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Smooth Muscle Cells, Interstitial Cells of Cajal and Myenteric Plexus Interrelationships in the Human Colon

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Abstract. The plane between longitudinal and circular muscle of human colon, as revealed on examination with ligl and electron microscopes, has no clear-cut border. Some groups of smooth muscle cells, obliquely oriented and wit features similar to both circular and longitudinal ones – the connecting muscle bundles – run from one muscle layer t another. Other groups of smooth muscle cells, possessing their own specific ultrastructural features – the myenter muscle sheaths –, make up envelopes of variable thickness around some myenteric ganglia and nerve strands, partial or completely embedding them in one or other muscle layer. Non-neuronal, non-muscular cells (interstitial cells Cajal, covering cells, fibroblast-like and macrophage-like cells) complicate the texture of the myenteric musc sheaths, creating an intricate, interconnected cellular network inside them, widespread among nerve bundles ar smooth muscle cells; however, only interstitial cells have cell-to-cell junctions also with the smooth muscle cells ar nerve endings. These data document the existence in this colonic area of two different types of muscle cell arrang ments, one of which, the myenteric muscle sheath, only contains putative pacemaker cells.

Introduction

It seems to be definitely accepted that slow waves can originate in the circular muscle layer of the colon of several mammals, man included, and data suggesting that the circular and longitudinal muscle layers are electrically coupled have been reported for the dog and pig proximal colon [Smith et al., 1987; Huizinga and Chow, 1988], and for the human distal colon [Huizinga and Chow, 1988]. It has also been reported [Smith et al., 1987] that in the canine proximal colon there are two interacting populations of pacemaker cells, one at the submucosal border and the other at the myenteric border of the circular muscle layer, generating the slow waves for both layers and coupling them. It remains, however, to be shown which kinds of cells are responsible for these events. Two putative pacemaker cell types can be proposed: the smooth

Many years ago, by light microscopy, Keith [191 described peculiar smooth muscle cells (that he call 'intermediate cells') in the rat colon which formed a coll together with the nerve elements of the myenteric plex and connected them with the typical smooth muscle ce of the muscle layers. This finding induced him to identi in this collar the nodal and conducting system of the colo He also found a large number of these cells in the descen ing human colon. Recently [Pace, 1968; Huizinga et a 1985], again with light microscopy, bundles of smoo muscle cells running from one muscle layer to anoth have been observed in both proximal and distal colon man, and it has been suggested [Huizinga et al., 1985] th they are responsible for the electrical coupling betwe the two muscle layers. By electron microscopy, in seve laboratory mammals (not including dogs and pigs), o fibroblast-like cells have been found running all along t colonic myenteric plexus and making up cell-to-cell ju

tions with the smooth muscle cells of the two muscle layers [Rogers and Burnstock, 1966; Gabella, 1972, 1979; Cook and Burnstock, 1976; Komuro, 1982, 1989; Faussone-Pellegrini, 1987]. This cell type was identified as interstitial cells of Cajal (ICC) and, because of their ultrastructural characteristics, interpreted as connective supporting cells [Gabella, 1979; Komuro, 1982]. More recently [Faussone-Pellegrini, 1987], it was possible, by electron microscopy, to reveal in the plane between the two muscle layers of the human colonic wall the presence of rare cells, interpreted as ICC, due to their close relationships with both nerve endings and smooth muscle cells, but not identifiable with the smooth muscle cells previously described in this area by light microscopy [Keith, 1915; Pace, 1968; Huizinga et al., 1985]. Moreover, the presence in this area of groups of cells recognizable as smooth muscle cells, but apparently not belonging to any muscle layer, has been suggested by the same author [Faussone-Pellegrini, 1987]. However, any peculiar structural characteristics and relationships between these cells and other cell types have not been reported.

The morphological data we have at present, with regard to the cell types present in this colonic area, seem at first glance to be in agreement with the physiological ones, indicating that, at least in man, the two cell types proposed as responsible for the electrical events recorded are present. However, it still has to be ascertained (1) whether these cells really possess specific structures or form peculiar arrangements that might be regarded as responsible for the electrical events recorded in this area; (2) if these cells are present and (3) if they are identical throughout the length of the colon, and (4) what relationships they have with each other, with nerves and with both muscle layers. We therefore studied by electron microscopy fragments of the ascending, transverse and descending colon obtained from patients undergoing surgery for cancer. These fragments were macro- and microscopically unaffected by disease.

Material and Methods

Fragments including both muscle layers (circular layer and taenia) and fragments of the intertaenial regions of the caecum, ascending colon, right and left transverse colon and descending colon, were studied. The specimens were obtained from 16 patients (aged between 48 and 75 years, mean age 59.5 years) undergoing surgery for cancer. The segments of excised colon had a normal appearance and were taken far from the carcinomatous areas (5 cm or more away from the site of cancer) and were histologically free of tumour and inflamma-

Immediately after surgery, specimens about 1–2 cm long and 0.5 cm large, macroscopically unaffected by disease, were clamped at both their extremities to avoid spiralling of the circular muscle layer and shortening of the longitudinal muscle layer. At this point, the two clamped extremities were tied with catgut thread. Then these specimens were immersed in a solution of 2% cacodylate-buffered glutaraldehyde pH 7.4, contained in a custom-made polystyrene box. At the time of fixative immersion they were also gently stretched and anchored at both their extremities by means of this thread to the opposite walls of this box. The segments were prefixed in this solution and kept in the controlled distension for 6–24 h, depending on the size and thickness of the muscle wall. Then both the extremities and the cut surfaces of each specimen were resected and the mucosa was removed by sharp dissection. A thin layer of submucosa remained attached to these segments. The remnant segments of the specimens were then cut into smaller and thinner strips of about 1 mm thickness and 2 mm length and rinsed in a buffered solution of saccharose. They were postfixed with 1% phosphate-buffered O_sO_4 pH 7.4, dehydrated with acetone and embedded in Epon, using flat moulds in order to obtain transverse or longitudinal sections.

The semithin sections were stained with a solution of toluidine blue and photographed at light microscopy. All areas showing a disarray of the muscle layers and unsuitable distension of their smooth muscle cells were excluded from both light- and electron-microscopic examination. The ultrathin sections, obtained with a Porter-Blum MT1 ultramicrotome using a sapphire knife, were stained with an alcoholic solution of uranyl acetate, followed by a solution of concentrated bismuth subnitrate. These sections were examined under Siemens Elmiskop IA and 102 electron microscopes.

Results

The plane between the longitudinal and circular muscle layers of the human colon is occupied by peculiar smooth muscle cell groups. Examination with the electron microscope revealed a complex organization due to intricate interrelationships between some of them and neuronal and non-neuronal cells. However, at variance with the submucosal border of the circular muscle layer of these same subjects [Faussone-Pellegrini et al., 1988], no differences were found between the taeniated and intertaenial regions and among the various levels of the colon (ascending, transverse and descending colon).

