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An ultrastructural study of the smooth muscle cells and nerve endings of the human stomach

M.S. FAUSSONE-PELLEGRINI, D. PANTALONE* and C. CORTESINI*

Dipartimento di Anatomia Umana e Istologia, Sezione di Istologia 'Enrico Allara'; *Clinica Chirurgica 3, Università di Firenze, Policlinico di Careggi, Italia

SUMMARY - The circular muscle layer of the human stomach was studied with the electron microscope. The smooth muscle cells differ structurally according to their location. In fact, the circular muscle layer of the fundus is made up of large, irregularly shaped smooth muscle cells, with long, thick, dense bands, and immersed in an abundant, elastic supporting connective tissue. In the circular muscle layer of the corpus (the two curvatures and the corpus between), those located in the outermost zone are identical to that commonly described in the alimentary tract, but are gradually substituted, first by larger irregularly shaped cells, and then, towards the innermost area, by flattened cells richer in sarcoplasmic reticulum, with thick and continuous dense bands on their submucosal surface. The smooth muscle cells of the antrum are all identical to the outermost located corporal ones. The gap junctions are absent in the fundus and in the innermost area of the corporal circular muscle layer, are present in the other areas, particularly frequent in the antrum and in the outermost area of the greater curvature. The number of the nerve endings is similar in the fundus, greater curvature and antrum, while it is two-three times more in the other parts of the corpus. They are located at the periphery of the muscle bundles in the fundus, antrum and greater curvature, sparsely scattered among the smooth muscle cells in the other areas of the corpus and contain small agranular and large granular synaptic vesicles, often mixed in the same axon. These nerve endings lie distant from the smooth muscle cells in the fundus, antrum and greater curvature and directly contact the smooth muscle cells in the other parts of the corpus.

Key Words *smooth muscle cells - nerve endings - stomach - man - ultrastructural study*

INTRODUCTION

As is well known, the gastric muscle wall in man and dog has different electrical and mechanical activities depending on whether it is part of the fundus, corpus or antrum. Further differences can be seen in these areas according to whether they are located in orad, caudad or even in the midcorpus, and in orad and caudad antrum (Kelly, 1981; Meyer, 1987, and Szurszewski, 1987, for review of the anatomical and physiological literature). However, fundamental questions regarding the site of slow wave origin and the rate and mechanism of their propagation through the thickness of the gastric wall are unclear.

Physiological data in canine gastric antral circular muscle suggest that smooth muscle cells must have structural differences depending on where they are located, even within the thickness of the circular muscle layer (Bauer and Sanders, 1985; Bauer *et al.*, 1985*a,b*). However, there is not yet any morphological information on this point. The only morphological data available upon the ultrastructure of gastric smooth muscle cells have been obtained in the dog (Oki and Daniel, 1974; Daniel *et al.*, 1984) and in the guinea pig (Moriya and Miyazaki, 1979). These data however, do not refer to peculiarities or structural differences among the gastric circular smooth muscle cells and between them and the intestinal or esophageal ones. The most relevant information these Authors have given us is that the smooth muscle cells of the gastric circular muscle have gap junctions, which (Daniel *et al.*, 1984) in the lesser curvature of the canine corpus are 30 per 100 cross-sectioned muscle cells.

On the other hand the role played by the nerve tissue upon gastric motility should not be disregarded. For example, it has been demonstrated that mechanical activity in the gastric smooth muscle is largely controlled by the nervous system (Szurszewski, 1977). In fact, a large number of neurotransmitters, which may be colocalized, have been demonstrated in the gastric nerve fibers of some mammals (see Furness and Costa, 1987, for review of the literature) and, recently (Wattchow *et al.*, 1988), the distribution of the peptide-IR nerve fibers along the length of the human gastrointestinal wall has been reported. According to these Authors, the number of these nerves in the gastric circular muscle layer is low in the fundus, increases in the corpus and reaches the maximal frequency in the antrum. Using the electron microscope Oki and Daniel (1974) and Daniel *et al.* (1984) found in the canine lesser curvature that the majority of nerve fibers are grouped outside the muscle bundles and that the nerve endings predominantly contain small agranular vesicles. No nerve ending was found in close proximity (≤20 nm) to a smooth muscle cell, and in fact most were several microns away.

We thought of examining the human stomach with the electron microscope because of the scarcity of morphological data on the gastric muscle wall, in humans in particular. We will refer to the organization of the circular muscle layer of the fundus, of a corporal ring, which includes the two curvatures and the corpus between, and of the antrum. These segments were chosen because they offer the possibility of comparing areas with different electrical and mechanical activities. Finding morphological differences or peculiarities among the various gastric areas can, therefore, assume a certain importance for a subsequent morphofunctional correlation and give some answer to the fundamental questions about the mechanisms which control gastric motility.

MATERIALS AND METHODS

Two fragments of the gastric fundus, two of the antrum, three rings from the corpus, including the two curvatures and the corpus between, were surgically obtained from 7 patients operated on total gastrectomy for 'early cancer' (IIa, IIc, with limited invasion into the mucosa). The patient age ranged from 37 to 71 years. None of these patients have taken drugs affecting gastric motility.

All the specimens were taken far (7-8 cm) from the carcinomatous regions and did not show any macroscopic and microscopic pathological changes. The mucosa was gently excised from all specimens and the strips of muscle coat 2 cm long were stretched out in a polystyrene box, attaching each end to the opposite walls of the box. They were completely immersed in a fixative solution of 2% cacodylate buffered glutaraldehyde pH 7.4 for 3-4 h. Then, the fragments were cut into thinner strips 1 mm thick and 5 mm long and fixed for another 2-4 h in the same fixative solution. After rinsing in a cacodylate buffer solution added to sucrose 0.44 M, the strips were postfixed with 1% phosphate buffered OsO_4 pH 7.4, dehydrated with acetone and embedded in Epon using flat moulds in order to obtain a suitable orientation.

Since the circular muscle layer in the human stomach is very thick, especially in the corpus and antrum, semithin sections, stained with toluidine blue, were observed at light microscope in order to verify their orientation and to choose the region to be examined with the electron microscope. In so far as possible the transverse sections of the circular muscle layer were preferred.

The ultrathin sections, obtained with a Porter-Blum MT1 ultramicrotome, were stained with uranyl acetate and an alkaline solution of bismuth subnitrate and examined under the Siemens Elmiskop 1A and 102 electron microscopes.

Morphometry and statistics

For each region of the stomach, two tissue blocks from each of two patients (i.e. four blocks per region) were used for morphometry.

1) The diameter of smooth muscle cells was measured on semithin sections at magnification × 600. Only the nucleated portions of the smooth muscle cells were measured. In regions where the transverse section of smooth muscle cells was elliptical rather than circular, both the major and minor transverse diameter was measured and from these values the area equivalent diameter (Weibel, 1979) and the ratio between the minor and major axis were computed. A total of 159 smooth muscle cells were measured. Each cell was considered to be one sample unit.

2) The number of smooth muscle cells, of gap junctions and of the nerve fibers located inside the muscle bundles (2-3 axons each and forming varicosities) were counted on ultrathin sections of cross-sectioned smooth muscle cells. Counts were done on fields of constant area (337,5 μm^2) at magnification × 4,000; therefore, the number of the smooth muscle cells varied among fields. Each field was considered to be one sample unit.

3) Distribution of values was checked for divergence from normality and lognormality with the X^2 test. Comparisons between different areas of the stomach were evaluated with Student's t test, with two tails. In all cases, $p<0.05$ was considered to be significant. Significance levels $p<0.001$ were also recorded (Lentner *et al.*, 1982; Bahr and Mikel, 1987).

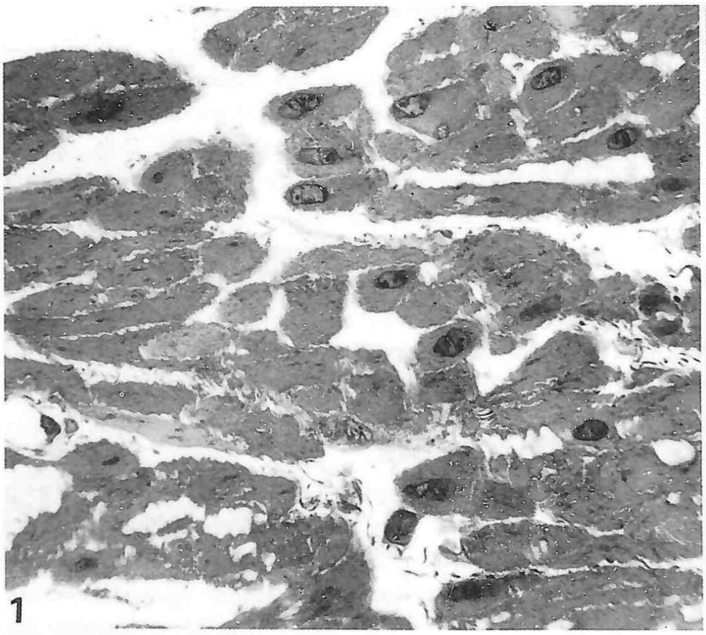
4) The distribution of the diameter of the smooth muscle cells was not significantly different from normal; therefore, means ± standard error were used for comparisons and will be indicated in the Results.

