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A histological and histochemical study of the cricopharyngeus muscle in the guinea-pig*

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INTRODUCTION

The cricopharyngeus muscle has baffled researchers for many years, such that Jackson & Jackson (1950) wrote, "This constantly closed gate is *Bab el Mandeb* (Gate of Tears) for the inexperienced esophagoscopist." The advent of manometry, cineradiography and electromyography has led to much debate with regard to the function and innervation of the muscle, although little has been written concerning its structure. The cricopharyngeus muscle was first distinguished from the rest of the inferior pharyngeal constrictor muscle, and marked out as an anatomic entity, in man, by Valsalva in 1717 (cited by Reichert & Faw, 1980). The current edition of *Gray's Anatomy* (Williams & Warwick, 1980) describes the muscle as the lower division of the inferior pharyngeal constrictor and states that it possesses no central raphe.

Although there is still some disagreement concerning the exact muscular components of the 'upper oesophageal sphincter' (a high pressure zone situated at the pharyngo-oesophageal junction), there is substantial evidence supporting the cricopharyngeus as a major component (Killian, 1907; Vantrappen & Hellemans, 1974; Goyal, 1984). It is also generally agreed that the cricopharyngeus remains in tonic contraction except during vomiting, eructation and deglutition (Killian, 1907; Ekberg & Nylander, 1982; Goyal, 1984), a fact demonstrable by manometric, radiographic and electromyographic studies. Moreover, the cricopharyngeus is reported not to act in concert with the thyropharyngeus (Palmer, 1976), the upper division of the inferior pharyngeal constrictor.

Because of its obvious functional importance in health and disease, and its role in efficient swallowing and in protection of the tracheopulmonary tree, this muscle demands further morphological analysis. Ryu (1981) examined the histochemistry of the proximal parts of the alimentary tract in the dog and reported the cricopharyngeus as consisting of approximately 57% Type I muscle fibres possessing a high oxidative activity upon investigation with reduced NADH and succinate dehydrogenase reactions. The remaining fibres were of Type II, demonstrating little oxidative activity. Ryu also noted that the adjacent thyropharyngeus and upper oesophageal muscles consisted of 20% and 16% Type I fibres respectively. He concluded that the predominance of Type I fibres in the cricopharyngeus muscle plays an important role in its function. In our previous morphological and physiological investigations of the proximal alimentary tract in the guinea-pig (Whitmore, 1982; Wareham & Whitmore, 1982; Whitmore, 1983), the unusual nature and fibre type characteristics of oesophageal striated muscle have been established.

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Accordingly, the present work is concerned with the structure and metabolic profile of the 'upper oesophageal sphincter' region of the proximal alimentary tract in the guinea-pig using histological and histochemical techniques.

MATERIALS AND METHODS

Tissues used in this study were obtained from 9 young adult guinea-pigs (8 females and 1 male), each weighing approximately 500 g. The animals were killed by chloroform overdose, and from each an intact preparation of larynx and laryngopharynx was removed. The whole of the soleus and extensor digitorum longus muscles were also excised and used for comparison.

Blocks of muscle were orientated under a dissecting microscope so that sections would cut through fibres transversely and provide complete cross sections of each muscle. Following immersion in dichlorodifluoromethane (Arcton 12, I.C.I. Ltd.) previously cooled to freezing (−160 °C) in liquid nitrogen, tissue blocks were subsequently stored at −70 °C until required for sectioning. Forty to fifty serial sections 10 μm thick were cut from each block in a cryostat maintained at −20 °C.

The general architecture of the region surrounding the cricopharyngeus muscle was revealed using Masson's trichrome stain. Myofibrillar adenosine triphosphatase (ATPase) activity was located using a technique modified from Guth & Samaha (1970). Unfixed sections were allowed to air-dry at room temperature for 2–5 hours, followed by pre-incubation at room temperature at either pH 4·35 or 4·6 for 15 minutes, or pH 10·2 for 25 minutes. Incubation at 37 °C lasted 45 minutes for acid pre-incubated sections and 30 minutes for alkali pre-incubated sections (Bonington, 1985).

