Ultrastructure of striated muscle fibers in the middle third of the human esophagus

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Summary. Striated muscle fibers and their spatial relationship to smooth muscle cells have been studied in the middle third of human esophagus. Biopsies were obtained from 3 patients during surgery. In both the circular and longitudinal layers, the muscle coat of this transition zone was composed of fascicles of uniform dimension (100-200 μm of diameter); some of these bundles were made up of striated muscle fibers, others were pure bundles of smooth muscle cells and some were of the mixed type. Striated muscle fibers represented three different types, which were considered as intermediate, with certain structural features characteristic of the fast fiber type. Of these, the most frequently-found fibers were most similar to the fast fiber type. Satellite cells were numerous; in mixed fascicles they were gradually replaced by smooth muscle cells. The gap between striated muscle fiber and smooth muscle cells was more than 200 nm wide. It contained the respective basal laminae and a delicate layer of amorphous conective tissue.

No specialized junctions were formed between consecutive striated muscle fibers, or between striated muscle fibers and smooth muscle cells. Interstitial cells of Cajal were never situated as close to striated muscle fibers as to smooth muscle cells.

Key words: Oesophageal musculature - Skeletal muscle - Satellite cells - Man

Introduction

Until recently, esophageal striated muscle fibers were considered to be similar to skeletal striated muscle fibers (Gruber, 1968; Samarasinghe, 1972). More recently Whitmore (1983) characterized the ultrastructural features of these striated muscle fibers in guinea pig and marmoset. These findings indicate that, although similar to skeletal muscle, the esophageal muscle displays significant differences as to its arrangement and motor innervation. It was also found impossible to classify these esophageal muscle fibers according to conventional ultrastructural criteria.

Classifications of the striated fibers in mammalian esophageal muscle coat have been made by histochemical techniques. The data obtained differ with respect to the species studied and, sometimes, also between animals of the same species. In man, for example, two types of fibers have been histochemically characterized, but the authors do not agree about their distribution and frequency. In particular, Shedlofsky-Des Champs et al. (1982) reported one type (the so-called type I-slow twitch no-fatiguable) to be present at the pharyngeal level only, whereas Whitmore (1982) found it also in the lowest esophageal levels. The latter stressed that this is the most frequent fiber type, whereas Shedlofsky-Des Champs et al. (1982) reported the other type identified (the so-called type 2B-fast twitch-fatiguable) to be the most frequent one.

Some authors also searched for structural relationships between striated muscle fibers and smooth muscle cells in some of those species having mixed esophageal musculature (mouse: Samarasinghe, 1972; guinea pig and marmoset: Whitmore, 1983). No connections or specialized contact areas were found between smooth and striated muscle. The human esophagus also has a mixed musculature, but only the ultrastructural and spatial arrangement of the smooth muscle bundles is known (Faussone-Pellegrini and Cortesini, 1985).

The aim of the present paper is, therefore, to report on the transitional zone (the middle third) of the human esophagus. Only the ultrastructural peculiarities of the striated muscle fibers and their spatial relationship to smooth muscle cells are described; their innervation will be considered in a separate paper.

Materials and methods

Biopsies of the esophageal muscle coat were obtained from 3 patients (2 males and 1 female, between 45 and 65 years of age) operated on for carcinoma of the esophagus. Two of the specimens included the middle third and part of the lower third; the third specimen was obtained from an area 20-24 cm apart from the dental arcade and some 10 cm from the carcinomatous area. All the specimens were cut into strips. These strips were fixed by immersion in 4% glutaraldehyde in cacodylate buffer pH 7.4 and postfixed with 1 To OsO_4 in phosphate buffer pH 7.4, dehydrated in acetone and embedded in Epon. For sectioning, the blocks were orientated so as to cut the esophagus transversely. Semithin sections were stained with toluidine blue. Thin sections were stained with uranyl acetate followed by lead citrate (according to Venable and Coggeshall, 1965) or with alcoholic solution of uranyl acetate followed by alkaline bismuth subnitrate (according to Riva, 1974). Identical results were obtained with both these methods, but the second staining better emphasized elastic material.

Electron microscope: Siemens Elmiskop 1 A.

Results

In the transition zone, both in the circular and longitudinal layers, each cylindric striated muscle fiber was 100-120 μm in diameter (Fig. 1). Long cylindric smooth muscle bundles were mixed among them, they too of 100-120 μm in diameter (Fig. 2). Smooth muscle cells partially or completely surrounded the thinning extremity of a striated muscle fiber and increased in number as the striated muscle fiber became thinner. In this case, the striated muscle fiber was gradually substituted by a cylinder of only smooth muscle cells, forming a mixed bundle of approximately the same diameter as shown by striated muscle fibers or pure smooth muscle bundles. Therefore, the muscle coat of the transition zone was made up of fascicles wedged between one another; some of these fascicles were formed by a single striated muscle fiber, others were bundles of pure smooth muscle cells, and some were of a mixed type.

