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Structural organization of enteric nervous system in human colon

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Summary. The organization of the Enteric Nervous System (ENS) was studied in the human colon. Fragments of the whole colonic wall were either routinely processed or Zinc-Iodide Osmium impregnated. Single-layer preparations were also obtained from some of the Zinc-Iodide Osmium-impregnated specimens. The results showed some differences in the organization of human colonic ENS from that of other mammals. In fact, the human submucous plexus was made up of three interconnected ganglionated networks arranged along three different planes. With respect to the myenteric plexus, its ganglia were large sized and irregularly shaped. Moreover, during the microdissection of the colonic wall, we found the absence of a cleavage plane between the circular and longitudinal muscle layers; on the other hand the cleavage plane between mucosa and submucosa was not immediately below the muscularis mucosae, but slightly deeper, since the innermost part of the submucosa remained adhering to overlying layers.

Key words: Colon, Enteric Nervous System, Man, Anatomy

Introduction

The Enteric Nervous System (ENS) has been investigated for more than a century (Meissner, 1857; Auerbach, 1862a,b, 1864; Henle, 1871; Dogiel, 1895, 1899; Cajal, 1911; Schabadasch, 1930a,b; Gunn, 1968; Furness and Costa, 1987; Hoyle and Burnstock, 1989; Scheuermann et al., 1989; Timmermans et al., 1992; Krammer et al., 1993), but most researches have been performed on the small intestine of laboratory mammals and, surprisingly, the information reported in the textbooks of Human Anatomy is mostly based on the assumption that the ENS of laboratory mammals and man have an identical organization. Actually the studies on the architectural organization of human ENS are fragmentary, possibly due to technical difficulties related to the thickness of the gut wall and the abundance of fatty tissue in comparison to the smaller sized animals.

In fact, literature data in man only refer to the total number of the ENS neurons (Furness and Costa, 1987; Hoyle and Burnstock, 1989) and to the presence of nonganglionated intramuscular plexuses (Furness and Costa, 1987; Faussone-Pellegrini et al., 1983, 1990a). Recently, the existence in man of an outer subdivision of the submucous plexus, made up of a ganglionated plexus running close to the submucosal border of the circular muscle layer, has also been described (Hoyle and Burnstock, 1989; Faussone-Pellegrini et al., 1990a). Moreover, a third ganglionated plexus, called «intermediate plexus» (Hoyle and Burnstock, 1989), has been found in the sigmoid colon of man.

The aim of this work was to study the organization of human ENS in the colonic segment, which appeared less extensively investigated even in other animal species. On the other hand, knowledge of the general architectural pattern of ENS in humans is fundamental in order to evaluate the possible neural alterations occurring in several gut diseases. The Zinc-Iodide Osmium (ZIO) method was used since it has previously given good results in laboratory mammals for both light and scanning electron microscopic investigation (Stach, 1972; Christensen and Rick, 1987; Jessen and Thuneberg, 1991; Ibba Manneschi et al., 1992). Findings obtained in ZIO-stained preparations were compared with those shown by routinely processed sections.

Materials and methods

Segments of human ascending, transverse and descending colon came from eight patients, ranging in age from 53 to 65 years, undergoing surgery for cancer. Informed consent was obtained from each patient. The fragments of excised colon had a normal appearance and were taken far from the carcinomatous areas (5 cm or more away from the cancer site) and were histologically free of tumor and inflammation. Before surgery the patients did not take any drug affecting colon motility.

After surgery, the intestinal content was washed out

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from each specimen and the specimen was divided into two cylinders of different length. A plastic tube, with a diameter of 4.5 or 3 cm, was inserted into the lumen of the longest segment obtained from the ascending, transverse and descending colon respectively. These specimens were fixed and stained in freshly prepared Zinc-Iodide Osmium tetroxide solution 3:1 (ZIO) for 24-36 h (Champy, 1913; Maillet, 1969; Rumessen and Thuneberg, 1982) at room temperature, and then submitted to two different procedures.