Oxidative enzyme activity was demonstrated by the nicotinamide adenine dinucleotide (reduced) tetrazolium reductasae (NADH-TR) reaction (Dubowitz & Brooke, 1973). Glycolytic activity was assessed by the phosphorylase technique of Takeuchi (1956). Finally, some sections were stained with either Sudan black B or the periodic acid–Schiff (PAS) technique to demonstrate metabolic storage products. Narrow fibre diameters of 60 fibres were measured in each cricopharyngeus and extensor digitorum longus muscles from each of three animals.

RESULTS

Histology

The most dorsal sections through the pharyngo-oesophageal region revealed the fibres of the thyropharyngeus inserting into the median raphe whilst the cricopharyngeus was seen to be continuous across the midline. In addition the latter muscle was seen to overlap slightly the proximal oesophagus where a fibrous septum was usually present separating these muscles. Another common feature was the presence of blood vessels (sometimes also a nerve) on the outer surface at the inferior and superolateral extremes of cricopharyngeus and a fibrous septum was observed in most sections, running from the 'superior' blood vessels to the lumen thus separating the cricopharyngeus from the thyropharyngeus. Nerves sectioned transversely and longitudinally were often observed within the cricopharyngeus and thyropharyngeus muscles.

Muscle fibre size appeared relatively uniform throughout the cricopharyngeus, thyropharyngeus, and proximal oesophagus, a fascicular arrangement being present in all. The fibres of extensor digitorum longus were found to be 41 μm (3·4 μm SE) in narrow diameter, approximately three times the size of cricopharyngeal fibres (14 μm, 0·3 μm SE). Muscle spindles, although observed frequently in extensor digitorum longus were never seen in the sections of cricopharyngeus.