Three types of striated muscle fibers were found, both in the circular and longitudinal muscle layers. The most frequent type was characterized by few, small mitochondria, small quantities of glycogen particles and poorly elaborate sarcoplasmic reticulum (Figs. 3, 4). Mitochondria were situated in pairs at the I-band level on both sides of the Z-lines. The other types of striated muscle fibers were, however, very rare.

One of these less frequent types could be identified by light microscope (Fig. 5), characterized by subsarcolemmal aggregates of mitochondria (Fig. 6). These aggregates were enormous, not only in tangential but also in transverse sections. The size of the mitochondria was slightly larger than that observed in the other fiber types. Moreover, this fiber type possessed more sarcoplasmic reticulum and more glycogen particles when compared to the other two fiber types. The glycogen particles were not only evenly dispersed but also associated to the clusters of mitochondria.

The third type of fiber had an intermediate position between the former with regard to glycogen particles and development of sarcoplasmic reticulum (Fig. 7). However, the mitochondria, still numerous, formed small clusters (Fig. 8).

All three types of fibers displayed numerous triads, scarce lipid droplets, Z-lines of similar thickness (100-130 nm), an opaque line (the so-called N-line) in the middle of the two hemibands I (Figs. 4, 7) and several small sarcoplasmic cisternae aligned alongside the plasmalemma (Figs. 8-10, 14, 16). Moreover, this plasmalemma was frequently reinforced by a dense material (Figs. 6, 10-12, 16) and some ring fibers and leptomeric fibrils could be observed in the peripheral sarcoplasmic areas.

Numerous satellite cells could be identified by light microscope (Fig. I). In the electron microscope various types of spatial relationship to striated muscle fibers were noted. Some satellite cells were close to the striated muscle fiber, with no basal lamina interposed (Fig. 9); others were partially (Fig. 10) or completely (Fig. I I) separated from the striated muscle fiber by one or two basal laminae. Amorphous connective tissue was also sometimes present in this gap (Fig. 11). Delicate cytoplasmic processes of the striated muscle fiber occasionally enveloped part of the surface of the satellite cell (Fig. 10).

Where smooth muscle cells enveloped and replaced the striated muscle fiber, single smooth muscle cells could be observed near to or at the place of a satellite cell (Fig. 15). The number of smooth muscle cells gradually increased when forming mixed fascicles, first a continuous mono-layer was formed and a pluristratified envelope thereafter (Fig. 12). The narrowest gap between a smooth muscle cell and a striated muscle fiber we could observe in a mixed fascicle was 200 nm, i.e. a gap occupied by the two basal laminae and a thin amorphous connective tissue interstice (Fig. 14). The gaps between the striated muscle fiber and the interstitial cells of Cajal were regularly found larger than that between smooth muscle cells and striated muscle fibers (Figs. 12, 16).

The individual striated muscle fiber was surrounded by the so-called basement membrane: i.e. one basal lamina externally reinforced by thin collagen fibrils (Figs. 6, 9, 11). Occasional oxytalan (Figs. 8, 9) and elastic fibers (Fig. 5) could be present. The endings of the striated muscle fibers were gradually thinning and formed only rare finger-like processes and crypts. Numerous plasmalemmal densities were present in these areas of myotendinous junctions and many thin collagen fibrils could be found
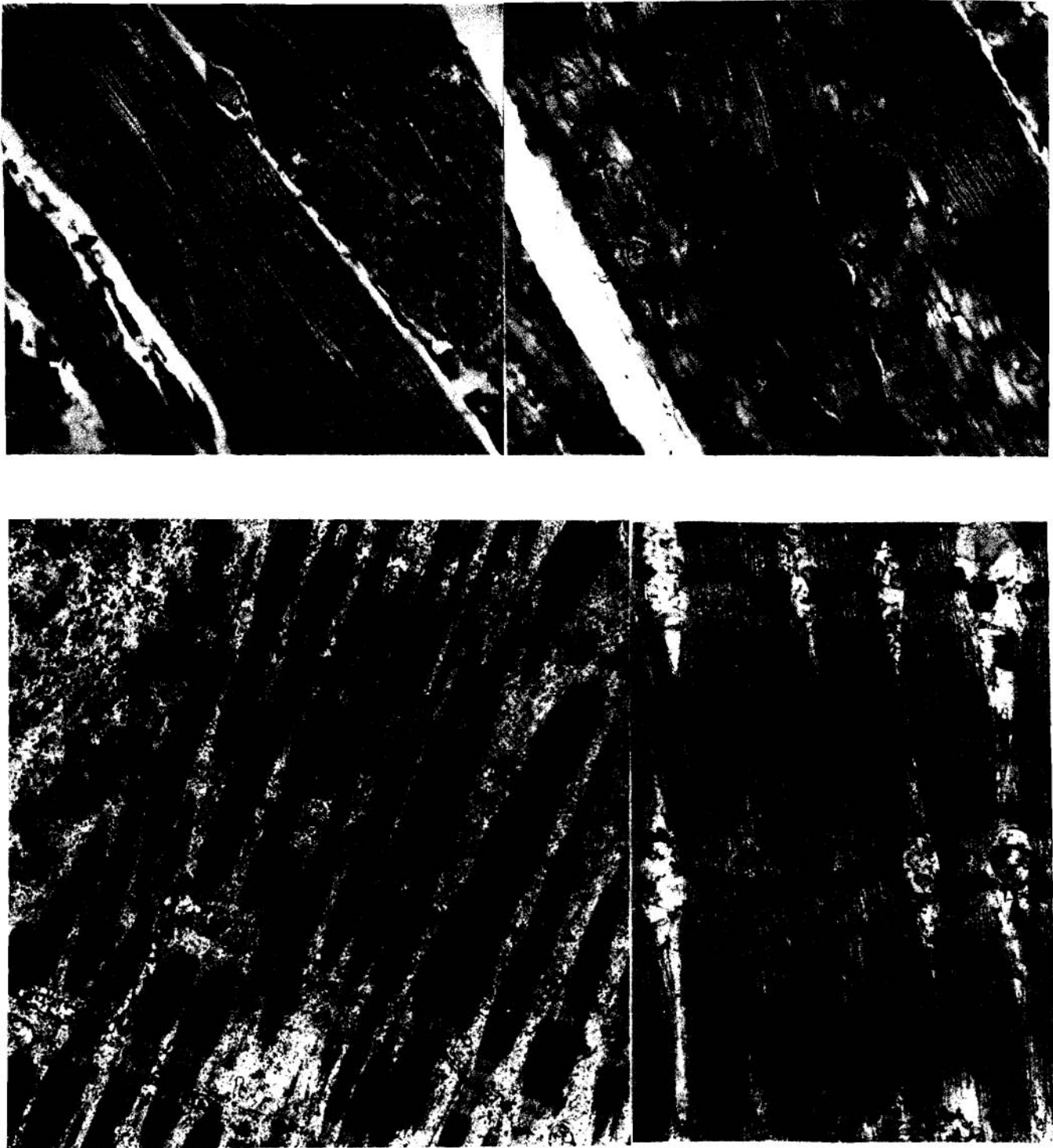
Figs. 1, 2. Muscle coat of the middle third of human esophagus. Semithin sections, toluidine blue.   1. Striated muscle fibers. A satellite cell (arrow)can be identified. × 840.   *2.* Striated muscle fibers and smooth muscle bundles wedged between one another. An interstitial cell of Cajal (arrow) can be identified. × 670