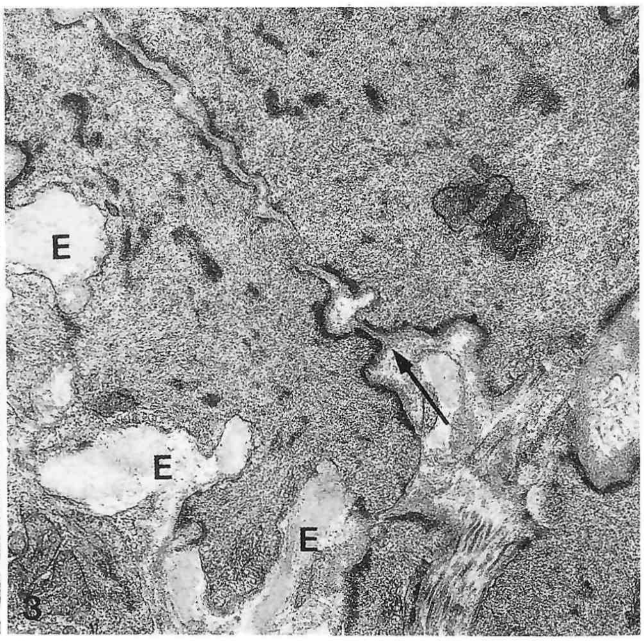
5) The distribution of the ratio between the number of nerve fibers and the number of smooth muscle cells and the distribution of the ratio between the number of the gap junctions and the number of smooth muscle cells were not significantly different from three-parametrical lognormal. Therefore, each observed value (X) was transformed into $\ln(X+C)$ for comparisons; C was 0.025 for the ratio between nerve fibers and smooth muscle cells and 0.05 for the ratio between gap junctions and smooth muscle cells. The medium values of these ratios per cent (derived from the above mentioned distribution) and their standard error will be given in the Results; the standard error is distributed asymmetrically around the medium, because of the observed distribution.

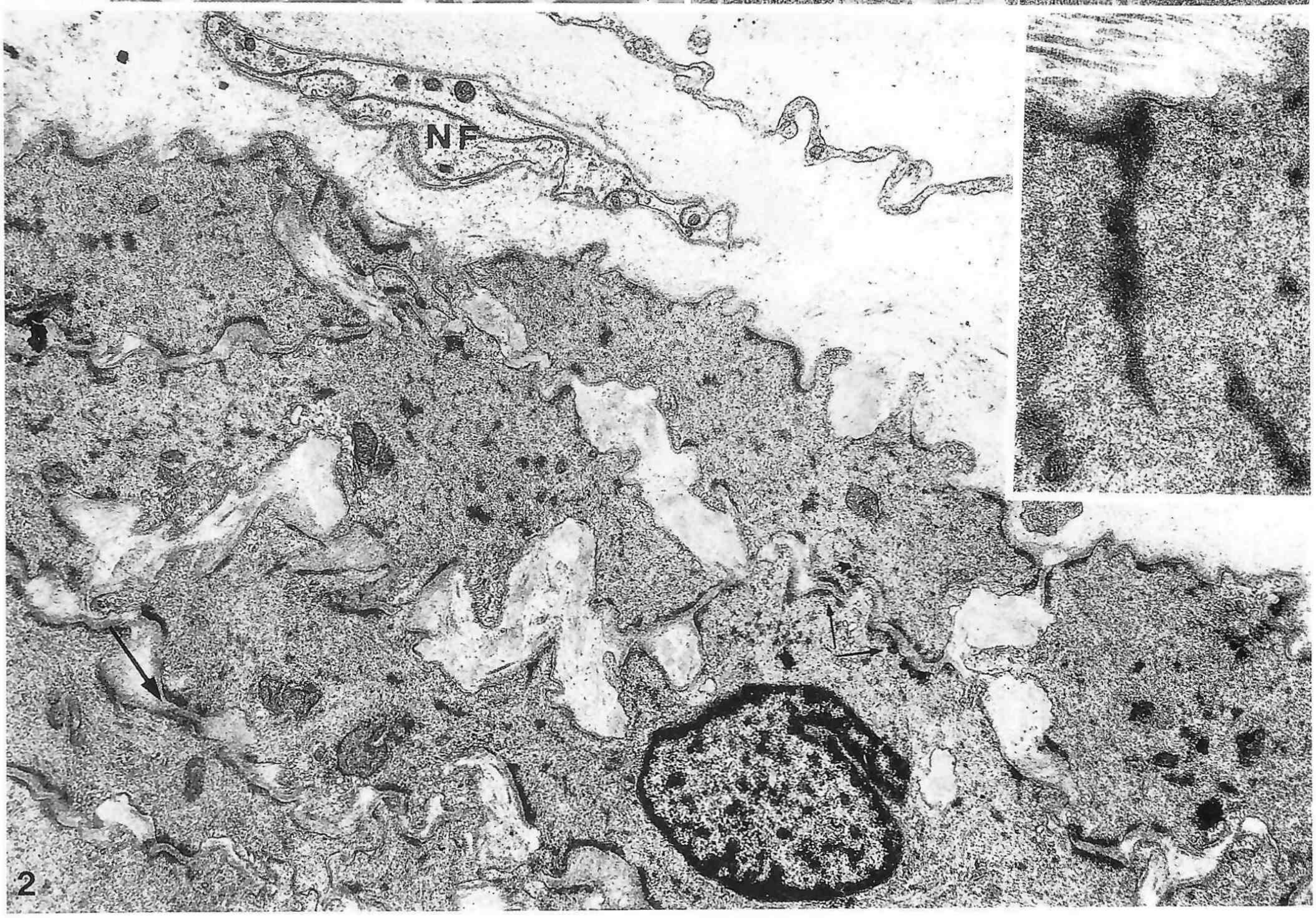
RESULTS

Smooth muscle cells

The smooth muscle cells of the gastric circular muscle layer show differences according to their location. In fact,







Figures 1 to 3 Human stomach. Circular muscle layer of the fundus.

FIGURE 1 Wide spaces of connective tissue separate the smooth muscle cells. Semithin section, toluidine blue. × 700.

FIGURES 2 and 3 Smooth muscle cells irregularly shaped with thick and long dense bands. The abundant elastic material present among these cells connects them together. The arrows indicate the filamentous material bridging two contiguous smooth muscle cells in areas similar to the 'intermediate' junctions. E: elastic fibers; NF: nerve fiber. Inset: detail of one dense band protruding about 1.5 μm inside the cytoplasm. Fig. 2: × 11,500; Fig. 3: × 13,000; inset: × 25,000.

those present in the fundus are different from those present in the corpus and antrum, and those of the corpus vary according to their location inside the thickness of the circular muscle layer itself.

