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Extractability and properties of the contractile proteins of vertebrate smooth muscle

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The vertebrate smooth muscles differ from the striated ones by their larger extracellular space, the smaller size of their cells and their high content in extracellular components. Furthermore, the smooth muscle cell is a bifunctional biological unit able to carry on also an important biosynthetic activity.

The contractile proteins of vertebrate smooth muscle are extractable at low ionic strength contrarily to those of striated muscle. The partition of the salt extractable nitrogen between the low and high ionic strength extracts is very different in these two cases. Acidification of low ionic strength extracts of vertebrate smooth muscle at pH 5 allows precipitation of the contractile proteins quantitatively together with a large amount of contaminants typical of the smooth muscles.

Comparison of the contractile proteins of vertebrate smooth muscle with their striated counterparts shows that actin is a very constant component of the contractile machinery, that tropomyosin holds an intermediate position, while myosin is the most variable. The smooth muscle myosin differs not only by some general properties as salting-out range and thermostability, but also by the behaviour of various parts of the molecule. The globular head has a different ATPase activity and is responsible for the very peculiar immunological behaviour of this myosin. The point along the myosin rod which is attacked by trypsin is much more resistant to proteolysis. The light meromyosin is more soluble and differs very much in amino acid composition. The comparative study of myosin reveals only minor variations from one species to the other but more or less wide ones within each species according to the type of muscle examined.

Vertebrate smooth muscle is very different from vertebrate striated muscle and a clear appreciation of this diversity is necessary in order to understand its peculiar properties. Some differences shown by these two types of muscle are given in table 1. The extracellular space of the myometrium or of the vascular wall amounts to one-third of the volume of the tissue; it is thus two to three times larger than in skeletal muscle. The smooth muscles contain a large amount of serum proteins. Incubation of various vessel preparations in Tyrode solution has been carried out in order to remove these contaminants (Hamoir 1967), but a large amount of serum albumin is still detected by electrophoresis after this treatment. Its percentage is difficult to assess as the creatine kinase of smooth muscle separates from serum albumin only after prolonged electrophoresis. It can be evaluated from 20 to 30 % of the protein content of the extracts.

The large extracellular space typical of these muscles, the very small size of their cells and the numerous pinocytic vesicles of the plasma membrane (cf. Somlyo & Somlyo 1968) favour exchanges between the cells and the external medium. These cells are less independent units than the skeletal fibres.

The external medium of the smooth muscles contains also a large amount of insoluble components: it includes not only the collagen and elastin networks representing a third to a half of the protein content of the myometrium and of the aorta respectively, but also the ill-defined ground substance which is apparently made of glycoproteins and acid mucopolysaccharides. These last components amount in whole aorta to 1 to 2 % of the dry tissue mass (Buddeke & Kresse 1969; Newmark, Malfer & Wiese 1972) or 0.3 to 0.6 % of the wet tissue mass. As the

liberation of the acid mucopolysaccharides requires a preliminary extensive proteolysis of the tissues, these components are not extracted by the salt solutions used to isolate the muscle proteins. The presence of extracellular components gives rise, however, to another complication. Ross has demonstrated (Ross & Klebanoff 1971; Ross 1971) that their biosynthesis is carried out by the smooth muscle cell. It is a bifunctional biological unit which contains the proteins responsible for the synthesis and excretion of these extracellular components as well as the contractile machinery.

Table 1. Heterogeneity of vertebrate muscles (in % of the wet mass)

		skeletal muscle	cardiac muscle	adult non-pregnant myometrium rabbit [1], human [8], rat [11]	vascular muscle (intima + media + adventitia)
extracellular space space (%, v/v)	12–15	9–12 interstitial space; 2–3 capillaries	25 [1]	35 [1]	39 (cow carotid) [2]; 35 (rat aorta) [3]
size of the cells					
diameter		80 μm [1]	20 μm [1]	6 μm [1]	3 μm [4]
length		several cm [5]	0.2 mm [1]	0.03 mm [1]	0.06 mm [4]
number of cells in a volume of 10 mm length by 1 mm depth and 0.1 mm height (1 mm^3)		17.5 [1]	12000 [1]	2.3×10^6 [1]	3.5×10^6 [6]
protein content (%, w/w)		16–21 [7]	—	17 [8]	24 ± 2 (aorta) [9]
collagen + elastin content (%, w/w)		1.3 [10]	—	4.8 [11]	11.7–14.8 (human aorta) [9]

(1) Kao (1967).
(2) Laszt (1960).
(3) Hagemeijer, Rorive & Schoffeniels (1965).
(4) Rhodin (1962).
(5) Walls (1960).
(6) Assuming as for uterus muscle the shape of a double cone.
(7) Hamoir (1961).
(8) Schwalm & Cretius (1958).
(9) Kirk (1962).
(10) Lowry, Gilligan & Katersky (1941).
(11) Needham & Cawkwell (1957).