Figs. 3, 4. Striated muscle fibers of the most frequent type.   3. × 6,600.   **4.** Detail of the sarcoplasmic reticulum; N:N-line; L:Lipid droplet. x 22,000
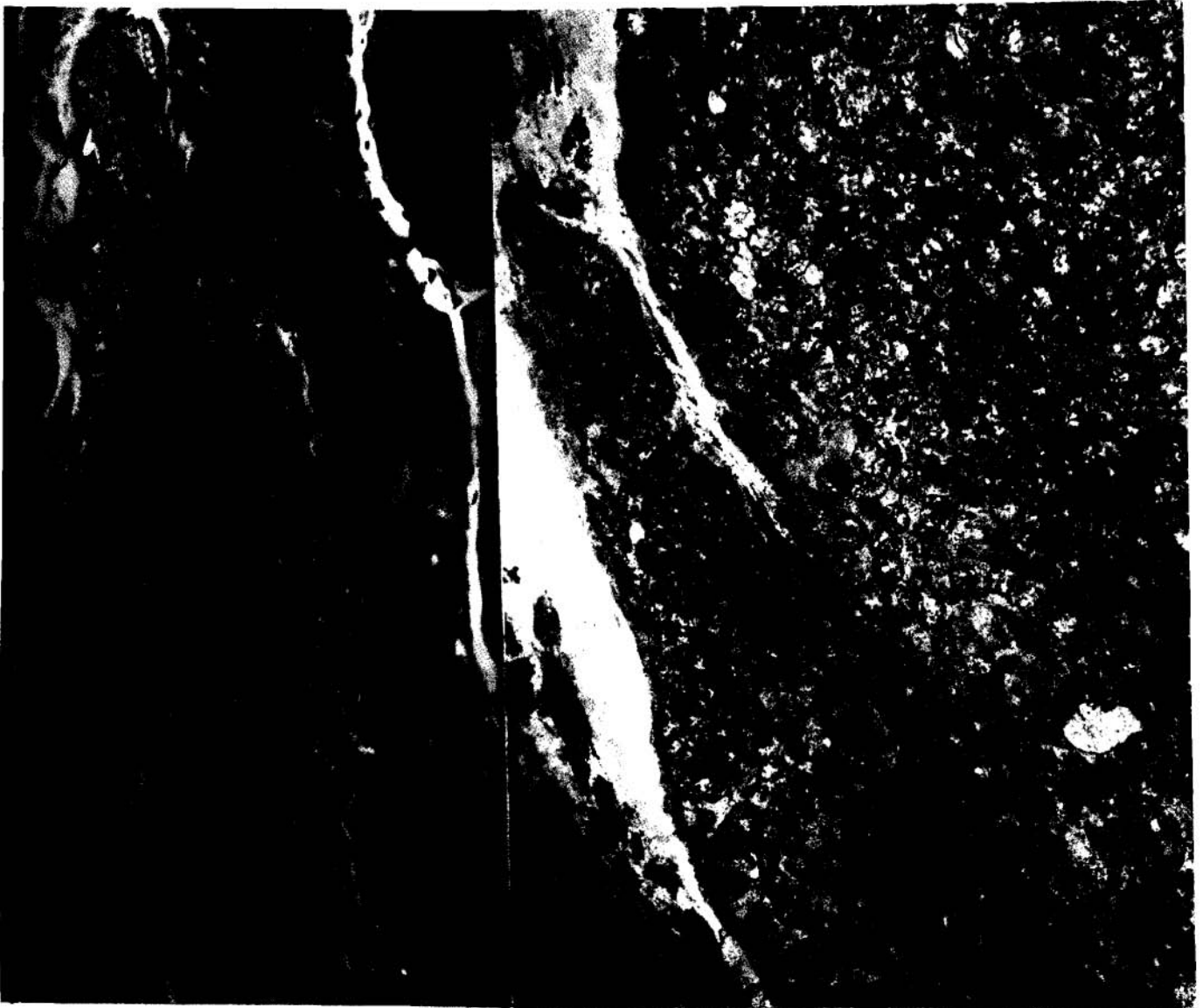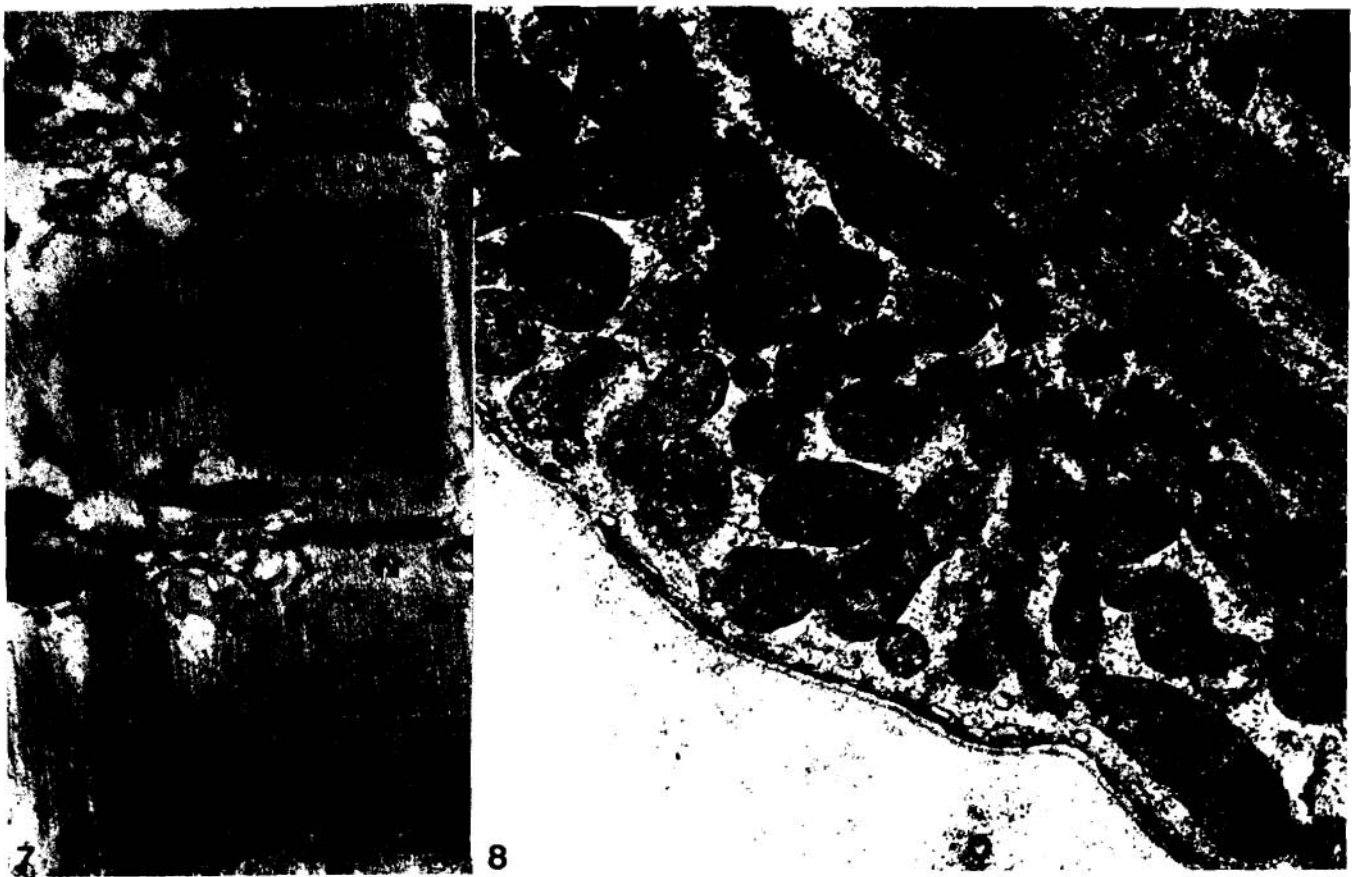
Figs. 5, 6. Striated muscle fibers rich in mitochondria and glycogen particles. 5. Semithin section, toluidine blue. The mitochondria are the small granules clustered in the subsarcolemmal region (arrows). x*670*. **6.** Subsarcolemmal area filled with mitochondria. x*6,600*

