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Oesophageal striated muscle arrangement and histochemical fibre types in guinea-pig, marmoset, macaque and man

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INTRODUCTION

Striated muscle fibres have been described in the muscularis externa of many mammals, and from these reports it is apparent that two basic arrangements exist. In the majority of mammals which have been studied (mouse, rat, guinea-pig, rabbit, bat, dog, sheep, horse, cow, elephant and giraffe), almost the whole length of the muscularis externa is composed of striated muscle fibres (Camp, 1935; Ingelfinger, 1958; Thomas & Trounce, 1960; Lawn, 1964; Code & Schlegel, 1968; Samarasinghe, 1972; Floyd, 1973; Francis, 1974; Floyd & Morrison, 1975; Weisbrodt, 1976; Asmussen & Gaunitz, 1978), the transition to smooth muscle occurring abruptly, just proximal to the cardia. In contrast, in primates, marsupials, cat and man this transition is spread throughout the middle third of the length of the muscularis externa (Ingelfinger, 1958; Code & Schlegel, 1968; Schofield, 1968; Floyd & Morrison, 1975; Weisbrodt, 1976; Enzmann, Harell & Zboralske, 1977; Dodds *et al.* 1978). The muscularis externa is described as being composed of two layers, the outer having fibres lying in a more longitudinal direction whilst those of the inner layer are arranged in a generally more circular orientation. Ingelfinger (1958) stated that in most animals there are only two layers, although some exhibit third and even fourth layers. Francis (1974) described four layers in the guinea-pig whereas Gruber (1968, 1978) and Samarasinghe (1972) observed only two layers in the rat and mouse, respectively.

Detailed histochemical studies of striated oesophageal muscle fibres have been performed in only a few species. In the rat, Asmussen (1974) observed high levels of myosin adenosine triphosphatase (ATPase) and evenly distributed succinic dehydrogenase, with moderate phosphorylase activity, lipids and periodic acid–Schiff (PAS)-positive deposits. Similar observations were made by Gruber (1978) whereas Bazhenov (1979) recorded that the oesophageal striated muscle of man and other mammals consisted solely of fast-acting, slow-fatiguing fibres.

As part of a detailed study into the functional anatomy of the mammalian oesophagus, the present paper reports on the form and histochemical characteristics of oesophageal striated muscle from the guinea-pig, marmoset, macaque and man.

MATERIALS AND METHODS

Tissues used in this study were obtained from 16 young adult guinea-pigs (of both sexes and weighing approximately 500 g total body weight), four adult marmosets (of both sexes), two macaques (sex unknown) and four humans (post mortem material, two males and two females aged between 55 and 76 years).

Material to be sectioned was orientated in the required plane and placed on macerated liver previously mounted on cryostat tissue holders. These were then quenched in either isopentane or dichlorodifluoromethane (Arcton-12) cooled in liquid nitrogen to melting point. Tissue was either transferred to a cryostat maintained at −20 °C for serial sectioning at 12–15 μm, or stored at −70 °C until required.

The distribution of muscle fibres was demonstrated using Masson's trichrome method. Myosin adenosine triphosphatase (ATPase) was localised using the technique described by Guth & Samaha (1970) on unfixed sections which were allowed to dry for 3 hours at room temperature before pre-incubation for 15 minutes at either pH 4·35 or pH 10·4. Incubation took place for 45 minutes at 37 °C. In order to demonstrate oxidative enzyme activity, the method for succinic dehydrogenase (SDH) after Nachlas *et al.* (1957) was followed, although in some cases Dubowitz & Brooke's (1973) nicotinamide adenine dinucleotide (reduced) diaphorase (NADH) method was employed in preference because it provided a denser reaction product. The glycolytic enzyme phosphorylase was visualised by the method of Takeuchi (1956). Finally, some sections were also stained with either Sudan black B or a periodic acid–Schiff technique. Control sections from the diaphragm, extensor digitorum longus or soleus (guinea-pig or marmoset) were incorporated in each of the histochemical methods.