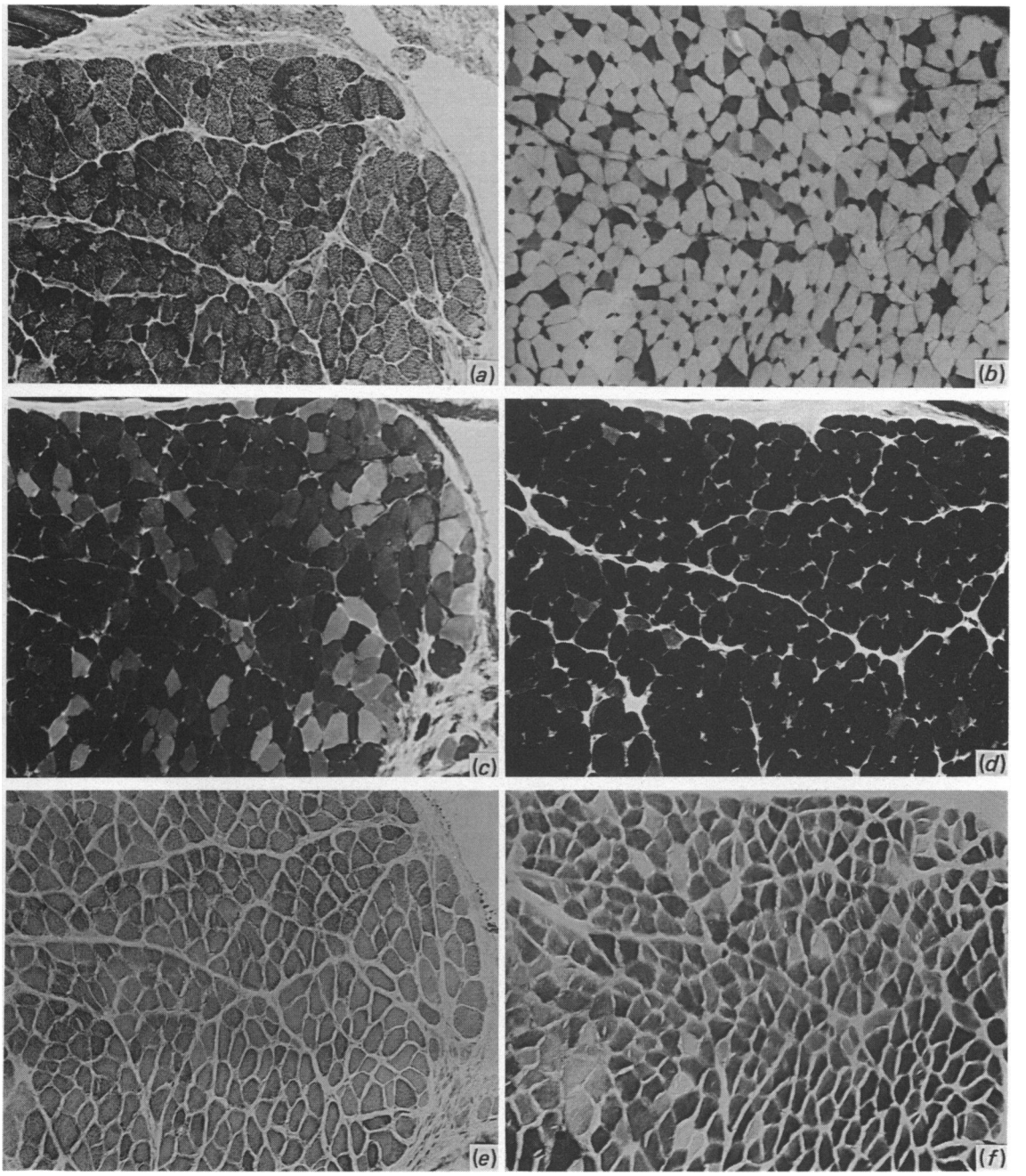

Fig. 1 (*a–f*). Guinea-pig cricopharyngeus. Stained for (*a*) NADH-TR, (*b*) ATPase preincubated at pH 4·35, (*c*) ATPase at pH 4·6, (*d*) ATPase at pH 10·2, (*e*) Sudan black B, (*f*) phosphorylase. × 130.