inserted in the basal lamina. As in tendons, these fibrils were parallel to each other and orientated with the long axis of the striated muscle fiber. Despite the fact that they were shorter, thinner and less numerous than in tendons, they could be regarded as similar. The endings of two consecutive striated muscle fibers did not interdigitate and connections between the two consecutive connective tissue envelopes (the so-called myo-myal junctions) were never observed.
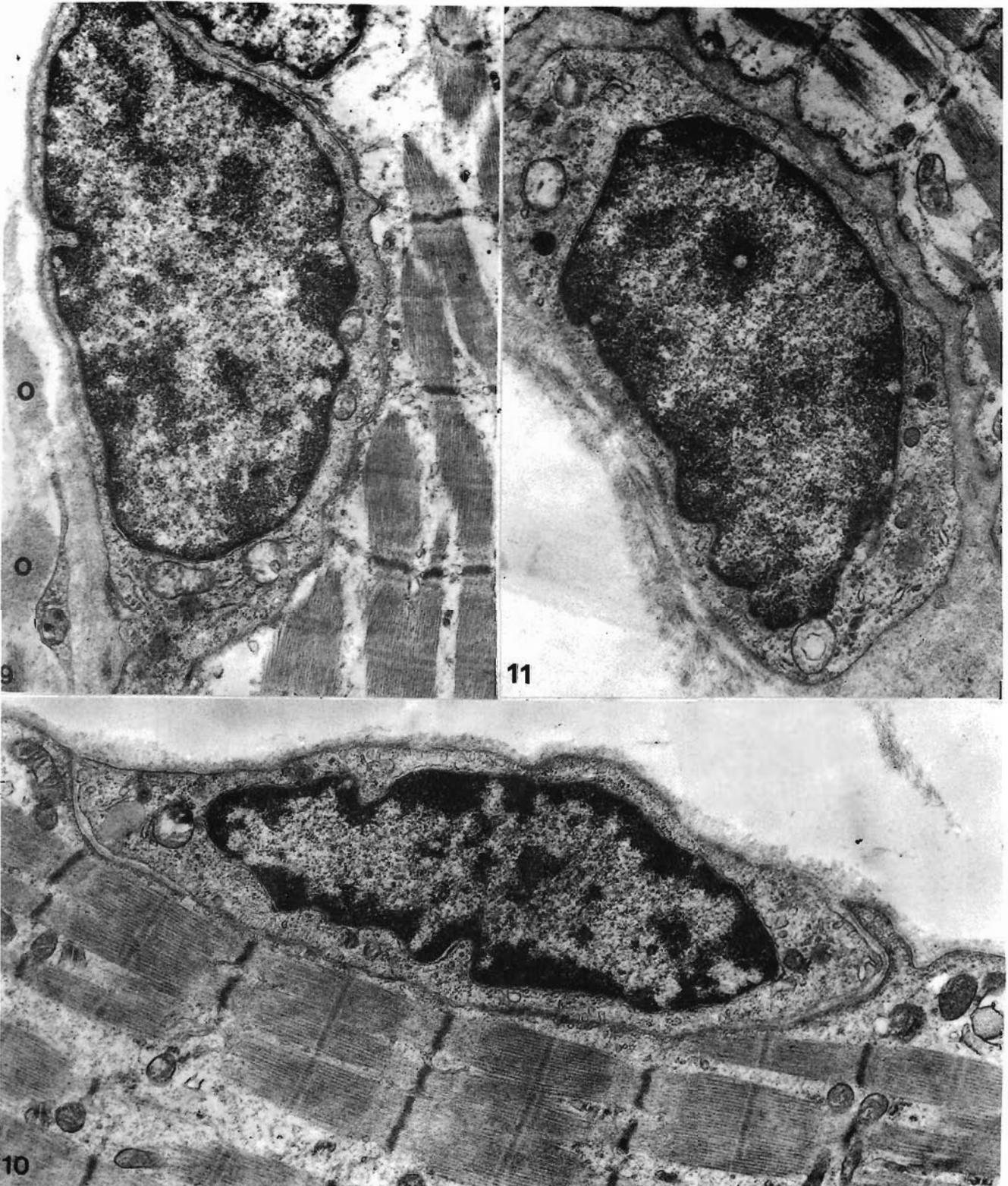
Striated muscle fibers and smooth muscle cells were joined together in the mixed fascicles by envelopes of connective tissue. These envelopes, both laterally and at the endings of the striated muscle fibers, were difficult to identify as separate entities (Fig. 13). In both these areas numerous, large elastic fibers could be found (Fig. 14).

