J. Anat. (1972), **113**, 2, pp. 213–240
With 17 *figures*
Printed in Great Britain

Postnatal changes in the histochemical fibre types of porcine skeletal muscle

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(*Accepted* 4 *August* 1972)

INTRODUCTION

When the innervation of fast- and slow-twitch crural muscles of the cat (Buller, Eccles & Eccles, 1960*a*), guinea-pig (Robbins, Karpati & Engel, 1969) and rat (Close, 1969) is exchanged, the ratio of the intrinsic speeds of contraction of these muscles is reversed. Contraction speed is directly related to the activity of myosin adenosine triphosphatase (myosin ATPase) in a variety of muscles (Bárány, 1967), and cross-innervation of fast and slow muscles alters the activity of myosin ATPase demonstrated biochemically (Buller, Mommaerts & Seraydarian, 1969, 1971; Samaha, Guth & Albers, 1970; Bárány & Close, 1971). The proportion of fibres shown histochemically to be high in myosin ATPase activity is related to the intrinsic speed of contraction of a muscle (Edgerton & Simpson, 1969), and following cross-innervation there is a change in this histochemical reaction. Thus when axons normally supplying fast-twitch muscles are made to innervate the slow-twitch soleus muscle of the rabbit (Dubowitz, 1967), guinea-pig (Karpati & Engel, 1967*a*; Robbins *et al.* 1969), cat and rat (Guth, Samaha & Albers, 1970), the proportion of myosin ATPase high fibres in soleus is increased. Similarly, when the nerve to soleus is made to innervate a fast-twitch muscle, there is a decrease in the proportion of myosin ATPase high fibres in the flexor hallucis longus muscle of the cat (Dubowitz, 1967; Guth *et al.* 1970) and the flexor digitorum longus muscle of the rabbit (Dubowitz, 1967). The type of innervation therefore has a direct influence on the contractile properties of the muscle fibre. As a corollary, any change in the speed of contraction of a muscle and the proportion of its fibres with high myosin ATPase activity should be the result of an innervation change.

However, the contractile properties of a muscle can be changed without direct interference with the nerve supply. When the amount of isometric exercise in a muscle is increased by eliminating the effect of synergistic muscles or by impairing the function of the opposite limb, there is a decrease in the speed of contraction (Vrbová, 1963; Lesch, *et al.* 1968; Olson & Swett, 1969; Gutmann, Schiaffino & Hanzliková, 1971). Gutmann & Hájek (1971) found that the ATPase activity of myosin extracted from the extensor digitorum longus muscle of the rat decreases when the tibialis cranialis muscle has been tenotomized for 7 days. Excision of synergists of the soleus and plantaris muscles of the rat was found after 10 weeks to result in fewer myosin ATPase high fibres in these muscles (Guth & Yellin, 1971). The manner in which isometric exercise affects the contractile properties of muscle

is unknown, but the evidence from cross-innervation studies given above suggests that work load may influence the type of innervation of individual fibres.

The concept that usage of a muscle may influence the nervous system is relevant to the understanding of adaptation of an animal to the environment, and in the interpretation of changes in muscle caused by disease and experimental procedures. In particular, it is relevant to the study of growth of an animal, in which the postural muscles must adapt themselves to support, by means of a force proportional to the transverse-sectional area of the muscles or the square of the body length, a weight proportional to the cube of the body length. In addition, we must consider the problem of growth in an animal such as the pig, which runs at approximately the same speed from birth to adult, even though its body length increases approximately fivefold in this time; the intrinsic speed of contraction of its propulsive muscles must consequently decrease as the pig grows (Hill, 1950).

This paper describes growth changes in the histochemical profiles of fibres of the longissimus and diaphragm muscles, and provides evidence that the mechanical and metabolic properties of muscles adapt to the changes brought about by an increase in body size. These muscles of the domestic pig were chosen because:

(1) The arrangement of fibre types in porcine muscles forms a pattern which is much less random than in other mammals.

(2) Both muscles permit sampling in a specific region of the muscle in each animal.

(3) The proportion of histochemical fibre types does not vary greatly in the regions near to sites of sampling.

(4) The muscles can be sampled from slaughter-house pigs with minimal mutilation of the carcasses.

(5) M. longissimus has a shape and fibre architecture that enables the measurement of the transverse section of the muscle, and an estimation of its total fibre population.

(6) The two muscles differ greatly in their function, patterns of usage, and proportion of histochemical fibre types.

MATERIALS AND METHODS

Sources and initial preparation of material

Pigs of the Large White breed were obtained from two different sources.

Series 1. Eighteen female pigs (Table 1) with live weights ranging from 1·3 to 60 kg (2–214 days of age) were obtained from a herd intensively selected for lean meat production for 6 years by the School of Agriculture, University of Newcastle-upon-Tyne. They were chosen to include as nearly as possible three pigs of each of the following live weights: 2, 4, 8, 16, 32 and 64 kg. Pigs of 32 and 64 kg live weight were killed near Newcastle. Samples of the left longissimus muscle from the dorsomedial region at the thoracolumbar junction (Figs. 1, 6), and of the diaphragm from the left costal region, were removed, chilled, and brought to Edinburgh with the carcasses. Smaller pigs were brought alive to Edinburgh where they were killed and eviscerated by a simulated abattoir procedure. Samples were removed as before.

Series 2. Ten female and six castrated male pigs with mean live weight of 93·0 kg (s.d. = 2·8 kg), carcass weight of 73·6 kg (s.d. = 3·7 kg), and age of 183 days

Table 1. *Measurements on m. longissimus of the pigs of Series* 1, *and means of measurements on the pigs of Series* 2

Pig. no.	Live weight (kg)	Age at slaughter (days)	Weight of m. longissimus (g)	TSA of m. longissimus (cm^2)	Depth/width m. longis-simus	Thoracic and lumbar length (cm)
Series 1						
1	1·27	2	12·6	1·61	0·45	16·0
2	3·69	12	49·5	3·75	0·41	22·6
3	3·72	12	53·3	3·32	0·47	23·7
4	3·98	10	49·4	3·00	0·41	23·9
5	4·16	13	56·8	2·68	0·43	24·1
6	7·33	19	123·0	5·90	0·38	30·5
7	7·69	53	94·8	4·29	0·36	32·9
8	8·11	48	96·2	5·68	0·37	33·6
9	9·46	49	125·0	5·68	0·36	34·8
10	13·0	56	200·0	8·15	0·47	38·8
11	13·5	56	208·0	10·40	0·47	41·6
12	15·0	56	209·0	7·94	0·29	42·1
13	25·0	101	376·0	11·05	0·37	51·3
14	27·8	100	456·0	16·19	0·43	54·1
15	28·9	100	486·0	12·33	0·36	52·6
16	57·4	158	1140·0	35·17	0·60	54·7
17	59·0	214	1091·0	25·74	0·40	64·2
18	59·6	170	990·0	22·09	0·40	62·9
Series 2 (N = 16)						
Mean	93·0	183·3	—	33·0	0·70	—
S.d.	2·8	10·2	—	2·8	0·12	—

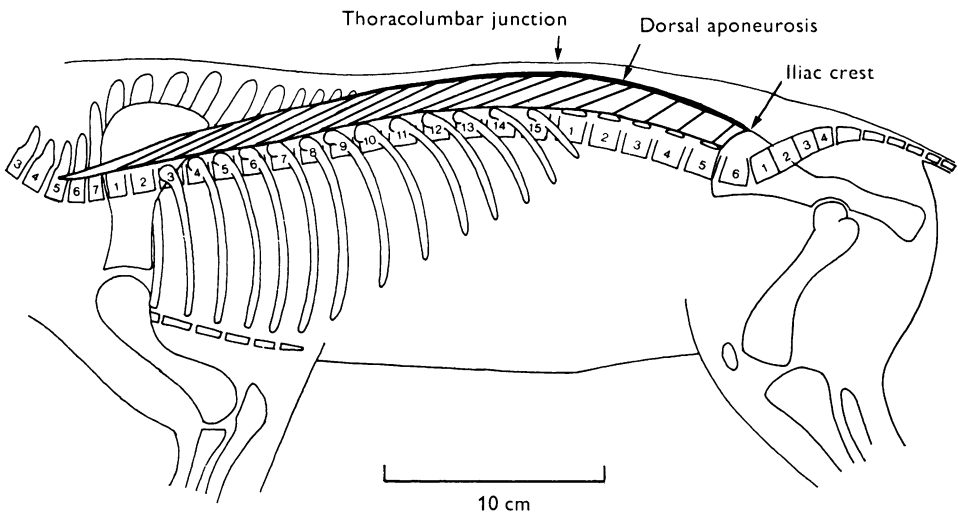


Fig. 1. Diagram of pig, live weight 6·0 kg, age 21 days, showing the location of m. longissimus and the angles of fibres to the vertebral axis.

(s.d. = 10 days), were sampled at a commercial abattoir at Stirling. These pigs had been reared under test conditions, and came from a variety of herds in which selection for improved carcass conformation was practised. Samples of longissimus and diaphragm were removed from similar regions to Series 1 within 45 minutes of slaughter, chilled, and brought to Edinburgh for processing. All muscle samples were removed pre-rigor and were therefore fully contracted.

In addition to the above two series of pigs, other pigs of mixed breeding and varying weights were used for studies not directly involved in the measurement of growth changes.

Measurements on m. longissimus

Series 1. The longissimus muscle was dissected from the right side of each pig, cleaned of superficial fat and weighed. An outline of the transverse sectional area (TSA) at the thoracolumbar junction was drawn on paper. This TSA was measured by a paper weighing method. The distance between the cranial end of the body of the first thoracic vertebra and the caudal end of the body of the sixth lumbar vertebra was measured. The combined length of the thoracic and lumbar vertebrae approximates to, and is proportional to, the length of m. longissimus.

