed AH, SL, PH fibres);

(iii) those having a low activity for myosin ATPase and a high activity for SDHase and GPase (termed AL, SH, PH fibres). (See Fig. 2.)

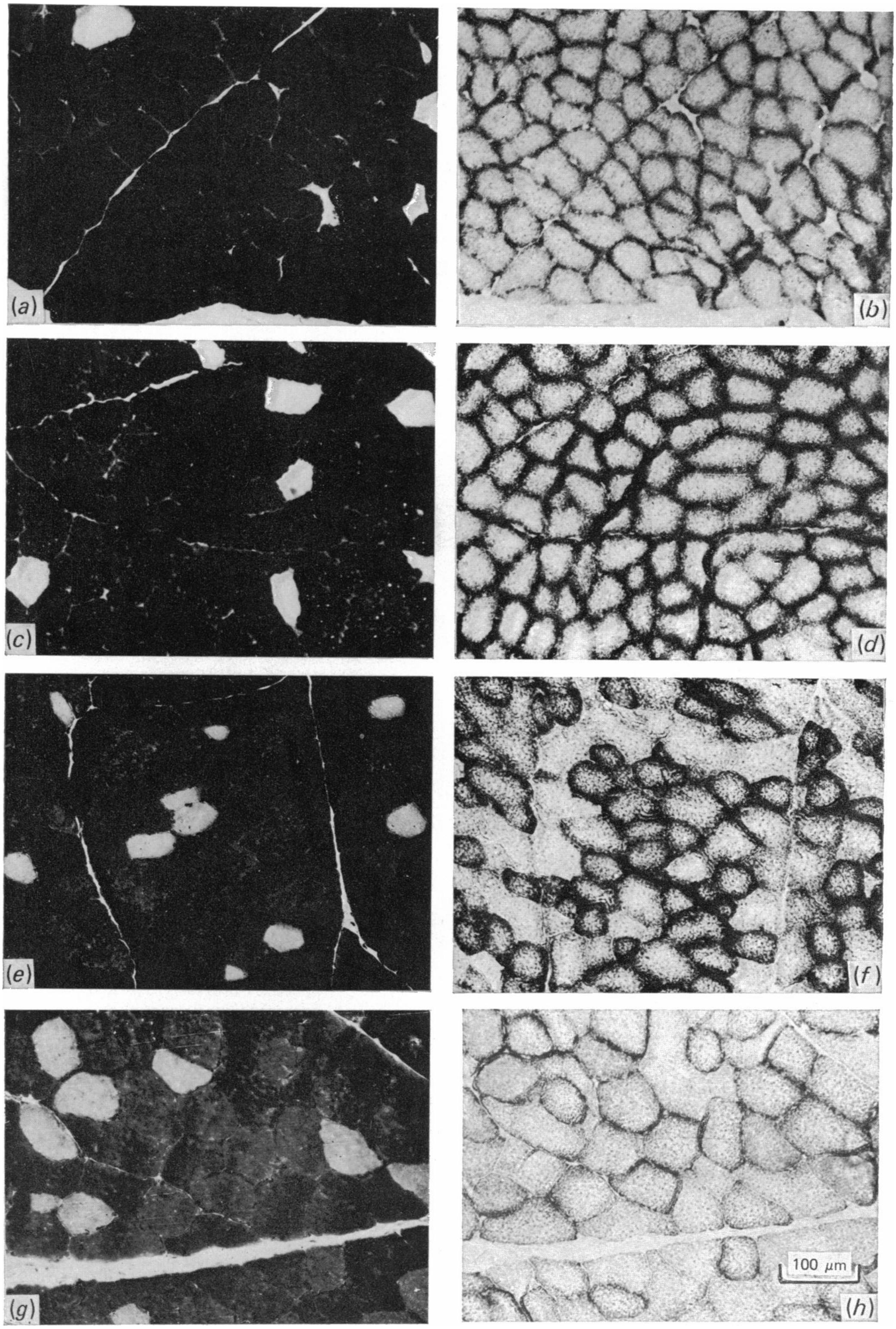


Fig. 2. (*a*, *b*) Superficial caudal region of semitendinosus of a 458 kg 3 years old thoroughbred mare. Myosin ATPase (*a*) and SDHase (*b*) activities. (*c*, *d*) Deep medial region of semitendinosus from the same animal as (*a*) and (*b*). Myosin ATPase (*c*) and SDHase (*d*) activities. (*e*, *f*) Superficial caudal region of semitendinosus of a 318 kg 6 years old piebald pony mare. Myosin ATPase (*e*) and SDHase (*f*) activities. (*g*, *h*) Deep medial region of semitendinosus from the same animal as (*e*) and (*f*). Myosin ATPase (*e*) and SDHase (*f*) activity.

