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A histological and histochemical study of the cricopharyngeus muscle in man*

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INTRODUCTION

In our previous paper (Bonington, Whitmore & Mahon, 1987) we reported on the histochemistry of cricopharyngeus in the guinea-pig. We also highlighted the importance of this muscle in man in relation to swallowing, eructation, vomiting and the prevention of aerophagia (Murakami, Fukuda & Kirchner, 1972; Goyal, 1984).

In the present study gross, histological and histochemical investigations into this unusual muscle are continued in the human species.

MATERIALS AND METHODS

The intact larynx and laryngopharynx were removed from several embalmed human cadavers and dissected further in order to observe the gross anatomy of the cricopharyngeus and the surrounding tissues.

A sample of human cricopharyngeus was obtained postmortem from each of 4 males and 2 females with no known cricopharyngeal abnormality (ages ranging from 36 to 62 years with a mean of 51·8) and used for histology and histochemistry. Samples of the lateral portion of quadriceps femoris were obtained from 3 of these individuals and a fourth postmortem sample of vastus lateralis muscle from a previously healthy 30 year male was also used for comparison.

Sections of several human cricopharyngeus muscles and the lateral portions of quadriceps femoris muscles, previously subjected to histological and histochemical techniques in another study (Froes, 1984) were also made available for the present study.

All blocks were frozen between 10 and 21 hours postmortem and processed for cryostat sections as outlined previously (Bonington, 1985; Bonington *et al.* 1987). In all, 40–50 serial sections were taken throughout each block.

Histological techniques (haematoxylin and eosin, Masson's trichrome and modified Gomori trichrome) were used to demonstrate the general architecture of the muscle and the presence of certain morphological features such as fibre splitting, central nuclei, hyaline fibres, ragged red fibres, nemaline rods and degenerating and regenerating fibres.

Histochemical demonstration of the activity of myofibrillar actomyosin adenosine triphosphatase (ATPase) at pH 4·35, pH 4·6 or pH 10·4, nicotinamide adenine dinucleotide (reduced) tetrazolium reductase (NADH-TR) and phosphorylase, and the Sudan black B and the periodic acid–Schiff (PAS) techniques were carried out to

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examine fibre type profiles as reported previously (Bonington, 1985; Bonington *et al.* 1987).

The results following procedures for the demonstration of the various histochemical reaction products were visually assessed and each fibre graded by the same observer, according to the order of staining density as + + +, + +, + or − (' − ' indicates the lightest density of staining). These four grades were used with the NADH-TR and Sudan black B methods. As only three grades were discernible with the actomyosin ATPase at pH 4·35, 4·6 and 10·4, phosphorylase and PAS techniques, these were graded as darkest + + +, intermediate + + and lightest −. The reproducibility of this grading scheme for each technique was verified by repeat grading of the same photomicrographs on different days.

Fibre size measurement

In 4 specimens of cricopharyngeus and 3 of the lateral portion of quadriceps femoris, photomicrographs from sections stained with haematoxylin and eosin and adjacent to those processed for fibre typing were used to evaluate fibre size by the measurement of narrow fibre diameter. Overall photomicrograph magnification was × 320. Narrow fibre diameters were measured on at least 100 contiguous fibres for cricopharyngeus and at least 60 fibres for quadriceps. Narrow fibre diameter was chosen to obviate the effects of section obliquity on fibre size estimation and corresponded to the shortest chord passing through the centroid of the fibre profile.

Data were analysed by parametric and non-parametric statistical tests and results were considered significant at $P < 0{\cdot}05$.

RESULTS

Gross anatomy

Careful dissection in the region of the distal pharynx revealed the horizontal (pars fundiformis) and oblique (pars obliqua) portions of the cricopharyngeus, separated by Killian's dehiscence. The oblique portion was attached to the lateral aspect of the cricoid cartilage directly posterior to the origin of the cricothyroid muscle, inserted into the midline raphe posteriorly where it blended above with the thyropharyngeus. The horizontal portion was attached to the cricoid cartilage postero-inferior to the attachment of the pars obliqua, and was continuous posteriorly across the midline. The pars fundiformis was observed to have a maximum vertical dimension of 7 mm. All the histological and histochemical tests were performed exclusively on the pars fundiformis.