Series 2. The TSA of the muscle was measured after the carcass had been bisected at the thoracolumbar junction. The weight of m. longissimus and the length of the thoracic and lumbar regions were not obtained.

Preparation of histological material

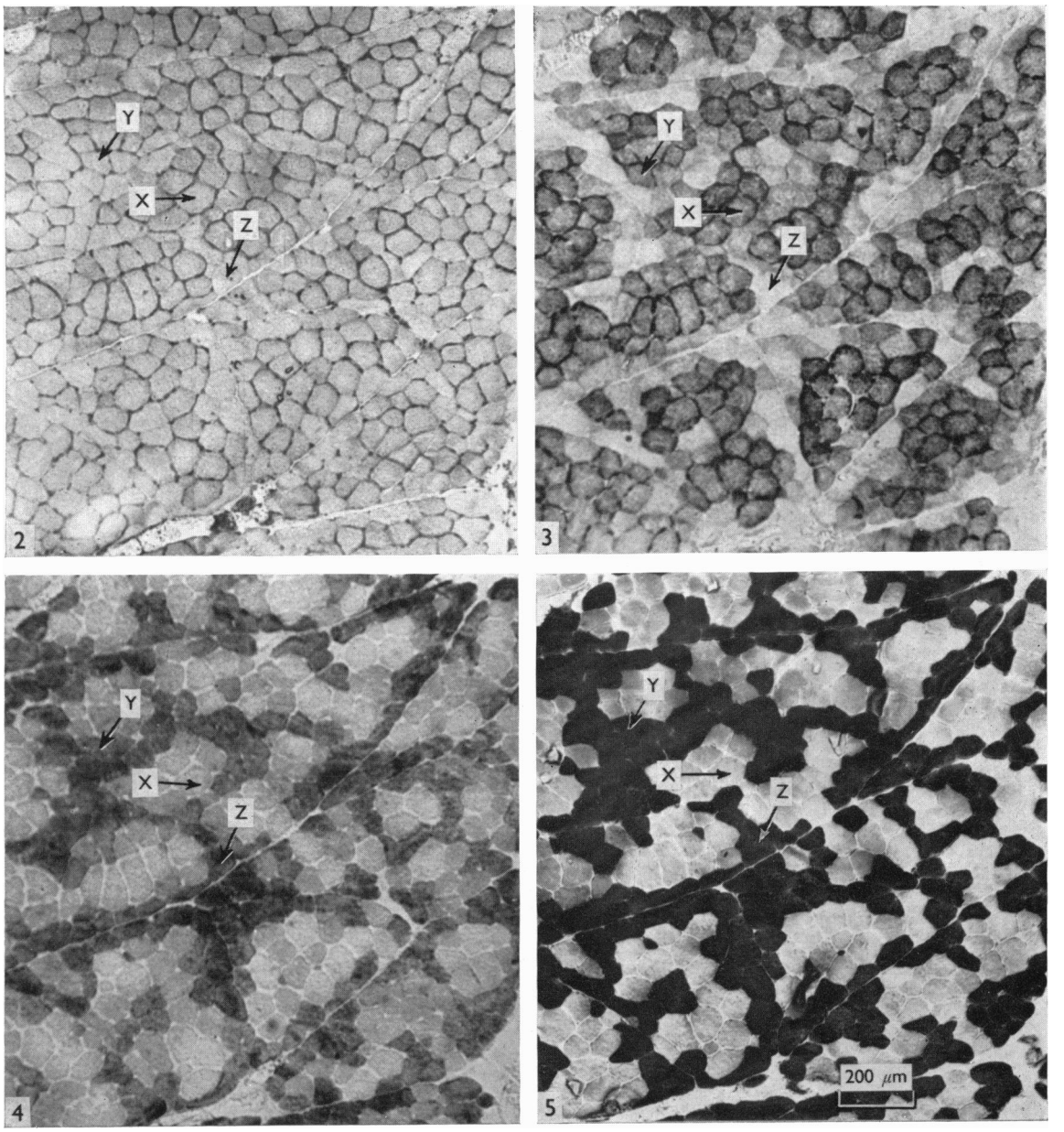
After rapid freezing of a block of fresh muscle, about ten adjacent serial sections were cut, 10 μm thick, transversely to the direction of the muscle fibres. Sections were treated to demonstrate the activity of the enzymes succinate dehydrogenase (SDHase), glycogen phosphorylase (GPase) and myosin ATPase, by modifications of the methods originally developed by Nachlas *et al.* (1957), Takeuchi (1956) and Padykula & Herman (1955) respectively. Cell outlines were demonstrated by staining with Ehrlich's haematoxylin. Details of the methods used in freezing and cutting the muscles, and the histochemical reactions, have been given in a previous communication (Davies & Gunn, 1972).

Determination of histochemical profiles of muscle fibres

Profiles of between 300 and 800 individual fibres in each sample were established by first back-projecting a haematoxylin stained section on to a glass screen, and then tracing the fibre outlines on to transparent paper. Each serial section was then projected in turn. Figs. 2–5 demonstrate the type of material used. The histochemical reaction of every fibre in the region sampled was estimated as either high or low relative to the other fibres in the same sample, and the reaction was indicated on the tracing. The numbers of each fibre type so determined were counted, and the proportions were calculated.

Estimation of mean TSA of muscle fibre types

The areas of paper representing the three main fibre types (myosin ATPase low, SDHase high; myosin ATPase high and SDHase either high or low), prepared as above, were weighed to give a measurement of the proportion of the TSA occupied by each fibre type.



Figs. 2–5. Transverse serial fresh frozen sections of the diaphragm of a Large White pig, live weight 25 kg, age 101 days, demonstrating fibre outlines with Ehrlich's haematoxylin (Fig. 2), and the activity of SDHase (Fig. 3), GPase (Fig. 4) and myosin ATPase (Fig. 5). X, Y and Z indicate (Al, Sh, Pl), (Ah, Sh, Ph) and (Ah, Sl, Ph) fibres respectively.

Measurement of the TSA frequency distribution of muscle fibre types

Sections of both longissimus and diaphragm from three pigs of live weights 4·0, 13 and 98 kg were chosen because they represented characteristic stages in the development of histochemical fibre types. Fibre type profiles, using the myosin ATPase and SDHase reactions only, were made on tracing paper. Individual TSAs of 300 to 400 fibres for each muscle were measured with a compensating planimeter, and frequency polygons for each fibre type were constructed.

Measurements on 'myosin ATPase low bundles' in longissimus

Low power back-projection of sections stained for myosin ATPase enabled a count of the number of myosin ATPase low bundles in an area whose magnification was chosen to include from 30 to 120 bundles. Using the measurement of TSA of the whole muscle, an estimate of the total number of bundles in the TSA was made. At the same time, the number of myosin ATPase low fibres in each bundle was recorded, and the mean number calculated. In addition, similar measurements were made on several regions of a complete section of longissimus of a 21 day old, 6·0 kg live weight, Large White X Landrace female pig, cut transversely to the direction of the muscle fibres at the thoracolumbar junction.

Statistical methods

Differences in the mean values of fibre type proportions between muscles, in estimates of the number of myosin ATPase low bundles in the transverse section of longissimus between groups of pigs, and in the number of myosin ATPase low fibres per bundle between groups of pigs, were tested for significance at the 5 % level by Student's *t* test. The significance of the difference in mean TSA between fibre types was tested on paired data within samples or within pigs at the 5 % level by Student's *t* test. Regression lines, significance of difference between regression coefficients at the 5 % level, and analysis of variance were calculated by methods outlined by Diem & Lentner (1970).

RESULTS

Aspects of the anatomy of longissimus and diaphragm of the pig

Fibre architecture (Fig. 1)

As in other mammals, fasciculi in the costal diaphragm of the pig run directly to the central tendon from their origins on the ribs and costal cartilages. Fasciculi within m. longissimus thoracis et lumborum of the pig originate on the ribs, transverse processes of the lumbar vertebrae, and intertransverse ligaments. They pass caudally at an angle to the vertebral axis that becomes greater towards the lumbar region (Fig. 1), and laterally at an angle to the sagittal plane of approximately 20° at the thoracolumbar junction, to end on a thick aponeurosis that covers the muscle dorsally before being inserted on to the iliac crest. These large paired muscles, that comprise 3·4 % of the weight of a pig of 64 kg live weight, are therefore powerful extensors of the thoracic, lumbar and lumbosacral intervertebral joints, and provide a propulsive thrust to the pelvic limbs.

Table 2. *Longissimus and diaphragm; Series* 2

(Mean proportions of fibre types based on three histochemical reactions.)

Histochemical reaction, h = high, l = low			Longissimus, 15 pigs; 5763 fibres		Diaphragm, 16 pigs; 7299 fibres		Significance of difference
Myosin ATPase	SDHase	GPase	Mean %	S.d.	Mean %	S.d.	
Al	Sh	Ph	2·0	3·34	5·0	6·2	N.S.
Al	Sh	Pl	15·8	6·0	31·5	6·8	$P < 0·001$
Ah	Sh	Ph	13·9	5·9	41·0	7·4	$P < 0·001$
Ah	Sh	Pl	3·0	4·8	4·2	3·0	N.S.
Ah	Sl	Ph	60·3	9·0	14·1	7·3	$P < 0·001$
Ah	Sl	Pl	5·0	7·2	4·2	5·0	N.S.

Table 3. *Longissimus and diaphragm; Series* 2

(Mean proportions of fibre types based on two histochemical reactions.)