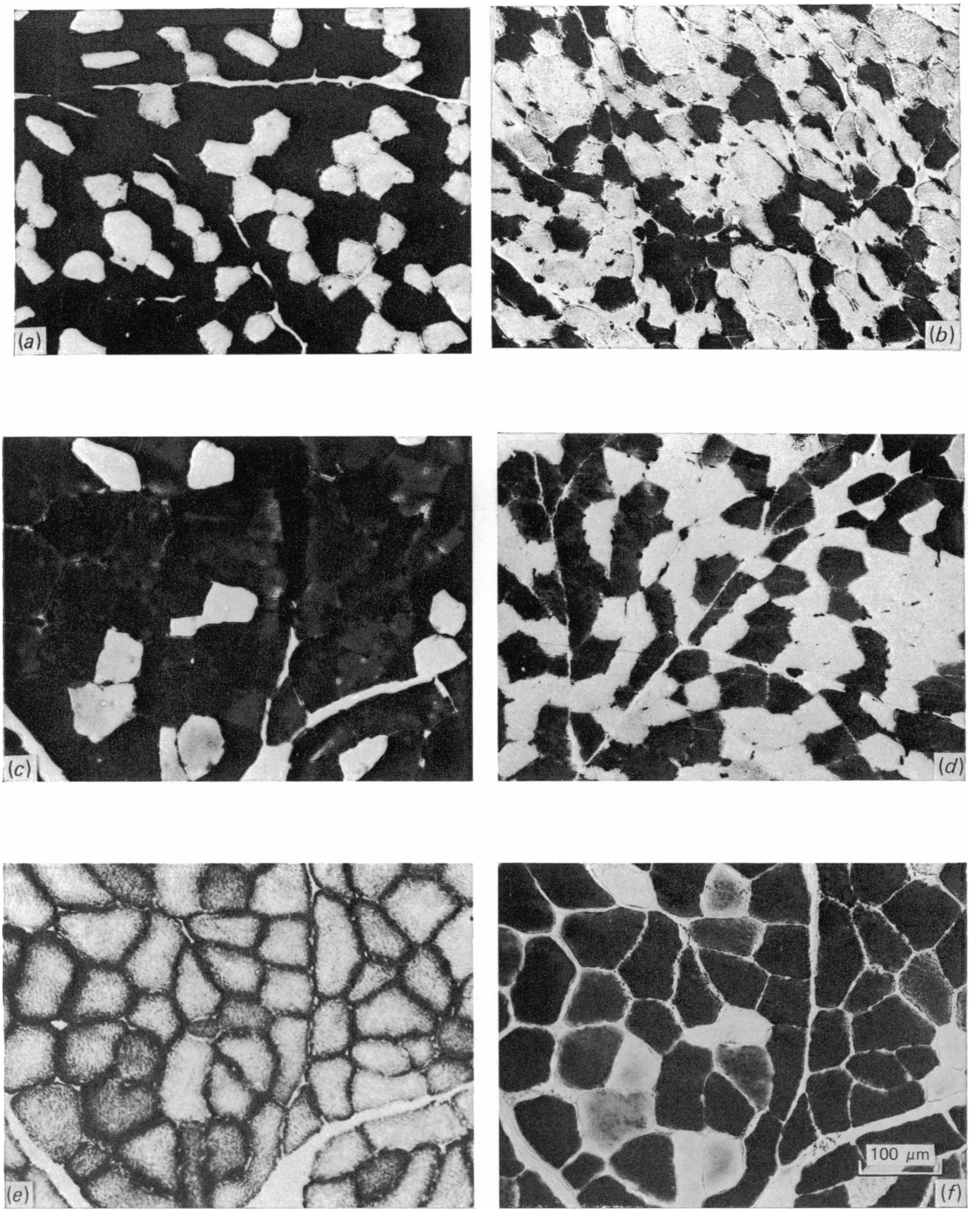


Fig. 3. (*a*) Diaphragm of a 25 kg adult male greyhound. Myosin ATPase activity. (*b*) Diaphragm of a 4 years old 32 kg adult male Afghan. Myosin ATPase activity. (*c, e, f*) Pectoralis transversus of a 25 kg adult male greyhound. Myosin ATPase (*c*), SDHase (*e*) and GPase (*f*) activities. Fibres are either AH, SH, PH or AL, SH, PL. Fig. 3(*a*) is also from the same animal. (*d*) Pectoralis transversus of a 10 years old 22 kg female collie. Myosin ATPase activity.