The ganglia and nerve strands of the myenteric plexus are not always located between the circular and longitudinal muscle layers. Very often, in fact, they are partially or completely embedded in one of the muscle layers (fig. 1a, b). Furthermore, sheaths (fig. 2a, b, 5a), mainly made up of smooth muscle cells, envelop some of them *(myenteric muscle sheaths)*. Moreover, smooth cells with an oblique orientation make up interconnecting bundles running from one muscle layer to another *(connecting muscle bundles)* (fig. 3a, b). Consequently, the two muscle layers

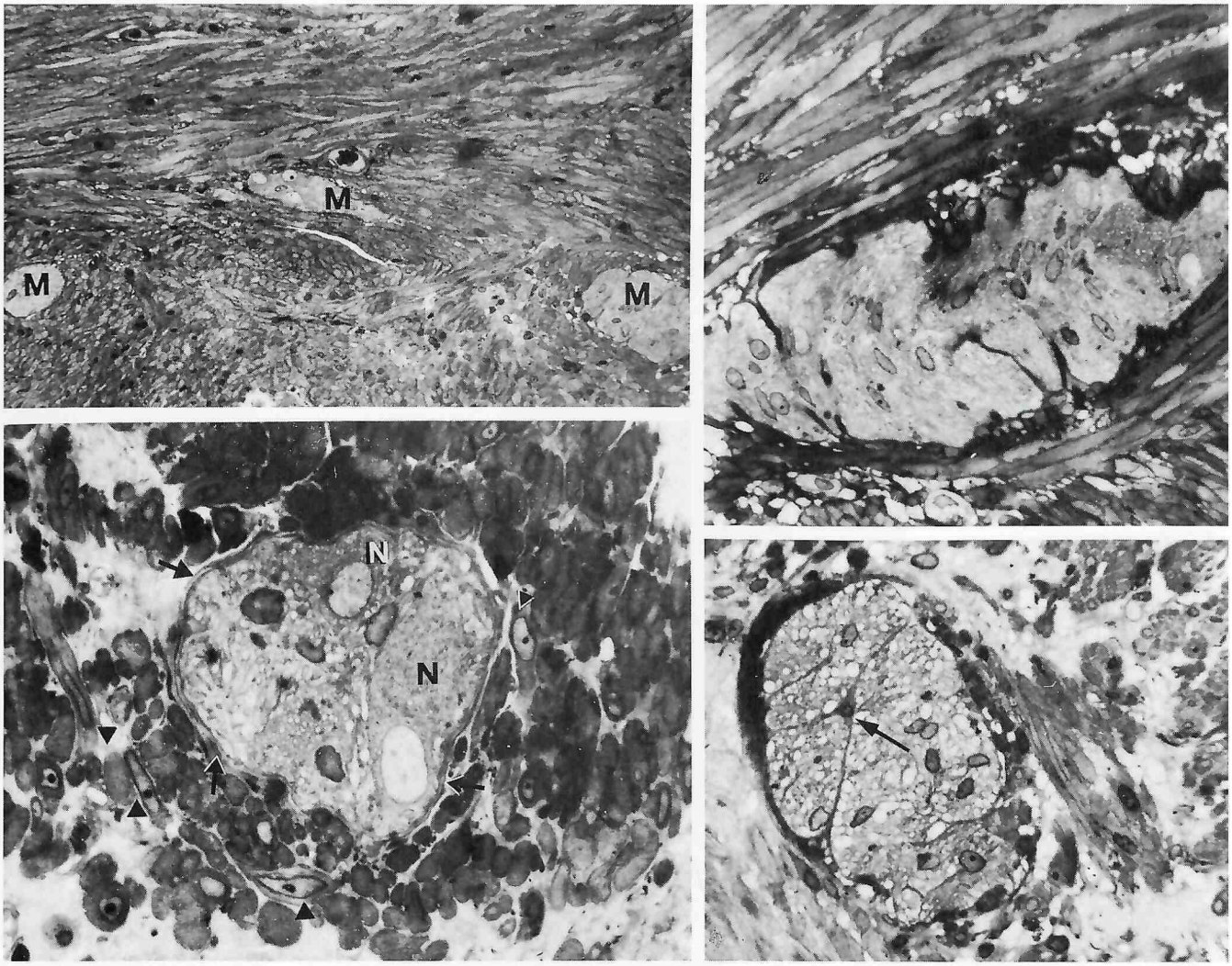

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Fig. 1. Semithin sections, toluidine blue. **a** Myenteric (M) ganglia and connective nerve strands located in both circular (upper side) and long tudinal (lower side) muscle layers. ×200. **b** A myenteric ganglion completely embedded in the circular muscle layer (longitudinally sectioned). O the lower side, the longitudinal muscle layer (transversely sectioned). ×480.

Fig. 2. Semithin sections, toluidine blue. **a** A myenteric ganglion surrounded by a muscle sheath. N = neuronal cells. The arrows indicate th layer made up by the covering cells. The arrowheads indicate the circularly oriented cells of the muscle sheath; the others are longitudinally or ented. On the lower side, the 'myenteric area' interposed between the longitudinal muscle layer (upper side, in continuity with the muscle sheath and the circular muscle layer (not included in the field of this micrograph). ×750. **b** A muscle sheath around a nerve strand. On the right side, th circular muscle layer; on the left side, the longitudinal muscle layer. The arrow indicates a covering cell. ×200.

ers is extremely variable, sometimes reaching a minimum of 20 nm, where the contiguous smooth muscle cells of the two muscle layers contact each other. In other areas the gap is occupied by connective tissue rich in elastic fibres, fibroblasts and mast cells; with aging, collagen fibres

Smooth Muscle Cells of Connecting Muscle Bundles

Examination with electron microscope revealed that th connecting muscle bundles (fig. 3a, b, 4) are exclusivel made up of smooth muscle cells running from one muscl layer to another, interconnected with each other and wit