Histochemical reaction, h = high, l = low		Longissimus, 16 pigs; 6151 fibres		Diaphragm, 16 pigs; 7299 fibres		Significance of difference
Myosin ATPase	SDHase	Mean %	S.d.	Mean %	S.d.	
Al	Sh	18·0	4·5	36·5	5·9	$P < 0·001$
Ah	Sh	17·3	4·0	45·2	7·4	$P < 0·001$
Ah	Sl	64·7	4·8	18·3	7·3	$P < 0·001$

Incidence and proportion of histochemical fibre types (Tables 2, 3)

In both longissimus and diaphragm, six types of muscle fibre could be identified by establishing profiles with the three histochemical reactions (Table 2). No fibres low in both myosin ATPase and SDHase activity were observed. The GPase reaction within individual fibres corresponds to the myosin ATPase reaction in all except 10 % of fibres in the longissimus and 13 % of fibres in the diaphragm of the pigs of Series 2; those which did not correspond are of three types, none of which exceeds 5 % of the total fibre population or differs significantly in proportion between the two muscles. In both muscles, regions were frequently seen where the incidence of GPase high fibres was reduced. In one sample of longissimus, these regions were too extensive for the GPase reaction to be used to determine fibre profiles. This sample has been excluded from the data in Table 2.

Fibres in which the myosin ATPase and GPase reactions correspond occur as three types; each exceeds 10 % of the total fibre population and differs significantly ($P < 0·001$) in proportion between the two muscles. When only the myosin ATPase and SDHase reactions are used to determine profiles, the standard deviation of these three fibre types is either reduced or remains constant (Table 3). Because the incorporation of the GPase reaction into a classification of muscle fibres introduces a slight variability that could confuse differences between muscles, many of the subsequent results use classifications under the three main fibre types.

Table 4. *Longissimus and diaphragm; Series* 2

(Mean TSA of all fibres sampled, of myosin ATPase low fibres (Al, Sh), and of fibres myosin ATPase high and either SDHase high (Ah, Sh) or low (Ah, Sl).)

	Longissimus			Diaphragm		
Fibre type	Mean TSA (μm^2)	S.d.	Coeff. of variance (%)	Mean TSA (μm^2)	S.d.	Coeff. of variance (%)
All fibres	5800	1100	19	4500	1100	24
Al, Sh	4700	900	19	4600	900	20
Ah, Sh	4500	1100	24	4100	1200	29
Ah, Sl	6400	1400	22	5200	1600	31

Comparison of the mean TSA of three types of fibres (Table 4)

In both muscles, the coefficient of variance of the TSA is lowest for the myosin ATPase low, SDHase high (Al, Sh) fibre type. The mean TSA of fibres high in myosin ATPase activity is significantly greater ($P < 0{\cdot}001$) when the SDHase activity is low. Fibres high in SDHase activity have a greater TSA when the myosin ATPase activity is low; the difference is significant in the diaphragm ($P < 0{\cdot}02$) but not significant in longissimus.

A comparison of fibre TSA between the two muscles shows that the mean TSA of all fibres is significantly greater ($P < 0{\cdot}01$) in longissimus. Since the mean TSA of Al, Sh and myosin ATPase high, SDHase high (Ah, Sh) fibres is not significantly different between the muscles, this difference must be mainly due to the significantly greater ($P < 0{\cdot}05$) mean TSA of the Ah, Sl fibres.

Organization of histochemical fibre types (Figs. 3–5)

There is a greater degree of organization of fibre types in porcine muscle than in other species. In the longissimus and diaphragm, one or more bundles of fibres characterized by low activity of myosin ATPase and GPase, but high activity of SDHase (Al, Sh, Pl fibres) are located within each fasciculus. In the diaphragm, these 'myosin ATPase low bundles' contain more fibres than in longissimus. Myosin ATPase low bundles are surrounded by a zone of fibres with high activity of myosin ATPase, GPase and SDHase (Ah, Sh, Ph fibres). Elsewhere, the fasciculus is occupied by fibres high in myosin ATPase and GPase, but low in SDHase activity (Ah, Sl, Ph fibres).

Distribution of myosin ATPase low (Al) fibres in m. longissimus (Fig. 6)

The number of Al bundles per mm^2, and the mean number of Al fibres per bundle, is greater in the medial and lateral regions of the muscle than in the middle region. There appears to be a direct relation between the density of the bundles and the number of Al fibres per bundle. Samples for the growth study were taken from the dorsomedial region of the muscle; sampling this region may introduce an error due to the variation in distribution of fibre types across the region. The mean number of Al fibres per bundle in sections of longissimus of the pigs of Series 2 was therefore

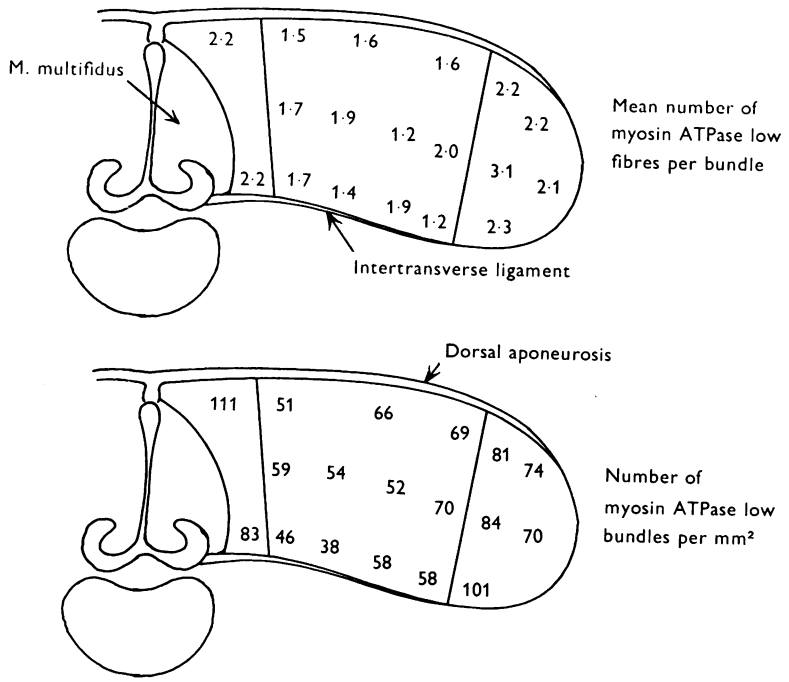


Fig. 6. Variation in the characteristics of myosin ATPase low bundles in a complete transverse section of m. longissimus at the thoracolumbar junction of a pig, live weight 6·0 kg, age 21 days. The vertical lines separate medial and lateral regions of the muscle containing myosin ATPase low bundles with a mean number of AI fibres per bundle greater than 2·0, and a density of AI bundles greater than 70 per mm².

tested by an analysis of variance. The variance within pigs, between two regions of each section approximately 1 cm apart, is significantly less ($P < 0{\cdot}05$) than the variance between pigs. Therefore it is unlikely that the method of sampling affects the interpretation of the results.

Growth changes in longissimus and diaphragm of the Large White pig

Relative growth of weight, length and TSA of longissimus (Table 1, Fig. 7)

The measurements of weight of longissimus, TSA of longissimus at the thoracolumbar junction, and the combined lengths of the thoracic and lumbar vertebrae for the pigs in Series 1 are shown in Table 1. These data are plotted as a double logarithmic regression in Fig. 7. The slopes of the two lines suggest that throughout the period of growth studied, the TSA is proportional to the 2/3 power, and the length is proportional to the 1/3 power of the weight of longissimus. The values of the ratio of depth to width of the muscle at the thoracolumbar junction do not appear to change with growth in Series 1. The mean ratio is 0·41, with a standard deviation of 0·07. The value of this ratio is significantly higher in Series 2 ($P < 0{\cdot}001$), possibly because the measurements were made on intact carcasses in Series 2, and on isolated muscles in Series 1.

Since the dimensions of the muscle in Series 1 maintain a constant proportion

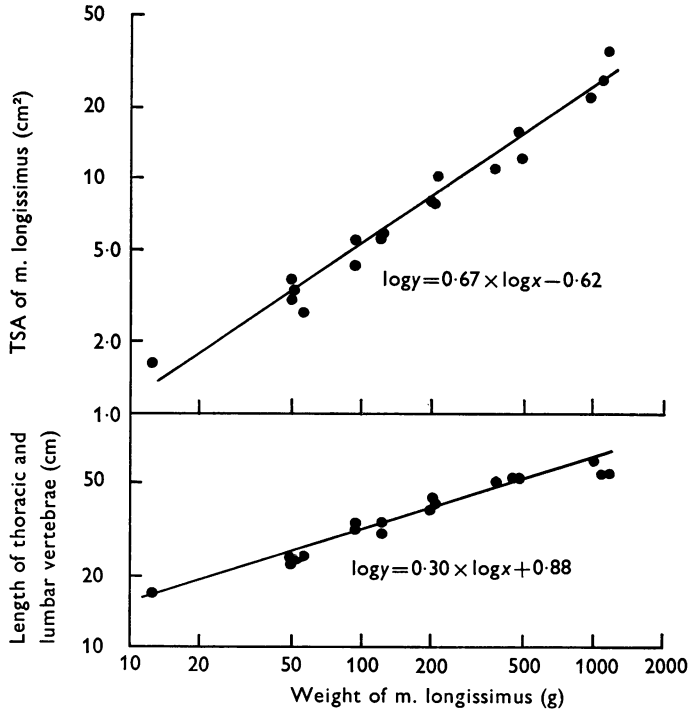


Fig. 7. M. longissimus of 18 Large White pigs (Series 1). Regression of log (TSA at thoracolumbar junction) and log (length of thoracic and lumbar vertebrae) on log (weight of m. longissimus).