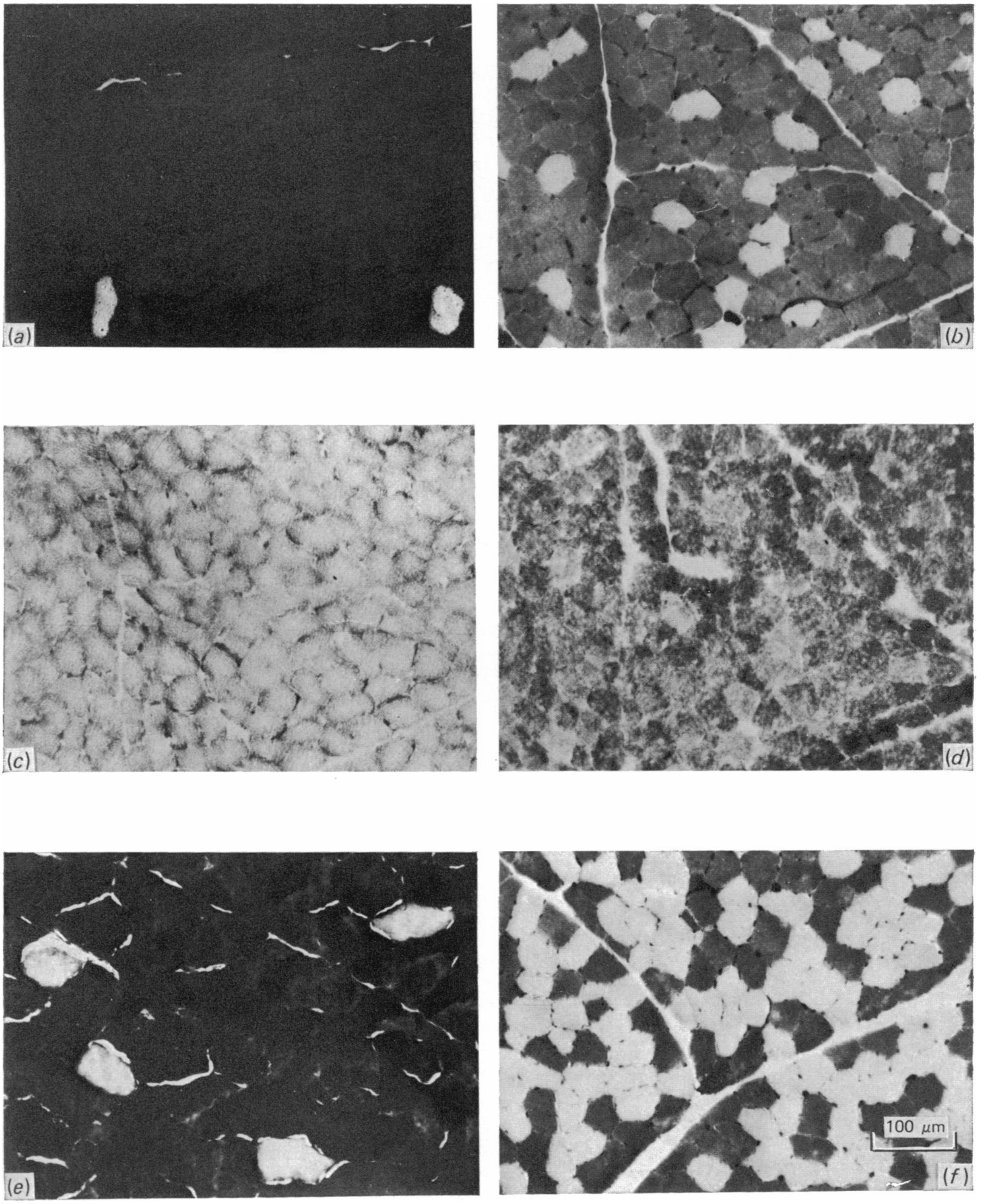


Fig. 4. (*a*, *e*) Superficial caudal (*a*) and deep medial (*e*) regions of semitendinosus of a 28 kg 3 years old male greyhound. ATPase activity. (*b*–*d*) Superficial caudal region of semitendinosus of an 8 kg 2 years old collie bitch. Myosin ATPase (*b*), SDHase (*c*) and GPase (*d*) activities. AH, SH, PH and AL, SH, Pl fibres are apparent. (*f*) Deep medial region of semitendinosus from the same transverse section as (*b*).

Table 2. *Quantitative histochemistry of equine skeletal muscle*

(Proportion of transverse section and transverse sectional areas of samples of m. semitendinosus, m. diaphragms and m. pectoralis transversus occupied by myosin ATPase low-reacting (AL) fibres.)

	Thoroughbreds				Other horses				
	Sex	Body weight (kg)	% AL numbers	% AL area	Breed	Sex	Body weight (kg)	% AL numbers	% AL area
					Musculus semitendinosus				
	♀	445	4·4	2·0	Shetland	♂	154	13	5·1
	♀	458	2·8	2·7	Welsh Mountain	♂	178	19	7·7
	♀	471	5·3	1·7	Shetland	♂	204	12	7·8
D*	♀	471	6·2	2·8	Welsh Mountain	♀	229	8·4	4·4
	♂	484	10	5·5	Shetland	♂	280	20	9·6
	♀	484	7·8	3·6	Connemara ×	♀	305	12	7·9
D*	♀	535	3·8	3·8	Arab	♀	305	15	7·0
D*	♀	560	0·15	0·25	Connemara ×	♀	318	12	11
D*	♀	598	5·7	3·4	Piebald	♀	318	13	7·1
					Thoroughbred ×	♀	356	11	6·5
					Exmoor ×	♂	356	15	13·0
					Thoroughbred ×	♂	369	12	5·0
					Thoroughbred ×	♂	433	10	4·7
					Connemara ×	♀	445	13	8·0
					Welsh Mountain	♂	458	12	9·1
					Exmoor ×	♂	480	4·6	2·5
					Connemara ×	♂	480	19	6·1
					Clydesdale	♀	496	14	9·3
					Thoroughbred ×	♂	522	9·1	2·9
					Thoroughbred ×	♀	535	6·6	3·0
		Mean	5·1	2·9			Mean	12·5	6·9
		S.D.	2·8	1·5			S.D.	3·9	2·8

Difference between means of % AL numbers and % AL areas is significant at the 0·1 % level.

	Thoroughbreds				Other horses				
					Musculus diaphragma				
	♀	445	73	73	Shetland	♂	154	73	83
	♀	458	58	61	Welsh Mountain	♂	178	68	73
	♀	471	61	70	Shetland	♂	204	79	82
	♂	496	67	69	Welsh Mountain	♀	229	63	75
	♂	509	74	78	Shetland	♂	280	74	84
	♀	585	68	69	Arab	♀	305	62	72
					Connemara ×	♀	318	76	81
					Piebald	♀	318	69	75
					Thoroughbred ×	♂	369	61	64
					Welsh Mountain	♂	458	83	84
					Connemara ×	♂	480	77	78
					Clydesdale	♀	496	76	76
					Thoroughbred ×	♂	522	70	73
					Thoroughbred ×	♀	535	67	30
		Mean	66·8	70			Mean	71·3	73·4
		S.D.	6·4	5·6			S.D.	6·7	13·8

There is no significant difference between the means of the % AL numbers and % AL of the two types of horse.