Histology

A typical transverse section through a human cricopharyngeus muscle is shown in Figure 1(*a*). A fascicular arrangement was evident in both the cricopharyngeus and the lateral portion of quadriceps femoris muscles and associated blood vessels and nerves were observed within both muscles. The endomysial connective tissue was particularly abundant in cricopharyngeus as compared to the lateral portion of quadriceps femoris. Muscle spindles were not observed in the cricopharyngeus sections examined.

Fibre size varied greatly in the cricopharyngeus muscle (Fig. 1*a*) although fibres were generally smaller than in the lateral portion of quadriceps femoris (Fig. 1*b*). Fibres of the cricopharyngeus resembled typical mammalian skeletal muscle fibres with peripheral nuclei and striations apparent on the few longitudinally sectioned

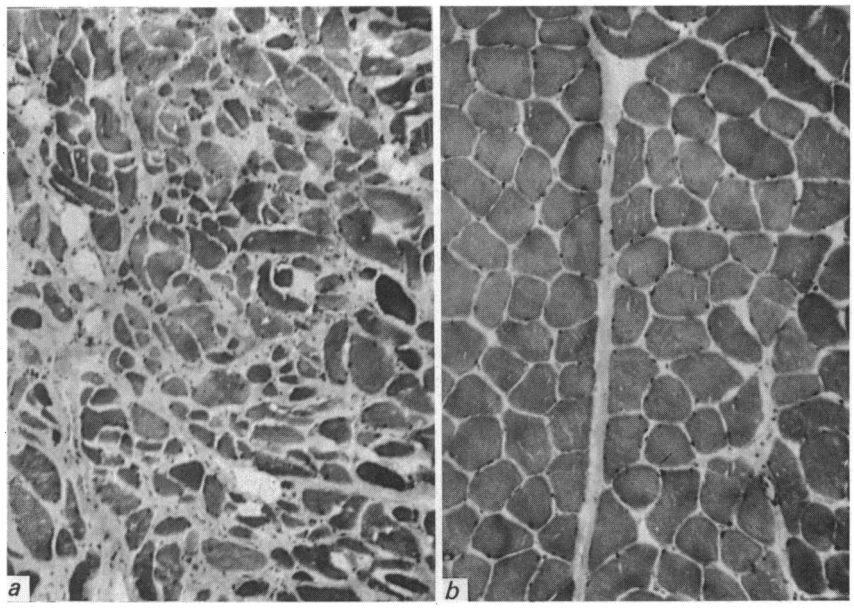

Fig. 1 (*a–b*). Sections of (*a*) cricopharyngeus and (*b*) quadriceps femoris. Masson's trichrome. × 70.

fibres examined. Obliquely sectioned fibres were also noted among predominantly transversely sectioned muscle.

For each high power field containing approximately 50–100 fibres of cricopharyngeus, 1–2 internal nuclei were observed. The periphery of a few fibres appeared to stain slightly more darkly than the central regions and occasional phagocytic infiltrates were seen. Most sections revealed the presence of an interfibre cellular infiltrate although few basophilic fibres were encountered.

Sections of cricopharyngeus stained with modified Gomori trichrome revealed 3–4 fibres per high power field characterised by relatively thin red-stained subsarcolemmal crescents. These corresponded with areas of high NADH-TR activity. The increased intermyofibrillar staining of typical 'ragged-red' fibres was usually absent.

Histochemistry

Two major fibre types were differentiated by the grading technique for cricopharyngeus whilst four major types were observed within the lateral portion of quadriceps femoris (Figs 2, 3; Table 1). Following preincubation of sections of cricopharyngeus at pH 10·2 (ATPase), all fibres appeared to be darkly staining. Increasing the pH of the preincubation medium to 10·4, however, revealed light staining Type I fibres. Since Type I fibres of the lateral portion of quadriceps femoris appeared light staining at pH 10·2, the pH sensitivities of the Type I ATPase in the two muscles are different.

Table 1 also indicates the presence of a few Type IIC fibres within the two muscles (0·7% in cricopharyngeus and 1·3% in the lateral portion of quadriceps femoris). A further 3–5% of fibres could not be placed into a single category and are classified as miscellaneous.