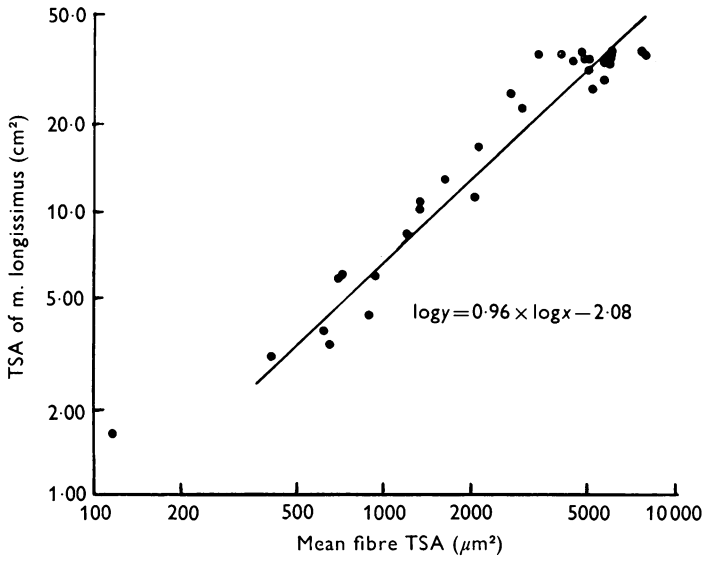


Fig. 8. M. longissimus of 34 Large White pigs (Series 1 and 2). Double logarithmic regression of TSA of the whole muscle on mean fibre TSA at the thoracolumbar junction.

with one another, and the angles between the fibres in a given region of the muscle and the vertebral axis (as shown in Fig. 1) do not appear to change with growth, it is possible at all stages of growth to relate the mean fibre TSA, as measured on a section cut transversely to the direction of the fibres, to the TSA of the whole muscle cut transversely to the vertebral axis.

Growth in mean fibre TSA and total fibre population of m. longissimus (Fig. 8)

The double logarithmic regression of the mean fibre TSA and the TSA of the whole muscle is linear ($r = 0{\cdot}97$) for all the pigs of both series except one at two days old. Since the regression coefficient of 0·96 (s.d. = 0·04) is not significantly different from 1, the mean fibre TSA is directly proportional to the TSA of the muscle. The histological appearance of the endomysium and perimysium does not suggest a disproportionate development of tissues other than muscle fibres. After 10 days of age, growth in TSA (and hence weight of the muscle) is therefore accounted for by the growth of a constant population of muscle fibres.

Changes in the TSA frequency distribution of three fibre types in longissimus and diaphragm (Figs. 9, 10)

TSA frequency polygons for histochemical fibre types of three pigs are shown for longissimus in Fig. 9 and for diaphragm in Fig. 10. The frequency intervals for pigs of live weight 4·0, 13 and 98 kg are 250, 500 and 1000 μm^2 respectively. Since the frequency intervals are represented by the same length of abscissa for each graph, and the frequency is shown as a percentage of fibres sampled, the sum of the areas under the polygons is the same for each of the six muscles. The area under each polygon indicates the proportion of the muscle occupied by each fibre type. The polygons indicate graphically the differences in the proportion of fibre types discussed in the next paragraph, both between the two muscles and between characteristic growth stages. In addition, they indicate that although there is some relationship between fibre type and TSA, there is considerable overlapping of these properties within individual fibres.

Changes in the proportion of fibre types in longissimus and diaphragm (Tables 2, 5; Figs. 9–12, 16)

Table 5 shows, for the pigs of Series 1, the proportion of fibres low in activity of each of the enzymes used. The data are derived from a classification of six fibre types for both muscles of each pig, similar to that in Table 2. In both muscles, the proportion of myosin ATPase low fibres increases with increasing body size (Figs. 9–12, 16).

At 2 days of age, both muscles are composed entirely of SDHase high fibres. Fibres low in the activity of this enzyme are differentiated in both muscles by 12 days after birth. In longissimus, the proportion of SDHase low fibres does not appear to change after this early differentiation process. The diaphragm has a lower proportion of SDHase low fibres in the pigs of Series 2, but since the grading of the reaction between fibres is not as clear-cut in this muscle as in longissimus (Figs. 9C, 10C), this difference cannot be regarded as significant. It is apparent, however, that in both muscles the proportion of SDHase low fibres does not increase with increasing body size.

Table 5. *Longissimus and diaphragm; Series* 1 *and* 2

(Fibres low in myosin ATPase (Al), SDHase (Sl) and GPase (Pl) as a percentage of total fibres sampled.)

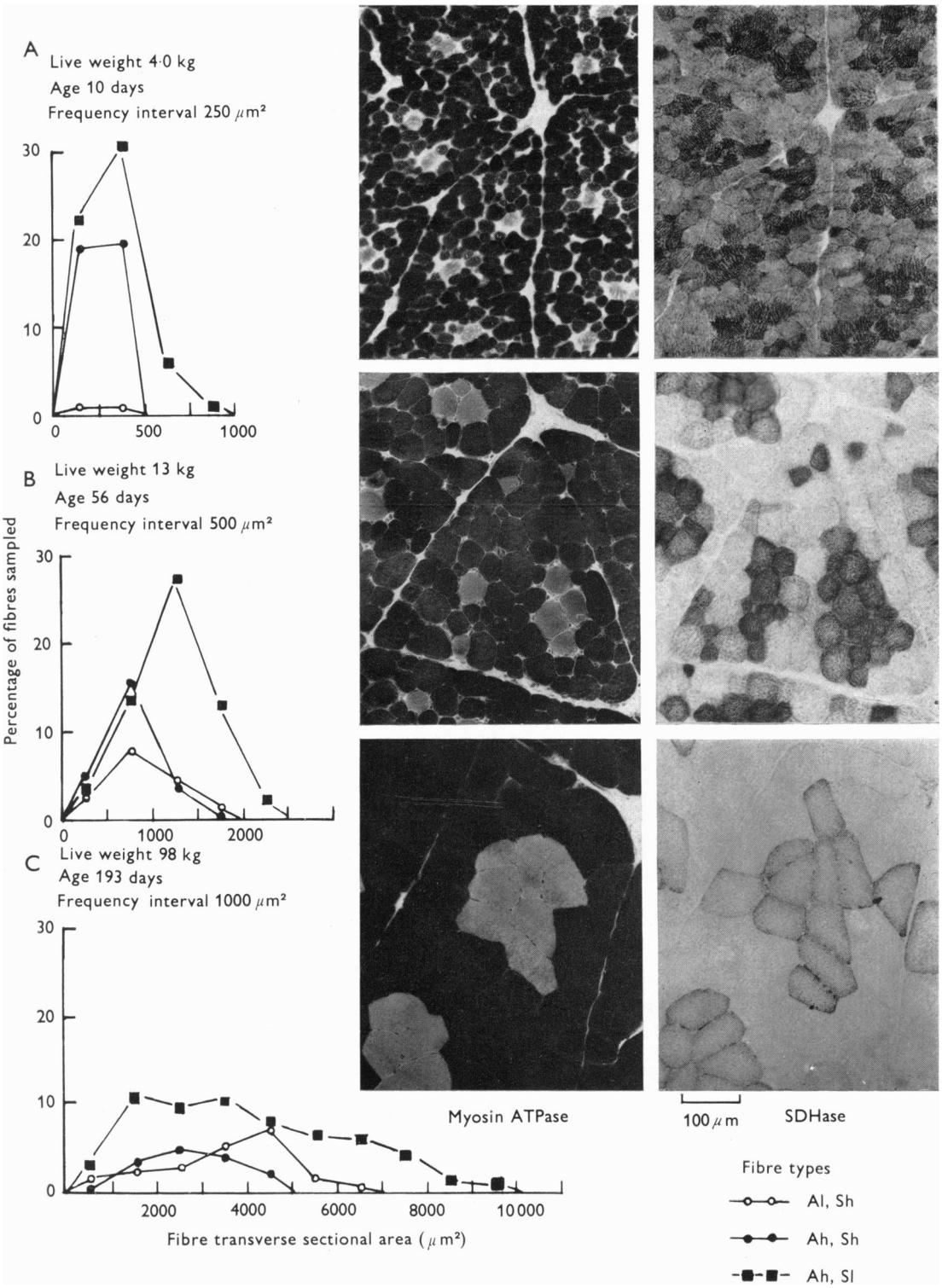
Pig no.	Live weight (kg)	Percentage of fibres: Longissimus Sl	Longissimus Pl	Longissimus Al	Diaphragm Sl	Diaphragm Pl	Diaphragm Al
1	1·27	*	*	2·0	*	*	10·4
2	3·69	63·8	5·8	4·3	*	*	18·5
3	3·72	62·7	5·6	4·0	42·3	36·6	20·7
4	3·98	63·3	4·0	3·2	*	24·6	20·0
5	4·16	62·1	2·9	2·9	38·1	38·1	24·3
6	7·33	64·1	8·2	5·5	32·6	37·0	26·6
7	7·69	62·2	7·1	5·3	41·8	33·1	25·7
8	8·11	58·5	16·1	9·2	23·8	12·2	22·5
9	9·46	61·2	11·6	5·5	38·4	23·5	22·7
10	13·0	58·4	13·5	13·9	20·5	31·6	29·9
11	13·5	61·0	7·5	7·7	29·2	47·7	36·9
12	15·0	57·4	15·3	9·3	24·7	31·6	28·7
13	25·0	56·5	—	14·1	34·3	41·1	39·9
14	27·8	63·4	—	8·9	22·1	35·1	36·5
15	28·9	74·5	14·3	7·4	32·9	28·1	36·2
16	57·4	82·4	14·5	3·3	38·8	30·0	33·9
17	59·0	55·6	14·7	13·6	31·0	34·5	36·2
18	59·6	72·6	14·3	9·3	28·4	34·1	36·9
Series 2 (N = 16)							
Mean	93·0	64·7	28·6	18·6	18·3	39·9	36·5
S.d.	2·8	4·8	11·6	4·5	7·3	8·1	5·9

* Indicates that fibres were insufficiently differentiated for quantitation by the histochemical reactions.

