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An ultrastructural study of the interstitial cells of Cajal of the human stomach

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SUMMARY - The interstitial cells of Cajal of the human stomach were studied at the electron microscope. These cells have an exceptionally elongated shape and several lateral branches. Their cytoplasm characteristically possesses cisternae of smooth endoplasmic reticulum and filaments. A capsular-like structure surrounds them and joins them to each other and to the neighbouring nerve endings and smooth muscle cells, and so they all make up anatomical units. Elastic fibres also make bridges between these cells and between smooth muscle cells and nerve endings. Despite these common characteristics, differences in cell number, distribution in respect of the muscle bundles and some cytoplasmic features have been found, depending on where these cells are located. In fact, there are few interstitial cells in the fundus, poor in filaments and branches, and only located inside the circular muscle layer. In the corpus and antrum they are many, rich in filaments and with their numerous branches make interconnected networks, one inside the circular muscle layer, another apposed to its myenteric surface and, in the antrum, a third one apposed to its submucosal surface, which accompany analogous nerve networks. Substantial differences in the contacts between the interstitial cells and the smooth muscle cells and nerve endings have not been found. For their ultrastructural characteristics a smooth muscle nature has been suggested for these cells. A correlation has also been attempted between the electrical and mechanical activities performed by the different gastric areas and the interstitial cell structure and arrangement.

Key Words *stomach - man - interstitial cells of Cajal - ultrastructural study*

INTRODUCTION

We have already reported (Faussone-Pellegrini *et al.*, 1989) the findings obtained by electron microscopy on smooth muscle cells and the nerve endings present in the circular muscle layer of the human stomach. According to the level examined, we found differences in the structure and relationships of both smooth muscle cells and nerve endings. A musculo-elastic and poorly innervated wall characterizes the fundus, which acts mainly as a reservoir. Moreover, its tonic muscle lacks in gap junctions. A noticeable richness in them, on the contrary, characterizes the rhythmic muscle of the corpus and antrum. Furthermore, in the corpus, where the function of food mixing and grinding is performed and where the peristaltic wave is non-occlusive, we found a circular muscle layer with a very peculiar complex organization. In fact, the structural characteristics of the smooth muscle cells gradually change from the outermost to the innermost portion of the circular muscle layer itself. Moreover, we found that the smooth muscle cells of the greater curvature are particularly rich in gap junctions but did not have an extensive nerve control, whereas in the other parts of the corpus the smooth muscle cells were under a direct nerve control (a gap of 20 nm devoid of basal lamina between the smooth muscle cells and the nerve endings) and possess a lower number of gap junctions. In the antrum, where the function to propel the food towards the pylorus is performed and where the peristaltic wave is occlu-

sive, the organization of the circular muscle layer is different from both the fundus and corpus. In fact, like in the fundus, its smooth muscle cells have a unique morphology, which is, however, identical to that described for the outermost portion of the corporal circular muscle layer. Moreover, the gap junctions are numerous and the nerve fibers few, as in the greater curvature.

The so-called interstitial cells of Cajal (ICC) represent an intriguing cell type characteristic of some areas of the gut muscle wall. At present they have been studied most extensively in the rodent intestine, both by light and electron microscopy. The possible presence of them in the stomach has not yet been demonstrated by light microscopy, whereas some, but incomplete, information we have from electron microscopy. In fact, the presence of these cells in the circular muscle layer of the canine lesser curvature (apparently few in number, about 1 per 900 smooth muscle cells) (Daniel *et al.*, 1984) and in the muscle coat of both human fundus and corpus (Faussone-Pellegrini, 1987) has been reported. As in other parts of the alimentary tract, this cell type showed ultrastructural features typical of the cells electron microscopically identified as ICC and was characteristically in contact with both the smooth muscle cells and the nerve endings. At variance with the small and large intestine of man (Faussone-Pellegrini and Cortesini, 1983) and of other mammals (Gabella, 1974, 1979; Thuneberg, 1982; Rumessen *et al.*, 1982; Faussone-Pellegrini, 1983*a,b*, 1984, 1985*a,b*) and similar to the esophagus of man (Faussone-Pellegrini *et al.*, 1977; Faussone-Pellegrini and Cortesini, 1985) and opossum (Daniel *et al.*, 1979; Daniel and Posey-Daniel, 1984) most of the gastric ICC were scattered throughout the inside of the circular muscle layer (the so-called S-ICC, Faussone-Pellegrini, 1987) and were not confined to a determined zone. However, a certain number of ICC, similar but not identical to the afore-mentioned ones (the so-called S-MP-ICC, Faussone-Pellegrini, 1987), were also found located in the myenteric plexus area of the guinea pig stomach (Cook and Burnstock, 1976) and human gastric corpus (Faussone-Pellegrini, 1987) as well as in the small and large intestine of man (Faussone-Pellegrini and Cortesini, 1983; Faussone-Pellegrini, 1987) and other mammals (Duchon *et al.*, 1974; Taylor *et al.*, 1977; Yamamoto, 1977; Gabella, 1979; Thuneberg, 1982; Komuro, 1982; Faussone-Pellegrini, 1985*a*).

However, from the available reports, it seems clear that ICC are not all identical in all species and in all organs and that the nature, as well as the role of these cells, has not yet been defined. We decided, therefore, to complete the description of the human gastric ICC extending their examination in all gastric areas. Since the arrangement of smooth muscle cells and nerve endings differs according to these same areas (Faussone-Pellegrini *et al.*, 1989), we have tried to see if there are also possible differences i the interstitial cell structure, localization and relationshi between them and smooth muscle cells and nerve enc ings. Moreover, we thought that the possibility of findin peculiar not yet described structural characteristics coul help to identify the nature of these cells. Another aim c this research was to see if possible structural peculiaritie according to the gastric areas performing different electr cal and mechanical activities (see Meyer, 1987; Szurszew ski, 1987, for review of the literature), might give clue for the role played by these cells.

MATERIALS AND METHODS

Fragments of the gastric fundus, of the antrum and rings from the cor pus including the two curvatures and the corpus between were surgical ly obtained from 7 patients operated on total gastrectomy for early can cer (IIa and IIc, with limited invasion into the mucosa). The patient ag ranged from 37 to 71 years. None of these patients have taken drug affecting gastric motility.

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

Since the circular muscle layer is very thick, especially in the corpus anc antrum, semithin sections, stained with toluidine blue, were observed a light microscopy in order to verify their orientation and to choose the region to be examined with the electron microscope. The transverse sections of the circular muscle layer were always preferred, even if for the purpose of this study the exact orientation is not so important.

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 number of the nuclei of interstitial cells of Cajal (ICC) and of smooth muscle cells 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; each field was considered to be one sample unit.

2) The surface to volume ratio of ICC was evaluated by intersection and point counting with a curvilinear isotropic test lattice (Weibel, 1979) on electron micrographs at magnification between × 5,000 and × 10,000; the exact magnification of each set of micrographs was checked with a test grid with 1,200 lines/mm (Balzers, Liechtenstein). All the cytoplasmic projections observed in one micrograph (which ranged in number between 1 and 6) were measured, each micrograph was considered to be one sample unit.