The extractability of the contractile proteins

The presence in smooth muscles of a large amount of insoluble components, particularly of a collagen and elastin meshwork, hinders the extraction of the muscle proteins. Grinding with sand has been used routinely in this case. As the homogenization of skeletal muscle is made under much more gentle conditions, it may be questioned if the more or less drastic procedure used does not influence the extraction of the proteins. This does not seem to be the case; skeletal muscle extracts have been prepared by grinding with sand and found similar to the usual ones. The flow birefringence and the high viscosity, decreasing in the presence of ATP, of the low ionic strength extracts of vertebrate smooth muscles observed ten years ago by Laszt (1961) and by Laszt & myself (Laszt & Hamoir 1961) are thus not due to the method of homogenization used. The contractile proteins of this type of muscle are extractable at low salt concentration contrarily to those of skeletal and cardiac muscle. This is true not only for myosin and actin but also for tropomyosin (Hamoir & Laszt 1962*a*). When the media of bovine carotid arteries is extracted with a dilute phosphate solution of neutral pH (1.5 ml/g), the extract contains about 1.3 % myogen together with 0.2 % of a soluble actomyosin which we have called

tonoactomyosin and 0.03 % tropomyosin. As the total amount of actomyosin of the media is 5 to 6 mg per gram of fresh tissue (Rüegg, Strassner & Schirmer 1965), about half of the actomyosin is present in this extract. The remainder is also extractable at low ionic strength if 1 mmol/l ATP is present in the extraction medium (Ruegg *et al.* 1965). The occurrence of two actomyosins, an easily soluble one and another less soluble one extractable only at high ionic strength, has, however, been suggested again by Christomanou (1971) in a recent work dealing with gravid and non-gravid rabbit uteri. In the case of tropomyosin, one-third is extracted at low ionic strength instead of a small percentage in the case of striated muscle (Hamoir & Laszt 1962*a*).

TABLE 2. PARTITION OF THE SALT EXTRACTABLE NITROGEN IN VARIOUS MUSCLES EXTRACTED AT NEUTRAL pH

			bovine vessels		
		adult non-pregnant human myometrium (2)	carotid		inferior vena cava (3)
	rabbit skeletal muscle (1)		(ref. 3)	(ref. 4)	
% extracted at low ionic strength	33.4	80	61	86	69
% extracted at high ionic strength	66.6	20	39	14	31

(1) Hasselbach & Schneider (1951).
(2) Schwalm & Cretius (1958).
(3) Hamoir (1967 and unpublished results).
(4) Rüegg *et al.* (1965).

The extraction of the contractile proteins at low ionic strength leads also to a different partition of the salt extractable nitrogen. We have compared in table 2 the percentage of protein isolated from various muscles which have been extracted exhaustively, first at low ionic strength and afterwards at high ionic strength. The nitrogen extracted at low ionic strength amounts to only one-third of the salt extractable nitrogen in the case of skeletal muscle, but to about two thirds in the smooth muscles.

TABLE 3. FRACTIONATION OF LOW IONIC STRENGTH MUSCLE EXTRACTS BY ACIDIFICATION AT pH 5.0

	ox white skeletal muscle	ox cardiac muscle	cow uterus	ox stomach	ox carotid
% soluble at pH 5.0	90	94	56	42	41
% insoluble at pH 5.0	10	6	44	58	59

