J. Anat. (1977), **123**, 2, pp. 467–486
With 5 figures
Printed in Great Britain

The distribution and relative sizes of fibre types in the extensor digitorum longus and soleus muscles of the adult rat

A. H. PULLEN*

M.R.C. Research Group in Applied Neurobiology, Institute of Neurology, 8/11, Queen Square, London, W.C.1

(*Accepted* 18 *April* 1976)

INTRODUCTION

While the physiology and general morphology of the rat extensor digitorum longus (EDL) and soleus muscles have been extensively studied (Edgerton & Simpson, 1969, 1971; Schiaffino, Hanzlikova & Pierobon, 1970; Close, 1972; Gauthier, 1974; Karparti, Eisen & Carpenter, 1975), variations in regional composition of these muscles have received less attention. Discrepancies between reported estimates of fibre type proportions for the rat EDL muscle (Edgerton & Simpson, 1969, 1971; Schiaffino *et al.* 1970; Ariano, Armstrong & Edgerton, 1973) could result from the use of different methods of quantitative analysis or it could be that histochemical classifications of fibres differ according to the enzyme systems used. This second point is particularly relevant when estimates of fibre type proportions based upon sections stained for oxidative enzymes are combined with estimates of 'mean' values of muscle composition based on ATPase studies (e.g. Close, 1972; Ariano *et al.* 1973). Estimates of the proportion of fibre types in a given muscle may vary even when studying a single enzyme system (e.g. ATPase), particularly if recognized modifications of the standard technique are employed. Despite the generally consistent estimates of composition obtained from sections of the soleus muscle stained for various oxidative enzymes, the proportions of the various fibre types seen in unfixed sections stained for myosin ATPase at pH 9·4 differ from those found in sections which have been subjected to prolonged fixation in dilute formaldehyde (Kugelberg, 1973*b*), or preincubated at pH 4·3–4·5 (Kugelberg, 1973*b*; Karparti *et al.* 1975).

This study presents a detailed analysis of the regional histochemical fibre composition of the rat EDL and soleus muscles. The results supplement those already given for the tibialis anterior muscle (Pullen, 1977), and, combined with morphometric analysis, demonstrate discrepancies between classifications based on different enzyme systems.

* Present address: Sobell Department of Neurophysiology, Institute of Neurology, The National Hospital for Nervous Diseases, Queen Square, London, W.C.1.

METHODS

Animals

Twelve adult (7♂, 5♀) CFHB-Wistar rats (2·5 months old; 250–300g), bred from an original stock supplied by Carworth (Europe) Ltd, were examined. These were fed a diet of Oxoid 41 B pellets and were allowed water *ad libitum*. No difference was detected between males and females in respect of the quantitative histochemical compositions of homologous hind limb muscles and so the results given here are based on pooled data from both sexes.

Preparation of muscle specimens

Animals were killed with an excess of anaesthetic ether. After death the muscles of either the anterior or posterior compartments of the right lower hind limbs were quickly removed and tied to wooden splints by means of ligatures at their tendons of origin and insertion before being coated with powdered talc and quenched directly in liquid nitrogen (−196 °C) for 5 minutes (Moline & Glenner, 1964). The specimens were then transferred to the cryostat and left for 1 hour to equilibrate with the higher chamber temperature (−20 °C). Transverse slices, 1 cm thick, cut at the mid-longitudinal level of either the EDL or soleus were mounted on cold microtome chucks (−20 °C) with 20 % gum acacia solution. Preliminary 10 μm sections stained with haematoxylin and eosin were used to check the orientation of the myofibres.

Histochemical methods

Serial and non-serial air dried sections were stained to demonstrate succinic dehydrogenase (SDH) (Pearse, 1968) and calcium activated myosin ATPase at pH 9·4 (Davies & Gunn, 1972). ATPase was demonstrated in sections that had been fixed at 4 °C for 2–4 minutes in 2 % cacodylate buffered formaldehyde (pH 7·2) before preincubation in a calcium-containing glycine buffer (pH 10·4) for 15 minutes at 37 °C. Subsequent incubation at pH 9·4 lasted 20 minutes at 37 °C.

Morphometric techniques

(*a*) *Determination of fibre type distributions*

Fibre type distributions were determined using the method previously described for analysis of the tibialis anterior muscle (Pullen, 1977).

EDL. The image of the EDL in complete transverse section was projected on to the screen of a demonstration microscope and magnified to a constant size. The deep to superficial (DS) and medial to lateral (ML) axes of the EDL were aligned beneath the mid-vertical and mid-horizontal axes of a square lattice counting grid drawn on the microscope screen. Each square thus covers an identical region of muscle in different specimens. The relative proportions of fibre types determined from direct counts of *total* fibre numbers in each square along a muscle axis were expressed as the ratio of the total number of fibres belonging to a given type (t) to the total number of fibres in that square (T). Histograms were compiled for the proportions of fibre types along the DS and ML axes, and the pooled data obtained for each grid square from groups of muscle stained for a given enzyme were used to determine the mean proportions of each fibre type (± S.D., ± S.E.) in grid squares defining a muscle axis (Bailey, 1959).

Soleus. A modified technique was necessary to accommodate the crescent shape of the soleus muscle as seen in transverse sections of the hind limb. While the DS axis was composed of six grid squares arranged in series along the axis, the ML axis was divided into a number of adjacent rectangular fields. Each field contained the complete depth of the muscle from its deep concave border abutting on to the fibula, to the superficial surface adjacent to the plantaris muscle. The total number of fibres along this axis thus represented the total fibre population of the muscle, but only at this particular level of section. All other procedures were identical with those applied to the EDL muscle.

(*b*) *Measurement of fibre cross sectional areas*

Fibre cross sectional areas were measured within representative areas in deep, superficial, medial, lateral and central regions of the two muscles, using the method of Aherne (1968). Measurements were made directly on the microscope image, using a calibrated Leitz micrometer eyepiece and a total magnification of × 500. The product of the distance between the most lateral points on a fibre contour ('diameter' = L) and the length of a random chord measured along the same axis (l) provided an estimate of the fibre cross sectional area ($A = L.l$). Fifty fibres per type were measured in each of 6 different specimens from each muscle (i.e. EDL and soleus), a total of 300 fibres per type being measured for each set of results. The direction along which the micrometer vernier travelled was altered randomly after measurements had been made on individual groups of ten fibres, by rotating the micrometer on the microscope eyepiece. This procedure reduces error due to differences in fibre shape, and improves the accuracy of the final estimate of mean fibre area, (Aherne, 1968).

Preliminary studies showed that a total of 200–300 fibres per type formed a statistically adequate sample. The size of this sample was derived using a cumulative frequency graph (Aherne, 1968; Saunders & Fleming, 1972). Individual fibre measurements were divided into groups of 50, and the size of a population was made to increase in a stepwise manner by adding together several groups, (i.e. n = 50, 100, 150, 200, etc). After each addition, the mean fibre cross sectional area was calculated for the population, together with values for S.D. and S.E. and the relative standard error (R.S.E.). The latter is given by the formula R.S.E. = 100 (S.D./$(n)^{\frac{1}{2}}$.mean area) %, and was considered to be within acceptable limits when at a level between 2 % and 5 %. The size of an adequate sample was considered to have been reached when (i) there was no further increase in the value of mean fibre area despite further increases in size of fibre population, (ii) the value of the S.D. was below 10 % of the value of the mean fibre area, and (iii) the value of the R.S.E. was between 2 and 5 %.

The pooled measurements obtained for each fibre type from the EDL and soleus muscles were used to compile frequency histograms of fibre cross sectional area. The range, mean area, S.D. and S.E. were calculated for each fibre type using the formulae given by Bailey (1959).

