J. Physiol. (1979), **296**, *pp.* 453–469
With 2 plates and 5 text-figures
Printed in Great Britain

CHANGES IN RODENT MUSCLE FIBRE TYPES DURING POST-NATAL GROWTH, UNDERNUTRITION AND EXERCISE

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(*Received 24 April 1978*)

SUMMARY

1. Using histochemical staining methods for myosin ATPase oxidative and glycolytic enzymes, three major types of muscle fibre could be identified in the skeletal muscle of hamsters and mice.

2. Muscle fibre counts showed that the proportions of the different fibres were not entirely stable with age. In the hamster biceps brachii which is predominantly composed of ATPase-high fibres there was a decrease in the number of ATPase-low fibres. In the soleus muscle which is predominantly composed of ATPase-low fibres there was a decrease in ATPase-high fibres with age.

3. Although there was a change in the proportions of fibre types there was no change in the total number of fibres within the muscles with age. It is suggested that some reinnervation may take place during growth and that this is why the less dedominant fibre type decreases.

4. The response of the different fibre types to partial starvation was studied. The ATPase-high fibres showed the greatest decrease in size. Of these, the ATPase-high glycolytic type responded more than the ATPase-high oxidative type. The effects of the under-nutrition on the different fibre types were found to be completely reversible. Starvation did not affect the total number of fibres or the numbers of any fibre type.

5. The response of the different fibre types to high intensity exercise (weight lifting) was studied. This type of exercise resulted in hypertrophy of all three major fibre types. However, the extent of the response varied according to the fibre type and the exact nature of the exercise. In most cases the ATPase-high fibres underwent hypertrophy more readily than the ATPase-low fibres. Where distinction was made between the two types of ATPase-high fibres, the ATPase-high glycolytic were found to hypertrophy more than the ATPase-high oxidative fibres. The effects of post exercise recovery (return to relative inactivity) were also studied and the changes in size of the fibres were found to be completely reversible.

INTRODUCTION

Skeletal muscle undergoes profound and rapid changes in the composition of its contractile, regulatory and energy yielding systems during growth, particularly just before birth and during early post-natal life (Goldspink, 1962; Goodfriend & Kaplan,

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0022-3751/79/5680-0418 $01.50

1964; Kendrick-Jones & Perry, 1967; Perry, 1970). For instance, there is good evidence that the myosin associated with fetal skeletal muscle is different enzymatically, immunochemically and in primary structure from its adult counterpart (Perry, 1970). However, differences in the myosin molecule can be demonstrated in mature muscle. Indeed, it is apparent that most mature muscles are not homogenous and that they consist of two or three different populations of fibres. Histochemical methods are particularly useful for distinguishing between the different types of fibres, especially the methods for myosin ATPase which when used with certain preincubation treatments can selectively stain the different types of myosins. The methods for oxidative enzymes are also useful as they can give information about the predominant metabolism of the individual fibres.

The fibre types in mature muscle can be described as slow twitch oxidative fibres, fast twitch glycolytic fibres and fast twitch oxidative-glycolytic fibres (Peter, Barnard, Edgerton, Gillespie & Stempel, 1970). Studies on the recruitment of muscle fibres during different types of activity (Gollnick, Piehl & Saltin, 1974*c*), and on the chemical energy utilization during different kinds of contraction (Goldspink, 1977) suggest that these types of fibres have different physiological roles. As the physiological properties of a muscle are dependent upon the number, size and type of its constituent fibres, it is important that we understand the post-natal differentiation of the different types and the factors that influence their development.

Muscle fibre size is known to be affected by the level of nutrition (Robertson & Baker, 1933; Joubert, 1964, 1965; Hagen & Scow, 1957; Goldspink, 1964, 1965; Rowe, 1968). Although a considerable amount of work has been carried out on the effects of starvation on muscle fibre size, virtually no attention has been paid to the responses of the different types of fibres within mixed muscle. Thus, in addition to studying the post-natal differentiation in the biceps brachii and soleus muscles the effects of undernutrition on the muscle fibre types were also examined.

Muscle undergoes changes with increased work-load and in some cases this involves muscle fibre hypertrophy (Goldspink, 1964; Gordon, Kowalski & Fritts, 1967), while decreased work may result in muscle fibre atrophy (e.g. Cooper, 1972; van der Meulen, 1974).

Hypertrophy of skeletal muscle fibres in response to increased work-load was described by Morpurgo in 1897 and since then a number of investigators have reported work-induced hypertrophy with a variety of animals and exercise regimens (Goldspink, 1964; Walker, 1966; Gordon, 1967; Kowalski, Gordon, Martinez & Adamek, 1969; Edgerton, Barnard, Peter, Gillespie & Simpson, 1972; Muller, 1975; Gonyea & Ericson, 1976). Although there have been a number of studies reported on the responses of different muscle fibre types to endurance exercise in laboratory animals, very little is known about their response to high resistance exercise. In view of this, it was decided to employ an exercise regimen (weight lifting) which was designed to induce increased muscle mass and strength rather than increased muscle stamina or endurance. The effect of post-exercise recovery (return to relative inactivity) has also been examined.

METHODS

Post-natal development

Animals. Albino hamsters of the CA strain and mice of the Re 129 strain were used.

As well as tracing the early post-natal histochemical differentiation of skeletal muscle fibre, measurements were made from the biceps brachii and soleus muscles of male and female animals at other post-natal ages. In hamsters this was done at a number of stages over a period ranging from 1 to 140 days (in males only) and in mice at 21 days, 140 days and 240 days (in males and females).

Effect of a low plane of nutrition

Animals. Male and female hamsters of the CA strain used for these experiments were selected at 30 and 50 days of age from a number of litters and matched for body weight with controls. Groups of male and female mice of the Re 129 strain were selected at 90 days of age from within a breeding colony and those to be used for the experiment were matched for body weight with control animals. Litter-mates were distributed evenly between the experimental and control groups for each experiment.

General procedure. Animals were placed singly in cages at commencement of the experiment and were transferred from a normal *ad libitum* diet, of Pilsbury's Rat and Mouse Pellets (approximately 21 % protein and 3875 kcal/kg), to a reduced food intake of the same diet. The hamsters received approximately 2·5–3·0 g per day and were maintained at this level of food intake for a period of 42 days. The mice received approximately 3·5–4 g per day. The amount of food required to maintain body weight was 5·0 g and 6·0 g for hamster and mice respectively. In one group of mice (male only) the period of inanition was 21 days whilst in the other two groups of mice (males and females) it was extended to 59 days. Control animals were fed the standard diet *ad libitum* during the period of the experiments. Water was available *ad libitum* to all animals.

A group of male hamsters was allowed to recover from the period of low food intake. In this case, food was available *ad libitum* for 30 days following the low plane of nutrition. Animals were weighed each week throughout the period of experiment. They were killed by cervical dislocation and their biceps brachii and soleus muscles removed, weighed and prepared for sectioning on the cryostat.

Exercise experiments

Animals. Male albino hamsters of the CA strain and mice of the Re 129 strain were used. Four groups of hamsters and one group of mice were exercised. Details of the age of animals, duration of the exercise and the weight lifted, are given with the results. The animals of the control groups were matched in pairs for body weight with those in the experimental groups.

Exercise regimen. The weight lifting procedure employed in this study was similar to that developed by Goldspink (1964). With this system, the animal has to pull down a counterweighted basket in order to obtain food. The number of pulls is recorded by an electrical counter attached to the pulley. In this series of experiments the static component of the exercise was increased by using a narrow mesh in the food basket, hence the animal has to hold the basket down for a considerable time in order to nibble the food pellets.