1) Some of these specimens were carefully dissected with fine forceps under a stereomicroscope in order to separate the various layers of the colonic wall. The isolated layers were flattened on glass-slides, dehydrated through an ethanol series, mounted and observed under a light microscope without any further staining.

2) Others were cut into several fragments, washed in four changes of distilled water, dehydrated through an ethanol series and embedded in paraffin using flat moulds. Serial sections, 12-14 µm thick, were cut according to the x, y and z planes; that is, along the longitudinal, transverse axes of the gut as well as parallel to the layers of the wall, with the aim of obtaining sections comparable to thin single-layer preparations.

The shortest colonic segments were fixed in Bouin's solution, embedded in paraffin, serially sectioned and routinely stained with haematoxylin-eosin. The observations were made under a Leitz Orthoplan light microscope.

Results

The whole Enteric Nervous network, consisting of both ganglia and nerve strands, were better detectable in ZIO-stained sections and single-layer preparations than in haematoxylin-eosin-stained ones. The latter, however, confirmed some of the neuronal details shown by the ZIO method.

Mucosa

The mucosal and muscularis mucosae plexuses, could be observed in the ZIO-stained specimens, whereas it was fairly difficult in haematoxilin-eosin-stained preparations.

A) In the ZIO-stained sections, the fine nerve fibres of the *mucosal plexus* were evident in the connective tissue of lamina propria among the tubular glands, close to the glandular epithelium and encircling their fundus like a nest, to form the interglandular, periglandular and subglandular networks respectively (Fig. 1a). In the single-layer preparations of lamina propria, the nerve fibres enveloping the glandular pits were very easily discriminated, intermingled with the vascular network (Fig. 1b). No ganglia were found in the mucosal plexus of the human colon.

B) In the ZIO-stained sections, the *muscularis mucosae plexus* appeared as a fine felt made up of a nonganglionated nervous network whose nerve bundles ran mostly parallel to the long axes of the smooth muscle cells (Fig. 2a). Only the single-layer preparations of the muscularis mucosae allowed this fine network to be detected among the muscle and vascular components (Fig. 2b).

Submucosa

The ganglia of the *submucous plexus* appeared arranged along three different planes in the sections stained with either ZIO or haematoxylin-eosin and in

