Electrophoretic analysis of proteins from single bovine muscle fibres

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A number of single fibres were isolated by dissection of four bovine masseter (ma) muscles, three rectus abdominis (ra) muscles and eight sternomandibularis (sm) muscles. By histochemical criteria these muscles contain respectively, solely slow fibres (often called type I), predominantly fast fibres (type II), and a mixture of fast and slow. The fibres were analysed by conventional sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and the gels stained with Coomassie Blue. Irrespective of the muscle, every fibre could be classed into one of two broad groups based on the mobility of proteins in the range 135,000–170,000 daltons. When zones containing myosin heavy chain were cut from the single-fibre gel tracks and ‘mapped’ (Cleveland, Fischer, Kirschner & Laemmli (1977) J. Biol. Chem. 252, 1102–1106) with Staphylococcus proteinase, it was found that one group always contained fast myosin heavy chain, whereas the second group always contained the slow form. Moreover, a relatively fast-migrating a-tropomyosin was associated with the fast myosin group and a slow-migrating form with the slow myosin group. All fibres also contained b-tropomyosin; the coexistence of a- and b-tropomyosin is at variance with evidence that a-tropomyosin is restricted to fast fibres (Dhoot & Perry (1979) Nature (London) 278, 714–718). Fast fibres containing the expected fast light chains and troponins I and C fast were identified in the three ra muscles, but in only four sm muscles. In three other sm muscles, all the fast fibres contained two troponins I and an additional myosin light chain that was more typical of myosin light chain 1 slow. The remaining sm muscle contained a fast fibre type that was similar to the first type, except that its myosin light chain 1 was more typical of the slow polymorph. Troponin T was bimorphic in all fast fibres from ra muscles and in at least some fast fibres from one sm muscle. Peptide ‘mapping’ revealed two forms of fast myosin heavy chain distributed among fast fibres. Each form was associated with certain other proteins. Slow myosin heavy chain was unvarying in the three slow fibre types identified. Troponin I polymorphs were the principal indicator of slow fibre types. The myofibrillar polymorphs identified presumably contribute to contraction properties, but beyond cud chewing involving ma muscle, nothing is known of the conditions that gave rise to the variable fibre composites in sm and ra muscles.

In recent years much effort has been directed at the characterization of muscle fibre types. Nomenclatures vary, but all authorities recognize a slow-twitch fibre type, often called type I (Peter et al., 1972), which can be distinguished histochemically (Padykula & Herman, 1955) from fast-twitch fibres, type II. Moreover, certain myofibrillar proteins in type I fibres can be distinguished immunologically from their fast-twitch analogues.

Abbreviations used: SDS, sodium dodecyl sulphate; sm, ma and ra, sternomandibularis, masseter and rectus abdominis (muscles) respectively.

Dhoot & Perry (1979), for instance, describe different forms of troponin and tropomyosin in type I and type II fibres, and slow and fast myosins have long been recognized. With classic histochemical techniques, type II fibres can be conveniently subdivided into those with both an oxidative and a glycolytic capacity, type IIA, and those with a low oxidative but a very high glycolytic capacity, type IIB (Brooke & Kaiser, 1970; Peter et al., 1972). In contrast with type IIB, type I fibres have a high oxidative and a low glycolytic capacity. Brooke & Kaiser (1974) also distinguish an undifferentiated fast fibre, type IIC.
The above studies have been performed largely by optical-microscopic observations on cross-sections of muscle tissue. An alternative approach has been to isolate single muscle fibres, either for enzymic analyses (Lowry et al., 1978) or for direct identification of proteins by SDS/polyacrylamide-gel electrophoresis (Weeds et al., 1975; Pette & Schnez, 1977a,b). This latter technique has had only limited success, partly because of the small amount of protein in a rabbit muscle fibre. Bovine muscle fibres are, however, relatively large, and have been analysed by this technique in the present study.