RESULTS

Classification of muscle fibre types

Myofibres were classified using the terminology given by Brooke & Kaiser (1970). *Type IIA* fibres are also known as 'C' (Stein & Padykula, 1962), red (Padykula & Gauthier, 1967; Guth & Samaha, 1969) and α fibres (Guth & Yellin, 1971). *Type I* fibres are equivalent to 'B' (Stein & Padykula, 1962), intermediate (Padykula & Gauthier, 1967; Edgerton & Simpson, 1969), and β fibres (Guth & Yellin, 1971). *Type IIB* fibres represent the 'A' or white fibre type, and in the rat are classified as $\alpha\beta$ fibres by Guth & Yellin (1971).

Histochemical fibre types in the EDL and soleus muscles

Three types were observed in the EDL muscle, but only two basic types in the soleus muscle. Fibre typing was based on the level of staining with a given enzyme, and on the sarcoplasmic distribution of the histochemical reaction product (Stein & Padykula, 1962; Guth & Samaha, 1969; Dubowitz & Brooke, 1973).

EDL

Types IIA, I and IIB were present in the EDL muscle.

Type IIA fibres possessed a high level of SDH activity, the reaction product appearing in the fibre as a coarse network (Fig. 1A), preferentially distributed in subsarcolemmal regions. This fibre type also exhibited intense floccular ATPase activity in formalin-fixed sections (Fig. 1B).

Type I fibres generally contained a moderately high oxidative enzyme activity, but less pronounced subsarcolemmal accumulations of reaction product (Fig. 1A). In addition to a moderate level of staining with SDH, many fibres possessed a low ATPase activity (Fig. 1B), like the Intermediate fibres described by Edgerton & Simpson (1969) and the β fibres defined by Guth & Yellin (1971).

Type IIB fibres were characterized by low oxidative enzyme, and moderately high ATPase, activities. Such fibres were not seen in the soleus muscle. While the reaction product for SDH was in the form of an open network (Fig. 1A), a fine precipitate typically occurred in fibres stained for ATPase (Fig. 1B).

The three fibre types observed in the EDL muscle were indistinguishable from those previously seen in the tibialis anterior muscle (Pullen, 1977).

Soleus

For the purposes of quantitation, only two basic fibre types were classified in the soleus muscle (Types IIA and I), although a limited spectrum of staining levels occurred in both types. Thus some Type I fibres exhibiting *diffuse* oxidative enzyme activity were as heavily stained as Type IIA fibres exhibiting characteristic *subsarcolemmal* accumulations of reaction product (Fig. 1E). Moreover, fibres possessing an ATPase activity slightly higher than usual in Type I fibres were found to contain a moderate and diffuse SDH activity when such fibres were individually identified in serial sections, and these were therefore included within the Type I fibre class for purposes of quantitative analysis. Other fibres were similar in appearance to Type

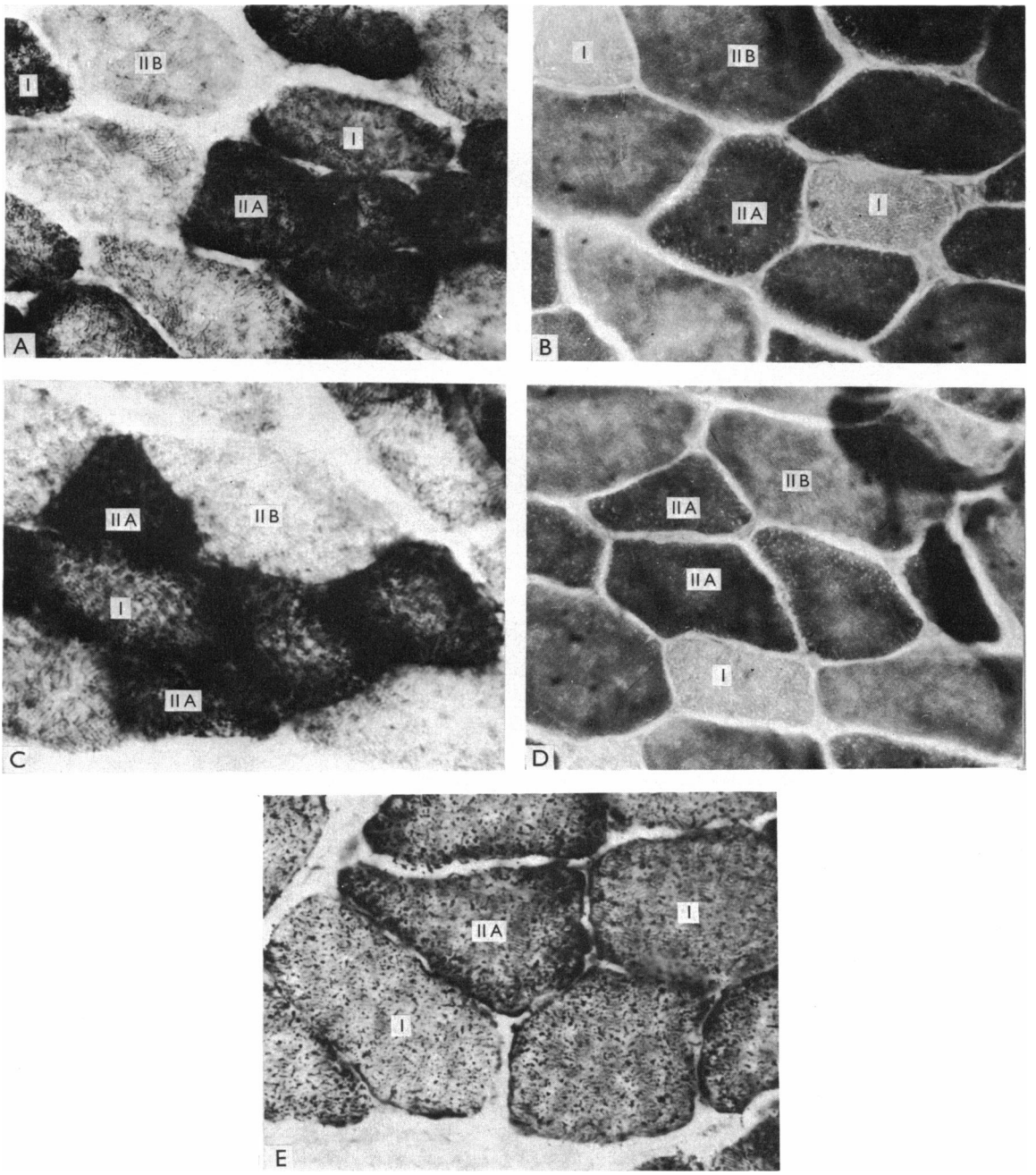

Fig 1. The serial sections shown in (A–B) and (C–D) were from the EDL muscle of the adult rat area stained for SDH (A, C) and myosin ATPase at pH 9·4 (B, D). The identities of some fibres are given. While no variation appears to occur between the classification of fibres in A and B, a single Type I fibre classified using SDH in C, conforms to the appearance of a Type II A fibre in a serial section stained for ATPase (D). The reverse situation also may arise, as seen by comparing individual fibres in C and D. (E). Some representative fibres in the rat soleus muscle (stained for SDH).