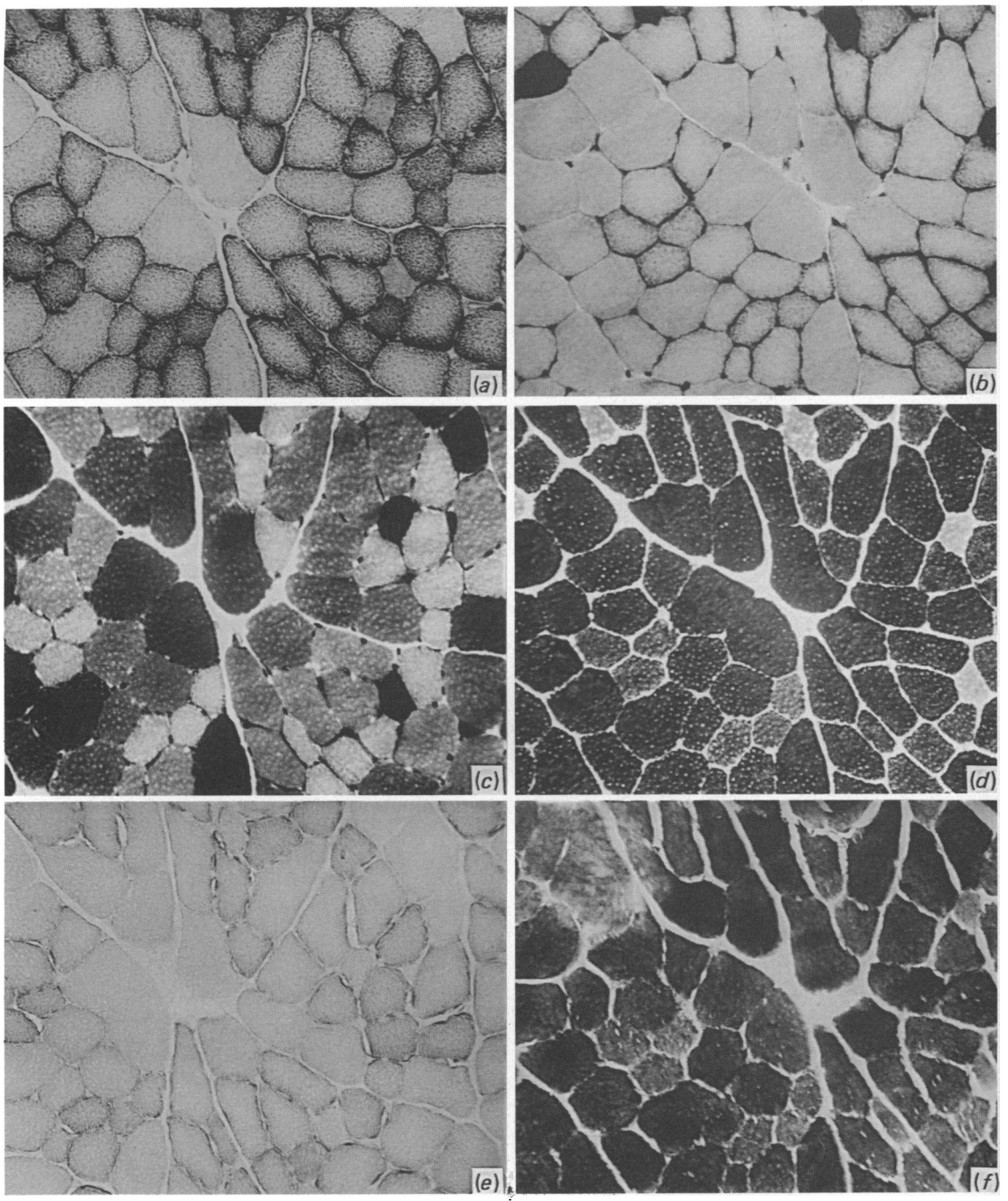

Fig. 2 (*a–f*). Guinea-pig extensor digitorum longus. Stained for (*a*) NADH-TR, (*b*) ATPase preincubated at pH 4·35, (*c*) ATPase at pH 4·6, (*d*) ATPase at pH 10·2, (*e*) Sudan black B, (*f*) phosphorylase. × 110.