Animals were acclimatized to the exercise cages for a week and initially taught how to obtain their food by using a small counterweight on the pulley. After the initial period of acclimatization, the load on the pulley was increased to the level of the formal exercise regimen. The exercise was regulated so that about the same total amount of work in joules was performed by each exercising animal. That is to say, those pulling half body weight were required to pull more times than those pulling full body weight. The extent of the downward movement of the counter-weighted food basket was restricted so that it could be pulled and held down at about shoulder height. This allowed the exercising animal to obtain food while standing on its hind limbs. Water was provided *ad libitum* throughout.

Identification and measurement of muscle fibre types. After the muscles were excised they were placed in a covered Petri-dish which was kept on ice, for approximately 30 min. They were subsequently arranged on microtome chucks so that experimental and control muscle were together in the same block. Liver tissue was used to pack the block so that the muscles remained upright.

The blocks were rapidly frozen by immersion in Freon (Arcton 12, I.C.I.) cooled to about −150 °C, and transferred to a refrigerated chamber at −20 °C. Serial sections were cut at 10 μm in a cryostat (Bright Co. Ltd.) in which the temperature was maintained between −15 and −20 °C. Sections were mounted on coverslips and air-dried for approximately 30 min before staining for a variety of enzyme reactions. These included NADH-tetrazolium reductase (Dubowitz & Brooke, 1973, after Farber, Sternberg & Dunlop, 1956), succinic dehydrogenase (Nachlas, Tsou, De Souza, Cheng & Seligman, 1957); menadione-linked α-glycerophosphate dehydrogenase (Dubowitz & Brooke, 1973, after Wattenberg & Leong, 1960); phosphorylase (Dubowitz & Brooke, 1973, after Eränkö & Palkama, 1961); and myosin ATPase. The myosin ATPase activity of muscle fibres was demonstrated at pH 9·4 with modifications of the classical method of Padykula & Herman (1955) and modified by Guth & Samaha (1970), Brooke & Kaiser (1970) and Hayashi & Freiman (1966). This method involves preincubation treatment with acid or alkaline buffers or fixation in buffered paraformaldehyde solution. In addition to the enzymes listed above, serial sections were also examined using the following substrate stains: glycogen, by the periodic acid–Schiff method (PAS; McManus & Mowry, 1960) and lipid with Sudan Black B (Dubowitz & Brooke, 1973).

Morphometry. Data on total muscle fibre number and fibre diameter were obtained from sections stained for myosin ATPase and correlated with information from other histochemical stains. Using a Leitz micro-projector, counts of the number of the different kinds of muscle fibres were made and recorded with an electrically operated pen counter. Previous work has shown that sections taken from the middle of the belly of the soleus and biceps brachii of the hamster contain all the fibres of those muscles. Muscle fibre sizes were measured with an eyepiece micrometer fitted to the microscope. The mean of two measurements at right angles to one another was used for each fibre. Where possible, 100 or more fibres of each type were measured for each muscle. These were sampled using equidistant sample lines, superimposed upon the muscle cross-section, thus giving an unbiased selection.

Statistical analysis. The mean and standard errors of muscle fibre diameter data were evaluated and compared using the Student's *t* test. In addition a test of independence of the distributions of muscle fibre size between control and experimental groups were compared by means of the G test (a test of association) according to Sokal & Rolf (1969).

RESULTS

Identification of muscle fibre types in mature muscle

For the purpose of this study, three types of muscle fibre were recognized:

(i) a fibre type possessing a myosin ATPase which is stable to alkaline pre-treatment and which possesses a moderate to low oxidative capacity, as indicated by the NAD tetrazolium reductase and succinic dehydrogenase staining, and a moderate to high glycolytic capacity as measured by the α-glycerophosphate dehydrogenase α-GPD staining.

(ii) a fibre type possessing similar, though not identical, stability to alkaline pre-treatment as the previously mentioned fibre type, and which possesses a relatively high oxidative capacity and a relatively low glycolytic capacity.

(iii) a fibre type possessing a myosin ATPase which is inactivated by alkaline pre-treatment (its myosin ATPase being stable to acid pretreatment, while that of the other two fibre types is much less stable in this respect). In addition, this third type possesses a moderate to high oxidative capacity and a low to moderate glycolytic capacity.

These three major categories of muscle fibre, distinguishable in the limb musculature of laboratory mammals, have been described elsewhere in the literature, e.g. Burke, Levine, Zajac, Tsairis & Engel (1971); Peter *et al.* (1970); Brook & Kaiser (1974). By virtue of their alkaline stability, the first two of the above mentioned

fibre types are denoted 'ATPase-high' in the text to follow. The third fibre type is denoted 'ATPase-low', following its inactivation after exposure to alkaline solution and consequent reaction product staining.

The histochemical profiles of the major categories of muscle fibre identified in the hamster and mouse species in this study are summarized in Table 1. From this Table it will be seen that in the mouse, the ATPase-high oxidative-glycolytic fibres (fast oxidative-glycolytic) have a higher alkaline stability than the ATPase-high glycolytic fibres (fast glycolytic). In our hands the distinction between the subtypes of the ATPase-high fibre category in terms of differential myosin ATPase staining was more difficult in the hamster biceps brachii muscle at different ages than in the same muscle in the mouse. However, it was evident that in the mouse biceps brachii the alkaline stability of the fast twitch glycolytic and the oxidative-glycolytic fibres was the converse of that in the biceps brachii in the hamster (Table 1).

TABLE 1. Histochemical profiles of the major fibre types in the biceps brachii and soleus muscles of hamster (Coomehurst Albino) and mouse (Re 129)

		Myosin-ATPase				
Species	Fibre type	Alkali pH 10·4	Acid pH 4·6	Acid pH 4·3	NADH-diaphorase	(M-) α-GPD
Mouse	FG	+ +	+ + +	+	+	+ + +
	FOG	+ + +	+	+	+ + +	+ +
	SO	+	+ + + +	+ + + +	+ +	+
Hamster	FG	+ + +	+ + +	+	+	+ + +
	FOG	+ +	+	+	+ + +	+ +
	SO	+	+ + + +	+ + + +	+	+

The three types of muscle fibre described in this Table are the major categories represented in the limb musculature of mammals; the existence of variants of these major types is recognized, but has been ignored in this simplified approach.

FG: fast glycolytic; FOG: fast oxidative-glycolytic; SO: slow oxidative.

Post-natal development

With careful staining for myosin ATPase two main populations of fibres of differing pH and prefixation stability could be identified in the skeletal muscles of fore and hind limbs of new-born mice (Pl. 1). Such a distinction was difficult in new-born hamster muscle, although at 24–36 hr post-partum, fibre type differentiation was readily demonstrable. After this time the distinction between the two major fibre types became increasingly clear using myosin ATPase staining, but only by the end of the second week of life could fibre be distinguished from one another using oxidative and glycolytic enzyme staining. The three major skeletal muscle fibre types could be distinguished from one another in the hamster and mouse on the basis of their staining for myosin ATPase at about 3 weeks after birth. This distinction was assisted most by the α-glycerophosphate dehydrogenase and NADH tetrazolium reductase reactions (see Table 1).

The quantitative information for muscle fibre types during growth is presented in Figs. 1 and 2. These show the proportions of the different fibre types within skeletal muscles are not entirely stable with age. In the hamster biceps brachii muscle, which

is predominantly composed of ATPase-high fibres (fast glycolytic and fast oxidative-glycolytic), there is a tendency for the number of ATPase-low (slow oxidative) fibres to decrease with age. On the other hand, in the hamster soleus muscle, which is composed predominantly of ATPase-low (slow oxidative) fibres, there is a tendency for the number of ATPase-high (fast oxidative-glycolytic) fibres to decrease with a corresponding increase in the number of ATPase-low fibres (Fig. 1).

