J. Anat. (1974), **118**, 1, pp. 171–186
With 14 *figures*
Printed in Great Britain

An ultrastructural and histochemical study of fibre types in the pectoralis thoracica and iliotibialis muscles of the fowl (*Gallus domesticus*)

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(*Accepted* 2 *April* 1974)

INTRODUCTION

Muscle fibres may be classified on a physiological, histochemical or histological basis. The integration of such classifications is difficult, although some correlations between histochemical reactions and ultrastructure have been reported. The functional differences between the different fibre types demonstrated histologically and histochemically are usually inferred from studies of whole muscles in which one fibre type predominates (Peter *et al.* 1972).

Gauthier & Padykula (1966) classified rat muscle fibres histologically into red, white and intermediate types, each of which gave distinctive histochemical reactions for succinate dehydrogenase (SDHase), lipid and mitochondrial adenosine triphosphatase. At the ultrastructural level these types differed in their content and distribution of mitochondria, thickness of Z disc and development of sarcoplasmic reticulum (SR) (Gauthier, 1969). However, in the extensor digitorum longus muscle of the rat, Schiaffino, Hanzlíková & Pierobon (1970) showed a continuous range of SDHase activity, mitochondrial content and thickness of Z disc so that discrete fibre types could not be differentiated on this basis.

In the domestic fowl the cranial (anterior) and caudal (posterior) latissimus dorsi are often used as examples of tonus and twitch muscles respectively (Ginsborg, 1960; Ginsborg & Mackay, 1961). These muscles have different patterns of innervation and fine structure (Hess, 1967; Bock & Hikida, 1968; Page, 1969), and Zelená & Jirmanová (1973) showed that the Z discs were significantly thicker and the mitochondrial content significantly higher in the cranial as compared with the caudal muscle.

Shafiq, Askanas & Milhorat (1971) differentiated four types of fibre in the sartorius muscle of the fowl by using histochemical reactions for myosin adenosine triphosphatase (myosin ATPase), glycogen phosphorylase (GPase) and SDHase. Their type I fibres (myosin ATPase low, GPase low, SDHase intermediate) had very thick Z discs (104 nm) and a moderate mitochondrial content; type IIb fibres (myosin ATPase high, GPase high, SDHase low) had thin Z discs (55 nm) and few mitochondria, and types IIa and c fibres (myosin ATPase high, GPase intermediate or high, SDHase intermediate or high) had moderately thick Z discs (80 nm) and numerous mitochondria. Krompecher *et al.* (1970) showed a difference in mito-

chondrial content between the pectoral and crural muscles, but did not compare fibres within either muscle.

George & Berger (1966) described two types of fibre in the pectoralis muscle of the pigeon which they called type 1 and type 2. They showed that the type 1 fibre was best suited for aerobic metabolism and the type 2 for anaerobic metabolism and suggested that the former was slow contracting and the latter fast contracting. However, they cited Bokdawala & George (1965) as demonstrating that a higher activity of the sulphydryl-dependent myofibrillar ATPase at pH 9·4 existed in the red fibre. Burke *et al.* (1973) stimulated individual motoneurons in the gastrocnemius muscle of the cat and differentiated two populations of fibre by their speed of contraction. They found that the fast contracting fibres had a high myofibrillar ATPase activity at pH 9·4.

The present investigation compares the ultrastructural and histochemical properties of two muscles of differing metabolic capacity in the fowl: the pectoralis thoracica and iliotibialis. The pectoralis thoracica, a wing muscle, depends on a predominantly anaerobic metabolism (Bass, Lusch & Pette, 1970) and, after a preliminary survey of several hind limb muscles, it was established by the author that the iliotibialis muscle has a high capacity for aerobic metabolism.

MATERIALS AND METHODS

Experimental material

Fourteen 9 to 12 week old Shaver female domestic fowl (*Gallus domesticus*), obtained from the Poultry Research Centre, Edinburgh, were used in this study. They were exposed to pure carbon dioxide until comatose (Scott & Ray, 1972) and killed by dislocation of the neck. Samples were removed immediately after death from the medial region of the pectoralis thoracica (Lucas & Stettenheim, 1965) and the cranial region of the iliotibialis muscle (Halvorson, 1972).

Histochemical methods

Immediately after death the samples were rapidly frozen in Arcton 12 (I.C.I.), cooled to its melting point of −158 °C with liquid nitrogen. Transverse serial sections 10 μm thick were cut on a cryostat at −20 °C, then mounted directly on to coverslips and allowed to thaw and dry at room temperature. The activities of succinate dehydrogenase, glycogen phosphorylase and myosin adenosine triphosphatase were demonstrated by modifications of the methods of Nachlas *et al.* (1957), Takeuchi & Kuriaki (1955) and Padykula & Herman (1955) respectively. The modifications used were those of Davies & Gunn (1972) except that sections were incubated for 30 minutes to demonstrate myosin ATPase activity. Outlines of the fibres were drawn on tracing paper from projections of sections stained with Ehrlich's haematoxylin so that histochemical profiles for individual fibres could be constructed from serial sections which had been stained histochemically.

Preparation of material for electron microscopy

Strips of muscle were splinted *in situ* at rest length and then removed and fixed immediately by immersion in a solution of 4% paraformaldehyde, 0·8% glutaraldehyde in 0·1 M sodium cacodylate at pH 7·4 and room temperature for 30 minutes.

Each strip was cut into 1 mm cubes, washed briefly in the cacodylate buffer, post-fixed in 1 % osmium tetroxide in cacodylate, and embedded in Araldite. Sections were cut with a Porter–Blum MT 1 ultramicrotome using a glass or diamond knife, and stained with a saturated solution of uranyl acetate in 50 % ethanol and lead citrate (Reynolds, 1963). Sections were examined in an AEI EM 6B electron microscope.

Measurement of Z disc thickness

Longitudinal sections, with an interference pattern of silver to grey, from each muscle in three birds were sampled by a systematic random technique, using the grid mesh as reference points (Weibel, 1969). One section from each sample from each bird was photographed in eight areas and the field adjusted if the sample straddled two fibres. The thicknesses of ten Z discs were measured on each print at a magnification of approximately 20000 as described by Patterson & Goldspink (1972). The values from the ten Z discs were averaged. In the iliotibialis, two sets of results were obtained by sampling from fibres with a high or low content of mitochondria.