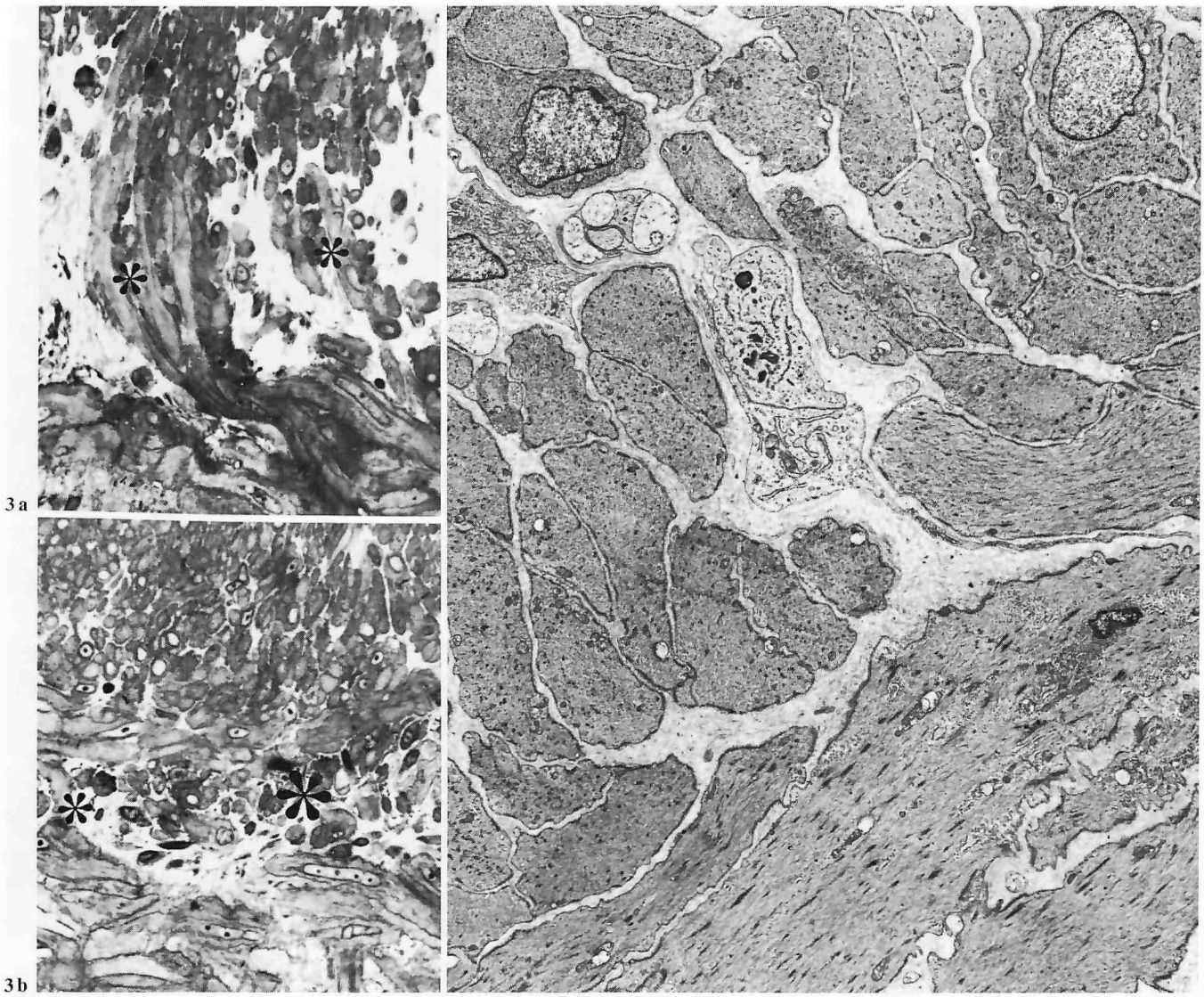


Fig. 3a, b. Connecting muscle bundles. Upper side, longitudinal muscle layer; lower side, circular muscle layer. In the middle, the obliquel oriented smooth muscle cells, most of which are similar to the circular ones (asterisks). Semithin sections, toluidine blue. ×480.

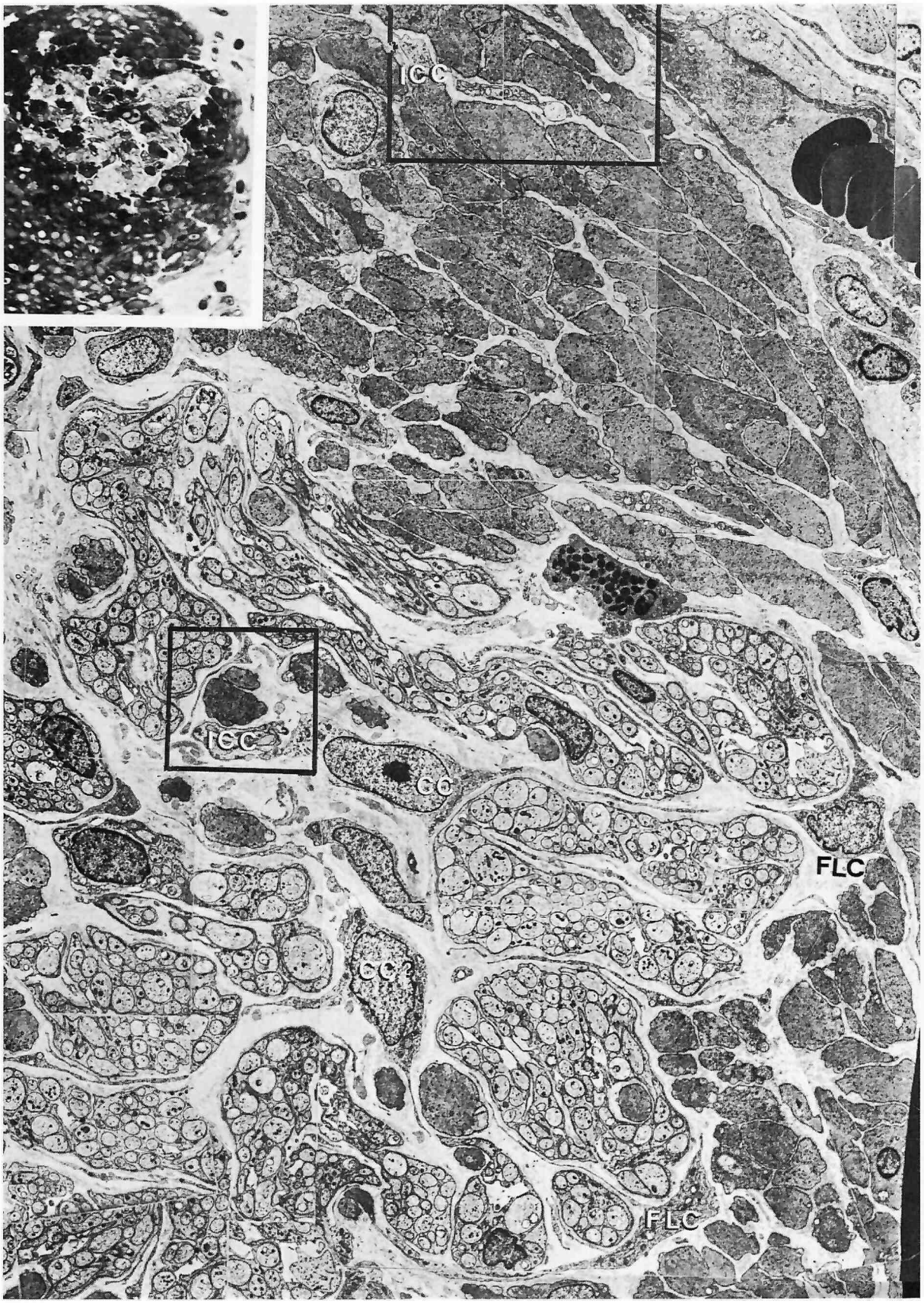
Fig. 4. Electron micrograph of the field shown in figure 3b. ×5,000.

mediate junctions and desmosome-like junctions. Most of these cells have a maximum diameter of 10 μm, like the circular cells; a few of them, however, have a maximum diameter of 6 μm, similar to the longitudinal cells.

Smooth Muscle Cells of Myenteric Muscle Sheaths

The myenteric muscle sheaths look like collars of variable thickness (1–10 smooth muscle cells thick) and are made up of smooth muscle cells assembled in layers all

plexus (fig. 2a, b, 5a, b). Some of the superficially locate smooth muscle cells come in contact with those of botl muscle layers by means of intermediate junctions an short appositions of their contiguous plasma membranes A few smooth muscle cells, mostly located at the periph ery of the sheaths, look like the circular ones (fig. 2a) whereas, most of these smooth muscle cells are very smal (no more than 6 μm in diameter) and mainly oriente longitudinally (fig. 2a, 5a, b, 6–10). These cells, more



