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Muscle fibre growth in five different muscles in both sexes of mice

I. Normal mice

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Understandably, much of the previous work concerned with muscle fibre growth has been carried out on human post-mortem and biopsy material (MacCallum, 1898; Walls, 1960; Fenichel, 1963) or on muscle from meat-producing animals (Hammond & Appleton, 1932; Robertson & Baker, 1933; McMeekan, 1942; Joubert, 1956). From this work much useful information has been obtained but as yet no coherent scheme of muscle fibre development has emerged. Several major difficulties are encountered in working with large muscles such as those of man and agricultural animals, including the difficulty of obtaining a ready supply of fresh material of known age and origin and the difficulty of devising a representative histological sampling technique. It seemed therefore, that a fundamental study of muscle fibre growth was required using a small laboratory mammal, such as the mouse, in which it has been shown that the muscles are ideal for such quantitative cytological procedures (Rowe, 1967).

It is well established that the constituent fibres of the different muscles of mammalian striated musculature are not uniform in size (Fernand, 1949; Walls, 1960), appearance (Ranvier, 1873; Needham, 1926; Denny-Brown, 1929; Walls, 1960), chemical composition (Stein & Padykula, 1962; George & Susheela, 1961; Drews & Engel, 1966; Dubowitz, 1963) or in their physiological characteristics (Denny-Brown, 1929; Buller, Eccles & Eccles, 1960a, *b*; Close, 1964). Some of these differences, as well as existing between the fibres of different muscles, appear to exist between the fibres of the same muscle, in so-called 'mixed' muscles. In view of this knowledge it was decided that the investigations should include muscles of mixed fibre constitution, different physiological characteristics and that they should be from different regions of the body.

MATERIALS AND METHODS

Normal homozygous males and homozygous females of the 129/Re strain were used. All mice were reared in this department and maintained under the same environmental conditions. They received food (Breeding diet cube, Messrs Heygate and Sons Ltd., Northampton) and water *ad libitum* and were maintained at 21° C.

A number of factors were taken into account during the selection of the muscles to be investigated; however, these have been reported elsewhere (Rowe, 1967). The five muscles finally selected were: m. tibalis anterior, m. biceps brachii, m. extensor digitorum longus, m. soleus and m. sternomastoideus.

Normal mice were sacrificed at the following ages: newborn, 1, 2, and 3 weeks and then at intervals of 3 weeks up to the age of 24 weeks. The muscles from the left side of the body provided material for histological examination and were fixed as soon after death as possible; however, in order to ensure that standard transverse sections were obtained, the treatment of muscles prior to and during fixation was regarded as being very important. A detailed description of these treatments has previously been reported (Rowe, 1967). Briefly they ensured that all muscles were fixed at their *in vivo* resting lengths. The muscles from the right side of the body were excised, cleaned and weighed.

All the muscles were fixed in Fleming's solution without acetic acid for 30 h. This fixative had previously been found to be particularly suitable for fixation of small muscles as it causes very little distortion of the muscle fibres (Goldspink, 1961). After fixation the muscles were washed in running water for 12 h, dehydrated in ethyl alcohol and embedded in ester wax (Steedman, 1960). Transverse sections were cut at 6 μm, stained in Mallory's triple stain and finally mounted using Canada Balsam.

Quantitative measurements. The total number of fibres was determined for each muscle by counting all the fibres in a transverse section taken from a region in which they were all known to be present (Rowe, 1967). As this study involved muscles from very young animals in which fibres at different stages of differentiation were encountered, it was necessary to establish a convention as to what would be counted as a muscle fibre. For this purpose a muscle fibre was considered to be any extrafusal structure containing myofibrils. Sections were either projected at a magnification of $\times 100$ or photographed at a magnification of $\times 250$ and the total number of fibres counted using an electric pen counter.

The fibre size distributions were obtained by measuring the diameters of a sample (100 fibres) from each muscle. It has been shown by other workers that it is statistically valid to use this size of sample and method of fibre measurement (Meara, 1947; Joubert, 1956). For muscles from mice 3 weeks of age and older the diameters were obtained by measuring the projected fibres at a magnification of approximately $\times 450$ using a pair of previously calibrated micrometer calipers. This measuring technique was not possible for muscles of mice 2 weeks old and younger because a higher magnification was necessary than could be obtained using the projection apparatus. In the case of these younger muscles, therefore, the fibre diameters were measured using an eyepiece micrometer in a Leitz Ortholux microscope.