Quantification of mitochondrial content

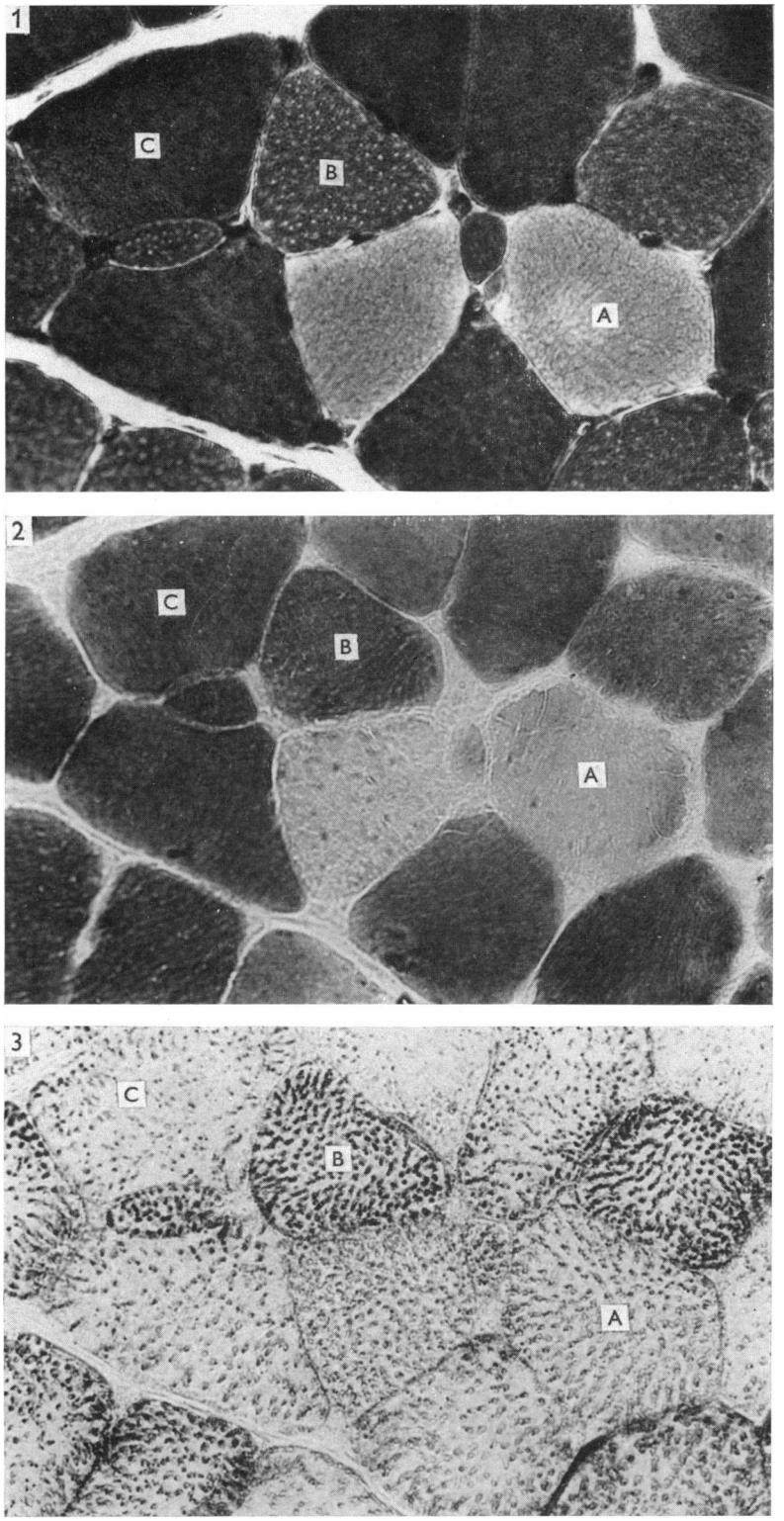
As described for the measurement of Z disc thickness, sections from both muscles in six birds were photographed in six areas. No adjustment was made if the field straddled more than one fibre, and sampling of the different fibres in the iliotibialis was random. Each micrograph was printed at a magnification of approximately 10000, any extracellular areas were trimmed off and the remaining area was weighed. The mitochondria were then cut out and weighed. From these measurements the percentage area of the fibre occupied by mitochondria was calculated, giving an estimate of the percentage volume of the fibre occupied by mitochondria. The results were treated in two ways: (i) data from the six micrographs were combined for each muscle in each bird to compare overall mitochondrial content between the two muscles, and (ii) the percentage area of fibre occupied by mitochondria was calculated for approximately 40 individual fibres above an arbitrarily defined minimum area; thus the variation in mitochondrial content within the two muscles was estimated.