Table 2 (*cont.*)

	Thoroughbreds				Other horses				
	Sex	Body weight (kg)	% AL numbers	% AL area	Breed	Sex	Body weight (kg)	% AL numbers	% AL area
				Musculus pectoralis transversus					
	♀	445	27	15	Shetland	♂	154	49	38
	♀	458	25	16	Welsh Mountain	♂	178	50	45
	♀	471	29	10	Shetland	♂	204	37	32
D*	♀	471	25	10	Welsh Mountain	♀	229	30	21
	♂	484	29	18	Shetland	♂	280	43	34
	♀	484	30	21	Pony	♀	305	38	28
D*	♂	496	36	21	Arab	♀	305	38	36
D*	♂	509	29	13	Connemara ×	♀	318	47	37
	♀	535	37	26	Piebald	♀	318	54	35
	♀	585	36	23	Thoroughbred ×	♀	356	37	29
					Pony	♂	356	46	34
					Thoroughbred ×	♂	369	47	32
					Pony	♀	407	55	58
					Pony	♀	420	54	50
					Thoroughbred ×	♂	433	33	21
					Connemara	♀	445	60	54
					Exmoor ×	♂	480	22	14
					Clydesdale	♀	496	42	31
					Thoroughbred ×	♂	521	45	38
					Thoroughbred ×	♀	535	41	20
					Percheron ×	♂	535	47	40
					Thoroughbred ×	♂	560	44	32
		Mean	30·3	17·3			Mean	43·6	34·5
		S.D.	4·5	5·5			S.D.	8·9	10·8

Difference between means of % AL numbers and % AL areas is significant at the 0·1 % level.
D*, Horses out of training.

Dogs

All fibres in the semitendinosus, diaphragm and pectoralis transversus of both types of dog have a high activity for SDHase and GPase. Although 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 (cf. Fig. 3), it is not possible to distinguish types of fibre by means of the histochemical reactions for SDHase or GPase. However, some AL fibres may also have a lower intensity of glycogen phosphorylase activity than AH fibres. For these reasons the myosin ATPase reaction was used to distinguish between fibre types in the dog.

Quantitative myology

Horses

Comparison of different regions of the same muscle

The proportion of AL and SH fibres increases towards the deep regions of both semitendinosus (see Fig. 2) and pectoralis transversus, but there is no apparent difference in the proportions of fibre types in the vicinity of the sampling site in the diaphragm.

Table 3. *The mean proportional numbers and areas of AL fibres in m. semitendinosus, m. diaphragma and m. pectoralis transversus of adult thoroughbreds, adult thoroughbred crosses and adult other horses and a comparison between corresponding values of the three types of horse using Student's 't' test*

	Number of observations		% AL numbers	% AL areas
M. semitendinosus				
Thoroughbreds	9	Mean	5·13[b]	3·03
		S.D.	2·85	1·58
Thoroughbred crosses	5	Mean	9·80[b]	4·60[a]
		S.D.	1·92	1·67
Other horses	15	Mean	13·5	7·73[a]
		S.D.	3·96	2·60
M. diaphragma				
Thoroughbreds	6	Mean	66·8	70·0
		S.D.	6·37	5·59
Thoroughbred crosses	3	Mean	66·0	55·7[b]
		S.D.	4·58	22·7
Other horses	11	Mean	72·7	78·3[b]
		S.D.	6·57	4·82
M. pectoralis transversus				
Thoroughbreds	10	Mean	30·3[d]	17·3[c]
		S.D.	4·50	5·46
Thoroughbred crosses	6	Mean	41·2[d]	28·7[c]
		S.D.	5·31	6·98
Other horses	16	Mean	44·5	36·7
		S.D.	9·87	11·3

a, b, c, d, Values followed by the same superscript within a muscle are significantly different ($P < 0·025$; $P < 0·01$; $P < 0·005$ and $P < 0·001$ respectively) from one another.

The effect of sex and training on fibre types

As Table 2 indicates, neither sex nor training introduced a significant difference in the percentage numbers and percentage area occupied by AL and SH fibres in the semitendinosus, diaphragm and pectoralis transversus. Adult thoroughbreds are therefore compared as a group with all the other adult horses as a group.

Comparison of muscle fibre type proportions between thoroughbreds and others

The percentage of, and the area occupied by, AL fibres in the semitendinosus and pectoralis transversus are less ($P < 0·001$) in thoroughbreds than in other horses. There is no significant difference in the diaphragm (Table 2).

The thoroughbred cross horses have values intermediate between those of thoroughbreds and other horses for the semitendinosus and pectoralis transversus (Table 3). When the percentage AL numbers and AL areas in the diaphragm of thoroughbreds are compared with those in other horses (but not including thoroughbred crosses) there is still no significant difference in the percentage number, but the percentage area is then less ($P < 0·01$) in the thoroughbreds than in the other horses.

The proportion of fibres in the sample which are both AH and SH is higher in thoroughbreds than in other horses (Table 4).