The anomalous composition of the low ionic strength extracts of the smooth muscles is also made obvious by their fractionation at pH 5. Acidification of striated muscle extracts of low ionic strength to pH 5.5 is carried out routinely before their analysis by starch gel electrophoresis, in order to remove any particulate material and some minor nucleoproteins (Scopes 1968). When smooth muscle extracts are brought to pH 5.5, the tonoactomyosin present is completely precipitated, but some tropomyosin remains in the supernatant. Skeletal muscle tropomyosin is slightly soluble at this pH (Dingle & Odense 1959) as well as cow carotid tropomyosin which is insoluble between pH 4.2 and 5.2 at the ionic strengths 0.1 and 0.2 (D. Matagne & G. Hamoir, unpublished). The pH of the smooth muscle extracts must be lowered of 5 in order to remove quantitatively the contractile proteins present. The different behaviours of the striated and smooth muscle extracts at this pH are shown in table 3. The extracts of striated muscle hardly precipitate in agreement with earlier results (Scopes 1964),

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but about half of the protein of the smooth muscle extracts becomes insoluble. I had the opportunity to examine also recently a fish smooth muscle at the Marine Laboratory of Plymouth. Its behaviour does not differ from that of the smooth muscles of warm-blooded vertebrates. The extractibility of the contractile proteins at low ionic strength appears to be a general property of the vertebrate smooth muscle. The large precipitate obtained by acidification is much too large to correspond to the tonoactomyosin and tropomyosin present; it contains also ill-defined components typical of the smooth muscles. It is well known that the usual actomyosin preparations of smooth muscle are heavily contaminated. Needham & Williams (1963*a*) have shown that half of the dilution precipitate corresponds to actomyosin in the pregnant uterus and a quarter only in a non-pregnant one. These preparations contain about 10 % RNA and DNA as well as some salt-soluble collagen (Needham & Williams 1963*b*). This low degree of purity is also shown by the tonoactomyosin isolated from cow carotids. It contains 50 % impurities according to Ruegg *et al.* (1965) and some salt-soluble collagen is also extracted at low ionic strength which coprecipitates with this soluble actomyosin (Gaspar-Godfroid, Hamoir & Laszt 1968). These various results make clear that the contractile proteins of smooth muscle are extracted very easily at low ionic strength and that they precipitate at low ionic strength more or less completely according to the pH, together with a large amount of ill-defined globulins typical of the vertebrate smooth muscle.

The extractibility of these contractile proteins appears to be related to the lability of the ultrastructure of vertebrate smooth muscle. The difficulties experienced by electron microscopists in the observation of the thick filaments of vertebrate smooth muscle are well known. These filaments probably dissociate after homogenization at low salt concentration. Shoenberg (1969) has examined smooth muscle homogenates prepared under many different conditions and found that the presence of 10 mmol/l Mg, 2 mmol/l ATP and traces of Ca is required to observe myosin filaments. Electron microscopic pictures of the tonoactomyosin extracted from cow carotid (Shoenberg *et al.* 1966) confirm also the low tendency of the smooth myosin to associate. When the complex was placed in a relaxing medium, the usual actin filaments were observed, but myosin filaments could not be seen.

The extractibility of actin is less understandable in view of the higher stability of its filaments *in situ* and after extraction at low ionic strength. It is relevant here to note that the stability of the actin filaments of striated muscle may differ widely: they disaggregate much faster at high salt concentration in the case of the cold-blooded vertebrates than in that of the warm-blooded ones. Homogenization of the smooth muscle at low ionic strength may induce the breakage of the actin filaments into fragments of shorter length. As sedimentation is fairly independent of the length of elongated particles and varies mainly with their width, the sharp peak of F-actin observed by ultracentrifugation of cow carotid tonoactomyosin in the presence of ATP (Laszt & Hamoir 1961) is in agreement with such a suggestion.

The high solubility of vertebrate smooth muscle myosin in the extracts assumed in this interpretation is, however, not found after isolation (cf. p. 174). Its abnormal extractability properties suggest that it occurs *in situ* in some kind of association with a more soluble component like tropomyosin and that this association is fairly labile.

But another way to look at this peculiar behaviour of the contractile proteins of vertebrate smooth muscle is to isolate them and to examine their properties, a field in which Dr Needham made important contributions. We shall mainly limit our present considerations to the differences shown by these proteins in regard to their skeletal counterparts.