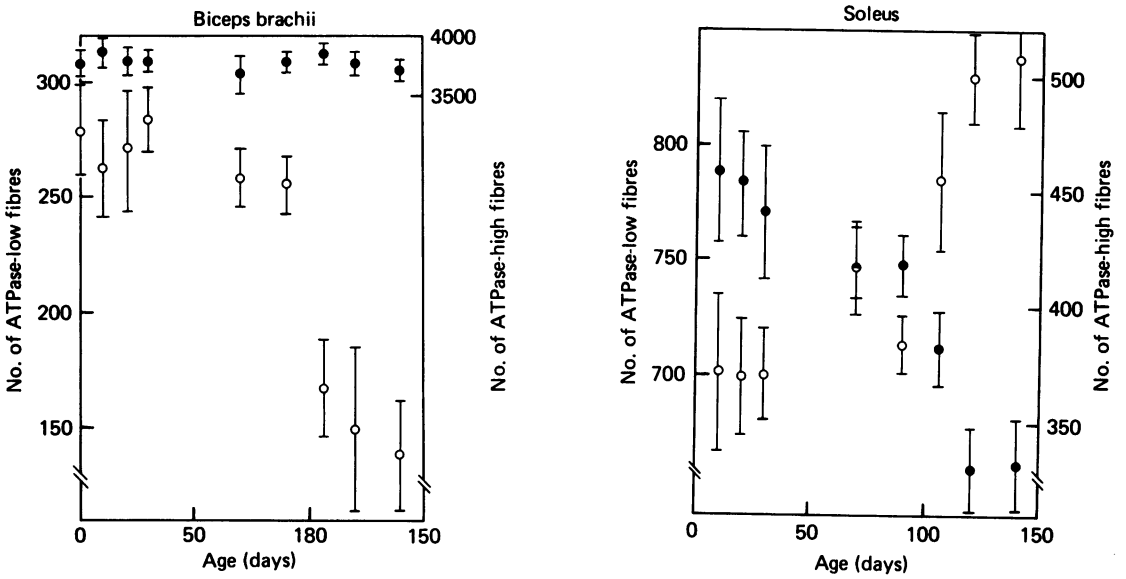


Fig. 1. The number of ATPase-low fibres (○, left vertical axis) and the number of ATPase-high fibres (●, right vertical axis) in the hamster biceps brachii muscle (left) and the soleus muscle (right) with growth. The ATPase-high fibres include both oxidative-glycolytic and glycolytic fibres. Each point is the mean of counts taken from ten animals. The standard errors are given on each side of each mean.

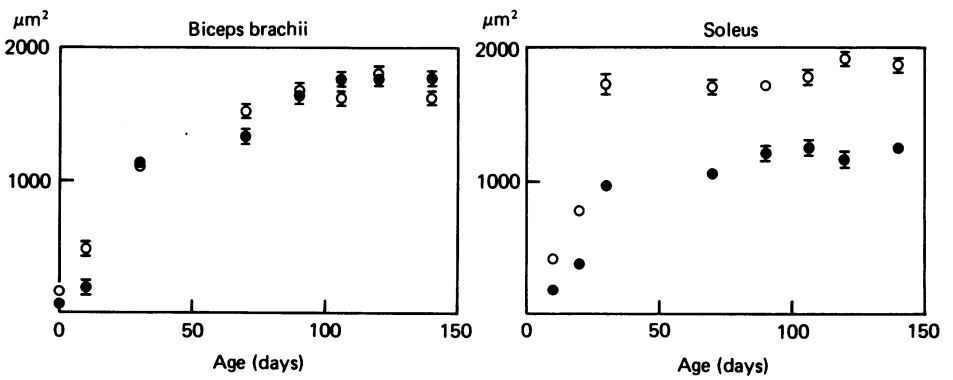


Fig. 2. The mean cross-sectional area of the ATPase fibres (○) and the number of ATPase-high fibres (●) in the hamster biceps brachii (left) and soleus (right) with growth. Each point is the mean of measurements made on ten animals with the standard errors given on each side of the points except when they were too small to plot.

Changes in the mean cross-sectional area of the different fibres types with growth are shown in Fig. 2. From this it will be seen that in the hamster biceps brachii the ATPase-high and ATPase-low fibres increased in size to about the same extent. In the soleus the ATPase-low fibres grew more than the ATPase-high fibres.

Effect of undernutrition

The undernutrition regimen used in these experiments resulted in a reduction of body weight, of approximately 20 %, in both hamsters and mice. The muscle weights were also considerably reduced; for the biceps brachii the reduction was of the order of 24–32 %, while for the soleus it was of the order of 10–20 %.

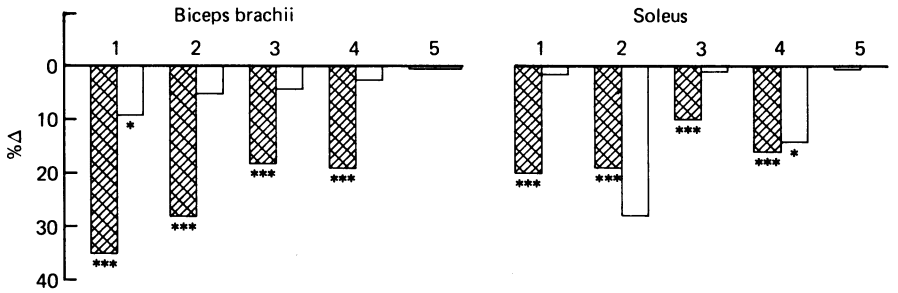


Fig. 3. The change in the mean cross-sectional area of the different fibre types following undernutrition for the hamster biceps brachii and soleus. Group 1: 30-day male hamsters, 42 days undernutrition. Group 2: 30-day female hamsters, 42 days undernutrition. Group 3: 50-day male hamsters, 42 days undernutrition. Group 4: 50-day female hamsters, 42 days undernutrition. Group 5: 50-day male hamsters, 42 days undernutrition followed by 30 days recovery (food *ad libitum*). Each group contained five animals. Statistical significance $***P < 0{\cdot}001$, $*P < 0{\cdot}05$, n.s., no significant difference. ▨, ATPase high fibres (fast-twitch oxidative-glycolytic and glycolytic fibres); □, ATPase low fibres (slow twitch oxidative fibres).

The effect of undernutrition on muscle fibre number is shown in Table 2 for the hamster muscles. As will be seen from these tables, neither the number of the different kinds of muscle fibre nor the total number of fibres was affected by the period of undernutrition.

The results concerning the change in size of the different kinds of fibre are shown in Fig. 3 for the hamster muscles and Fig. 5 for the mouse muscles. It will be seen from Figs. 3 and 5 that the reduction in the cross-sectional area of the ATPase-high fibres was much greater than that of the ATPase-low fibres. This was true for the soleus as well as the biceps brachii muscle, indeed in most cases there was not significant change in the size of the ATPase-low fibres. In the mouse biceps brachii muscle which contains few if any ATPase-low fibres, the ATPase-high (fast glycolytic) fibres were reduced in area to a greater extent than the ATPase-high (fast oxidative-glycolytic) fibres (Fig. 5).