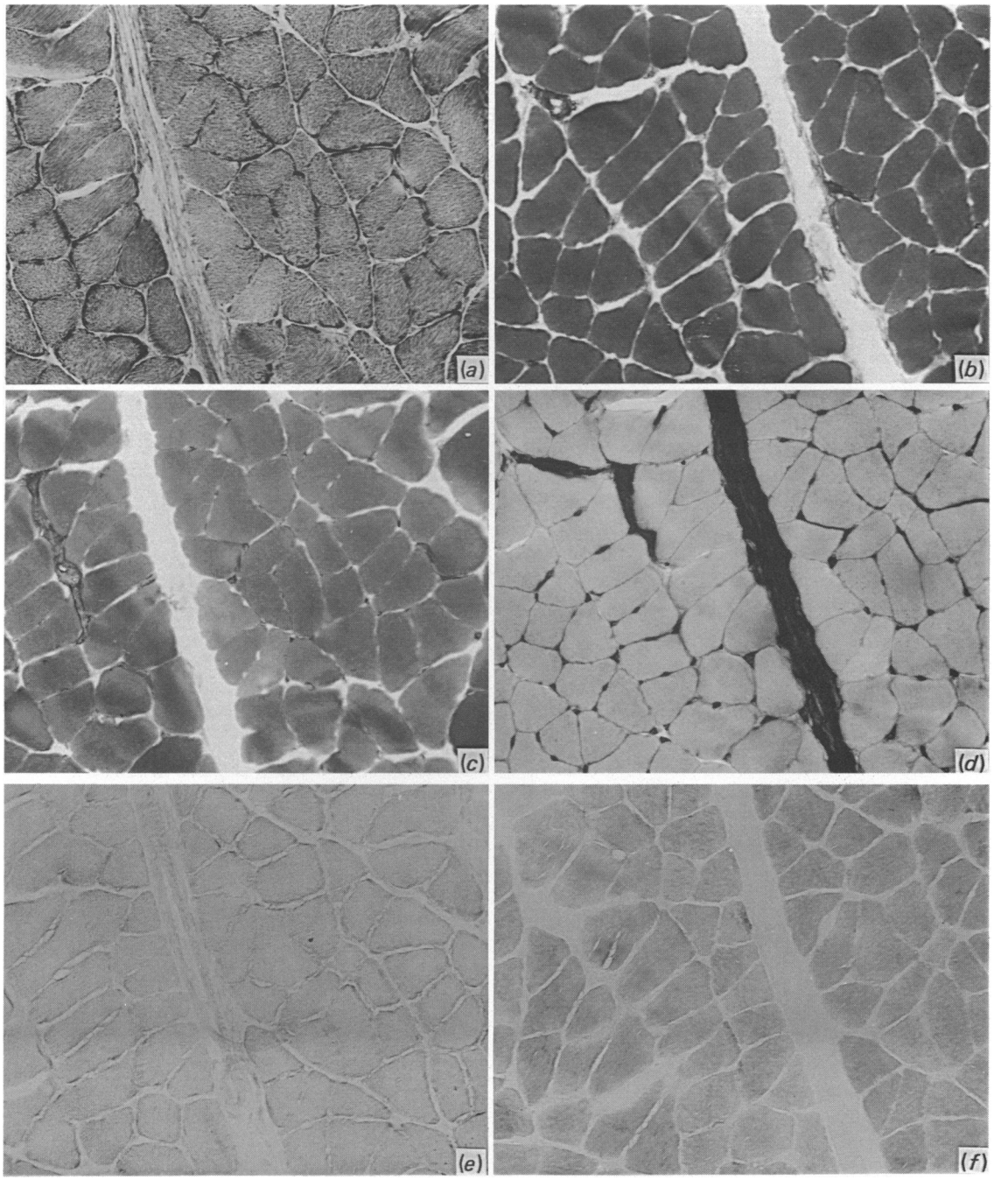

Fig. 3 (*a–f*). Guinea-pig soleus. Stained for (*a*) NADH-TR, (*b*) ATPase preincubated at pH 4·35, (*c*) ATPase at pH 4·6, (*d*) ATPase at pH 10·2, (*e*) Sudan black B, (*f*) phosphorylase. × 130.

