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MECHANICAL PROPERTIES AND MYOSIN LIGHT CHAIN COMPOSITION OF SKINNED MUSCLE FIBRES FROM ADULT AND NEW-BORN RABBITS

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SUMMARY

1. The maximum velocity of shortening, V_{max}, and stiffness were measured in skinned single fibre segments from psoas and soleus muscles of adult rabbits and psoas muscles of new-born rabbits, and the myosin light chain composition was also determined in the same segments used in the mechanical studies.

2. V_{max} was obtained at 15 °C during maximal activation at pCa 5·49 using a method involving measurement of the time required to take up various amounts of slack imposed on the segments. Stiffness was measured during activation at 10 °C by application of length steps complete in 0·6 msec. The myosin light chain composition of the segments was then determined by SDS-polyacrylamide gel electrophoresis.

3. Only fast type light chains were found to be present in the psoas fibre segments, though the relative amounts of myosin LC_{1f}, LC_{2f} and LC_{3f} in these segments was somewhat variable. In most instances, the sum of the amounts of LC_{1f} and LC_{3f} present was equivalent to the amount of LC_{2f}. Only slow type light chains were found in the soleus segments and the sum of the amounts of LC_{1as} and LC_{1bs} was about equal to the amount of LC_{2s}.

4. The results indicate that there are no consistent relationships between V_{max}, tension development or stiffness and LC_{1f}/LC_{2f} in the segments from adult and new-born psoas muscles, or between these mechanical parameters and LC_{1as}/LC_{2s} or LC_{1bs}/LC_{2s} in the adult soleus segments. However, the psoas segments, which had light chains of the fast type, had V_{max} values that were consistently higher than those of the soleus segments, which had light chains of the slow type.

5. The stiffness values obtained in each of the three kinds of muscle were similar, suggesting that cross-bridge stiffness is similar in rabbit skeletal muscles of different type and age. Moreover, the results indicate that the amount of end compliance introduced by the connections to the fibre segments has a marked influence on the stiffness that is measured.

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INTRODUCTION

It has long been known that mammalian skeletal muscles can generally be separated into two main classes according to colour: white and red (see Needham, 1971; Granit, 1970, for a history of this subject). Compared with red, white muscles have shorter twitch contractions in which the rates of rise and fall of tension are greater (Close, 1972). This has led to the practice of designating white muscles 'fast-twitch' and red muscles 'slow-twitch'. Fast-twitch muscles also have higher speeds of shortening (Close, 1972) and myosin ATPase activities (Bárány, 1967) than do slow-twitch muscles.

At the molecular level, the myosin molecule of vertebrate striated muscles consists of six subunits: two heavy chains each of about 200,000 molecular weight (Gershman, Stracher & Dreizen, 1969; Gazith, Himmelfarb & Harrington, 1970) and two pairs of light chains with molecular weights in the 20,000 range (Weeds & Lowey, 1971). Fast-twitch skeletal muscle myosin contains two classes of light chains. The first of these can be dissociated by treatment with DTNB and is variously termed LC_{2f} or the DTNB light chain (Weeds & Lowey, 1971; Gazith *et al.* 1970). The other class of light chains can be removed by alkali treatment, and these are thus called the alkali light chains (Weeds & Lowey, 1971). Two alkali light chains have been distinguished using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), LC_{1f} (or the A1 light chain) and LC_{3f} (or A2). The light chain pattern of slow-twitch muscle is different (Weeds, 1976; Lowey & Risby, 1971; Sarkar, Sreter & Gergely, 1971). These chains migrate on SDS-PAGE with a lower velocity so that the bands appear in the gels somewhat above those from fast-twitch muscle. Slow-twitch muscle does not contain a light chain which appears in a gel in the same position as LC_{3f}, but it does contain an LC_{2s} subunit which is of about the same molecular weight as the corresponding light chain found in fast-twitch muscle. In contrast to the single LC_{1f} band appearing in gels of the fast-twitch muscle, slow-twitch muscle gels show a doublet which appears just above the level of the LC_{1f} band. The bands in the doublet have been designated LC_{1as} and LC_{1bs}, and these light chains are considered to be homologous to the alkali light chains of fast-twitch myosin (Weeds, 1976).

There has been much interest recently in the study of the myosin light chains and their possible role in determining the kinetics of the actin-myosin interaction. Wagner & Weeds (1977) found that the actin-activated ATPase activity of homogeneous preparations of myosin sub-fragment-1 (S-1), in solutions of low ionic strength, differed substantially depending on whether the A1 or A2 light chain was present. The work described here was done to investigate whether the mechanical properties of single muscle fibres from the rabbit were in some way related to their myosin light chain composition. Mechanical V_{max} and stiffness were measured in fibre segments obtained from psoas and soleus muscles of adult and from psoas muscles of 1 day old rabbits. The light chain contents of these fibres was then determined by SDS-PAGE using techniques similar to those described by Weeds, Hall & Spurway (1975). Fibres from new-born rabbits were included in the study because of evidence showing that the relative amounts of LC_{1f} and LC_{3f} change in a reciprocal fashion during normal muscle development (Syrovy, 1979; Roy, Sréter & Sarkar, 1979; Sréter, Balint & Gergely, 1975*a*).

The muscle fibres that were studied had light chain compositions typical either of fast or slow muscle. The results indicate that for all fibres studied, neither V_{max}, which is believed to correlate with myosin ATPase activity (Bárány, 1967), nor stiffness correlated with the relative amounts of light chains present. This suggests that while the particular light chains that are present correlate with muscle type, i.e. fast or slow, the relative amounts of these light chains do not directly determine V_{max}. Further results indicate that the stiffness of these three muscle types are similar, though there is a clear suggestion that the stiffness obtained from any given fibre segment depends on the magnitude of compliance in the end connections. These findings are interpreted to mean that cross-bridge stiffness is similar in rabbit new-born psoas and adult psoas and soleus muscles.

A preliminary report of some results of this study were presented at a meeting of the Biophysical Society (Moss & Julian, 1979).

METHODS

Dissection. Psoas and soleus muscles were dissected from adult male rabbits (2·0–3·0 kg), and psoas muscles from new-born rabbits (35–95 g) within 24 hr of birth. Bundles of about fifty fibres were stripped from the central portion of the psoas muscles and were tied with thread to glass capillary tubes. Muscle length was adjusted to remove any slack. The length of muscle between the threads was 3–4 cm for the adult and 1–1·5 cm for the new-born muscles. Fibre bundles with attached tendons were cut from the adult soleus muscles using knives broken from razor blades, and were then tied to capillary tubes. Fibre lengths in these muscles were 0·8–1·3 cm.

Procedure for obtaining skinned fibres. The fibre bundles were then placed in cold skinning solution similar to that described by Wood, Zollman, Reuben & Brandt (1975) containing the following (mM): K proprionate, 180; EGTA, 4; ATP, 4; $MgCl_2$, 1; imidazole, 10 (pH 7·0). After 1–2 hr, the bundles were transferred to cold skinning solution containing 47 % (w/v) glycerol, and were stored in a freezer at − 15 to − 20 °C for 3–15 days before use. Single fibres could then be relatively easily stripped end-to-end from the psoas bundles. Isolation of soleus single fibres was made difficult by the presence of dense connective tissue surrounding the fibres. However, fibres were frequently found which would easily slip out of the bundle when pulled from the end. Care was taken during the isolation to avoid undue stretching of the fibres. Physiological measurements were done using a 3–5 mm segment of each fibre, and then the entire fibre was used for the determination of the myosin light chain content, as described below.