RESULTS

Histology

In the guinea-pig, the muscularis externa was found to be composed of two basic layers, the inner layer having the long axis of its fibres lying in a more circular direction whilst the outer layer consisted of fibres with a more longitudinal orientation. Bundles of muscle fibres were frequently observed extending between these two layers and were orientated in various directions giving an appearance which has been interpreted as additional layers by some authors. The transition from striated to smooth muscle occurred just proximal to the stomach, striated muscle fibres being present in the upper part of the lower oesophageal sphincter, only 3–4 mm above the mucosal gastro-oesophageal junction. The whole oesophagus was 9 cm in length whereas the muscular transition was complete within 3 mm, being more abrupt in the inner, more circular layer.

The marmoset oesophagus exhibited two clearly defined layers within the muscularis externa, the outer layer containing longitudinally orientated fibres and the inner layer circular fibres. The transition to smooth muscle began 2 cm above the stomach and occupied approximately 4 cm of the total length of the oesophagus (10 cm). There was little difference in the length of the transitional zone between the two layers.

In the macaque and in man, it was not possible to examine the whole length of the oesophagus, but those portions which were available demonstrated that the transition zone occupied a considerable proportion of its total length. In both species, the muscularis externa consisted of two layers, an outer layer of mainly longitudinally orientated muscle and an inner layer of mainly circularly arranged cells. However, as in the guinea-pig, there was a less obvious separation between the two muscle layers and bundles of differently orientated fibres frequently occurred between them.

In all species examined, ganglion cells were observed between the two muscle layers within that portion of the oesophagus which contained striated muscle cells.