Most fibres were dissected from bovine sterno-mandibularis, a ventral neck muscle used for lowering the head. By histochemical criteria this muscle contains a mixture of fibre types (Leet & Locker, 1973; Davey & Winger, 1979). By contrast, bovine cheek muscle, masseter, is a homogeneous slow muscle (R. H. Locker, personal communication) adapted in this species for cud chewing; masseter was used as a reference standard for slow-twitch fibres. Rectus abdominis is located in the lower belly and is involved in flexing the lower spine. It is composed predominantly of fast fibres (Leet & Locker, 1973) and was also used as a reference standard.

In the present study, fibre types have been characterized by myofibrillar protein composition. The definitive variations were seen in the light and heavy chains of myosin, tropomyosin, troponin and a group of proteins around 160000 daltons. Among other results, the study demonstrates two kinds of fast myosin heavy chain, and fibre types where a fast myosin heavy chain is associated with light chains one of which is more characteristic of slow fibres.

### Experimental

**Dissection of muscles**

Table 1 summarizes the origins of the muscles used. The fibres were usually dissected from the muscles within 6 h of slaughter, but in some cases up to 24 h after slaughter. Such post-slaughter delays did not affect gel-staining patterns. Sm fibres were dissected from the core and the periphery of the mid-region. The fibres from ma and ra muscles were dissected from the central region of the muscles.

The dissection medium was phosphate-buffered saline (137 mM NaCl/3 mM KCl/2 mM KH₂PO₄/8 mM Na₂HPO₄, pH 7.3), containing 5 mM-EGTA (sodium salt), pH 7.3, to minimize contraction. The fibre bundles were teased apart under a stereomicroscope with zoom facility, and only fibres at least 8 mm long were selected; a code number was assigned (A1, A2 etc.) and any peculiar characteristics were noted.

Wide differences were encountered in the ease of isolating single fibres, presumably through variations in amounts of endomysial collagen and in fibre diameter. The largest fibres isolated were from sm muscles of old and large animals. Such fibres were typically between 2 and 3 cm long and about 70 µm in diameter. In no case could a complete muscle cell be isolated, but each fibre was part of a single cell, except for M7 and M12 (ra muscle) and 20 of the 28 ma fibres. These were selected as clumps of two or three fibres because of dissection difficulty. Each resulting gel pattern could, however, be clearly equated with a particular single fibre type from its respective muscle.

Fibres were transferred to the bottom of Pyrex

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Code</th>
<th>Breed</th>
<th>Animal age (years)</th>
<th>Fibres sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>sm</td>
<td>A</td>
<td>Angus</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Angus</td>
<td>&gt;1</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>Friesian</td>
<td>1.5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>Friesian</td>
<td>2.5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>Friesian</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>—</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Hereford x Angus</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>Hereford</td>
<td>4 or 7</td>
<td>16</td>
</tr>
<tr>
<td>ma</td>
<td>H</td>
<td>Friesian</td>
<td>1.5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>Friesian</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Hereford</td>
<td>&gt;1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Q</td>
<td>—</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>ra</td>
<td>M</td>
<td>—</td>
<td>—</td>
<td>10</td>
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<td></td>
<td>O</td>
<td>Angus</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>Friesian</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1. Sources of bovine muscle fibres

All animals were bulls, but muscles M, O and R were from castrated animals. Muscles H and I were from the same animal, as were muscles J and K. —, information not available.
Electrophoresis of muscle fibre proteins

Fractionation range was extended from the reported 50–55% to 35–55% saturation. Desmin was purified from sm muscle (Young et al., 1981).

Sigma Chemical Co. supplied horse heart myoglobin and the following rabbit muscle enzymes: creatine phosphokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphorylase a and a number of glycolytic enzymes. A marker mix from Pharmacia Fine Chemicals containing phosphorylase (94,000 daltons), serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100) and a-lactalbumin (14,400) was routinely used.