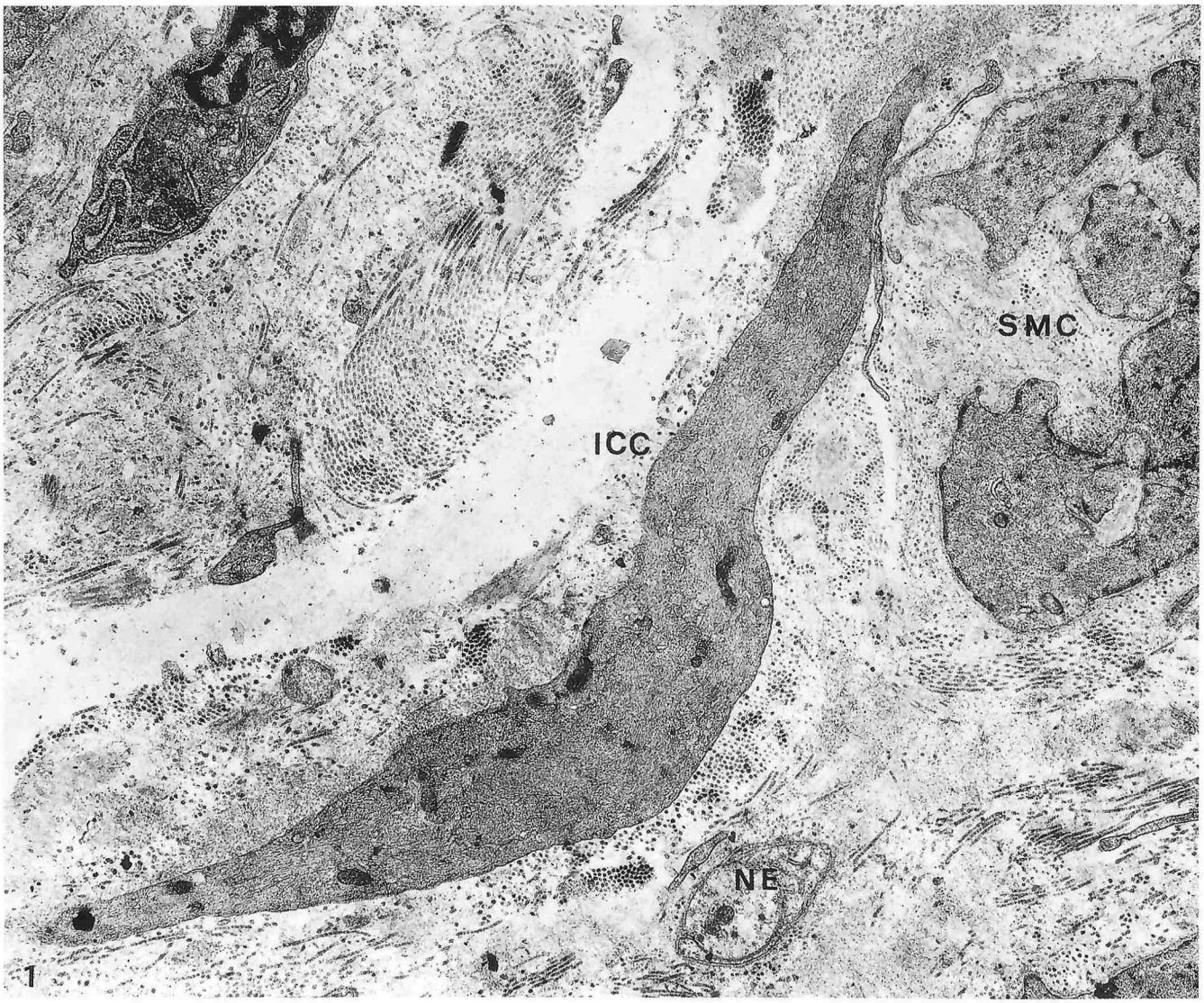
3) Distribution of values was checked for divergence from normality and lognormality with X^2 test. Comparisons between different areas of the stomach were evaluated with Student's t test, with two tails. In all cases, $p<0.01$ was considered to be significant. Significance levels $p<0.001$ were also recorded (Lentner *et al.*, 1982; Bahr and Mikel, 1987).

4) The surface to volume ratio of ICC was not significantly normal from lognormal; therefore, the logarithms of the observed values were used for comparisons and the medium values and their standard errors will be given in the Results.

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

RESULTS

In the interstitium among the smooth muscle cells, few types of cells are present: scarce fibroblasts, mainly located in the largest connective tissue septa, several mast cells, occasional macrophages near the blood capillaries and cells showing the ultrastructural features characteristic for the cells unequivocally identifiable as ICC (Faussone-Pellegrini, 1987). These cells were found in all areas of the corpus in two different locations: 1) scattered inside the circular muscle layer (S-ICC) and 2) grouped in the myenteric plexus area (S-MP-ICC), facing the circular muscle layer. In the fundus, they were mainly found inside the circular muscle layer (S-ICC). In the antrum they



Figures 1 to 3 Human stomach. Interstitial cells of Cajal located in the myenteric plexus area (S-MP-ICC).

FIGURE 1 A longitudinal section of one interstitial cell of Cajal (ICC). NE: nerve ending; SMC: smooth muscle cells of the circular muscle layer. × 10,000.

were mainly found at the submucosal border of the circular muscle layer (S-ICC). No other types of cells, as migrating cells or carcinomatous cells, were identifiable. Presumptive differences reliable to the age of the subjects were not noted.

Interstitial cells of Cajal located in the myenteric plexus area (S-MP-ICC)

The ICC located in the myenteric plexus area are all closer to the circular muscle layer than to the longitudinal one. They showed identical features in all the gastric areas examined. These ICC are very long fusiform cells (Fig. 1), with numerous lateral branches. They possess an oval clear nucleus, a large Golgi apparatus and numerous mitochondria mostly located at the cell periphery (Figs. to 8, 10 and 11). The cisternae of the rough endoplasmi reticulum are small, whereas the cisternae of the smoot endoplasmic reticulum are extremely ramified and di: tributed everywhere (Figs. 1 to 11). Most of them ru parallel to the major axis of the cell (Figs. 1 and 2), som are aligned along the plasma membrane (Fig. 3), an some lie close to the mitochondria (Fig. 3). Filaments fi both the cell body and the origin of the lateral branche (Figs. 1 to 11). They have a preferential orientation para lel to the major axis of the cell (Fig. 2). These filament seem to be of two types (Fig. 2). In fact, most of ther have a diameter of 6-7 nm, identical to that of the 'thir filaments contained in the neighbouring smooth muscl cells (are they thin or intermediate filaments?) and a fe\