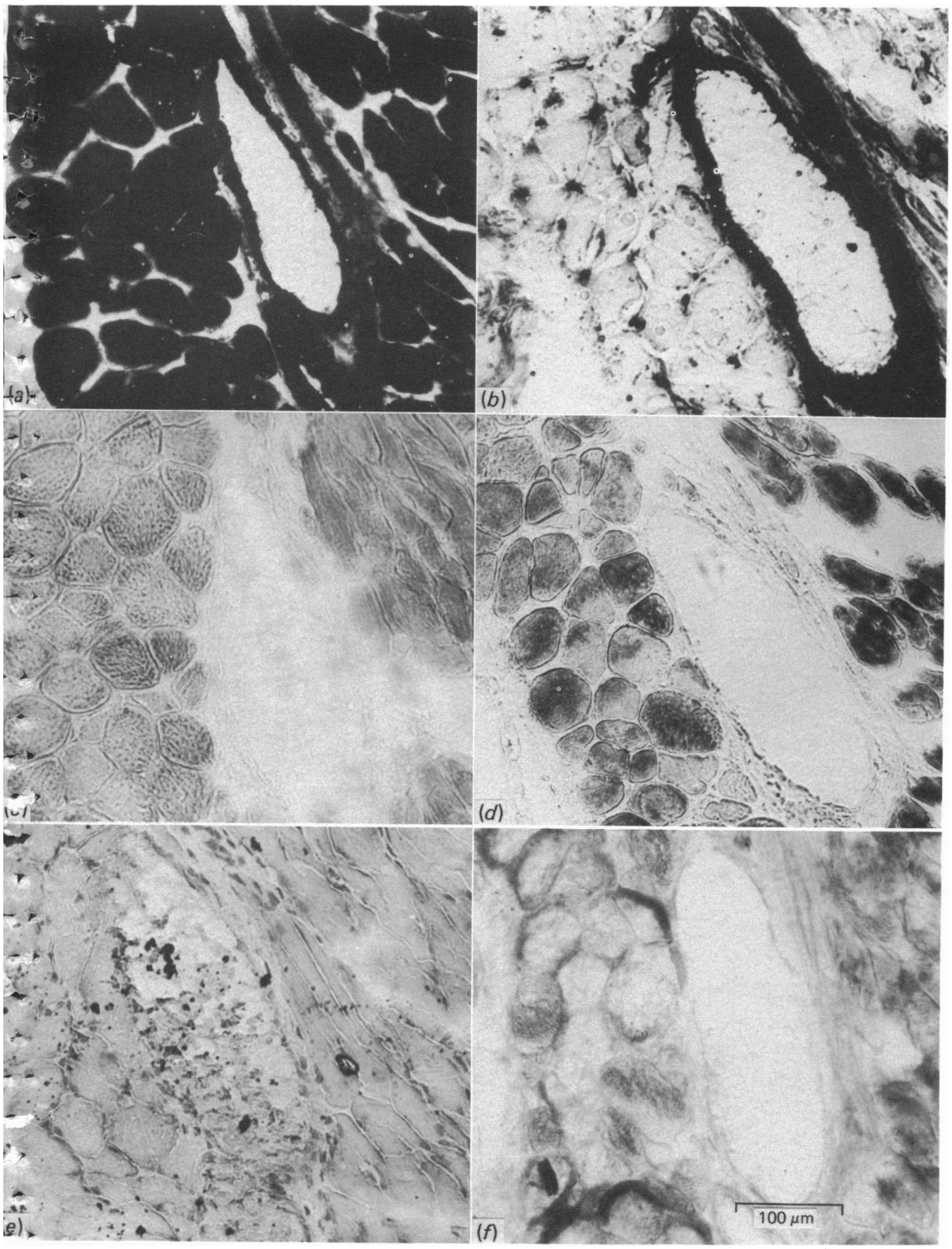

Fig. 1. Guinea-pig oesophageal muscularis externa after reaction for (*a*) Myosin ATPase at pH 10·4; (*b*) Myosin ATPase at pH 4·35; (*c*) SDH; (*d*) Phosphorylase; (*e*) Sudan black B; (*f*) PAS.