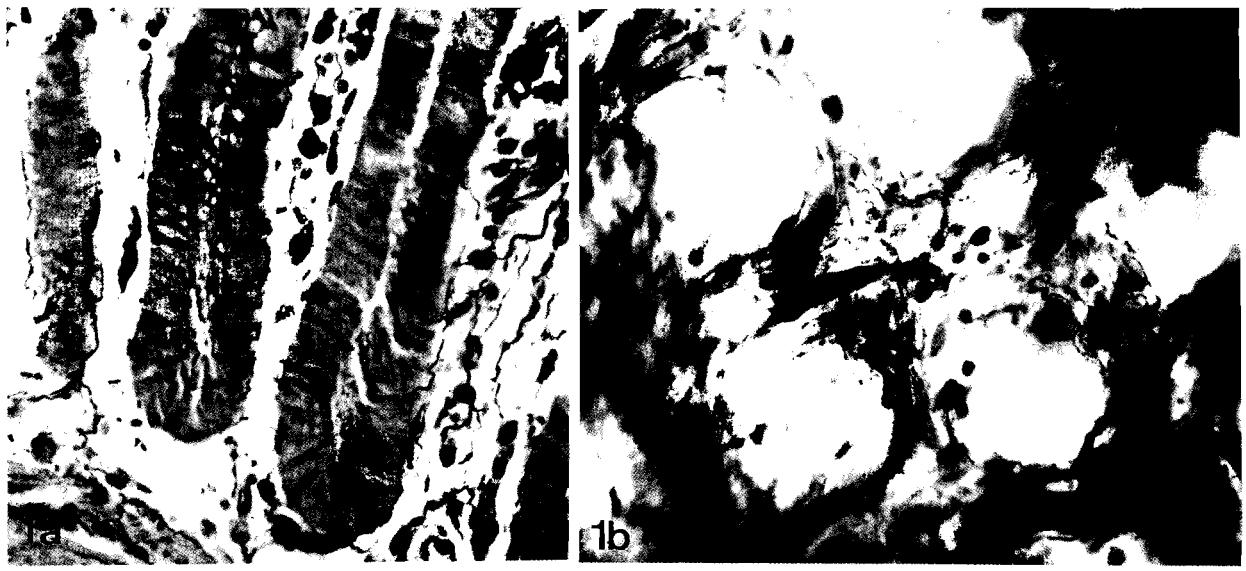


Fig. 1. Mucosa of human colon. Nerve fibres of the mucosal plexus. ZIO method. **a.** Deparaffinized section. x 300. **b.** Single-layer preparation of lamina propria. x 300

single-layer preparations.

The ganglia of the innermost network were small sized and regularly arranged in a single row immediately below the muscularis mucosae as well as at the level of the submucosal border of solitary lymphoid nodules (Fig. 3a,b). These ganglia could be observed in the single-layer preparations of the muscularis mucosae only, since the innermost part of the submucosa remained adherent to muscularis mucosae during microdissection (Fig. 3c,d). Nerve strands from these ganglia ran towards the mucosal plexus and the middle part of the submucosa (Fig. 3e).

A second series of ganglia was deeply located, often at the same level as the large blood vessels (Figs. 3f, 4a,b), and a third one was closely apposed to the circular muscle layer (Fig. 4c). The microdissection of the intestinal wall performed along the cleavage plane, between the mucosa and submucosa allowed the observation of these two ganglionated networks. One network displayed wide meshes made up of large ganglia connected by thick nerve strands (Fig. 4d,e), while the one closely apposed to the circular muscle layer showed smaller meshes and the nerve strands appeared considerably thinner than the first one (Fig. 4f). Furthermore, both ZIO-stained sections and single-layer preparations showed nerve strands connecting all these ganglia to each other and presumably forming a three-dimensional network. The shape of the nerve cells was well defined in both haematoxylin-eosin (Fig. 4b) and ZIO- (Fig. 4e,f) stained preparations.

In the single-layer preparations of the submucosa the *submucosal perivascular plexus* was particularly well recognizable (Fig. 5).

Muscle coat

Wide areas of single-layer preparations of the muscle coat could not be obtained in man, probably due to toughness of muscle tissue with a concurrent fragility of connective tissue framework after ZIO impregnation.

The intramuscular nerve fibre bundles could be detected in the ZIO-stained sections only, running parallel to the long axes of the smooth muscle cells and forming the nonganglionated *circular* and *longitudinal muscular plexuses* respectively (Fig. 6).

On the contrary, the *myenteric plexus* was well detectable in the sections stained with either ZIO or haematoxylin-eosin. This plexus was made up of a network of internodal nerve strands and large-sized and irregularly-shaped ganglia (Fig. 7a). In the ZIO-stained sections (Fig. 7b) the shape of the nerve cells was more evident than in the haematoxylin-eosin ones (Fig. 7c). It was impossible, however, to follow the whole network, since the ganglia and connecting nerve strands were located in different planes and were enveloped by sheaths of smooth muscle cells in continuity with those of both circular and longitudinal muscle layers.