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Actin

Actin appears as the most constant contractile protein of the muscle and of the living cells able to change shape or to move (*Nature* 1970, 1971). Microfilaments reacting with heavy meromyosin as skeletal actin, have been observed in many primitive cells. The presence of such thin filaments in the smooth muscles have been described by many electron microscopists (cf. Burnstock 1970). Actin has been isolated from human and sheep uterus (Carsten 1965) and from cow carotids (Ruegg *et al.* 1965; Gaspar-Godfroid *et al.* 1968; Gosselin-Rey, Gerday, Gaspar-Godfroid & Carsten 1969). The great resemblance of these actins between themselves and with skeletal and cardiac actins of various origins (Carsten & Katz 1964) confirm the electron microscopic observations. No difference in amino acid composition was detected except some minor variations corresponding to a more or less extensive contamination with tropomyosin. The peptide maps of the smooth and skeletal actins also closely agree (Gosselin-Rey *et al.* 1969). The sluggishness of polymerization and depolymerization pertaining to the smooth muscle actins, thus appear due to a minor change in molecular structure.

Tropomyosin

Comparative work carried out by Tsao, Kominz and their collaborators around 1955 has shown that tropomyosin is present in vertebrate smooth muscle. It has been isolated from human uterus, from cow and rabbit uterus, from bovine urinary bladder (Sheng & Tsao 1955; Kominz, Saad, Gladner & Laki 1957) and from duck gizzard (Tsao, Tan & Peng 1956). We also described cow carotid tropomyosin ten years ago (Hamoir & Laszt 1962*a*). All these tropomyosins were found very similar to the skeletal ones, showing only minor variations in electrophoretic mobility. But in 1968, Carsten made a more detailed investigation of the tropomyosins from human and pregnant sheep uterus. She found the human and sheep uterus tropomyosins highly similar to one another but quite different from rabbit and sheep skeletal tropomyosins, from the point of view of their amino acid compositions. This discrepancy was confirmed by the peptide maps, the uterus preparations giving six to eight less peptides than the skeletal ones. Thus the primary structure of the smooth muscle tropomyosin is different from that of the skeletal one.

Our observation that a third of the total amount of tropomyosin is extracted from cow carotid at low ionic strength instead of a small percentage in the case of skeletal muscle remains, however, difficult to explain so far. Its slightly higher electrophoretic mobility at neutral pH suggests a higher net charge which may favour its release from actin during the extraction, but does not seem large enough to explain such a contrasting behaviour. In view of the progressive liberation observed when the extraction is repeated several times, its solubilization seems to be related to the disruption of the actin filaments during extraction.

Smooth muscle differs, however, not only by the occurrence of a more extractable genuine tropomyosin, but also by its higher content in this protein. Needham & Shoenberg (1967) have compared the ratio tropomyosin/actomyosin + myosin in skeletal and uterus muscles and found it 1/17 and 1/4 respectively. In cow carotid the amount of tropomyosin present per gram of fresh tissue does not differ from that of skeletal muscle (Hamoir & Laszt 1962*a*) but as the muscle cells make about 50 % of the preparation, their tropomyosin content can be evaluated to twice as much as that of skeletal fibres.

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Troponin

Evidence in favour of the occurrence of troponin in smooth muscle has been produced by Ebashi and co-workers in 1966 (Ebashi *et al.* 1966). These authors obtained a crude native tropomyosin-like protein from chicken gizzard which showed properties similar to that of skeletal muscle. It slowed the superprecipitation of synthetic actomyosin of skeletal muscle, and its action was suppressed in the presence of calcium. It acted in the same way on chicken gizzard actomyosin treated with trypsin in order to abolish its sensitivity to Ca action. The occurrence in smooth muscle of regulatory proteins has also been shown by Bohr and collaborators (Sparrow, Maxwell, Rüegg & Bohr 1970), who have prepared a calcium ion-sensitive Mg-activated actomyosin from arteries. The isolation of pure troponin of smooth muscle is however recent. It was prepared from cow uterus last year by Carsten (1971), following a procedure described previously for skeletal troponin. The preparation was devoid of tropomyosin and differed from the bovine skeletal troponin by a slightly higher electrophoretic mobility in 6 % acrylamide gel at pH 7.5. It combines with uterine tropomyosin, the reduced viscosity of the mixture being considerably higher than would be expected for two non-interacting proteins. It inhibits skeletal as well as uterine Mg-actomyosin ATPase, but the maximum inhibition which corresponds to a ratio: troponin/tropomyosin of 1.5 is approximately half of that found for the skeletal troponin complex. On the other hand, when skeletal tropomyosin replaces uterine tropomyosin in the complex with uterine troponin, some inhibition is also observed, but it is much lower. Thus although troponin and tropomyosin differ in smooth and skeletal muscle, they can cross-associate and maintain partially their biological function.