Experiments were attempted on skinned fibres of new-born soleus muscles, but fibre length was found to be too short (1·5–2·5 mm) to permit either mechanical measurements or the determination of light chain composition.

Physiological measurements

Solutions. The solutions for activating and relaxing the fibre segments were those described previously by Julian (1971). The relaxing solution contained the following (mM): KCl, 100; EGTA, 4; ATP, 4; $MgCl_2$, 1; imidazole, 10 (pH 7·0). The activating solution was similar in composition with the exception that calcium was added to a final pCa of 5·49 which resulted in maximal activation of the segments. The apparent stability constant for the Ca-EGTA complex was assumed to be $10^{6\cdot68}$ (Julian, 1971). Creatine phosphate (Sigma Chemical Co., St Louis, Mo.) was in several instances added to the bathing solution to a concentration of 14 mM (see Godt, 1974), though such addition was without an apparent effect on the results of this study obtained at 10 and 15 °C. Tension development at 20 °C required the presence of creatine phosphate in order to be maximal. Addition of creatine phosphokinase (1 mg/ml.) did not influence the above results with creatine phosphate, suggesting that the amount of endogenous enzyme in the skinned segments was sufficient for the regeneration of ATP.

Apparatus. The experimental chamber, force transducer and servo motor have previously been described in detail (Moss, 1979). The chamber was modified for this study to include a small

well into which the motor arm was placed. The well contained glycerol which provided fluid damping for the arm, making it possible to achieve length steps that were complete within 0·6 msec and that were free of oscillation. The force transducer had a resonant frequency of 3·3–4·2 kHz depending on the stylus employed.

The fibres were viewed and photographed through a microscope, as previously described (Moss, 1979). High power (approx. 460 ×) optics were used, so that single photographs included a view of a 0·3 mm length of a segment. The segments could be quickly scanned from end to end during activation by translating the microscope stage. Segments having regions in which the striations were grossly non-uniform were discarded. Examples of photographs of the soleus muscle fibres used in this study are shown in Pl. 1.

Procedure. A fibre segment was transferred to an experimental trough containing relaxing solution, and was attached to wires from the force transducer and servo motor (see Moss, 1979). A piece of the segment from 1 to 2·5 mm long remained exposed to the bathing solution between the connectors. Solution temperature was lowered to 15 °C, or sometimes to 20 °C, and overall segment length was adjusted to achieve an average striation spacing of about 2·7 μm during maximal activation. D. L. Morgan & F. J. Julian (unpublished result) have found this sarcomere length to be on the descending limb in tetanically stimulated mammalian muscle fibres. This length was chosen in the present study to ensure that the segment did not shorten over the plateau and on to the ascending limb, below a sarcomere length of about 2·3 μm in rabbit skeletal muscle, during a velocity measurement. Gordon, Huxley & Julian (1966) have shown previously in living frog fibres that V_{max} remains constant on the plateau and descending limb but falls off progressively on the ascending limb.

V_{max} was then measured in a series of contractions using a method (Hill, 1970; Julian, 1971; Edman, 1978) that will be called the slack test. Once a steady active tension was attained the segments were rapidly released to shorter lengths thereby introducing various amounts of slack. Following the release, force remained at zero for a period of time (Fig. 1), dependent on the amount of imposed slack. In each segment, various amounts of slack were introduced in from three to six succeeding contractions, and the amount of release was then plotted against the time of the unloaded shortening (Fig. 1). A straight line was fitted to the plotted points by the least-squares method and V_{max} was measured as the slope of this line. V_{max} is expressed in muscle lengths per second, where a muscle length is the equivalent segment length at an average striation spacing of 2·4 μm. Extrapolation of the fitted line to zero time yielded an estimate of the rapid length change required to reduce tension to zero. This value represents the extension of series elastic elements, mainly the ends of the segments at the points of attachment, during steady tension development.

The bathing solution was then lowered to 10 °C for measurement of the stiffness of each segment. This temperature was used in order to minimize the amount of tension recovery which occurred while the step was in progress (see Huxley & Simmons, 1971). At lower temperatures, tension was generally too low to allow accurate stiffness measurements. Small, rapid releases and stretches complete within 0·6 msec were applied to the segments during successive contractions. The tension responses were analysed in the manner previously described by Huxley & Simmons (1971) and Julian & Sollins (1975) for living skeletal muscle fibres of the frog. The tension value reached during the rapid tension change coincident with the length step was measured as T_1 and was expressed as a fraction of the maximum isometric tension, T_0. T_1/T_0 was plotted against the amount of length change per half-sarcomere. Because of the masking effect of tension recovery for greater extents of release (Huxley & Simmons, 1971; Julian & Sollins, 1975) the amount of rapid length change (Y_0') required to reduce tension to zero, i.e. if there were no tension recovery during the step, was estimated by linear extrapolation from T_1 values obtained for stretches and small releases. When the mechanical measurements were completed, the fibre segment was removed from the apparatus and pooled with the unused portion of the fibre for analysis of the myosin light chain content.

Myosin light chain analysis

Preparation of samples for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Fibre segments were prepared for electrophoresis using the procedure described by Weeds *et al.* (1975), with a few modifications. Fibres were transferred to capillary tubes, to which 5 μl. 10 % (w/v) SDS was added. The samples were ultrasonicated for 5 min and then placed

in a boiling water bath for 5 min. After this, samples were kept frozen for one to several days. On the day of the gel run the samples, containing 0·5–1·0 μg protein, were thawed, 2 μl. sample buffer was added and the samples were again placed in boiling water for 5 min before being applied to polyacrylamide gels. Sample buffer composition was modified slightly from that described by Laemmli (1970), that is, 62·5 mM-Tris-HCl (pH 6·8), 2 % (w/v) SDS, 10 % (v/v) glycerol, 5 % (v/v) 2-mercaptoethanol and 0·05 % (w/v) bromophenol blue.

Electrophoresis. SDS-PAGE was performed using a modification of the procedure of Laemmli (1970). Gels were prepared from a stock solution of 30 % (w/v) acrylamide and 0·8 % (w/v) *N,N'*-bis-methylene acrylamide. The final concentrations in the 12 % acrylamide separating gels were: 375 mM-Tris-HCl (pH 8·8); 0·1 % (w/v) SDS; 0·14 % (v/v) tetramethylethylenediamine (TEMED); and 2·5 mg ammonium persulphate/10 ml. gel solution. The final concentrations in the 3 % acrylamide stacking gels were: 125 mM-Tris-HCl (pH 6·8), 0·1 % (w/v) SDS, 0·14 % (v/v) TEMED and 2·5 mg ammonium persulphate/10 ml. gel solution. A freshly prepared ammonium persulphate solution (5 mg/ml.) was added to the gel solutions after de-aerating them for 10–15 min.