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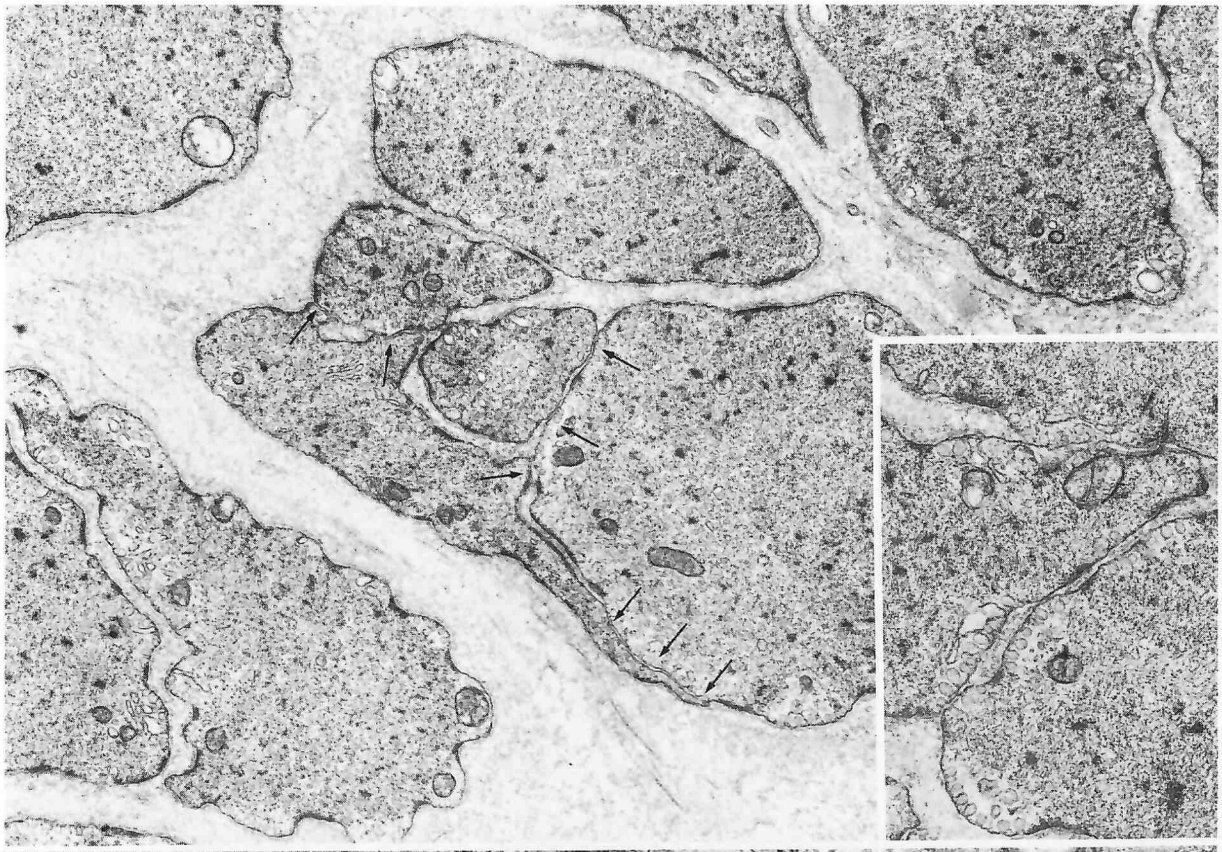

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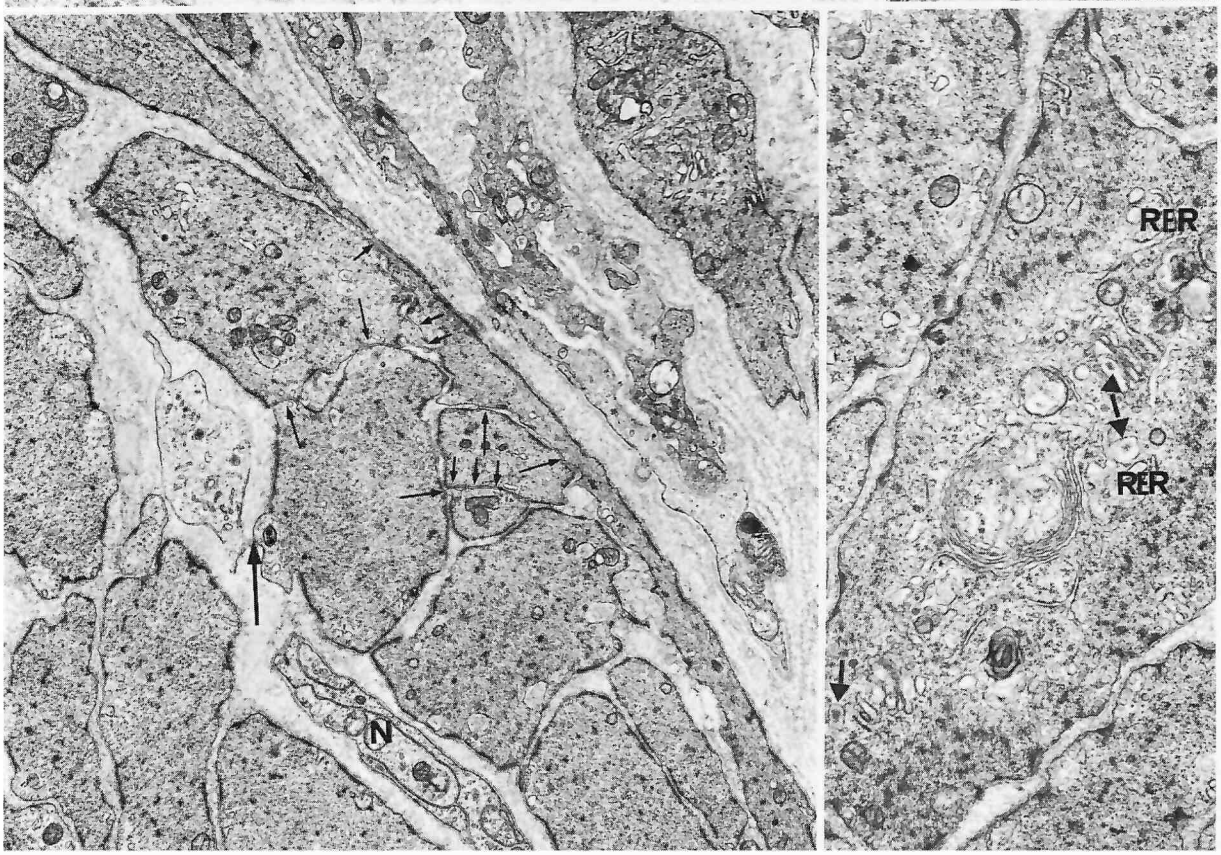