Fernand (1949) and other workers have shown that different muscles have fibres of different cross-sectional area arranged in various spatial configurations. Therefore the method of sampling the fibres of a muscle was considered to be important. The fibre sample was taken in the same way for all five muscles. The 100 fibres were taken as those that were transected by parallel lines drawn across the projected image of the section or comparable lines across the eyepiece field. The measurements of individual fibres were assigned to appropriate size groups, as follows: 2·6–7·5 μm (midpoint 5 μm); 7·6–12·5 μm (midpoint 10 μm); etc.

RESULTS

It is hoped that the method of presentation of the results will render them reasonably self-explanatory and therefore only the more important aspects will be mentioned below.

Body weights. The curves obtained for the body weights with age (Fig. 1, top) were of the typical growth curve pattern for both sexes of mice.

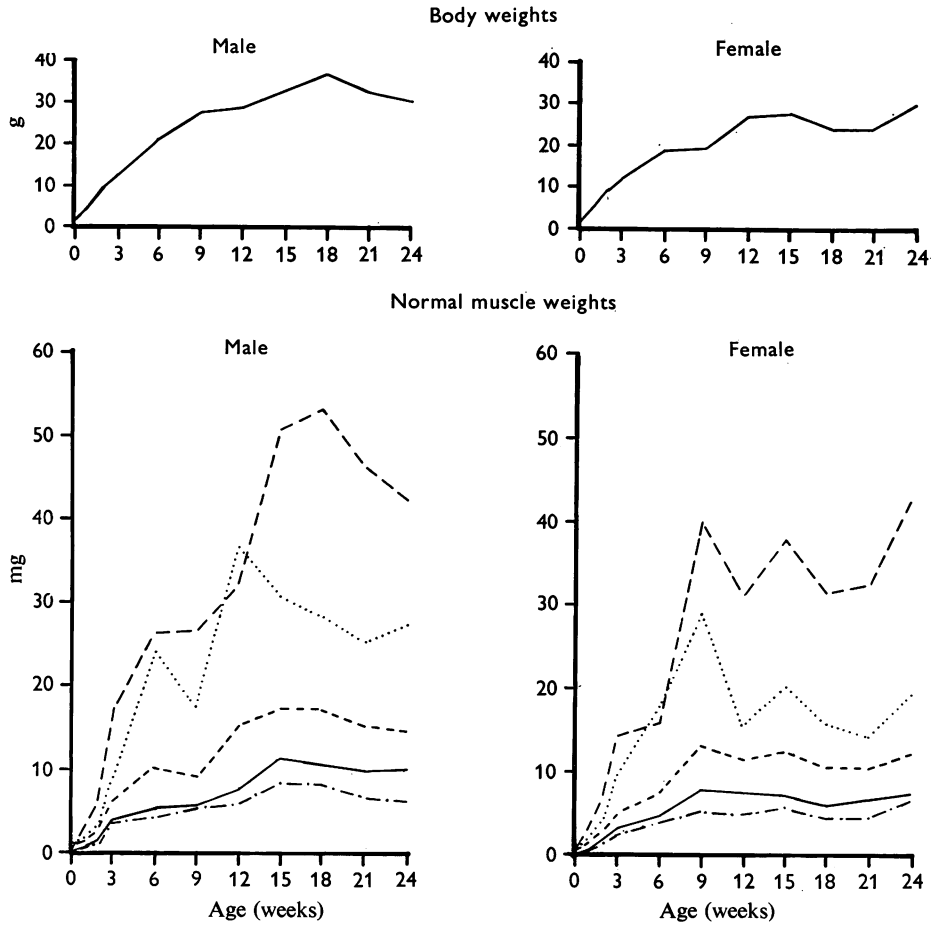

Fig. 1. Body weights of mice in g plotted against age in weeks. Also shown are the muscle weights in mg for mice of both sexes. — — — M. tibialis anterior, - - - - m. biceps brachii, ——— m. extensor digitorum longus, -·-·-·- m. soleus, ... m. sternomastoideus.