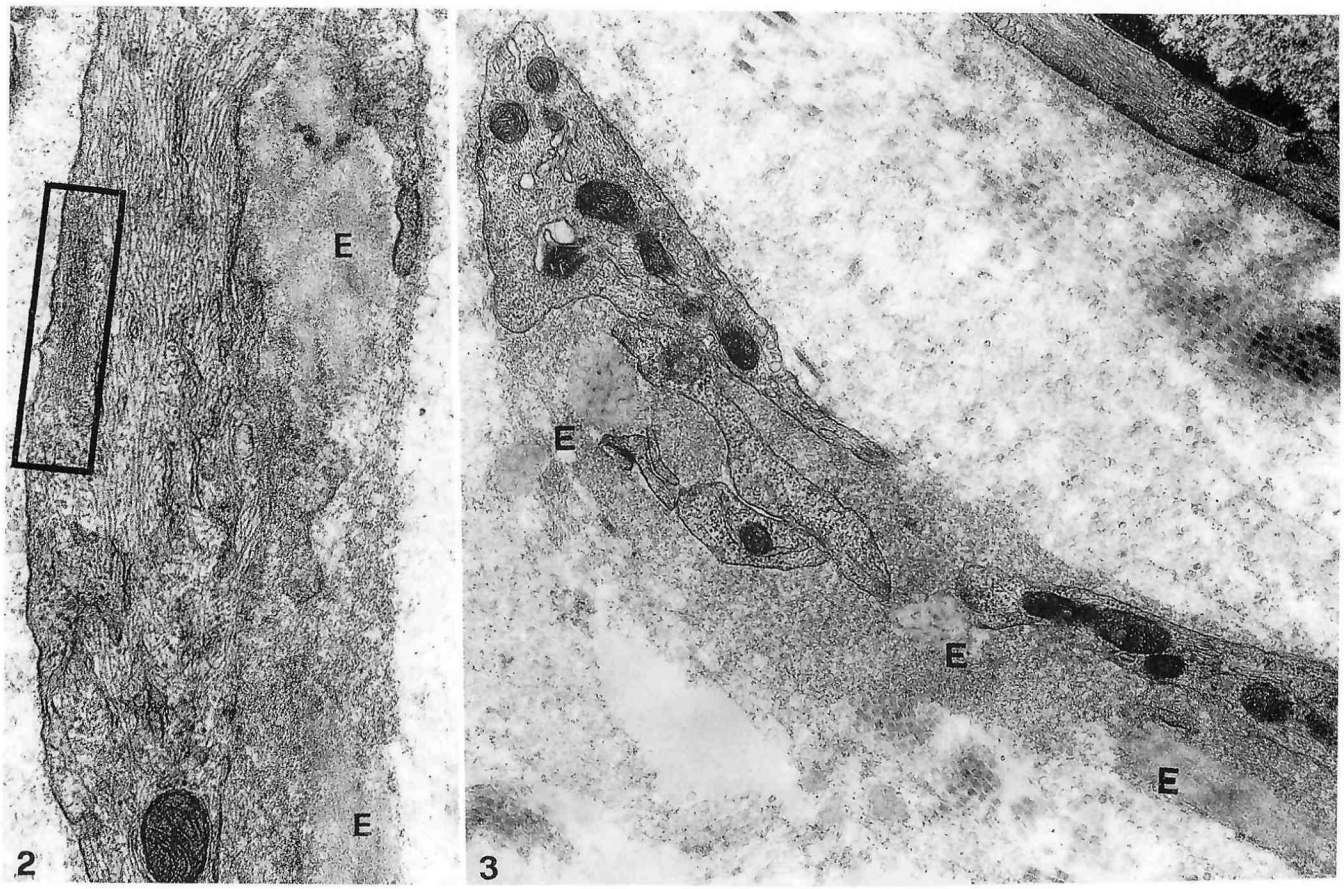


FIGURE 2 Detail of the smooth endoplasmic reticulum and of the two types of filaments present in the interstitial cell cytoplasm. In the square, the filaments 4-5 nm thick. E: elastic fibres. × 37,500.

FIGURE 3 S-MP-ICC either directly contacting each other or bridged by elastic fibres (E). These cells are embedded in a discontinuous capsular-like material. × 20,000.

of them are thinner (a diameter of about 4-5 nm; definitely thin filaments?). The latter may present dense bodies along their length and insert into the plasma membrane in correspondence with structures similar to the dense bands (Fig. 11 and inset). Some caveolae can be found along the plasma membrane (Fig. 3).

Cytoplasmic processes, often in number of 3-5 at the same level, leave the cell body at right angles all along its length and go towards other ICC and towards the smooth muscle cells to contact them (Figs. 4 to 10). These processes may already have a very small diameter in their proximal region, looking like filiform protrusions devoid of organelles, or may be thicker at their origin from the cell body and contain cisternae of smooth endoplasmic reticulum, mitochondria and filaments. Not far from their origin, however, all of them ramify dichotomically, immediately acquiring a small diameter (0.05-0.1 μm) (Figs. 4 to 10). Their length is variable and difficult to define.

The S-MP-ICC often contact each other, by means of extended and complicated interdigitations (Figs. 3, 9 and 11), and with the smooth muscle cells of the circular layer (Figs. 7 and 8). Gap junctions and 'intermediate' junctions have been rarely observed. These ICC form rows, both parallel and perpendicular to the major axis of the stomach. Each row is made up of one to three elements apposed side by side. Probably these rows are interconnected, forming a network apposed to the myenteric surface of the circular muscle layer.

Nerve fibres, with large varicosities filled with synaptic vesicles, are always present near these ICC and contain large granular vesicles only (Figs. 4 to 6) or a mixture of large granular and small agranular vesicles. Few of these nerve endings are directly contacting the ICC (Figs. 5 and 6), whereas most of them lie at the same distance ('distant' type of contact) from the ICC (Figs. 4 to 6) as from the smooth muscle cells (Figs. 5 and 6). Therefore, another network, a nerve network, is apposed to the myenteric surface of the circular muscle layer, intermingled with the ICC network.

Figs. 4 to 8 are photographs of one selected area chosen from serial sections. The consecutive ribbons of about 6-8 sections each were spread over three grids (parallel bar grids). Fig. 4 is one micrograph from the first section of the first grid, showing a group of three ICC contacting each other and located far from the smooth muscle cells; several nerve endings are present, all around them. Figs. 5 and 6 are micrographs of the first two sections of the ribbon of the second grid. These micrographs clearly demonstrate that the apparently numerous nerve endings supplying the ICC group are in reality only one nerve ending (or varicosity?), also supplying the smooth muscle cells. This nerve ending is very long and winds around