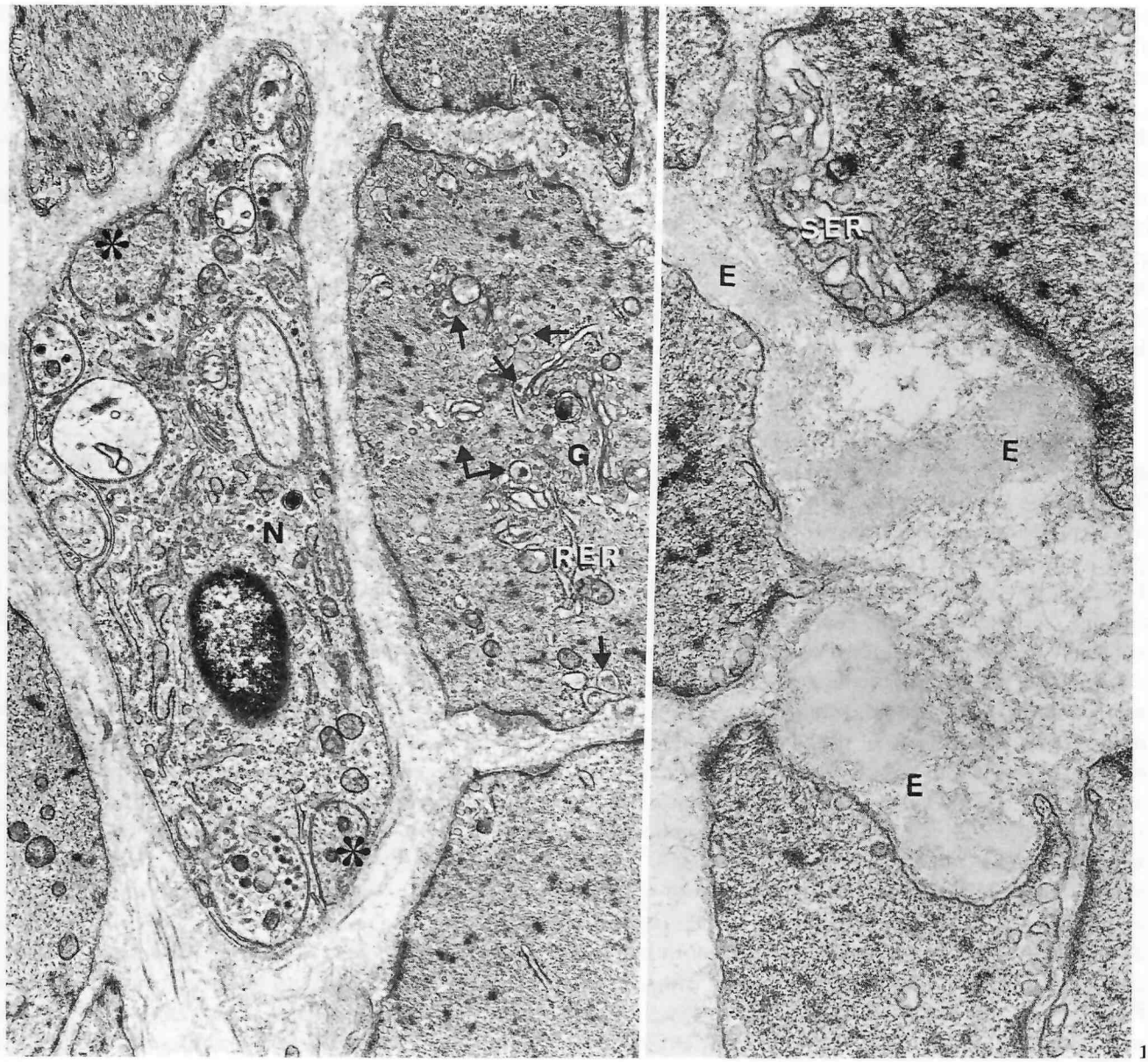

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Fig. 9, 10. Smooth muscle cells of the myenteric muscle sheaths. **9** The smooth muscle cells on the right side have several cisternae of RER an a large Golgi apparatus (G). The arrows indicate the vacuoles sprouting from the Golgi cisternae and containing a granular material. N = nerv bundle. Two axons contain small agranular vesicles only (asterisks). × 15,000. **10** E = elastic fibres linking two contiguous smooth muscle cell SER = clusters of cisternae of the sarcoplasmic reticulum. × 37,500.

Fig. 5. A muscle sheath around a non-capsulated nerve strand. **a** Semithin section, toluidine blue. × 945. **b** Montage illustrating by electro microscopy the field shown in **a**. In the square: a group of ICC, reproduced in detail in figure 14. The field in the rectangle is reproduced in detail figure 7. FLC = Fibroblast-like cells; CC = covering cells. × 2,500.

Fig. 6–8. Smooth muscle cells of the myenteric muscle sheaths. **6** The arrows indicate the contact areas between the smooth muscle cell × 15,000. **Inset** A detail of the contact areas made up by two contiguous plasma membranes provided with caveolae. × 25,000. **7** Detail of the supe ficial region of the sheath indicated in the rectangle in figure 5b. The smooth muscle cells peripherally located are interconnected with each oth (arrows) and with an ICC (thick arrow). N = nerve bundle. × 10,000. **8** Central cytoplasm of a smooth muscle cell where a large Golgi apparatus located and from whose cisternae sprout vacuoles containing a granular material (arrows). RER = cisternae of the rough endoplasmic reticulun

fact, they come into contact with each other also by means of extended appositions of their contiguous plasma membranes, along which several caveolae are aligned (fig. 5b, 6, inset fig. 6, 7). Furthermore, the cisternae of the sarcoplasmic reticulum (SER) may form clusters along the cell periphery (fig. 6, 10), and some cisternae of the rough endoplasmic reticulum (RER) may be present, preferentially located in the central cytoplasm (fig. 8, 9). The Golgi apparatus is large and vacuoles 0.15 μm wide containing a granular material (fig. 8, 9) sprout from its cisternae. The sheath smooth muscle cells may be linked together by elastic fibres (fig. 10).

Myenteric Plexus

Nerve Elements. The general architecture and histology of the myenteric plexus is well known [Gabella, 1972, 1979; Furness and Costa, 1987]. In the human colon, the ganglia appear as compact masses (fig. 1a, b, 2a) enveloped by a connective capsule (fig. 1b), rich in elastic fibres, and by a discontinuous layer of so-called covering cells (fig. 2a). The connecting nerve strands are devoid of neuronal cells, but always have a capsule and covering cells (fig. 2b). The finer nerve strands still look like compact structures, apparently devoid of connective capsule and covering cells (fig. 5a). The electron microscope, however, revealed that these nerve strands are no longer compact and purely nerve structures. In fact, some smooth muscle cells enter among the nerve bundles, and the major number of ICC are located here (fig. 5b). These smooth muscle cells are identical to the smallest ones of the muscle sheaths, of which they are branchings (fig. 5b). The gap between these deepest located smooth muscle cells and the nerves is 0.4 μm minimum, often without any other cell type interposed, but the axons in these nerve bundles never contain synaptic vesicles. Their further ramifications enter the muscle sheaths, run among their smooth muscle cells in smaller nerve bundles (a maximum of 10 axons), and their axons possess synaptic vesicles (fig. 7, 9). These intermuscular axons contact the sheath smooth muscle cells with a gap of only 20 nm. The synaptic vesicles are mainly small agranular vesicles; some large granular vesicles alone or a mixture of both large granular and small agranular vesicles may be present in a few axons. Nerve bundles with 1–3 axons each have also been found repeatedly contacting a group of 2–4 smooth muscle cells (fig. 11).

Covering Cells. These cells form a discontinuous monolayer around ganglia and finer nerve strands as well (fig. 2a, b, 5b). They have a triangular body and an oval

Golgi apparatus are located near the nucleus (fig. 5b) Long, thin cytoplasmic protrusions emerge from the ce body, envelop the ganglia and the nerve strands and ente them, always carefully following their border and definin their subdivisions (fig. 2a, b, 5b, 13). These cell protru sions contact each other, overlapping (with a gap of 2 nm) for variable lengths (fig. 5b, 12, 14), and they are als in contact with ICC (fig. 5b, 14).