RESULTS

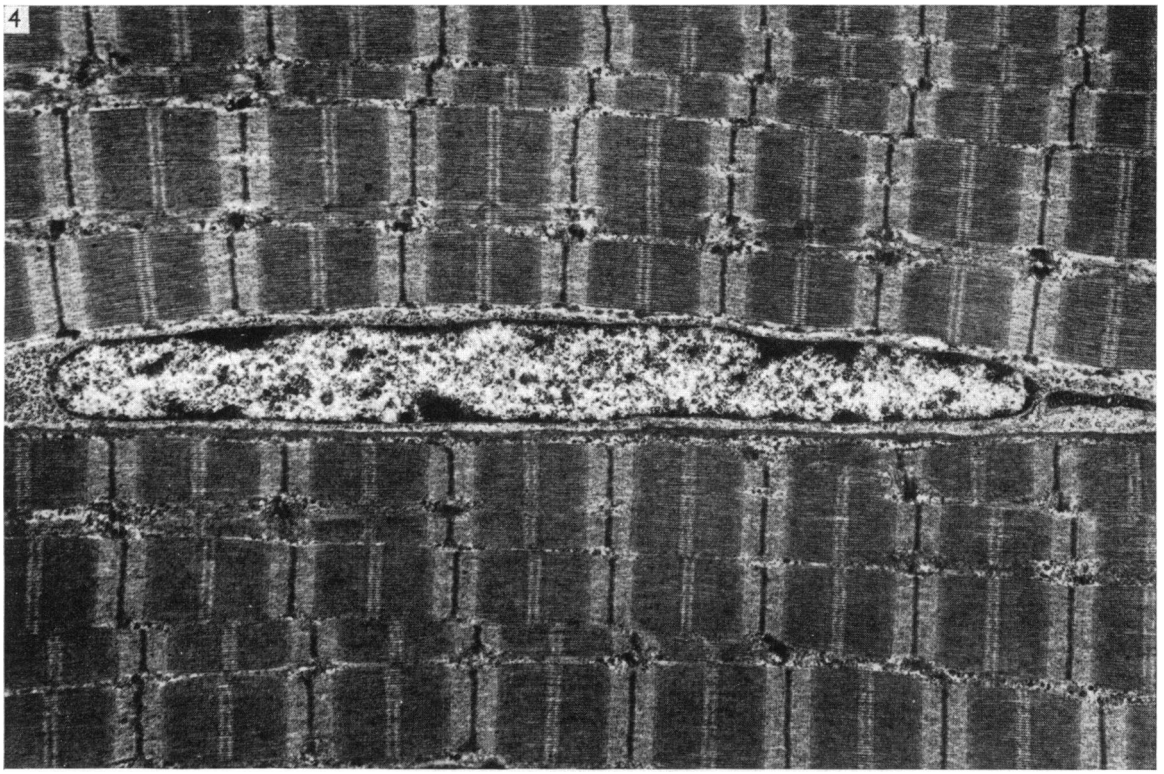
Histochemistry

The iliotibialis consisted of a heterogeneous population of fibres in which two fibre types could be differentiated by their myosin ATPase activity. Seven per cent of the fibres were low in myosin ATPase and GPase activity and intermediate or occasionally high in SDHase activity. These were the type I or slow-twitch-oxidative (SO) fibres, using the classification of Peter *et al.* (1972). The remaining fibres were high in ATPase and GPase activity and embraced a complete range of SDHase activities (Figs. 1–3), so it was not possible to differentiate these type II fibres into the fast-twitch-glycolytic (FG) and fast-twitch-oxidative-glycolytic (FOG) types of Peter *et al.* (1972).

The pectoralis muscle contained only fast-twitch-glycolytic (FG) fibres as their SDHase activity was uniformly low and all fibres reacted strongly for myosin ATPase and GPase.



Figs. 1–3. Transverse serial sections of the iliotibialis muscle stained for myosin ATPase (Fig. 1), GPase (Fig. 2) and SDHase (Fig. 3). Fibres low in myosin ATPase and GPase activities and intermediate in SDHase activity are seen (A). Fibres high in myosin ATPase and GPase activities may be high (B) or low (C) in SDHase activity. Note the range of SDHase activities in Fig. 3. ×450.



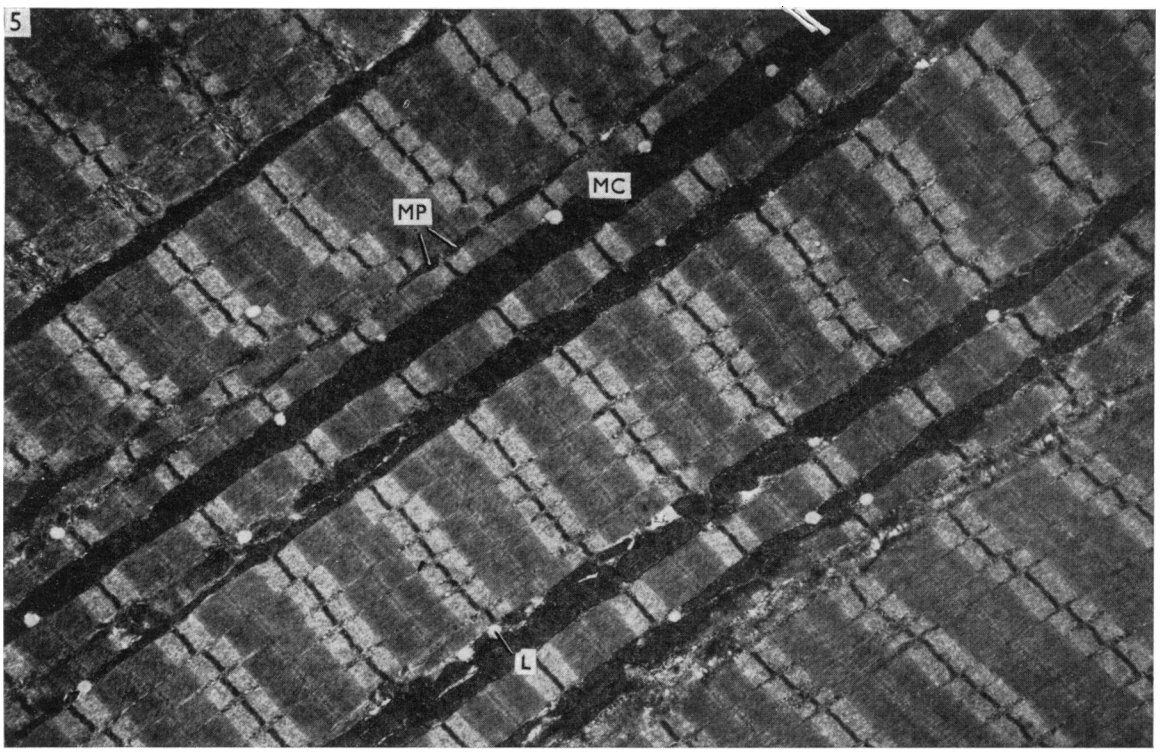
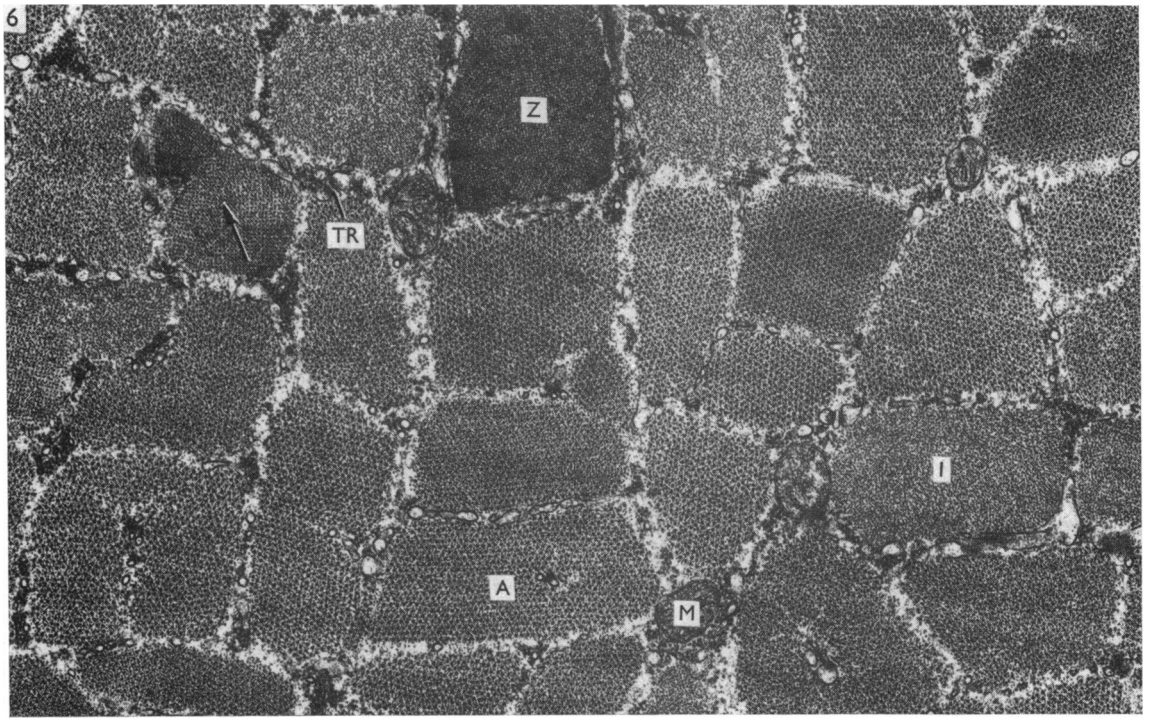