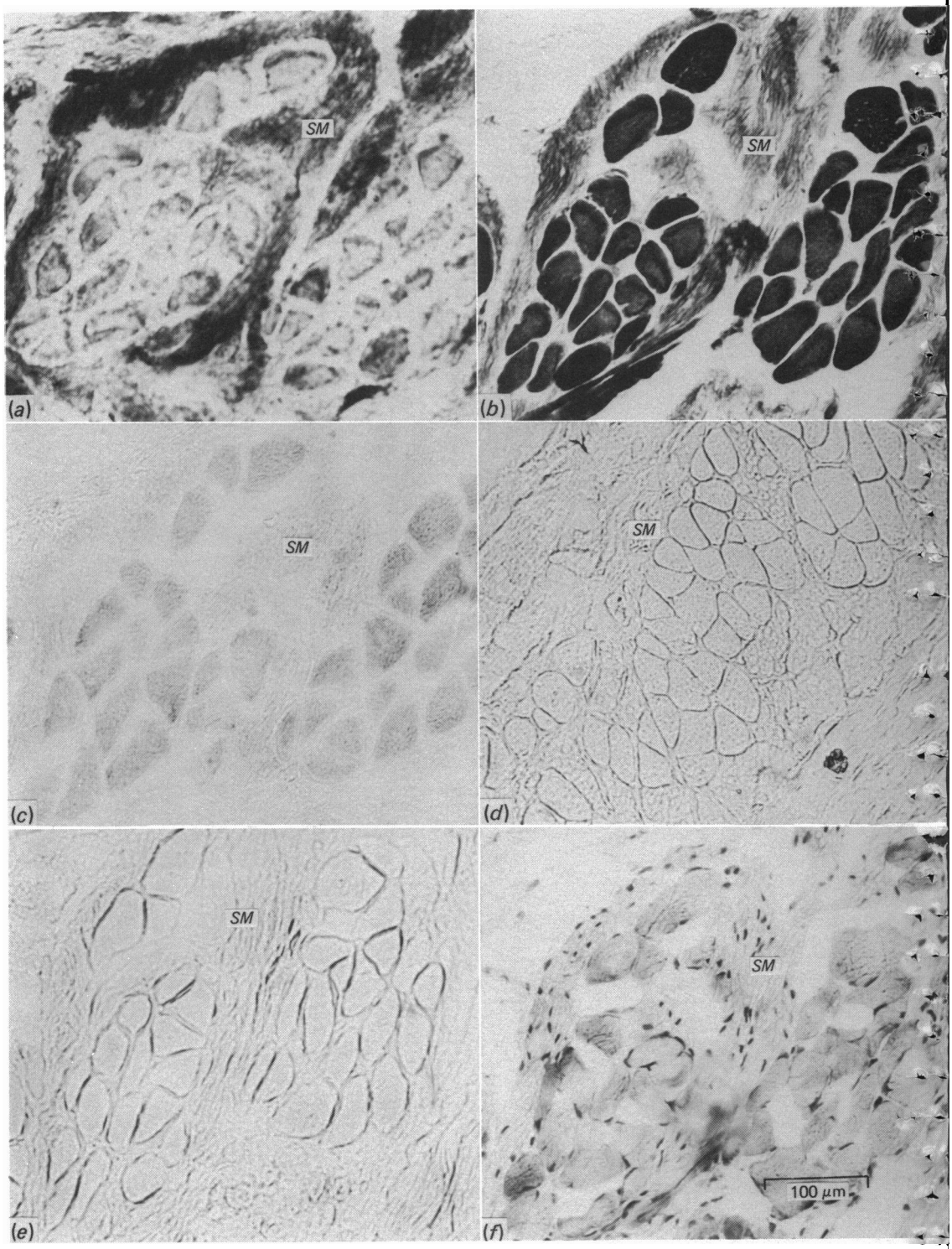

Fig. 2. Marmoset oesophageal muscularis externa after reaction for (*a*) Myosin ATPase at pH 10·4; (*b*) Myosin ATPase at pH 4·35; (*c*) SDH; (*d*) Phosphorylase; (*e*) Sudan black B; (*f*) PAS. Smooth muscle cells (*SM*) are present.