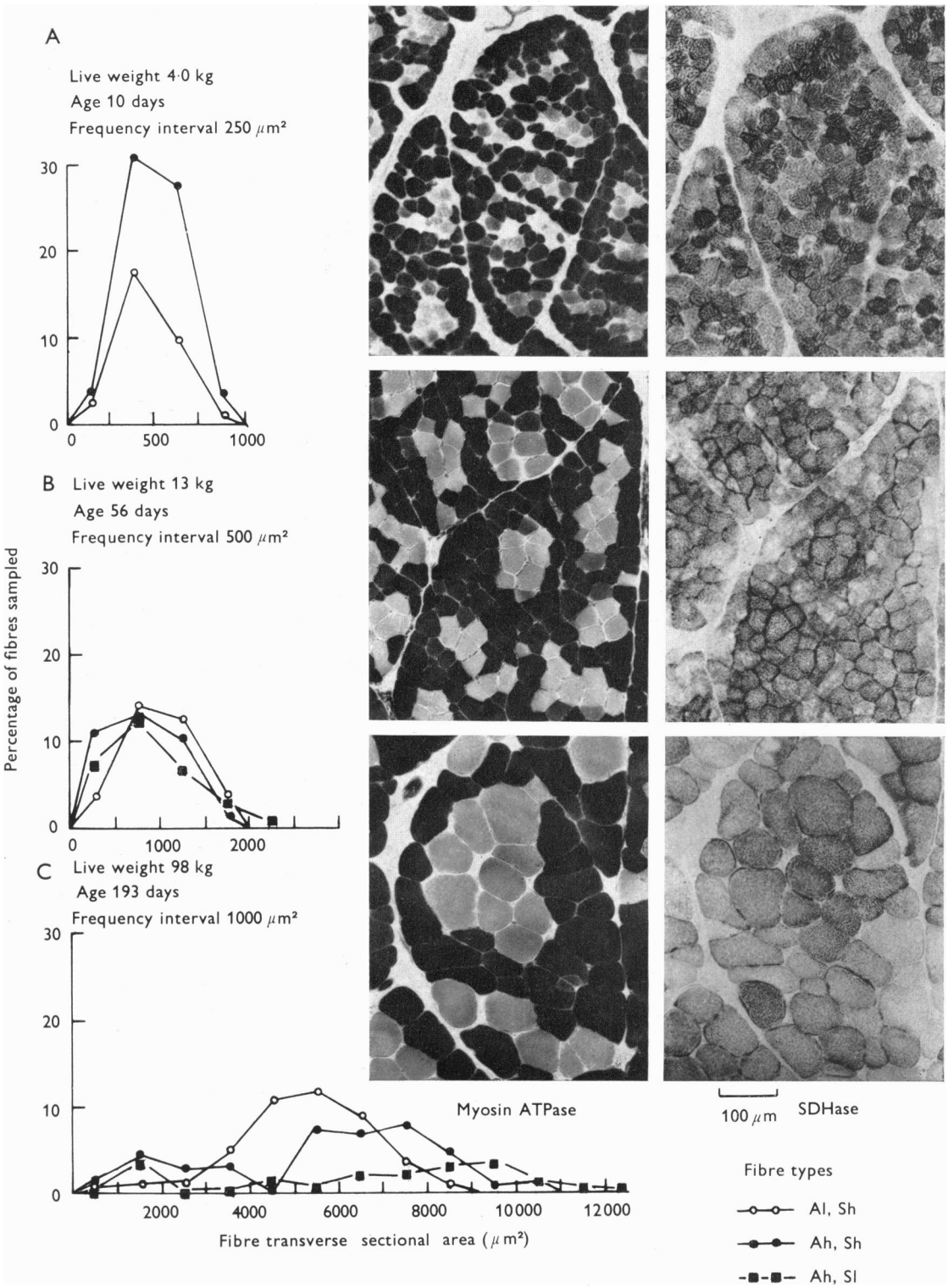
At two days, the activity of GPase in all fibres is low. By 10 days of age, activity of this enzyme has usually developed sufficiently to distinguish fibres with different levels of activity, but before 13 days of age, iodine stains the GPase high fibres pink or purple rather than the blue colour characteristic of the GPase high fibres of more mature pigs. In the diaphragm of the smaller pigs of Series 1, a fibre type in which the GPase and myosin ATPase reactions do not correspond occurs in significant proportions. This is the Ah, Sh, Pl fibre type that averages 7·4 % of the total fibre population in 10 pigs from 10 to 56 days of age. This fibre is responsible for the higher proportion of GPase low fibres than myosin ATPase low fibres over this period (Table 5). The diaphragms of the older pigs of Series 1, and longissimus muscles of all pigs subsequent to the early differentiation process, are principally composed of the three main fibre types previously described for the pigs of Series 2.

Changes in the 'myosin ATPase low bundles' in longissimus (Table 6)

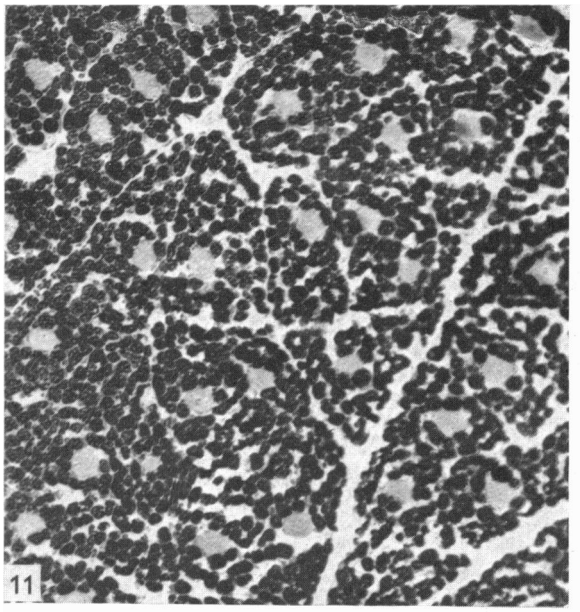
The pigs of Series 1 are divided into four groups, A–D. Group A consists of only one pig of 1·3 kg live weight. Groups B–D comprise pigs of live weights ranging from 3·0 to 7·5 kg, 7·5 to 15 kg and 15 to 60 kg respectively. Group E includes all the pigs of Series 2. Although the estimate of the total number of myosin ATPase low bundles

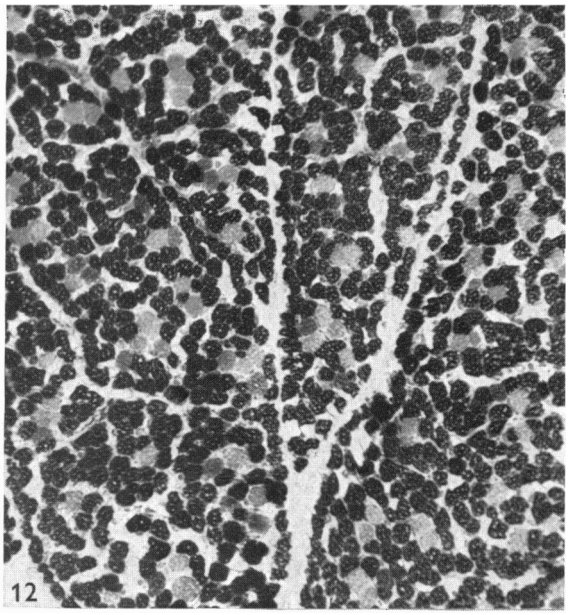


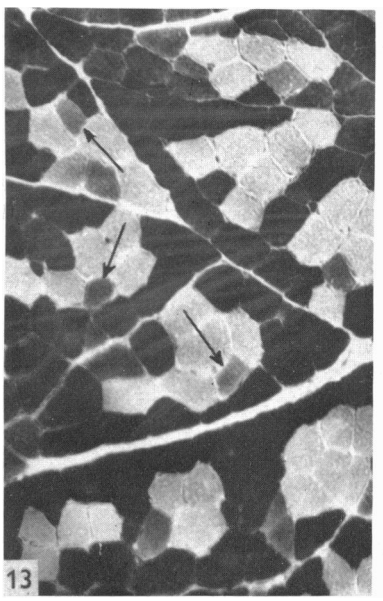
Figs. 9A–C. Fibre types in m. longissimus at three stages of growth.

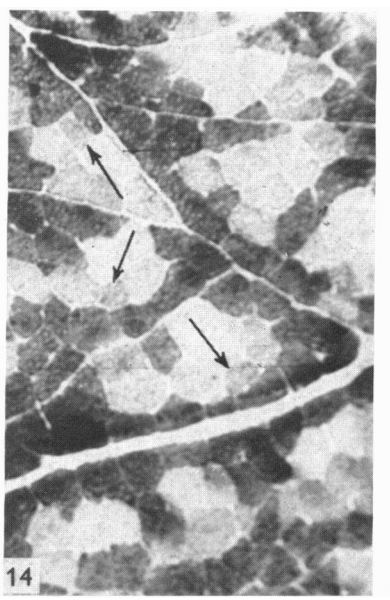


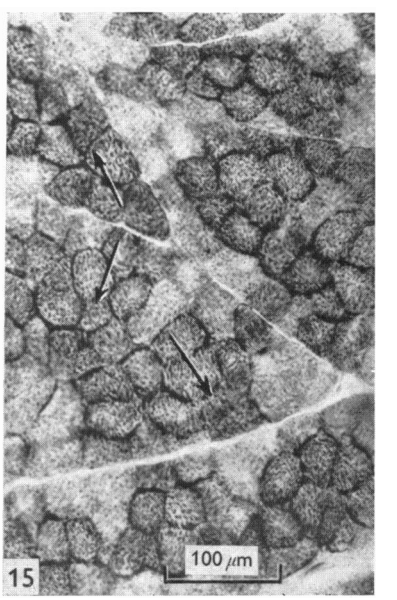
Figs. 10A–C. Fibre types in the diaphragm at three stages of growth.