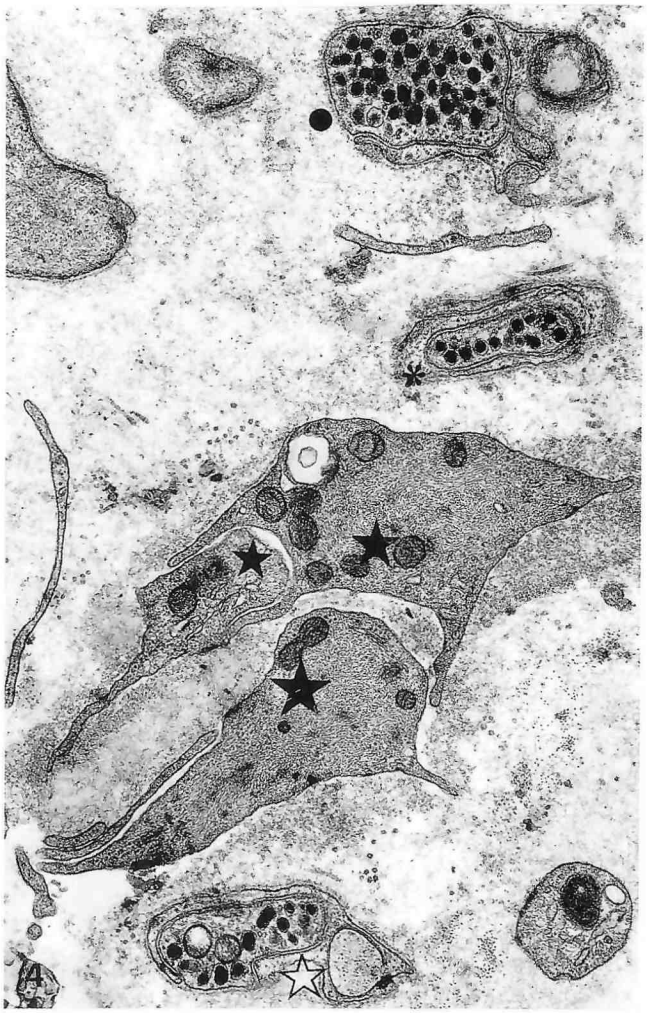
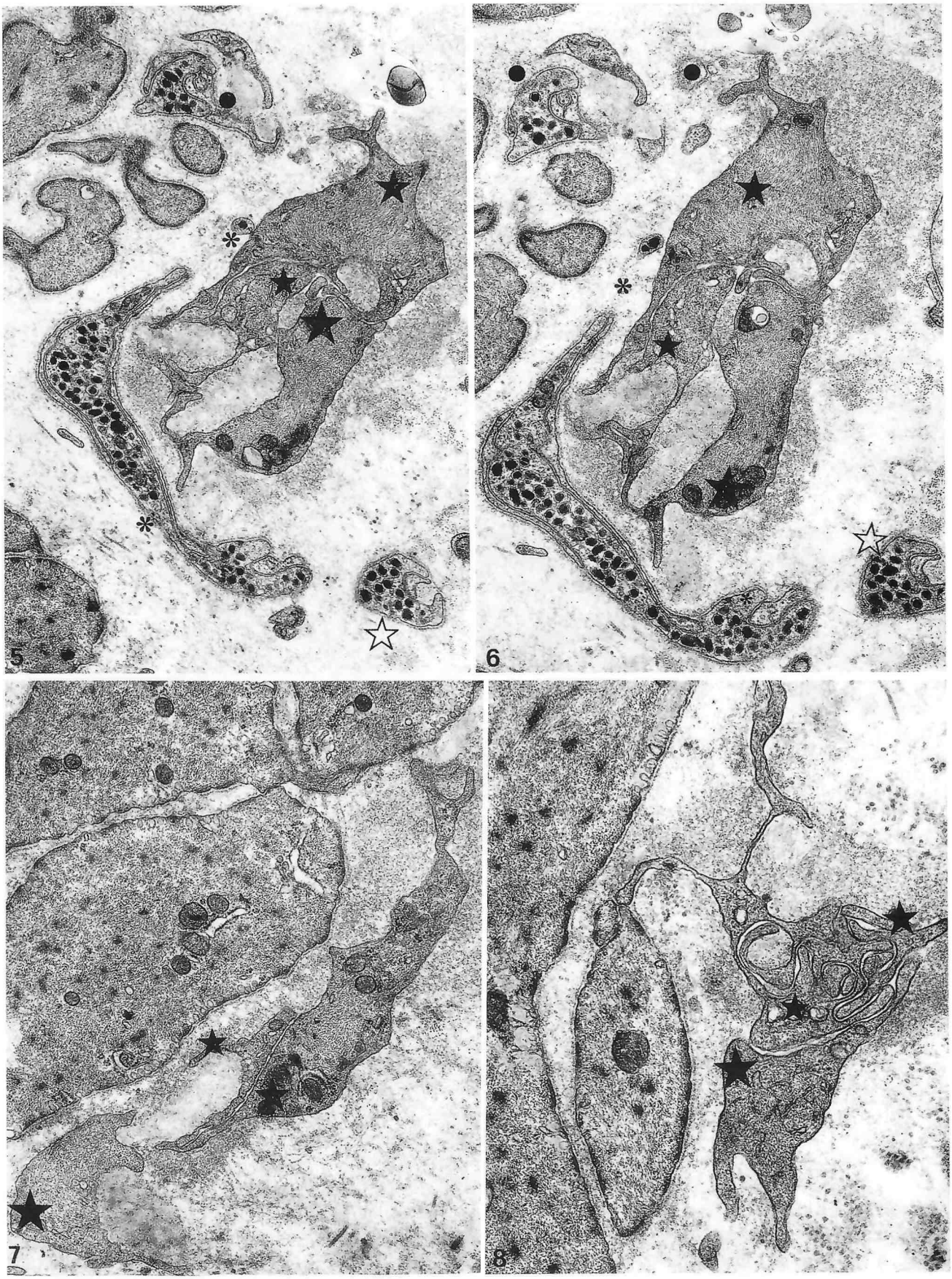


FIGURE 4 *Human stomach. Interstitial cells of Cajal located in th myenteric plexus area (S-MP-ICC). Serial sections.* Micrograph from th first section of the first grid, showing a group of three ICC contactin each other and located far from the smooth muscle cell; several nerv endings are present all around them. × 17,000.

the ICC group and 'closely' contacts it (Figs. 5 and 6 This nerve ending is filled all along its length with larg granular vesicles and covered by a partial glial sheath which at one extremity of the nerve ending opens on th side facing the ICC and at the other on the side facin the smooth muscle cells. This nerve fibre, therefore, ir nervates the three cells of the ICC group and the neare smooth muscle cells. Figs. 7 and 8 are photographs of th two sections at the opposite extremities of the ribbon c the third grid and show the 3 ICC no longer contactin the nerve endings, but now contacting the smooth muscl cells of the circular muscle layer. In Fig. 8 this contact i probably a gap junction, whereas in Fig. 7 the contac type is unclear (a presumed 'intermediate' junction).

Occasionally, short fragments of a basal lamina-like mate rial, often in correspondence with structures similar t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the dense bands, can be identified on the ICC surface (Figs. 4, 8 and 11). Characteristically an electron-dense material, partly amorphous and partly structured (Figs. 1 to 9 and 11), incompletely surrounds these cells, forming a capsular-like structure and linking the ICC together and with the neighbouring smooth muscle cells. Its thickness varies from 0.1 to 1.6 μm. At high magnification it has a filamentous appearance (Fig. 11) and, near the ICC, a periodic structure made up of bands alternately more or less electron-dense, 30-35 nm each, can be observed (Fig. 9). Peripherally, single or grouped collagen fibrils with variable orientation and in continuity with the collagen bundles of the surrounding connective tissue are present (Fig. 9). This capsule has a reduced thickness or is missing on the ICC side facing the nearest smooth muscle cell (Figs. 3 to 9) and in proximity of the contact with the nerve ending (Figs. 4 to 6).

Another peculiar feature we observed is represented by the fact that the ICC processes partially envelop elastic fibres (Figs. 2 and 4 to 10) which run either from one ICC to another (Figs. 3 and 7), or towards the nearest nerve ending (Figs. 8 and 9) inserting themselves onto the Schwann cell, or to the smooth muscle cells (Fig. 10) inserting themselves onto their covering. These elastic fibres are 0.5 μm thick, have a core of moderate electron-density and microfibrillae both inside and around this core. Some of them can also be embedded in the capsular-like material (Figs. 2 to 8).