Fig. 4. Longitudinal section of pectoralis muscle showing thin, straight Z discs and prominent M lines. ×10000.

Fig. 5. Longitudinal section of iliotibialis muscle showing thick Z discs, many of which cross the myofibril erratically, M lines and numerous mitochondria in chains (MC) and couplets (MP). L, lipid droplet. ×6500.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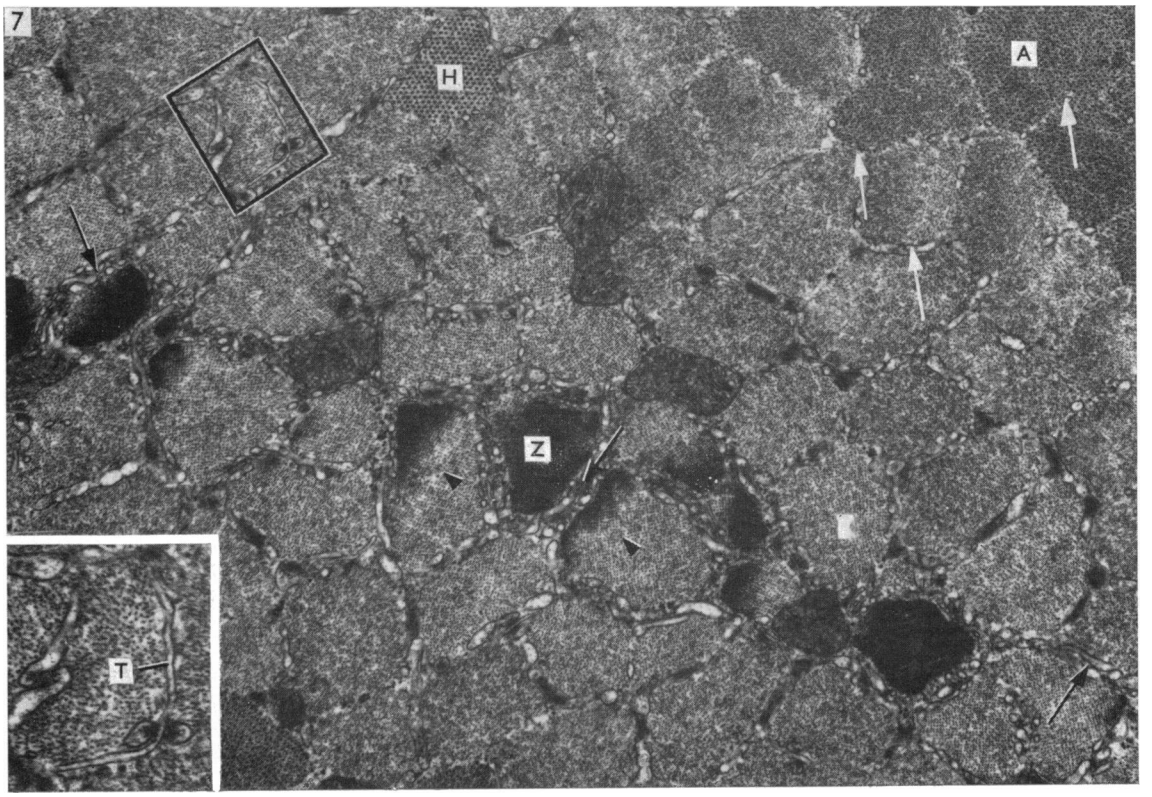
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Ultrastructure

Myofibrils

In the pectoralis muscle (Fig. 4) the Z discs were thin and ran straight across the myofibrils and there was a prominent M line. In transverse section (Fig. 6) the myofibrils had polygonal outlines and were clearly delineated by sarcoplasm at all levels. The typical hexagonal packing of the thick and thin filaments was observed in the A band and cross-bridges linked adjacent thick filaments at the M lines (Fig. 8). The thin filaments were haphazard in the I bands but acquired a quadrilateral lattice mmediately adjacent to the Z discs (Fig. 6).

In the iliotibialis muscle (Fig. 5) the Z discs in all the fibres were about twice as thick as in the pectoralis muscle and they sometimes crossed the myofibrils erratically. The M lines were present and the N line, a fine granular line traversing the I band close to the A band, was sometimes seen (Fig. 12). In transverse section (Fig. 7) the myofibrils were usually confluent at the level of the A band where there was no intervening sarcoplasm, but otherwise the arrangement of the myofibrils and myofilaments was similar to the pectoralis.

Z disc

The variation in thickness of the Z disc between and within the two muscles was quantified. There was little difference ($P < 0{\cdot}1$) in thickness of the Z disc between fibres rich (77 ± 12 nm) and poor (72 ± 12 nm) in mitochondria in the iliotibialis, but in both groups the thicknesses of the Z discs were significantly different ($P < 0{\cdot}0005$) from the fibres of the pectoralis muscle (39 ± 6 nm).

A histogram showing the distribution of Z disc thicknesses was plotted (Fig. 9). Within each muscle the spectrum of Z disc thicknesses was continuous, except for a small group of fibres with very thick Z discs in the iliotibialis.

Sarcotubular system

In the pectoralis muscle, the transverse tubule (T-tubule) ran at the level of the Z disc and at intervals along its length formed one triad per sarcomere with the terminal cisternae of the SR (this arrangement, which was found also in the iliotibialis muscle, is shown in Fig. 7). The terminal cisternae contained a dense granular material concentrated at their junction with the T-tubule, and were continuous with the longitudinal tubules of SR which ran to a fenestrated collar at the H zone (Fig. 10).