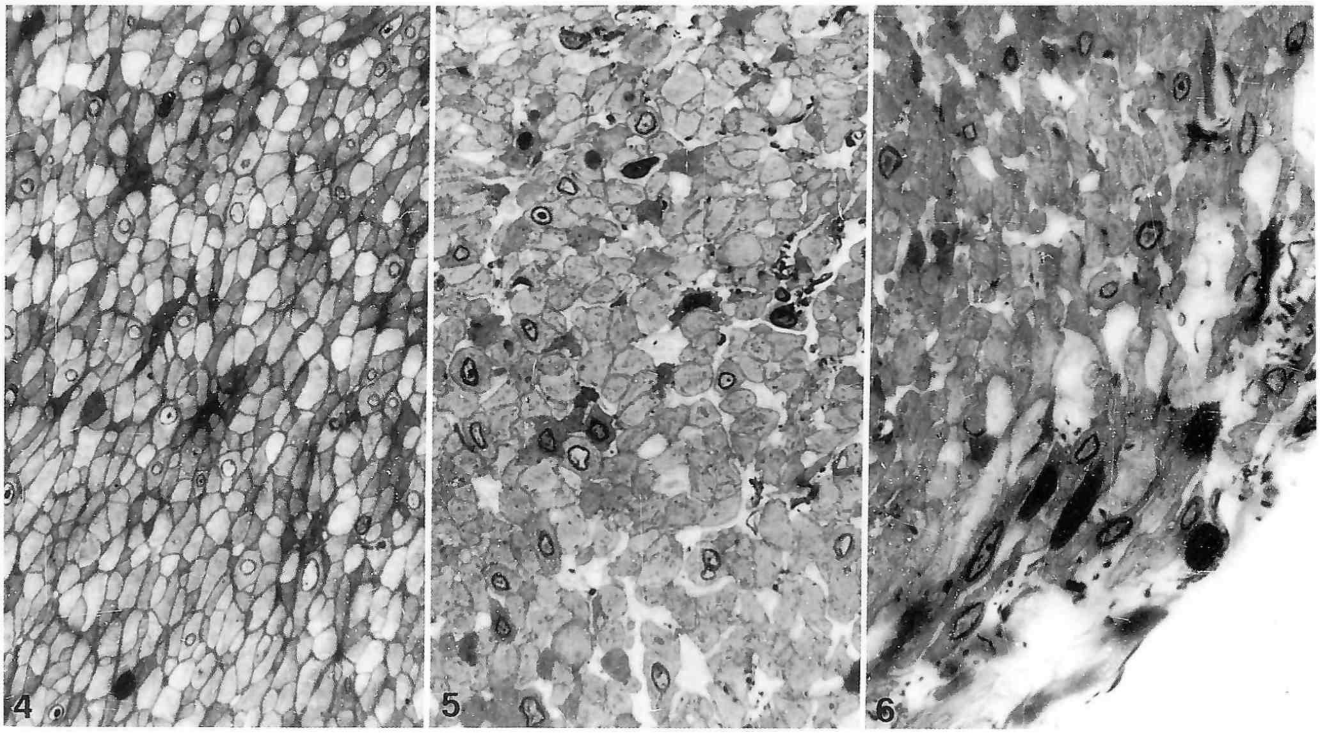
Fundus

The circular muscle layer of the fundus shows several structural characteristics which make this muscle layer peculiar as compared to the other visceral muscles. In fact, wide spaces of connective tissue separate the fundic smooth muscle cells one from another (Figs. 1 to 3). The shape of these cells can be considered fusiform, with a transverse diameter of about 7.97 ± 0.26 μm, but their profile is extremely variable, due to the presence of deep invaginations and long evaginations (Figs. 2 and 3). The dense bands are strongly electron-dense, 1-2 μm long and irregularly distanced (Figs. 2 and 3). Their thickness is also noticeable (0.2 micron) and they can protrude into the cytoplasm for 1 μm or more (inset Fig. 2). The dense bodies, on the contrary, are only rarely so large a thick.

The connective tissue among the smooth muscle cells abundant and rich in elastic fibers (Figs. 2 and 3), wh often link two contiguous smooth muscle cells, like bridge, in the areas of the plasma membrane witho dense bands and provided with caveolae (Fig. 3). W this same appearance, but inserted in correspondence two dense bands, a filamentous material identical to t basal lamina, can also be found (Figs. 2 and 3). No g junctions can be observed.

Corpus

Everywhere in the corpus the circular muscle layer made up of smooth muscle cells which gradually chan their aspect from the outermost to the innermost area this layer. Therefore, even if no definite demarcation c be made inside its thickness (Figs. 4 to 6), to simplify t following description, the circular muscle layer has be

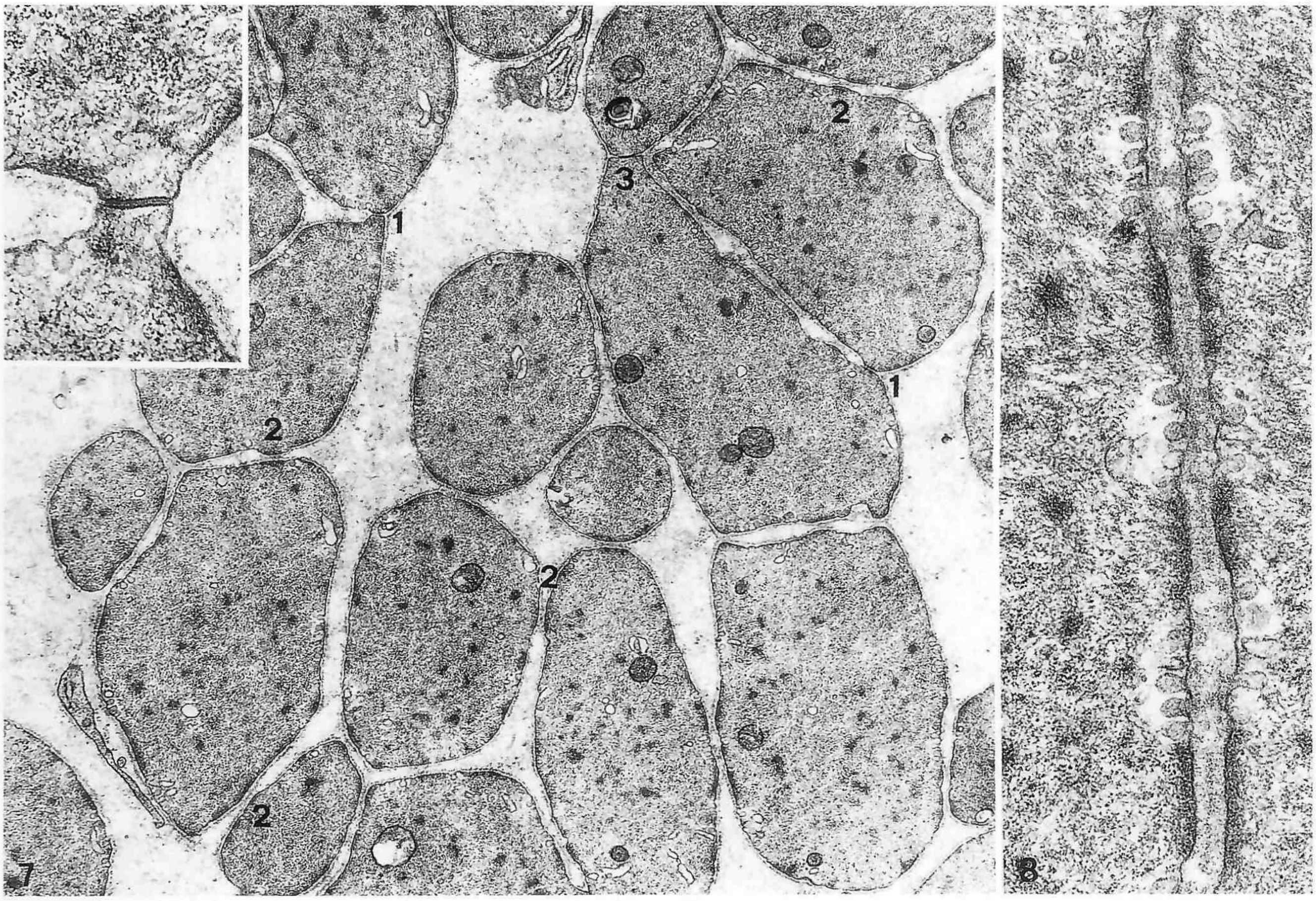


Figures 4 to 6 Human stomach. Circular muscle layer of the corpus.

FIGURE 4 Outermost (myenteric) area. × 700.

FIGURE 5 Intermediate area. × 700.

FIGURE 6 Innermost (submucosal) area. Semithin sections, toluidine blue. × 700.



Figures 7 and 8 Human stomach. Circular muscle layer of the corpus, outermost (myenteric) area.

FIGURE 7 Smooth muscle cells with a regular outline, connected each other by 'desmosome-like' junctions (1), 'intermediate' junctions (2) and gap junctions (3). Inset: detail of one gap junction. × 15,000; inset: × 37,500.

FIGURE 8 Detail of four 'intermediate' junctions of the same length and facing two contiguous smooth muscle cells, perfectly corresponding each other; areas provided with caveolae are located between every 'intermediate' junction and they too are of the same length and perfectly corresponding each other. × 37,500.

divided into three areas: an outermost one, facing the myenteric plexus, an innermost one, facing the tela submucosa and a third one, intermediate between the two.

Outermost area (myenteric area) - This zone is the thickest and is made up of typical smooth muscle cells 4.80 ± 0.15 μm in diameter and with a regular profile (Figs. 4 and 7). The supporting connective tissue is not abundant. Collagen and elastic fibrils can be present, especially in older subjects (aged 70 years). The smooth muscle cells contact each other by means of: 1) gap junctions (Fig. 7 and inset); 2) 'intermediate' junctions (Figs. 7 and 8); 3) 'desmosome-like' junctions (Fig. 7) and 4) interlockings. The 'intermediate' junctions are regularly spaced and their length (about 0.5 μm) identical to the interposed areas with caveolae; this arrangement can also perfectly correspond to that of the opposite cell (Fig. 8). The gap junctions are more frequent (see Table 1) in the greater curvature. Sparse, single, small cisternae of the smooth endoplasmic (sarcoplasmic) reticulum are only peripherally located.