Figs. 11, 12. Transverse frozen sections of longissimus (Fig. 11) and diaphragm (Fig. 12) of a Large White pig, live weight 1·3 kg, age 2 days. Myosin ATPase.

Figs. 13–15. Transverse serial frozen sections of the diaphragm of a Large White pig, live weight 13 kg, age 56 days. Myosin ATPase (Fig. 13), GPase (Fig. 14) and SDHase (Fig. 15). Arrows indicate examples of 'transition' fibres.

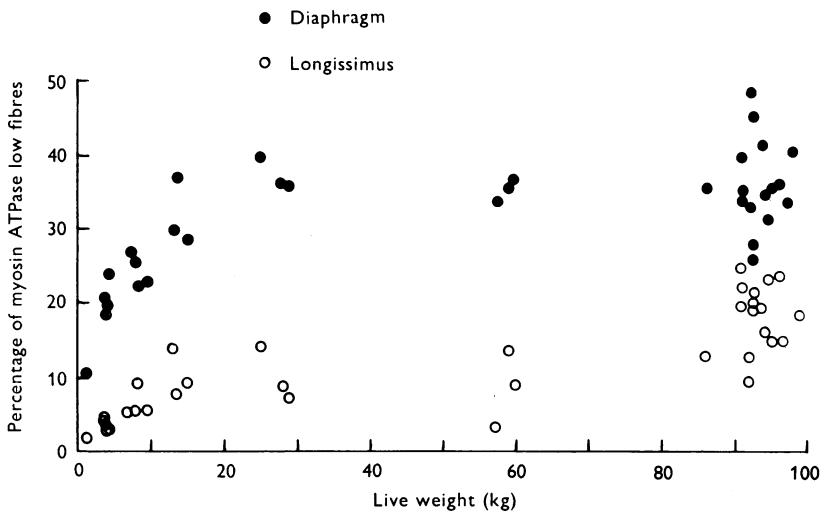


Fig. 16. Longissimus and diaphragm of 34 Large White pigs (Series 1 and 2). Growth changes in the proportion of myosin ATPase low fibres.

Table 6. *Growth changes in the myosin ATPase low bundles of m. longissimus*

Group	Range of live weights (kg)	No. of pigs	Estimate of no. of bundles in T.S. of muscle ($\times 10^4$)		No. of myosin ATPase low fibres per bundle	
			Mean	S.d.	Mean	S.d.
A	1·3	1	2·35	—	1·0	—
B	3·0–7·5	5	1·64	0·42	1·20	0·20
C	7·5–15	6	2·60	0·73	1·99	0·40
D	15–60	6	2·22	0·67	2·21	0·61
E	85–97	16	2·70	0·42	3·24	0·50

in a complete transverse section of longissimus is significantly lower in group B than in group C, the estimate of the number of bundles does not vary significantly between the other groups, suggesting that the number of myosin ATPase low bundles in longissimus does not change during the period of growth studied. There is, however, a steady increase in the mean number of myosin ATPase low fibres per bundle between all stages of growth; the difference is significant between groups B and C, and between groups D and E. This change is also seen by comparing Figs. 9A and 11 with Figs. 9B and 9C.

Occurrence of 'transitional' fibres (Table 7; Figs. 13–15)

Especially in pigs between birth and 15 kg live weight, fibres with intermediate reaction for myosin ATPase are seen in both longissimus and diaphragm (Figs. 13–15). These fibres are always adjacent to myosin ATPase low bundles. A large number of these fibres was seen in a sample of diaphragm from a 13 kg, 56 day old pig.

Table 7. *Histochemical profiles of 'transitional' and other fibre types in the diaphragm of a Large White pig, live weight 13 kg*

Fibre type	Percentage of fibres (N = 1232)	Mean TSA (μm^2)	Percentage area of muscle (area sampled = 1·2 mm^2)
Myosin ATPase high, SDHase high or low, GPase high or intermediate	64·6	957	63·3
Myosin ATPase intermediate, SDHase high, GPase intermediate ('transitional' fibre)	6·3	773	5·0
Myosin ATPase low, SDHase high, GPase intermediate	10·6	934	10·2
Myosin ATPase low, SDHase high, GPase low	18·4	1142	21·5

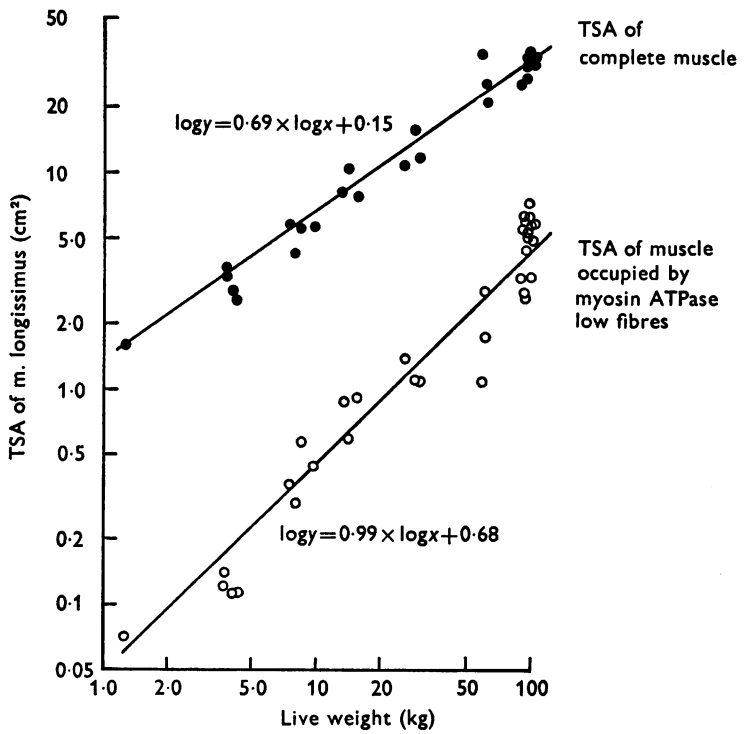


Fig. 17. Growth changes in m. longissimus of 34 Large White pigs at the thoracolumbar junction (Series 1 and 2). Total transverse sectional area compared with the area occupied by fibres low in myosin ATPase activity.

Fibre profiles were determined for this sample, and areas were measured by the paper weighing method. Details of the fibre types are shown in Table 7. In this classification, myosin ATPase high fibres are not differentiated by the SDHase reaction. Myosin ATPase high, intermediate and low fibres are classified as high, intermediate and low by the GPase reaction; elsewhere in this study GPase intermediate fibres are classified as GPase low (see p. 216).

The following observations may be listed:

(i) 'Transitional' fibres have an SDHase activity as high as that of myosin ATPase low fibres, but a smaller mean TSA.

(ii) Large myosin ATPase low fibres have a lower GPase reaction.

(iii) The gradation in mean TSA and in the myosin ATPase and GPase reactions between these fibre types suggests a transition from one type to another in the order shown in Table 7.

(iv) The 'transitional' fibres appear to originate from those fibres of the first type that are myosin ATPase and SDHase high, are GPase intermediate, have a low mean TSA, and are adjacent to the myosin ATPase low bundles.

Changes in the TSA of longissimus occupied by myosin ATPase low fibres (Fig. 17)

The relationship between live weight and both the complete TSA of longissimus, and the TSA occupied by myosin ATPase low fibres, is represented in Fig. 17 as a double logarithmic regression for the pigs of both series. The slopes of the two regression lines are significantly different ($P < 0{\cdot}001$) from one another. The regression coefficient for the TSA of the whole muscle is not significantly different ($P > 0{\cdot}05$) from 0·67, and the regression coefficient for the TSA occupied by myosin ATPase low fibres is not significantly different ($P > 0{\cdot}05$) from 1.

These results support the hypotheses that the TSA of a muscle, which is growing proportionately to the rest of the body, varies as the 2/3 power of the body weight; and that, in such a muscle, the TSA occupied by myosin ATPase low fibres bears a direct proportion to body weight.

DISCUSSION

Fibre type classification

The evidence that the histochemical reactions for SDHase and GPase demonstrate the capacity of a muscle fibre to use aerobic and anaerobic metabolism respectively, and that the myosin ATPase activity is related directly to its intrinsic speed of contraction has been discussed by Davies & Gunn (1972). This evidence is accepted in the present discussion. Muscle fibres are classified, first by their reaction for myosin ATPase as fast- or slow-twitch fibres, and secondly by their reaction for SDHase and GPase as aerobic, anaerobic, or combined aerobic and anaerobic fibres.

Histochemical fibre types in porcine muscle

The grouping of histochemical fibre types into bundles within the fasciculi of porcine muscle is demonstrated by methods for reduced nicotinamide adenine dinucleotide tetrazolium reductase ($NADH_2$-TR) and GPase (Moody & Cassens, 1968), lipids (Todorov & Petrov, 1969), myoglobin and $NADH_2$-TR (Morita, Cassens

& Briskey, 1969), SDHase, cytochrome oxidase and lipids (Sair *et al.* 1970) and $NADH_2$-TR, GPase and myosin ATPase (Cooper, Cassens, Kastenschmidt & Briskey, 1970). Although the distribution of fibre types is probably not random in any species, as shown by James (1971*a*, *b*, 1972) for rabbit and guinea-pig muscles and by Jennekens, Tomlinson & Walton (1971*b*) for human muscles, the muscles of the pig so far examined exhibit a much more orderly arrangement than those of any other species described. No reports are available on the muscle histochemistry of other species within the suborder *Suiformes*.