Fig. 6. Transverse section of pectoralis. The myofibrils are clearly delineated by SR at all levels. The I-filaments form a quadrilateral lattice adjacent to the Z disc (arrow). The SR forms a single, discontinuous layer between the myofibrils and is reduced at the level of the A band. Myofibrils sectioned through their Z discs, A and I bands are labelled. TR, triad; M, mitochondrion. × 18000.

Fig. 7. Transverse section of iliotibialis. The myofibrils are confluent at the level of the A band. Cross-bridges between thick filaments are present at the M line (H). The I-filaments form a quadrilateral lattice adjacent to the Z disc (arrow heads). Double layers of SR are present between adjacent Z discs and I bands (black arrows). Isolated single profiles of SR separate A bands and adjoining parts of I bands (white arrows). Inset – note a length of T-tubule free of terminal cisternae (T). × 18000.

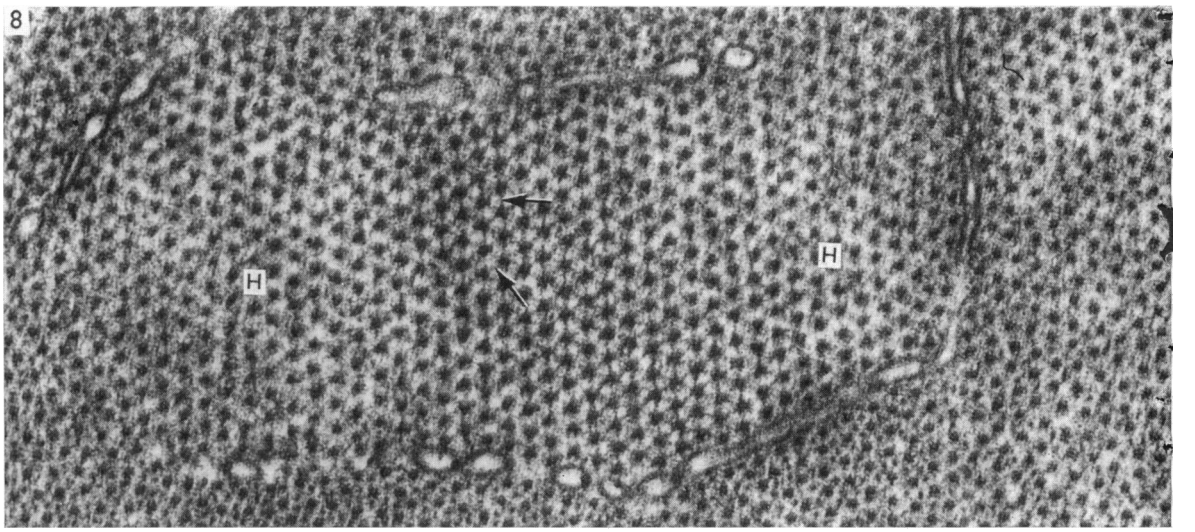


Fig. 8. Transverse section of pectoralis. A myofibril cut at the level of the H zone (H) is almost surrounded by SR. Cross-bridges between adjacent thick filaments at the M line can be seen (arrows). × 79000.

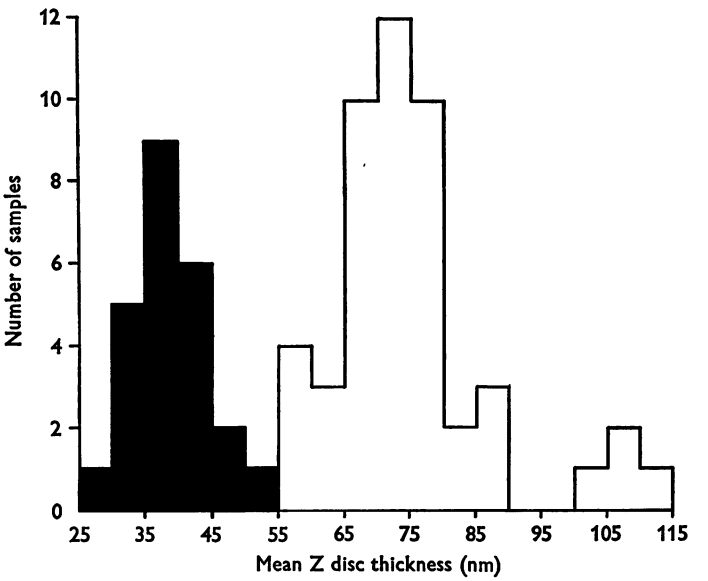
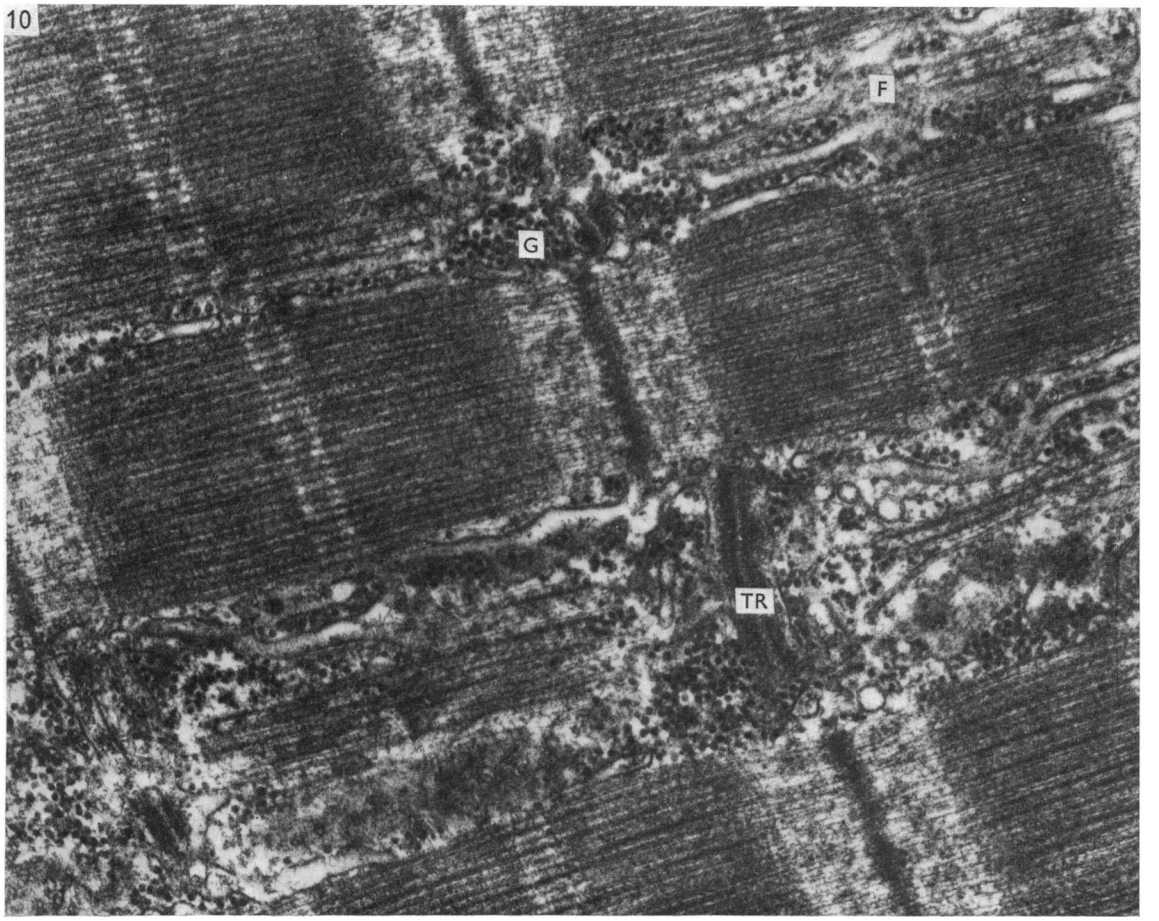