Discussion

Three types of striated muscle fibers have been discerned in the transition zone (middle third) of the human esophagus. One of these three fiber types is frequent, whereas the other two types are rarely found. The three fiber types we found mainly differ in mito-chondrial number and amounts of glycogen particles. The most frequently found fibers were characterized by few mitochondria and small quantities of glycogen particles. One of the two rare fiber types, on the contrary, possessed large clusters of mitochondria in a subsarcolemmal position and many glycogen particles; the second rare fiber types should be interpreted as intermediate between the other two, its main differences being the intermediate number of mitochondria. There were also some differences


Figs. 7, 8. Striated muscle fibers of intermediate type between those of Figures 3 and 4 and of Figures 5 and 6. 7. Detail of the sarcoplasmic reticulum. N:N-line; L:lipid droplet. x 22,000. 8. Subsarcolemmal region. A cluster of mitochondria and numerous small sarcoplasmic cisternae along the sarcolemma. O:Oxytalan fibers. x 33,000

Fig. 9-11. Satellite cells. **9.** The satellite cell is close to the striated muscle fiber. O:Oxytalan fibers. × 13,200. **10.** The satellite cell is partly close to the striated muscle fiber and partly separated by a basal lamina. × 13,200. **11.** The satellite cell and the striated muscle fiber are separated by a connective tissue gap. × 11,000
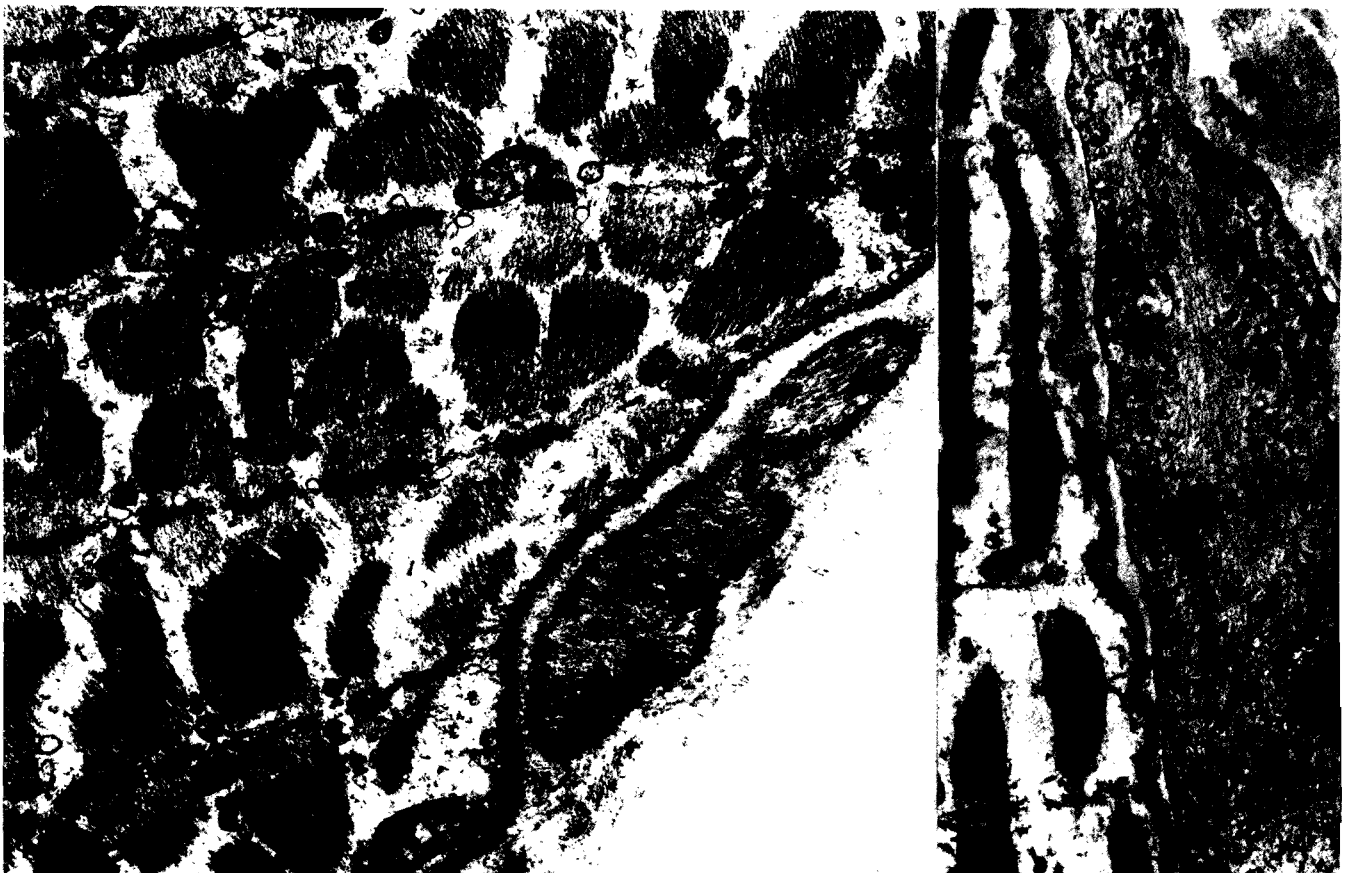
12