Results

Identification of major myofibrillar proteins

Each gel track in Figs. 1–5 displays a marker(s) or the proteins from usually a single muscle fibre. Actin and the heavy chain of myosin were easily and unequivocally identified. a-Actin was identified by its staining intensity, ubiquity, relative migration and solubility properties (Young et al., 1981). Troponomyosin was identified by comparisons with the migrations of purified bovine tropomyosins; as the study progressed, two forms of bovine a-tropomyosin (Cummins & Perry, 1973) were identified (Fig. 1). Each was coincident on electrophoresis with its respective form purified from slow (ma) and fast (ra) muscles. Troponin components were similarly identified and are considered in detail below.

The light chains of myosin were identified by using markers of purified myosin and by the migrations of light chains relative to troponins I and C from fast and slow muscles. Troponin I was particularly useful as a reference, since both troponin I fast (TI1) and slow (TI1) (Dhoot et al., 1979) were turquoise when stained, whereas other proteins were purple–blue. Thus it was possible to distinguish between TI1 and light chain 1 fast, which had identical migrations (Fig. 1a). By such criteria, three so-called fast and two slow light chains analogous to those described by Lowey & Risby (1971) were identified (Figs. 1 and 2). For example, fibre C10 (Fig. 2) contains light chain 1 fast (LC1f), LC2, and lesser amounts of LC3, whereas in fibre A1, LC3 is more strongly expressed.

An overview of fibre types

In an analysis of the protein patterns of single fibres, one is faced with the problem of what importance to attach to each character. Leaching of sarcoplasmic proteins, which could otherwise be considered in analysis, compounds the problem. In this respect, myoglobin, which migrated just below LC3 (Fig. 1a), was completely absent, or present only in traces, yet is found in significant con-
Fig. 1. SDS/polyacrylamide-gel electrophoresis of single sm muscle fibres and of slow and fast tropomyosins
(a) SDS/polyacrylamide-gel electrophoresis with 12.5% (w/v) polyacrylamide was performed as described in the text. The markers were myoglobin (MYG) and enolase (EN). When myoglobin was re-run at a lower concentration (result not shown), its migration relative to myosin light chain 3 fast (LC3f) was unchanged. The other two fast myosin light chains (LC1f, LC2f) are also indicated, as are the two slow light chains (LC1s, LC2s). Note that muscle G fast fibres (G12, G2) lack LC1s, but contain a protein more typical of LC1f. Other myofibrillar proteins are: myosin heavy chain (MHC) (gel zones have been removed for peptide ‘mapping’), α-actinin (α-AC), actin and α- and β-tropomyosin (α/β-TM). Slow and fast troponins I and C (TI, TC etc.) are indicated, whereas troponin T polymorphs are examined in Fig. 5. A turquoise band is indicated by ‘t’ and a purple–blue band by ‘p’. LC1f in fibre A4 and TI, in G7 have identical migrations, but can be distinguished by colour, respectively purple–blue and turquoise. (b) A close-up view of slow (from a ma muscle) and fast (from a ra muscle) tropomyosins analysed in alternate fashion on a 12.5%-polyacrylamide gel. Each track contained 0.4 μg of tropomyosin. The tracks f and s display the tropomyosin regions of a fast and a slow fibre dissected from the two muscles before tropomyosin purification. (The relatively broad β-tropomyosin band in f also contains a troponin T polymorph). Slow α-tropomyosin has a relatively slow migration. This difference is also observed between slow (G7) and fast (A4, G2) sm fibres in (a) above.

Fig. 2. SDS/polyacrylamide-gel electrophoresis of single sm muscle fibres
Fibres from three animals were analysed on a 12.5%-polyacrylamide gel. All were fast (see later), but those from muscle G lacked LC1f. When LC3f was strongly expressed, as in muscle A fibres, certain other proteins including phosphorylase (PE), creatine phosphokinase (CPK), aldolase (AL) and glyceraldehyde 3-phosphate dehydrogenase (GPDH) were likewise expressed. Other abbreviations are defined in Fig. 1.