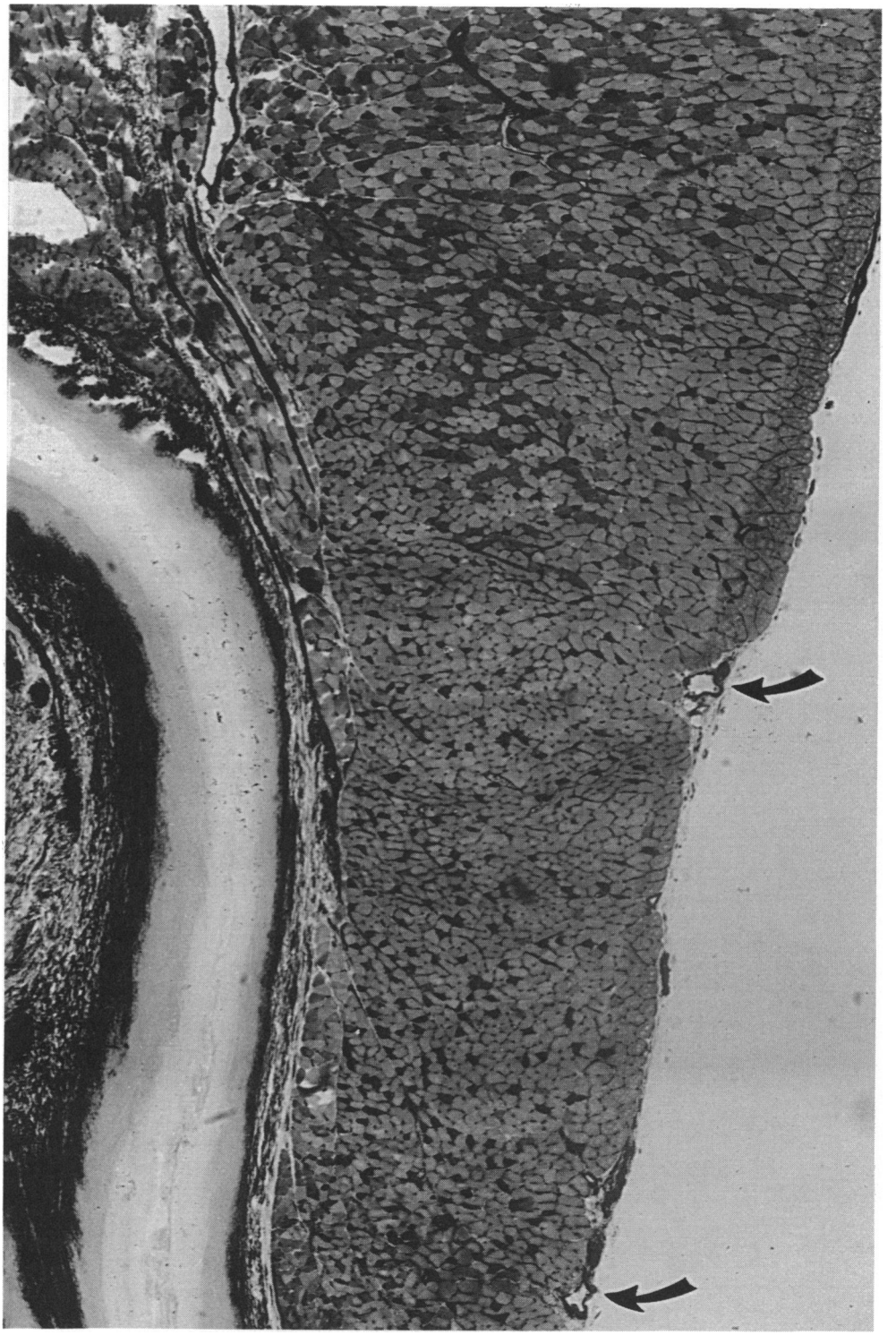

Fig. 4. Guinea-pig cricopharyngeus. Stained for ATPase preincubated at pH 4·35. The arrows indicate the blood vessels marking the upper and lower borders of cricopharyngeus. × 60.

Table 1. *Histochemical reactions in muscles of the guinea-pig*

Muscles	ATPase pH 4·35	ATPase pH 4·6	ATPase pH 10·2	PAS	Phosph-orylase	NADH	Sudan black B	Fibre type	% of fibres
	—	++	+++	+++	+++	++	++	IIB FOG	54·5
	—	++	+++	—	+++	++	++	IIB FOG	9·7
	—	++	+++	—	—	++	++	IIB FO	8·5
	—	—	+++	—	—	++	++	IIA FO	4·3
Cricopharyngeus	—	—	+++	+++	+++	++	++	IIA FOG	8·0
	+++	+++	—	—	—	+++	++	I SO	1·4
	+++	+++	++	—	—	++	++	?I SO	8·2
	++	+++	+++	—	—	++	++	IIC FO	0·8
	miscellaneous							various	4·7
	—	++	+++	—	+++	—	—	IIB FG	22·2
	—	+	+++	+++	+++	++	+	IIB FOG	17·9
	—	—	+++	+++	++	+++	+++	IIA FOG	27·1
Extensor digitorum	—	—	+++	—	—	+++	+++	IIA FO	5·8
longus	—	+	+++	+++	+++	++	+++	IIAB FOG	11·6
	+++	+++	—	—	—	++	++	I SO	10·2
	++	+++	++	na	na	na	na	IIC F	0·4
	miscellaneous							various	4·8
Soleus	+++	+++	—	—	—	++	+	I SO	100·0

na, not available

Higher power magnification revealed the fibres of the cricopharyngeus to be polygonal in outline with nuclei positioned at the periphery. This appearance is typical of normal mammalian extrafusal muscle fibres and confirmation of their striated nature was revealed in the few longitudinally sectioned fibres.