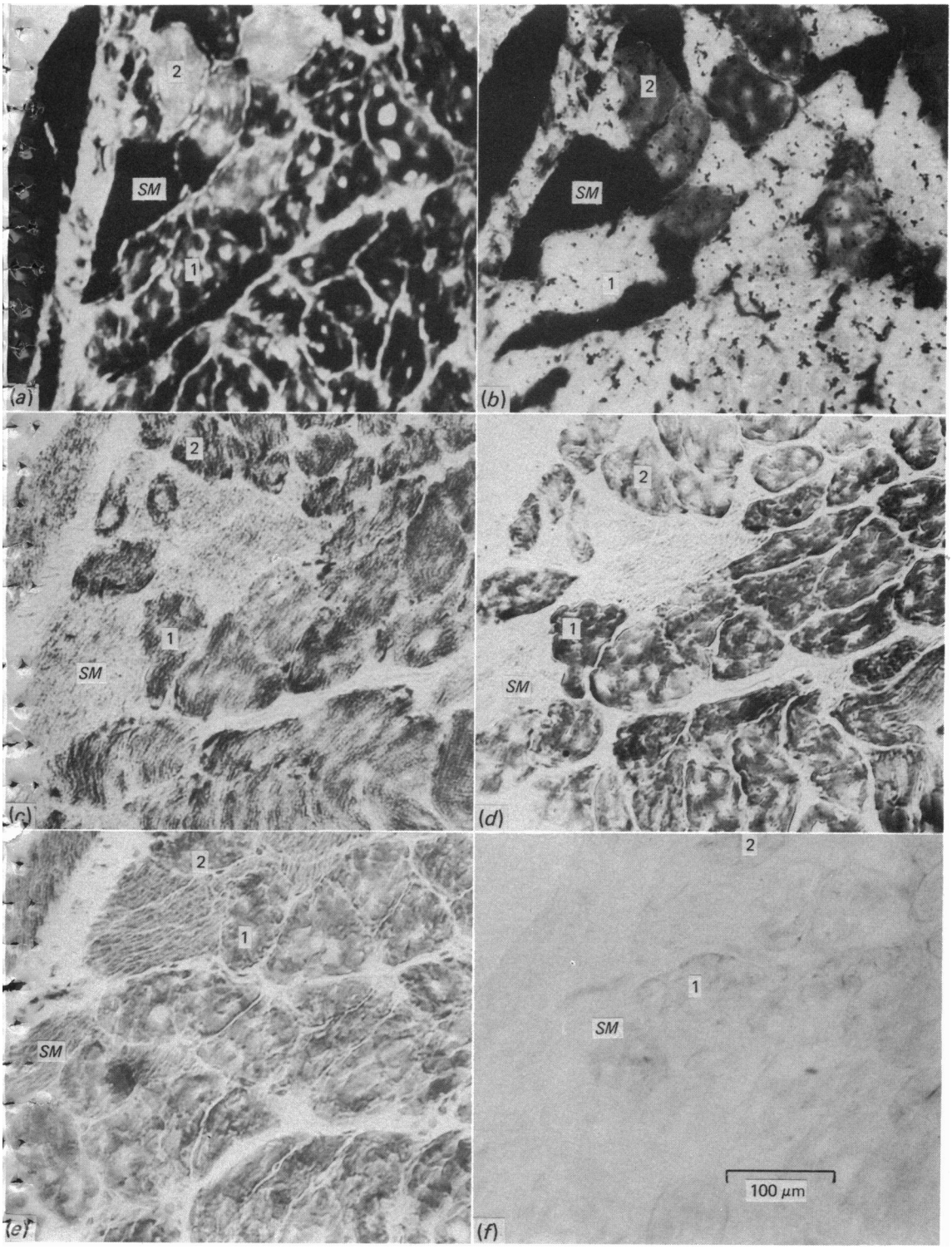

Fig. 3. Macaque oesophageal muscularis externa after reaction for (*a*) Myosin ATPase at pH 10·4; (*b*) Myosin ATPase at pH 4·35; (*c*) SDH; (*d*) Phosphorylase; (*e*) Sudan black B; (*f*) PAS. Smooth muscle cells (SM) are present. (1) Indicates fibres numbered 1 in Table 1. (2) Indicates fibres numbered 2 in Table 1.