The homogeneous appearance of the cricopharyngeus with the NADH-TR technique (Fig. 2) contrasts with the chequerboard distribution found within the lateral portion of quadriceps femoris (Fig. 3). The distribution of phosphorylase activity

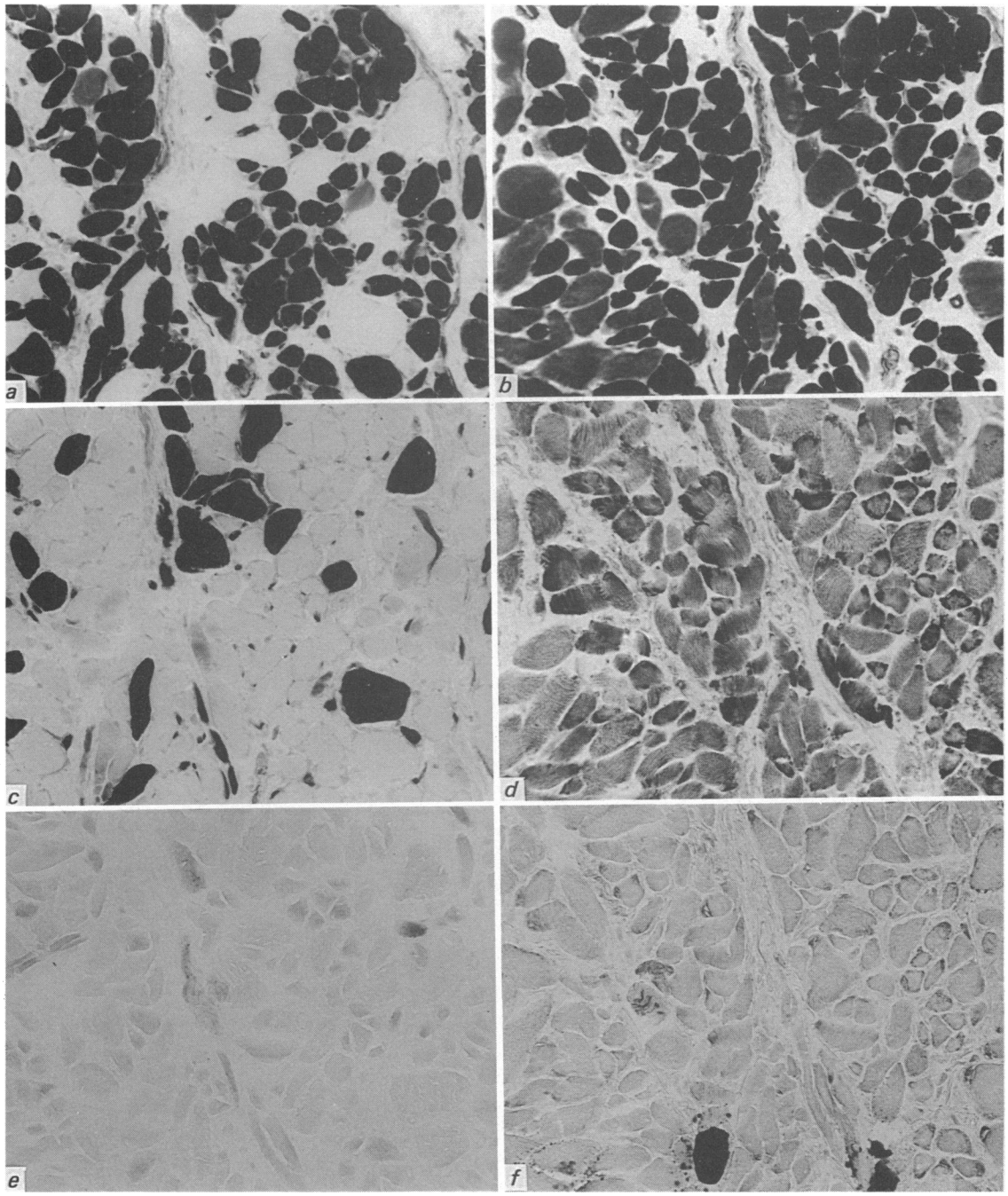

Fig. 2 (*a–f*). Adjacent sections of cricopharyngeus stained for the demonstration of myosin ATPase activity preincubated at pH (*a*) 4·35, (*b*) 4·6, (*c*) 10·4, (*d*) NADH-TR, (*e*) phosphorylase activity and with (*f*) Sudan black B. × 140.