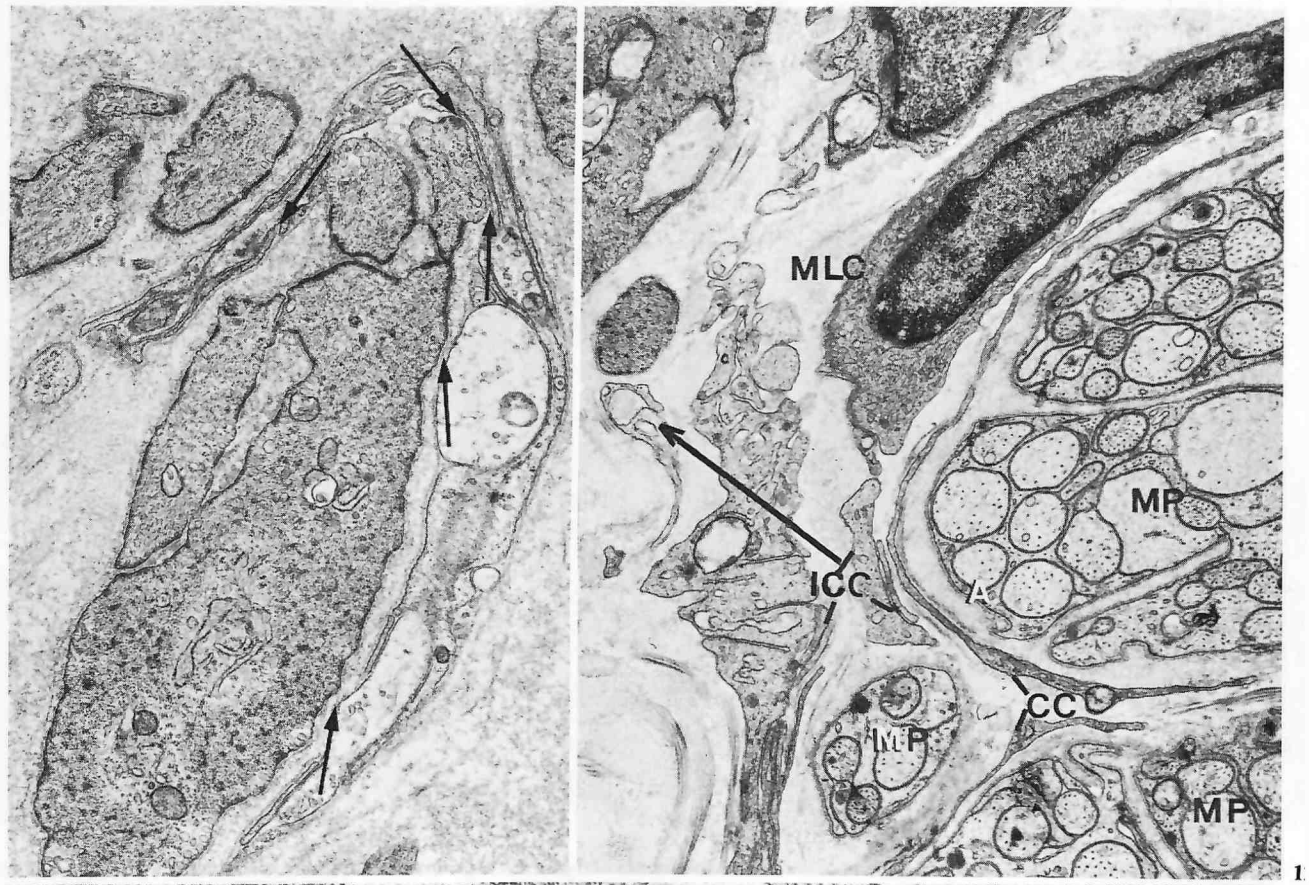
Non-Neuronal, Non-Muscular Cells Related to the Myenteric Plexus and the Muscle Sheaths

Most of these cells are located around the myenteri plexus elements and among the sheath muscle cells. Thes cells show different morphologies and have therefor been grouped in three different categories.

Cells with Structural Characteristics of ICC. A few cell are identifiable as ICC. Like the ICC described in othe parts of the gut, these ICC are characterized by richness i SER and the presence of intracytoplasmic filaments an some caveolae along the plasma membrane (fig. 12–15 17). These ICC also possess some cisternae of REI (fig. 13) and some granules 0.1–0.2 μm in diameter with a electron-dense core surrounded by a clear halo (fig. 12 14). Among the ramifications of the nerve strands devoi of the connective capsule, the ICC make up groups of 2– cells each, connected with analogous groups of 2– smooth muscle cells (fig. 12–14). Inside the muscl sheaths, the ICC run singly or in rows (fig. 15, 17). Th ICC are connected with each other and with the smoot muscle cells by overlappings and/or interlockings of thei contiguous plasma membranes and by elastic bridge (fig. 12–15, 17). 'Close' contacts (a gap of 20 nm) with th nerve endings are rare and have been found only insid the muscle sheaths. The ICC also contact the coverin cells and fibroblast-like and macrophage-like cells (fig. 12 14, 15, 17, 18).

Fibroblast-Like Cells. As well as the covering cells with which they can be easily confused, some of these cell are located around the ganglia and nerve strands an among their subdivisions, delineating their border wit their long, thin protrusions (fig. 5b). However, a

Fig. 11–13. Myenteric muscle sheath. **11** A small group of smoot muscle cells surrounded by a nerve fibre. The arrows indicate the con tact areas between axons and smooth muscle cells. ×15,000 **12** ICC = interstitial cell of Cajal; CC = covering cell; MLC = macro phage-like cell; MP = nerve bundles of a myenteric strand. ×13,000 **13** A small group of smooth muscle cells (asterisks), surrounded b



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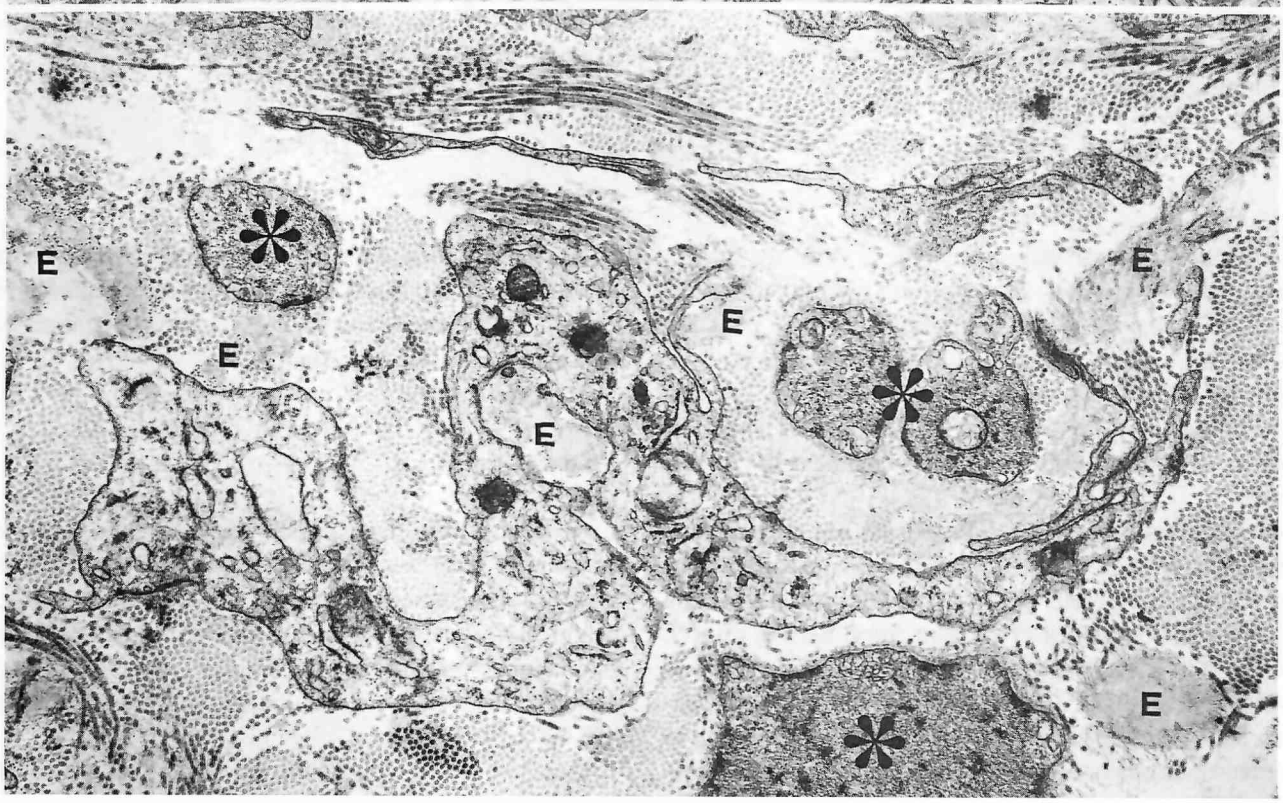