Moody & Cassens (1968) describe the histochemistry of longissimus and trapezius of the Chester White pig at 90 kg live weight by the use of reactions for $NADH_2$-TR and GPase. They find that the fibre population of longissimus is composed of 30·5 % (s.d. = 5·0) aerobic fibres and 17·0 % (s.d. = 4·3) GPase low fibres. These findings are similar to the present estimations of 35·3 % (s.d. = 4·8) of aerobic fibres and 28·6 % (s.d. = 11·6) of GPase low fibres (Table 5). These workers did not, however, establish histochemical profiles of individual fibres, and so could not assess the GPase activity of individual aerobic fibres; hence their conclusion that the activity of these two enzymes is reciprocal is questionable, and is not supported by the present observations or by those of Davies & Gunn (1972). The 'intermediate' (fast-twitch, combined aerobic and anaerobic) fibre described by Moody & Cassens (1968) is not equivalent to the slow-twitch aerobic fibre described as having 'intermediate' aerobic capacity in crural muscles of the rat and guinea-pig by Edgerton & Simpson (1969). In the diaphragm of the pig, slow-twitch fibres have a higher SDHase activity than fast-twitch fibres (Davies & Gunn, 1972). The term 'intermediate' in the context of fibre type classification is therefore misleading.

Meijer (1968*a*) showed that the histochemical demonstration of GPase depends on the presence of tissue glycogen. Therefore when complete ante-mortem or post-mortem glycolysis has occurred in a muscle fibre, GPase cannot be demonstrated by the method used in this study. Regions with very few GPase high fibres are seen in some samples from commercially slaughtered pigs. Their focal nature suggests that they are due to post-mortem glycolysis, since this phenomenon can occur to a different extent in closely adjacent regions of the same muscle (Lawrie, Gatherum & Hale, 1958). The existence of GPase activity in these affected fibres could be tested by comparing them with serial sections incubated in a medium containing dextran (Meijer, 1968*b*).

Relation between size, metabolism and rate of energy conversion of muscle fibres

The time taken for oxygen to diffuse into the centre of a fibre is proportional to its transverse sectional area (Hill, 1965). Hence, the TSA of a fibre would be expected to be influenced both by its dependence on aerobic metabolism and by the rate of energy conversion within the fibre.

An inverse relationship between fibre TSA and aerobic capacity is well established. In the biceps brachii of mice, predominantly aerobic fibres are narrower (Goldspink, 1969) and, in the present study, myosin ATPase high fibres in the longissimus and diaphragm have smaller TSAs if the SDHase activity is high. Fibres converting energy at the same rate, as determined by their myosin ATPase activity, are more slender and have more adjacent capillaries (Romanul, 1965; Cooper, Cassens &

Briskey, 1969) if they depend on oxygen and blood-borne nutrients for their energy source.

The influence of rate of energy conversion on the TSA of a fibre has not received the same attention. In the diaphragm of the pig the myosin ATPase low fibres have the densest reaction for SDHase and a greater mean TSA than the surrounding myosin ATPase high fibres, which react for both aerobic and anaerobic enzymes. Thus, it appears that when the energy demands are low, a large diameter fibre can obtain sufficient fuel for a predominantly aerobic metabolism.

Only fast-twitch anaerobic fibres are significantly different in TSA between muscles as diverse in function as the diaphragm and longissimus of the pig. Jennekens, Tomlinson & Walton (1971*a*) showed that slow-twitch aerobic fibres are relatively larger in the rectus femoris and gastrocnemius than in the deltoid and biceps brachii muscles of man. Engel, Brooke & Nelson (1966) demonstrated the relative susceptibility to atrophy of slow-twitch fibres following tenotomy and fast-twitch fibres following experimental denervation. The extent to which the differences in mean fibre TSA between muscles of mature animals, and between individuals of the same species, are due to the relative hypertrophy of different types of fibres is relevant to the effect of breed, sex, nutrition, exercise and disease on muscle development.

Neonatal fibre type differentiation

Changes in the histochemical fibre types of mammalian muscles are observed during the immediate postnatal period. The findings of Wirsen & Larsson (1964), Dubowitz (1965), Beatty, Basinger & Bocek (1967), Dorn (1969), Ommer (1971) and Ashmore, Tompkins & Doerr (1972) demonstrate a variation in the time of onset of differentiation of an anaerobic fibre type in muscles of the mouse, rat, hamster, guinea-pig, rabbit, cat, rhesus monkey, pig, sheep, man and ox. Dubowitz (1965) considers that this variation depends on the relative maturity of the species at birth. Nyström (1968) observes topographical differences in the onset of differentiation in individual muscles of the cat. Thus fibres with low activity of SDHase have already appeared in forelimb muscles, intercostal muscles and diaphragm at birth, at a time when hindlimb muscles retain a uniformly high reaction. Cooper, Cassens, Kastenschmidt & Briskey (1970) state that fibre types in longissimus of the neonatal pig are undifferentiated, although their published photographs of serial sections from a 1 day old pig appear to show several fibres of large TSA that also differ from surrounding fibres by their low activity of myosin ATPase and GPase. Their statement is contrary to the findings both of the present investigation and to that of Nyström (1968) that the gastrocnemius and soleus of the relatively immature neonatal kitten is differentiated with respect to myosin ATPase and GPase.

Wohlfart (1937), who studied fixed and stained preparations of a wide variety of human fetal and neonatal muscles, describes the presence of 'b' fibres that differ from the surrounding 'a' fibres because of their relatively large size. They occur singly, but two or three can be seen in the same fasciculus. They form between 0·5 and 2·5 % of all fibres at birth. Fenichel (1963) showed that Wohlfart's 'b' fibre is low in myosin ATPase activity and there is little doubt that this fibre type is the myosin ATPase low fibre seen in neonatal porcine muscles.

The above studies were concerned with changes due to the different functional

requirements of muscle in a prenatal and a postnatal environment, rather than with the adaption of muscle to meet the mechanical and metabolic demands of increased body size.

Mechanical adaption of muscles to increasing body size

Welcker & Brandt (1903) suggested that larger species of animals need a higher proportion of muscle and bone than smaller species, in order to support and move their bodies with structures whose strength is proportional only to their TSA. However, the data they recorded for the mouse, bat, hedgehog, guinea pig, monkey, ox and elephant does not support their hypothesis. Jackson & Lowry (1912) reviewed other findings and concluded that comparatively small animals, such as the rabbit and cat, have the highest proportions of muscle, although these workers demonstrated that growth in the rat results in an increase in the weight of muscle as a proportion of body weight from 23 % at one week to 45 % at one year of age. In the pig, the proportion of muscle decreases slightly as liveweight increases from 23 kg to 118 kg (Cuthbertson & Pomeroy, 1962; Stant, Martin, Judge & Harrington, 1968; Richmond & Berg, 1971).

As an increase in body size is not associated with an increase in the proportion of muscle, it is to be expected that the contractile properties of postural muscles must adapt to the changing demands placed upon them. For animals maintaining the same dimensional proportions, Hill (1950) predicted that the intrinsic speed of contraction of muscles would decrease with increasing body size. No physiological studies have been made specifically to test Hill's hypothesis. Both fast- and slow-twitch muscles of neonatal kittens (Buller, Eccles & Eccles, 1960*b*; Buller & Lewis, 1965; Mann & Salafsky, 1970) and rats (Close, 1964) are initially slow contracting; the subsequent differentiation of contraction speed may be associated with the development of normal muscle usage in animals born in an immature state. These workers also showed that the time to peak tension of soleus of both the rat and cat increases between 40 and 100 days of age; the rat soleus from $28{\cdot}5 \pm 2{\cdot}3$ ms at 35 days to $36{\cdot}0 \pm 2{\cdot}3$ ms at 100 days (Close, 1964), and the cat soleus from 59 ms at 42 days to 75 ms at 126 days (Mann & Salafsky, 1970). They did not, however, comment on the significance of this later change.

The developing soleus muscle of the rat, guinea-pig and cat was studied histochemically by Karpati & Engel (1967*b*). Their findings for the cat were confirmed by Nyström (1968). At birth, the soleus of the guinea-pig has approximately equal numbers of myosin ATPase high and low fibres. The rat soleus, undifferentiated at birth, resembles the cat and guinea-pig soleus by 10 days. The soleus of the adult cat and guinea-pig is almost entirely composed of myosin ATPase low fibres; about 90 % of the fibres in the adult rat soleus are myosin ATPase low. A similar increase in the proportion of myosin ATPase low fibres occurs with increasing body size in the pectineus muscle of the dog (Cardinet *et al.* 1969), and in an interspecies comparison of m. semitendinosus (Davies & Gunn, 1971) and the diaphragm (Davies & Gunn, 1972). However, the observations of Cooper *et al.* (1970) on fibre types in m. longissimus of the pig from birth to 90 kg live weight contradict the above findings, and those of the present study. They reported a decrease in the proportion of the TSA of the muscle occupied by myosin ATPase low fibres with increasing body size,

and their illustrations did not show an increase in the number of fibres per myosin low bundle.

Edgerton & Simpson (1969) suggest that the proportion of myosin ATPase low fibres demonstrated histochemically in a muscle is related directly to the contraction time. This is confirmed by Barnard, Edgerton, Furukawa & Peter (1971), Cardinet, Tunell & Fedde (1971) and Davies & Gunn (1972). The histochemical changes seen in the muscles of growing animals show that changes in the contractile properties of muscle depend on body size. The direct relationship demonstrated between body weight and the TSA of a muscle occupied by myosin ATPase low fibres (Fig. 17) supports the concept that the histochemical change occurs in response to a demand on a muscle for prolonged isometric contraction, directly proportional to the weight it must support.

This response has not been studied biochemically. Trayer & Perry (1966) report that the ATPase activity of myosin extracted from muscles of the fetal rat, guinea-pig and rabbit is lower than that of adult myosin. Guth & Samaha (1972) confirm this observation on rabbit muscle. They also claim that all fibres show an intense histochemical reaction for myosin ATPase in rabbit limb muscles at birth, although large fibres with a less intense reaction are apparent in their illustration of a rat hind limb muscle. Since the histochemical method demonstrates a difference between fibres in the alkali stability of myosin ATPase (Guth & Samaha, 1969) rather than the overall activity of this enzyme, the difference between the biochemical and histochemical findings does not necessarily belittle the ability of the histochemical method to differentiate fibre types on a functional basis. In any case, this apparent biochemical difference between fetal and adult myosin may not be directly associated with its ATPase activity (Dow & Stracher, 1971).