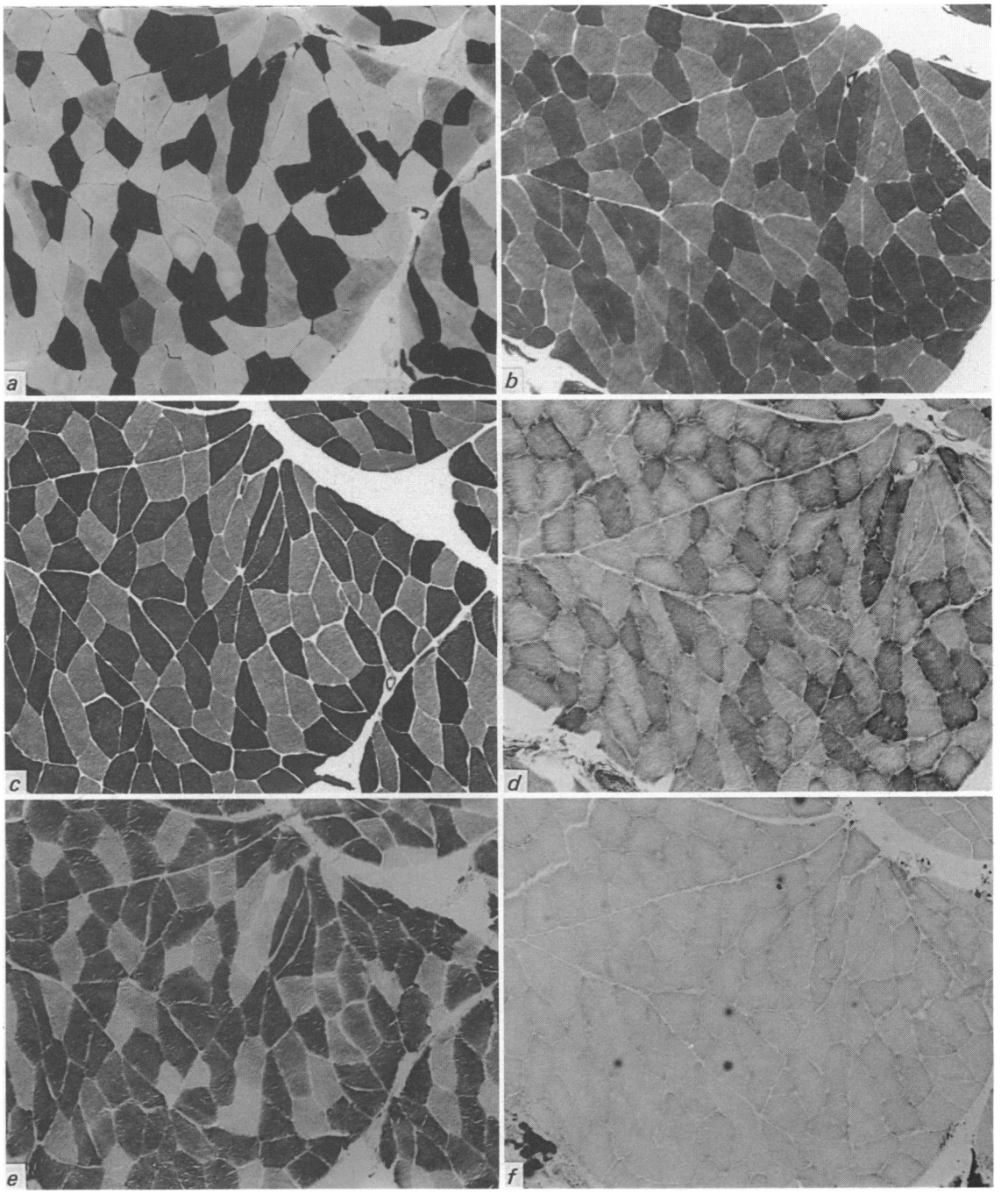

Fig. 3 (*a–f*). Adjacent sections of quadriceps femoris stained for the demonstration of myosin ATPase activity preincubated at pH (*a*) 4·35, (*b*) 4·6, (*c*) 10·4, (*d*) NADH-TR, (*e*) phosphorylase activity and with (*f*) Sudan black B ×70.