Intermediate area - Gradually, from thin smooth muscle cells, with a regular profile, we pass to cells similar to the fundic ones, with a larger diameter (7.75 ± 0.30 μm) and a very irregular profile (Figs. 5 and 9). The supporting connective tissue also gradually increases (Figs. 5 and 9). Collagen and elastic fibrils are present and the amount of

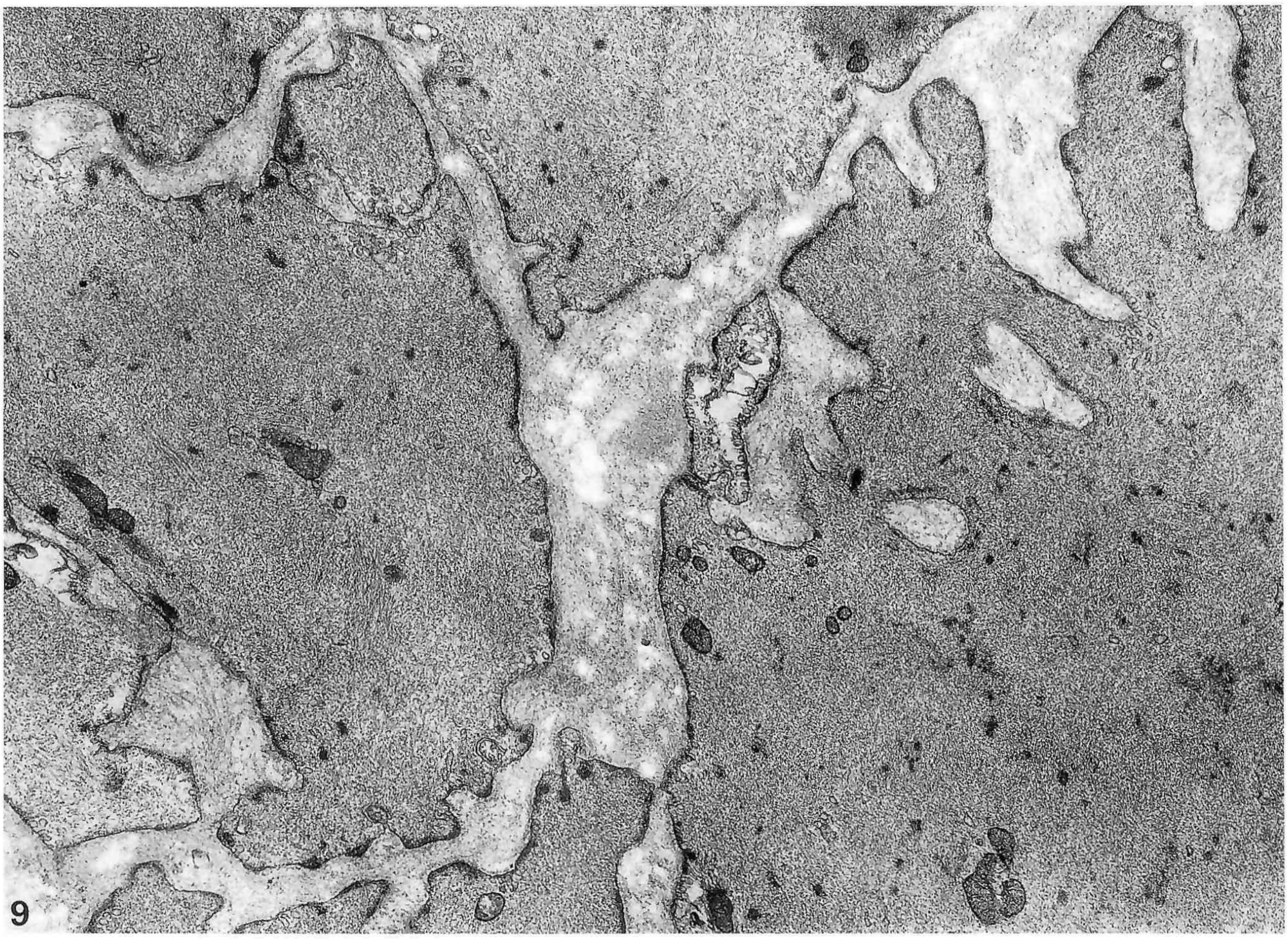


FIGURE 9 *Human stomach. Circular muscle layer of the corpus, intermediate area.* Large and irregularly shaped smooth muscle cells (compare w Fig. 7) separated by wide connective tissue spaces. × 15,000.

TABLE 1

Number of intramuscular nerve fibers and gap junctions per 100 cross-sectioned smooth muscle cells. Medium values ± standard errors

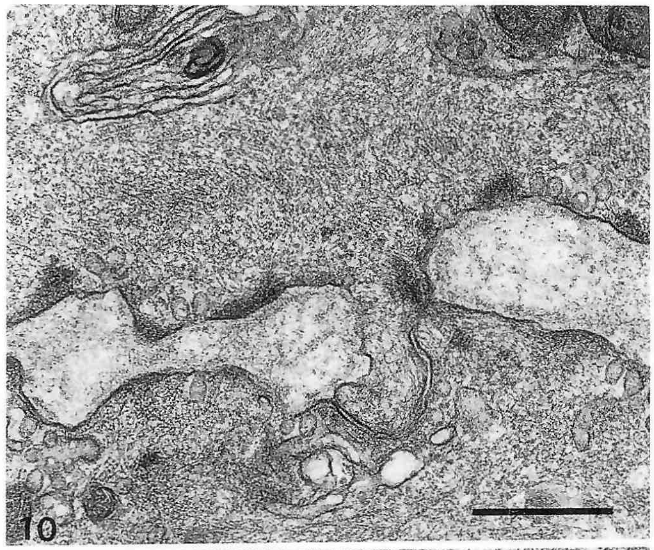
	Fundus	Corpus			Antrum
		Between curvatures + lesser curvature	Greater curvature		
			Intermediate region	Outermost region	
Number of observed fields	24	41	16	24	36
Nerve fibers	$2.2^{+1.0}_{-0.8}$ *	5.9±0.7	$1.5^{+0.8}_{-0.7}$ *	1.5±0.4 *	1.7±0.4 *
Gap junctions	None	6.6±0.9	$9.8^{+2.6}_{-2.2}$ *	$16.5^{+1.4}_{-1.3}$ *°	$15.5^{+1.6}_{-1.5}$ *°

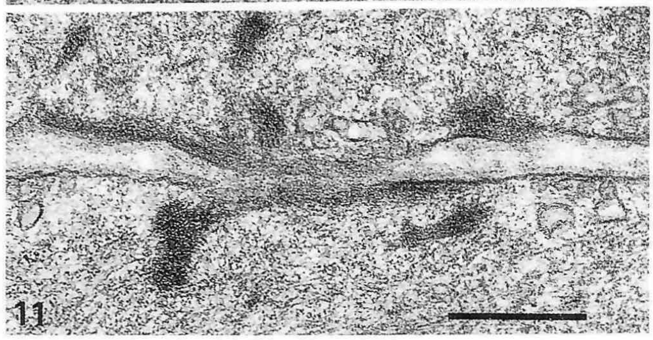
* p<0.001 compared with corpus (between curvatures plus lesser curvature); °p<0.05 compared with corpus (greater curvature, intermedi region).

collagen increases with age. Another peculiar characteristic of these smooth muscle cells is the aspect of their dense bands. These are, in fact, very short (0.15, 0.25 μm) and located at a short distance (0.2 μm) from one another (Figs. 9 to 11). Some of them protrude into the cytoplasm (Figs. 9 to 11) for 0.25 μm, others have a normal thickness (0.05 μm). Long, ramified cisternae of the sarcoplasmic reticulum form clusters at the periphery of the cells nearest to the tela submucosa (Fig. 10). The types of contact areas are the same as those described for the outermost cells, but the gap junctions are less frequent (see Table 1). Those present in the greater curvature, however, are also here more numerous than in the other parts of the corpus.

Innermost area (submucosal area) - This area is made up of extremely flattened, irregularly shaped smooth muscle cells, forming a net with large meshes filled with a supporting connective tissue rich in collagen and elastic fibers (Figs. 6 and 12 to 14). Also in this area the collagen fibrils increase with age. It is impossible to define exactly where this area begins, since the smooth muscle cells of the intermediate area gradually assume the features typical of the innermost area, but it is surely made up of no more than 10-12 layers of cells. Their area equivalent transverse diameter was 4.68 ± 0.20 μm; the ratio between the minor and major axes was 0.45 ± 0.03 μm. The innermost smooth muscle cells have on their submucosal face dense bands 0.2 μm thick, strongly electron-dense and several microns long or continuous (Figs. 13 and 14). Moreover, projections at varying distances from each other protrude for a considerable length (about 0.5 μm) into the cytoplasm (Fig. 13). On the opposite surface, on the contrary, the dense bands look similar to those observed in the intermediate area. Portions of the plasma membrane rich in caveolae can also be wide and the elastic fibers are often inserted at this level (Fig. 15). However, the elastic fibers or amounts of basal lamina-like material may bridge two contiguous smooth muscle cells, also inserted at the level of corresponding dense bands (Fig. 14).

The contact areas are: 1) 'intermediate' junctions (Figs. 13 and 14); 2) 'desmosome-like junctions (Fig. 14) and 3) apposition or interlockings (Figs. 13 to 16). No gap junctions were observed. The 'intermediate' junctions can be found at short intervals (0.05 μm), one following another (Fig. 14). The interlockings are made up of the apposition for a variable length of the plasma membranes of two-three contiguous cells; caveolae can be present at this level, sometimes perfectly corresponding with each other (Figs. 15 and 16). The sarcoplasmic reticulum, as in the intermediate area, is made up of clusters of long ramified cisternae, but which are now located either along the plasma membrane, also in correspondence with the contact areas (Figs. 13 and 16), or in the innermost cytoplasm (Fig. 16).