Table 4. *The incidence of fibre types in musculus semitendinosus of the adult horse as differentiated by the succinate dehydrogenase reaction*

	Thoroughbreds				Other horses				
	Sex	Body weight (kg)	% SH†	AH.SH‡	Breed	Sex	Body weight (kg)	% SH†	% AH.SH‡
	♀	458	100	97	Shetland	♂	154	54	41
	♀	471	63	58	Welsh Mountain	♂	178	55	36
	♂	484	58	48	Shetland	♂	204	34	22
D*	♀	535	42	38	Welsh Mountain	♀	229	46	38
D*	♀	560	99	99	Shetland	♂	280	51	31
D*	♀	598	74	68	Connemara ×	♀	305	46	34
					Arab	♀	305	58	43
					Piebald	♀	318	62	49
					Thoroughbred ×	♀	356	52	41
					Exmoor ×	♂	356	82	67
					Thoroughbred ×	♂	369	38	26
					Thoroughbred ×	♂	433	76	66
					Connemara	♀	445	80	67
					Welsh Mountain	♂	458	100	88
					Exmoor ×	♂	480	43	38
					Connemara ×	♂	480	83	64
					Clydesdale	♀	496	65	51
					Thoroughbred ×	♂	522	79	70
					Thoroughbred ×	♀	535	51	44
All adult thoroughbreds					All adult other horses				
		Mean	72·7	68·0			Mean	60·8	48·2
Detrained adult thoroughbreds					Thoroughbred cross adults				
		Mean	71·7	68·3			Mean	59·2	49·4
Trained thoroughbreds									
		Mean	73·7	67·7			Mean	61·4	18·6

D* Horses out of training.
† Fibres having a high reaction for succinate dehydrogenase.
‡ Fibres having a high reaction for both myosin ATPase and succinate dehydrogenase.

Comparison of fibre type proportions between muscles

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 ($P < 0{\cdot}001$) AL fibres and a smaller ($P < 0{\cdot}001$) area of AL fibres:

(i) in the semitendinosus than in either the diaphragm or the pectoralis transversus, and

(ii) in pectoralis transversus than in the diaphragm.

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

Dogs

Comparison of different regions of the same muscle

There is no apparent variation in the proportion of fibre types in the vicinity of the sampling site in the diaphragm. Samples of pectoralis transversus were assessed at a constant distance – 20 fibre-breadths deep from the surface of the muscle – because the proportion of AL fibres increases towards the deep regions of the muscle. This variation was not investigated further. In the semitendinosus there is

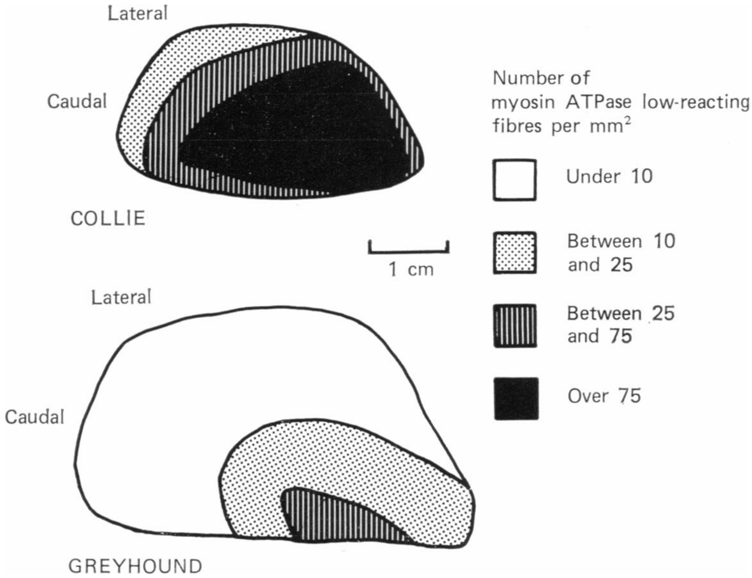


Fig. 5. Incidence of myosin ATPase low-reacting (AL) fibres in the complete transverse section of the semitendinosus of a greyhound and collie, both adapted for a live weight of 30 kg.

a marked gradation across the transverse section of the muscle; the relative incidence of AL fibres in the semitendinosus of a greyhound and of a collie, which was assessed by sampling the muscle intervals across the entire transverse section, is indicated in Figure 5. The proportion of AL fibres is initially lower in the deeper part of the greyhound than in the collie muscle, and progressively diminishes towards the lateral part (Fig. 5). The greyhound, unlike the collie, has no AL fibres at the exterior of the semitendinosus, although some are present near the periphery (Fig. 3). The mean of the differing proportions of fibre types across the whole muscle, or part of the muscle sampled, was taken to represent the whole muscle. The method of sampling largely compensates for the variation in distribution in fibre types in the pectoralis transversus and semitendinosus.

The effect of detraining and sex

The percentage numbers of AL fibres and their percentage areas in the transverse section of the semitendinosus, diaphragm and pectoralis transversus were assessed in various groups of dogs. No sex differences were found within either the greyhounds or other dogs and, hence, males and females have been grouped together in subsequent calculations. Detraining has a small effect on the results. There is no significant difference between trained and detrained adult greyhounds in the percentage number or area of AL fibres in the semitendinosus and pectoralis transversus, although in the diaphragm the percentage area of AL fibres is greater ($P < 0{\cdot}005$) in trained animals.

Comparison of the incidence of fibre types in greyhounds and other dogs

In all three muscles the percentage AL numbers and areas are less ($P < 0{\cdot}001$) in the greyhounds – irrespective of training status or sex – than in all the other dogs (Table 5, Figs. 3, 4).

Table 5. *Quantitative histochemistry of canine skeletal muscle*

(Proportion of transverse section and transverse sectional areas of samples of m. semitendinosus, m. diaphragma and m. pectoralis transversus occupied by myosin ATPase low-reacting (AL) fibres.)