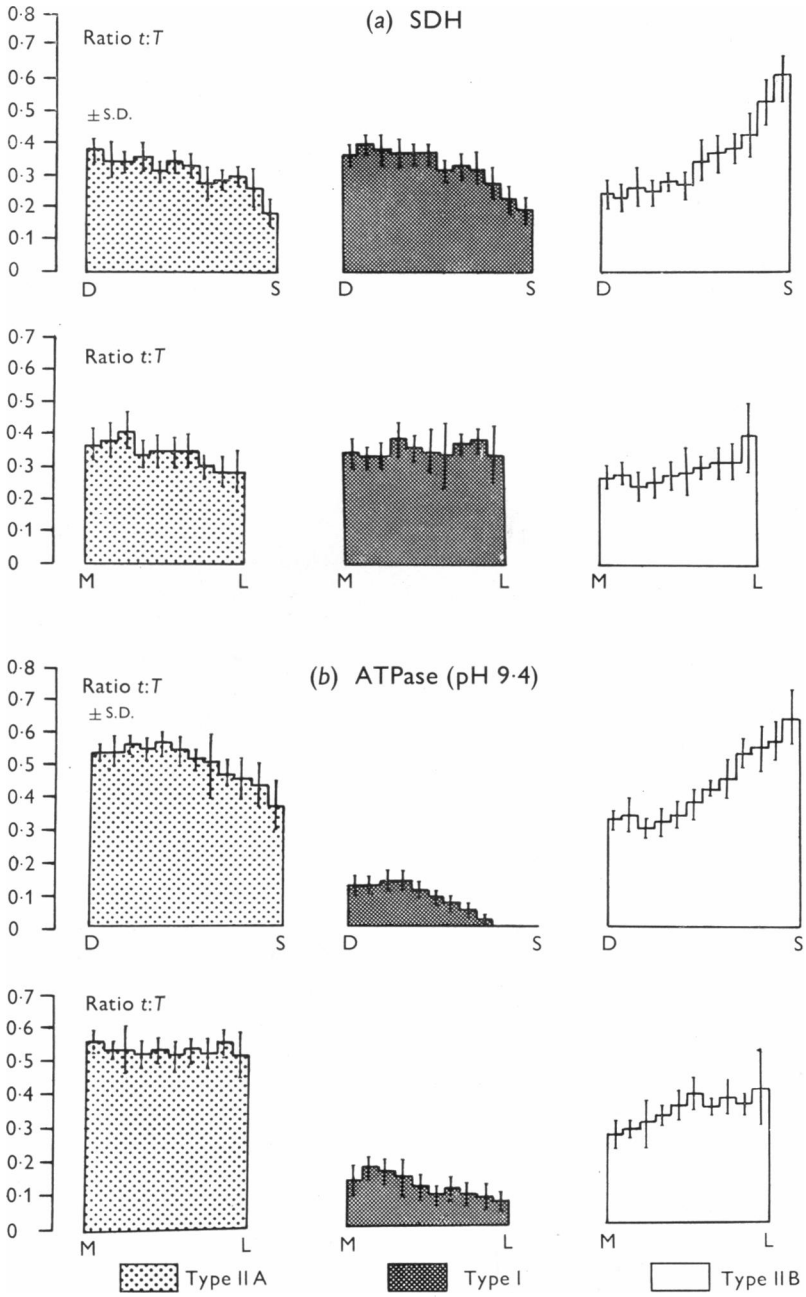

Fig. 2. Distribution profiles for fibres in the EDL muscle classified on the basis of their SDH (*a*) and myosin ATPase activities. (*b*). The variation in proportions of fibres along the deep to superficial (D–S) and medial to lateral (M–L) axes of the muscle is shown in each case. Bar lines indicate values of ±S.D.

IIA and Type I fibres of the EDL muscle. Unfortunately in this study it was impossible to classify fibres in the soleus muscle into further subclasses, as has been attempted by Brooke & Kaiser (1970), Kugelberg (1973*b*) and Karparti *et al.* (1975), since their acid preincubation techniques were not employed as preliminary steps in the ATPase method used here.

Distribution of fibre types in the EDL muscle

General morphology

The marked regional variation in histochemical composition seen in the tibialis anterior muscle (Pullen, 1977) was not so apparent in the EDL. Deeper regions contained a higher proportion of Type IIA and Type I fibres than superficial and lateral aspects of the muscle. Fibres possessing a low ATPase activity were scattered throughout the muscle, and were not so restricted in distribution as those in the tibialis anterior muscle.

Succinic dehydrogenase

Distribution profiles for fibres classified by the intensity and sarcoplasmic distribution of SDH activity are shown in Figure 2, and the corresponding statistical data appear in Table 1. Type IIA and Type I fibres progressively decreased in proportion along the DS axis of the muscle, while Type IIB fibres showed the reverse pattern of distribution. A smaller variation in fibre type proportions occurred along the ML axis (Fig. 2*b*; Table 1), Type IIA fibres predominating in the medial and central regions while Type IIB fibres occurred mainly in lateral aspects. Little variation in the proportions of Type I fibres was found along this axis.

ATPase (pH 9·4, formalin-fixed sections)

The relative distribution of fibre types along both muscle axes is illustrated in Figure 2 and is analysed in Table 1. Although Type 1 fibres were present in the most superficial regions of the EDL in sections stained for SDH, they were absent from similar regions of the muscle when fibres were classified using ATPase (Fig. 2, Table 1). Type IIB fibres again predominated in superficial regions and progressively decreased in prevalence towards the deep pole of the muscle. The distribution of fibre types along the ML axis was different from that found in muscles exhibiting SDH activity. Type IIA fibres showed little variation in proportion along this axis, but Type I fibres decreased in number towards the lateral pole, and their overall proportion was markedly lower than in sections stained for SDH (Table 1). Type IIB fibres progressively increased in number towards lateral regions. The greater overall proportion of Type IIA fibres in sections stained for ATPase was offset by a smaller number of fibres containing a low ATPase activity (Tables 1, 5).

Results obtained for the EDL muscle were subjected to a two-way analysis of variance (Bailey, 1959) to test the significance of any variation present, attributable to (i) differences in regional muscle composition and (ii) differences in fibre type classification in SDH and myosin ATPase-stained preparations. The results of the analysis are given in Table 3. Significant differences in regional composition occurred

Table 1. *The mean proportions of fibre types along the deep to superficial and medial to lateral axes of the rat EDL muscle in sections stained for SDH and myosin ATPase (pH 9·4)*

(The proportions are expressed as the ratio *t:T* (see text), and the number of muscles examined in each case is given by the value *N*.)

	Deep to superficial axis							Medial to lateral axis					
	S.D.H. $N = 6$			ATPase $N = 6$				S.D.H. $N = 6$			ATPase $N = 6$		
	t:T	S.D.	S.E.	*t:T*	S.D.	S.E.		*t:T*	S.D.	S.E.	*t:T*	S.D.	S.E.
							Type II A fibres						
D	0·38	0·024	0·010	0·53	0·018	0·007	M	0·37	0·061	0·025	0·56	0·034	0·014
	0·35	0·052	0·022	0·53	0·062	0·025		0·38	0·059	0·024	0·53	0·032	0·013
	0·35	0·034	0·014	0·56	0·020	0·008		0·41	0·067	0·027	0·52	0·077	0·031
	0·37	0·056	0·023	0·54	0·034	0·014		0·34	0·047	0·017	0·52	0·047	0·019
	0·32	0·030	0·012	0·56	0·033	0·013		0·35	0·061	0·025	0·53	0·039	0·016
	0·35	0·030	0·012	0·54	0·043	0·017		0·36	0·041	0·016	0·51	0·047	0·019
	0·34	0·043	0·017	0·51	0·025	0·010		0·35	0·064	0·026	0·54	0·027	0·011
	0·28	0·050	0·020	0·50	0·090	0·037		0·30	0·037	0·015	0·51	0·041	0·016
	0·29	0·028	0·011	0·46	0·037	0·015		0·29	0·038	0·016	0·55	0·028	0·011
	0·30	0·020	0·003	0·45	0·069	0·028	L	0·29	0·072	0·029	0·51	0 099	0·041
	0·26	0·078	0·032	0·43	0·070	0·031							
S	0·19	0·043	0·017	0·36	0 083	0 041							
							Type I fibres						
D	0·37	0·036	0·014	0·13	0·035	0·010	M	0·35	0·066	0·027	0·15	0·036	0·015
	0·40	0·031	0·012	0·13	0·015	0·006		0·34	0·033	0·013	0·18	0·027	0·011
	0·39	0·052	0·021	0·14	0·021	0·008		0·34	0·033	0·013	0·17	0·043	0·017
	0·38	0·044	0·018	0·14	0·025	0 010		0·39	0·054	0·022	0·15	0·050	0·020
	0·38	0·030	0 012	0·11	0·035	0·014		0·36	0·045	0·018	0·12	0·050	0·020
	0·38	0·033	0·013	0·09	0·022	0·009		0·35	0·087	0·035	0·09	0·030	0·012
	0·32	0·033	0·013	0·070	0·016	0·006		0·34	0·098	0·040	0·11	0·016	0·006
	0·34	0·043	0·017	0·050	0·019	0·008		0·37	0·033	0·013	0·10	0·034	0·014
	0·32	0·046	0·019	0·020	—	—		0·38	0·043	0·017	0·09	0·035	0·014
	0·28	0·068	0·028	0·00	—	—	L	0·32	0·100	0·044	0·07	0·035	0·014
	0·23	0·034	0·014	0·00	—	—							
S	0·20	0·041	0 016	0·00	—	—							
							Type II B fibres						
D	0·25	0·046	0·019	0·33	0·029	0·012	M	0·28	0·052	0·021	0·29	0·038	0·015
	0·24	0·037	0·015	0·34	0·057	0·023		0·28	0·051	0·021	0·29	0·020	0·008
	0·26	0·061	0·024	0·30	0·019	0·008		0·25	0·028	0·011	0·31	0·084	0·034
	0·26	0·034	0·014	0·32	0·043	0·017		0·26	0·062	0·025	0·33	0·031	0·012
	0·29	0·024	0·010	0·34	0·044	0·018		0·28	0 050	0·020	0·37	0 041	0·016
	0·27	0·041	0·016	0·37	0·048	0·019		0·29	0·092	0·037	0·40	0·050	0·020
	0·35	0·067	0·027	0·42	0·016	0·006		0·31	0·044	0·018	0·35	0·026	0·010
	0·38	0·061	0·025	0·45	0·050	0·043		0·32	0·042	0·017	0·38	0·052	0·021
	0·39	0·052	0·021	0·53	0·042	0·017		0·32	0·067	0·027	0·36	0·027	0·011
	0·43	0·088	0·036	0·55	0·069	0·028	L	0·38	0·110	0 046	0·41	0·123	0·050
	0·54	0·073	0·030	0·57	0·073	0·033							
S	0·62	0·073	0·030	0·64	0·083	0·041							