Fig. 9. Frequency distribution of the thickness of Z discs in the pectoralis (black) and iliotibialis (white) muscles.



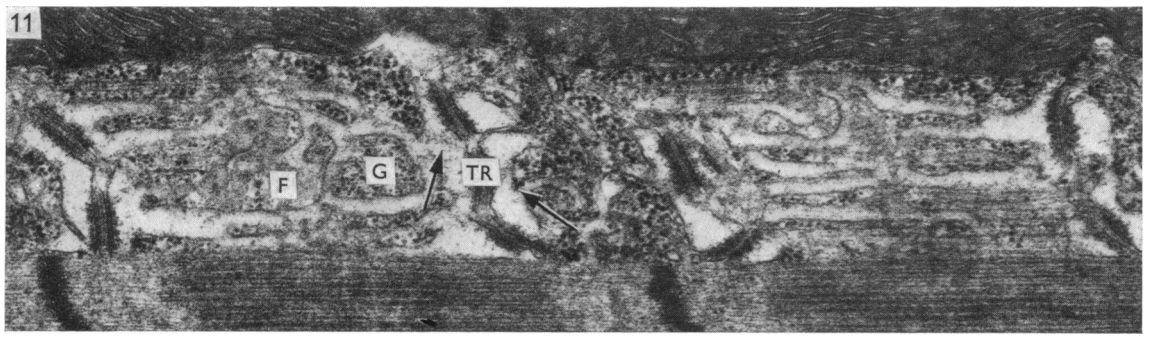


Fig. 10. Longitudinal section of pectoralis. A single triad (TR) is situated near the Z disc. Note the dense, granular content of the terminal cisternae. Longitudinal tubules of SR run along the myofibril to the fenestrated collar (F). G, glycogen. × 41 500.

Fig. 11. Longitudinal section of iliotibialis. The triads are situated near the A–I junctions, and their terminal cisternae are continuous with the Z disc reticulum and the longitudinal tubules of SR which run to the fenestrated collar (F) (arrows). G, glycogen. × 35 500.

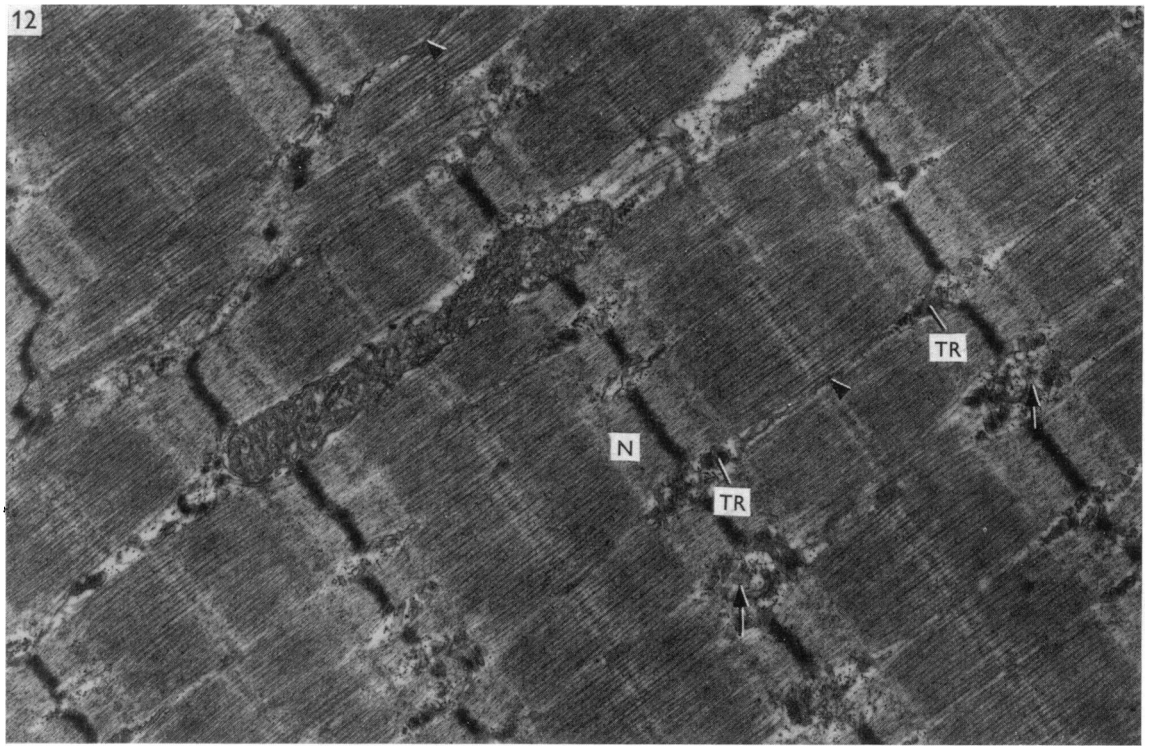


Fig. 12. Longitudinal section of iliotibialis showing the complex Z disc reticulum (arrows), the triads (TR) and the longitudinal tubules of SR (arrow heads). Note the N line (N). ×18500.