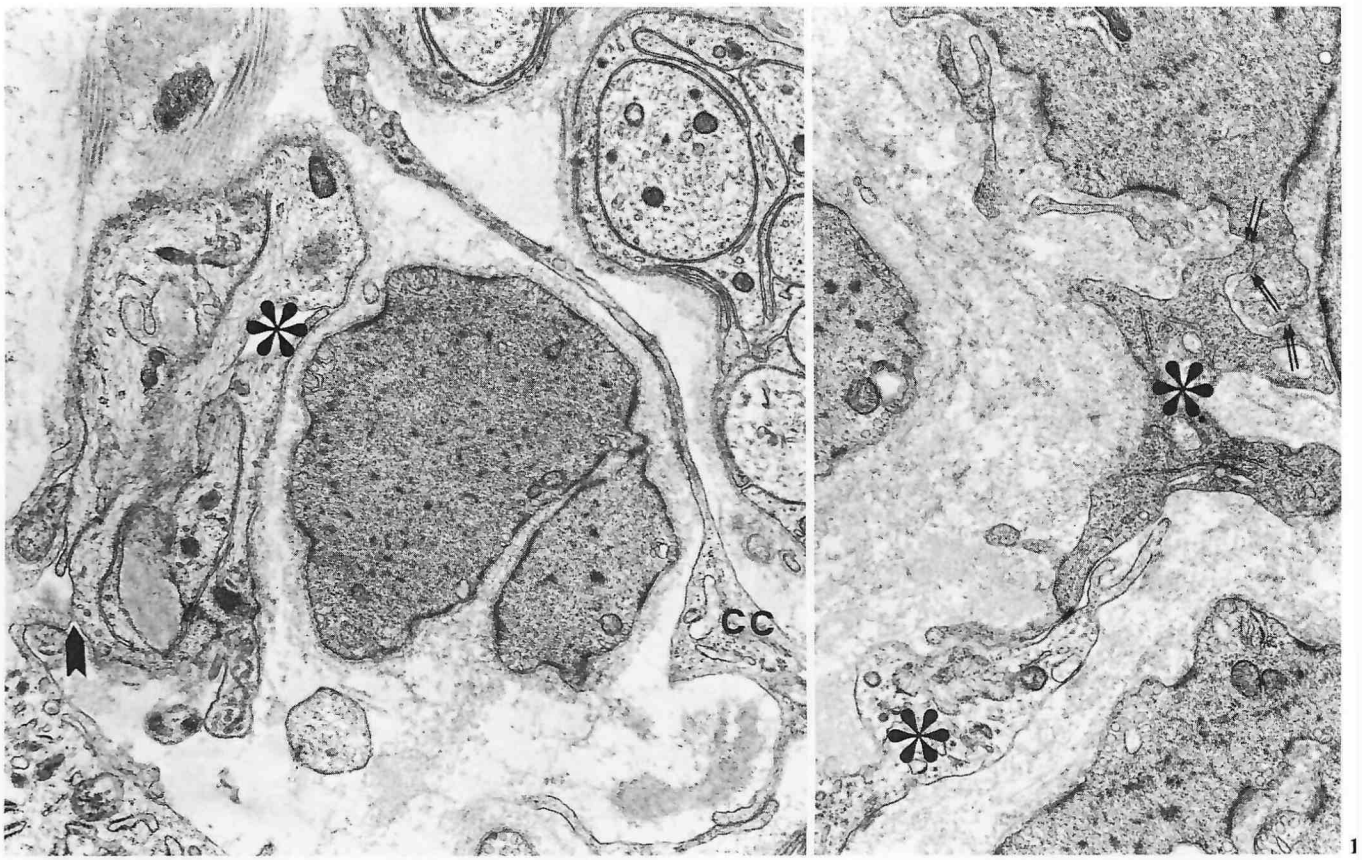

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Fig. 14, 15. Myenteric muscle sheath. **14** Detail of the field in the square of figure 5b. A group of ICC (asterisk) near a group of smooth muscle cells and contacting a covering cell (arrow). CC = covering cells. × 15,000. **15** Two consecutive ICC (asterisks) running inside a myenteric muscle sheath, among its smooth muscle cells, and one of which directly contacts one smooth muscle cell (double arrow). × 17,000.

variance with the covering cells, these cells are larger, richer in RER and possess a nucleus with an irregular outline (fig. 5b, 16). Some other fibroblast-like cells are located inside the muscle sheaths and at the myenteric border of the circular muscle layer (fig. 4, 16, 17). All these cells (with a gap of 20 nm) are in contact with each other, with ICC and with the smooth muscle cells (fig. 5b, 16, 17).

Macrophage-Like Cells. These cells are occasionally found, but only at the level of the finer nerve strands. These small, oval cells possess an oval nucleus with a dense chromatin and a cytoplasm filled with a smooth tubular reticulum, with oval-round dense bodies and coated vesicles (fig. 12, 18). These structural characteristics, similar to those described for the cells belonging to the macrophage system, induced us to use the term mac-

to the fact that these cells may be in direct contact (with a gap of 20 nm) with the ICC and the covering cells (fig. 12, 18) and, by means of elastic bridges, are also linked with the smooth muscle cells (fig. 18).

Discussion

All along the length of the human colon (caecum, ascending, right and left transverse and descending colon), in the plane between the two muscle layers, both in and between the taeniated regions, peculiar smooth muscle cell groups and complex interrelationships between some of them and neuronal and non-neuronal cells have been found.

Bundles, exclusively made up of obliquely oriented

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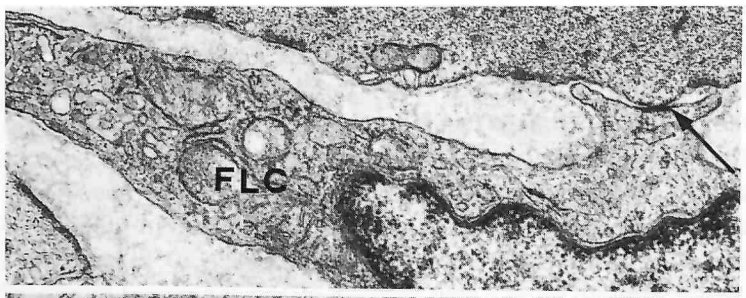

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Fig. 16–18. Myenteric muscle sheath. **16** A fibroblast-like cell (FLC) contacting (arrow) a smooth muscle cell. ×25,000. **17** A fibroblast-lik cell (FLC), two ICC and smooth muscle cells (SMC), all interconnected with each other. ×15,000. **18** A macrophage-like cell near the myenter plexus bundles. The arrows indicate the contact areas between this cell and a covering cell (thin arrow) and an ICC (thick arrow). E = elastic fibr connect the macrophage-like cell and the ICC with each other and with the smooth muscle cells. ×20,000.

another, connecting them. Sheaths, mainly made up of smooth muscle cells assembled in several layers (up to 10), envelop both myenteric ganglia and nerve strands. Some of the smooth muscle cells, mostly located at the periphery of the sheaths, look like the circular ones. On the other hand, most of the cells are very small in size, are

ultrastructural features. In fact, they have the characte istic of coming into contact with each other also by mear of extended appositions of their contiguous plasma mem branes, which are rich in caveolae, and possess clusters c cisternae of SER at the cell periphery and some cisterna of RER and granules in the perinuclear region. More

The presence of connecting muscle bundles has already been reported in the human colon [Keith, 1915; Pace, 1968; Huizinga et al., 1985] and ileum [Faussone-Pellegrini and Cortesini, 1983]. Our data confirm their presence throughout the length and circumference of the human colon, and the electron-microscopic examination revealed that these bundles are, as in the human ileum [Faussone-Pellegrini and Cortesini, 1983], exclusively made up of smooth muscle cells with typical structural features. These bundles have been considered responsible for the electrical coupling between the two muscle layers [Huizinga et al., 1985], and our ultrastructural findings do not seem in contrast with such a hypothesis.