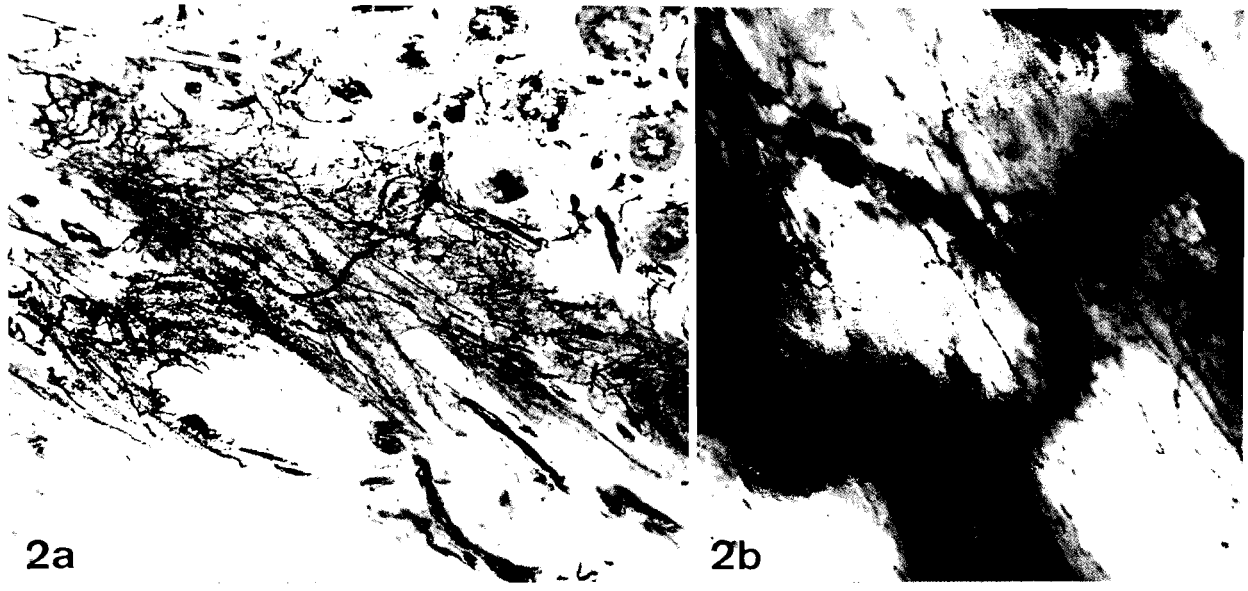