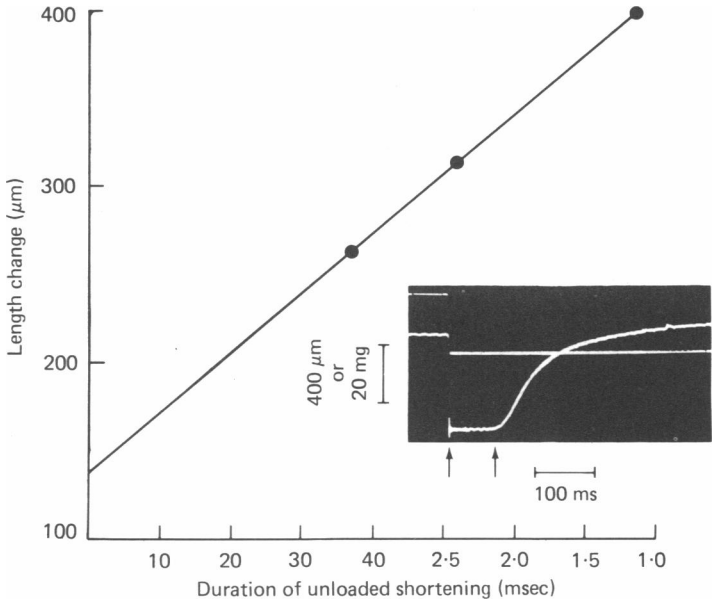

Fig. 1. Plot of data, length change *vs.* duration of unloaded shortening, obtained by the slack test method for measuring V_{max}. As shown in the inset, slack was introduced into the fibre segment (first arrow) to lower tension to zero. Tension remained at zero for a period of time, during which the segment underwent unloaded shortening, and then began to redevelop (second arrow). In this instance, various amounts of slack were introduced in three successive contractions. The amount of length change was plotted against the duration of unloaded shortening, a straight line was fitted to this data by the least-squares error method, and the slope of this line was calculated as V_{max}. Data obtained from an adult psoas segment at 15 °C. V_{max} was 1·88 *ML*/sec. Initial sarcomere length was about 2·7 μm, and end-to-end length was 2·04 mm.

Gels were cast in a vertical slab electrophoresis cell (Bio Rad, Model 220), 1·5 mm thick. A Teflon (DuPont Co.) comb was used which made it possible to form wells approximately 1·5 mm in width. The running, or electrode, buffer consisted of 25 mM-Tris, 192 mM-glycine and 0·1 % (w/v) SDS, pH 8·3 as described by Laemmli (1970). Electrophoresis was carried out at room temperature, using tap water circulation in order to prevent overheating of the gels. The runs were done at a constant current (24–30 μA/slab gel while samples were in the stacking gel and at 40 μA/slab gel in the separating gel) for a total of about 4 hr. The gels were stained with Coomassie brilliant blue as described by Weber & Osborn (1969) for 2 h at 50–60 °C or overnight at room

temperature (1·25 g Coomassie brilliant blue was dissolved in a mixture of 454 ml. 50 % methonal and 46 ml. glacial acetic acid; insoluble material was removed by filtration through filter paper). Destaining solution composition was as described by Weber & Osborn (1969), that is 5 % methanol and 7·5 % acetic acid. The processes of staining and destaining were both done with shaking of the bath solutions.

Analysis. The destained gels were scanned with a Joyce-Loebl automatic recording microdensitometer, Model MK 111 CS. A low density wedge (Wedge B, 0·36 D/cm) and a wide band filter (550 nm, Ditric Optics Inc.) were used to enhance sensitivity. The portion of the scannings containing the light chain peaks were photographed using 35 mm film, and then projected on a photographic enlarger. Tracings were made of the light chain peaks, and the areas under these peaks were determined by planimetry. Staining of the protein bands of the gels was assumed to be uniform (see Weeds *et al.* 1975), so that the measured areas were taken to indicate directly the relative amounts of the different light chains present in a given fibre.

RESULTS

Tension development. The time courses of tension development at 20 °C are shown in Fig. 2 for adult psoas and soleus fibre segments which were maximally activated with calcium. The time to peak tension was similar in both fibre types, though the rate of tension rise was probably limited by the diffusion of calcium-containing EGTA buffer into the cores of the fibres (see Moisescu, 1976), and this could serve to conceal any real differences in the rate of rise of tension. Similar tension time courses were observed during the activation and relaxation of the new-born psoas fibre segments. The average tensions developed by the fibre segments used in this study, as well as their mean physical dimensions, are listed in Table 1. The end to end lengths (L_{ee}) of the segments which were exposed to solution between the connectors, corrected to an average striation spacing of about 2·7 μm, were similar in the three kinds of preparations used. The cross-sectional areas of the segments were highly variable, and the relatively large average value for the new-born psoas segments reflects the presence in some of these segments of two and sometimes three fibres in parallel. There was no correlation between either the maximal velocities of shortening or the stiffness and the cross-sectional areas of the fibre segments. This finding rules out the possibility that these mechanical parameters were significantly limited by the diffusion of either calcium or ATP into the cores of the segments. The maximal isometric tension developed by the adult soleus and new-born psoas muscles averaged 0·55 and 0·64, respectively, of the tension developed by the adult psoas fibres at the two temperatures that were extensively studied. The Q_{10} value calculated for each different kind of fibre was about 2.

Light chain content of fibres from adult and new-born muscles. Densitometric scans of the light chain gel patterns obtained from single fibre samples of each of the kinds of muscle used in this study are shown in Pl. 2. The adult and new-born psoas fibres contained only LC_{1f}, LC_{2f} and LC_{3f} as has been reported previously for rabbit fast muscle by several investigators (Sarkar *et al.* 1971; Lowey & Risby, 1971; Sréter, Elzinga, Mabuchi, Salmons & Luff, 1975; Weeds *et al.* 1975; Pette & Schnez, 1977; Syrovy, 1977; Roy *et al.* 1979). There was no suggestion that any of the new-born psoas fibres used in this study contained any of the slow myosin light chains. The amount of LC_{1f} present relative to the amount of LC_{2f} is shown in Table 2 for 9 adult and 11 new-born psoas fibres. The ratios LC_{1f}/LC_{2f} and LC_{3f}/LC_{2f} (which is not shown

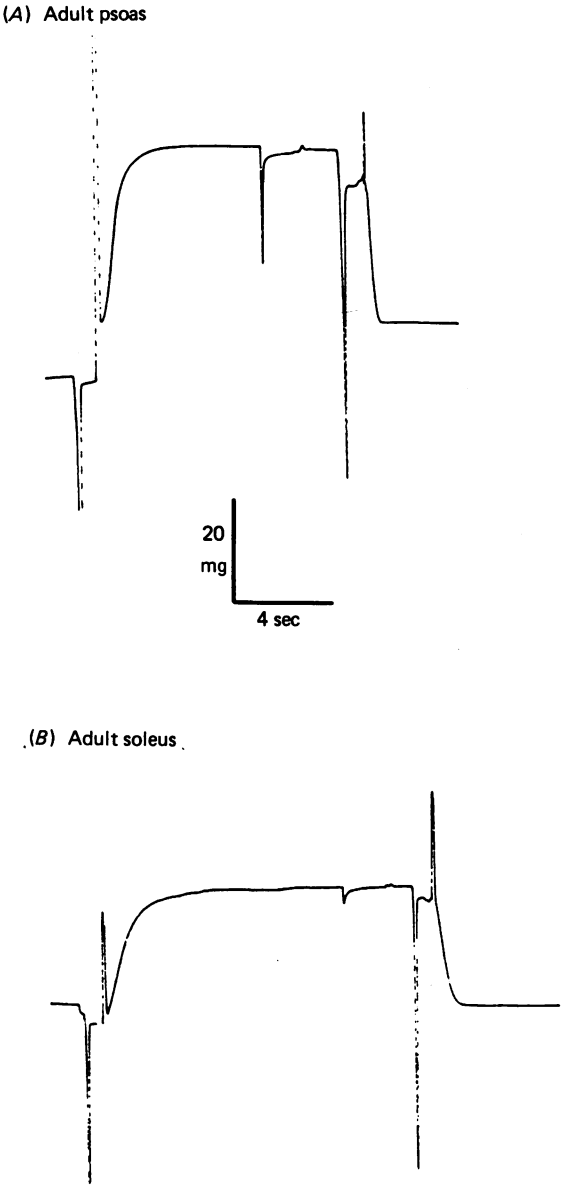

Fig. 2. Tension records obtained from adult psoas (part *A*) and adult soleus (*B*) fibre segments. In each case, the segment was transferred from relaxing to maximally activating solutions and a steady tension was allowed to develop. (The tension offset in part *A* occurred as the transducer wire passed through the air-fluid interface.) A length step (not shown) was applied in each case, resulting in a rapid drop in tension followed by tension recovery. The segments were then relaxed. Temperature was 20 °C.