	Greyhounds				Other dogs				
	Sex	Body weight (kg)	% AL numbers	% AL area	Breed	Sex	Body weight (kg)	% AL numbers	% AL area
				Musculus semitendinosus					
	♀	19	1·8	1·6	Collie	♀	8	22	22
	♀	22	1·3	0·92	Terrier ×	♀	10	20	14
D*	♀	24	9·7	5·0	Collie	♀	11	28	18
	♂	25	3·4	3·3	Collie	♀	12	34	27
	♀	25	1·7	1·1	Collie	♂	14	19	13
	♂	25	2·5	1·9	Collie	♀	22	12	10
D*	♂	25	2·1	1·6	Afghan	♀	25	15	11
	♂	27	4·3	3·2	Afghan	♂	32	10	7·0
	♂	28	2·1	1·6	Labrador	♀	33	12	15
	♂	28	2·6	1·1	Great Dane	♂	47	8·9	5·4
D*	♀	29	2·7	2·1					
	♂	29	0·97	0·82					
	♀	30	0·52	0·58					
	♂	30	0·33	0·32					
D*	♂	30	2·5	1·5					
	♂	31	5·3	5·1					
	♂	31	2·5	2·8					
	♀	34	4·8	3·3					
	♀	34	3·1	1·2					
	♂	34	4·5	2·8					
	♂	37	4·8	3·3					
		Mean	3·00	2·2			Mean	18·1	14·2
		S.D.	2·1	1·3			S.D.	8·2	6·7
				Musculus diaphragma					
D*	♀	24	39	25	Terrier ×	♀	10	46	47
	♀	25	29	23	Collie	♀	11	55	72
	♂	25	27	31	Collie	♀	12	52	42
D*	♂	25	16	13	Collie	♂	14	54	41
D*	♀	29	23	13	Collie	♀	22	59	48
	♀	30	20	27	Afghan	♀	25	42	49
D*	♂	30	19	16	Afghan	♂	32	60	59
	♀	34	29	30					
	♂	34	30	27					
	♂	37	34	29					
		Mean	26·6	23·4			Mean	52·6	51·1
		S.D.	7·1	6·9			S.D.	6·6	10·9
				Musculus pectoralis transversus					
D*	♀	24	42	22	Terrier ×	♀	10	56	56
	♀	25	14	12	Collie	♀	11	60	78
	♂	25	16	13	Collie	♀	12	56	56
D*	♂	25	12	9	Collie	♂	14	47	38
D*	♀	29	13	9	Collie	♀	22	56	48
	♀	30	12	6	Afghan	♀	25	42	36
D*	♂	30	17	17	Afghan	♂	32	51	48
	♀	34	18	11	Great Dane	♂	47	50	44
	♂	37	25	20					
		Mean	18·8	13·2			Mean	52·3	50·5
		S.D.	9·6	5·4			S.D.	5·9	13·3

Difference between the means of % AL numbers and % AL areas for the same muscle in the two types of dog are significant at the 0·1 % level.

D*, Detrained greyhounds.

Comparison of the incidence of fibre types in different muscles

In the greyhounds the percentage number and area of AL fibres in the complete cross section of the semitendinosus is less ($P < 0.001$) than in samples from the diaphragm and pectoralis transversus. There is no significant difference between diaphragm and pectoralis transversus as regards percentage number, but the percentage area is significantly smaller ($P < 0.005$) in pectoralis transversus. In other dogs the percentage number and area of AL fibres in the complete transverse section of the semitendinosus is again less ($P < 0.001$) than in the diaphragm and pectoralis transversus. However, there is no significant difference between diaphragm and pectoralis transversus.

Comparison of fibre type proportions between species

When all the dogs and all the horses, irrespective of type, are compared, the proportional number and area of AL fibres in samples of diaphragm is greater ($P < 0.001$) in horses than in dogs. There is no significant difference in pectoralis transversus between the two species. The proportions of fibre types in semitendinosus were not comparable in the two species because of the differing methods of sampling.

DISCUSSION

There is considerable evidence that the succinate dehydrogenase reaction indicates the capacity of an individual fibre for aerobic metabolism, and that the phosphorylase reaction establishes the rate at which a fibre can derive energy for contraction anaerobically (see Davies & Gunn, 1972, for discussion). However, the significance of the myosin ATPase reaction requires further consideration, although there is little doubt that myosin ATPase high-reacting fibres may be described as fast-twitch and myosin ATPase low-reacting fibres as slow-twitch.

Treatment with acids, alkalies or formalin prior to the histochemical demonstration of myosin ATPase activity (Guth & Samaha, 1970; Samaha, Guth & Albers, 1970*a*, *b*; Stein & Padykula, 1962; Yellin, 1972; Khan, Papadimitriou & Kakulas, 1974) has not been undertaken in this study. It is suggested by Khan *et al.* (1974) that the different 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.

There is much evidence that the contraction times of whole muscles are directly related to the proportion of fibres having a low reaction for myosin ATPase at pH 9·4 (Peter *et al.* 1972; Cardinet, Fedde & Tunell, 1972; Johnson, Polgar, Weightman & Appleton, 1973); and that the histochemical reaction for myosin ATPase at pH 9·4 differentiates two populations of fibres, one of which contracts faster than the other (Burke *et al.* 1971; Burke, Levine, Tsairis & Zajac, 1973; Burke, Levine, Salcman & Tsairis, 1974; Stephens & Stuart, 1974).