The hamster data in Table 2 on growth and in Fig. 3 concerning the effects of undernutrition was not extended to the two subtypes of ATPase-high fibres because of the initial difficulties distinguishing between the fast glycolytic and the fast oxidative-glycolytic fibres (oxidative enzyme levels are reduced in starvation,

TABLE 2. The number of fibres of different types in the m. biceps brachii and m. soleus of male and female hamsters subject to a low plane of nutrition, compared with normally fed animals

Details and treatment				Biceps brachii			Soleus		
Group	Age (days) sex	Starved (days)	Recovery (days)	ATPase high	ATPase low	Total	ATPase high	ATPase low	Total
1	30 M	42	None	3755 ± 28	280 ± 6	4035 ± 32	439 ± 14	715 ± 7	1154 ± 12
Control	30	None	None	3802 ± 98	273 ± 8	4075 ± 106	470 ± 6	707 ± 9	1178 ± 3
2	30 F	42	None	3567 ± 74	186 ± 38	3753 ± 71 *	334 ± 42	639 ± 32	973 ± 48
Control	30	None	None	3666 ± 28	273 ± 11	3939 ± 36	381 ± 7	680 ± 11	1066 ± 6
3	50 M	42	None	3747 ± 48	241 ± 8	3988 ± 44	446 ± 20	686 ± 28	1131 ± 22
Control	50	None	None	3723 ± 29	241 ± 17	3964 ± 27	458 ± 16	693 ± 13	1151 ± 6
4	50 F	42	None	3558 ± 50	230 ± 8	3789 ± 50	359 ± 11	679 ± 7	1038 ± 14
Control	50	None	None	3564 ± 51	236 ± 8	3800 ± 52	367 ± 8	687 ± 6	1054 ± 3
5	50 M	42	30	3665 ± 49	227 ± 7	3892 ± 42	372 ± 14	780 ± 30	1152 ± 24
Control	50	None	None	3700 ± 39	229 ± 5	3929 ± 38	379 ± 22	784 ± 10	1162 ± 10

1. Each value is the mean and S.E. from five muscles.
2. Statistical significance (Student's t test) $*P < 0{\cdot}05$; the differences between control and experimental.

TABLE 3. Showing the total numbers of muscle fibres and the numbers of the different types of fibres in the biceps brachii and soleus muscle of exercised and non-exercised hamsters

				Biceps brachii				Soleus		
	Training		Recovery	ATPase-high		ATPase-low				
Group	Days	Weight	(days)	FG	FOG	SO	Total	ATPase-high	ATPase-low	Total
1	60	½	None	1655 ± 73	2131 ± 70	271 ± 9	4057 ± 76	404 ± 17	724 ± 17	1128 ± 25
2	60	Full	None	3865 ± 67		236 ± 16	4102 ± 79	375 ± 19	727 ± 13	1102 ± 33
Control	—	—	None	1752 ± 52	2016 ± 37	271 ± 13	4039 ± 13	418 ± 13	713 ± 12	1131 ± 25
3	38	½	None	1733 ± 87	1921 ± 64	255 ± 9	3810 ± 128	401 ± 18	740 ± 12	1141 ± 20
Control	—	—	None	1711 ± 41	2049 ± 122	276 ± 12	4036 ± 164	419 ± 15	749 ± 21	1168 ± 35
4	38	½	38	3751 ± 125		101* ± 10	3852 ± 98	358 ± 17	788 ± 9	1146 ± 24
Control	—	—	—	3848 ± 106		167 ± 21	4015 ± 95	383 ± 16	785 ± 31	1168 ± 43

* Significance $P < 0{\cdot}05$, all other values not significant from controls.

Goldspink & Waterson, 1971) in sufficient numbers to give statistically valid results. In the exercise studies it was somewhat easier to make the distinction between fast glycolytic and the fast oxidative-glycolytic, however, because of the time involved in comparing serial sections; this was only carried out for certain representative groups.

In the hamsters (group 5, Fig. 3) allowed to feed *ad libitum* after the low plane of nutrition, it was found that the muscles had completely recovered within the time period used (Fig. 3).

Effects of weightlifting exercise

Data for the number of the different fibre types in exercised and control biceps brachii and soleus muscles of both hamster and mice are summarized in Table 3. Data for the change in mean cross sectional areas of the fibre types are given in Fig. 4 for the hamster experiments and in Fig. 5 for the mouse experiments.

Total muscle fibre number. The total fibre number of the biceps brachii and soleus muscles did not change as a result of the exercise treatment. There was, however, a decreased number of ATPase-low fibres in the biceps brachii muscle of animals of group 4, i.e. muscles that were allowed to recover from the effects of exercise. This result, which may have been fortuitous, is discussed later. In the case of the mouse muscles there was no change in the total number of fibres or the number of the different fibre types with exercise. As the data were essentially the same for the hamsters they are not presented.

Muscle fibre size. There was a significant hypertrophy in both the ATPase-high and the ATPase-low fibres of the biceps brachii and soleus muscles of all exercised groups with the exception of the ATPase-low fibre category of the soleus muscle of group 3 animals. For reasons which are not known, the variation in this latter muscle fibre category was large and the mean fibre diameter was lower than that of the control group. In the group that was allowed to recover from the exercise, the cross-sectional area of both ATPase-high and ATPase-low muscle fibres returned to the control levels and even decreased below that of the controls. This was more marked in the ATPase-high fibres.

In group 1 and group 3 animals, in which the distinction was made between the two categories of ATPase-high fibre in the biceps brachii muscle, the fast glycolytic fibres hypertrophied to a greater extent than the fast oxidative glycolytic fibres.

The longer duration of exercise experienced by group 1 animals compared to group 3 animals was reflected in the greater hypertrophy of the three muscle fibre-types in the biceps brachii muscle of the former group. This trend was also apparent in the soleus muscle, except for the inconsistent response of the ATPase-low fibres of group 3 animals.

In the biceps brachii and soleus muscles of group 2 animals made to pull against their full body weight, the heavier load produced a greater response of the ATPase-low fibres as compared with that shown by this fibre type in group 1 animals. The increase in the cross-sectional area of the ATPase-high fibres in these two muscles did not however follow this pattern. Not only did this latter fibre type hypertrophy to a lesser extent than the ATPase-low fibre in group 2 animals, it also hypertrophied less than the ATPase-high fibre in group 1 animals.

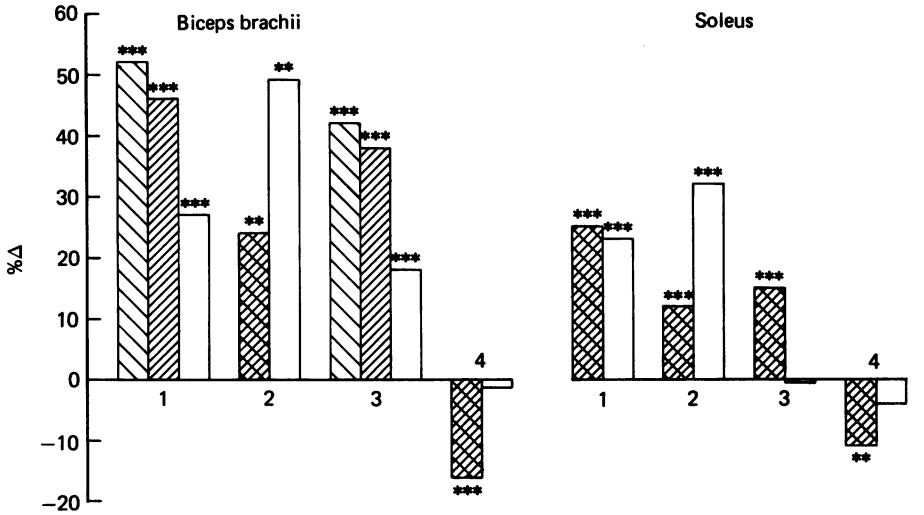


Fig. 4. The change in the cross-sectional area of the fibres of different types in exercised (E) and control (C) muscles of hamsters. □, Slow oxidative fibres; ▨, fast glycolytic fibres; ▨, fast oxidative-glycolytic fibres; ▩, both fast glycolytic and fast oxidative-glycolytic fibres. Group 1: exercised for 60 days pulling half body weight. Group 2: exercised for 60 days pulling full body weight. Group 3: exercised for 38 days pulling half body weight. Group 4: exercised for 38 days pulling half body weight and allowed to rest (recover) for 38 days. Each group contained at least five animals. $***P < 0{\cdot}001$; $**P < 0{\cdot}01$; $*P < 0{\cdot}5$.