in Table 2) were calculated directly from the areas measured under each light chain peak on the densitometric tracings. In most of the adult psoas samples, the sum of the amounts of LC_{1f} and LC_{3f} was roughly equal to that of LC_{2f} (Table 2), suggesting that in these samples there was no significant contamination of the light chain peaks by histones (Pette, Vrbova & Whalen, 1979). However, samples 10, 11 and 14 con-

tained relatively small amounts of LC_{2f}, a finding that we are unable to explain on the basis of a systematic procedural error. These samples were run in parallel and on the same gels as fibres having the expected higher quantity of LC_{2f}, which seems to preclude a selective proteolytic break-down in the gel of LC_{2f} in these cases. However, the amounts of LC_{3f} in these three samples was somewhat high relative to the amounts of LC_{1f} present and may indicate the presence of some LC_2 break-down products which co-migrated with the LC_{3f} peak (Pinset-Harstrom & Whalen, 1979).

TABLE 1. Summary of the salient features and average maximal tensions of the fibres used in this study. The values are reported ± 1 S.D. L_{ee} stands for segment length end-to-end between the connectors. *XSA* stands for cross-sectional area and was determined using the method of Gordon, Huxley & Julian (1966). Several of the new-born psoas preparations contained more than one fibre segment

	n	L_{ee} (mm)	*XSA* ($\times 10^{-5}$ cm^2)	P_{10} °C (kN/m^2)	P_{15} °C (kN/m^2)	Q_{10}
Psoas	18	1·78 ± 0·23	2·9 ± 1·2	117 ± 61	163 ± 78	1·94
New-born psoas	17	1·97 ± 0·31	4·4 ± 1·5	75 ± 32	104 ± 35	1·92
Soleus	11	2·00 ± 0·44	3·5 ± 1·2	63 ± 24	91 ± 35	2·09

TABLE 2. Summary of the V_{max} measurements at 15 °C and myosin light chain ratios of psoas fibres. Data from a given fibre were included only if both the mechanical measurements and most of the light chain determinations could be done. In some instances (*), especially with the new-born psoas fibres, it was difficult to determine accurately the area under the LC_3 peak

	Sample no.	LC_1/LC_2	$(LC_1+LC_3)/LC_2$	V_{max} (*ML*/sec)
Adult psoas	1	0·92	1·20	3·83
	5	0·70	0·89	2·56
	6	0·58	0·78	3·86
	7	1·12	*	1·88
	10	1·74	2·85	2·06
	11	1·67	2·45	1·52
	12	0·76	1·04	1·65
	13	0·64	0·73	1·80
	14	1·77	2·89	1·70
Mean ± 1 S.D.				2·32 ± 0·92
New-born psoas	1	1·54	1·72	2·05
	2	0·37	*	3·65
	3	0·55	0·68	2·88
	4	0·35	*	1·24
	6	0·39	*	0·92
	7	0·29	*	2·31
	8	0·95	*	1·90
	10	0·64	0·76	1·01
	11	0·88	0·92	1·47
	12	1·22	1·64	0·93
Mean ± 1 S.D.				1·84 ± 0·91

None of these three fibres were found to have mechanical properties which differed markedly from those of the other fibres. The LC_{1f}/LC_{2f} ratios for the new-born psoas fibres indicate a great variability in the relative amounts of these two light chains which were present in each of the fibres. The source of this variability is unknown, but it is possible, for example, that proteolytic activity was greater in the new-born

than in the adult fibre segments. The LC_{3f} peak was usually very small for the new-born fibres, and in several cases it was not possible to obtain a measurement of the area under this peak. For those values that were obtained, the LC_{3f}/LC_{2f} ratio again indicated some variability in the relative amounts of these light chains. It is likely that a major part of this variability in light chain ratios represents fibre to fibre variation in the proportions of the various light chains present. Previous measurements of light chain content which were obtained from pooled whole muscle preparations (e.g. Lowey & Risby, 1971; Roy *et al.* 1979) would tend to mask such variation. The adult soleus fibres contained myosin light chains LC_{1as}, LC_{1bs} and LC_{2s} of the slow type (Weeds, 1976; Pette & Schnez, 1977). Both of the ratios LC_{1as}/LC_{2s} and LC_{1bs}/LC_{2s} are listed in Table 3. Also listed in Table 3 are the ratios $(LC_{1as}+LC_{1bs})/LC_{2s}$, and it can be seen that in most cases this ratio is near unity.

TABLE 3. Summary of the V_{max} measurements at 15 °C and myosin light chain ratios of adult soleus fibres. Data from a given fibre were included only if both the mechanical measurements and light chain determinations could be done

	Sample no.	LC_{1a}/LC_2	LC_{1b}/LC_2	$(LC_{1a}+LC_{1b})/LC_2$	V_{max} (ML/sec)
Adult soleus	1	0·20	0·17	0·37	0·53
	4	0·70	0·60	1·30	0·70
	5	0·84	0·28	1·12	0·81
	6	0·57	0·29	0·86	0·71
	8	0·77	0·28	1·05	0·72
	9	1·05	0·65	1·70	0·70
Mean					0·70
±1 S.D.					±0·09

Relation of V_{max} to light chain content. The V_{max} data for all of the fibre segments for which measurements of light chain content were obtained are listed in Tables 2 and 3. The mean V_{max} values for the new-born and adult psoas fibres do not differ significantly, though in several instances V_{max} values from the new-born fibres were below the lowest values obtained from the adult fibres (these findings from new-born fibres are in contrast to those of Close (1964) who found living whole muscles of new-born rat to have a uniformly slow V_{max}). The V_{max} values for the soleus fibres were consistently below those obtained from either kind of psoas fibre. The ratio of the mean V_{max} for the adult psoas to that of the adult soleus fibres was approximately 3:1, which is near the value of about 2·5 calculated from the data of Close (1965) obtained from whole fast and soleus muscles of the cat. Thus, the presence of fast type myosin light chains in the psoas fibres, as reported above, was correlated with a relatively high V_{max}, and the presence of slow type light chains in the soleus fibres was correlated with a low V_{max}.

The measured V_{max} of each of the adult and new-born psoas fibres is plotted in Fig. 3*a* against the LC_{1f}/LC_{2f} ratio determined from the same fibre. The V_{max} data from the adult soleus fibres are plotted in Fig. 3*b* against both the LC_{1as}/LC_{2s} and LC_{1bs}/LC_{2s} ratios for each fibre. In none of these cases was a consistent relationship found between V_{max} and any of these light chain ratios.