Muscle weights. From Fig. 1 it will be seen that there is a considerable sex difference for all five muscles in the normal mature mice ($P < 0{\cdot}01$). It will also be noted that the male muscles took longer to attain their plateau or mature values (12–15 weeks) than did the female muscles (9 weeks).

Total fibre numbers. The results for the counts of the total fibre number are given

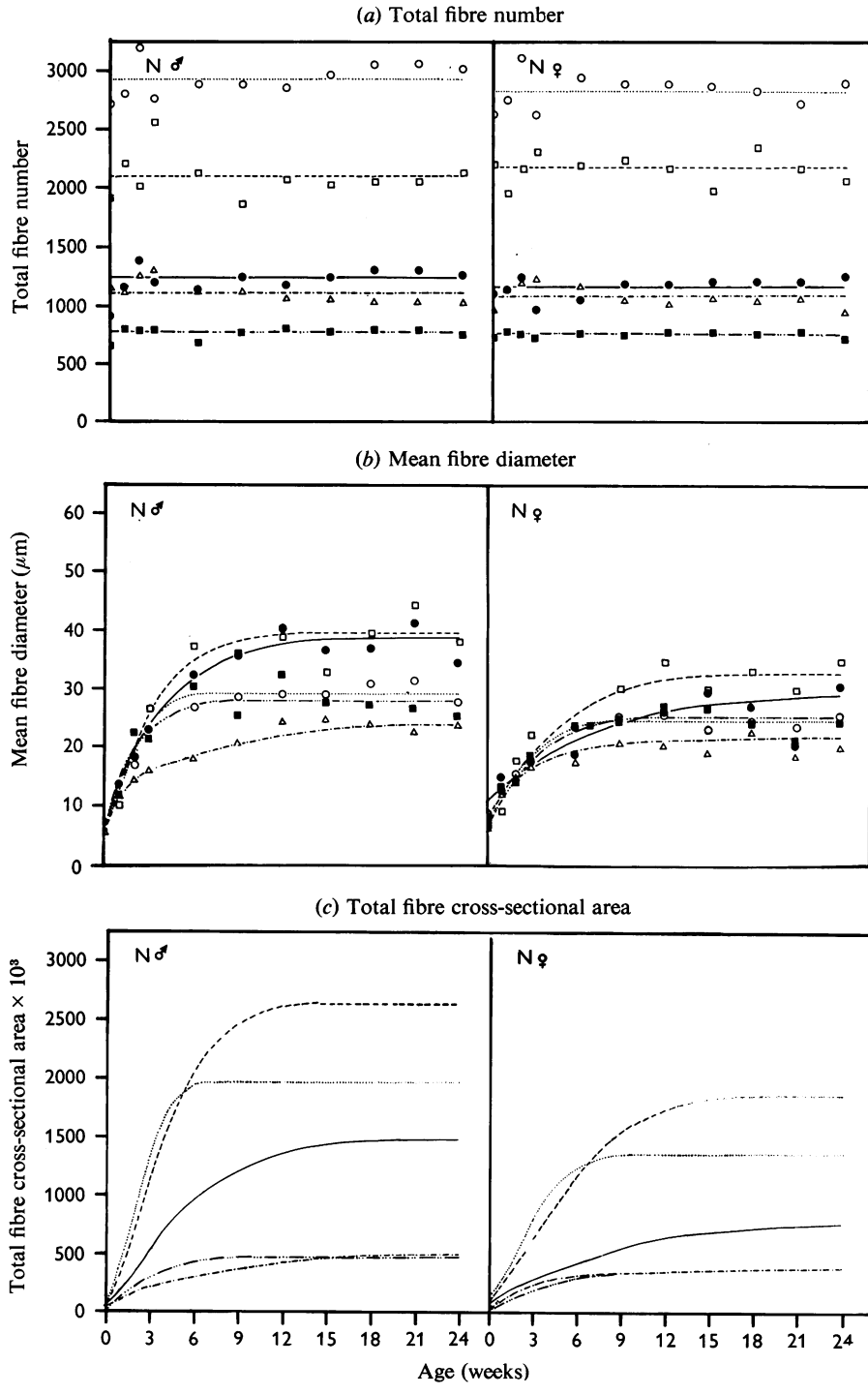

Fig. 2. (*a*): the total fibre number plotted against age; (*b*): the mean fibre diameter; (*c*): the total fibre cross-sectional area. ○ ..... M. anterior tibialis, □ ----- m. biceps brachii, △ -·-·- m. extensor digitorum longus, ■ ···—···— m. soleus, ● ——— m. sternomastoideus.