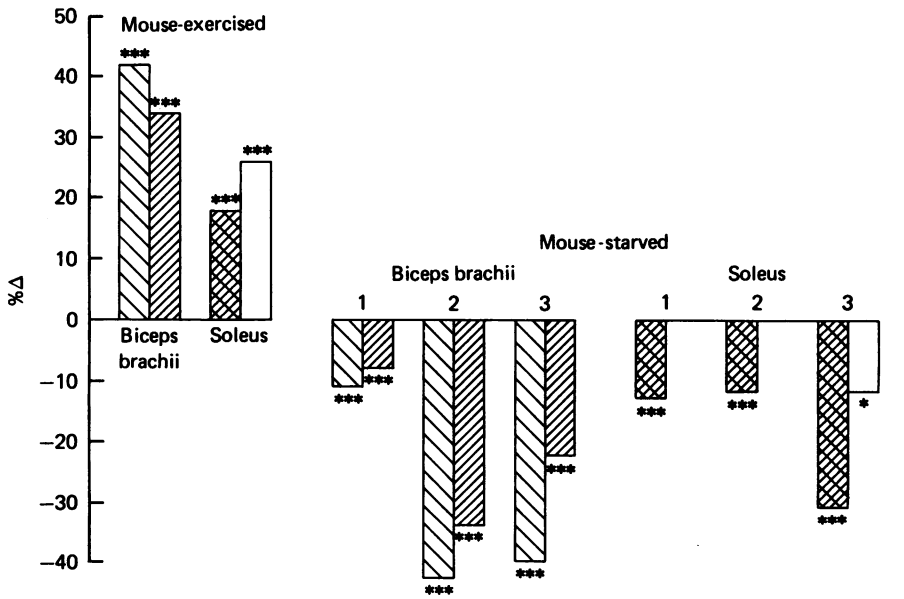


Fig. 5. The change in the cross-sectional area of the different types of fibres in the biceps brachii and soleus of mice that have been exercised or starved. Group 1: 90-day-old male mice, 21 days restricted food. Group 2: 90-day-old male mice, 59 days restricted food. Group 3: 90-day-old female mice, 59 days restricted food. The slow oxidative fibres of groups 1 and 2 showed no change in size.

The data from the mouse experiments are shown in Fig. 5. The cross-sectional areas of all the major fibre types distinguished in the biceps brachii and soleus muscles of mice were increased by the exercise stimulus. In the biceps brachii muscle, those showing the greatest hypertrophy were the fast glycolytic fibres, whilst the fast oxidative glycolytic fibres hypertrophied to a lesser extent. However, in the soleus muscle, the ATPase-high fibre showed less hypertrophy than the ATPase-low fibres.

DISCUSSION

Perhaps the most interesting finding as far as the growth studies are concerned is that the populations of the different muscle fibre types are not completely static. For example, in the hamster biceps brachii muscle, the number of ATPase-low fibres decreases while in the hamster soleus the ATPase-low fibres increase in number. As the total number of fibres in the muscle was unchanged, this presumably means that one muscle fibre type is being converted into another. Since the total number of fibres of each type was determined for each muscle, the recorded change in the respective muscle fibre population cannot be attributable to any sampling errors (anomalous results can be obtained when sampling methods are used particularly when the different fibre types change size to different extents and thus occupy different areas). The change in the proportion of muscle fibre types which occurs post-natally is in agreement with the work of Kugelberg (1976) who reported an increase in the per cent of ATPase-low fibres in the soleus muscle of the rat with growth and Tomanek (1975) who reported a decrease in the number of ATPase-high fibres in the soleus muscle of the cat with growth.

Such findings raise the question of how one type of muscle fibre can be converted into another. The interconversion of fast glycolytic to fast oxidative-glycolytic fibres may be expected to follow quantitative and qualitative changes in the mitochondrial population and may only involve alterations in the organization of energy metabolism. However, the conversion of fast fibres into slow fibres is rather different as this necessitates a change in the myosin species. The mechanism by which muscle fibre interconversion could take place may involve alterations in the innervation of muscle fibres. Alternatively it may involve a change in the pattern of impulses received by the fibres. Certainly, there is some evidence of alterations in the motor input of skeletal muscles with increasing age and this may involve a turnover of the neuromuscular connexions themselves (e.g. Barker & Ip, 1965; Tuffery, 1971) or in the pattern of neuromuscular transmission (e.g. Gutmann, 1976). In the context of reinnervation during growth, it is interesting to note that in the hamster biceps brachii muscle, the number of least predominant fibre type, ATPase-low fibres, decreases with age. The converse is true for the soleus muscle where the ATPase-high fibre is the least predominant type, and it is this fibre type that decreases in number during growth. If reinnervation is taking place, the chances of a fibre being reinnervated by a branch of the predominant type of neuron are greater than it being reinnervated by other types of neurons. On this basis, therefore, the number of the predominant fibre type might be expected to increase.

In contrast to the events which occurred during growth, undernutrition did not significantly affect the number of fibres of any type in the muscles studied. However,

it did have a profound influence on the size of the different types of muscle fibre. Muscle fibre size was considerably reduced but the extent of atrophy varied between the different muscle fibre categories in the different muscles studied. In general, the ATPase-high fibres were more severely affected than the ATPase-low fibres. In the mouse biceps brachii muscle, which is without ATPase-low fibres, of the two categories of ATPase-high fibres, the ATPase-high (fast glycolytic) fibres seemed to be more susceptible than the ATPase-high (fast oxidative-glycolytic) fibres. It is not known why the different types of fibre are affected to different extents. It may be that the ATPase-low fibres are spared more because they have a rich blood supply and it follows from this that the fast oxidative-glycolytic fibre, which has a high oxidative capacity (Baldwin, Klinkerfuss, Terjung, Mole & Holloszy, 1972) and a rich blood supply (Reis & Wooten, 1970), would be spared in comparison with the fast glycolytic fibre. However, it is perhaps more likely that the mechanism producing the differences in response is related to an intrinsic property of the muscle fibres which could operate through the protein degradation mechanisms. The ATPase-low fibres are believed to be used for both postural activities and for slow isotonic movements (Goldspink, 1975) and hence they are in much more frequent use than the ATPase-high (fast contracting) fibres. Certainly, there would seem to be a selective advantage in sparing the muscle fibres whose function is primarily to maintain body posture.

Weight training of the kind used in these experiments was found to be an effective stimulus for inducing hypertrophy of the fibres of both fast-twitch and of slow-twitch muscle. Although all the fibre types in the biceps brachii and soleus muscles of hamsters and mice hypertrophied in response to the exercise training, the extent of this response varied among the different fibre types according to the exact nature of the exercise regimen and to the muscle in question. The response of the ATPase-high (fast) muscle fibres of the biceps brachii of exercised hamsters was greater than that of the ATPase-low (slow) fibres in this muscle, with the exception of the group made to pull against their full body weight for the duration of the exercise. The rather small degree of hypertrophy of the ATPase-high fibres of the biceps brachii muscle of the hamsters in group 2 may be attributable to the stress associated with producing maximum effort. A study by Woods & Routtenbury (1971) has indicated that exercise may result in overactivity of the hypothalamus which in turn may result in a decreased food intake. Marked losses of body weight during exercise training have been reported in previous exercise studies (Gordon *et al.* 1967; Faulkner, Maxwell & Lieberman, 1972). Certainly, if this is a 'self-starvation effect' (Spear & Hill, 1962) which may be an important aspect of strenuous exercise, then there would be two counteracting effects particularly as far as the ATPase-high fibre are concerned. The exercise would tend to make them hypertrophy but the 'self starvation' would tend to make them atrophy. The results of animal and human studies have shown that motor units containing oxidative fibres (slow oxidative and fast oxidative glycolytic) are primarily involved at light to moderate work loads. Fast glycolytic fibres seem to make a major contribution only when the intensity of work is increased or when the oxidative fibres become fatigued during prolonged activity (Gollnick *et al.* 1973*a*, *b*, 1974*a*, *b*; Edgerton *et al.* 1972; Baldwin, Reitman, Terjung, Winder & Holloszy, 1973). Indeed, neurophysiological studies have indicated a hierarchy of motor unit recruitment

with the slow oxidative fibres having the lowest threshold (Henneman & Olson, 1965). Therefore in any exercise study it is important to consider what type of fibres are being recruited. The type of exercise regimen employed here involved quite high isometric tensions, at least in the biceps brachii muscle. Consistent with this is the fact that it was the fast-twitch fibres, particularly the fast glycolytics, which tended to undergo the greatest hypertrophy. However, a considerable involvement of slow-twitch muscle fibres would also be expected and this was indicated by the extent of their hypertrophy.