Concentrations in sm-muscle extracts (Young et al., 1981). The present study therefore considers mainly the myofibrillar proteins, which remain insoluble in the dissection medium.

All fibres from the three muscles could be placed into one of two broad classes based on a cluster of proteins identified on 12.5%-polyacrylamide gels between 135,000 and 170,000 daltons (Fig. 3). One class is characterized by sets of bands at each end of this range, whereas in the other class, bands in this region are closer together, although it is not often clear that each set of bands is comprised of more than one protein. However, numbers of proteins were always observed when the gel composition was changed to 7.8% polyacrylamide (Fig. 4). At both gel concentrations, classification was complicated by variations in loading and, concomitantly, intensity of staining, but fibres could always be consistently classed into the two broad groups. The usefulness of this somewhat arbitrary classification became evident when gel zones incorporating myosin heavy
Electrophoresis of muscle fibre proteins

Fig. 3. SDS/polyacrylamide-gel electrophoresis of bovine muscle fibres

Fibres from a number of animals and muscles were analysed on a 12.5%-polyacrylamide gel. Fibres can be classed into two groups on the basis of a cluster of proteins between 135,000 and 170,000 daltons (135 and 170k). The class characterized by two sets of bands separated by some 35,000 daltons, e.g. J5, contained slow myosin heavy chain, whereas the other class, e.g. M12, contained fast heavy chain. It is not always clear on 12.5% gels that numbers of proteins occur in this molecular-size range, but it is evident on gels designed to resolve this range more clearly (Fig. 4). Myosin light chains and troponins are indicated, as is the migration position of desmin (DS). MIX refers to Pharmacia marker mix, its heaviest component protein being phosphorylase (PE). The relatively broad bands of, respectively, slow and fast troponins I in J5 and J11 are presumed to be doublets and are indicated thus: = t. In fibre J11, but not in J2, LC3 f and certain glycolytic enzymes identified in Fig. 2 are strongly expressed. Comparisons between other fibres are made in the text. Other abbreviations are defined in Fig. 1.

chain were ‘mapped’ with Staphylococcus proteinase (see below); it was clear that the widely spaced type (e.g. fibre J5, Fig. 3) always contained slow myosin heavy chain, whereas the other class (e.g. M12, Fig. 3) always contained fast heavy chain. Fibres will be referred to as ‘slow’ or ‘fast’ by these criteria.

No attempt has been made to identify the proteins in the 135,000–170,000-dalton range, which are likely to include C-protein (Offer et al., 1973), M-protein (Trinick & Lowey, 1977) and others (Etlinger et al., 1976).

Considering now the tropomyosin bands, two forms of α-tropomyosin (Cummins & Perry, 1973) were recognized, one form exemplified by the slow fibre G7 and the other by the fast fibres A4 and G2 (Fig. 1a). It should be noted that light chain 1 fast is absent from muscle G fast fibres. The G7 α-tropomyosin has a slightly slower migration. In routine analysis the difference between the two forms was clear only when a slow and a fast fibre of similar staining intensity were adjacent. In all instances the slower-migrating form of α-tropomyosin was associated with slow fibres and the faster form with fast fibres. Tropomyosin purified from slow (ma) and fast (ra) muscles clearly showed this difference (Fig. 1b).

In contrast with the usually clear correlations observed above, the relationships between fast and slow fibres, myosin light chains and troponins were more complex. On the basis of these relationships the fibres have been broadly classed into three fast and three slow types (Scheme 1). The scheme is intended only as a general guide to the most obvious differences observed in the present study and should be referred to as the various types are now examined in detail.

Fast fibres from bovine muscles

Fast fibres from the three ra muscles were closely similar in the distribution of their myosin light chains and their troponins I and C. Light chain 1 fast (LC1 f) and LC2 f were clearly identified, whereas LC3 f was either absent or faintly expressed; TI and TC r were clearly expressed (see, for example, M7, Fig. 3). Sm muscles A, C, I and L yielded fast fibres qualitatively similar to those from ra muscles. Comparison between the four fibres at the left of Fig. 3 suggests that the proteins in question are identical.
This fibre type will be referred to as fast1, only for convenience of discussion.