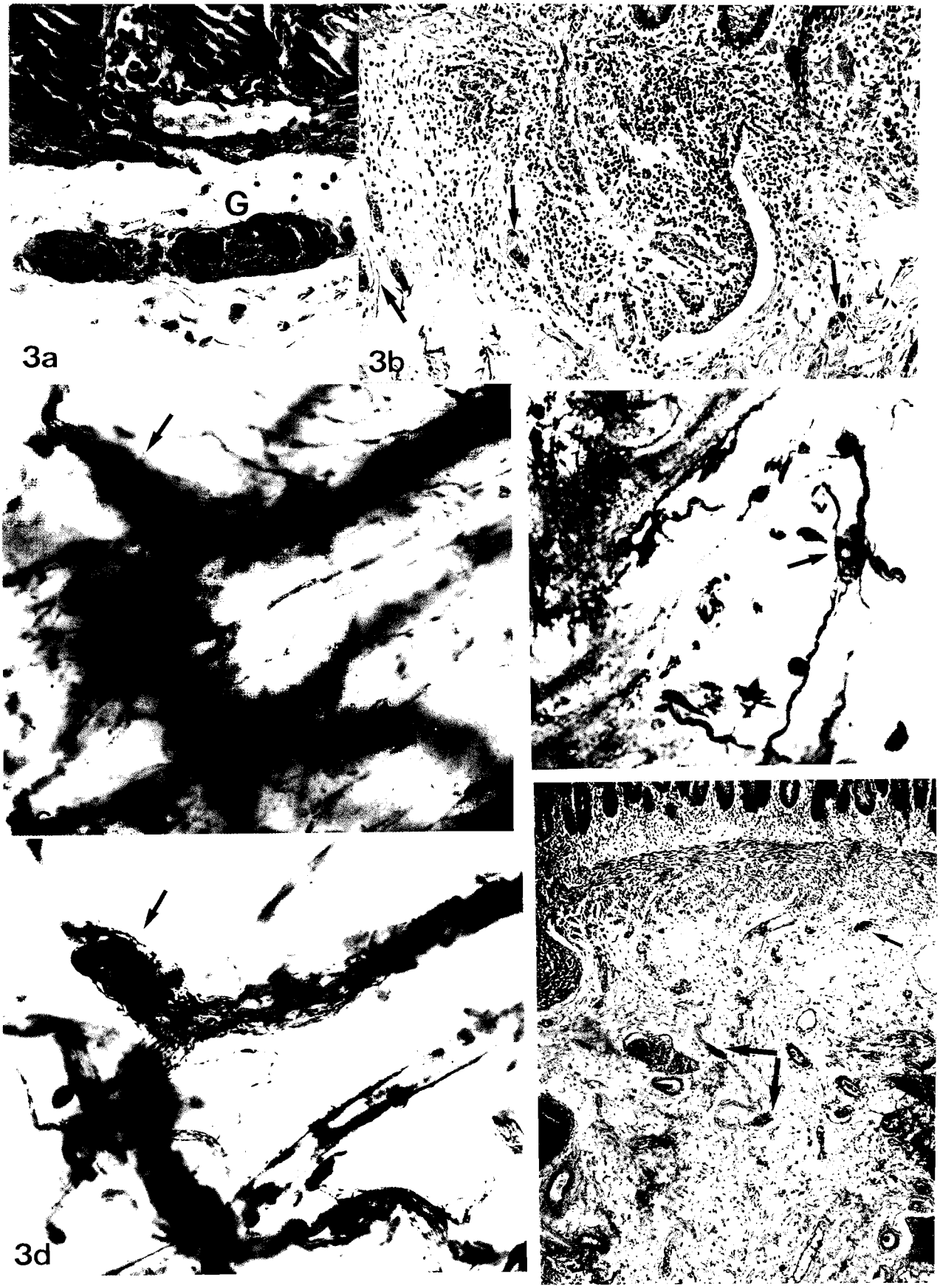