Table 2. *The mean proportions of fibre types along the deep to superficial and medial to lateral axes of the rat soleus muscle in sections stained for SDH and myosin ATPase*

(The number of muscles examined is indicated in each case (*N*).)

	Deep to superficial axis							Medial to lateral axis					
	S.D.H. $N = 4$			ATPase $N = 6$				S.D.H. $N = 4$			ATPase $N = 6$		
	t:*T*	S.D.	S.E.	*t*:*T*	S.D.	S.E.		*t*:*T*	S.D.	S.E.	*t*:*T*	S.D.	S.E.
								Type II A fibres					
D	0·36	0·012	0·001	0·28	0·030	0·012	M	0·16	0·037	0·018	0·14	0·037	0·015
	0·35	0·020	0·010	0·25	0·058	0·023		0·21	0·025	0·012	0·18	0·048	0·018
	0·39	0·026	0·013	0·22	0·066	0·027		0·29	0·060	0·030	0·19	0·046	0·018
	0·33	0·073	0·036	0·20	0·036	0·014		0·23	0·025	0·012	0·18	0·035	0·014
	0·29	0·052	0·026	0·14	0·055	0·021		0·27	0·082	0·041	0·17	0·041	0·016
S	0·21	0·067	0·034	0·12	0·048	0·026		0·29	0·095	0 047	0·20	0 069	0·028
								0·25	0·078	0·039	0·26	0·064	0·026
								0·31	0·075	0·037	0·25	0·070	0·028
								0·34	0·110	0·051	0·26	0·048	0·019
								0·30	0·021	0·018	0·25	0·058	0·024
								0·38	0·106	0·053	0·28	0·042	0·017
								0·29	0·075	0·038	0·25	0·046	0·019
								0·35	0·058	0·029	0·25	0·042	0·017
								0·35	0·057	0·028	0·30	0·064	0·024
								0·33	0·025	0 012	0·39	0·113	0·046
							L	0·30	0·016	0·001	0·46	0·076	0·031
								Type I fibres					
D	0·64	0·012	0·006	0·72	0·030	0·012	M	0·84	0·037	0·018	0·85	0·048	0·019
	0·64	0·025	0·012	0·75	0·058	0·023		0·79	0·025	0·012	0·85	0·019	0·007
	0·57	0·021	0·010	0·78	0·063	0·026		0·71	0·064	0·032	0·85	0·019	0·007
	0·67	0·073	0·036	0·80	0·036	0·014		0·77	0·025	0·012	0·82	0 035	0 014
	0·71	0·052	0·026	0·85	0·053	0·021		0·73	0 082	0·041	0·84	0·045	0·018
S	0·80	0·045	0·027	0·86	0·061	0·025		0·71	0·092	0·043	0·82	0·058	0·024
								0·75	0·078	0·039	0·75	0·071	0·029
								0·69	0·075	0·037	0·74	0·073	0·054
								0·68	0·086	0·043	0·74	0·057	0·023
								0·69	0·020	0·014	0·74	0·061	0·024
								0·62	0·106	0·053	0·74	0·060	0·024
								0·71	0·075	0·037	0·74	0·044	0·018
								0·65	0·058	0·029	0·77	0·128	0·011
								0·65	0·057	0·028	0·62	0·094	0·038
								0·67	0·023	0·016	0·58	0·113	0·046
							L	0·70	0·010	0·008	0·57	0·108	0·044

for all three fibre types along the DS axis, but the less marked variation in regional distribution observed along the ML axis was reflected in the smaller values of variance ratio for this particular axis (Table 3). Profiles based on sections stained for SDH were also significantly different from those obtained with ATPase ($P = 0{\cdot}001$ for most fibre types along both the DS and ML axes).

Table 3. *The results of an analysis of variance carried out to test the possible significance of any variations in observed fibre type distributions which might be attributable to (i) differences in regional muscle composition, (ii) differences in classification resulting from the use of SDH and myosin ATPase preparations for fibre typing*

(Levels of significance are interpreted as follows: $P = 0{\cdot}001$, highly significant; $P = 0{\cdot}01$, significant; $P = 0{\cdot}05$, possibly significant.)

Muscle	Axis†	Fibre type	Source of variation	D.F.‡	Variance ratio	P
EDL	D–S	IIA	Between regions	11	16·36	< 0·001 (Sig)
			Between enzymes	1	521·77	< 0·001
			(Residual)	11		
		I	Between regions	11	16·65	< 0·001
			Between enzymes	1	935·04	< 0·001
			(Residual)	11		
		IIB	Between regions	11	41·67	< 0·001
			Between enzymes	1	48·37	< 0·001
			(Residual)			
EDL	M–L	IIA	Between regions	9	1·19	< 0·05 (Sig)
			Between enzymes	1	194·8	< 0·001
			(Residual)	9		
		I	Between regions	9	1·07	> 0·05 (Possibly Sig)
			Between enzymes	1	298·4	< 0·001
			(Residual)	9		
		IIB	Between regions	9	5·12	0·01/·05
			Between enzymes	1	25·46	< 0·01 (Sig)
Soleus	D–S	IIA	Between regions	5	11·42	< 0·01
			Between enzymes	1	67·50	< 0·001
			(Residual)	5		
		I	Between regions	5	5·54	< 0·05
			Between enzymes	1	31·99	< 0·001
			(Residual)	5		
Soleus	M–L	IIA	Between regions	15	3·17	> 0·05
			Between enzymes	1	5·32	< 0·05
			(Residual)	15		
		I	Between regions	15	3·01	> 0·05
			Between enzymes	1	4·69	< 0·05
			(Residual)	15		

† D–S, Deep to superficial; M–L. medial to lateral.

‡ Expressed in the usual manner; i.e. (no muscle regions − 1) = $(n-1)$; (no. enzyme methods − 1) = $(k-1)$; residual variation = $(n-1)(k-1)$.