In transverse section a few profiles of SR formed a single, discontinuous layer at the level of the Z disc and I band and although the H zone was almost surrounded, elsewhere opposite the A band very little SR was seen (Figs. 6, 8).

In the iliotibialis, two triads per sarcomere were observed lying near the A–I junction. As in the pectoralis muscle, longitudinal tubules of SR connected the triads and the fenestrated collar within the same sarcomere, but there was also a complex reticulum of SR tubules crossing the Z disc to connect triads from adjacent sarcomeres (Figs. 11, 12). In transverse section this development of the SR opposite the Z discs and adjacent parts of the I bands was evident. Each myofibril was completely surrounded with SR so a double layer of SR lay between adjacent Z discs and I bands. Only isolated single profiles of SR separated A bands and adjoining parts of I bands, except at the level of the H zone which was almost surrounded by SR (Fig. 7).

Mitochondria

The few mitochondria in the pectoralis muscle usually occurred singly between the myofibrils, but short chains were also seen. In the iliotibialis muscle the mitochondrial content was extremely variable, ranging from fibres poor in mitochondria, comparable with the fibres of the pectoralis muscle, to fibres rich in mitochondria in which

Table 1. *Estimated percentage of the volume of the fibre occupied by mitochondria in the iliotibialis and pectoralis muscles of the fowl*

Bird no.	M. pectoralis (%)	M. iliotibialis (%)
1	1·6	8·8
2	0·4	2·1
3	0·9	8·9
4	0·8	2·8
5	0·4	5·4
6	0·3	2·7

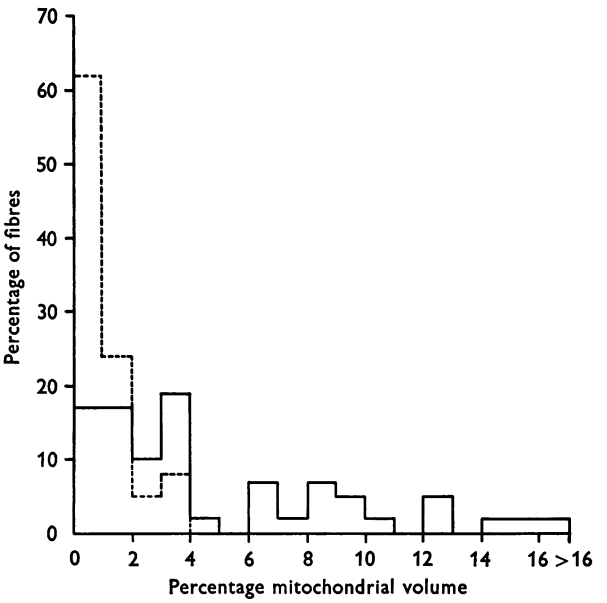


Fig. 13. Frequency distribution of the estimated percentage of the volume of the fibre occupied by mitochondria expressed as a percentage of the total number of fibres sampled, in the pectoralis (dotted line) and iliotibialis (solid line) muscles.

tightly packed long intermyofibrillar chains and perinuclear and subsarcolemmal aggregations of mitochondria were observed (Fig. 5). Mitochondria running transversely round the myofibril at the level of the I band were sometimes seen.

The quantitative data on the overall mitochondrial content of the two muscles was compared by a Student's t test for paired samples; the estimated percentage volume of the fibre occupied by mitochondria was significantly higher ($P < 0{\cdot}01$) in the fibres of the iliotibialis than the pectoralis muscle (Table 1).

The histogram in Fig. 13 shows the distribution of the fibres with respect to mitochondrial content within the two muscles. In the pectoralis there was little variation as most fibres have < 1 % of their volume occupied by mitochondria. Wide variation was observed in the iliotibialis and it is likely that the fibres sampled were

drawn from a series whose mitochondrial content varied continuously from < 1 % to nearly 20 % of their volume.

Glycogen

The amount of glycogen varied in both muscles, and this may depend on ante-mortem conditions (Bendall, 1960). Single particles were concentrated between the myofibrils at the level of the I band, but they also occurred opposite the A band and even between the thin filaments of the I band (Figs. 10, 11).

Lipid

In the iliotibialis muscle lipid droplets were often found among the mitochondria between the myofibrils (Fig. 5); however in the fibres of the iliotibialis which were poor in mitochondria and in all the fibres in the pectoralis, lipid droplets were rare and not necessarily associated with mitochondria.

DISCUSSION

The ultrastructure of the iliotibialis and pectoralis muscles is evidently similar to that of vertebrate twitch muscles, but there are differences between the two muscles which are relevant to the classification of fibre types.

The fibre types in fowl muscle were described by Ashmore & Doerr (1971*b*) in the adductor muscle and by Shafiq *et al.* (1971) in the sartorius muscle. These authors employed different nomenclatures, but the reactions indicated that it was possible to equate slow-twitch-oxidative (SO) fibres with βR (Ashmore & Doerr, 1971*b*) and type I (Shafiq *et al.* 1971); fast-twitch-glycolytic (FG) with αW and type IIb; and fast-twitch-oxidative-glycolytic (FOG) with αR and types IIa and c. In the iliotibialis muscle, slow-twitch-oxidative (SO) and fast-twitch fibres could be differentiated by their myosin ATPase and GPase activities, but the latter could not be subdivided into fast-twitch-glycolytic (FG) and fast-twitch-oxidative-glycolytic (FOG) as a complete range of SDHase activities was observed. Ashmore & Doerr (1971*a*) also found that the pectoralis muscle consists of a uniform population of FG fibres.

The relationships between these fibre types and their ultrastructure are confused. It can be assumed that SDHase activity is directly related to mitochondrial content; thus the sparsity of these organelles in the pectoralis conforms to the uniform population of FG fibres, while in the iliotibialis the spectrum of activity matches the range of mitochondrial content. Schiaffino *et al.* (1970) also observed a continuous range of mitochondrial content in the extensor digitorum longus fibres of the rat, but most classifications of fibre types emphasize sharp differences in mitochondrial content (Gauthier & Padykula, 1966; Shafiq *et al.* 1971; Tomanek *et al.* 1973).