Fig. 2. Muscularis mucosae of human colon. Nerve network of the muscularis mucosae plexus. ZIO method. **a.** Deparaffinized section. x 300. **b.** Single-layer preparation of the muscularis mucosae. x 300

Fig. 3. Submucosa of human colon. Inner subdivision of the submucous plexus. **a.** A ganglium (G) lying below the mucosa. x 210. **b.** Small ganglia (arrows) in the vicinity of a solitary lymphoid nodule. Haematoxylin-eosin-stained sections. x 130. **c, d.** Single-layer preparations of the muscularis mucosae (c) and inner part of the submucosa (d) photographed at two different focal planes. Arrows indicate a ganglium of the inner subdivision of the submucous plexus. ZIO method. x 330. **e.** The arrow indicates a small ganglium. ZIO method, deparaffinized section. x 300. **f.** The first series (thin arrow) and the second series (thick arrows) of submucous ganglia. Haematoxylin-eosin-stained section. x 60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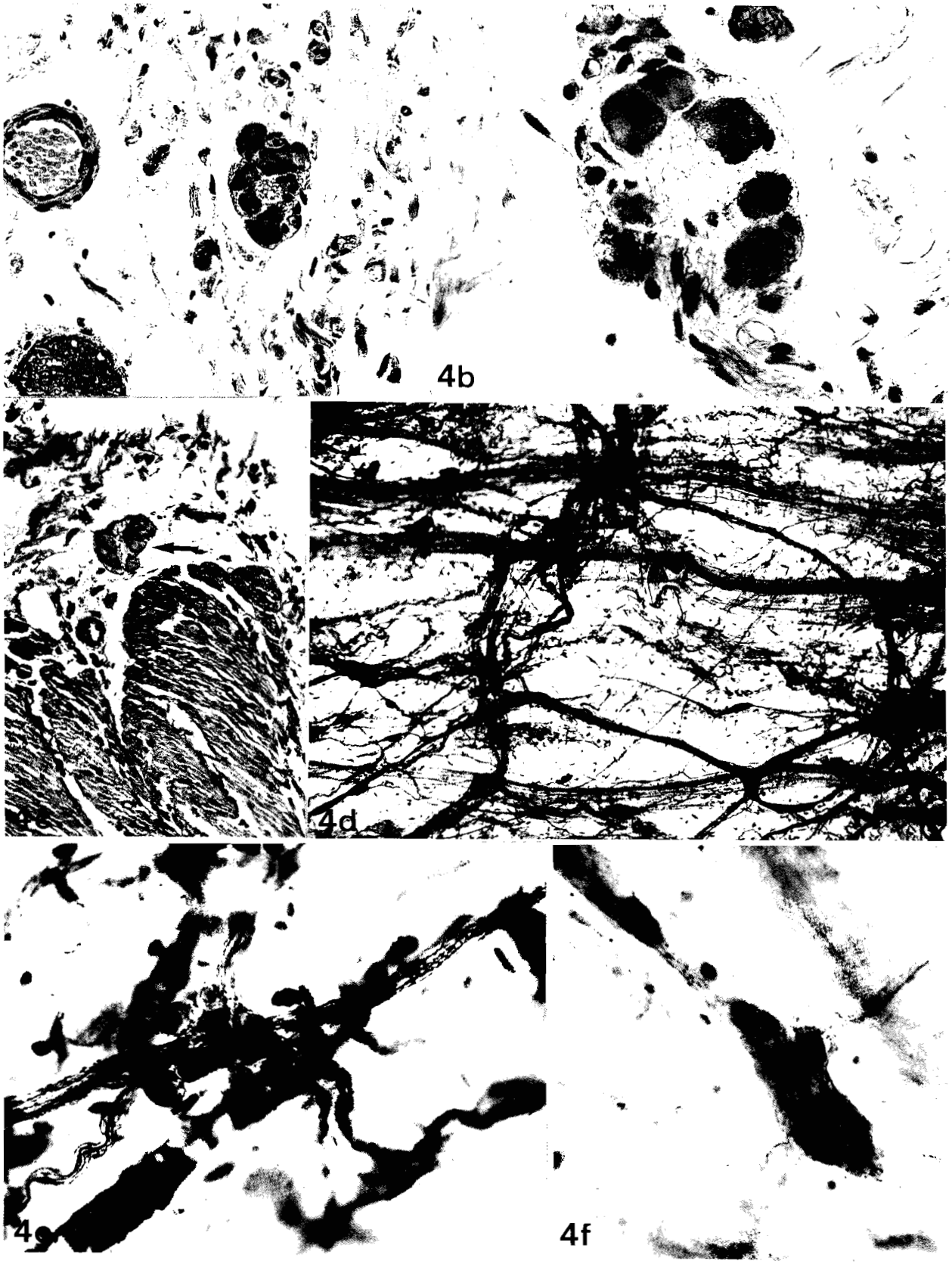
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Fig. 4. Submucosa of human colon. **a, b.** Ganglia of the submucous plexus located in the middle part of the submucosa. Haematoxylin-eosin-stained sections. a, x 210; b, x 530. **c.** A small ganglium (arrow) of the outer subdivision of the submucous plexus. Haematoxylin-eosin-stained section. x 210. **d.** Low magnification of the submucous ganglionated nerve networks. x 50. **e, f.** Ganglia of the submucous plexus located in the middle and outer part of the submucosa respectively. d-f. ZIO method. Single-layer preparation of the submucosa. e, x 300; f, x 300

Serosa

The ZIO-stained sections showed nerve fibres, mostly of large calibre, passing through the serosa and penetrating the longitudinal muscle layer. None of these fibres were surrounded by an osmiophilic sheath, thus indicating that in the colonic regions examined the subserosal plexus was made up of amyelinated nerve fibres only. Neuronal cells were never found at this level.

Discussion

The general architecture of ENS in the human colon is different from that of other mammals in some respects. The main difference is the presence in man of three networks of ganglia throughout the thickness of the submucosa, instead of two, as usually described in small-sized mammals, and corresponding to the inner (Meissner) and the outer (Schabadasch, Henle, Stach) submucous plexuses (see review of literature: Furness and Costa, 1987; Scheuermann et al., 1987a,b).