In this study no increase in muscle fibre number was found. In contrast to this, an increase in the apparent muscle fibre number has been reported following a surgically induced overload (Van Linge, 1962; Hall-Craggs, 1972). Such increases were attributed to longitudinal splitting of existing muscle fibres. Recently Vaughan & Goldspink (1978) have shown that under these conditions fibre splitting is incomplete and occurs mainly at one end of the muscle. Therefore the situation is still unclear as to whether fibre splitting is an adaptive response or whether it is merely due to physical damage. It may be that in very strenuous exercise such as human weight training where the athletes lift several times their own body weight that total muscle fibre number does increase. Unfortunately it is very difficult, if not impossible, to obtain this sort of information. Generally speaking, no significant changes in the number of any of the major different types of muscle fibre occurred as a result of the weight training employed here. The reduction in the number of the ATPase-low fibres in the biceps brachii muscle of one of the hamster recovery groups is a possible exception. This may constitute an acceleration of the age-related trend reported in this paper. It may well be that in other kinds of exercise, for example long distance running or other very repetitive kinds of exercise, that the percentage of fibre type may change. This possibility is now being investigated at the present time in this laboratory.

The finding of an approximate return to control values after a period of recovery (relative inactivity) is consistent with that of Goldspink & Howells (1974) following weight training and that of Faulkner *et al.* (1972) following endurance running. The study of the persistence of muscle fibre hypertrophy following exercise training has been rather neglected and it would be interesting to investigate the effects of post-training bouts of exercise with a view to sustaining the hypertrophy of the different fibre types.

REFERENCES

AZIZULLA (1973). Studies on muscle development with particular reference to the effects of protein malnutrition. Ph.D. Thesis, University of Hull.

BALDWIN, K M., KLINKERFUSS, G. H., TERJUNG, R. L., MOLE, P. A. & HOLLOSZY, J. O. (1972). Respiratory capacity of white, red and intermediate muscle: adaptive response to exercise. *Am. J. Physiol.* **222**, 373–378.

BALDWIN, K. M., REITMAN, J. S., TERJUNG, R. L., WINDER, W. W. & HOLLOSZY, J. O. (1973). Substrate depletion in different types of muscle and in liver during prolonged running. *Am. J. Physiol.* **255**, 1045–1050.

BARKER, D. & IP, M. C. (1965). The probable existence of motor end-plate replacement. *J. Physiol.* **176**, 11–12*P*.

BROOKE, M. H. & KAISER, K. K. (1970). Muscle fibre types: how many and what kind? *Archs Neurol., Chicago* **23**, 369.

BROOKE, M. H. & KAISER, K. K. (1974). The use and abuse of muscle histochemistry. *Ann. N.Y. Acad. Sci.* **228**, 121–144.

BURKE, R. E., LEVINE, D. N., ZAJAC, F. E., TSAIRIS, P. & ENGEL, W. K. (1971). Mammalian motor units: physiological–histochemical correlation in three types of cat gastrocnemius. *Science, N.Y.* **174**, 709–712.

COOPER, R. R. (1972). Alterations during immobilisation and regeneration of skeletal muscle in cats. *J. Bone Jt. Surg.* **54A**, 919–953.

DAWSON, M., GOODFRIEND, T. L. & KAPLAN, N. O. (1964). Lactic dehydrogenase: functions of the two types. *Science, N.Y.* **143**, 929–933.

DOBOWITZ, V. & BROOKE, M. J. (1973). *Muscle Biopsy: A Modern Approach*, p. 29. London: W. B. Saunders.

EDGERTON, V. R., BARNARD, R. J., PETER, J. B., GILLESPIE, C. A. & SIMPSON, D. R. (1972). Overloaded skeletal muscles of non-human primate (*Galago senegalensis*). *Expl Neurol.* **37**, 322–339.

ERANKO, O. & PALKAMA, A. (1961). Improved localisation of phosphorylase by the use of polyvinyl pyrolidine and high substrate concentration. *J. Histochem. Cytochem.* **9**, 585.

FARBER, R., STERNBERG, W. H. & DUNLAP, C. E. (1956). Histochemical localisation of specific oxidative enzymes. *J. Histochem. Cytochem.* **4**, 254–263.

FAULKNER, J. A., MAXWELL, L. C. & LIEBERMAN, D. A. (1972). Histochemical characteristics of muscle fibres from trained and detrained guinea pigs. *Am. J. Physiol.* **222**, 836–840.

GOLDSPINK, G. (1962). Biochemical and physiological changes associated with the post-natal development of the biceps brachii. *Comp. Biochem. Physiol.* **7**, 157–168.

GOLDSPINK, G. (1964). The combined effects of exercise and reduced food intake on skeletal muscle fibres. *J. cell. comp. Physiol.* **63**, 209–216.

GOLDSPINK, G. (1965). Cytological basis of decrease in muscle strength during starvation. *Am. J. Physiol.* **209**, 100.

GOLDSPINK, G. (1970). The proliferation of myofibrils during muscle fibre growth. *Jl cell Sci.* **6**, 593–606.

GOLDSPINK, G. (1975). Biochemical energetics for fast and slow muscles. In *Comparative Physiology – Functional Aspects of Structural Materials*, ed. BOLIS, L., MADRELL, H. P. & SCHMIDT-NEILSEN, K. Amsterdam: North Holland.

GOLDSPINK, G. (1977). Muscle energetics and animal locomotion. In *Mechanics and Energetics of Animal Locomotion*, chap. 3, ed. ALEXANDER, R. McN. & GOLDSPINK, G. London: Chapman & Hall.

GOLDSPINK, G. & HOWELLS, K. F. (1974). Work-induced hypertrophy in exercised normal muscles of different ages and the reversibility of hypertrophy after cessation of exercise. *J. Physiol.* **239**, 179–193.

GOLDSPINK, G. & WATERSON, S. E. (1971). The effect of growth and inanition on the total amount of Nitroblue tetrazolium deposited in individual muscle fibres of fast and slow skeletal muscle. *Acta Histochemica* **40**, 16–22.

GOLLNICK, P. D., ARMSTRONG, R. B., SALTIN, B., SAUBERT, C. W., SEMBROWICH, W. L. & SHEPHERD, R. E. (1973*a*). Effect of training on enzyme activity and fibre composition of human skeletal muscle. *J. appl. Physiol.* **34**, 107–111.

GOLLNICK, P. D., ARMSTRONG, R. B., SEMBROWICH, W. L., SHEPHERD, R. E. & SALTIN, R. E. (1973*b*). Glycogen depletion pattern in human skeletal muscle fibres after heavy exercise. *J. appl. Physiol.* **34**, 615–618.

GOLLNICK, P. D., KARLSOON, J., PIEHL, K. & SALTIN, B. (1974*a*). Selective glycogen depletion in skeletal muscle fibres of man following sustained contractions. *J. Physiol.* **241**, 59–67.

GOLLNICK, P. D., PIEHL, K. & SALTIN, B. (1974*b*). Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *J. Physiol.* **241**, 45–57.

GONYEA, W. J. & ERICSON, G. C. (1976). An experimental model for the study of exercise-induced skeletal muscle hypertrophy. *J. appl. Physiol.* **40**, 630–633.