Myosin

Myosin has been isolated from several vertebrate smooth muscles: cow carotid (Hamoir & Laszt 1962*b*; Huriaux, Pechère & Hamoir 1965), chicken gizzard (Barany, Barany, Gaetjens & Bailin 1966), horse oesophagus (Kotera, Yokoyama Yamaguchi & Miyazawa 1969; Yamaguchi, Miyazawa & Sekine 1970), rabbit uterus (Needham & Williams 1963*c*; Wachsberger & Kaldor 1971) and human uterus (Gröschel-Stewart 1971). All physicochemical determinations carried out on these myosins suggest that their size and gross shape are similar to those of the skeletal and cardiac myosins. The smooth muscle myosins differ however in several aspects from those of striated muscle. Some differences are given in tables 4 and 5.

The salting-out range of the smooth muscle myosins differs from that of skeletal myosin. This higher solubility is, however, not shown in the salting-in range. When purified, the smooth muscle myosin behaves at low salt concentration as the skeletal one. We could detect only a small variation in the presence of traces of ATP. A new small sedimenting peak appears under these conditions with a sedimentation coefficient of 10 to 12 *s*. Thus the cow carotid myosin gives rise to an aggregate which is not observed in the case of rabbit skeletal myosin, but the amount of myosin solubilized by these traces of ATP is quite small.

The tendency towards aggregation of the uterine and arterial myosins is larger than that of the skeletal mammalian myosin. Although the smooth muscle myosins aggregate quickly and show an extensive denaturation at about 50 °C, the denatured protein does not become insoluble by heating to a higher temperature unlike skeletal myosin.

The part of the molecule which is particularly susceptible to tryptic digestion is also different:

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the rates of tryptic digestion of the cow arterial (Huriaux 1965) and chicken gizzard (Barany *et al.* 1966) myosins are very slow, much slower than that of red skeletal myosin.

On the other hand, the study of the ATPase activity has made obvious that structural differences occur at the level of the active centre of the molecule (table 5). The low ATPase activity of the smooth muscle myosins is well known. Recent experiments on cow carotid myosin have shown, however, that its activity is very dependent of the integrity of the SH groups (Gaspar-Godfroid 1968). The activities given in the literature are usually too low in view of the reducing environment occurring in muscle.

TABLE 4. SOME DIFFERENCES SHOWN BY COW CAROTID MYOSIN AND SKELETAL MYOSIN

	cow carotid myosin	skeletal myosin
salting-out range at neutral pH ($(NH_4)_2SO_4$)	47–60 %†	35–45 %
salting-in range measured by ultracentrifugation at 60 000 rev./min		
(*a*) $I = 0.10$ pH 7.1 (phosphate, NaCl) without ATP	insoluble	insoluble
(*a*) $I = 0.10$ pH 7.1 (phosphate, NaCl) with 60 μg ATP/ml	a very small peak of 10–12 *s*	insoluble
(*b*) $I = 0.20$ pH 7.1 (phosphate, NaCl) without ATP	a small peak of 6 *s*	a small peak of 6 *s*
(*b*) $I = 0.20$ pH 7.1 (phosphate, NaCl) with 60 μg ATP/ml	a supplementary peak of 10–12 *s*	a small peak of 6 *s*
tendency towards aggregation after reprecipitation at low I and neutral pH	larger‡	small
thermostability at $I = 0.35$ pH 7.1 or 6.3	no precipitation after 10 min heating at 100 °C†	80 % precipitation after 10 min heating at 60 °C
tryptic digestion	slow	fast

† Uterus myosin behaves similarly. ‡ This tendency is still more pronounced with uterus myosin.