The innermost row of ganglia, similarly to that described in the sigmoid colon of man (Hoyle and Burnstock, 1989), runs closely to the muscularis mucosae and solitary lymphoid nodules. These ganglia are easily identifiable in the full-thickness sections of the colonic wall, stained with either ZIO or haematoxylin-eosin, and in the single-layer preparations of muscularis mucosae. Close relationships between submucosal ganglia and lymphoid nodules (Peyer's patches) have also been described in the porcine small intestine (Krammer and Kuhnel, 1993).

The outermost ganglionated plexus, similarly to that found in the sigmoid colon (Hoyle and Burnstock, 1989), lies close to the circular muscle layer, as previously demonstrated at other colonic levels both electron microscopically and immunohistochemically (Faussone-Pellegrini et al., 1990a, 1993) and should correspond to the Henle-Schabadasch-Stach plexus described in both small- and large-sized mammals.

The third plexus is located at the same level as the large blood vessels. This means that it approximately lies in the middle part of the submucosa, between the afore-mentioned two ganglionated networks. It shows ganglia, nerve strands and neurons larger than those of the other two submucous plexuses. This plexus should correspond to Hoyle and Burnstock's «intermediate plexus» (Hoyle and Burnstock, 1989). However, in our specimens this plexus has been found not so close to the muscularis mucosae as was reported for the sigmoid colon. Notwithstanding the different depth at which this «intermediate plexus» is located according to the colonic levels, it might correspond, together with the innermost one, to the Meissner plexus.

All submucous plexuses are interconnected and the innermost one is clearly connected to both the muscularis mucosae and mucosal plexuses. The outermost one is connected to the myenteric plexus, similarly to the other submucosal plexuses, and with the circular muscle plexus. Unfortunately, single-layer preparations of the whole submucosa of the human colon cannot be obtained, due to its thickness and adhesion to the muscularis mucosae and circular muscle respectively. Therefore, single-layer preparations of submucosa contain no more than two rows of ganglia (the middle one plus the outer one) or a single row of ganglia (in this case the middle one), thus confirming for the submucous plexus of the human colon the distinction in three ganglionated plexuses. Consequently, the distinction in three plexuses proposed by Hoyle and Burnstock for the sigmoid colon submucous plexus, can be extended to the entire length of human colon.

The subdivision of the submucous plexus into three ganglionated plexuses might be due to the remarkable thickness of human colonic submucosa and should be important from a functional point of view. In fact, considering the relationship each plexus has with a specific submucosal zone (submucosal border of the muscularis mucosae and of circular muscle layer respectively, solitary lymphoid nodules, large blood vessels) a different population of neurons might be postulated. In this sense, the presence within each plexus of a heterogeneous distribution of motor-, sensory-, and inter-neurons might be related to the role each submucous plexus plays. Variations in the distribution of the different types of neurons might occur and be at the root of several gut diseases.

The myenteric plexus in man differs from that of other mammals in its extremely irregularly-shaped and variably-sized meshes, most of which are larger than in guinea pig and rat (Hanani, 1992). Moreover, both

Fig. 5. Submucosa of human colon. Ganglium and fibres of perivascular plexus. ZIO method. Single-layer preparation of the submucosa. x 300

Fig. 6. Intramuscular plexus of human colon. ZIO method. Deparaffinized section. x 480

Fig. 7. Myenteric plexus of human colon. **a.** Low magnification of the myenteric plexus. x 63. **b.** The arrow indicates a muscle sheath around a ganglium. ZIO method. Deparaffinized sections. x 130. **c.** High magnification of a ganglium. Haematoxylin-eosin-stained section. x 210