Stiffness measurements. Examples of the tension responses of the new-born and adult fibre segments to length changes of about -5 nm/half-sarcomere are shown in

Fig. 4. The form of the tension responses in the adult psoas fibre segment was similar to that seen previously in frog living muscle fibres (Huxley & Simmons, 1971; Julian & Sollins, 1975), though the time-course of tension recovery is somewhat slower in the fibres of the present study. Similar responses have been seen previously in rabbit skinned psoas fibres (Abbott & Steiger, 1977; Guth & Kühn, 1978) and frog skinned muscle fibres (Heinl, Kühn & Ruegg, 1974; Goldman & Simmons, 1977; R. L. Moss & F. J. Julian, unpublished results). Tension recovery in the soleus fibre segments

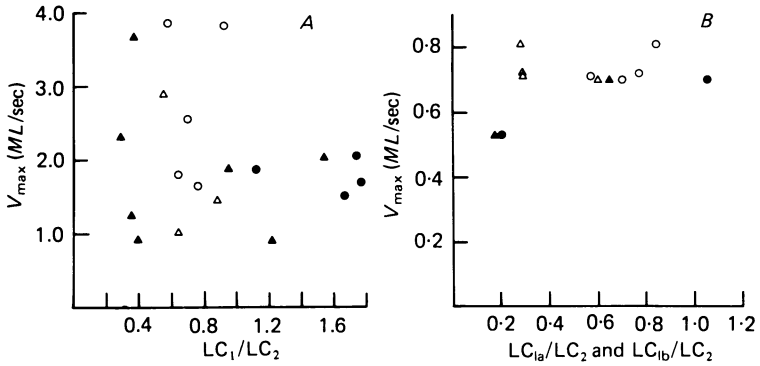

Fig. 3. *A*, plot of V_{max}, in muscle lengths/sec (*ML*/sec), *vs.* the ratio LC_1/LC_2 of adult psoas fibres (○, ●) and of new-born psoas fibres (△, ▲). The open symbols correspond to fibres in which the ratio $(LC_1+LC_3)/LC_2$ was between 0·68 and 1·20; the filled symbols refer to fibres in which the ratio was outside this range. *B*, plot of V_{max} *vs.* the ratios LC_{1a}/LC_2 (○, ●) and LC_{1b}/LC_2 (△, ▲) of adult soleus fibres. The open symbols correspond to fibres in which the ratio $(LC_{1a}+LC_{1b})/LC_2$ was between 0·86 and 1·30; the filled symbols refer to fibres in which the ratio was outside this range.

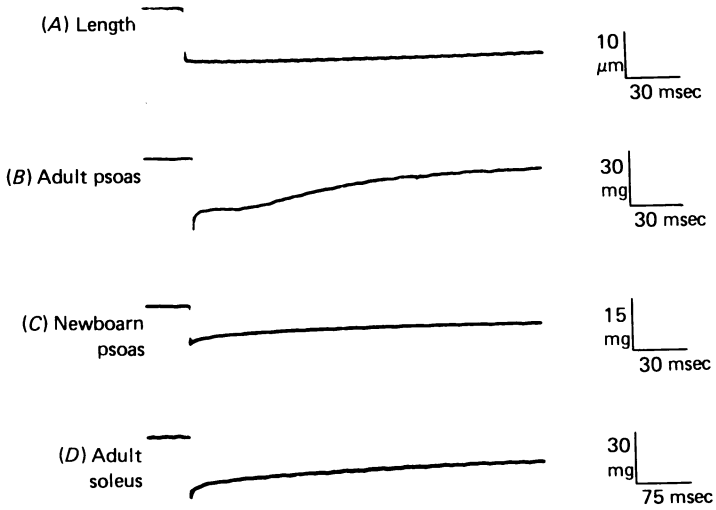

Fig. 4. Records of the tension responses of the skinned fibre segments to length steps (uppermost) applied during steady activation in solution of pCa 5·49. The amplitude of length step (nm/half-sarcomere) and T_1/T_0 value obtained in each case were: adult psoas: 5·1, 0·69; new-born psoas: 4·4, 0·71; adult soleus: 4·3, 0·74. Note the different time and tension calibrations that were used. Temperature was 10 °C.

occurred at a much slower rate, and the extent of recovery was less than in the adult psoas segments. The tension response of the new-born psoas segments were more like the adult soleus in form, rate and extent of recovery. However, in one instance a new-born psoas segment showed a clear reversal in the record of tension recovery similar to that seen in the adult psoas segments.

The T_1 values for all the fibre segments are plotted in Fig. 5 against the amount of length change calculated in nm per half-sarcomere. The range of data from the three different kinds of muscle segments show considerable overlap. The average extrapolated length changes per half-sarcomere (Y_0') required to rapidly lower tension to zero were similar in the three muscle types studied. A value of $12{\cdot}8 \pm 4{\cdot}1$ nm ($n = 14$) was obtained for the adult psoas, $11{\cdot}2 \pm 1{\cdot}9$ nm ($n = 9$) for the adult soleus and $14{\cdot}3 \pm 3{\cdot}6$ nm ($n = 11$) for the new-born psoas segments.

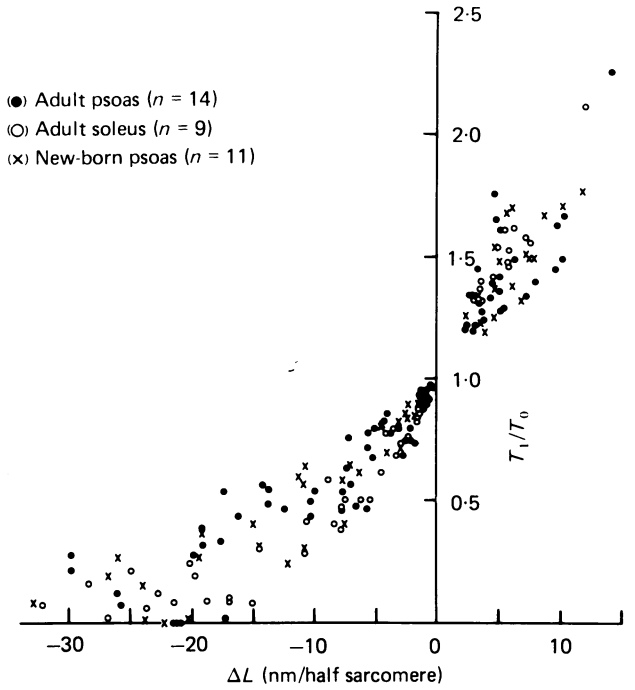

Fig. 5. Plot of the relative magnitude of T_1 (i.e. T_1/T_0) *vs.* the size of the applied step (nm/half-sarcomere). The filled circles are data from adult psoas fibre segments ($n = 14$); the open circles, from adult soleus segments ($n = 9$); and the crosses from new-born psoas segments ($n = 11$).

Influence of the end connections on instantaneous stiffness. Previous efforts in this laboratory (see Moss, 1979) have attempted to improve the method of attachment to the ends of the fibre segments in order to reduce end compliance and striation non-uniformity during activation. Still, the points of attachment are likely sources of extra compliance in these preparations. The value of Y_0' for each fibre segment is plotted in Fig. 6 *vs.* the extension of series elements, measured from the slack test data obtained from the same segments, expressed relative to L_0, the segment length at an average striation spacing of 2·4 μm during steady activation. The relative

extension of series elements during steady activation was variable from segment to segment though the ranges of values obtained were similar in each of the three muscle types. Attempts were made to determine whether the measured series extension correlated with other relevant physical properties of the segments. No discernible relationships were found between the relative series extension and either L_{ee} or the absolute tension developed by the segments. This finding suggests that the absolute compliance introduced by the end connections can vary significantly in different segments.