TABLE 5. DIFFERENCES IN ATPASE ACTIVITY SHOWN BY COW CAROTID MYOSIN AND RABBIT SKELETAL MYOSIN

	cow carotid myosin	skeletal myosin
optimal activity at $I = 0.05$ pH 7 (ATP 2 mmol/l, Ca^{2+} 10 mmol/l, 25 °C)		
SH unprotected	0.12 μmol P mg^{-1} min^{-1}	0.75 μmol P mg^{-1} min^{-1}
SH protected	0.20 μmol P mg^{-1} min^{-1}	0.85 μmol P mg^{-1} min^{-1}
Ca^{2+} activation at $I = 0.2$ pH 9.2 (glycine, NaCl) or 6.5 (tris, NaCl)	unchanged in the Ca^{2+} range 5–15 mmol/l	large increase in the Ca^{2+} range 5–15 mmol/l
influence of increase in the ionic strength	increase	decrease
actin activation at low I in presence of Mg^{2+}	low	large

The change of activity in the presence of increasing concentrations of calcium differs. When the calcium concentration exceeds 5 mmol/l, a plateau region is observed with cow carotid myosin, while a large increase of activity occurs in the cases of the cow and rabbit skeletal myosins. The smooth muscle myosins can also be distinguished from the skeletal ones by their higher Ca-ATPase activity at higher ionic strength.

Another important difference is shown when this myosin is associated with actins of smooth or skeletal muscle. It has been repeatedly observed that the complex is hardly activated by magnesium. This Mg-actomyosin-ATPase activity is not influenced by the method of preparation of the myosin. The mammalian smooth muscle myosin has a low Mg-actomyosin-ATPase activity corresponding to the low speed of shortening of this type of muscle (Barany 1967).

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During these last years the amino acid compositions of a few mammalian smooth muscle myosins and of skeletal myosins of the same species have been determined. Data are now available on the cow carotid (Huriaux *et al.* 1965) and longissmus dorsi myosins (J. Thomas & N. Frearson, unpublished results), the chicken gizzard and breast myosins (Barany *et al.* 1966); the horse oesophagus and horse skeletal myosin (Yamaguchi *et al.* 1970), the pectoral and uterus human myosins (Gröschel-Stewart 1971). Variations occur in all cases which exceed the experimental errors and make clear that the smooth myosins differ in amino acid composition from their skeletal counterparts. The histidine content is lower in all smooth muscle myosins, but the other variations are less constant. An increase in aspartic and glutamic acids is usually observed, together with a decrease of lysine. The negative net charge of the smooth myosins at neutral pH is probably higher than that of the skeletal myosins. Lower contents in proline and glycine are typical of all smooth muscle myosins except that of the chicken gizzard which shows the reverse variation. The smooth muscle myosins thus differ much between themselves, more apparently than the skeletal ones, and chicken gizzard myosin does not appear to represent the most usual type of smooth muscle myosin.

The content of mammalian smooth muscle myosin in 3-methylhistidine is still unsettled. Johnson & Perry (1970) claim in a recent comparative study that the 3-methylhistidine/histidine ratio of cow uterus myosin is 1/68.3. As the 3-methylhistidine is located in the subfragment 1 (Johnson, Harris & Perry 1967; Kuehl & Adelstein 1969) and cow carotid subfragment 1 does not contain this amino acid (Huriaux 1970), the results available seem contradictory.

Immunology has been used recently by several authors in order to compare the different types of myosin (Aita, Conti, Laszt & Mandi 1968; Becker & Murphy 1969; Gröschel-Stewart & Doniach 1969; Gröschel-Stewart 1971) Their results illustrate very clearly the degree of differentiation of the myosin isoenzymes. The antibodies against the red and white skeletal myosins and against the smooth muscle myosin are not species specific, but they are specific for each of these three types of muscle. The antisera against the two skeletal and the cardiac myosins cross-react but that against the smooth myosin does not. Some relatedness is thus observed between the myosins of striated muscles but not in the case of the smooth one. As the antibody does not react with the M line (Becker & Murphy 1969) and combines with the heavy meromyosin but not with the light one (Gröschel-Stewart 1971), the specificity observed by immunology lies in the globular part of the molecule.

The myosin subunits

The subfragments of cow carotid myosin have been investigated in some detail. The light chains have been separated by succinylation (Huriaux, Hamoir & Oppenheimer 1967). The light meromyosin-1 (LMM-1) has been isolated in 1965 (Huriaux 1965); its properties have been re-examined and compared to the LMM-1 of a white skeletal muscle (musculus cutaneus trunci) of the same species (Huriaux 1972). The subfragment 1 has been described (Huriaux 1970). Some work has also been carried out on subunits of chicken gizzard myosin. Its subfragment 1 has been investigated (Bailin & Barany 1971); the rod portion has been isolated by papain digestion, but it has only been examined from the point of view of its association into segments (Kendrick-Jones, Szent-Györgyi & Cohen 1971).