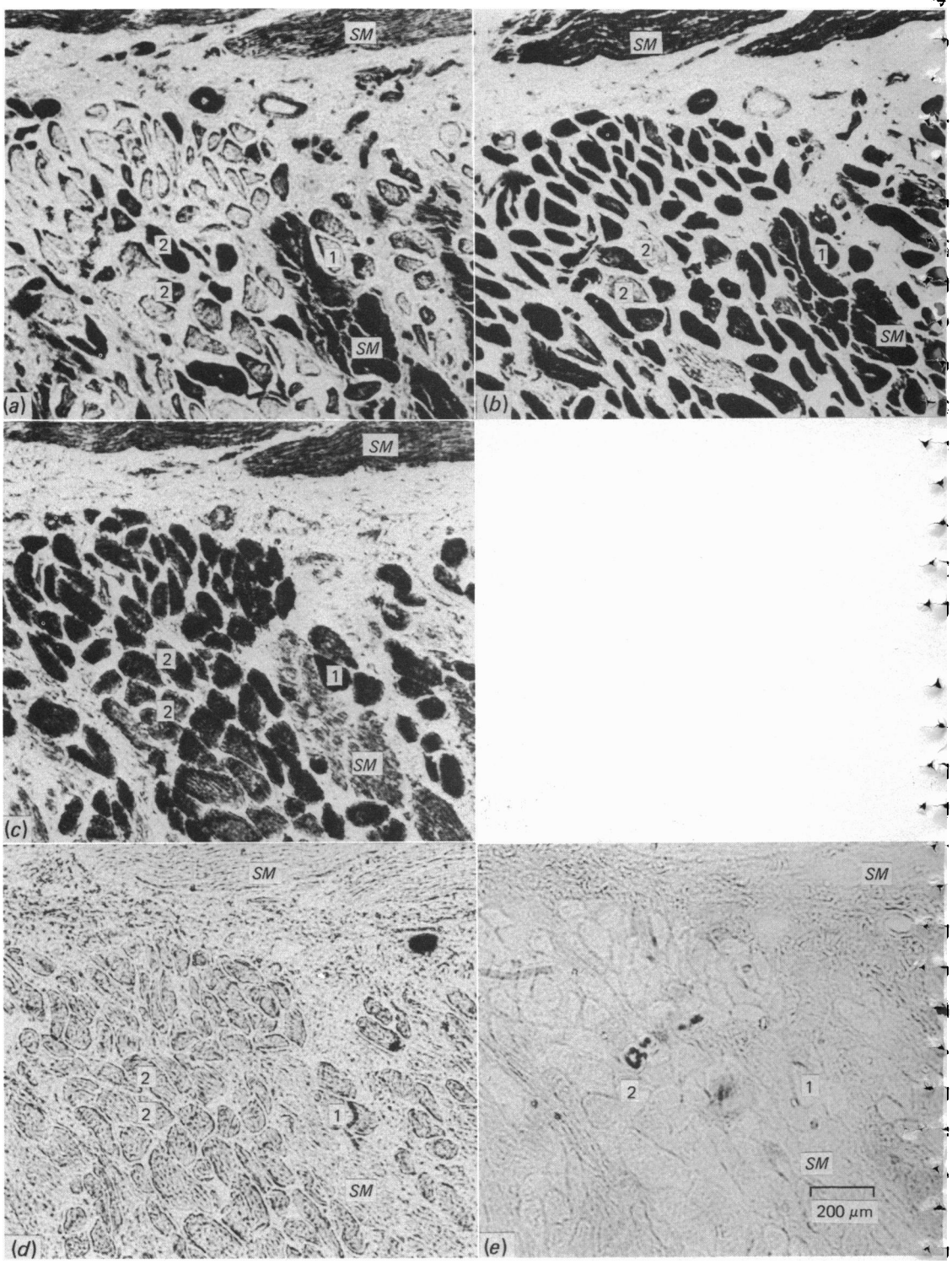

Fig. 4. Human oesophageal muscularis externa after reaction for (*a*) Myosin ATPase at pH 10·4; (*b*) Myosin ATPase at pH 4·35; (*c*) NADH; (*d*) Sudan black B; (*e*) PAS. Smooth muscle cells (SM) are present. (1) Indicates fibres numbered 1 in Table 1. (2) Indicates fibres numbered 2 in Table 1.

Table 1. *Histochemical reactions of oesophageal striated muscle*

Species	ATPase pH 10·4	ATPase pH 4·35	Phosphorylase	SDH/NADH	Sudan black B	PAS
Guinea-pig	++	−	++	+	−	−
Marmoset	−	++	−	+	−	+
Macaque						
(1)	−	++	++	+	−	+
(2)	++	−	++	+	−	+
Human						
(1)	−	++		+	−	−
(2)	++	−		−	−	++

Histochemistry

The histochemical reactions of individual fibres from the different species were graded as ++, + or −. Using the techniques for myosin ATPase, PAS and Sudan black B, fibres staining darkest were graded ++, those lightest − and those of intermediate staining +. NADH and SDH were assessed as ++ only if there were subsarcolemmal aggregates of reaction product, high levels of evenly distributed reaction product being given a grade of +, and low levels a grade of −. It was not possible to detect an intermediate grade of fibre following the localisation of phosphorylase. The results of these procedures are summarised in Table 1 and illustrated in Figures 1–4.

Using the technique for the demonstration of myosin ATPase, only one type of fibre was observed in guinea-pig oesophageal striated muscle. The marmoset revealed a different reaction, but once again all the fibres were of the same type. In contrast, the macaque possessed two fibre types, those few fibres that stained darkly after acid pre-incubation being distributed throughout that part of the oesophagus containing striated muscle fibres, usually as bundles of several fibres.