The light chain and troponin distributions of other fast fibres from sm muscles J, E and B were distinctly different from the fast1 type. The fibres of muscles B and E were thin and difficult to dissect out, producing only faint staining patterns. The patterns were, however, sufficiently clear to be identified with the larger muscle-J fast fibres exemplified by J11, J2 and J4 (Fig. 3) fast2 fibres. Present were two light chains, two troponins I (both turquoise), one troponin C, one light chain 2 and various amounts of light chain 3. Comparisons between appropriate fibres in Fig. 3 and other results not shown suggested that all but one form of troponin I and the slowest-moving light chain had their equivalents in fast1 fibres. Other comparisons suggested that the latter chain was equivalent to light chain 1 of slow fibres (LC1s).

The light-chain distribution of muscle-G fast fibres (Figs. 1 and 2) was distinct from both fast1 and fast2 fibres. In these fast3 fibres, the slowest-moving light chain appeared identical with LC1s, and a comparable amount of LC2r was present. In other respects these fibres were similar to the fast3 type.
Electrophoresis of muscle fibre proteins

Determined by closely spaced protein bands between 135000 and 170000 daltons, and a relatively fast-migrating α-tropomyosin. All contained fast myosin heavy chain, although two variants were identified.

<table>
<thead>
<tr>
<th>Fast₁</th>
<th>Fast₂</th>
<th>Fast₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found in ra muscles and sm muscles A, C, I, L</td>
<td>Found in sm muscles J, E and B</td>
<td>Found in sm muscle G</td>
</tr>
</tbody>
</table>

\[ \begin{align*}
\text{TT}_f^* \quad \text{TT}_f^* \quad \text{TT}_f^* \\
\text{LC1}_f \quad \text{LC1}_f \quad \text{LC1}_f \\
\text{TI}_f \quad \text{TI}_f(2) \quad \text{TI}_f \\
\text{TC}_f \quad \text{TC}_f \quad \text{TC}_f \\
\text{LC2}_f \quad \text{LC2}_f \quad \text{LC2}_f \\
\text{LC3}_f \quad \text{LC3}_f \quad \text{LC3}_f \\
\end{align*} \]

* As is explained in the text, TTₕ may be present in a number of forms.

Scheme 1. An outline of the fibre classification

The key characteristics of the six fibre types broadly identified in the three muscles (sm, ma, ra) are summarized. The drawing approximately locates the relative migrations of the myosin light chains and troponin components associated with each type. The abbreviations LC₁ₕ, LC₂ₙ, etc. refer to the fast and slow light chains, whereas TTₕ, TIₙ, etc. refer to the fast and slow troponin components. A bar (———) represents a protein and a broken bar (-----) indicates variable expression of a protein; (2) means a protein is bimorphic, and a question mark indicates uncertain identity.

The overall staining pattern of fast fibres dissected from a particular muscle was normally distinctive and was irrespective of whether sampling was delayed post mortem or whether, in sm muscles, the core or the periphery was sampled. One notable exception was fibre J11, which among 18 fast fibres from muscle J was alone in its high content of LC₃ₕ (Fig. 3).

The data do not indicate a relationship between fast fibre type and animal breed or age (Table 1).

**Slow fibres from bovine muscles**

Within a given sm muscle there was a rough bimodal distribution of fibre diameters; although the gel tracks of thinner fibres (as judged by eye) did not stain strongly, their characterization according to protein banding was unequivocally slow. Out of a total of 114 sm-muscle fibres, 21 (18%) were classified as slow. The proportions varied among muscles; from muscles A, C, I and L (the source of fast₁ fibres) 8% of the total were slow, with the highest proportion in L (two of seven fibres). In contrast, 34% of fibres from muscles J, E and B (the source of fast₂ fibres) were slow, the lowest proportion being 22% in J. From muscle G only one out of 16 was slow. Out of 25 ra fibres, 36% were slow, significantly more than expected from the composition of tropomyosin (Fig. 1) and troponin (see below) purified from ra muscle. This selection bias may have arisen from the different diameters of ra fast and slow fibres; in contrast with the situation in sm muscles, ra slow fibres were markedly wider than fast fibres and were perhaps more readily isolated. In sm muscles, similar pressures may have biased fibre selection.