in Fig. 2*a*. The total number of fibres in each of the muscles was found to be statistically unchanged throughout the animal's life. There were no significant differences between the sexes (P greater than 0·2 for all five muscles).

Mean fibre diameters. The values for the mean fibre diameters are given in Fig.

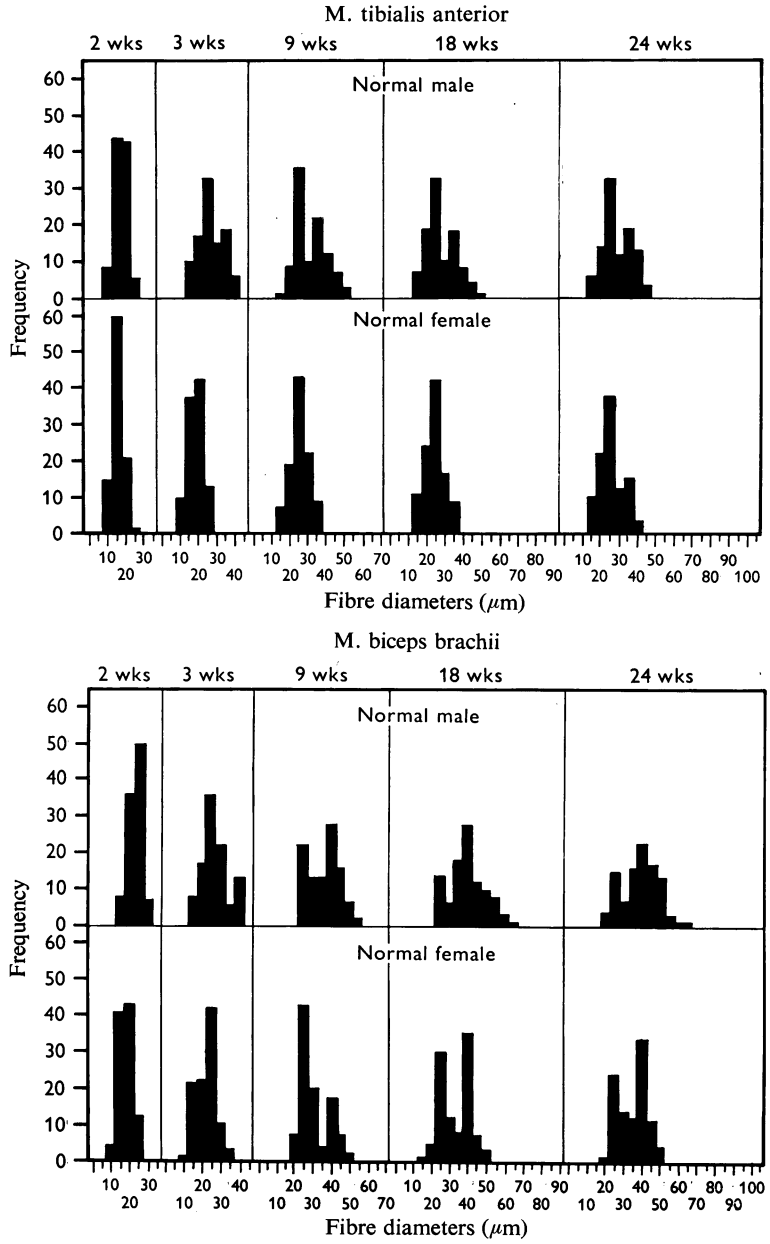

Fig. 3. Fibre diameter distributions for the m. tibialis anterior and m. biceps brachii. The number of fibres occurring in each size group is plotted against the individual size groups.

2*b*. In all five muscles the mean fibre diameter increased gradually as post-natal growth progressed. There were considerable differences between the different muscles and between the muscles of the two sexes.