Hence, it is considered that a classification of muscle fibres using the histochemical myosin ATPase reaction at pH 9·4 to differentiate two populations of fibres, one of which is faster contracting than the other, is at present the best criterion we have for differentiating fibres in the dog and horse.

Classification of fibre types

The system of classification of muscle fibres used in this study, which is based on their capacity to synthesise and degrade ATP, has been advanced previously (Gunn & Davies, 1971); Peter *et al.* (1972) have since adopted a similar classification.

Histochemical fibre types in equine muscle

In the present study of the pectoralis transversus and diaphram, the myosin ATPase and SDHase reactions differentiate fibres into two types only: namely, slow-twitch and fast-twitch, both with combined aerobic and anaerobic metabolism. In semitendinosus, however, all the fibres have a high anaerobic capacity, and the myosin ATPase and SDHase reactions differentiate fibres into slow- and fast-twitch, the fast-twitch fibres being subdivided further by their aerobic capacity into fast-twitch fibres with both a high aerobic and anaerobic capacity and fast-twitch fibres with a high anaerobic capacity.

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

Histochemical fibre types in canine muscle

Trevino, Demaree, Saunders & O'Donnell (1973) claim a differentiation into three types of fibre, 'red', 'white' and 'intermediate' in the triceps muscle of German shepherd dogs. This agrees with reports on other species, although 'white' and 'intermediate' fibres cannot be distinguished in their photomicrographs. Three groups of investigators have used the Type I/Type II classification of Engel (1962) to describe both normal and pathological dog muscle (Cardinet *et al.* 1969; Cardinet *et al.* 1972; Griffiths *et al.* 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. Similarly, therefore, in this study the myosin ATPase reaction is used to differentiate fibre types in the dog.

Quantitative differences in fibre type between individual animals

The increase in the proportion of slow-twitch fibres in the diaphragm and semitendinosus which appears to occur with increasing live weight in mammals *pari passu* with decreasing respiratory rate and speed of limb movement (Davies & Gunn, 1971), receives some confirmation in this study. Thus, the diaphragm of the horse has more AL fibres, although this is not evident in the semitendinosus or pectoralis transversus (Tables 2, 5). In the case of the semitendinosus this may be due to comparison of the cross section of the whole muscle in the dog with only the superficial portion of the muscle in the horse; in the case of pectoralis transversus the muscle may function differently in the two species.

However, within either species, factors associated with breed may override 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 the diaphragm.

In 'doubled muscle' cattle also, there may be a lower proportion of slow-twitch fibres than in normal cattle (Holmes & Ashmore, 1972; Hendricks, Aberle, Jones & Martin, 1973; West, 1974). However, it is possible that the two types of cattle could still have the same absolute number of slow-twitch fibres in the same muscle, the larger number of muscle fibres in 'double muscled' cattle (Ouhayoun & Beaumont, 1968) reducing the proportion. Although this feature was not investigated in the studies of 'double muscled' cattle, or in the horses in the present study, it is apparent that the semitendinosus of the adult greyhound (Fig. 5) has a smaller total area of slow-twitch fibres than in dogs of similar weight.

The smaller number of slow-twitch fibres in the limb muscles both of greyhounds and thoroughbreds suggests that they have a greater speed of sarcomere contraction than their fellows. This would indicate a capacity for a greater rate of work per gramme of muscle in athletic animals, and suggests subtle differences in their nervous system, since the chemical properties of myosin and speed of contraction of muscle fibres are governed by their pattern of electrical stimulation or nerve supply (Buller, Eccles & Eccles, 1960; Close, 1969; Robbins, Karpati & Engel, 1969; Dubowitz, 1967; Karpati & Engel, 1967; Guth, Samaha & Albers, 1970; Sréter, Gergely, Salmons & Romanul, 1973; Sréter, Gergely & Luff, 1974; Salmons & Vrbová, 1969; Lømo, Westgaard & Dahl, 1974; Weeds, Trentham, Kean & Buller, 1974).

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 any case, while similar intensities and durations of (short-term) exercise may be limited by muscular ATP utilization in some individuals, in others the limiting factor may be the ATP supply to muscles. The rate of ATP utilization, i.e. the speed of sarcomere contraction, is only one factor dictating stride frequency and, hence, speed of running. However, although differences between athletes and non-athletes in the proportion of fast-twitch and slow-twitch fibres in similar muscles must be kept in perspective, a high proportion of fast-twitch fibres undoubtedly enhances stride frequency and appears to be the most significant attribute of fleet runners (Gunn, 1975*b*).

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 differences in the proportions of fibre types between muscles support this belief. The distribution of fibres suitable for isometric work (AL fibres) within a muscle (Guth & Samaha, 1969; Davies & Gunn, 1971; Gunn, 1973; Johnson *et al.* 1973), confirmed by this study, suggests that different parts of the same muscle function differently. On these grounds the deep medial region of the semitendinosus appears to be adapted for a postural function, while the superficial lateral region of the muscle is suited for a predominantly propulsive function. As in pig muscle (Beecher, Cassens, Hoekstra & Briskey, 1965; Tarrant, Hegarty & McLoughlin, 1972), the proportion of succinate dehydrogenase high-reacting (SH) fibres increases towards the deep medial region of the equine semitendinosus. Biochemical studies (Gunn, 1975*b*) of the greyhound semitendinosus have shown a higher activity of this enzyme in the deep medial region than in the superficial portion, although histochemically all fibres in both regions had a high SDHase activity. The enhanced aerobic capacity of the deep region shown biochemically is probably not confined to slow-twitch fibres but may occur in fast-twitch fibres as well; certainly this would be compatible with the histochemical observation. These findings support the suggestion by Gollnick, Karlsson, Piehl &

Saltin (1974) that slow-twitch fibres are used predominantly for postural or low intensity isometric functions. If propulsion is then needed, the fast-twitch fibres with the highest aerobic capacity may be recruited next and fast-twitch fibres with low aerobic capacity thereafter; this hypothesis requires further investigation.