All fibres from ma muscles were uniformly thin and gave remarkably similar patterns (compare H and K fibres, Fig. 3), which were characteristically slow-type in their distribution of light chains and troponin components. This slow₁ type contains light chain 1 slow (LC₁ₕ), LC₂ₙ, troponin T slow (TTₕ), TIₙ and TCₙ (see Q5, Fig. 5). The claim from
histochemistry that bovine ma muscle is homogeneous and slow is clearly justified by the present analyses.

Three slow fibre types were represented in sm muscles. Only one example of a slow fibre was identified, the sole slow fibre isolated from muscle G (G7, Fig. 1). Slow2, which was widespread in sm muscles, was closely similar to slow1, but its troponin I slow was present as a doublet. This doublet, which was consistently observed in slow2 fibres, is indicated in J5 (Fig. 3); slow1 and slow2 also differ in the relative staining intensities of LC1s and troponin I.

Slow3 was encountered only once in sm muscle (J12, Fig. 3). J12 might have been overlooked as a distinct type had slow3 not been clearly identified in ra muscles, where it was the only slow fibre type. The slowest-moving light chain 1 (nominally LC1,) was more typical of LC1s, though not identical (compare, for example, O5 and O10, Fig. 5). Light chain 2 of slow1 appeared identical with LC2s common to the other slow fibre types. Similarities and differences relating to the troponins are examined below. (Heavier proteins also provide some interesting comparisons. In Fig. 4, Q4, which is a slow fibre, is different from ra slow fibres R4 and R7 in the region between 170 000 daltons and myosin heavy chain. By contrast, H11, also a slow fibre, is identical with J10, a slow sm fibre. Since R4 and R7 are probably slow2 fibres, it is likely that J10 is more akin to a slow1 type and is not a second example of a slow3 fibre in sm muscle.)

The specific expression of the troponins

Troponin of ma muscle was resolved into troponin T slow (TTs), TI and TC (Dhoot et al., 1979; Harrington, 1979) and thus presented no surprises (Fig. 5). Comparison of ma troponin with a slow1 fibre (Q5) and a slow2 fibre (R1) in Fig. 5 shows that troponins I of the two fibre types are distinct. Other comparisons throughout the present study suggested that these two troponins I comprise the doublet troponin I of slow2 fibres. As judged by relative migration, all slow troponins C are identical, and in all slow fibres a protein equivalent to TTs from ma muscle was identified between α- and β-tropomyosin.

Fast troponins I and C from ra muscle were clearly resolved between LC1s and LC2s as expected, but three major bands were present in the general region of TTs (Fig. 5). The relative staining intensities of the two lower bands were the same in the two ra troponin preparations (RAT1, RAT2), whereas the upper band varied. In contrast with the upper band, which might be aldolase, the two lower bands have clear equivalents in the fast ra fibre O4, and are presumed to represent two troponin T fast coexisting in ra-muscle cells. In fast sm-muscle fibres there was sometimes clear evidence for a troponin T doublet akin to that from fast ra fibres, exemplified in J20 (Fig. 5). However, in other cases the doublet was poorly resolved from β-tropomyosin; doublet troponin T may therefore be present in other sm fast fibres, being obscured in some by β-tropomyosin. In certain fast fibres, such as L4 (Fig. 3), however, there is no evidence for a doublet, and a possible troponin T is identified just below α-tropomyosin.