Table 1. *Fibre types in cricopharyngeus and quadriceps femoris*

Tissue	ATPase pH 4·35	ATPase pH 4·6	ATPase pH 10·4	PAS	Phos-phorylase	NADH	Sudan black B	Fibre	Type	% of fibres
Cricopharyngeus	−	++	+++	++	++	++	++	IIB	FOG	10·2
	+++	+++	−	−	−	+++	++	I	SO	85·9
	++	+++	+++	++	++	various		IIC	F	0·7
	miscellaneous							various		3·2
Quadriceps	−	++	+++	++	+++	−	−	IIB	FG	22·3
	−	−	+++	+++	+++	+	+	IIA	FOG	30·3
	+++	+++	−	−	−	+++	++	I	SO	34·0
	+++	+++	−	++	++	+++	++	I	SO	7·2
	++	+++	+++	++	++	++	++	IIC	FOG	1·3
	miscellaneous							various		4·9

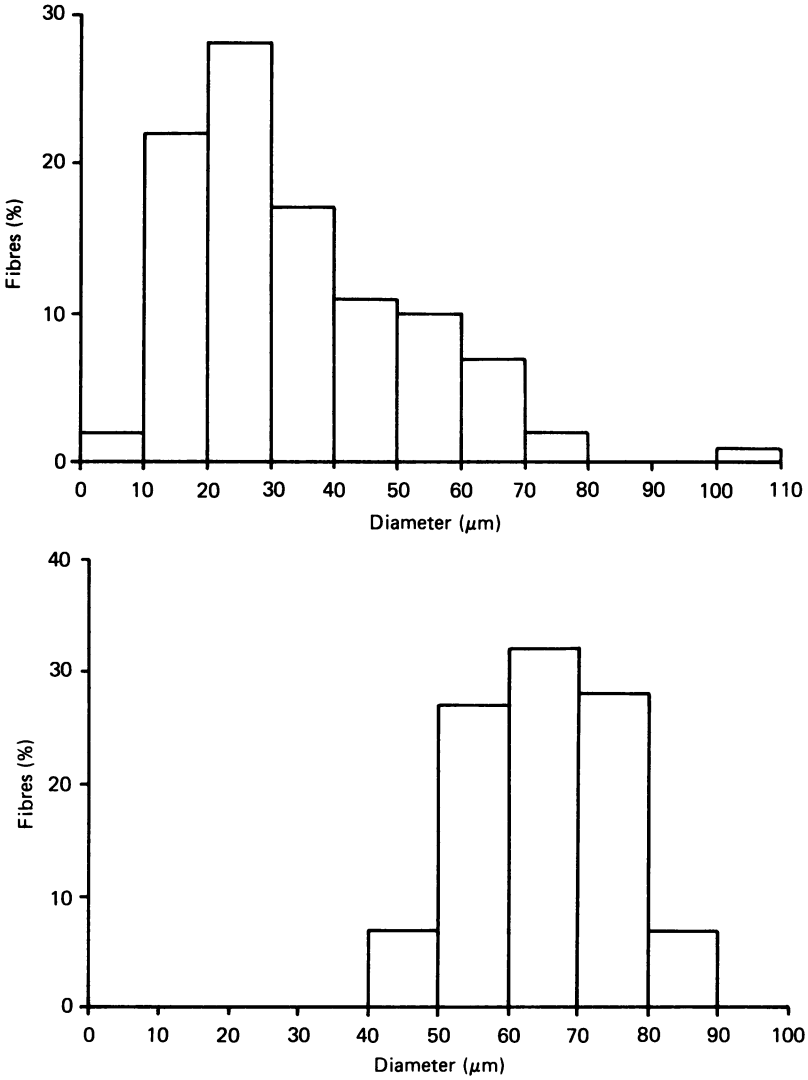

Fig. 4 (*a–b*). Histograms of narrow fibre diameter from specimen 5: (*a*) cricopharyngeus and (*b*) quadriceps femoris.

within the lateral portion of quadriceps femoris presents a similar pattern to that following NADH-TR. Very few fibres within the cricopharyngeus revealed intermediate (+ +) or high (+ + +) phosphorylase activity, this being limited to the few (10·2%) Type II b FOG fibres present (Fig. 2). No Type II a fibres (light staining when preincubated at pH 4·6 for the ATPase technique) were observed within the cricopharyngeus muscle.

Fibre sizes

Cumulative mean graphs of narrow fibre diameter revealed that the measurement of about 40 fibres for the lateral portion of quadriceps femoris and 90 fibres for cricopharyngeus were required to give a mean value within 5% of the final mean. Histograms of narrow fibre diameter showed a positively skewed distribution for

Table 2. *Narrow fibre diameters (μm) of cricopharyngeus and quadriceps femoris*

Specimen	Cricopharyngeus			Quadriceps		
	n	Mean	SD	n	Mean	SD
2	133	20·6	6·2	—	—	—
3	108	24·4	10·6	78	55·8	11·0
4	326	23·9	10·1	—	—	—
5	184	33·1	16·6	63	62·0	9·4
7	—	—	—	63	60·5	11·9
Combined means	751	25·5	—	204	59·4	—

n, number of fibres; SD, standard deviation.

cricopharyngeus which was significant at the 1% level (Fig. 4*a*) and positive kurtosis ($P < 0·05$).