GORDON, E. E. (1967). Anatomical and biochemical adaptations of muscle to different exercises. *J. Am. med. Ass.* **201**, 755–758.

GORDON, E. E.; KOWALSKI, K. & FRITTS, M. (1967). Adaptations of muscles to various exercise studies in rats. *J. Am. med. Ass.* **199**, 139–144.

GREENBAUM, A. L. & YOUNG, F. G. (1953). A comparison of the difference in the total nitrogen content of the muscles of the rat resulting from treatment with growth hormone and from inanition. *J. Endocr.* **9**, 127.

GUTH, L. & SAMAHA, F. J. (1970). Procedure for the histochemical demonstration of actomyos in ATPase. *Expl Neurol.* **28**, 365.

GUTMANN, E. (1976). Age changes in the neuro-muscular system and aspects of rehabilitation medicine. In *Neurophysiological Aspects of Rehabilitation Medicine*, ed. BUERGER, A. A. & JÖBIS, J. S., pp. 42–61. Springfield, Ill.: C. S. Thomas.

GUTMANN, E., HAJEK, I. & HORSKY, P. (1969). Effect of excessive use on contractile and metabolic properties of cross-striated muscle. *J. Physiol.* **203**, 46–47*P*.

HAGEN, S. N. & SCOW, R. O. (1957). Effect of fasting on muscle proteins and fat in young rats of different ages. *Am. J. Physiol.* **188**, 91.

HALL-CRAGGS, E. C. B. (1972). The significance of longitudinal fibre division in skeletal muscle. *J. Neurol. Sci.* **15**, 27–33.

HAYASHI, M. & FREIMAN, D. G. (1966). An improved method of fixation for formalin-sensitive enzymes with special reference to myosin-adenosine triphosphatase. *J. Histochem. Cytochem.* **11**, 577.

HELANDER, E. A. S. (1961). Influence of exercise and restricted activity on the protein composition of skeletal muscle. *Biochem. J.* **78**, 478–482.

HENNEMAN, E. & OLSON, C. B. (1965). Relations between structure and function in the design of skeletal muscles. *J. Neurophysiol.* **28**, 581–598.

HERBISON, G. J. & GORDON, E. E. (1973). Exercise of normal muscle: biochemical effects. *Archs phys. med. Rehab.* **54**, 409–416.

HOLLOSZY, J. O. & BOOTH, F. W. (1976). Biochemical adaptations to endurance exercise in muscle. *A. Rev. Physiol.* **38**, 273–291.

JOUBERT, D. M. (1956). An analysis of factors influencing post-natal growth and development of the muscle fibre. *J. agric. Sci., Camb.* **47**, 59.

KENDRICK-JONES, J. & PERRY, S. V. (1967). The enzymes of adenine nucleotide metabolism in developing skeletal muscle. *Biochem. J.* **103**, 207.

KOWALSKI, K., GORDON, E. E., MARTINEZ, A. & ADEMEK, J. (1969). Changes in enzyme activities of various muscle fibre types in rat induced by different exercises. *J. Histochem. Cytochem.* **17**, 601–607.

KUGELBERG, E. (1976). Adaptive transformation of rat soleus motor units during growth. Histochemistry and contraction speed. *J. neurol. Sci.* **27**, 269–289.

MACKOVA, E. & HINK, P. (1973). Compensatory muscle hypertrophy induced by tenotomy of synergistic muscles is not true working hypertrophy. *Physiologia bohemoslov.* **22**, 43–49.

MCMANUS, J. F. A. & MOWRY, R. W. (1960). *Staining Methods: Histological and Histochemical.* New York: Harper & Row.

MCMEEKAN, C. P. (1940). Growth and development in the pig with special reference to care as quality characters. *J. agric. Sci., Camb.* **30**, 276.

MONTGOMERY, R. D. (1962). Growth of human striated muscle. *Nature, Lond.* **195**, 194.

MÜLLER, W. (1975). Isometric training of young rats – effects upon hind limb muscles. *Cell and Tissue Res.* **161**, 225–237.

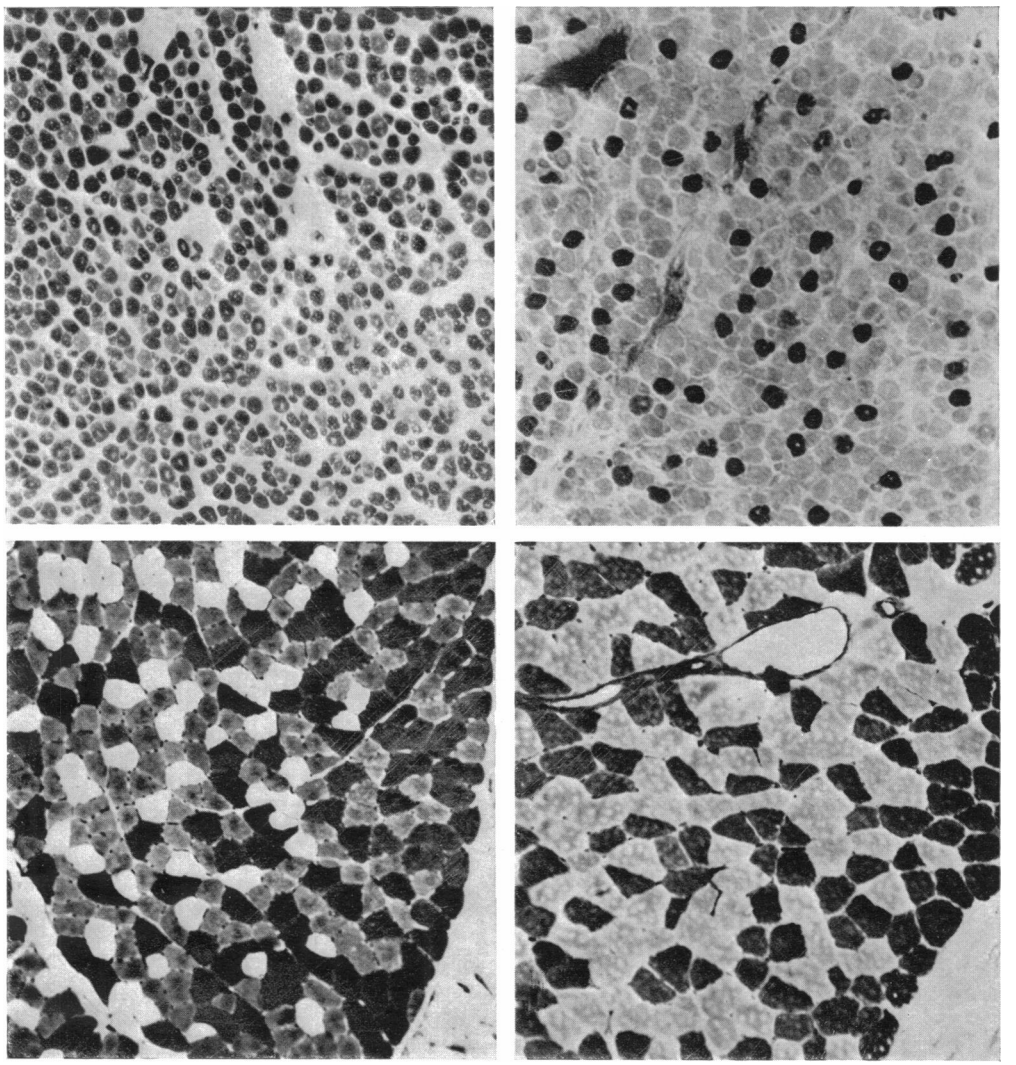
NACHLAS, M. M., TSOU, K. C., DE SOUSA, E., CHENG, C. S. & SELIGMAN, A. M. (1957). Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. *J. Histochem. Cytochem.* **5**, 420–436.