Cross sectional areas for fibres in the EDL muscle

Despite the similar ranges of size exhibited by Type IIA (and Type IIB) fibres in SDH and ATPase preparations, Type I fibres identified in ATPase preparations were generally smaller than Type I fibres identified in SDH preparations (Fig. 3, Table 4). While Type I fibres were intermediate in size between Types IIA and IIB using SDH (Fig. 3*a*), they were similar in size to the Type IIA fibres in sections stained for ATPase (suggesting that two distinct groups of fibres make up the Type I class, which differ in their histochemical properties as well as in their size).

Table 4. *Ranges of size and mean cross sectional areas of fibres classified in the EDL and soleus muscles according to their succinic dehydrogenase and ATPase properties*

Muscle	Fibre type	*N*	Range (μm^2)	Mean area (μm^2)	S.D.	S.E.
			SDH			
EDL	IIA	200	126·6–2008·7	812·7	360·3	25·4
	I	200	356·1–3839·1	1339·1	550·5	38·9
	IIB	200	524·6–5396·5	1996·8	825·5	58·3
Soleus	IIA	200	615·6–4712·4	2012·3	807·1	57·1
	I	200	1049·4–7196·6	2834·7	1072·6	75·8
			ATPase			
EDL	IIA	200	294·7–2374·9	1126·15	499·3	35·31
	I	200	130·1–2459·4	804·87	388·5	27·47
	IIB	200	823·1–6231·5	2416·60	934·1	66·05
Soleus	IIA	200	447·0–6076·7	2100·3	1055·0	74·60
	I	200	925·1–8443·1	3277·3	1403·6	99·25

Distribution of fibre types in the soleus muscle

General morphology

While an initial examination of the soleus muscle suggested that both Type I and Type IIA fibres were randomly distributed in both SDH and ATPase preparations, a more careful analysis revealed the presence of a consistent pattern of distribution. Type I fibres predominated both in sections stained for SDH and also in those demonstrating ATPase activity (pH 9·4). Groups of Type IIA fibres were particularly noticeable in the lateral pole of muscles stained for ATPase.

Succinic dehydrogenase

A higher number of Type IIA fibres were found in deep and lateral aspects than in superficial or medial regions of the soleus muscle (Fig. 4, Table 2). A marked decrease in the number of Type IIA fibres was evident between the central and superficial regions, with a more gradual reduction in numbers towards the lateral pole. Approximately 60 % Type I and 40 % Type IIA fibres occurred in muscles stained for SDH, but the general organization was not as regular as that previously found in the EDL and tibialis anterior muscles.

ATPase (pH 9·4)

The difference in overall proportions of fibre types between sections stained for SDH and ATPase was particularly apparent in the soleus muscle, since it only contains two basic fibre types. Only 20 % of the fibres were classifiable as Type IIA, using ATPase, the remainder being Type I. Despite the discrepancy in numbers in the SDH and ATPase muscles, the relative pattern of distribution was similar in both cases, although a more extreme pattern was seen with ATPase (Fig. 4*b*).

Table 3 shows the results of an analysis of variance performed on the data obtained for the soleus muscle. Significant differences in regional distribution of fibre types occurred along the DS axis, but the distributions along the ML axis were on the borderline of significance (between P values indicating variations that were 'possibly significant' and those indicating a higher level of significance). Significant differences between profiles based on SDH and ATPase were detected.

Cross sectional areas of fibres in the soleus muscle

The overall ranges of size for both fibre types were greater in sections stained for SDH. Nevertheless, two populations of Type I fibres were thought to be present since the largest Type I fibres with ATPase were even larger than those seen using SDH (Table 4). Mean fibre sizes for Type I fibres were $2834{\cdot}6 \pm 1072$ μm^2 (SDH) and $3277{\cdot}6 \pm 1403$ μm^2 (ATPase).

Analysis of serial sections stained for SDH and ATPase

Differences between the relative distributions and sizes of fibre types classified according to SDH and ATPase criteria suggested that these enzyme systems do not give compatible results. A limited examination of individual fibres in serial sections showed that some individual fibres would be classified differently in SDH and ATPase preparations. The results of a quantitative analysis using these two enzymes, based on counts of fibre numbers in fascicles of the muscle which could be easily recognized in serial sections, are given in Table 5.

In both the EDL and soleus muscles, fibres were found which, although possessing the appearance of Type II A fibres when stained for SDH (high activity with peripheral accumulations of reaction product), were classifiable as Type I with ATPase, since they contained a low activity of this enzyme (Fig. 1A–D). In addition, other fibres appeared to be classifiable as Type I with SDH (Fig. 1A, C), but contained the high floccular ATPase activity characteristic of Type II A fibres (Fig. 1B, D). Type II B fibres failed to show any anomalous classifications with these particular histochemical techniques.

When the incidence of fibre type mis-matching found in the soleus muscle was compared with the proportional difference between the fibre type numbers found along the ML axis in sections stained for SDH and ATPase, an identical number of fibres in each case exhibited anomalous classifications (8 %). A similar comparison carried out for fibres in the EDL muscle resulted in 24·7 % of Type I fibres being found to possess distributions which differed according to whether they had been classified with ATPase or SDH, while 23·7 % of all fibres counted in fascicles identified in serial sections were equivocal in their classification.

DISCUSSION

The extensor digitorum longus (EDL) and soleus muscles of the adult rat are both composed of a mixture of different histochemical fibre types which are distributed in each muscle in a characteristic manner. The distributions and numbers of Type II A and Type I fibres classified according to SDH activity differ from those based upon ATPase histochemistry. Two subclasses of each of these fibre types can be distinguished and this is confirmed when individual fibres are examined in serial sections.

Table 5. *Numbers of fibres counted in some fascicles of the soleus and EDL muscles classified in serial 10μm sections stained for* S.D.H. *and ATPase (pH 9·4)*

(These results demonstrate that the classification of individual fibres may differ according to which particular histochemical method is used for fibre 'typing')

	Observed fibre type (no fibres)					
Technique: ATPase	1	II A	I	II A	II B	
Technique: SDH	1	II A	II A	I	II B	
	Soleus muscle					
Animal (*a*)	40	19	8	0	0	
(*b*)	50	28	4	3	0	
(*c*)	51	17	3	4	0	
(*d*)	57	20	4	5	0	
(*e*)	81	92	6	2	0	
Totals	279	176	25	14	0	494
% total fibres counted	56·5	35·6	5·1	2·8		
	No. anomalous fibres = 39 (7·9 %)					
	Muscle					
Animal (*b*)	1	12	1	4	5	
(*f*)	0	5	2	1	4	
(*g*)	2	8	1	0	6	
(*h*)	0	8	4	5	7	
Totals	3	33	8	10	22	
% total fibres	3·9	43·4	10·5	13·2	28·9	
	No. anomalous fibres = 18 (23·7 %)					

These observations are in agreement with those obtained in an earlier study of the rat tibialis anterior muscle (Pullen, 1977), and this validates the quantitative techniques used in both studies for investigating fibre type populations in complete transverse sections of mammalian muscles.