13   14   E
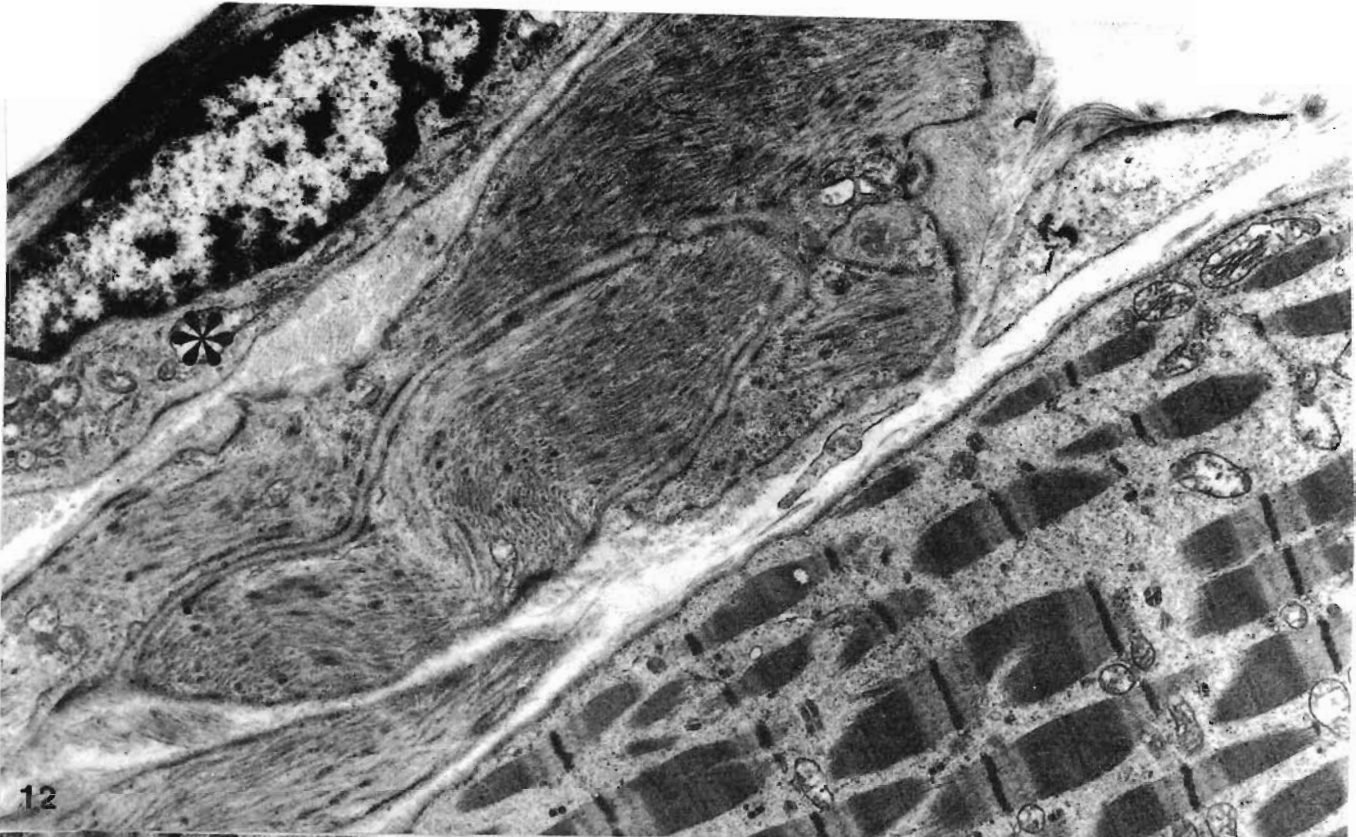
between the three fiber types as to the extension of the sarcoplasmic reticulum. In fact, the sarcoplasmic reticulum, even if never found to be prominent, was widest in the fibers richest in mitochondria.

On the other hand, the thickness of the Z-lines, the number of triads and the number of lipid droplets did not significantly differ. Some features common to all three fiber types were characteristic of these esophageal striated muscle fibers (presence of N-lines, cisternae of

Figs. 12- 14. Mixed muscle fascicles. Relationship between the striated muscle fiber and the smooth muscle cells. 12. An interstitial cell of Cajal can be recognized (asterisk). x 6,600. 13. The connective tissue envelopes of the two types of muscle components of the mixed fascicle are difficult to identify as separate entities. x 8,800. 14. The narrowest gap between a smooth muscle cell and the striated muscle fiber (arrow); an elastic fiber (E) in the lower part of the micrograph. x 17.600

sarcoplasmic reticulum aligned along the plasmalemma, dense plasmalemmal areas scattered everywhere, inconspicuous myotendinous junctions) and unusual in skeletal muscles (see Schmalbruch, 1985, for review of literature).