Figures 10 and 11 Human stomach. Circular muscle layer of the corpus, intermediate area.

FIGURE 10 Detail of one contact area between two smooth muscle cells. The protrusion of one cell is lodged in a groove of the second one. Just below this groove, clusters of cisternae of the smooth endoplasmic reticulum are located. × 37,500.

FIGURE 11 Detail of the dense bands characteristic of the smooth muscle cells of this area: they are short and protrude for a variable length inside the cytoplasm. × 37,500.

Antrum

The smooth muscle cells of this area have all the same features (shape, size, types of contact areas, sarcoplasmic reticulum, dense bands) as those of the outermost area of


12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13


E
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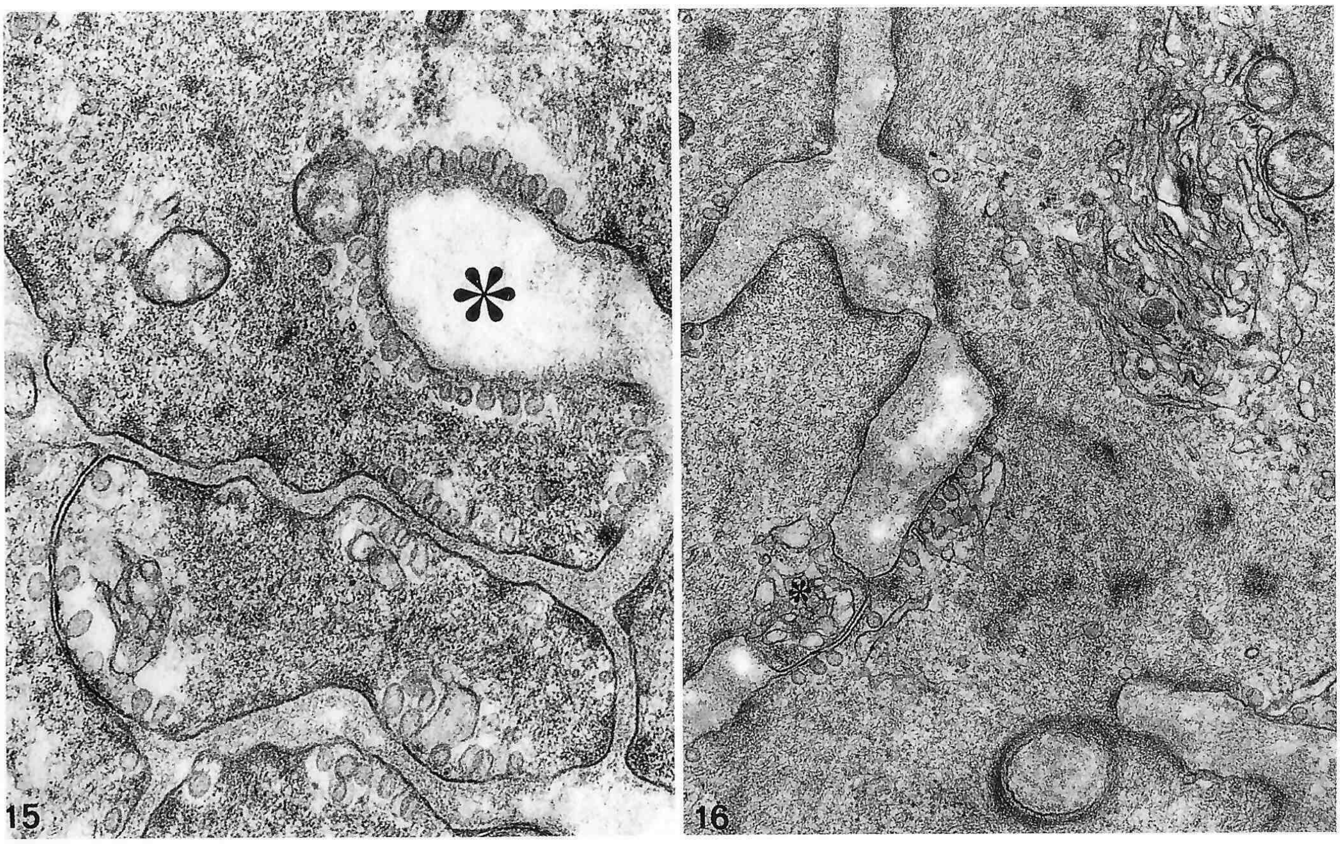
14



Figures 15 and 16 Human stomach. Circular muscle layer of the corpus, innermost (submucosal) area.

FIGURE 15 Detail of a long contact area between two smooth muscle cells, where both the two contiguous plasma membranes are provided with caveolae. The asterisk indicates one elastic fiber partially surrounded by a wide area of plasma membrane full of caveolae. × 37,500.

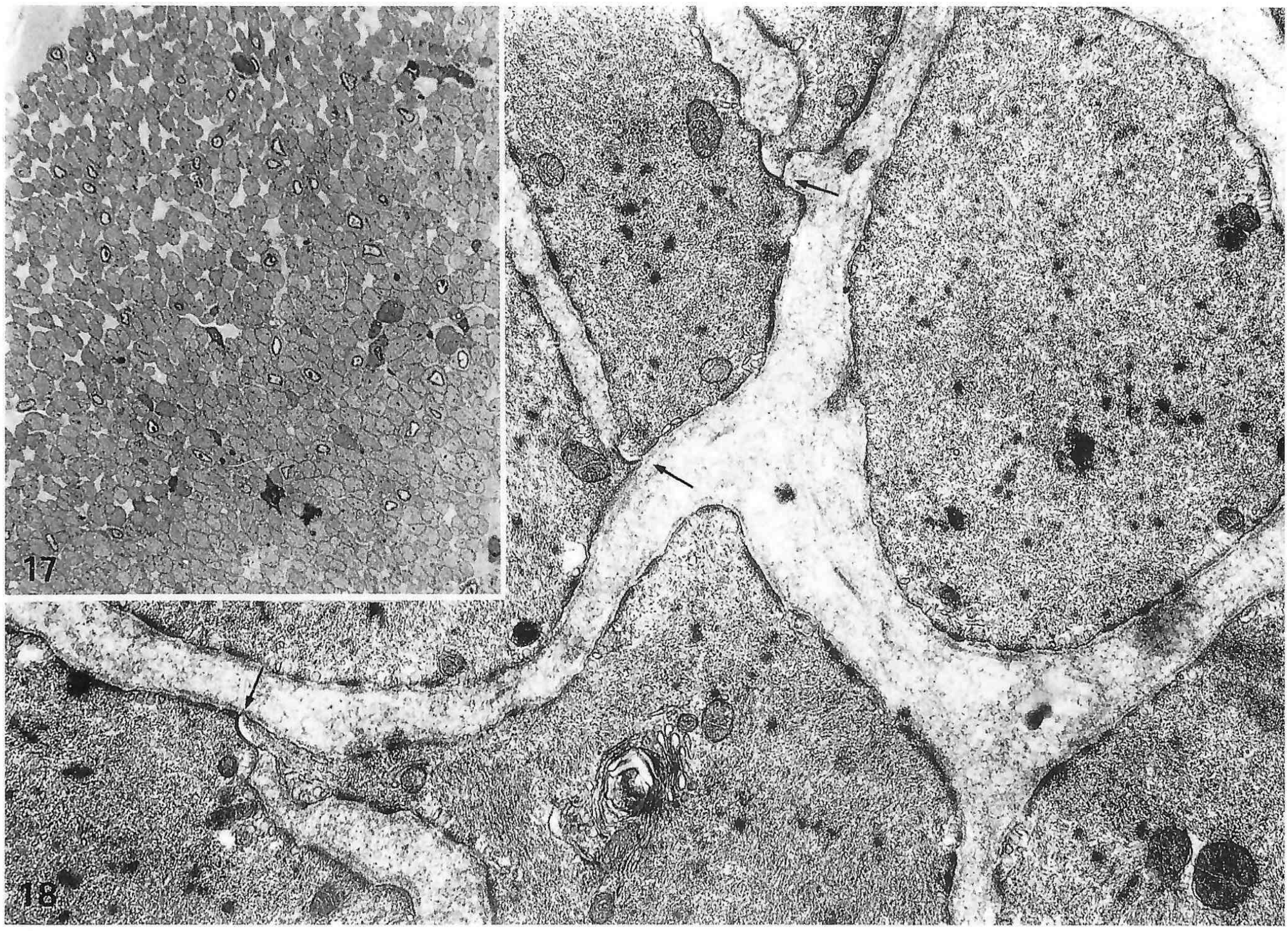
FIGURE 16 Clusters of cisternae of the smooth endoplasmic reticulum are distributed both in the center and the periphery of the smooth muscle cells. These cisternae are also grouped along the contact areas (asterisk). × 25,000.

Figures 12 to 14 Human stomach. Circular muscle layer of the corpus, innermost (submucosal) area.

FIGURE 12 On the left side: the submucosa. The smooth muscle cells of the first rows are flattened and separated by abundant connective tissue. × 11,250.