Histochemistry

The cricopharyngeus showed a heterogeneous checkerboard distribution of fibre types according to the ATPase reaction but was homogeneously dark staining with respect to NADH-TR (Fig. 1). This differed from the mixed (extensor digitorum longus) and homogeneous (soleus) fibre type distribution found in the guinea-pig hind limb (Figs. 2, 3). Cricopharyngeal fibres possessing an acid-stable myofibrillar ATPase were consistently observed to be more numerous deep within the muscle (Fig. 4). Following procedures for the demonstration of the various histochemical reaction products, results were obtained by visual assessment by a single observer. Each fibre was graded for each stain as $+++$, $++$, $+$ or $-$, according to the density of the reaction product, with $+++$ representing the darkest staining and $-$ the lightest. Using the technique for myofibrillar ATPase with pre-incubation at pH 4·35, 4·6 or 10·2, only three grades were discernible in cricopharyngeus, whereas four were seen in extensor digitorum longus.

Eight clearly different combinations of histochemical staining properties were differentiated in cricopharyngeus muscle fibres, whereas seven were seen in extensor digitorum longus and only one in soleus (Figs. 2, 3, 4, and Table 1). Although fibres staining deeply with the ATPase technique at all pH levels were observed in both cricopharyngeus and extensor digitorum longus, these were rare.

Although approximately 15% of fibres within cricopharyngeus appear dark with the ATPase technique preincubated at pH 4·35, very few (1·4%) of these had the appearance of the classical Type I SO fibre when pre-incubated at pH 10·2. As shown in Table 1, about 8% of fibres fall into a single category, staining darkly at pH 4·35 and at an intermediate level at pH 10·2. The remaining 4·7% of fibres possessing acid-stable ATPase fit into none of these categories, yet do not appear themselves to represent a single fibre type. Such cricopharyngeal fibres are represented as 'miscellaneous' in Table 1.

DISCUSSION

The striated nature of the guinea-pig cricopharyngeus muscle has been demonstrated, and found to be in agreement with the situation reported in man (Murakami, Fukuda & Kirchner, 1972), and with that observed in other skeletal muscles.

Muscle spindles were not observed in the cricopharyngeus although several were detected in extensor digitorum longus. The absence of these structures, specialised for providing elaborate feedback information to the CNS about the length and the rate of change of length of a muscle, is consistent with their absence from the human cricopharyngeus (Bonington, 1986), the striated portion of the mouse oesophagus (Samarasinghe, 1972), guinea-pig oesophagus (Whitmore, 1982), and the human external urethral sphincter (Gosling, Dixon, Critchley & Thompson, 1981). As all of these structures possess a tubular arrangement and the nature of their peripheral myoneural feedback is unknown, luminal pressure receptors may be more appropriate as a source of sensory information. The intraluminal pressure would be of more relevance to the normal function of such tubular structures than the stretch characteristics of their intramural muscle fibres.

A combination of two systems of nomenclature has been used in this study to allow a more accurate description of the muscle fibres. The system proposed by Brooke & Kaiser (1970) was based purely on the myofibrillar ATPase reaction and classified fibres as I, IIA, IIB and IIC; the classification of Peter *et al.* (1972) was selected for its descriptive nature as it also takes into account the metabolic profiles of the fibres. The results for the ATPase technique were interpreted by Peter *et al.* (1972) as follows: an alkali-stable and acid-labile actomyosin ATPase is indicative of 'fast-twitch' fibres (Barany, 1967; Guth & Samaha, 1969; Barnard, Edgerton, Furukawa & Peter, 1971; Gutmann & Melichna, 1979). Conversely, an acid-stable and alkali-labile ATPase is indicative of 'slow-twitch' fibres. Thus a full description of a muscle fibre might be IIA, Fast Oxidative Glycolytic (IIA FOG).