Expression of light chain 3 fast (LC3f)

Many fast and slow fibres show a band in the region of LC3f. This was unexpected in slow fibres, but whatever its true identity in slow fibres, it was
never a dominant protein in this group, unlike in certain fast fibres. Despite leaching of sarcoplasmic proteins, fast fibres rich in the putative LC3, were also rich in proteins migrating in the same way as phosphorylase, creatine phosphokinase, aldolase and glyceraldehyde 3-phosphate dehydrogenase (muscle A fibres, Fig. 2; J11, Fig. 3). The coincidence of LC3, creatine phosphokinase and these glycolytic enzymes probably confirms these identities. However, the glycolytic enzymes phosphoglycerate mutase, phosphoglucone isomerase, enolase, pyruvate kinase and lactate dehydrogenase could not be positively identified for a number of reasons, such as co-migration of proteins or possible leaching. For instance, rabbit muscle phosphoglucose isomerase (Scopes & Penny, 1971) was coincident with bovine desmin, so the relatively intense desmin band in J11 (Fig. 3) might contain this enzyme. The strongly staining band immediately above desmin in J11 is unidentified.

**Peptide 'mapping' of myosin heavy chains**

The identification of myosin light-chain heterogeneity in a number of fibre types prompted a study of myosin heavy-chain composition by peptide ‘mapping’.

Fig. 6 shows typical results obtained by *Staphylococcus* proteinase digestion of myosin heavy chains judged slow or fast by criteria described above. Within each of several experiments, all heavy-chain zones from slow-fibre myosins produced identical peptide ‘maps’. Since myosin from ma fibres was included in these experiments, it was clear that this type of ‘map’ represented slow myosin heavy chain. As a group, fast myosin ‘maps’ were distinct from the slow type, but there was minor variation within the group. For instance, the ‘map’ of ra fibre O8 myosin is subtly different from the other fast ‘maps’ in Fig. 6, which are identical with each other irrespective of staining intensity. Similar subtle differences were observed in other ‘mapping’ experiments involving fast myosin heavy chain from sm muscles I, J and L, and ra muscles M, O and R. Considered collectively, it was concluded that one of two myosin heavy-chain forms was associated with certain ra fast fibres, but never with the sm fast fibres, whereas the other form had the converse distribution. Myosin from ra fibre O6, for instance,
was identical with the type in sm J and I fibres (Fig. 6).

The difference between 'maps' of O6 to J23 on the one hand, and O8 on the other, is reflected in Fig. 4, where of the fast fibres, O8 alone is clearly distinct, particularly in the region between 135,000 and 170,000 daltons.

In another series of 'mapping' experiments (results not shown) with *Staphylococcus* proteinase and papain, it was established that fast, and fast, fibres contained fast myosin heavy chain. Since both these fibre types contain a myosin light chain more typical of slow fibres, it follows that at least some fast, and fast, fibre myosins are heterogeneous with respect to this light chain(s) (Lowey, 1979).

Discussion

Scheme 1 outlines the classification determined in the present study. It is a functional classification, since it centres on proteins that are intimately associated with the contraction mechanism, although not all have been considered, and the detailed contraction characteristics of the various types are not known. Nonetheless, the fibre types broadly identified here may be compared with previously described types.

By comparison with the considerable literature on muscle fibre types, it is clear that at least fast, fibres are equivalent to the previously described type II fibres (Peter et al., 1972). Fast, and fast, fibres are previously undescribed, but, as judged by the characteristics of all but one of their myofibrillar proteins, are presumably also type II fibres. Further, it is likely that fibres where LC, and certain glycolytic enzymes are strongly expressed represent type IIb, although it is not known whether oxidative capacity is concomitantly low in these instances. All other fast fibres might represent type IIA.

Fast, and fast, fibres both contain a light chain 1 that is more typical of, and may be identical with, LC, whereas myosin heavy chain from these fibres is fast. Lutz et al. (1979) and Gauthier & Lowey (1979) have demonstrated the simultaneous presence of fast and slow myosins in single muscle fibres. In their studies, a proportion of fibres reacted with both fast and slow anti-myosins, whereas in the present study all the fast fibres in question contained the mixture of light chains. This result and the immunological results are thus qualitatively different and their relationship is not clear.