The structures that we named myenteric muscle sheaths have not been described before. We have found that these sheaths are made up of several types of interconnected cells, most of which are in reality smooth muscle cells (possessing specific features), but some of which are myenteric elements and at least three types of non-neuronal, non-muscular cells. The possible role played by these structures might, therefore, be more complicated than that played by the connecting muscle bundles, since this role is the result of the interaction of all ensheathing cell types. The functional interpretation of these sheaths may be further complicated by the fact that (1) the outermost smooth muscle cells are connected with those of both muscle layers; (2) some of the innermost ones, together with the covering cells of the nerve strands, penetrate inside the subdivisions of the non-capsulated nerve strands, and (3) between the deepest located smooth muscle cells and the main muscle component of the sheaths there is an interwoven network of non-neuronal, non-muscular cells. The sheath smooth muscle cells, moreover, are innervated by the proximal branches of the myenteric plexus, with which they also form 'close' contacts (with a gap of 20 nm).

Several years ago, by light microscopy, peculiar smooth muscle cells, called 'intermediate cells' and considered 'non-neuronal, non-connective, perhaps muscular or ICC in nature', were found in rat and human colon [Keith, 1985]. These cells were described as forming collars closely linked with the myenteric plexus, together with which they were thought to make up the 'nodal tissue', providing for the rhythmic activities of the colon [Keith, 1915]. We think it likely that our 'myenteric muscle sheaths' might be identified as Keith's 'collars' and that 'intermediate cells' represent at least one part of the cell population of the myenteric muscle sheaths we observed, even if it is difficult to decide which, among the sheath cell types, they could be. However, considering the interrelationships all sheath cell types have with each other and with the myenteric elements, Keith's interpretation could still be formulated nowadays. This interpretation could be based upon the fact that putative pacemaker cell types are effectively present at this level. In fact, the sheath smooth muscle cells, as Keith's 'intermediate cells', specifically envelop the myenteric plexus elements, and their ultrastructural features are quite identical to those of the smooth muscle cells located at the submucosal border of the circular muscle layer at the level of the human descending colon [Faussone-Pellegrini and Cortesini, 1984], where, according to recent findings [see Smith et al., 1987 for review of the literature], a population of pacemaker cells seems to be located. These data might be sufficient to consider them as constituting Keith's 'nodal tissue'. Moreover, in addition to these peculiar smooth muscle cells, other cell types (the non-neuronal, non-muscular cells) are present in the myenteric sheaths, which might be responsible for the 'nodal' function; one of these might be identified in the putative pacemaker cells of the gut, the ICC [Faussone-Pellegrini et al., 1977; Thuneberg, 1982; Hara et al., 1986; Suzuki et al., 1986]. These cells, however, even if quite identical to those found in the myenteric area of the human stomach [Faussone-Pellegrini et al., 1989] and small intestine [Faussone-Pellegrini and Cortesini, 1983], are rare in the human colon and rarely 'closely' contact with the nerve endings. At variance with those described in the guinea pig [Rogers and Burnstock, 1966; Gabella, 1972, 1979; Cook and Burnstock, 1976], rabbit [Komuro, 1982], mouse and rat [Faussone-Pellegrini, 1987; Komuro, 1989], these ICC possess scarce RER elements, whereas they are linked together by elastic bridges, similar to human gastric ICC [Faussone-Pellegrini et al., 1989].

However, since also the other types of non-neuronal, non-muscular cells are interconnected with both ICC and/or smooth muscle cells, their influence upon the myenteric sheath role cannot be excluded. Unfortunately, their nature and/or function is at present uncertain. Some of them, which we called 'fibroblast-like cells', have a similar morphology to the cells identified as ICC in the intestine of laboratory mammals [Rogers and Burnstock, 1966; Gabella, 1972, 1979; Cook and Burnstock, 1976; Komuro, 1982, 1989; Faussone-Pellegrini, 1987], but, at variance with ICC, do not come into contact with nerve endings. Some of them, furthermore, are also intercalated between the covering cells of the nerve strands, mimicking their layout; they are different from the latter, however, in the number of RER elements, a large Golgi apparatus and an irregular outline of the nucleus. The

doubt remains, therefore, as to whether these cells are to be considered as ICC, as covering cells or as common connective tissue cells. Another intriguing finding we observed in human colon is that also the covering cells come into contact with the cells identified as ICC. Furthermore, macrophage-like cells have occasionally been observed to be interposed between the covering cells and the ICC. This finding has already been reported in mouse small intestine and an important, even if not yet ascertained, function in the gastro-intestinal motility has been suggested for this cell type, too [Mikkelsen et al., 1985, 1988]. In summary, the most important information that results from the analysis of all these cell interconnections is that, among all the non-neuronal, non-muscular cell types, only ICC come into contact with all others and this seems to support for ICC only, as previously suggested, a pacemaker role.

Morphological evidence in favour of identifying the myenteric muscle sheaths with the 'nodal tissue' proposed by Keith [1915] seems, therefore, supported by the fact that the two putative pacemaker cells types (ICC, sheath smooth muscle cells) reside at their level. Slow waves might propagate to both muscle layers directly through the sheath smooth muscle cells or with an interaction of the other cell types, and under the nerve control. It cannot be excluded, however, that the myenteric muscle sheaths intervene in other electrical and possible mechanical activities recorded in the colon.

In conclusion, our findings in the plane between the circular and longitudinal muscle layers of human colon might account for the presence of muscle bundles and of an interconnected cell network which, at variance with other regions of the gut [Faussone-Pellegrini, 1987], possess both cell types (i.e. the smooth muscle cells and the ICC), which may have a function in the electrical coupling between the two muscle layers and the slow wave origin. However, other cell types have been identified which are interconnected with smooth muscle cells, ICC and each other, whose functions might influence the aforementioned. Similarly, three different cell types, all interconnected with each other, have recently been described around the myenteric plexus elements of rat small intestine [Komuro, 1989). In this animal species, however, no sheath smooth muscle cells or connecting muscle bundles have been found, ICC directly bridging the two ileal muscle layers.

At least it should be taken into account that in man both the smooth muscle cells of the connecting muscle bundles (all of them) and muscle sheaths (those superfi-

layers. Thus the myenteric nerve elements are partially or completely embedded in one or other muscle layer and it is probably impossible to define exactly for a reasonable length the 'myenteric border' of each muscle layer. It is, therefore, with some caution that physiological and morphological data obtained in man have to be interpreted and compared with those obtained in the other animal species, which do not possess connecting muscle bundles or myenteric muscle sheaths.

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