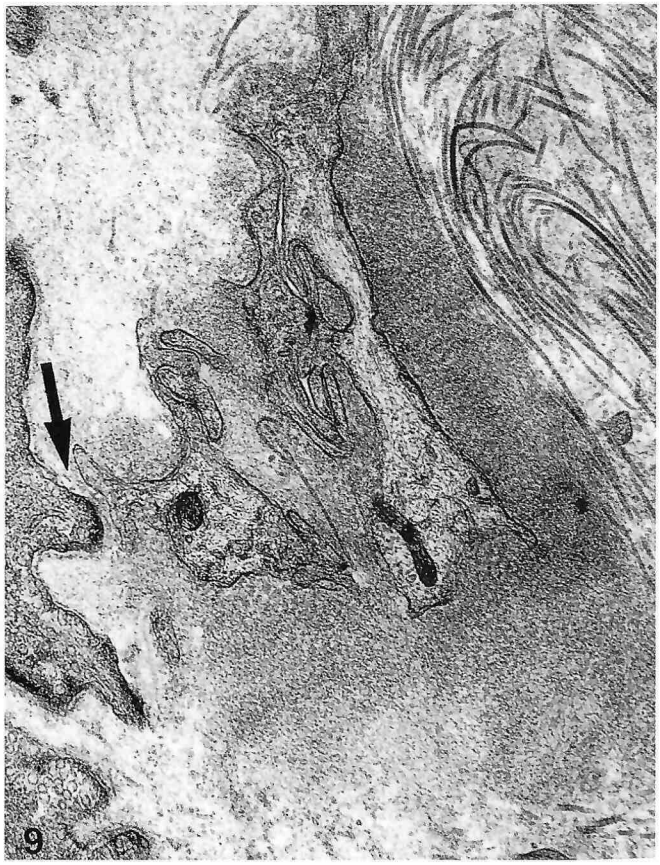
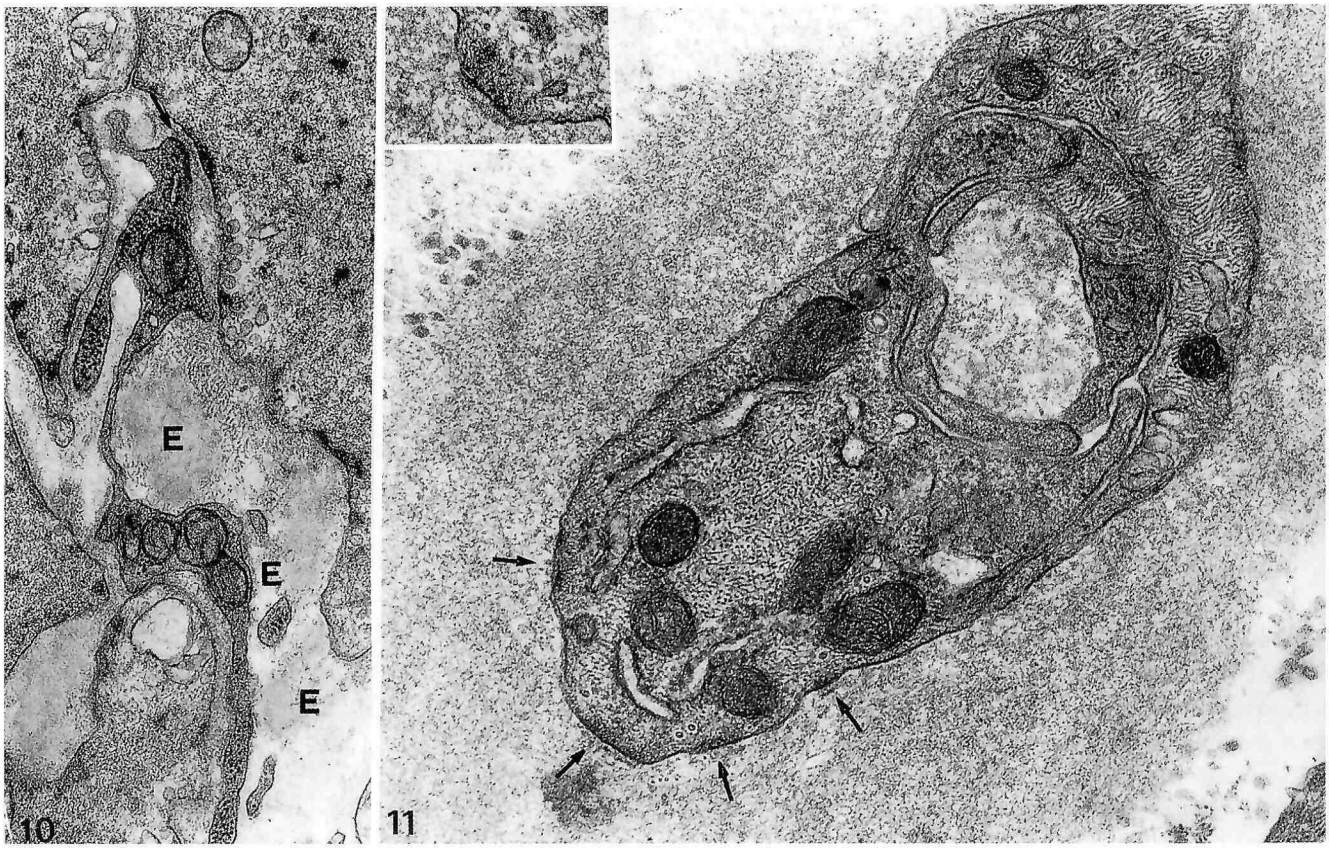


FIGURE 9 *Human stomach.* One group of S-MP-ICC enveloped by a thick capsular-like material. The arrow indicates one probable contact area between one S-MP-ICC of the group and one smooth muscle cell of the circular muscle layer. × 20,000.

FIGURES 5 to 8 *Human stomach.* Serial sections in which are shown the relationships between three interstitial cells of Cajal located in the myenteric plexus area (S-MP-ICC) and both the smooth muscle cells of the circular muscle layer and their related nerve endings. The capsular-like material is always thick, but incomplete; elastic fibres connect the S-MP-ICC and these with both nerve endings and smooth muscle cells. The asterisks indicate the nerve fibre which is supplying the S-MP-ICC group. The black stars indicate the S-MP-ICC; since these cells are three in number, black stars of different size have been used to recognize them better in all sections. The white star indicates one nerve ending, 'distant' from the S-MP-ICC group and which is probably the continuation of the nerve ending indicated by the asterisks. The black ball mark indicates one nerve ending which lies at a certain distance from the S-MP-ICC group and which innervates the smooth muscle cells of the circular muscle layer (see Figs. 5 and 6). Also this nerve ending is probably in continuation with those indicated by the asterisks. For further explanation of these figures see the text. × 17,000.



Figures 10 and 11 Human stomach. Interstitial cells of Cajal located in the myenteric plexus area (S-MP-ICC).

FIGURE 10 Elastic fibres (E) bridge one S-MP-ICC to one smooth muscle cell. The arrow indicates one 'intermediate' junction between these tw cells. × 25,000.

FIGURE 11 Detail of the material forming the capsular-like envelope and of the insertion of the thin filaments into the plasma membrane (arrow: Inset: detail of one bundle of filaments peripherally located. × 37,500.

Interstitial cells of Cajal located inside the circular muscle layer (S-ICC)

As previously reported for the fundic and corporal ICC (Faussone-Pellegrini, 1987), also the antral ICC located inside the circular muscle layer (S-ICC) have a structure similar but not identical to the myenteric ones (S-MP-ICC). Moreover, some as yet undescribed differences in S-ICC morphology and distribution have been observed among those of the fundus, the antrum, the greater curvature and other parts of the corpus. Peculiar features of their supporting connective tissue have also been found. We will, therefore, make a detailed description of these cells according to their location.

Fundus - The fundic ICC are few (about 2.2%) and, a variance with the other gastric ICC, are poor in filamen and branches (surface to volume ratio: about 0.75, Tabl 1). These ICC are exclusively located in the wide an elastic intermuscular connective spaces, closely appose to the smooth muscle bundles. The fundic S-ICC posses short and thick branches by means of which contact eac other and smooth muscle cells (Fig. 12 and inset). Larg elastic fibres and a basal lamina-like material also connec the ICC with the smooth muscle cells, inserting at th level of their plasma membranes (inset Fig. 12). A discor tinuous basal lamina is present around these ICC (Fig. 1 and inset), whereas the capsular-like material is thin an rarely seen.

TABLE 1

	Fundus	Corpus		Antrum	
		Between curvature + lesser curvature	Greater curvature	Submucosal extramuscular region	Intramuscular region
Surface to volume ratio of ICC	+0.19 0.75 −0.15	+0.13 1.60** −0.12	+0.23 1.29 −0.19	+0.19 1.27* −0.16	+0.19 2.02** −0.17
Number of observed fields	14	50	21	14	16
Nuclei ICC/100 nuclei smooth muscle cells (in 16 sample units)	+0.9 2.2*** −0.8	8.3±1.0	8.3±1.0		+0.5 1.0*** −0.4

Number of the interstitial cells of Cajal, related to the circular muscle layer (S-ICC), per 100 cross-sectioned smooth muscle cells and their surface to volume ratio. Medium values ± standard errors.* $p < 0.01$ versus the antral intramuscular region; ** $p < 0.01$ versus the fundus; *** $p < 0.001$ versus the corpus.