The overall substructure of the smooth myosins does not differ from that of the skeletal and

cardiac myosins, but as some properties of the subunits are different, their study may contribute to a better understanding of the functional behaviour of the smooth muscle myosin.

The smooth muscle LMM-1 is more soluble at low as well as at high ionic strength. As this fragment is responsible for the association of the myosin molecules into thick filaments, the stability of the smooth muscle thick filaments should be lower. The higher net charge of this subfragment at neutral pH is also in favour of a lower ionic interaction (table 6). The amino

TABLE 6. PROPERTIES OF THE COW ARTERIAL AND COW SKELETAL LMM-1 (HURIAUX 1972)

	cow carotid	skeletal white muscle
solubility at neutral pH	very soluble at $I = 0.075$	precipitates at $I = 0.075$
salting-out range at 4 °C in presence of $(NH_4)_2SO_4$	49–52 %	37–45 %
$10^5 \times$ descending electrophoretic mobility ($I = 0.35$ pH 7.1)	−2.9	−2.2

TABLE 7. AMINO ACID COMPOSITIONS OF VARIOUS LMM-1 (IN RESIDUES PER 10^5 g OF PROTEIN)

	cow carotid LMM-1†	cow white skeletal muscle LMM-1†	rabbit skeletal muscle LMM-1‡
Lys	104	95	94
His	7.5	19	21
NH_3	100	—	108
Arg	54	58	60
Asp	79	85	83
Thr	27	37	33
Ser	39	31	34
Glu	249	228	210
Pro	0	0	0
Gly	17	20	18
Ala	94	82	81
Cys	0.76	3.2	4.0
Val	30	27	38
Met	14	17	19
Ile	14	48	39
Leu	115	103	96
Tyr	6.3	7.7	9
Phe	9.6	4.3	4
total	860	865	843

† Huriaux (1972). ‡ Lowey & Cohen (1962).

acid composition reveals the occurrence of a higher glutamic content which appears responsible for the increased negative electrophoretic mobility observed. Other striking differences are shown by the histidine, half cystine, isoleucine and phenylalamine contents (table 7) which contrast with the fairly constant amino acid composition of the skeletal LMM-1s. According to our results, the half cystine content should amount to only one residue per molecule; if this is correct, the two polypeptide chains should not be identical or two populations of myosin should exist.

The smooth muscle heavy meromyosin has been much less investigated. In the case of cow carotid myosin, it aggregates in the course of the digestion into a faster sedimenting component

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of higher intrinsic viscosity (Huriaux 1965). Further work has shown that its isolation must be carried out in a reducing medium or in the presence of actin which protects the globular part of the myosin molecule (Gaspar-Godfroid 1968). Chicken gizzard HMM is more resistant to tryptic digestion than the skeletal HMM (Bailin & Barany 1971). It has been isolated in order to prepare subfragment 1 so that its properties have not been investigated except its rate of digestion by trypsin and papain. A comparative study of these two meromyosins should be of great interest in order to assess the variations which may occur in this part of the smooth muscle myosin.

Our knowledge of the corresponding subfragments 1 is better. Both components have been extensively purified. Cow carotid subfragment 1 (Huriaux 1970) has an intrinsic viscosity which is somewhat lower than that of other subfragments 1 isolated so far and seems therefore to have a more globular shape; its composition differs from that of cow heart subfragment 1 by higher aspartic and phenylalanine contents and lower glutamic and leucine contents. The ATPase activity remains remarkably undamaged after this treatment. Bailin & Barany (1971) have observed that the removal of the myosin rod increases the activity per milligram of protein according to the patterns found for the rabbit skeletal and bovine heart myosins. Furthermore, two characteristics of the smooth muscle myosin ATPase, its low activity and the increase of this activity with the ionic strength are also shown by its subfragment 1 (Huriaux 1970; Bailin & Barany 1971).