FIGURE 13 Detail of the extremely long dense bands of the submucosal surface of the smooth muscle cells of the first row. SM: submucosa. The arrows indicate some protrusions inside the cytoplasm of the dense bands. Cisternae of the smooth endoplasmic reticulum are grouped everywhere. × 20,000.

FIGURE 14 Detail of the dense bands and of connections between the smooth muscle cells. E: elastic fibers bridging these cells. Simple arrows: short 'intermediate' junctions; double arrows: long 'intermediate' junctions. × 20,000.



Figures 17 and 18 Human stomach. Circular muscle layer of the antrum.

FIGURE 17 On the upper side the tela submucosa. Semithin section, toluidine blue. × 500.

FIGURE 18 Smooth muscle cells with a conventional aspect of both cell profile and dense bands. The arrows indicate three gap juncti× 20,000.

the corporal circular muscle layer (Figs. 17 and 18). The mean diameter was 4.57 ± 0.09 μm. This diameter and that found for the outermost area of the corpus were significantly lower than those of the fundus and the intermediate area of the corpus ($p < 0.001$). Moreover, also in this area, the supporting connective tissue gradually increases from the outermost to the innermost portion of the circular muscle layer. The gap junctions are very numerous (see Table 1), as in the greater curvature. Their number increases from the outermost to the innermost area of the circular muscle layer, although this increase is not significant. Medium values and standard errors of gap junctions/100 smooth muscle cells were 17.7 (+2.3/−2.1) close to the submucosa and 12.9 (+2.1/−1.9) deep in the muscle layer.

Nerve fibers and nerve endings

Differences can be observed in the distribution of ne fibers and nerve endings according to the area examin Differences have also been found in the relationships l tween nerve endings and smooth muscle cells. We h grouped together fundus, antrum and greater curvat on one side and corpus and lesser curvature on the ot side since, even if not identical, they show so many si larities that only 2 separate descriptions are enough.

Fundus, antrum and greater curvature

The nerve fibers (Fig. 19) containing a large number axons (15-20) are mainly located at the periphery of la

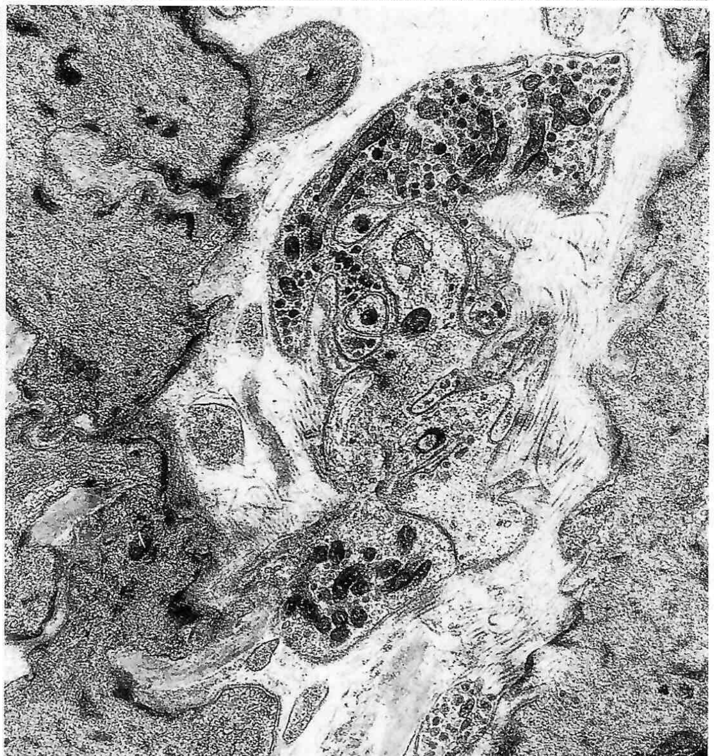


FIGURE 19 *Human stomach. Circular muscle layer of the corpus.* Varicose axons, one of which filled with synaptic vesicles also between one varicosity and another. The varicosities (or nerve endings) lie 'distant' (arrows) from the smooth muscle cells. × 19,000.

FIGURE 20 *Human stomach. Circular muscle layer of the greater curvature.* Detail of one axon showing three varicosities (1, 2, 3) filled with synaptic vesicles and lying 'distant' (1, 2) or 'near' (3) one smooth muscle cell. × 25,000.

FIGURE 21 *Human stomach. Circular muscle layer of the fundus.* The nerve endings are irregularly shaped, enormous, devoid of the glial sheath to a wide extent and rich in synaptic vesicles. × 13,000.

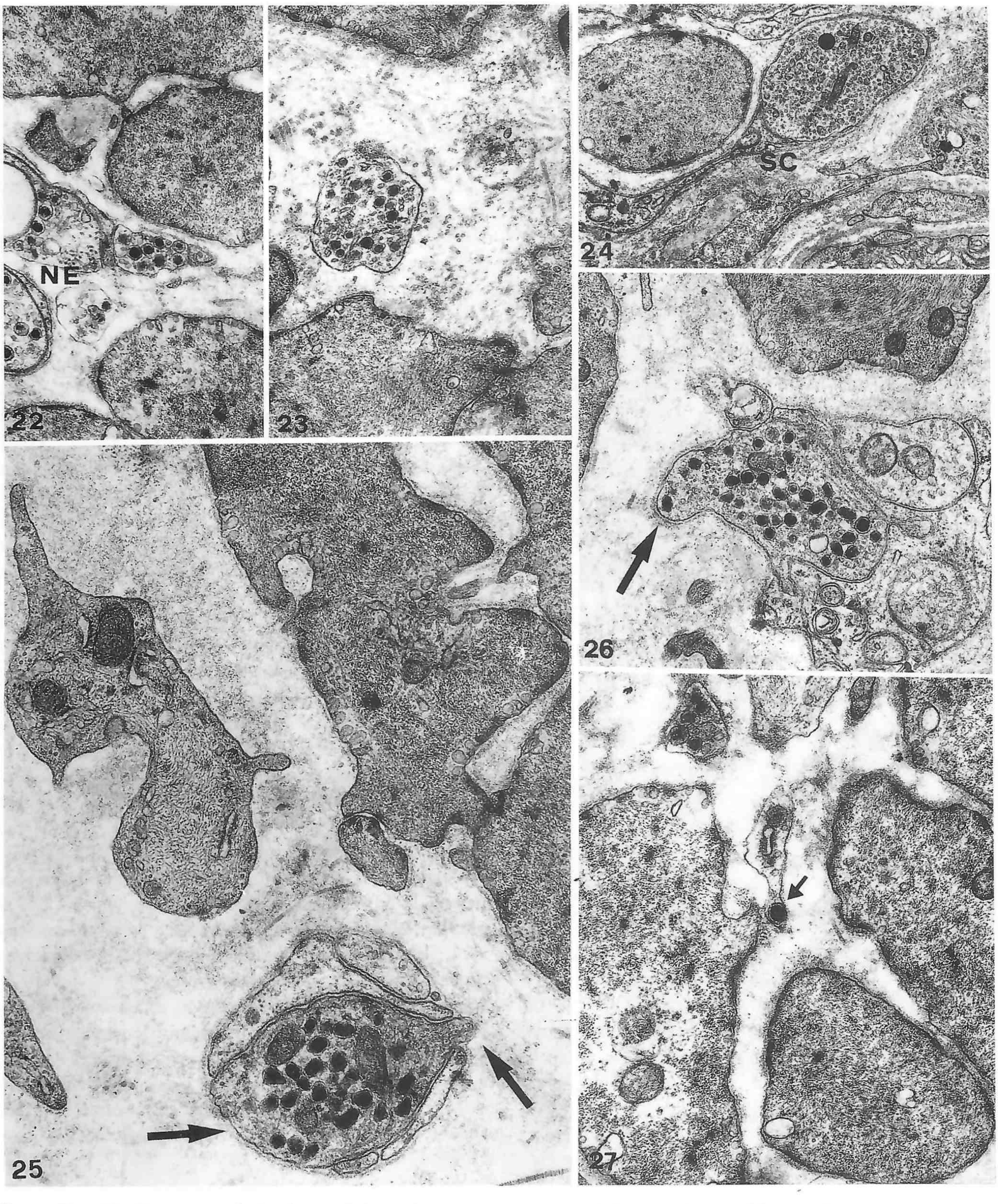
groups of smooth muscle cells, either scattered everywhere inside the thickness of the circular muscle layer (fundus and greater curvature), or along its submucosal border (antrum). A few small nerve fibers (2-3 axons) also enter the muscle cell group, among the smooth muscle cells (Fig. 20). These nerve fibers possess varicosities rich in synaptic vesicles and are only partially devoid of the glial sheath (Figs. 19 and 20), except in the fundus, where the nerve endings may be large, with an irregular profile and devoid of the glial sheath for a wide extent (Fig. 21). These varicosities (or nerve endings) are always 200 nm or more distant from the smooth muscle cells (Figs. 19 to 21), and, therefore, show the same relationships with the smooth muscle cells already described for those present in other segments of the alimentary tract.
The synaptic vesicles we could observe were of two types: small agranular ones, 50 nm in diameter (Fig. 19), and large granular ones, 150 nm in diameter (Figs. 20 and 21). The latter may differ in their profile, spherical or oval, and in the electrondensity of their granules (Figs. 20 and 21). The granules of a moderate density may have a discontinuous outer delimiting membrane and a non homogeneous content. Usually, the nerve endings contain a mixture of these vesicles but, rarely, may contain only the large granular vesicles with spherical dense core (Fig. 20). The synaptic vesicles can also be contained in the restricted parts of the axon between two varicosities (Fig. 19).
The number of the nerve fibers is almost similar in all these areas, only slightly lower in the greater curvature than in the other parts. This number, on the contrary, is significantly lower (two-three times less) than in the other parts of the corpus (see Table 1).