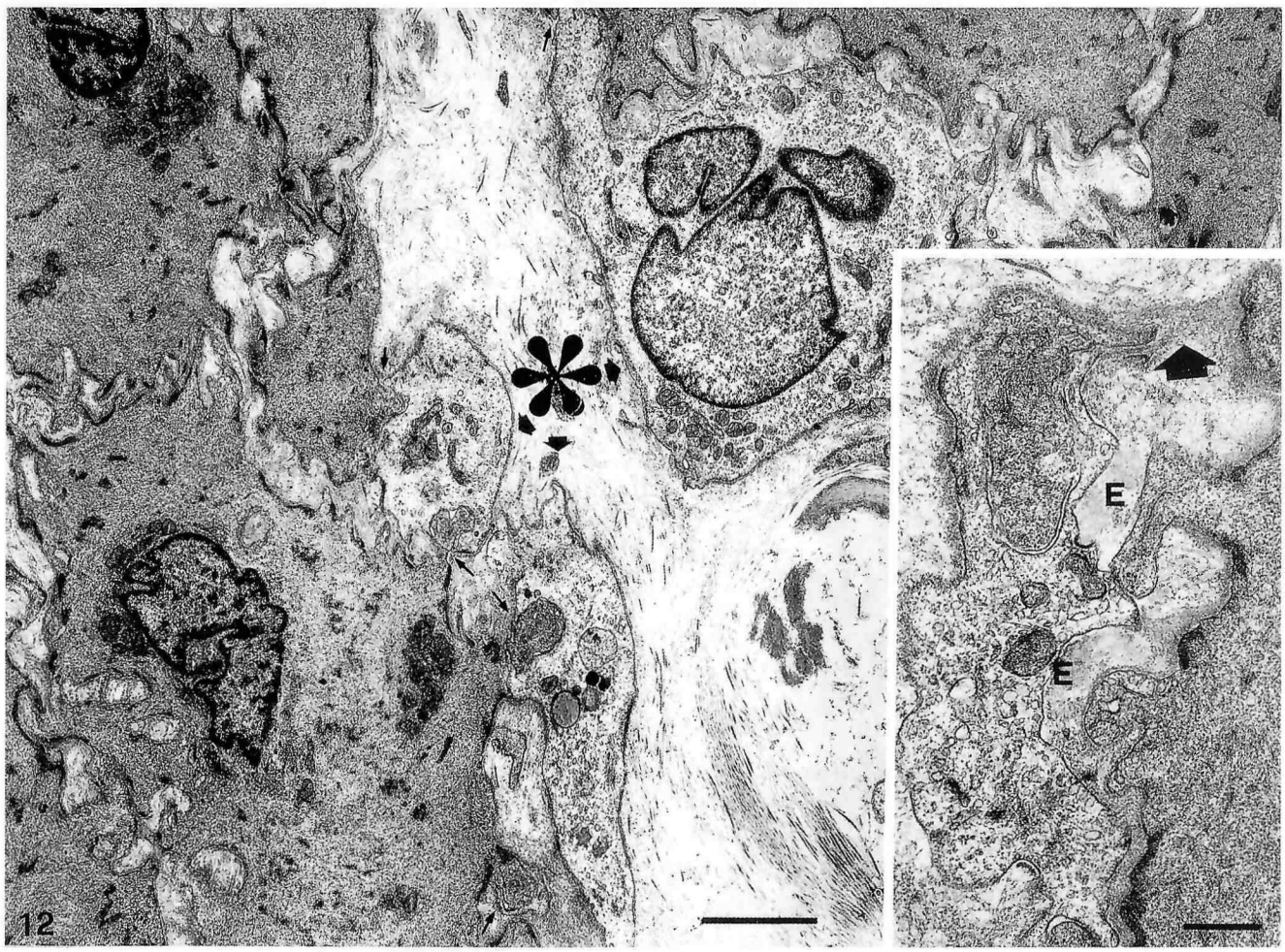


FIGURE 12 *Human stomach. Circular muscle layer of the fundus.* Three interstitial cells of Cajal (S-ICC) located outside two muscle bundles. The thin arrows indicate the contact areas between these cells and the smooth muscle cells. Inset: detail of the contact areas between the S-ICC and the smooth muscle cells. E: elastic fibres. The arrow indicates a basal lamina-like material attaching the S-ICC to the smooth muscle cell. × 7,500; inset: × 20,000.

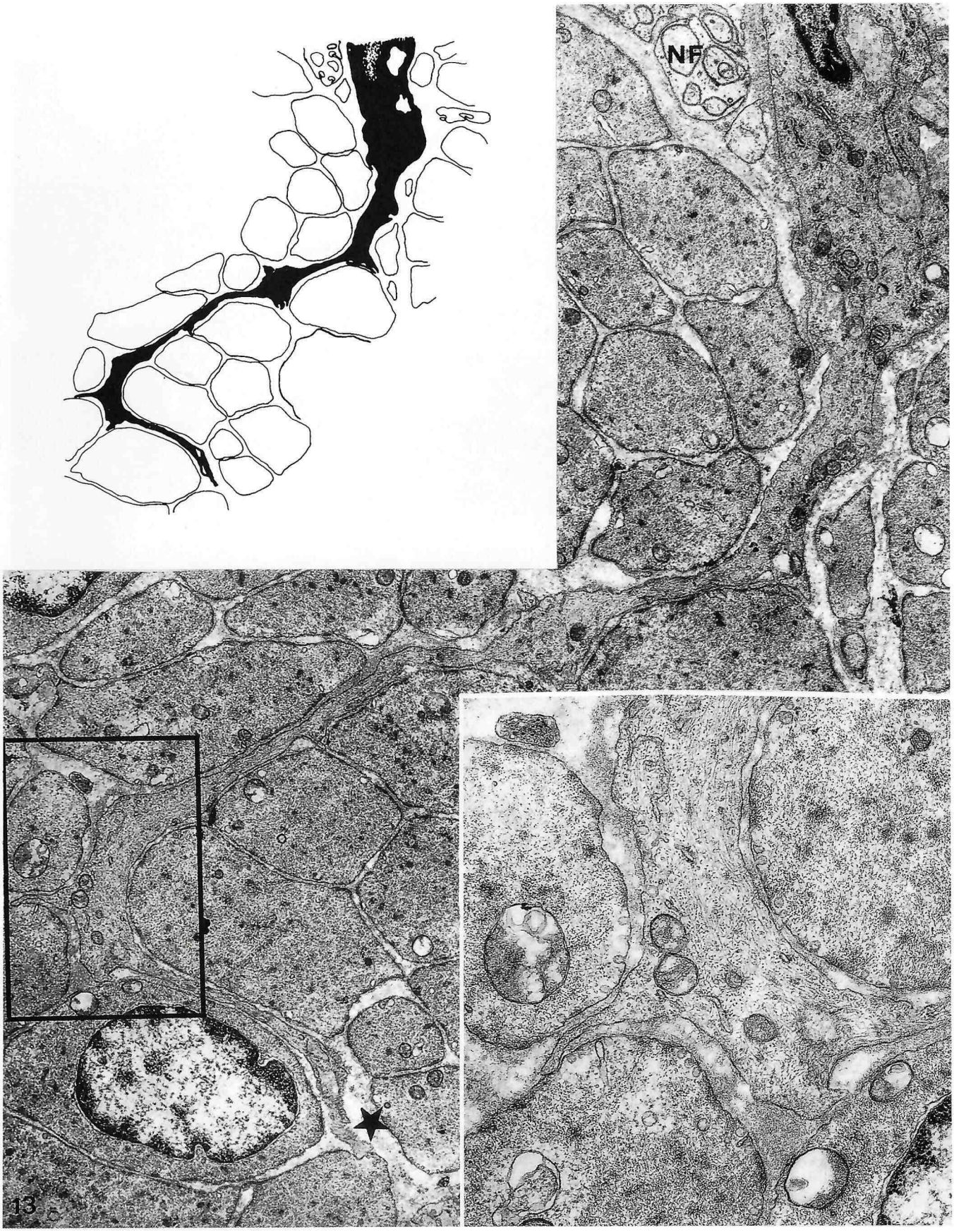
The nerve endings present inside the circular muscle layer of the human fundus are mostly near the ICC. We did not, however, observe any 'close' contact (a gap of 20 nm devoid of basal lamina) between them.