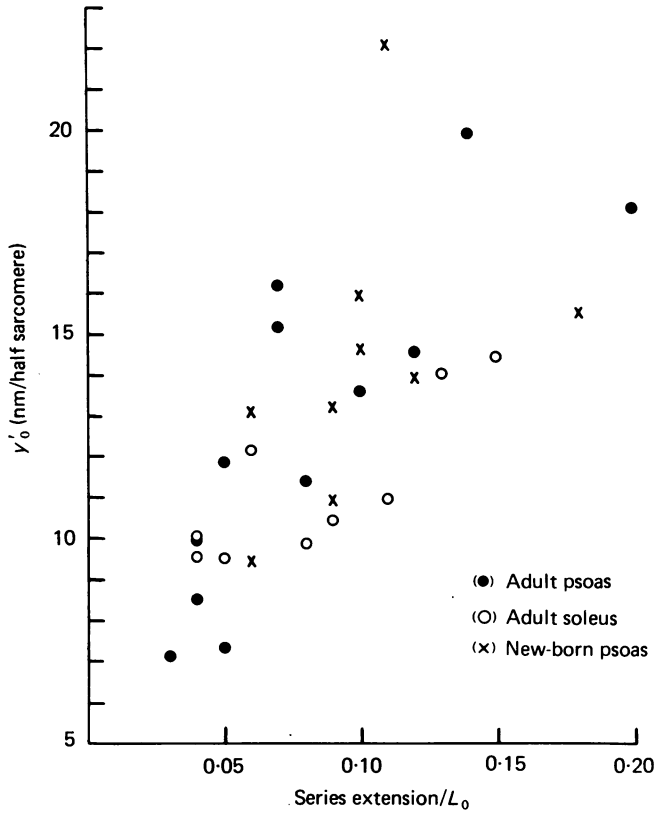

Fig. 6. Plot of the extrapolated length change (Y'_0) required to lower tension rapidly to zero *vs.* the relative series extension measured for each fibre segment. The absolute series extension was measured as the axial intercept at zero time of a straight line fit to the data obtained by the slack test method to determine V_{max} (see Fig. 1). The filled circles are data from adult psoas fibre segments, the open circles from adult soleus segments; and the crosses from new-born segments.

The relationship between Y'_0 and the relative series extension shows clearly that the value of Y'_0 increases as the amount of series extension increases, i.e. the preparations become less stiff. The correlation coefficient for these two variables was 0·69. A straight line fit to all of the data using the least squares method (r^2 = 0·49) yields by extrapolation a Y'_0 value of 7·6 nm/half-sarcomere for a relative series extension of zero.

Relation of the extrapolated stiffness and developed tension to light chain content. The

tension developed by each fibre segment, as well as segment stiffness was plotted *vs.* the light chain ratios, LC_{1f}/LC_{2f} and LC_{3f}/LC_{2f}, measured for that fibre segment (plots not shown). No consistent relationships between developed tension or stiffness and either of these light chain ratios were found for any of the different fibre types.

DISCUSSION

Mechanical properties of adult and new-born muscle fibres

Tension development. The fibre segments in this study developed absolute tensions in solutions of pCa 5·49 and 15 °C which were less, by a factor of 2 or 3, than the tensions developed by frog skinned twitch fibres at the same pCa and 5 °C (Moss, 1979). However, the rabbit fibres were found to have a Q_{10} of about 2·0 while living frog fibres have been observed to have a Q_{10} of around 1·0 for isometric tetanic tension (see, for example, Ramsey & Street, 1940). This difference in Q_{10} presumably results from a basic difference in some aspect of the actin-myosin interaction in the muscles of these animals. If tension development in the rabbit fibres were extrapolated to a more physiological temperature range, based on a Q_{10} of 2, better agreement with the tension data from frog muscle would be obtained.

The tensions developed by the adult and new-born psoas and adult soleus fibres of the present study were somewhat variable. The observed variation did not consistently correlate with segment diameter and did not depend on the presence or absence of an ATP regenerating system. Similar variations in tension development have been reported previously for other skinned fibre preparations (see, for example, Moss, 1979; Thames, Teichholz & Podolsky, 1974). Comparisons of the tensions developed by the three kinds of muscle used in this study are thus made difficult. Generally, the adult psoas segments developed larger tensions, normalized to cross-sectional area, than either the new-born psoas or adult soleus segments. Such a difference would occur if the amount of tension generated by individual cross-bridges varied according to muscle type and age. Our own studies suggest that this is not the case, in that the relative stiffness measured in each of the different fibre types are similar. Another possibility to explain the disparities in tension development is that the percentage of fibre cross-section composed of contractile material differs in each of the muscles used. If this is the case, comparisons of tension normalized to cross-sectional area would not be valid.

Maximum velocity of shortening. The velocities measured in the present study indicate that V_{max} for the adult psoas is about 3 times greater than that of the adult soleus, in good agreement with data obtained from intact muscles of the cat (Close, 1965). Velocity data for skinned mammalian muscle has also been obtained by Gulati (1976) who found a V_{max} of about 0·2 ML/sec (muscle length per second) for soleus and 0·5 ML/sec at 10 °C for extensor digitalis longus muscles of the guinea-pig. These values are low with respect to the values measured from the rabbit muscle fibres of the present study, even when the temperature difference between the two studies is taken into account, perhaps suggesting a species variation in shortening velocity. It is possible that Gulati's measurements tended to underestimate V_{max} in his preparations in that no loads less than about 0·2 P_0 were used in the extrapolation to velocity at zero load.

The range of V_{max} values obtained from the soleus fibres of the present study was small; however, V_{max} for the psoas muscle fibres showed greater variability. V_{max} for the adult psoas fibres varied between about 1·7 and 3·8 ML/sec, and for the new-born fibres between and 0·9 and 3·8 ML/sec. Attempts were made to determine whether some part of this variability was artifactual. No correlation was found between V_{max} and fibre cross-sectional area. Further, the value obtained for V_{max} did not depend in a consistent way upon the relative magnitude of the end compliance estimated for each preparation. We conclude that the observed variation in V_{max} is for the most part a real property of these fibres, and reflects variability in the kinetics of the actin-myosin interaction cycle. If, as suggested by the work of Bárány (1967), actomyosin ATPase and V_{max} are directly related, our results on single fibres would indicate that there is fibre to fibre variation in the actomyosin ATPase activity, though measurements of actomyosin ATPase are not yet available for single muscle fibres of the rabbit. The finding that many of the new-born psoas fibres had lower V_{max} values than the adult suggests that the development of the higher V_{max} characteristic of the adult occurs at a rate which varies from fibre to fibre.

We have assumed in selecting fibres from the central one third portion of the psoas muscles that the fibres comprise a histochemically uniform population. Some variation in V_{max} could result if the fibre population was non-uniform in this regard. However, this possibility seems unlikely, at least for the adult fibres, in that Weeds *et al.* (1975) found that 98 % of the fibres in the central two-thirds of the rabbit psoas muscle were of the 'fast twitch glycolytic type'.