Total fibre cross-sectional area. Presented in Fig. 2*c* are values for the total fibre

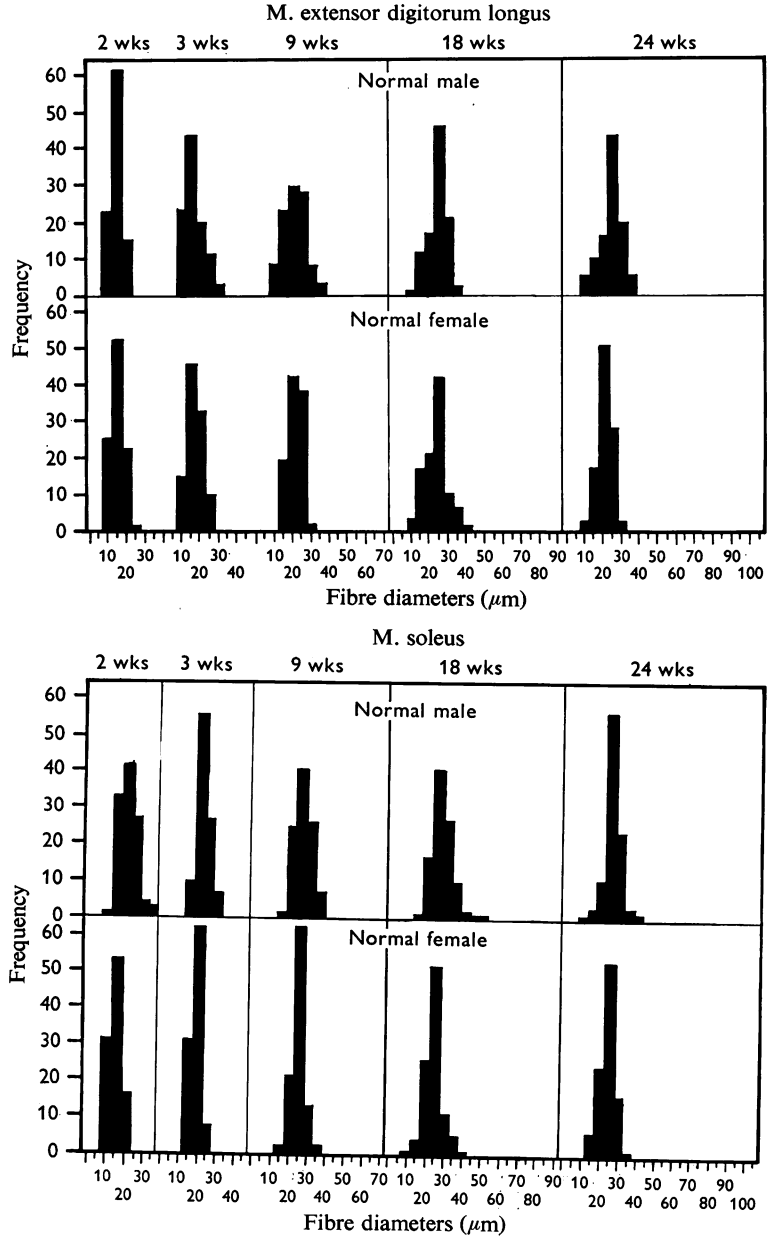

Fig. 4. Fibre diameter distributions for the m. extensor digitorum longus and m. soleus. The number of fibres occurring in each size group is plotted against the individual size groups.

cross-sectional area for each muscle, plotted against age. These were derived by multiplying the total fibre number of the muscle by the mean fibre cross-sectional area (calculated from the mean fibre diameter). This measurement is useful as it gives some indication of the strength of the muscles. It is realized, however, that it provides only an estimate, as the strength of muscle fibres is in some cases disproportionate to their cross-sectional area (Goldspink, 1965; Rowe, 1967). The total fibre cross-sectional areas for the normal muscles follows the same trends as the mean fibre diameters and require little comment.