Lesser curvature and corpus - These S-ICC can be frequently found (about 8.3%) and are mainly located inside the smooth muscle cell bundles forming a three-dimensional dense network and always following an analogous nerve fibre network. These cells are very elongated and ramified (surface to volume ratio: about 1.60, see Table 1). In a longitudinal section of one ICC including the nucleated portion and its polar extremities we were able to measure a length of about 70 μm. Thin processes of an undefinible length, at right angles and along the whole cell body, went towards similar ICC processes and towards the smooth muscle cells penetrating among them. Each of these processes could simultaneously contact 2 or more smooth muscle cells (Fig. 13, diagram and inset Fig. 13) and often also partially envelop them. In the plan of a thin section we were able to count about 30 smooth muscle cells contacting one ICC. These ICC possess filaments as the S-MP-CC (Figs. 13 to 21). The contact areas between ICC and between ICC and smooth muscle cells are mostly interlockings and appositions (Figs. 13 to 16), but 'intermediate' junctions between ICC and smooth muscle cells (Figs. 14 to 16) and gap-junctions and 'desmosome-like' junctions between ICC (Figs. 14 and 15) and between them and smooth muscle cells (Figs. 14 to 16) were also found.

The numerous nerve endings present in these areas lie near the ICC, either at the level of their body or their processes; many of these nerve endings 'closely' contact the ICC; sometimes, they simultaneously contact both ICC and smooth muscle cells (Fig. 17). In the lesser curvature, the nerve endings contacting the ICC contain mainly or only small agranular vesicles (Fig. 18).

A definitive basal lamina around these ICC is always absent, whereas elastic fibres bridging the ICC or the ICC and the neighbouring smooth muscle cells (Fig. 13) are frequently seen. Even if rarely, it is also possible to observe in the outermost portion of the circular muscle layer 'complexes' (Figs. 19 to 21) made up of nerve fibres which surround one-three ICC and one-five smooth muscle cells, interconnected by elastic fibres. A thick capsular-like material envelops the 'complexes' located at the periphery of the muscle cell groups (Figs. 19 and 20), whereas this is rare or absent around the 'complexes' located inside the muscle cell groups (Fig. 21). The ICC of these 'complexes' contact each other (Figs. 19 and 20) and incompletely envelop the smooth muscle cells of each 'complex', contacting them by means of gap junctions, 'intermediate' junctions or simple appositions. The nerve fibres, usually, lie outside the capsular-like material (Figs. 19 to 21), the nerve varicosities (or endings) of which may 'closely' contact the ICC (Fig. 20).

FIGURE 13 *Human stomach. One interstitial cell of Cajal (S-ICC) located inside the circular muscle layer of the corpus.* This extremely long S-ICC has been sectioned from its nucleated portion (upper right side) to one of its extremities (lower left side). One extremity of this S-ICC is connected with one smooth muscle cell by an elastic bridge (black star). NF: nerve fibres. The S-ICC portion included in the square is reproduced at higher magnification in the inset, where the relationships between this S-ICC and the smooth muscle cells are clearer. The diagram reproduces the same field as Fig. 13 and the S-ICC is black stained. × 10,625; inset: × 25,000.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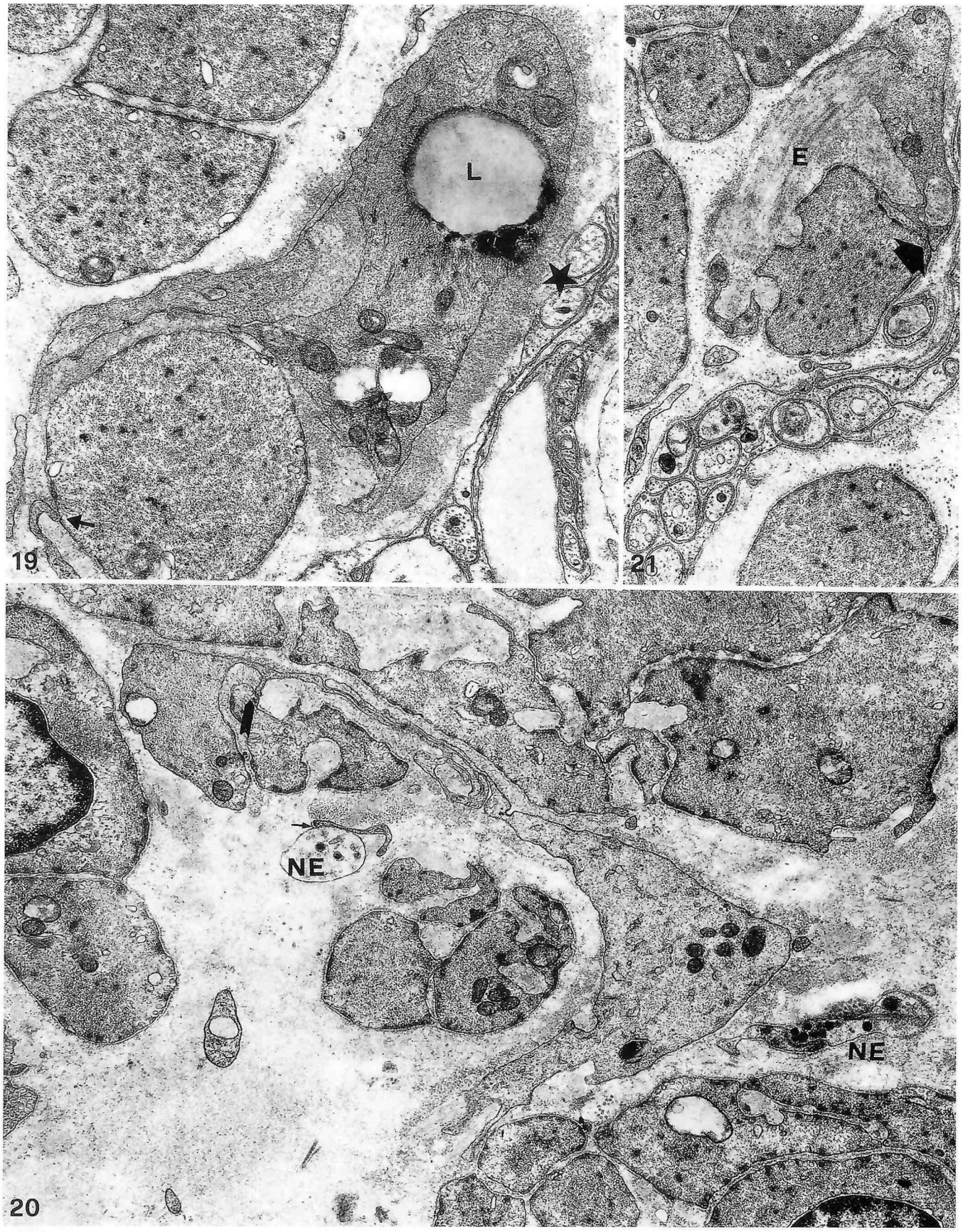
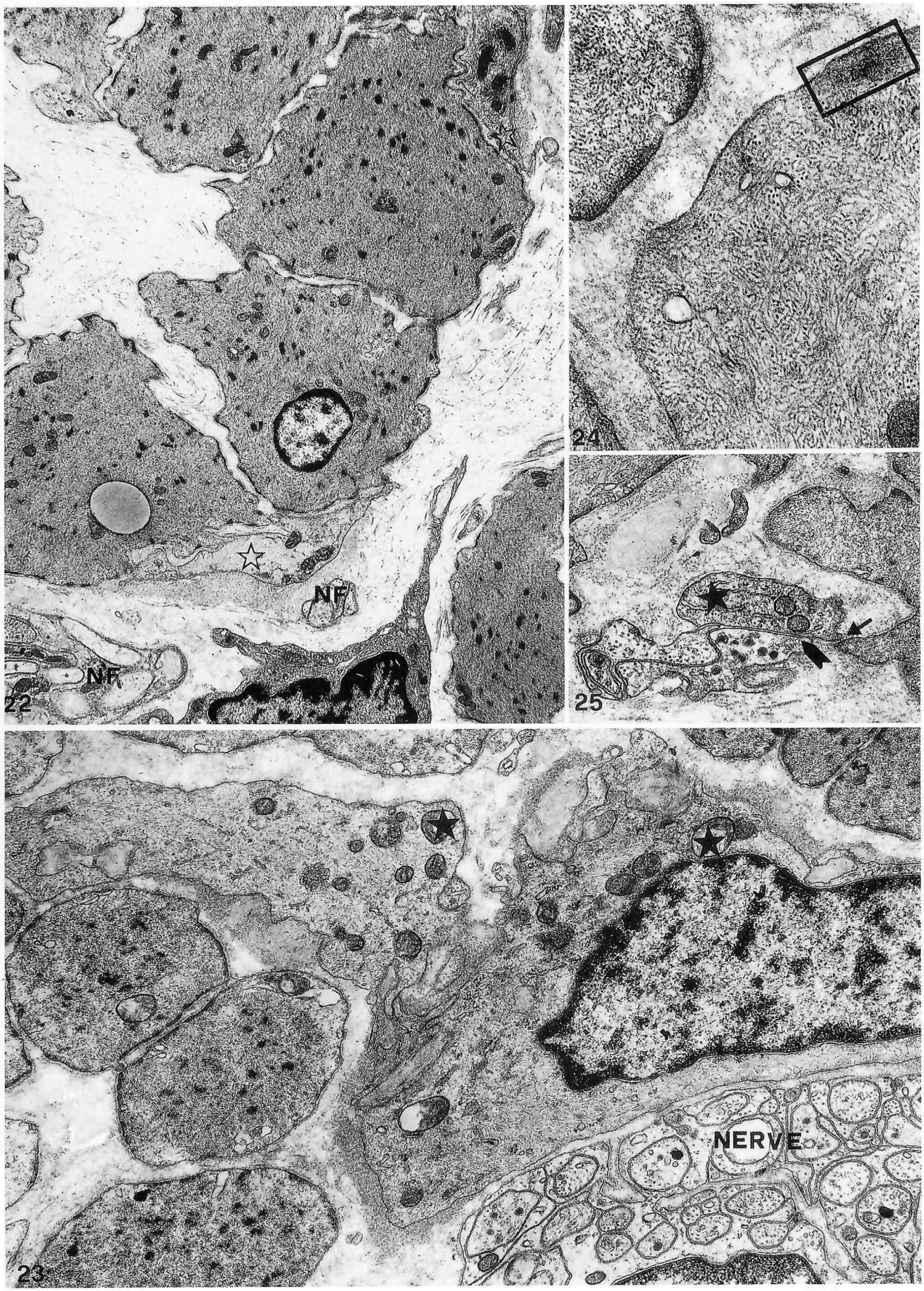