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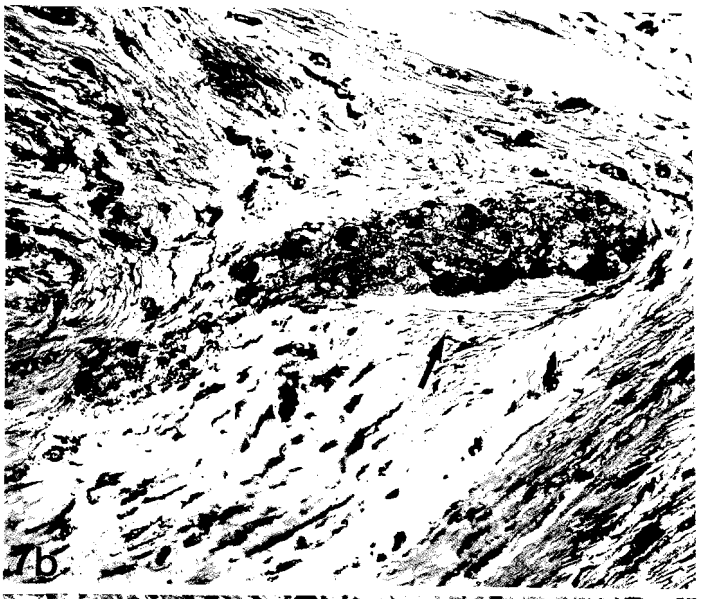
7a



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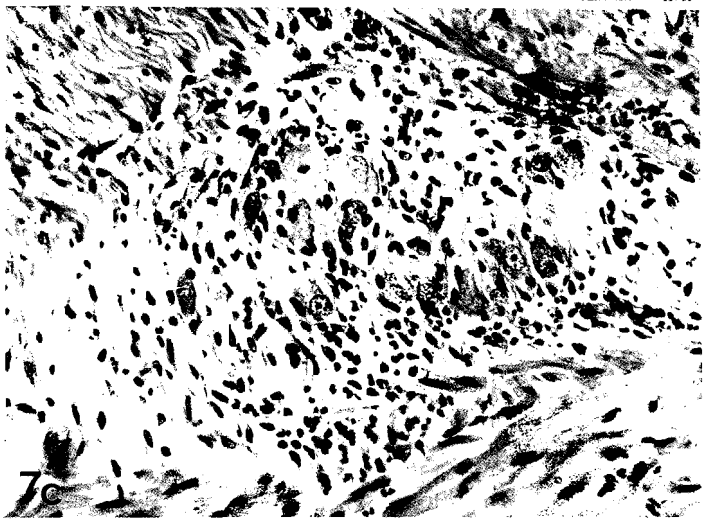

7c

ganglia and nerve strands, enveloped by sheaths of smooth muscle and interstitial cells, lie in different planes and oblique muscle bundles run from the circular to the longitudinal muscle layer and vice versa (Faussone-Pellegrini et al., 1990b). Consequently, single-layer preparations of large areas of myenteric plexus are difficult to obtain in man.

The colonic intramuscular, muscularis mucosae and mucosal plexuses in man are similar to those described in other mammals. However, when compared with rat colon (Mestres et al., 1992a, b), the lack of ganglia in the human colonic mucosa is noteworthy. The subserosal plexus at all colonic levels examined, except the sigmoid colon (Crowe and Burnstock, 1990), has no ganglia and all nerve fibres are amyelinated.

Neurons count and evaluation of their diameter can be performed using both ZIO- and haematoxylin-eosin-stained specimens, since both shape and contour of neuronal body are well defined. In the ZIO-stained specimens, moreover, nerve processes are also well revealed and can be traced far from perikarya. ZIO staining also permits the distinction between myelinated and amyelinated nerve fibres.

In conclusion, the organization of human colonic ENS shows some differences from that of other mammals. In fact, the human submucous plexus is made up of three interconnected ganglionated networks arranged along three different planes. With regard to the myenteric plexus, its ganglia are large sized and irregularly shaped. Moreover, during the microdissection of the colonic wall, we have found the absence of a cleavage plane between the circular and longitudinal muscle layers; on the other hand the cleavage plane between mucosa and submucosa was not immediately below the muscularis mucosae, but slightly deeper, since the innermost part of the submucosa remained adhering to overlying layers.

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