Lesser curvature and corpus

The nerve fibers are mainly located inside the muscle cell groups (Figs. 22 to 27) and most of them contain a small number of axons (3-4). Moreover, it should be mentioned that in these areas the nerve endings have several unusual peculiarities. In fact, they can be: 1) enormous (1-2 μm) (Fig.,24); 2) devoid of the glial sheath for such a large extent (Figs. 22, 23 and 27) to frequently seem in section to be completely free of the Schwann cell covering (Figs. 23 and 27); 3) completely (Figs. 22, 23 and 27) or partially (Figs. 22 and 27) devoid of the basal lamina; 4) can directly contact the smooth muscle cells with a gap of 20 nm devoid of any basal lamina (Figs. 28 and 29).
The Schwann cell, in correspondence with the varicosity (or the nerve ending), often opens on one (Fig. 26) or on two opposite sides (Fig. 25). In this case only the centre of the varicosity (or nerve ending) is full of synaptic vesicles and the uncovered portions of the axon protru out of the glial sheath with 1-2 pseudopod-like projectior covered by a basal lamina and containing neurotubul and an amorphous substance (Fig. 25). These projectio run from the nerve fiber towards the neighbouri smooth muscle cells (Fig. 27) and, reaching them, cor in direct contact with them (Figs. 28 and 29). In the co tact area, the smooth muscle cell forms a groove in whi the nerve ending, now provided with synaptic vesicl fits perfectly. The synaptic vesicles we observed in t corpus are mostly of the large oval (Figs. 25 and 26) round (Figs. 22, 23 and 25 to 29) granular type, bo with a core of high (Figs. 22, 23 and 25 to 29) and mo erate (Figs. 22 and 26) electron-density; whereas, in t lesser curvature (Figs. 23 and 24), the nerve endings co taining a prevalence of the small agranular vesicles can l frequently found. Everywhere all the nerve endings rectly contacting the smooth muscle cells contain mair or only large granular vesicles.
In both these areas the small nerve fibers are significant more numerous (see Table 1), two-three times more th in the other parts of the stomach.

DISCUSSION

The circular muscle layer of the human stomach sho several peculiarities when viewed with the electron micr scope which differentiate it from that already describ in other parts of the human alimentary canal (Fausson Pellegrini and Cortesini, 1983, 1984, 1985). Moreover, t structure and distribution of the smooth muscle cells a nerve endings differ in the various areas examined.
The smooth muscle cells in the *fundus* have unusual fe tures in respect of the visceral ones and in particular all the antral and most of the corporal ones, showing irregular profile, a large diameter (7.97 μm) and an enc mous extension (1-2 μm instead of 0.5 μm) and thickne of the dense bands (1-2 μm instead of 0.05 μm). Mor over, the gap junctions are absent in this area and t supporting connective tissue is abundant and rich in ela tic fibers; peculiar connections between it and t smooth muscle cells have also been observed. The ner endings in the fundic area are, as in the antrum and gre er curvature, few, distant 200 nm or more from t smooth muscle cells, but unusually wide and have exte sive areas devoid of glial sheath and filled with lar granular vesicles.
As the fundic one, also the arrangement of the circul muscle layer of the *corpus* is different from that of t other parts of the gut. Moreover, this layer is more co plex than that of the fundus and antrum. In fact, consi ering that the smooth muscle cells in all areas examin differ according to their location inside the thickness



Figures 22 to 27 Human stomach. Circular muscle layer of the lesser curvature and of the corpus interposed between the two curvatures.

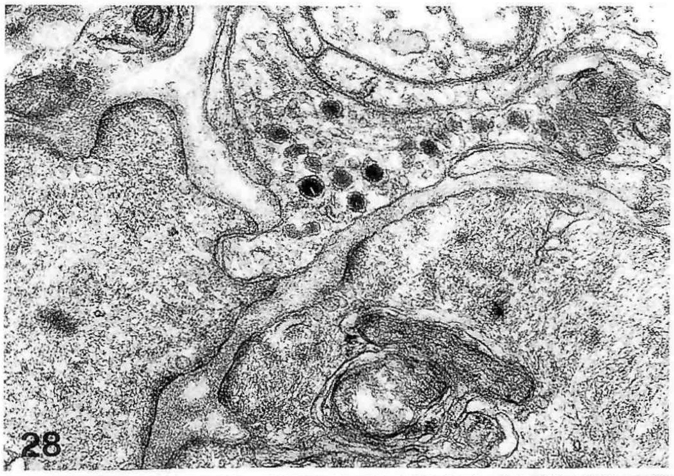
FIGURE 22 Nerve endings (NE) devoid of the glial sheath or only partially covered by it. × 20,000.

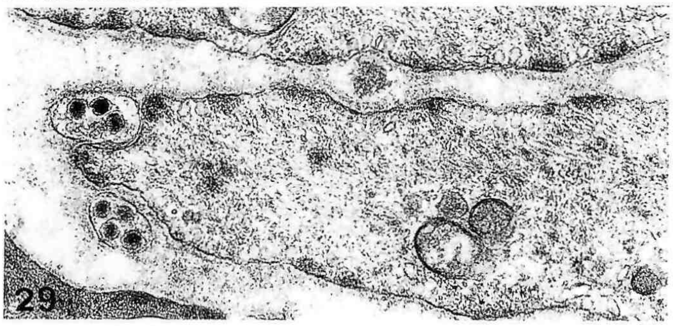
FIGURE 23 One nerve ending completely devoid of both glial sheath and the basal lamina. × 20,000.

FIGURE 24 Lesser curvature. One enormous nerve ending (round mark) filled with small agranular vesicles and hardly covered in a very limite part by the Schwann cell (SC). × 12,000.

FIGURE 25 The nerve ending protrudes on one side by means of one pseudopod-like projection towards the neighbouring smooth muscle cells and is devoid of the glial sheath for a large extension in the opposite side of the protrusion (arrows). The large granular vesicles contained in th nerve ending are either round or oval shaped. × 32,500.

FIGURE 26 One nerve ending protrudes by means of a pseudopod-like projection (arrow) towards the neighbouring smooth muscle cells. Th large granular vesicles contained in this nerve ending show different electron density and shape. × 20,000.





Figures 28 and 29 Human stomach. Circular muscle layer of the lesser curvature and the corpus interposed between the two curvatures.

FIGURE 28 Corpus. One protrusion of one nerve ending (varicosity?) goes towards one smooth muscle cell and 'closely' contacts it, perfectly lodged in a smooth muscle cell groove. × 25,000.

FIGURE 29 Lesser curvature. Two nerve endings, one 'close' and one 'near' the same smooth muscle cell. × 25,000.

the circular muscle layer, this has been ideally subdivided in three parts. These parts, however, cannot be anatomically delimited from each other, since the structural differences of the smooth muscle cells we found (profile and size, number of gap junctions, size, shape and extent of the dense bands and richness in the sarcoplasmic reticulum), gradually change from the myenteric to the submucosal surface. Similarly, the connective tissue increases. Moreover, it has been found that the gap junctions are about two times more frequent in the greater curvature than in the other parts of the corpus.

The nerve endings also differ in several ways according to their location in the various corporal areas and according to their location inside the thickness of the circular muscle layer itself. *In the greater curvature*, most of the nerve bundles contain a large number of axons (15-20) and are mainly located at the periphery of large groups of smooth muscle cells; only rarely do nerve fibers (2-3 axons each) provided with varicosities enter the smooth muscle ce groups. All nerve endings lie 200 nm or more distar from the smooth muscle cells and most of them contain mixture of small agranular and large granular vesicle: with a prevalence of the large granular ones. On the cor trary, *in the lesser curvature and corpus*, most of the nerv fibers contain few axons (3-4), are mainly located insid the muscle bundles and are two-three times more than i the other parts of the stomach. Some nerve endings ar devoid of the glial sheath for wide extensions. It is, there fore, easy to find nerve endings appearing in one sectio completely devoid of the glial sheath. Moreover, thes nerve endings may directly contact the smooth muscle cell (with a gap of 20 nm devoid of basal lamina). The syn aptic vesicles here too are of two types, often mixed i the same axon. In the lesser curvature, however, severa nerve endings possess a prevalence of small agranula vesicles, but those directly contacting the smooth muscl cells always contain mainly or only large granular vesicle: In the *antrum*, the smooth muscle cells of the circula muscle layer, at variance with those of the corpus, bu similarly to those of the fundus, have all the same feature This feature, however, and the cell size are identical t those of the outermost smooth muscle cells of the corpu and, therefore, identical to that commonly described fc the other parts of the alimentary canal. The gap junction are very numerous, as in the greater curvature, with gradual decrease from the submucosal to the myenteri regions. Moreover, characteristically, the nerve fibers cor taining both a large and a low number of axons ar mainly distributed along the submucosal border of th circular muscle layer. However, similarly to the other ga: tric areas, some of them are scattered inside the smoot muscle bundles. These nerve fibers contain 2-3 axor each, possess the same size, features and relationshir with the smooth muscle cells and are few in number a those found in the greater curvature.