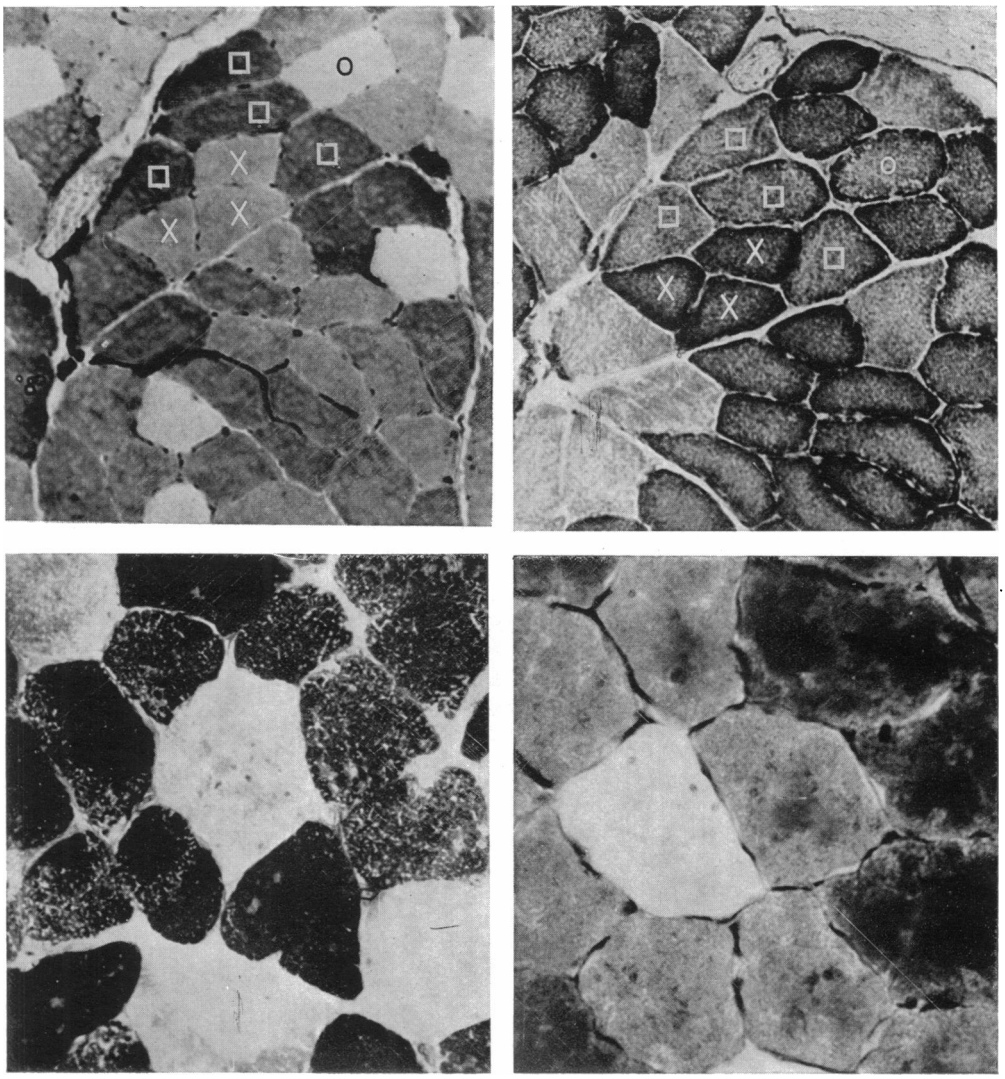
PADYKULA, H. A. & HERMAN, E. (1955). Factors affecting the activity of adenosine triphosphatase and other phosphatases as measured by histochemical techniques. *J. Histochem. Cytochem.* **3**, 161.

PERRY, S. V. (1970). Biochemical adaptation during development and growth in a skeletal muscle. In *The Physiology and Biochemistry of Muscle as a Food* 2, ed. BRISKEY, E. J., CASSENS, R. G. & MARSH, B. B. Madison: University of Wisconsin Press.

PETER, J. B., BARNARD, R. J., EDGERTON, V. R., GILLESPIE, C. A. & STEMPEL, K. E. (1970). Metabolic profiles of three fibre types of skeletal muscle in guinea-pigs and rabbits. *Biochemistry, N.Y.* **11**, 2627–2633.

REIS, D. J. & WOOTEN, F. (1970). The relationship of blood flow to myoglobin, capillary density and twitch characteristics in red and white skeletal musle in cat. *J. Physiol.* **210**, 124–135.





ROBERTSON, D. D. & BAKER, D. D. (1933). Histological differences in the muscles of full, half and rough red steers. *Missouri Agricultural Experimental Station Research Bulletin* **200**.

ROWE, R. W. D. (1968). Effect of low nutrition on size of striated muscle fibres in the mouse. *J. exp. Zool.* **167**, 353.

SOKAL, R. R. & ROLF, F. J. (1969). *Biometry*. San Francisco: W. H. Freeman.

SPEAR, N. E. & HILL, W. F. (1962). Methodological note: excessive weight loss in rats in activity wheels. *Psychol. Rep.* **11**, 437.

STAUN, H. (1963). Various factors affecting number and size of muscle fibres in the pig. *Acta agric. scand.* **13**, 293.

TOMANEK, R. J. (1975). A histochemical study of post-natal differentiation of skeletal muscle with reference to functional overload. *Devl Biol.* **42**, 305.

TUFFERY, A. R. (1971). Growth and degeneration of motor end-plates in normal cat hind limb muscles. *J. Anat.* **110**, 221–247.

VAN DER MEULEN, J. P., PECKHAM, P. H. & MORTIMER, J. T. (1974). Use and disuse of muscle. *Ann. N.Y. Acad. Sci.* **228**, 177–189.

VAN LINGE, B. (1962). The response of muscle to strenuous exercise. An experimental study in the rat. *J. Bone Jt Surg.* **44B**, 711–721.

VAUGHAN, H. & GOLDSPINK, G. (1979). Fibre number and fibre size in a surgically overloaded muscle. Accepted for publication *J. Anat.* (in the Press).

WALKER, M. G. (1966). The effect of exercise on skeletal muscle fibres. *Comp. Biochem. Physiol.* **19**, 791–797.

WATTENBURG, L. W. & LEONG, G. (1960). Effects of coenzyme Q_{10} and menadione on succinic dehydrogenase activity as measured by tetrazolium salt reduction. *J. Histochem. Cytochem.* **8**, 296.

WOODS, D. J. & ROUTTENBURG, A. (1971). 'Self-starvation' in activity wheels, developmental and chlorpromazine interactions. *J. comp. physiol. Psychol.* **76**, 84–92.

EXPLANATION OF PLATES

PLATE 1

Shows examples of the histochemical methods used in identifying the different muscle fibre types in animals of different ages.

Top left: biceps brachii muscle of the fore-limb of a new-born mouse stained for myosin ATPase following alkaline preincubation and treatment with paraformaldehyde. × 100.

Top right: the same muscle stained myosin ATPase but the staining in this case is after acid preincubation. × 100.

Bottom left: the biceps brachii of the adult hamster stained for myosin ATPase following alkaline preincubation and pretreatment with paraformaldehyde. This illustrates the three muscle fibre types in this muscle. × 100.

Bottom right: the soleus muscle of the adult hamster stained for myosin ATPase following alkaline preincubation and paraformaldehyde treatment. This illustrates the two fibre types of this muscle. × 100.

PLATE 2

Top left: ATPase staining of the hamster biceps muscle following incubation at alkaline pH. × 500.

Top right: an adjacent section from the same muscle showing the same fibres after staining with the NADH tetrazolium reductase method. × 500. ○ = SO; X = FOG; □ = FG.

Bottom left: biceps brachii muscle of the hamster after a period of undernutrition stained for ATPase after alkaline preincubation. Note the sparing of the slow twitch oxidative fibres (lightly stained fibres). × 750.

Bottom right: control muscle for one: bottom left. × 750.