In the human oesophagus, two distinct populations of striated muscle fibre could be discerned in the muscularis externa. These two types occurred roughly in the proportion of 2:1, being distributed throughout the length and thickness of that part of the muscularis externa which contained any striated muscle.

DISCUSSION

The guinea-pig oesophagus has been shown to have a muscularis externa composed predominantly of striated muscle, although previous workers (Irwin, 1931; Thomas & Trounce, 1960; Francis, 1974) have disagreed as to its precise distribution. The results of the present study concur with the latter two reports, striated muscle fibres having been observed within a few millimetres of the gastro-oesophageal junction. The presence of only two layers of striated muscle has also been confirmed in this species. The observation that bundles of fibres of different orientation lay both nearer the lumen and between the two layers was in agreement with the descriptions of Ingelfinger (1958) and Francis (1974). However, such bundles are not considered to form additional muscle layers.

In the marmoset, macaque and human, the more gradual transition from striated to smooth muscle in the muscularis externa, as reported by previous authors and summarised by Ingelfinger (1958) and Weisbrodt (1976), was confirmed in this study. In the marmoset, the transition was observed to occupy the middle one third of the length of the oesophagus, in accordance with the accepted pattern for man and other primates (Ingelfinger, 1958; Weisbrodt, 1976). Although observation of the whole length of the macaque and human oesophagi was not possible, it was confirmed that the transition zone occupied an extended portion of the oesophagus. Whereas in the marmoset the transition zone was seen to occur within the inner circular and outer longitudinal muscles at the same level, in the macaque and man, smooth muscle fibres were observed more proximally in the inner circular layer, as observed by Marklin, Krause & Cutts (1979) in the opossum. This arrangement conforms with the pattern in the human described by Ingelfinger (1958) and Christensen & DeCarle (1974) who have suggested that oesophageal morphology in humans and primates concurs with an accepted concept that primates exhibit a more primitive level of evolution than other mammals, and may be progressing towards an oesophagus with a greater striated muscle component.

Histochemistry

Using the classification of Peter *et al.* (1972), the results of the various histochemical procedures may be interpreted as follows:

(i) Myosin ATPase staining which is stable after alkaline pre-incubation (pH 10·4) and labile at acid pH (4·35) is indicative of 'fast twitch' fibres (Bárány, 1967; Guth & Samaha, 1969; Bárány & Close, 1971; Barnard, Edgerton, Furukawa & Peter, 1971; Burke *et al.* 1971; Close, 1972; Gutman & Melichna, 1979). Conversely, the localisation of myosin ATPase which is stable at acid pH (4·35) and labile at alkaline pH (10·4) is indicative of 'slow twitch' fibres.

(ii) Blue coloration after the application of Gram's iodine in the technique for phosphorylase is indicative of glycolytic activity.

(iii) Oxidative capacity is indicated by the level and disposition of diformazan deposition following incubation for SDH or NADH-diaphorase.

(iv) Amounts of intracellular substrate are indicated by staining with the periodic acid–Schiff and Sudan black B techniques.

Human material was not subjected to the phosphorylase technique because this was obtained at post mortem, with an unavoidable delay of 12 to 24 hours between interruption of the blood supply and collection of the tissue. However, the results from the other techniques were held to be valid in accordance with Eriksson, Eriksson, Ringqvist & Thornell (1980) who have shown that ATPase and NADH muscle typing remains normal for up to 3 days after death, even at 21 °C.

By referral to Table 1, the following descriptive classifications (Peter *et al.* 1972) can be made for the present experimental results in purely histochemical terms:

Guinea-pig oesophageal striated muscle:	'Fast twitch' oxidative glycolytic (FOG) with low levels of lipid and glycogen.
Marmoset oesophageal striated muscle.	'Slow twitch' oxidative (SO), with low level of lipid and moderate level of glycogen.
Macaque oesophageal striated muscle.	(1) 'Slow twitch' oxidative glycolytic (SOG) with low level of lipid and moderate level of glycogen. (2) 'Fast

	twitch' oxidative glycolytic (FOG) with low level of lipid and moderate level of glycogen.
Human oesophageal striated muscle.	(1) 'Slow twitch' oxidative (SO), with low levels of lipid and glycogen. (2) 'Fast twitch' low oxidative (F), with low level of lipid and high level of glycogen.