The EDL contains three fibre types which differ in their histochemical and ultrastructural properties (Schiaffino *et al.* 1970; Edgerton & Simpson, 1969, 1971). Differences in the levels of SDH activity and in the patterns of intracellular staining between fibre types can be correlated at ultrastructural levels with differences in the relative content and regional distribution of mitochondria (Stein & Padykula, 1962; Padykula & Gauthier, 1967; Schiaffino *et al.* 1970). Type II A (red) fibres for example, possess a high mitochondrial content concentrated peripherally, and these properties are reflected in high oxidative enzyme activity with subsarcolemmal accumulations of reaction product. There was no apparent difference between the fibre types identified in the present study of the EDL muscle, and those described in the gastrocnemius muscle (Stein & Padykula, 1962), and in the semitendinosus, diaphragm and tibialis anterior (Gauthier & Padykula, 1966; Padykula & Gauthier, 1967; Pullen, 1977). Nevertheless, while a comparison of distribution profiles showed that the larger proportion of Type II A fibres seen in ATPase preparations was offset by a lower number of Type I fibres (low ATPase activity), analyses of serial sections

Table 6. *A comparison of previous estimates of muscle composition with the results obtained in the present study*

(Fibre type classifications have been equated with one another as far as possible and are tabulated using the nomenclature introduced by Brooke & Kaiser (1970))

Author(s)	Technique	Type IIA	Type I	Type IIB
	1. Extensor digitorum longus muscle			
Edgerton & Simpson (1969–1971)	Oxidative enzymes/ α-GPDH/ATPase, pH 9·4	?	3·8 %	60 %
Schiaffino *et al.* (1970)	Histochemical/ Ultrastructural	(IIA+1 = 58 %) 25–29 %	?	42 %
Ariano *et al.* (1973)	SDH/ATPase/ Phosphorylase	37 % (FOG)†	6·0 % (SO)†	57 % (FG)†
Present study	SDH	32·77 %	34·32 %	33·10 %
	ATPase (pH 9·4)	51·18 %	9·61 %	39·34 %
	2. Soleus muscle			
Karparti & Engel (1967)	ATPase (pH 9·4) Unfixed material	10–15 %	85–90 %	—
Edgerton & Simpson (1969)	NADH-D/ATPase (pH 9·4)	20 %	80 %	—
Schiaffino *et al.* (1970)	SDH/Ultrastructural	20–25 % (10–15 %‡)	80–75 %	—
Ariano *et al.* (1973)	SDH/ATPase/ Phosphorylase	16 %	84 %	—
Gauthier (1974)	Ultrastructural (mitochondrial content)	25 %	75 %	—
Present study	Overall fibre no. in sampled level of muscle			
	SDH	30 %	70 %	—
	ATPase (pH 9·4)	23·7 %	76·3 %	—

† FOG, fast-oxidative-glycolytic; SO, slow-oxidative; FG, fast-glycolytic. Classification terminology of Barnard, Edgerton & Peter (1970).

‡ Fibres possessing a particularly rich sarcoplasmic reticulum.

revealed that some Type IIA fibres, classified as such because of their high floccular ATPase activity, possessed the appearance of Type I fibres with SDH. Furthermore, some fibres classifiable as Type I because of low ATPase activity could be classified as Type IIA on account of their high SDH activity and peripheral accumulations of reaction product. Evidently Type IIA and Type I fibre classes are not homogeneous but are each divisible into two subclasses. Other subclasses of the basic fibre types have been demonstrated on quantitative analysis by Davies & Gunn (1972) in porcine muscle, and by Kugelberg (1973*a*), Schmalbruch & Kamieniecka (1975) and Pullen (1977) in the rat. Although Edgerton, Gerchman & Carrow (1969) and Guth & Yellin (1971) referred to variations in the combinations of enzyme activities in muscle fibres stained respectively for oxidative enzymes, phosphorylase and ATPase, no quantitative analyses were undertaken by these authors.

Unfortunately no previous accounts of the regional distribution of fibre types within the EDL muscle exist for comparison with the present observations. Overall fibre type proportions calculated from the combined data for both muscle axes

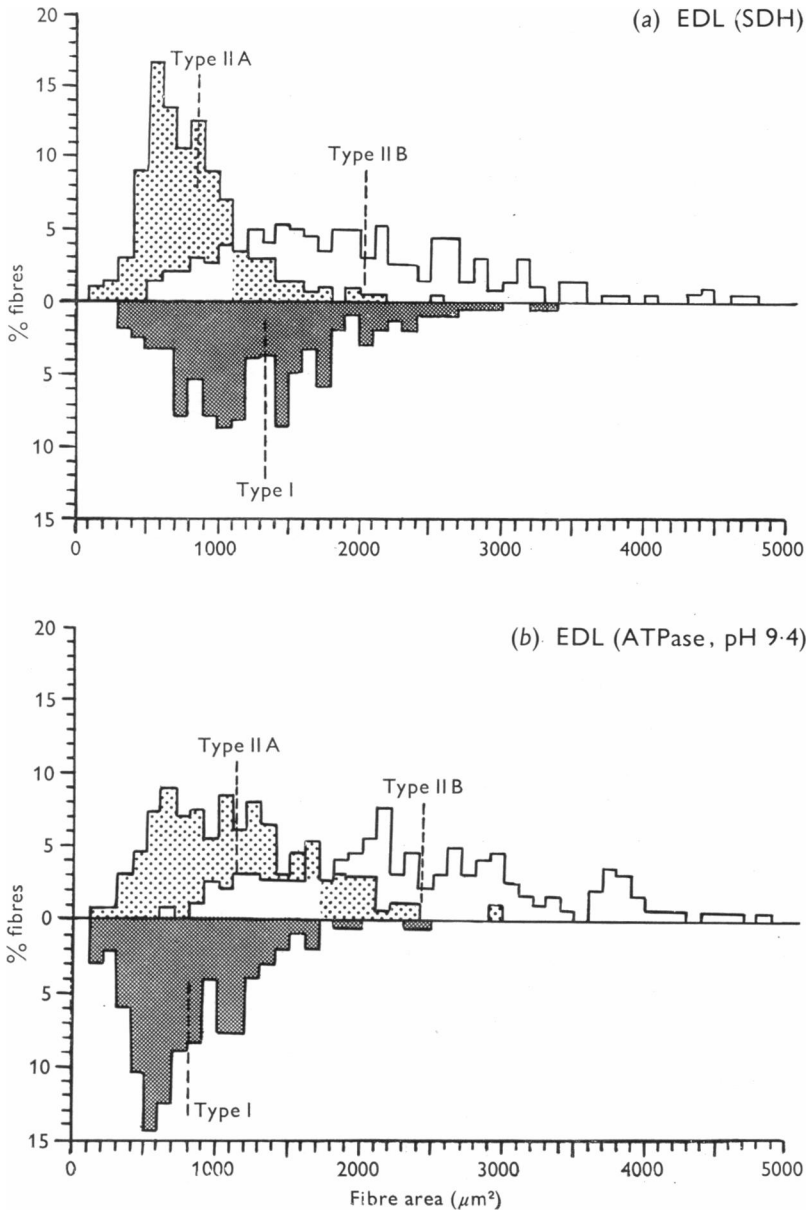

Fig. 3. Frequency histograms of fibre cross sectional areas for fibres classified according to their SDH (*a*) and ATPase (*b*) properties, and measured in the EDL muscle of the rat. While comparable ranges of size may be seen in either graph for Type II A and Type II B fibres, a marked difference in range of size occurs for Type I fibres.

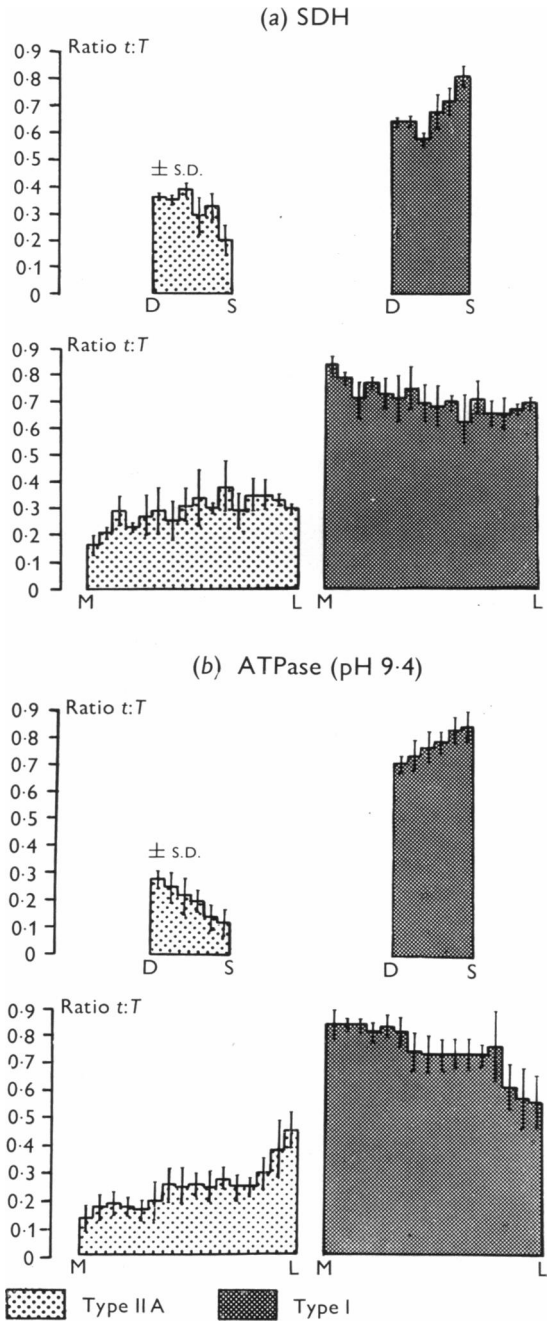

Fig. 4. Distribution profiles for fibres in the rat soleus muscle classified on the basis of their SDH (*a*) and ATPase, (*b*) properties. Fibre type proportions are shown for each fibre type along the deep to superficial (D–S) and the medial to lateral (M–L) axes of the muscle.