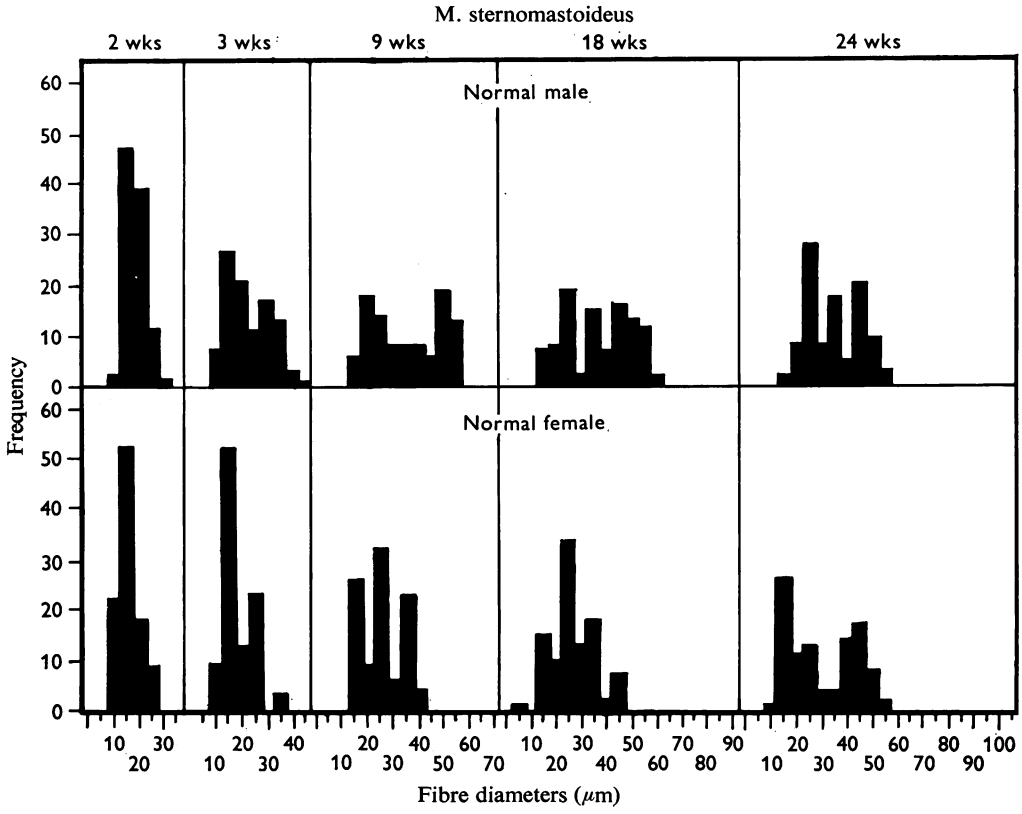

Fig. 5. Fibre diameter distributions for the m. sternomastoideus. The number of fibres occurring in each size group is plotted against the individual size groups.

Fibre diameter distributions. The distribution of fibre diameters in a 100-fibre sample was plotted for each muscle studied. Owing to restricted space the fibre diameter distributions of male and female muscles for the ages 2, 3, 9, 18 and 24 weeks only are given (Figs. 3–5). These ages were considered to be reasonably representative of the period studied.

At birth all five muscles in normal animals consisted almost entirely of muscle fibres with diameters of 5–10 μm. However, as growth progressed, differences in the fibre distribution emerged between the different muscles and in some cases between the sexes for the same muscle.

Statistical analysis. Multiple regression analyses (linear and curvilinear) were carried out on these data, to establish the following:

(1) The relationship of muscle weight to fibre number and fibre diameter.
(2) The relationship of fibre number, fibre diameter and muscle weight to age.
(3) The validity of using a sample mean as compared with a sample mode.

Table 1. *The results of the multiple regression analyses*

(The determining variables considered in each analysis are listed below the relevant dependent variable (in italics). For each analysis the significance of the relationship (slope of the regression line) is indicated by asterisks, * = significant at the 5% level; ** = significant at the 1% level; *** = significant at the 0·1% level. Also given is the amount of variation in the dependent variable that could be explained by the appropriate determining variable (V. Exp.))