The histochemical findings require physiological and biochemical confirmation and should be supplemented by histochemical observations on a wide variety of muscles.

Metabolic adaptation of muscles to increasing body size

Increase in body size imposes restrictions on the ability of the respiratory and circulatory systems to supply oxygen to muscle. The total surface area of the lung alveoli is shown by Tenney & Remmers (1963) to be proportional to the rate of oxygen consumption of the whole body, or the 3/4 power of body weight (Kleiber, 1947), of different mammalian species. This area is also proportional to the surface area of the human body during growth (Dunnill, 1962), rather than directly to body weight. Similarly, the TSA of the aorta, and hence the ability of the heart to supply oxygenated blood to the body tissues, cannot maintain a direct proportionality to the body weight (Hill, 1950). Thus, although small vital organs and postural muscles demanding relatively low energy conversion rates for isometric contraction can retain a purely oxidative metabolism during growth, the muscles which a large animal uses mainly for brief periods of intensive propulsive force must adapt to obtain energy anaerobically.

Studies that might support or refute this hypothesis are difficult to interpret. The *in vitro* respiration experiments of Bertalanffy & Pirozynsky (1953) on the diaphragm of the mouse, Bertalanffy & Estwick (1953) on the diaphragm and leg muscles of the mouse and rat, Latzkovits & Domonkos (1965) on longissimus, abdominal muscles

and soleus of the rabbit, and van Den Hende, Muylle, Oyaert & de Roose (1971) on longissimus of the pig did not examine the complete metabolic capability of muscle in the intact animal. The studies by Goldspink (1962) on the activity of SDHase in the biceps brachii of the mouse, by Markert & Ursprung (1962) on the changing proportions of lactate dehydrogenase isoenzymes during growth of the mouse diaphragm and 'skeletal muscle', by Kendrick-Jones & Perry (1967) on enzymes of adenine nucleotide metabolism in diaphragm and leg muscles of the rabbit, and by Bocek, Basinger & Beatty (1969) on enzymes concerned with glycogen synthesis and breakdown in limb muscles of the rhesus monkey were not dissociated from the adaptive changes inherent in the transition from a maternal dependent to an independent environment. Cooper *et al.* (1971) studied lactate dehydrogenase (LDHase) and GPase in the developing longissimus and trapezius muscles of Poland China and Yorkshire pigs from birth to 90 kg live weight. The changes occurring in neonatal pigs are also difficult to interpret, but from 8 weeks of age (presumably about 5 kg live weight) to 90 kg, their results appear relevant to the present study. Over this weight range, both muscles show the same trend; the total LDHase activity increases, and the proportion of this activity (LDHase isoenzyme 5) favouring lactate production from pyruvate increases, while the total GPase activity decreases. The activity of LDHase and GPase is lower, and the proportion of aerobic fibres is higher (Moody & Cassens, 1968), in trapezius. These results are consistent with the concept that with increasing body size, the pyruvate produced from glycolysis in both muscles is converted increasingly to lactate, rather than passing to the respiratory chain via the citric acid cycle. Lactate production is greatest in the propulsive longissimus muscle.

Although it is possible by histochemical methods to estimate the relative capacity of adjacent fibres for a particular metabolic process, the proportion of fibres with low activity of a particular enzyme does not necessarily indicate the overall enzymic activity of different muscle samples. Therefore the results of the present study, suggesting that subsequent to an initial neonatal differentiation the proportion of SDHase low fibres of both longissimus and diaphragm does not increase with growth (Table 5), cannot be considered as evidence that the aerobic capacity of the muscle does not change. The results are in any case contrary to the observations of van Den Hende, Muylle, Oyaert & de Roose (1972), who reported a steady increase in the proportion of SDHase low fibres in the longissimus of Pietrain and Landrace pigs, from 55 % in pigs weighing 2 kg to 85 % in pigs of 105 kg live weight. The number of fibres in the SDHase high bundles in their published photograph appears even less than that published by Cooper *et al.* (1970) for the longissimus of the Poland China and Yorkshire breeds. The difference between these two reports and those of Moody & Cassens (1968) and the present study suggests a large source of variation in the histochemical properties of this muscle between pigs of different breeding and environment.

The present study shows that in Large White pigs between 10 and 56 days of age, a fast-twitch, purely aerobic fibre type is present in the diaphragm in greater proportions than in more mature pigs. This fibre type is predominant in the mouse diaphragm, occurs as 25 % of fibres in the rat diaphragm, but does not occur in significant proportions in the diaphragms of larger animals (Davies & Gunn, 1972).

These intraspecies and interspecies observations suggest that the diaphragm of larger animals increases its capacity for anaerobic metabolism. However, the problem of metabolic adaption of muscle to increased body size awaits a comprehensive biochemical study of postnatal growth over a wide weight range.

Mechanism of fibre type changes

After an initial differentiation of fibres with respect to the extent to which they derive energy for contraction by aerobic or anaerobic metabolism, the most significant postnatal histochemical changes seen in this study are an increase in the proportion of myosin ATPase low fibres, and a concomitant increase in the proportion of GPase low fibres (Table 5). A relative increase in a particular fibre type could occur either by:

(i) the addition of new fibres to the population;

(ii) the subtraction of fibres of a different type from the population;

(iii) a rearrangement of fibre architecture to enable more fibres of one type to be seen in a transverse section than previously; or

(iv) a conversion of a fibre of one type into another.

Unless (i) and (ii) occur simultaneously, or the rearrangement in (iii) is particularly complex, only (iv) can occur without alteration to the number of fibres in a muscle. Estimates of the fibre populations of muscles during growth suggest that although fibre numbers may increase for a short neonatal period, subsequent postnatal growth of muscle is due to hypertrophy of fibres present at birth. This is evident in m. radialis of the rat (Morpurgo, 1898), for the human sartorius (MacCallum, 1898; Montgomery, 1962), the biceps brachii of the mouse (Goldspink, 1962), the biceps brachii, extensor carpi radialis, gastrocnemius and tibialis anterior of the rat (Enesco & Puddy, 1964), and the extensor carpi radialis, soleus and plantaris of the rat (Chiakulas & Pauly, 1965). Similarly, the present results suggest that the postnatal fibre population of the longissimus of the pig is constant. Karpati & Engel (1967*b*) showed that while the proportion of myosin ATPase low fibres in the guinea-pig soleus increases from 60 to 100 % of the fibre population between birth and adulthood, the total fibre population increases only from 3384 to 3531.

Although the addition, subtraction or rearrangement of fibres is not disproved, it appears much more likely that a conversion of myosin ATPase high to myosin ATPase low fibres occurs with growth. The following aspects of the present study support this. In both longissimus and diaphragm, the TSA distribution and morphology of myosin ATPase low fibres at any stage of growth does not suggest a development from small, immature fibres. The splitting of aerobic fibres and degeneration of anaerobic fibres seen in the longissimus of growing pigs by Todorov & Petrov (1969) has not been seen in the material used in present study. However, in pigs from birth to 15 kg live weight, coincident with the most rapid increase in the proportion of myosin ATPase low fibres (Fig. 16), many fibres of intermediate staining for the myosin ATPase reaction are seen in both muscles (Fig. 13). When the pH of the incubation medium is held at 9·5, intermediate reactions are seldom seen in more mature porcine muscle (Fig. 5). The histochemical profile and mean TSA of these fibres suggest that they are in transition from fast-twitch combined aerobic and anaerobic fibres to slow-twitch purely aerobic fibres. They are located adjacent to

myosin ATPase low bundles; the process would therefore augment the number of fibres in these bundles.

The possibility that a change in the characteristics of a muscle fibre as basic as the ATPase activity of its myosin and its intrinsic speed of contraction occurs with growth is of great interest. It implies a considerable readjustment of the motor unit, and warrants further morphological and physiological study.

SUMMARY

The adaption of skeletal muscle to meet the changing mechanical and metabolic demands of an increase in body weight during growth has been studied by establishing histochemical profiles of individual muscle fibres using the myosin ATPase, succinate dehydrogenase (SDHase) and glycogen phosphorylase (GPase) reactions. Samples of longissimus and diaphragm muscles from a series of 18 Large White pigs between birth and 60 kg live weight, and from a series of 16 Large White pigs of mean live weight of 93 kg were used.

In both muscles, the number of fibres low in myosin ATPase activity increased with growth, and these fibres were grouped into bundles. In m. longissimus, the estimate of the total fibre population and the number of myosin ATPase low bundles in a complete transverse section of the muscle remained constant, while the mean number of myosin ATPase low fibres per bundle increased from one at birth to 3·2 at 93 kg live weight. Whereas the complete transverse sectional area of the muscle increased in proportion to the 2/3 power of the body weight, the area occupied by myosin ATPase low fibres increased in direct proportion to the body weight. This observation suggests the mechanism by which larger animals are supported without a relative increase in their muscle mass. Some histochemical evidence was obtained that this is achieved by a transformation of the physiological properties of certain fibres.

The diaphragm of smaller pigs contained a greater proportion of a myosin ATPase high, SDHase high and GPase low fibre type than that of more mature pigs. After initial neonatal differentiation, the muscles studied did not change their proportion of SDHase high fibres during growth.

In both longissimus and diaphragm, the mean transverse sectional area of myosin ATPase high fibres was greatest when the SDHase activity was low. Also, the mean transverse sectional area of SDHase high fibres was greatest when the myosin ATPase activity was low, but this difference was significant only for the diaphragm.

I am indebted to Professor A. R. Muir for advice in all stages of this study, to Dr W. C. Smith for supplying material, and to the Meat and Livestock Commission who made material available and provided financial support.

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