(i.e. deep to superficial and medial to lateral) indicate that while 32·7 % Type IIA, 34·3 % Type I and 33 % Type IIB fibres occurred in muscles stained for SDH, 51·2 % Type IIA. 39 % Type IIB but only 9·6 % Type I fibres occurred when classification was carried out using ATPase. Neither set of values agrees with previous estimates of fibre type composition for the EDL (Table 4). Although adult animals were studied in each case, Sprague–Dawley rats were studied by Edgerton & Simpson (1969, 1971) and Ariano *et al.* (1973), but Wistar rats were examined here and by Schiaffino *et al.* (1970). Despite possible variation in muscle composition between different strains of rat, the major causes of variation between the estimates given in Table 6 may be attributed to (i) differences between the methods of quantitative analysis used by the various authors, and (2) the calculation of mean fibre type proportions by combining data obtained from different enzyme preparations to classify myofibres. Thus in this study samples were taken along the major and minor axes of the muscles, and this method contrasts with others based on selected regions which may not be fully representative of the muscle as a whole. Discrepancies are inevitable between results based on oxidative enzyme and myosin ATPase classifications (at pH 9·4).

It is generally accepted that the soleus muscle of the rat contains two basic fibre types (Schiaffino *et al.* 1970; Gauthier, 1974), and the present findings agree with this. The predominant fibre type – variously called the 'B' (Stein & Padykula, 1962), Intermediate (Padykula & Gauthier, 1967; Edgerton & Simpson, 1969, 1971; Schiaffino *et al.* 1970; Gauthier, 1974), or Type I fibre (Brooke & Kaiser, 1970) – although histochemically similar to the corresponding fibre type in other muscles, possesses some atypical ultrastructural properties. The Z-line is often wider than in Intermediate fibres of other muscles (Gauthier, 1974), and Schiaffino *et al.* (1970) report the accumulation of branched mitochondria in subsarcolemmal regions. Although these latter features are typical of Type IIA fibres, the less well developed sarcoplasmic reticulum and the sparse transverse tubule system confirm that such fibres do indeed belong to the Intermediate fibre class (Schiaffino *et al.* 1970). The soleus muscle thus possesses a class of fibre peculiar to itself, and although it has distinct ultrastructural properties, its histochemistry is confusing. The soleus muscle does, however, possess some distinctive histochemical features. Although Kugelberg (1973*b*) and Karparti, Eisen & Carpenter (1975) employed acid preincubation techniques in conjunction with ATPase histochemistry, their observations are relevant to the quantitative data presented here. These authors showed that acid preincubation enabled at least three histochemically different classes of fibre to be distinguished (Types I, IIA and IIC). Furthermore, while three types were evident after preincubation at pH 4·3, Karparti *et al.* (1975) found that Type I fibres could be divided into two subclasses on the basis of differences in their staining after preincubation at pH 4·5. Although ATPase activity was totally inhibited in Type IA fibres when preincubation was carried out at pH 4·5, it was enhanced in Type IB fibres. Both subclasses appear to be contained within the Type I fibre population described by Kugelberg (1973*b*), for the properties of Type IIA and IIC fibres are similar in both reports. Type II fibres observed in sections stained for ATPase at pH 9·3 by Kugelberg (1973*b*) may be equated with the Type IIA fibres described in this study, while fibres exhibiting a moderate SDH, but low ATPase activity are classified as Type I in both studies. The relationship between the fibre classes given in

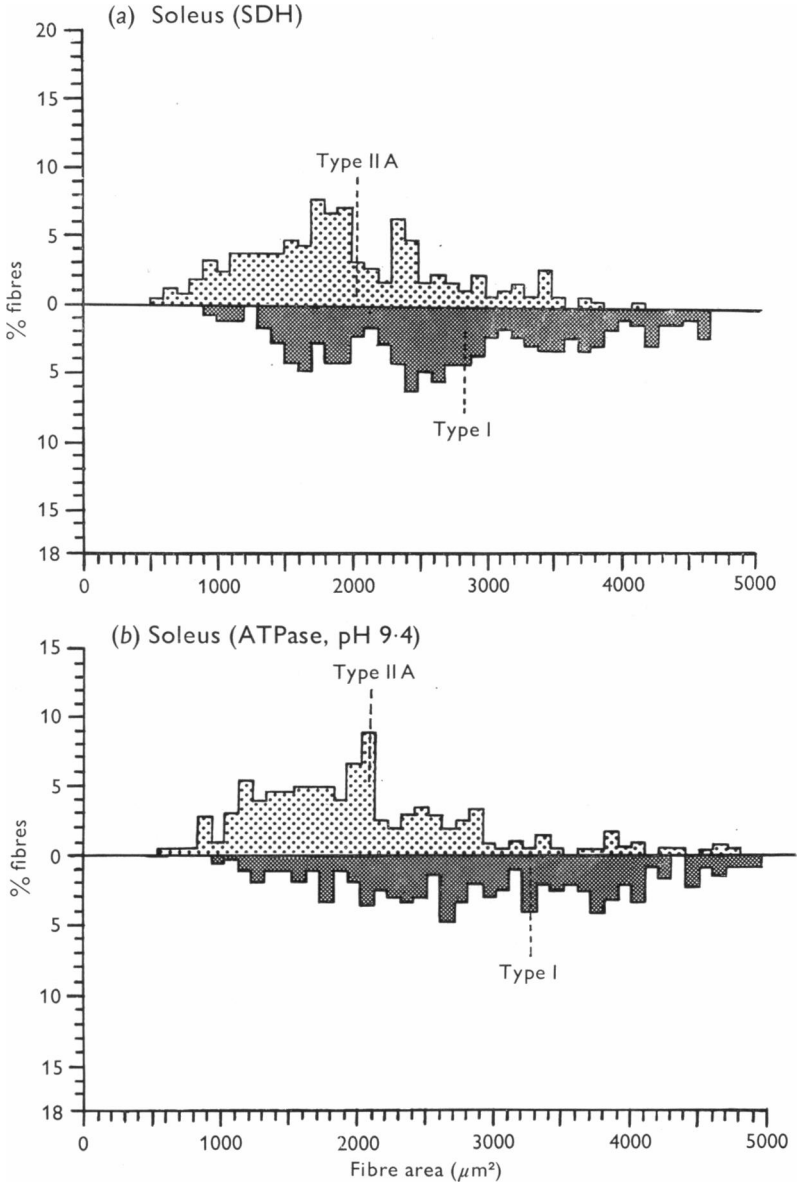

Fig. 5. Fibre cross sectional areas for fibre types in the rat soleus muscle measured in sections stained for SDH (*a*) and ATPase (*b*).

this study, and those defined by Karparti *et al.* (1975) is more difficult to assess, but it may be determined by reference to the published photographs in Karparti *et al.* The Type IB fibres illustrated there in Figure 1 would be classified within the Type II A fibre class of the present study since they contain a high floccular ATPase activity at pH 9·3–9·4. Type I fibres in this study are similar to the Type I A fibres described by Karparti *et al.* on the basis of their low ATPase activity at pH 9·4. Similar classifica-

tions occur if the fibres shown in Figure 1*c* of Karparti *et al.* are classified using the criteria applied in the present study to fibres stained for oxidative enzymes. Type IIC fibres were identified both by Kugelberg (1973*b*) and Karparti *et al.* (1975), but are not classified as a separate group here; they are probably included within the Type IIA fibres.