The results of Straub *et al.* (1975) suggest that there is greater aerobic capacity in the semitendinosus of trained than untrained thoroughbreds. However, in the present study both trained and detrained adult thoroughbreds have higher proportions of aerobic fibres in the semitendinosus than do the other horses (Table 4). Histochemical analysis of samples from 'double muscled' cattle also indicate that they have a smaller proportion of aerobic fibres than normal (Hendricks *et al.* 1973; West, 1974). 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.

Sex differences and cross breeding

Vaughan, Azizi-Ullah, Goldspink & Nowell (1974) suggest that in the mouse the male soleus (believed not to be an androgen-sensitive muscle) has fewer slow-twitch fibres than the female. However, since no sex differences were found in the present study, this factor is unlikely to affect the proportions of fibre types in 'athletes' and 'non-athletes'.

It is commonly observed that the crosses of thoroughbreds with non-athletic horses are faster than 'non-athletes' but slower runners than thoroughbreds over short distances. This correlates well with the evidence of this study: thus, the values for the proportions of slow-twitch fibres in the limb muscles of thoroughbred crosses lie between those of thoroughbreds and non-thoroughbreds. However, other cross breeding effects have not been so obvious, nor have they shown statistically significant differences.

The effect of training

Several earlier studies have been carried out concerning the effect of exercise on myosin ATPase activity as demonstrated either histochemically in tissue sections or biochemically in homogenates of muscle. Barnard, Edgerton & Peter (1970*a*, *b*) showed that, although the proportion of aerobic fibres in the crural muscles of the adult guinea-pig increases with exercise, the ratio of fast- to slow-twitch fibres remains unaltered. In agreement with other workers (Maxwell, Faulkner & Lieberman, 1973; Walker, 1968; Bagby, Sembrowich & Gollnick, 1972; Syrový, Gutmann & Melichna, 1972), contrary findings may be attributed to the immaturity of the animals. However, in adult pigs, Campbell *et al.* (1971) demonstrate that the proportion of total fibre area composed of fibres with myosin ATPase (AH fibres in this study) increases with exercise. In human adults also, Gollnick *et al.* (1973) find that a prolonged training period causes an increase in the relative area occupied by slow-twitch (AL) fibres in vastas lateralis. Such contrasting results (between pigs and men) may be due to species differences or to the type of exercise regime. In general, myosin ATPase activity of homogenates has been shown to remain constant after exercise, as indicated by the initial studies in this field (Rawlinson & Gould, 1959; Hearn & Gollnick, 1961). While it may be true that training does not increase the absolute number of a particular type of fibre (as differentiated by the myosin ATPase reaction) in the adult, this may occur in the young animal.

In the present study only one year could be devoted to detraining greyhounds, and the detrained throughbreds had also been out of training for one year or more. Such specimens had to be utilized to represent untrained adult thoroughbreds and greyhounds, which are rare and difficult to obtained. The lack of a significant difference in the histochemical properties of limb muscles between detrained and trained adult athletes suggests that the histochemical difference between athletic and non-athletic horses and dogs cannot be attributed to training, at least within the adult period. Whether the smaller fractional area of the diaphragm occupied by slow-twitch fibres in trained greyhounds is associated with a higher respiratory rate in those animals was not investigated.

In conclusion, the most noticeable attribute of the limb muscles of athletes of both species is the higher proportional area of fast-twitch fibres. This indicates a greater intrinsic speed of sarcomere contraction favouring an enhanced stride frequency in these animals. The development of these characteristics in adults requires further investigation.

SUMMARY

Histochemical profiles of individual muscle fibres were established using myosin adenosine triphosphatase (myosin ATPase), succinate dehydrogenase (SDHase), and glycogen phosphorylase (GPase) reactions in three muscles (semitendinosus, diaphragm, and pectoralis transversus) of the horse and dog.

The major histochemical difference between fibres lies in their myosin ATPase activity; fibres can be subdivided into those with a high and those with a low activity. In horse muscle, all fibres have a high activity of GPase. In the diaphragm and pectoralis transversus, all fibres have a high SDHase activity, but fibres with a low activity of SDHase are also present in samples of the semitendinosus. In dog muscle, all fibres have a high SDHase activity; myosin ATPase low-reacting fibres also have a low activity of GPase.

There is a greater fractional area of myosin ATPase high-reacting fibres in the pectoralis transversus and semitendinosus of thoroughbred horses and greyhounds (breeds selected for high speed running) and in the diaphragm of greyhounds. In adults this feature does not appear to be due to training, as are the differences in aerobic and anaerobic capacity (shown in other studies). The preponderance of myosin ATPase high-reacting fibres suggests that there may be differences in the nervous systems of athletes and non-athletes.

It is concluded that the proportions of fibre types in muscles are related to the functions of muscles and of their parts. No sex differences or detraining effects were apparent, although the value for the proportion of fibre types (as differentiated by the myosin ATPase reaction) in the limb muscles of thoroughbred crosses lies between those of thoroughbreds and non-thoroughbreds.

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