Stiffness. The stiffness measurements, obtained with rapid length steps, indicate that the mean stiffness of the adult psoas and soleus and new-born psoas muscles are similar. Similar measurements obtained in living frog fibres reflect the stiffness of the elastic part of the cross-bridges (Huxley & Simmons, 1971; Julian & Sollins, 1975; Ford, Huxley & Simmons, 1977). Thus, the findings of the present work would indicate that cross-bridge stiffness is similar in muscle fibres of different types and age.

The average values of Y_0' obtained from the rabbit fibres were between 11 and 14 nm/half-sarcomere. Values of 10–11 nm/half-sarcomere have been obtained in similar measurements on skinned frog muscle fibres (Goldman & Simmons, 1977; R. L. Moss and F. J. Julian, unpublished results). These values are high relative to the value of about 6 nm obtained in living frog muscle fibres at 0 °C (Julian & Sollins, 1975; Ford *et al.* 1977). Some of the discrepancy between this value and that of the rabbit fibre segments is probably a result of the higher temperatures used in the present study, which would result in a more rapid tension recovery following the length step. The data of Fig. 6 suggests that the most likely explanation for the apparently lower stiffness of the rabbit fibre segments is that the compressed ends of the preparation give rise to a relatively large and variable amount of compliance. In contrast, the ends of the frog fibres are very stiff due to the presence of a stout tendon for attachment to the apparatus. The tension-extension characteristic of the compliant regions at the ends of the skinned fibre segments is very likely non-linear (see Fung, 1967). During steady tension development, extension of these regions would result in a greatly increased stiffness, which for small length changes would be approximately constant. The disparity, by about a factor of 2, between the Y_0' values of the skinned fibres of the present study and those previously measured in

living fibres could be explained if the stiffness of the end regions of the skinned segments during steady activation was less than that of the central portion.

The large extents of release (3–20 % L_0) estimated to just lower tension to zero by the slack test method would clearly include some amount of additional length change to offset tension recovery during the length step. In living muscle fibres, the amount of length change (about 12 nm/half-sarcomere) which would be necessary to attain a T_2 level of zero is about twice that required for a T_1 of zero (e.g. Ford *et al.* 1977). In the present study, the length change to zero tension estimated by the slack test method was usually several times greater than the zero tension estimate for T_1 based on small stretches and releases. This indicates that another factor in addition to tension recovery is the cause of the observed large releases necessary to reduce tension to zero. Examination of the data of Fig. 6 reveals that as the amount of estimated relative series extension decreased, the variability, as well as the magnitude, of Y_0' decreased. Variability in Y_0' for a particular relative series extension presumably reflects variability in the tension-extension characteristics of series elastic elements in addition to the cross-bridges. The reduced variation in Y_0' at lower values of series extension would thus represent the tendency for the measured Y_0' to converge on the value that would be obtained if the muscle preparations were free of relatively large end compliances.

The Y_0' value for some segments was about 7 nm/half-sarcomere. In these cases the series extension was small. Linear regression analysis of the data of Fig. 4 yielded an extrapolated value of about 7·6 nm/half-sarcomere for Y_0' for the ideal situation in which there is no compliance in series with the sarcomeres. This value is close to that of the living frog fibre and suggests that the stiffnesses of the cross-bridges in the muscles of the two animals are similar. The value obtained for the coefficient of determination (r^2) indicates that factors in addition to end compliance influence the value of Y_0'. A likely possibility is segment length, though the observed lack of correlation of Y_0' with this parameter argues that the end compliance effects dominate in determining Y_0'.

This study also qualitatively describes the tension recovery phase of the adult and new-born muscles following attainment of T_1. The phase of rapid recovery was less extensive in the soleus than in the adult psoas fibre segments. In terms of the Huxley & Simmons (1971) cross-bridge model, this result would suggest that the extent of cross-bridge rotation following the length step was less in the soleus fibre segments. The subsequent slower phase of recovery occurred at a slower rate in the soleus muscle and this presumably results from the slower kinetics of interaction of actin and myosin which in the slow muscle is marked by a relatively low mechanical V_{max}. The new-born psoas fibres showed variable extents and rates of tension recovery, appearing in some cases like that of the adult soleus and in others more like the adult psoas fibre segments. This finding supports the idea that the developing muscle, which will be fast in the adult, is in transition at birth in terms of its physiological and biochemical characteristics.

Relation of light chain content to mechanical properties. The psoas and soleus fibres used in this study had clearly different myosin light chain components. The presence in the psoas fibres from both adult and new-born rabbits of LC_{1f}, LC_{2f} and LC_{3f} was associated with a relatively high mechanical V_{max}, while LC_{1as}, LC_{1bs} and LC_{2s} were

found in adult soleus fibres which had uniformly low V_{max} values. The ratio (LC_{1f} + LC_{3f})/LC_{2f} was near unity in most psoas fibres from adult rabbits. There was no correlation between mechanical V_{max} and the ratio LC_{1f}/LC_{2f} in fibres from either adult or new-born rabbits. In the soleus fibres from adult rabbits, the ratio (LC_{1as} + LC_{1bs})/LC_{2s} was usually near unity. In the soleus fibres, V_{max} did not vary much and was not correlated with either of the ratios LC_{1as}/LC_{2s} or LC_{1bs}/LC_{2s}. We have further found that tension development and stiffness are also independent of the relative proportions of light chains present in each type of fibre. The most straightforward conclusion based on these findings is that the relative proportions of the alkali light chains present do not directly influence the interaction of myosin with actin as revealed mechanically by tension development, muscle stiffness or unloaded shortening velocity.

Results have been obtained by several investigators in which correlations between the structural and functional changes in myosin have been shown to exist during the development of predominately fast muscle. The amount of LC_{1f} decreases and that of LC_{3f} increases during development of these muscles from the embryonic to the adult stages (Roy *et al.* 1979; Syrovy, 1979; Sréter, Luff & Gergely, 1975*c*). At the same time, the myosin ATPase activity is observed to increase somewhat (Syrovy, 1979; Sréter *et al.* 1975*c*), though the extent of this increase has in some instances been found to be small (Sréter *et al.* 1975*c*). In the present study, the ratio LC_{1f}/LC_{2f} was found to vary substantially among the adult psoas fibres with no apparent correlation with the measured V_{max}. Since V_{max} is thought to be correlated with myosin ATPase activity (Bárány, 1967), the correlation of the LC_{1f} content, and, reciprocally, LC_{3f}, with myosin ATPase activity during development would seem on the basis of the present results to be coincidental.

More recent work by Wagner, Slater, Pope & Weeds (1979) indicates that when the ionic strength is increased towards more nearly physiological levels the maximum rate of actin-activated ATP hydrolysis is the same for both S-1 (A1) and S-1 (A2). In addition, Wadzinski *et al.* (1979) have shown through the use of time-resolved fluorescence depolarization techniques that the affinity constants for the binding of S-1 (A1) and S-1 (A2) to F-actin in the absence of ATP are very similar. These new biochemical data, taken together with the results presented here concerning the lack of correlation between mechanical properties and light chain ratios within each class of muscle, make it difficult to arrive at any immediately apparent role for the light chains in skeletal myosin function. However, in our work, fibres from fast-twitch and slow-twitch muscles could clearly be distinguished on the basis of their light chain patterns and maximum speeds of shortening (V_{max}). Further work is needed to elucidate the nature of this intriguing association, particularly with regard to determining how a particular pattern – rather than a proportion – of light chains could influence V_{max}.