In conclusion, from our findings it results that th smooth muscle cells of the human gastric circular muscl layer show many structural differences in respect to thos commonly described for the other parts of the alimentar tract in man (Faussone-Pellegrini and Cortesini, 198: 1984, 1985) and other mammals (see Gabella, 1987, fc review of the literature).

In particular, the smooth muscle cells present in the fur dus are very peculiar. They look similar, in the thicknes and asymmetric distribution of their dense bands, t those described in the arteriolar wall (Gabella, 1983). we consider this together with the absence of gap junc tions, the scarcity of innervation and the enormous quar tity of elastic fibers present in the fundic wall, we se that in fact the fundus could well function as a reservoi Moreover, the irregular profile of the fundic smooth mu:

cle cells we observed could be explained by the fact that the smooth muscle cells need to maintain their contacts during the fundic dilatation ('the receptive relaxation' described by Cannon and Lieb, 1913) in the presence of an abundance of supporting connective tissue. This irregularity, however, might be enhanced in our specimens by an ineffective distension of the fundic fragment, since we stretched it out only in one direction, not considering its elastic content.

In the circular muscle layer of the corpus, smooth muscle cells identical to those described in the other parts of the alimentary tract are present in the myenteric portion and smooth muscle cells with some unusual features are present in its submucosal portion. It is not a new finding that in the circular muscle layer of the alimentary tract different areas having smooth muscle cells with different features and with a constant location might be identified. But, at variance with the human small and large intestine (Faussone-Pellegrini and Cortesini, 1983, 1984), where the circular muscle layer can be subdivided into two easily identifiable portions, in the gastric corpus these portions seem to be three and without any clear boundary. In the circular muscle layer of the antrum on the contrary, all the smooth muscle cells are of a unique morphology, quite identical to that described in the other parts of the gut.

Structural differences between the gastric smooth muscle cells and the ileal, colonic or esophageal ones have not been reported in the literature. The only peculiarity mentioned is that reported for those of the lesser curvature of the canine gastric corpus (Daniel *et al.*, 1984) where a high number of gap junctions has been found. We can confirm this also for the smooth muscle cells of the human corpus and antrum even if we counted a low number of them. Moreover, in our specimens, the greater curvature and the antrum are richer in them than the other parts of the stomach. We can also add that in the greater curvature the gap junctions gradually decrease from the myenteric to the submucosal portion and that they are not found at all in the innermost portion of the corporal circular muscle layer and in the fundus.

The possibility of the existence of structural differences between the gastric smooth muscle cells according to their location inside the thickness of the circular muscle layer was proposed by Bauer and Sanders (1985) and Bauer *et al.* (1985*a,b*) in the canine antral circular muscle, since they found that the slow waves originating at different depth of the circular muscle layer behave in different ways and propagate from the myenteric towards the submucosal portion of it with a gradual decrease of velocity. It seems to us that a correlation may be suggested between these data and our findings on the human antrum considering that we found some differences between the myenteric and the submucosal portion (number of ga junctions and amounts of the supporting connective ti: sue). While a morpho-functional correlation between ca nine antral physiological data and human corpor: morphological data may be difficult, since it has to cor sider the different electrical and mechanical events occu ring in the antrum and corpus and the different anim: species examined (man and dog); we cannot exclud however, that a similar mechanism is at the basis of th electrical wave propagation in the three dimensions i these different gastric areas and in these two animal sp cies. But we have to remember that, at variance with th other gastric areas, the smooth muscle cells of the corpu possess structural differences within the thickness of th circular muscle layer itself which may influence both th electrical and mechanical behaviour of this area. It is po: sible, for example, that the not uniform thickness an distribution of the dense bands of the innermost locate smooth muscle cells, together with the major abundanc of their supporting connective tissue and their differer diameter, is at the basis of both the mechanisms whic regulate the circumferential propagation of the contracti waves and the diameter variation of the gastric lumen.

The findings we obtained on a different distribution c the nerve endings present in the various areas of the hu man gastric circular muscle may agree with the data re ported by Wattchow *et al.* (1988) in these same areas ob tained from patients operated for pathologies similar t ours. These Authors, in particular, found important di ferences in the number of immunoreactive nerve fibre supplying the muscle that exhibits the electrical beh: viour of tonic muscle (the fundus) and the muscle th: exhibits the electrical behaviour of rhythmic muscle (co pus and antrum). These Authors also reported that th human gastric nerve endings contain several types of ne rotransmitters, some of which are colocalized in the sam axon; indeed, with electron microscopy we found mo than one type of synaptic vesicle, often mixed in th same axon (see the axons of Figs. 22 and 26, each cor taining three different types of synaptic vesicles). Simil: data have been reported in the canine stomach (Oki an Daniel, 1974; Daniel *et al.*, 1984).

However, we must add more information about the ir nervation of the human stomach. In particular, we foun important differences in the nerve ending distributio and in the relationships between them and the smoot muscle cells not only between tonic (fundus) and rhyth mic (corpus and antrum) muscle, but also according t the various parts of the stomach performing rhythmic a tivity. One example is represented by the greater curv: ture, considered as the site of origin of the driving pac maker (slow waves with highest intrinsic frequency; Kell and Code, 1971), and the antrum which our finding showed to be less innervated than the other parts of the corpus (which, on the contrary, are richly innervated, about 1.5%-1.7% versus 5.9%). It seems to us important to point out also that the afore-mentioned areas (greater curvature and antrum) have, at variance with the other parts of the corpus, a higher number of gap junctions (9.8%-16.5% versus 6.6%) and that the fundus lacks in them; whereas both the highest rhythmic areas and the tonic fundus are poorly innervated.

Furthermore, we found that in the corpus, except the greater curvature, the nerve endings are also large, devoid of the glial sheath for a broad extent and, often, directly contacting the smooth muscle cells. Their great size and the existence of wide surfaces devoid of the glial sheath may suggest that the neurotransmitters (possibly released in large quantity) can simultaneously reach a great number of smooth muscle cells. Moreover, where the nerve endings and the smooth muscle cells directly contact, the gap to cross (20 nm) and the delay between the release of the neurotransmitter and its effect upon the smooth muscle cells are minimum. These data suggest that in these areas the smooth muscle cells are under intensive and direct nerve control.

In summary, from our research, structural differences in both smooth muscle cells and nerve endings have been observed among the gastric areas performing different electrical and mechanical activities; most of these differences have been found between the fundus, which is usually considered as a reservoir electrically silent (smooth muscle cells with a spontaneous tone and no spontaneous fluctuations in their potentials), and both the corpus and antrum, which possess both tonic and rhythmic activities. However, differences are present also between corpus and antrum. Therefore, we think that the human stomach can be really divided, not only from both an anatomical and a physiological point of view (Kelly, 1981; Meyer, 1987; Szurszewski, 1987), but also from a microscopical point of view, in at least three parts: fundus, corpus, antrum, each part possessing a specific structure and performing a specific function.

The differences in the structure of the smooth muscle cells we have found among the three gastric areas and inside the thickness of the corporal circular muscle layer can be hypothetically related to the differences in the velocity of contractile wave propagation and to the mechanical requirements for the diameter variation of the gastric lumen. The differences in the distribution of the nerve endings and in their relationships with the smooth muscle cells between the tonic fundus and the rhythmic corpus and antrum, and their structural and numerical differences between the corpus and antrum and among the various parts of the corpus, seem to indicate that the electrical activities between these areas depend on a different nerve control of the smooth muscle cell activity. Howe er, before accepting that the smooth muscle cell electri activities are influenced by the nerve tissue only, it shou be remembered that the cells, the so-called interstit cells of Cajal, present in the intestinal and esophag muscle wall and which show special relationships to bo nerve endings and smooth muscle cells, have been fou in the fundus and corpus of man (Faussone-Pellegri 1987). In a future paper the presence of these cells al in the antrum will be ascertained and their structure a possible different relationships and distribution inside t circular muscle layer in the gastric areas here examin will be reported and discussed, especially bearing in mi that several Authors (Faussone-Pellegrini *et al.*, 197 Thuneberg, 1982; Faussone-Pellegrini and Cortesi 1983, 1985; Suzuki *et al.*, 1986; Hara *et al.*, 1986) ha suggested that this is the cell type which is able to gen ate the slow waves usually recorded in the gastrointestir muscle wall.

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