Recent experiments (Wagner & Weeds, 1977; Hoh, 1978; Lowey, 1979) have demonstrated a lack of specificity in the interaction between the alkali light chains (LC, LC,) and fast heavy chain of myosin. The question of specificity is now compounded by the hybrids, fast, and fast, described here. In fibre J11 (Fig. 3), for instance, a large number of myosin isoenzymes are possible; is the distribution of light chains in complete myosin molecules random or otherwise? Whatever the answer, it is clear that some flexibility of association extends to hybrids between fast-type heavy chains and the aberrant light chain(s) of fast, and fast, fibres.

In their staining patterns, bovine slow fibres were less variable than fast fibres, although three types were distinguished; histochemically, however, all three might well be classed as slow-twitch oxidative, often called type I.

The present study has identified a number of troponin, tropomysin and myosin polymorphs by the criteria of relative migration, stain colour and peptide 'mapping'. Two troponins I fast were identified (fast, fibres). At least two troponins I slow were distributed among slow fibres, and troponin T was bimorphic in some fast fibres. a-Tropomyosin was present in two forms that presumably represent the bovine equivalent of two of the four or more rabbit tropomyosin polypeptides recognized from sequence data (Hodges et al., 1972). The a- and b-forms of tropomyosin were both present in all fibres examined, fast or slow. This finding is at variance with the results of Dhoot & Perry (1979), who have shown by immunological tests on human semispinalis capitis muscle that a-tropomyosin is probably restricted to fast fibres, and have suggested that b-tropomyosin is associated only with slow fibres. Moreover, considerable reported evidence supports the notion that fast muscles are richer in a-tropomyosin. In contrast, the a/b ratio of cross-innervated rabbit soleus muscle remained unchanged, even though troponin I slow was largely replaced by troponin I fast (Amphlett et al., 1975), a finding more consistent with the present results. In resolving these differences, single-fibre analysis techniques might be very useful.

Turning now to the polymorphs of myosin, the five previously described myosin light chains (Lowey & Risby, 1971) have been identified in the present study, along with a probable sixth (in slow, fibres). Moreover, the exact identities of the aberrant light chains in fast, and fast, fibres are unknown. Of further interest has been the identification of an additional fast myosin heavy chain in certain fast fibres from rat muscles. From amino-acid-sequence data, Starr & Offer (1973) have demonstrated two fast myosins heavy chain in rabbit muscle. The two variants observed in the present study may represent the bovine equivalent of this polymorphism. It is clear that they can coexist in one muscle, and there is an indication (Figs. 4 and 6) that each variant occurs with certain other proteins. However, the two variants do not appear to dictate the expression of myosin light chains, since several instances were observed where the light-chain composition was identical in fibres containing either one heavy chain
or the other. This suggests there is no restriction on association between each variant and the light chains. In support of this hypothesis, Pope et al. (1977) and d’Albis et al. (1979) have reported that there is no selectivity in the interaction between individual (alkali) light chains and the two heavy-chain variants in rabbit muscle.

In view of the known specificity of Staphylococcus proteinase, it is likely that the fast heavy-chain variants of bovine myosin differ at least in some of their glutamyl or aspartyl environments. Had another enzyme been used, the variants might not have been detected. Conversely, another enzyme might expose variants, but would they be coincident with those discovered here? In this respect single-fibre analysis combined with the 'mapping' method of Cleveland et al. (1977) should provide fertile ground for experimentation.

The present study has demonstrated myofibrillar polymorphs that presumably contribute, as do the metabolic enzymes, to the characteristic physiological properties of individual fibre types. As a variable composite of these fibre types, each muscle is evidently fine-tuned to a particular role under a given set of conditions.

Beyond cud chewing involving ma muscle, however, nothing is known of the conditions that gave rise to the various fibre composites in sm and ra muscles.

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References


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