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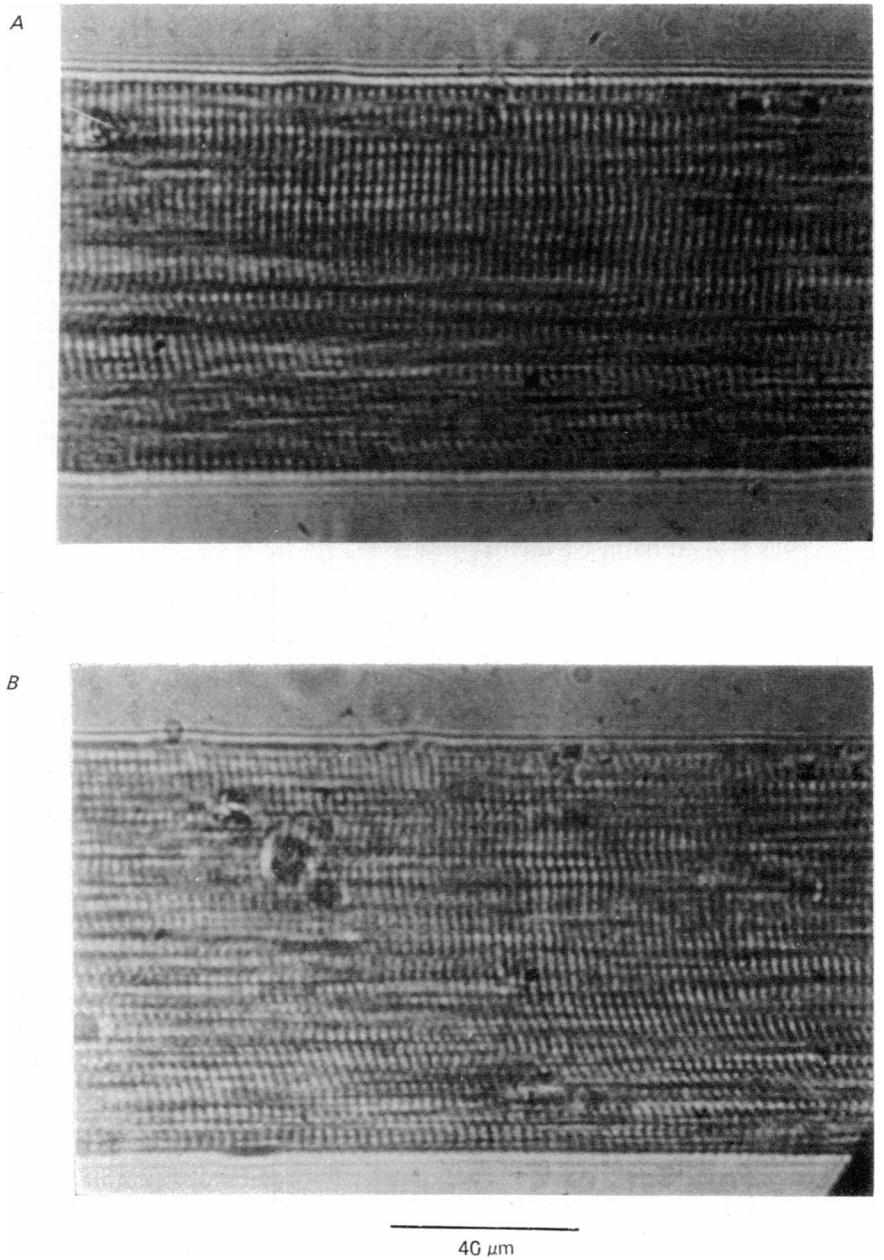
EXPLANATION OF PLATES

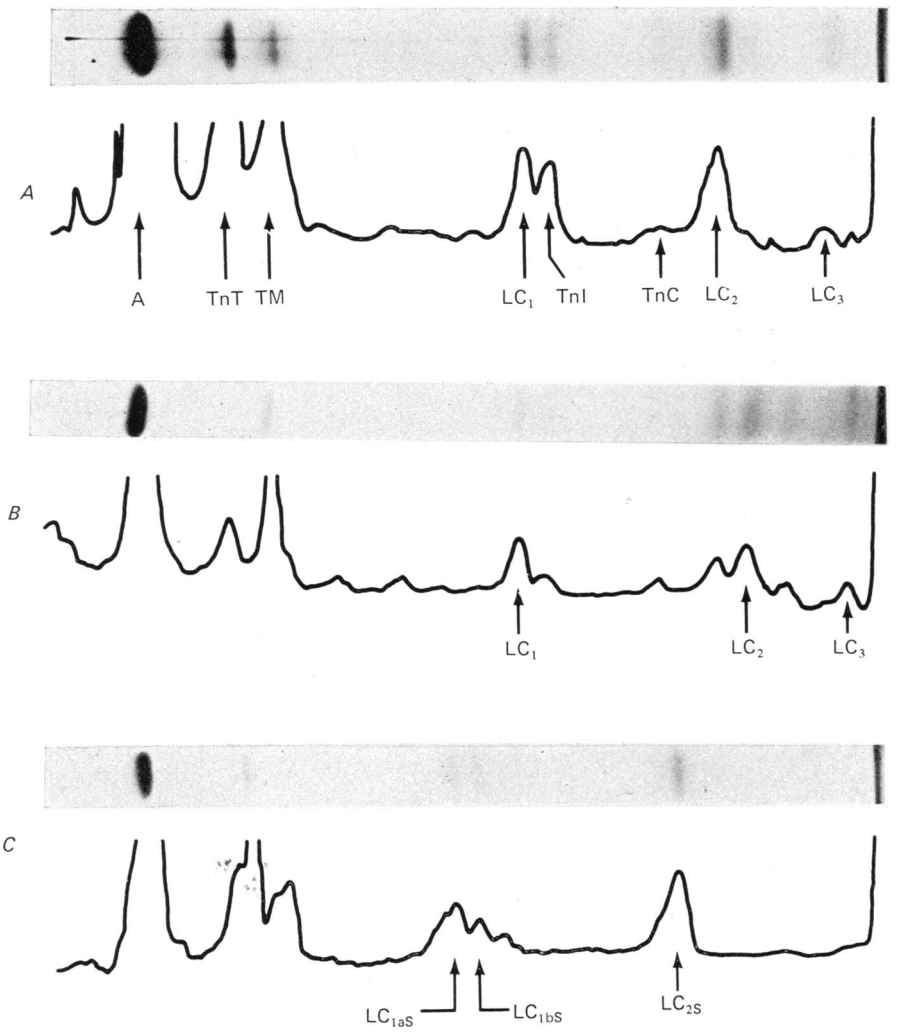
PLATE 1

Light photomicrographs of a segment from a soleus muscle fibre, while relaxed (part *A*) and during maximal activation (part *B*). The striation spacing in part *A* was 2·47 μm and in part *B*, 2·32 μm. Temperature was 15 °C.

PLATE 2

Photographs of the SDS-PAGE patterns obtained from single fibre segments of adult psoas (part *A*) and soleus (part *C*) and new-born psoas (part *B*) muscles. An ink tracing of the densitometric scan of each gel is positioned below the appropriate gel, and the peaks are labelled as to their compositions. A represents actin; TnT, troponin-T; TM, tropomyosin; LC_1, LC_2 and LC_3 are the myosin light chains typical of fast muscle; TnI, troponin-I; TnC, troponin-C; LC_{1s}, LC_{2s} and LC_{2s} are the myosin light chains typical of slow muscle. The gels and scans are cut just above the actin peak.


A
B
40 μm



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