In the human oesophagus, the 'fast twitch' fibres were probably glycolytic because they exhibited only low oxidative activity and contained high levels of glycogen, features typical of glycolytic activity in skeletal muscle (Peter *et al.* 1972).

Thus, the present results obtained from the guinea-pig concur with the reports in rat oesophageal striated muscle of only one fibre type, namely 'fast twitch' oxidative glycolytic (Asmussen, 1974; Gruber, 1978). However, in later work with Gaunitz, Asmussen (1978) noted that rat oesophageal muscle exhibited features of 'slow twitch' striated muscle in a physiological preparation for measuring isometric contraction parameters. This inconsistency remains unexplained, although Floyd & Morrison (1975) reported similar findings in isolated oesophageal muscle from cat and sheep.

The three primate species examined in the present study possess somewhat different characteristics. In the marmoset, all the fibres showed the same histochemical reaction, and can be classified as 'slow twitch' oxidative in type. However, in the macaque and man, two types of striated muscle fibre occur in the muscularis externa. In the macaque, the majority of fibres are similar to those seen in the marmoset, except that they exhibit glycolytic activity as well as oxidative activity. Thus, they would be typified as 'slow twitch' oxidative glycolytic fibres. However, such a classification does not fit with the commonly accepted types of striated muscle fibre. The second type of fibre, which was observed only in very small quantities, is identical to that found in the oesophagus of the guinea-pig and reported in the rat (Asmussen, 1974; Gruber, 1978).

In the human oesophagus, the majority of fibres were of the 'slow twitch' oxidative type similar to those seen in the marmoset, whereas the smaller group were 'fast twitch' and putatively glycolytic. In both the latter species, the distribution of the smaller group of fibres amongst the larger group appeared to be random, fibres of both types being seen in similar proportions both close to the pharynx and more distally, mixed with smooth muscle bundles. Although the description by Marklin *et al.* (1979), of fibres from the pharynx contributing to oesophageal musculature in the opossum, suggests that the least frequent fibres may be pharyngeal in origin, the even distribution in man and macaque observed in the present study tends to refute this theory.

The significance of the different histochemical characteristics of striated oesophageal muscle from the guinea-pig, marmoset, macaque and man are difficult to comprehend. They obviously represent species variation, and support the warnings given by Yellin & Guth (1970) and Yellin (1972) with regard to extrapolation of histochemical fibre typing between species. In addition, the present findings disagree with Bazhenov's (1979) conclusion that all mammalian oesophageal striated muscle is of only one type, namely 'fast twitch' and slow-fatiguing. Using the Peter *et al.*

(1972) classification, such fibres would be 'fast twitch' oxidative glycolytic in type (Burke *et al.* 1971) and thus conform only with those of the present study seen in guinea-pig and macaque striated oesophageal muscle.

SUMMARY

The muscularis externa of the oesophagus was examined histologically and histochemically in the guinea-pig, marmoset, macaque and man. It was found that the transition from striated muscle to smooth occurred more gradually and at a more proximal level in the primates than in the guinea-pig. In addition, minor differences in fibre lay-out were observed between the four species examined.

Guinea-pig oesophageal striated muscle was all found to be of one type, 'fast twitch' oxidative and glycolytic. The marmoset oesophageal muscle, also unitypical, was 'slow twitch' and oxidative. Both the macaque and man each revealed two types: 'slow twitch' oxidative glycolytic and 'fast twitch' oxidative glycolytic, and 'slow twitch' oxidative and 'fast twitch' putatively glycolytic, respectively. It is concluded that these differences represent species variation.

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