Histograms for the lateral portion of quadriceps femoris (Fig. 4*b*) showed no significant skewness or kurtosis. Fibres of this muscle were considerably larger (Table 2) than those of cricopharyngeus (Mann–Whitney 'U' test, $P < 0·01$).

DISCUSSION

The specimens examined by dissection revealed that the cricopharyngeus, which refers to the pars fundiformis in this work, conformed to the accepted anatomical descriptions (Zaino, Jacobson, Lepow & Ozturk, 1970; Williams & Warwick, 1980).

As reported by Murakami *et al.* (1972), human cricopharyngeus muscle was found to be striated in nature. In the present study, the human cricopharyngeus possessed large quantities of endomysial connective tissue. This connective tissue framework may be the cause of the small remaining intraluminal pressure (10 mmHg) recorded by Asoh & Goyal (1978) following motor nerve section of the cricopharyngeus in the opossum. Goyal (1984) stated that this remaining pressure may be due to passive elastic forces acting on the sphincter wall. The presence of such a supporting framework provides a mechanism whereby this muscle, reported by other authors (Killian, 1907; Jackson & Jackson, 1950; Fyke & Code, 1955; Negus, 1957; Kirchner, 1958; Lund, 1965; Winans, 1972; Nanson, 1974; Berlin, Tedesco, Fierstein & Ogura, 1977; Devgan, Gross, McCloy & Smith, 1978; Goyal & Cobb, 1981; Ekberk & Nylander, 1982) to be tonically contracted, is firmly anchored.

An alternative explanation for the large quantities of connective tissue is that the muscle fibres do not travel the entire length of the muscle but may insert into the connective tissue framework. In support of this is the large variation in fibre size noted in the present work, where the smallest profiles may represent the tapering ends of fibres inserting into connective tissue. Electron microscopy would be needed to substantiate this view.

No muscle spindles were detected in the sections of human cricopharyngeus examined (Bonington, 1986), which is in agreement with findings in the guinea-pig (Bonington *et al.* 1987). Muscle spindles have not been identified in the striated portion of other tubular structures such as the mouse oesophagus (Samarasinghe, 1972), guinea-pig oesophagus (Whitmore, 1983), nor in the human external urethral sphincter

(Gosling, Dixon, Critchley & Thompson, 1981). The peripheral myoneural feedback mechanisms of these tubular structures are unknown, although some form of peripheral feedback is presumed to be present to enable their complex functions to be performed and the presence of luminal pressure receptors has been suggested as an alternative to muscle spindles (Bonington *et al.* 1987).

Dense red subsarcolemmal crescents in the modified Gomori trichrome stain were found in cricopharyngeal muscle fibres. These features are common in 'mitochondrial myopathies' (Kremzier, 1984); however, ultrastructural confirmation (Carpenter & Karpati, 1984) is neccessary to ascertain their exact nature in the human cricopharyngeus. Taken in conjunction with the presence of an interfibrous cellular infiltrate, a few basophilic fibres and a few internal nuclei, the appearance seen in cricopharyngeus would be classed as abnormal if seen in a limb muscle. Since these features were observed in all of the 6 cricopharyngeus muscles used in the main part of the present study and also those pre-stained sections of another 14 muscles available from a previous work (all from individuals considered to have no cricopharyngeal abnormality at the time of death), they may be representative of 'normal' human cricopharyngeus muscle. Alternatively these changes may be due to the age of the patient, or the time between death and freezing of the tissue. Histological features of degeneration and regeneration in the cricopharyngeus muscle fibres and interstitial fibrosis have been described by Cruse, Edwards, Smith & Wyllie (1979). These authors speculated on restrictive fibrosis as the cause of dysphagia and considered the fibrosis to be secondary to muscle fibre damage, the cause of which was obscure. They rarely observed any muscle damage in controls and fibrosis was never present.