The extensor digitorum longus muscles examined were found to contain approximately 85% histochemically 'fast' fibres and 10% 'slow' fibres, the remaining 5% being unclassifiable. Approximately 73% of fibres were classified as oxidative, 57% of these also showing glycolytic activity. Only 22% of fibres were purely glycolytic, not demonstrating oxidative activity. Less than 1% of the fibres fell in the IIC group. The soleus muscle was found to consist of 100% Type I 'slow' oxidative (SO) fibres.

The guinea-pig cricopharyngeus was similar to the extensor digitorum longus and was found to contain approximately 85% histochemically 'fast' fibres and 10% 'slow' fibres. Preliminary physiological studies (Bonington, 1985) confirmed this similarity with respect to twitch contraction times. All cricopharyngeal fibres were classified as oxidative, 72% of these also possessing glycolytic activity. Less than 1% of fibres in cricopharyngeus were IIC fibres and slightly less than 5% could not be classified as any of the fibre types listed ('miscellaneous'). Thus in the guinea-pig the cricopharyngeus is a predominantly 'fast-twitch' muscle with a high metabolic activity. This is in agreement with the predominantly oxidative activity noted in the dog (Ryu, 1981) and human (Bonington, 1986) cricopharyngeus, although in these species 'fast-twitch' fibres account for 43% and 10% of the fibres respectively.

In the guinea-pig, cricopharyngeus was found to contain 8% of fibres which stained darkly at both alkaline pH levels and at an intermediate level at pH 10·2. These fibres, which were oxidative, had a similar myofibrillar ATPase pH sensitivity to those reported by Rowlerson, Mascarello, Veggetti & Carpene (1983). They found these fibres, which they called IIM, in the first branchial arch muscles of most species they examined. The jaw-closer muscles were found to contain IIM fibres in all carnivores except the lesser panda and in all primates except man. These authors correlated IIM fibres with an aggressive bite, both panda and man being lacking in this respect. Rowlerson, *et al.* (1983), in addition to standard histochemical techniques, used myosin type-specific antisera (immunocytochemistry) and it is believed that such techniques, possibly combined with electron microscopy will be needed to determine the exact nature of the various types of fibre in cricopharyngeus. It should be noted that the cricopharyngeus is neither a jaw-closer muscle, nor is it derived from the first pharyngeal arch, and the role of these fibres is yet to be determined.

The present study has disclosed the presence of an intermediate to high oxidative activity in all fibres of the guinea-pig cricopharyngeus. Examination of the surrounding musculature has revealed a checkerboard pattern of oxidative activity within thyropharyngeus and a low to intermediate homogeneous distribution within the circular muscle of the proximal oesophagus. The latter is in agreement with the data of Whitmore (1982). Similarly, low oxidative enzyme concentrations have been found in the middle pharyngeal constrictor and in the oesophageal muscles of the rabbit (Mahon & Lendon, 1982). Of these three muscles located in the vicinity of the upper

oesophageal sphincter, the cricopharyngeus muscle demonstrates the highest level of oxidative activity and thus the highest fatigue resistance. This makes it the most suitable candidate for the maintenance of tonic contraction and thus it would appear to be well suited to play an important role in the upper oesophageal sphincter.

SUMMARY

Histological, histochemical and morphometric methods were used to investigate the cricopharyngeus muscle in the guinea-pig and to compare it with the extensor digitorum longus and soleus muscles. The cricopharyngeus comprised uniformly small diameter fibres otherwise similar in appearance to those found in skeletal limb muscles. Several fibre type profiles were distinguished within the cricopharyngeus, all of which had homogeneously high oxidative activity, whilst the majority were histochemically fast (Type II). Muscle spindles were not observed in the cricopharyngeus muscles.

Compared to the surrounding musculature the cricopharyngeus has a higher oxidative activity and may thus be suitably adapted for the maintenance of tonic contraction, forming a part of the upper oesophageal sphincter.

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