The study of the myosin rod may also contribute to reveal some differences characteristic of the smooth muscle myosin. According to electron microscopic measurements, the length of the chicken gizzard myosin rod is about 156 nm while that of chicken breast muscle is 145 nm (Kendrick-Jones *et al.* 1971). The precipitation of this subunit with 50 mmol/l calcium gives rise to compound bipolar segments made of oppositely directed pairs of molecules with a 43 nm overlap. In the case of the striated muscle rods, the extent of the overlap is of 130 and 90 nm (Cohen *et al.* 1970; Harrison, Lowey & Cohen 1971). Both types of rod show an assembly which fits with the sliding filament mechanism.

The light chains of skeletal myosin aroused much interest in the last five years. In the case of the smooth myosins, they have not been investigated much so far. They are liberated from cow carotid myosin by succinylation and may be isolated by ammonium sulphate fractionation (Huriaux *et al.* 1967). The low molecular mass fraction obtained is very similar to the corresponding fractions isolated by the same method from the rabbit and chicken skeletal myosins (Oppenheimer, Barany, Hamoir & Fenton 1967). Both are characterized by a proline content twice as high and a much higher ratio of phenylalanine to tyrosine than in the case of the parent molecule. Some preliminary experiments have been made in our laboratory in order to separate these light chains by polyacrylamide electrophoresis in the presence of sodium dodecylsulphate. The light chain of 25 000 found in the rabbit white, red and cardiac myosins (Sarkar, Sreter & Gergely 1971) and in the white and red ox myosins (J. Keller, unpublished) has apparently a lower molecular mass of 20 000 in this case. A more detailed study of these light chains is under way.

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Conclusion

In the last ten years, the main proteinic components of the contractile mechanism of vertebrate smooth muscle have been isolated and characterized. Although this work has been limited so far to cow carotid, chicken gizzard as well as cow, sheep and human uteri, the main properties of these contractile proteins seem now well determined.

Their sizes and shapes do not differ significantly from their skeletal and cardiac counterparts; the only discrepancies noticed so far are the slightly greater length of the chicken gizzard myosin rod (107 %) (Kendrick-Jones *et al.* 1971) and the more globular shape of the cow carotid myosin subfragment 1 (Huriaux 1970).

Larger divergences occur in the biochemical properties. As far as one can judge from the scarce comparative work available, actin appears as the most constant contractile protein, tropomyosin holds an intermediate position and myosin is the most variable. While the antisera against the two skeletal myosins and the cardiac myosin cross-react, that against the smooth myosin does not. As this behaviour is furthermore not species specific, the smooth muscle myosin appears from the immunological point of view to be the most divergent myosin isoenzyme. This result is not unexpected in view of teh large differences in biochemical properties summarized in tables 4 and 5. This peculiar position of the vertebrate smooth muscle myosins is not reflected by their amino acid compositions. The various results obtained do not indicate a definite trend in regard to the other myosins except perhaps an increase in the ratio Glu + Asp/His + Lys + Arg related to a higher net negative charge at neutral pH. The variations observed suggest that these myosins vary more between themselves than the skeletal ones do. As differences in amino acid composition occur in subfragment 1 as well as in light meromyosin-1 (Huriaux 1970, 1972), the comparison of the compositions of the same subunits will perhaps allow one to draw better conclusions when further analytical data become available.

The extractibility of the vertebrate smooth muscle myosin at low ionic strength remains puzzling. After isolation, its solubility properties at low ionic strength do not differ from those of skeletal myosin (Huriaux *et al.* 1965) and it forms filaments similar to those of striated muscle (Hanson & Lowy 1964; Kaminer & Bell 1966). Furthermore the myosin rods prepared by papain digestion precipitate with calcium in the presence of KSCN as compound segments displaying the bipolar and polar bonding patterns characteristic of the striated myosin thick filaments (Kendrick-Jones *et al.* 1971). However, the LMM-1, which corresponds to the portion of the rod responsible for the self-association of the myosin molecules in the case of the striated muscle myosins, is much more soluble and more negatively charged at neutral pH than its skeletal counterpart (Huriaux 1972). It is obvious that these properties favour the easy disruption of the smooth muscle thick filaments and the extractibility of this myosin at low salt concentration. But as the isolated myosin is insoluble at low ionic strength, the high solubility of LMM-1 is obviously not the sole factor which causes the peculiar behaviour of the smooth muscle myosin. The higher instability of its self-association *in situ* than after isolation constitutes a challenge to the muscle biochemist.

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