Some striking similarities were noted between the histological appearance of cricopharyngeus in the present study and that of the intrinsic laryngeal muscles reported by Rosenfield, Miller, Sessions & Patten (1982). These authors mentioned increased amounts of endomysial connective tissue and longitudinal extensions of fibres that were intermingled with other fibres sectioned transversely. They failed to find any muscle spindles and reported the presence of basophilic and 'ragged red' fibres, and the large variation in fibre size. All these features compare well with those reported in the present paper for cricopharyngeus.

In order to elucidate the functional properties of the cricopharyngeus muscle fibres, they were classified on the basis of a combination of two systems of nomenclature: Brooke & Kaiser (1970), using only the ATPase reaction (I, IIA, IIB, IIC), and the classification of Peter *et al.* (1972) (SO, FOG, FG) which takes into account the metabolic profiles of the fibres.

The lateral portions of quadriceps femoris muscles examined were found to contain a mixture of histochemically 'fast-twitch' fibres and 'slow-twitch' fibres. In addition, these fibres displayed a range of oxidative and glycolytic activity, thus conforming to previous reports on this well studied muscle. A large inter-subject variation was noted with respect to fibre type proportions in the lateral portion of quadriceps femoris, similar to those reported by Mahon, Toman, Willan & Bagnall (1984).

While the human cricopharyngeus muscle consists of a few histochemically 'fast-twitch' fibres and many 'slow-twitch' fibres, all fibres were classed as oxidative. Additionally a few fibres also demonstrated glycolytic activity. As the lateral portion of quadriceps femoris muscles displayed expected levels of phosphorylase activity, it is felt that the low level of glycolytic activity in cricopharyngeus reflects reality and is not the result of postmortem changes. Less than 1% of fibres were classed as IIC in cricopharyngeus.

A uniform high-intermediate oxidative activity has been found in the crico-

pharyngeus and this is in agreement with the findings in the guinea-pig cricopharyngeus (Bonington *et al.* 1987). Human cricopharyngeus contains a predominance of histochemically 'slow-twitch' fibres comparable with those of the external urethral sphincter which was found to consist of a single population of Type I fibres (Gosling *et al.* 1981). The large proportion of Type I oxidative fibres in the cricopharyngeus indicates structural adaptation to the maintainance of tone over prolonged periods, and is therefore ideally suited to its suggested function within the upper oesophageal sphincter.

The population of Type II FOG fibres (10%) may also play an important role in maintaining the closure of the sphincter against increased pressure of short duration. For instance, on inspiration, the cricopharyngeus has been found further to contract (Levitt, Dedo & Ogura 1965; Parrish, 1968; Goyal, Sangree, Hersh & Spiro, 1970; Winans, 1972; Vantrappen & Hellemans, 1974; Roed-Petersen, 1979; Goyal & Cobb, 1981; Goyal, 1984) and it may be that the Type II fibres are responsible. Alternatively the small population of Type II fibres may be responsible for the rapid contraction of the cricopharyngeus immediately following deglutition when the intraluminal pressure rises to approximately twice the resting level for about one second (Devgan *et al.* 1978; Goyal, 1984) before returning to the resting level.

The small size of the cricopharyngeal fibres in contrast to those of the lateral portion of quadriceps femoris is consistent with other studies reported on striated muscle fibres lacking skeletal attachment (Gosling *et al.* 1981; Lendon & Mahon, 1982). The great variation in fibre size could reflect heterogeneity of function within the cricopharyngeal muscle fibre population. Equally, variations along the length of the fibre, for example tapering close to points of attachment, may be responsible for these findings. However, the latter suggestion is not supported by observations of myo-tendinous or myo-myal junctions. Furthermore the wide variation in fibre size together with the histological 'abnormalities' could indicate pathological features of this muscle associated with ageing and requires further investigation.

SUMMARY

The human cricopharyngeus muscle was investigated by dissection and by histological, histochemical and morphometric methods. Muscle fibres in the cricopharyngeus were found to be similar in appearance to those of the lateral part of the quadriceps femoris, although they were generally much smaller and more variable in size. The endomysial connective tissue was markedly increased in the cricopharyngeus and muscle spindles were not found. Certain features normally considered to be pathological were also noted in the cricopharyngeus muscles.

The fibre type population consisted mainly of histochemically 'slow-twitch' richly oxidative fibres. This finding is consistent with the proposed function of this muscle in its sphincteric role in deglutition, vomiting, eructation and in the control of aerophagia.

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