	Anterior tibialis		Biceps brachii		Extensor dig. long.		Soleus		Sterno-mastoideus	
	Slope	V. Exp. (%)	Slope	V. Exp. (%)	Slope	V. Exp. (%)	Slope	V. Exp. (%)	Slope	V. Exp. (%)
Muscle wt.										
Age	+**	88	+**	89	+**	86	+**	85	+**	72
Sex	+	2	+	4	+	3	+	4	+	6
Fibre no.										
Age	+	21	−	7	−	41	+	11	+	24
Sex	+	21	−	7	+	4	+	11	+	7
Mean diam.										
Age	+**	77	+**	82	+**	86	+**	79	+**	} 86
Sex	+	6	+	17	+	2	+	4	+*	}
Mode 1										
Age	+**	77	+*	66	+**	85	+**	77	+*	} 63
Sex	+	1	+	9	+	2	+	2	+*	}
Mode max.										
Age	+*	75	+**	88	+**	85	+**	77	+**	} 81
Sex	+	5	+	1	+	2	+	2	+*	}
Muscle wt.										
Fibre no.	+	.	−	.	−	.	−	.	−	.
Mean diam.	+*	76	+**	84	+***	84	+*	74	+**	80
Muscle wt.										
Fibre no.	+	.	−	.	−	.	+	.	−	.
Mode 1	+*	68	+*	76	+**	85	+*	71	+*	58
Muscle wt.										
Fibre no.	+	.	−	.	−	.	+	.	−	.
Mode max.	+*	71	+**	83	+**	85	+*	71	+**	79
Mean diam.										
Mode 1	+***	94	+**	88	+***	98	+***	99	+*	75
Mean diam.										
Mode max.	+**	91	+***	94	+***	98	+***	99	+***	95
Fibre no.										
Mean diam.	+	16	−	0	−	11	+	7	+	31

The regression analyses were carried out using the general strategy of Robinson & Taylor (1960). The approximation used for the critical *F*-ratio is based on equation (16.42) of Kendall & Stuart (1958).

The results of the multiple regression analyses are presented in Table 1. Initially the sex of the mouse was taken into consideration as one of the determining variables, but in the majority of cases it was found to have no statistically significant effect on the other regressions. For this reason the results presented in Table 1, except where stated, are the results of the analyses after recomputation excluding sex.

DISCUSSION

One of the main functions of skeletal muscle is to enable the animal to move and to do this the muscles have to work against the load imposed on them. This load must bear some relationship to the gross body weight especially in the case of the limb muscles. A study of the body weights, therefore, provides essential background knowledge. The data collected here revealed that there was a considerable difference between the sexes.

As the muscle fibres constitute about 80–90 % of the muscle (Creese, D'Silva & Hashish, 1955; Goldspink, 1966), any appreciable change in the weight of a muscle during growth must presumably be due to a change in the bulk of the fibres. The magnitude of the weight increases presented here varied from muscle to muscle. It was also interesting to note that the difference between the normal males and normal females was more pronounced in some muscles than in others. The multiple regression analyses that were carried out showed that there was no significant difference in the correlation coefficient of the two sexes. However, there was a significant difference between the mature muscle weights, even when they were expressed as mg/g body weight. In other words, the males had a higher mature muscle weight but took longer to attain it.

The results presented here showed that the post-natal increase in size and weight of the muscles was brought about by an increase in the size of the constituent fibres, the fibre number normally remaining constant. The finding that the number of fibres in normal muscles does not change significantly throughout the animal's lifetime is in agreement with the findings of other workers (human muscle: MacCallum, 1898; pig muscle: McMeekan, 1940; Staun, 1963; rat muscle: Elliot, Wigginton & Corbin, 1943; rabbit muscle: Meara, 1947). Apparently contradictory are the reports of Morpurgo (1895), Goldspink (1962) and Chiakulas & Pauly (1965). These latter three workers reported an increase in the number of fibres in rodent muscles during the first few weeks after birth. However, they included only fully differentiated fibres in the counts.

The mean fibre diameters of the five muscles were found to increase in a curvilinear manner from birth to maturity in a fashion closely related to the increase in muscle weight. The multiple regression analyses showed that 75 % of the variation in weights between muscles of different ages could be explained by the variation in mean fibre diameter. Presumably most of the remaining variation can be attributed to a change in fibre length.