The quantitative results obtained for the soleus muscle are compared with those of other authors in Table 4. Again, no previous detailed analysis of fibre type distribution has been reported for the soleus. Indeed, it has been claimed that fibre types are randomly distributed in this muscle (Schiaffino *et al.* 1970) although Kugelberg (1973*b*) found that while Type I fibres predominated the medial regions, both Type I and Type IIA fibres were present within the lateral parts of the muscle. Considerably less variation occurs between estimates of fibre type proportions for the soleus than for either the EDL or tibialis anterior muscles. In the present study approximately 6 % more fibres were found to possess the appearance of Type IIA fibres with SDH than with ATPase, while an equivalent proportion of fibres were classified as Type I with ATPase, over and above the number similarly classified in sections stained for SDH. It is unlikely that this discrepancy arises from poor sampling since these proportions were calculated from the total number of fibres present in the muscle at a given level, and not from the number of fibres found within restricted regions along a muscle axis. Furthermore, the results based on an assessment of non-serial sections taken from different animals agree with those obtained from an independent study of individual fibres in serial sections.

Analyses of complete transverse sections of mammalian muscles have provided some useful information regarding fibre type populations in both adult and developing muscles (Pullen, 1974, 1977). It would be interesting to apply similar techniques to muscles undergoing temporary stress (e.g. temporary denervation with subsequent reinnervation, or excessive exercise) in order to elucidate further the capacity of muscle fibres to adapt their properties in response to changed conditions.

SUMMARY

Histochemical fibre types classified in sections stained for succinic dehydrogenase (SDH) and myosin ATPase at pH 9·4 were found to be distributed in a consistent manner within the extensor digitorum longus (EDL) and soleus muscles of the adult rat. Simple morphometric techniques applied to complete transverse sections of both muscles showed that the relative distributions and proportions of fibre types along their deep to superficial, and medial to lateral, axes varied according to the histochemical method used for fibre typing. Similar differences occurred when the relative ranges of size exhibited by each fibre type were compared in sections stained for SDH and ATPase, and the discrepancies in fibre classification were confirmed by an analysis of individual fibres in serial sections. The findings are discussed in relation to those previously reported for the EDL and soleus muscles of the rat.

The author wishes to express his appreciation to Professor J. B. Cavanagh for the facilities made available in the MRC Research Group in Applied Neurobiology during this study, and to Dr D. N. Landon for his valuable guidance during preparation of the manuscript.

REFERENCES

AHERNE, W. (1968). A method for determining the cross-sectional area of muscle fibres. *Journal of the Neurological Sciences* **7**, 519–528.

ARIANO, M. A., ARMSTRONG, R. B. & EDGERTON, V. R.. (1973). Hindlimb muscle fiber populations of five mammals. *The Journal of Histochemistry and Cytochemistry* **21**, 51–55.

BAILEY, N. T. J. (1959). *Statistical Methods in Biology*. London: English Universities Press.

BARNARD, R. J., EDGERTON, V. R. & PETER, J. B. (1970). Effect of exercise on skeletal muscle. 1 Biochemical and histochemical properties. *Journal of Applied Physiology* **28**, 762–766.

BROOKE, M. H. & KAISER, K. K. (1970). Muscle fiber types–how many and what kind? *Archives of Neurology* **23**, 369–379.

CLOSE, R. I. (1972) Dynamic properties of skeletal muscle. *Physiological Reviews* **52**, 129–197.

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

DUBOWITZ, V. & BROOKE, M. H. (1973). *Muscle Biopsy: A Modern Approach. Problems in Neurology* Series, no. 2. Philadelphia: Saunders.

EDGERTON, V. R. & SIMPSON, D. R. (1969). The intermediate fiber of rats and guinea-pigs. *Journal of Histochemistry and Cytochemistry* **17**, 828–838.

EDGERTON, V. R. & SIMPSON, D. R. (1971). Dynamic and metabolic relationships in the rat extensor digitorum longus muscle. *Experimental Neurology* **30**, 374–376.

EDGERTON, V. R., GERCHMAN, L. & CARROW, R. (1969). Histochemical changes in rat skeletal muscle after exercise. *Experimental Neurology* **24**, 110–123.

GAUTHIER, G. & PADYKULA, H. A. (1966). Cytochemical studies of fiber types in skeletal muscle: a comparative study of the mammalian diaphragm. *Journal of Cell Biology* **28**, 333–354.

GAUTHIER, G. F. (1974). Some ultrastructural and cytochemical features of fiber populations in the soleus muscle. *Anatomical Record* **180**, 551–564.

GUTH, L. & SAMAHA, F. J. (1969). Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. *Experimental Neurology* **25**, 138–152.

GUTH, L. & YELLIN, H. (1971). The dynamic nature of the so-called 'fiber types' of mammalian skeletal muscle *Experimental Neurology* **31**, 227–300.

KARPARTI, G. & ENGEL, W. K. (1967) Neuronal trophic function: a new aspect demonstrated histochemically in developing soleus muscle. *Archives of Neurology* **17**, 542–545.

KARPARTI, G., EISEN, A. A. & CARPENTER, S. (1975). Subtypes of the histochemical Type I muscle fibers. *The Journal of Histochemistry and Cytochemistry* **23**, 89–91.

KUGELBERG, E. (1973*a*). Properties of rat hindlimb motor units. In *New Developments in Electromyography and Clinical Neurophysiology*, vol. 1 (ed. J. E. Desmedt), pp. 2–13. Basle: Karger.

KUGELBERG, E. (1973*b*). Histochemical composition, contraction speed and fatiguability of rat soleus motor units. *Journal of the Neurological Sciences* **20**, 177–198.

MOLINE, S. W. & GLENNER, G. C. (1964). Ultrarapid freezing of tissues in liquid nitrogen. *Journal of Histochemistry* **12**, 777–783.

PADYKULA, H. A. & GAUTHIER, G. F. (1967). Morphological and cytochemical characteristics of fibre types in normal mammalian skeletal muscle. In *Exploratory Concepts in Muscular Dystrophy and Related Processes* (ed. A. T. Milhorat), pp. 117–128. Amsterdam: Excerpta Medica Foundation International Congress Series, no. 147.

PEARSE, A. G. E. (1968). *Histochemistry – Theoretical and Applied* 3rd ed., vol. 1. London: Churchill.

PULLEN, A. H. (1974). Quantitative analysis of myofibre differentiation in the rat. *Third International Congress on Muscle Diseases*, Newcastle-Upon-Tyne. Abstract 379, p. 163. Amsterdam: Excerpta Medica Foundation International Congress Series, no. 334.

PULLEN, A. H. (1977). The distribution and relative sizes of three histochemical fibre types in the rat tibialis anterior muscle. *Journal of Anatomy* **123**, 1–19.

SAUNDERS, L. & FLEMING, R. (1972). *Mathematics and Statistics – for Use in the Pharmaceutical Sciences*. London: Pharmaceutical Press.

SCHIAFFINO, S., HANZLIKOVA, V. & PIEROBON, S. (1970). Relations between structure and function in rat skeletal muscle fibers. *Journal of Cell Biology* **47**, 107–119.

SCHMALBRUCH, H. & KAMIENIECKA, Z. (1975). Histochemical fiber typing and staining intensity in cat and rat muscles. *Journal of Histochemistry and Cytochemistry* **23**, 395–401.

STEIN, J. M. & PADYKULA, H. A. (1962). Histochemical classification of individual skeletal muscle fibers of the rat. *American Journal of Anatomy* **110**, 103–124.