The histochemical data obtained in the human esophagus (Whitmore, 1982; Shediofsky-Des Champs et al., 1982) revealed only two fiber types, but the authors did not agree about their frequency. None of the three fiber types we discerned in the electron microscope unequivocally can be identified as corresponding to the histochemically defined fiber types in man. In fact, when correlating ultrastructural features of the more frequent fibers with histochemical characteristics of frequently occurring fibers (Whitmore, 1982; slow-twitch type) some are in agreement (few glycogen particles and lipid droplets) but some are not (number of mitochondria). The contrary is seen when correlating the distribution frequency with the findings of Shedlofsky-Des Champs et al. (1982): some criteria agree (number of mitochondria), whilst others do not. Neither could correlation between histochemical and ultrastructural data of the less frequently occurring fiber types be obtained. All three fiber types

Figs. 15, 16. A smooth muscle cell in the same location as a satellite cell. 15. x 17,600. 16. Mixed muscle fascicle. The closest relationship between an interstitial cell of Cajal and the striated muscle fiber. x 11,000

described in the present paper displayed criteria of slow and fast fiber types in varying relation.

Electron microscopy has confirmed the presence of several types of striated muscle fibers in the human esophagus (three types in the transition zone); but at present it is impossible to correlate morphological and histochemical results in order to classify them.

Therefore, we attempted to classify with respect to ultrastructural peculiarities only, according to classical features of skeletal muscle fibers (see Schmalbruch, 1985, for review of literature). The parameters of Mukuno (1968) of classification of extraocular muscle fibers in man were also considered as these muscles have close similarities with striated muscle fibers of the human esophagus. With respect to all these data, we consider that the striated muscle fibers of human esophagus are neither slow nor fast fibers, but are intermediate type and, according to morphology, we suggest that are rather fast fibers.

Identifying the type of esophageal striated muscle fibers presents no problem in mouse or rat. Samarasinghe (1972), in the mouse, described a single fiber type (identified as "fast"), although sometimes enormous aggregates of mitochondria were present in the subsarcolemmal region. Also Gruber (1968) described only one fiber type in the rat, but did not classify it. However, as judged from his illustrations, striated muscle fibers seem to be of the fast type. Whitmore (1983) also found only one type of fiber in guinea pig and marmoset. This author stressed the fact that electron microscopic criteria for fiber classification did not agree with histochemical and physiological ones. In fact, fine structurally he found "fast" fibers, whilst histochemical data (Whitmore, 1982) indicated them as "fast twitch" fibers and physiological ones (Wareham and Whitmore, 1980, 1982) as "slow twitch, fatigue-resistant" fibers. In conclusion, each species examined so far presents different kinds of striated muscle fibers in the esophagus, even if the "fast" type seems to predominate. In addition, the esophageal striated muscle fibers display several differences to skeletal muscles, probably due to the fact that the esophagus moves differently and has a non-voluntary striated muscle.

Satellite cells have not been found in mouse and rat esophagus. However, Whitmore (1983) occasionally observed satellite cells in guinea pig and marmoset esophagus and described them as being regularly surrounded by the basal lamina of the striated muscle fibers. Numerous satellite cells are present in the human esophagus. Their spatial relationship to the striated muscle fibers varies. Some satellite cells are situated close to the striated muscle fiber, without any basal lamina interposed; others are partially or completely separated from the plasmalemma of the striated muscle fiber by a single or by two basal laminae, even though the satellite cells are always within the basement membrane of the striated muscle fiber. Similar situations are commonly described in voluntary skeletal muscles (see Campion, 1984, for review of literature) and, therefore, we do not consider them of any significance.

An interesting finding concerns the possibility that smooth muscle cells may replace satellite cells inside the basement membrane surrounding each striated muscle fiber. It seems relevant that the connective tissue envelope of a striated muscle fiber and its related satellite cell is continuous with the envelope of a mixed fascicle and the smooth muscle bundle emerging from it. The narrowest gap between a smooth muscle cell and a striated muscle fiber, in a mixed fascicle, is only 200 nm wide. This distance is smaller in man than in other species (Samarasinghe, 1972; Whitmore, 1983), where mixed fascicles have not been described. The narrow gap between striated and smooth muscle and the continuity of the respective enveloping connective tissue probably enables a mixed fascicle to act as an individual unit. Far reaching, continuous anatomical (and perhaps functional) units are, therefore, undertaken in the muscle coat of the human esophagus; these units include striated fibers at their beginning, striated and smooth elements in the middle and smooth muscle cells near to their ending. The transition from the striated into the smooth muscle in the human esophagus occurs in a gradual manner.

The fairly large gap between striated muscle fibers and the interstitial cells of Cajal may support the hypothesis that these cells "belong" to smooth muscle bundles (Faussone-Pellegrini and Cortesini, 1985) and do not directly influence the motor behaviour of striated muscle fibers.

We could not find the so-called myo-myal junctions, i.e. interdigitations between consecutive striated muscle fibers, as described in rat (Gruber, 1968), guinea pig and marmoset (Whitmore, 1983). We only examined the transition zone and therefore cannot exclude that similar connections may exist in the upper third of the human esophagus.

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