The mean fibre diameter measurements provided only a limited amount of information about the growth of individual muscle fibres. The data for the distribution of fibre diameters were much more informative. At birth, differentiation was found to be incomplete in all the normal muscles. For the sake of the investigation the myotubes

were regarded as muscle fibres for the reasons specified above. The first stage in the post-natal development of a fibre, therefore, was the completion of differentiation. This involved a gradual increase in diameter to approximately 25 μm accompanied by the synthesis of more myofibrils and the movement of the nuclei to a subsarcolemmal position. This level of development was common to all normal muscle fibres examined and it is thought that the development to this stage might be independent of work load and/or functional activity.

Two main forms of fibre distributions were seen to develop over the 24 weeks period investigated: the unimodal distributions of the m. extensor digitorum longus and the m. soleus consisting almost exclusively of small fibres with diameters approximately 25 μm, and the bimodal distributions of the m. biceps brachii and m. tibialis anterior. This type of distribution was found to develop by a proportion of the 25 μm diameter fibres undergoing hypertrophy into larger fibres with diameters of approximately 40 μm. The unusual type of distribution found in the m. sternomastoideus was brought about by the persistence in the female of fibres of lower than usual diameter (approximately 15 μm) and the presence of larger fibres in the male (approximately 45 μm). The reasons why this muscle showed this type of distribution are not clear; however, as this muscle is the one that shows a marked sex difference, it is possible that it is due to the more direct influence of the endocrine system on this particular muscle.

From the results it is apparent that there is a basic level of normal fibre development (25 μm). By considering the functions of the five muscles studied it seems reasonable to postulate that any subsequent development is dependent on the work-load per fibre. As the body weight of the mouse increases, so presumably must the work-load per fibre and in the case of the m. tibialis anterior, m. biceps brachii and m. sternomastoideus the increase appears to be such as to require or induce more of the basic fibres to undergo hypertrophy. On the basis of this hypothesis the m. extensor digitorum longus and m. soleus must presumably be able to carry out their work with their fibres at the basic level of development. In the case of the m. sternomastoideus there may be superimposed on this pattern of development a direct influence of sex. Sex also has an indirect influence on this and the other four muscles but possibly only by hormonal control of the growth of body tissues in general.

The reason why the fibres of normal bimodal muscles show distinct distribution peaks is most probably related to the fact that hypertrophy when it takes place is relatively rapid in individual fibres. The physiological significance of the hypertrophy of basic fibres into larger fibres lies in the fact that there is a disproportionately large increase in contractile material. Some recent experiments have been carried out that strongly support the hypothesis that it is the work-load that induces fibre hypertrophy (Rowe & Goldspink, 1968). These experiments have shown that the m. soleus, which normally had a unimodal distribution of fibre diameters, can be converted into a bimodal muscle by subjecting it to an increased work-load. It appears therefore that all fibres have the same potential to develop irrespective of the muscle to which they belong. The maximum size to which they are able to develop may be restricted in normal muscles by several physical factors, for example, the supply of oxygen and metabolites.

The two levels of fibre development found in bimodal muscles may be of the same

identity as the red and white fibres in so-called mixed muscles (Denny-Brown, 1929) and probably some of the histochemical types of fibres described by Dubowitz (1963), Stein and Padykula (1962), George & Susheela (1961), Ogata (1958), Romanul (1964). These two levels of post-natal development certainly have quantitative chemical differences and they also have a slightly different structural appearance (Goldspink, 1964; 1965). These are now being investigated in more detail.

SUMMARY

Muscle fibre size and number were measured in five different muscles from both sexes of normal mice at different ages from birth to maturity. Data for body weights and muscle weights were also presented. The total fibre number in all muscles was found to remain constant throughout the animal's life; there was no significant difference between the sexes. The mature mean fibre diameters were found to differ considerably from muscle to muscle and also between the sexes. Distribution histograms of fibre diameters showed that the m. soleus and m. extensor digitorum longus exhibited unimodal distributions. The m. tibialis anterior and m. biceps brachii, although unimodal at birth became distinctly bimodal as growth progressed. The bimodal distribution was due to the fact that some of the fibres remain at a basic level of growth (diam. = 20–25 μm), whilst others undergo hypertrophy to a diameter of about 40 μm. The work-load per fibre was thought to be the factor bringing about hypertrophy.

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