A correlation between mitochondrial content and Z disc thickness was proposed by Gauthier (1969). Her red fibres (FOG; Peter *et al.* 1972) had thick Z discs, while white (FG) and intermediate (SO) fibres had thin Z discs. Such a correlation of Z disc thickness with fibre type was also shown by Shafiq *et al.* (1971) in the sartorius muscle of the fowl and Tomanek *et al.* (1973) in the vastus lateralis and soleus muscles of the guinea-pig. They found the thickest Z discs in SO fibres (type I, slow-twitch inter-

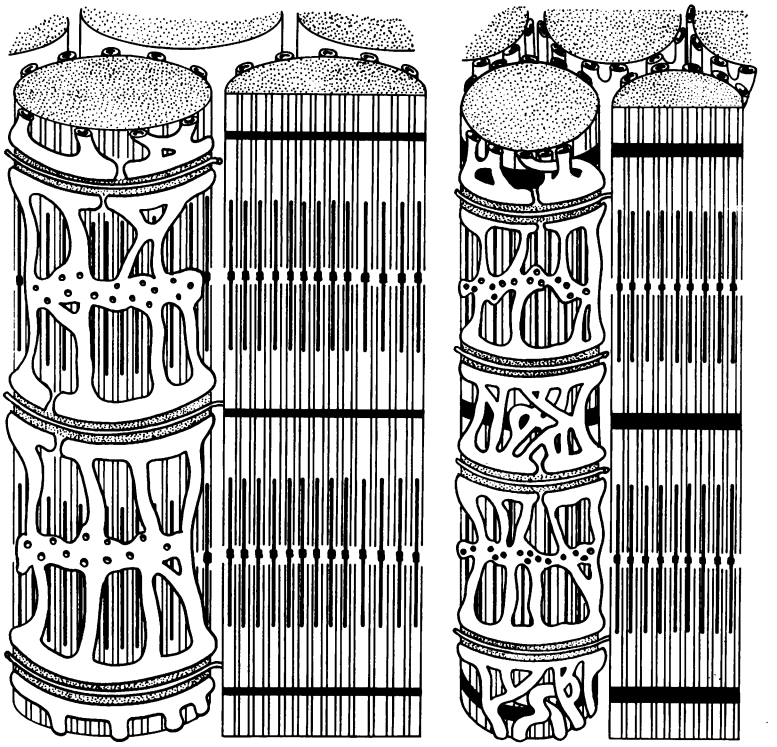


Fig. 14. A diagrammatic interpretation of the arrangement of the sarcotubular system. Myofibrils are represented in surface view and cut longitudinally and transversely. In the pectoralis muscle the triad is situated at the level of the Z disc, and successive triads are connected by longitudinal tubules of SR which run to the fenestrated collar at the H zone. In transverse section there is a single layer of SR between adjacent myofibrils. In the iliotibialis muscle there are two triads per sarcomere located near the A–I junctions, connected within each sarcomere by the longitudinal tubules of SR and the fenestrated collar, and between sarcomeres by the complex Z disc reticulum. In transverse section the myofibrils have been cut through the I bands, close to the level of the Z disc, therefore they are separated by two layers of SR.

mediate), the thinnest in FG fibres (type 11 b, fast-twitch white), and an intermediate value in the FOG fibres (type IIa and c, fast-twitch red).

Both Shafiq *et al.* (1971) and Tomanek *et al.* (1973) used regions of muscle of fairly homogeneous fibre type. In the present investigation the Z discs in the FG fibres of the pectoralis were very thin. The Z discs were thicker but more variable in the fibres of the iliotibialis: however, thickness was not related to the content of mitochondria, and so the Z discs cannot be used to differentiate discrete populations of FG and FOG fibres. Nevertheless it is likely that the few fibres observed with very thick Z discs were SO fibres. The observations of Schiaffino *et al.* (1970) on the extensor digitorum longus and soleus muscles of the rat are in agreement with this conclusion.

It has been suggested that the development of the SR is related to speed of

contraction (Revel, 1962; Bergman, 1965; Page, 1965, 1969). The existence of very different arrangements of the sarcotubular system in two twitch skeletal muscles of the same animal is unusual. The single triad, the longitudinal tubules of SR and the fenestrated collar of the pectoralis are shown diagrammatically in Fig. 14. The SR network is shared by adjacent myofibrils; thus in transverse section only one layer of tubules is observed between myofibrils. The two triads per sarcomere and two systems of SR tubules seen in the iliotibialis muscle are also represented in Fig. 14. However, in this muscle each myofibril is separately ensheathed with a network of SR; thus in transverse section two layers of tubules are seen between adjacent myofibrils except where tubules are sparse.

This description of the sarcotubular system in the pectoralis muscle agrees with earlier accounts of the fowl (Bennett & Porter, 1953; Mendell, 1971) and pigeon (Ashhurst, 1969). The sarcotubular system in the M. serratus superficialis metapatagialis of the pigeon is similar to that in the iliotibialis, except that double layers of SR are not seen between adjacent myofibrils in transverse section (Hikida, 1972).

Schiaffino *et al.* (1970) and Tomanek *et al.* (1973) have shown that the sarcotubular system is more highly developed in fast-twitch than slow-twitch fibres. However, within the iliotibialis, no consistent difference was found between fibres, although SO fibres may have been overlooked, as they constitute only 7 % of the total fibre population. The marked difference between the development of the SR in the pectoralis and iliotibialis appears anomalous, as in both muscles over 93 % of the fibres gave strong reactions for myosin ATPase. This histochemical reaction is not, however, quantitative and so the relationship between speed of contraction, myosin ATPase activity and development of the SR in avian muscle fibres remains to be elucidated.

SUMMARY

The histochemical and ultrastructural properties of the pectoralis and iliotibialis muscles of the fowl (*Gallus domesticus*) have been examined.

All the fibres in the pectoralis muscle showed high myosin adenosine triphosphatase (myosin ATPase) and glycogen phosphorylase (GPase) activities and low succinate dehydrogenase (SDHase) activity. Two types of fibre could be differentiated in the iliotibialis muscle, one low in myosin ATPase and GPase activities and moderate to high in SDHase activity, the other high in myosin ATPase and GPase activities with a very wide range of SDHase activity in different fibres.

The ultrastructure of both muscles was typical of vertebrate twitch muscle. The pectoralis fibres had thin Z discs, few mitochondria, a single triad per sarcomere situated at the level of the Z disc and, compared with the iliotibialis, a poorly developed sarcoplasmic reticulum. In the iliotibialis the Z discs were thicker and more variable and the overall content of mitochondria was higher than in the pectoralis, but there was wide variation between fibres. There were two triads per sarcomere located near the A–I junction, and the sarcoplasmic reticulum was extensive.

A correlation between the histochemical and ultrastructural features of different fibre types was attempted.

I am deeply indebted to the late Professor A. R. Muir for assistance at all stages of this study and to Mr N. A. Smith for photographic assistance. Fig. 14 was drawn by Mr I. Lennox of the Medical Illustrations Department, University of Edinburgh. This work was supported by a grant from the Agricultural Research Council.

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