Figures 19 to 21 Human stomach. Circular muscle layer of the corpus. 'Complexes' made up of S-ICC, smooth muscle cells and nerve endings.

FIGURE 19 Lesser curvature. L: one large lipofuscinic body in the S-ICC cytoplasm. The arrow indicates one presumptive gap junction between one branch of one interstitial cell and the smooth muscle cell of the complex. Two nerve endings (black star) contain small agranular vesicles only. × 17,000.

FIGURE 20 Corpus interposed between the two curvatures. NE: nerve endings, one of which is in 'close' contact with a thin S-ICC process (arrow). The arrowhead indicates a presumptive gap junction between one S-ICC and one smooth muscle cell. These same two cells also contact by means of 'intermediate' junctions. × 15,000.

FIGURE 21 Lesser curvature. The thick arrow indicates one gap junction between the S-ICC and one smooth muscle cell of the 'complex'. These two cells are also connected by an abundant elastic material (E). × 13,000.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NERVE
23

Greater curvature - At variance with the previous ones, the bodies of these ICC are mainly located at the periphery of large groups of smooth muscle cells (Figs. 22 and 23), always together with the nerve fibres. The number of ICC is the same as in the other parts of the corpus (see Table 1) but their branches are few (surface to volume ratio: about 1.29) and enter the muscle bundles making, together with the few nerve fibres present, a network at ample meshes. These ICC also are rich in filaments (Figs. 23 and 24) and cisternae of smooth endoplasmic reticulum (Fig. 23) and contact each other (Fig. 23) and smooth muscle cells (Figs. 22, 23 and 25) as previously described. The basal lamina is discontinuous (Fig. 25) or absent, whereas the elastic fibres (Fig. 23) and a capsular-like material (Figs. 22 and 23), with an identical arrangement to that previously described, are present. The few nerve endings present in the circular muscle layer of this area lie near the ICC (Figs. 22 and 23) and some of them 'closely' contact with the ICC (Fig. 25). All these nerve endings contain a prevalence of large granular vesicles.

Antrum - The morphology of the antral S-ICC, their connective envelope, as well as the contact areas they have each other and with both smooth muscle cells and nerve endings, are identical to those of the corporal ones. These cells have, however, a distribution different in respect of both fundus and corpus. In fact, few of them (about 1.0%) run together with the nerve fibres inside the circular muscle layer, mostly peripherally to the muscle bundles, and with their numerous branches make a network with ample meshes similarly to the greater curvature one; the others, always together with the nerve fibres, border the submucosal surface of the circular muscle layer (Figs. 26 to 28), making up a very dense network. In fact groups of 1-3 ICC each, distanced each other minimum 20 μm, by means of their branches form the interconnected rows of this superficial ICC network.
All these ICC are immersed in a thick capsule identical to that described for the corporal ones (Figs. 26 and 27). Moreover, these cells, together with the nerve fibres, may also be partially enveloped by the perineural sheath (Figs. 26 and 27). The capsule lacks on the side facing the smooth muscle cells, where the contacts between these two cell types can be found (Fig. 28). The branches of these ICC are thin, not numerous (see Table 1) and always following the nerve fibres enter the circular muscle layer. Therefore, contacting the ICC deeply located, they connect the superficial with the inner ICC network.

DISCUSSION

Several findings result from this study. 1) We can confirm what has already been reported by one Author (Faussone-Pellegrini, 1987), i.e. that cells with peculiar ultrastructural features and contacts with each other and with the smooth muscle cells and nerve endings, as the so-called interstitial cells of Cajal (ICC), are present in the fundus and corpus of the stomach of man. 2) These cells

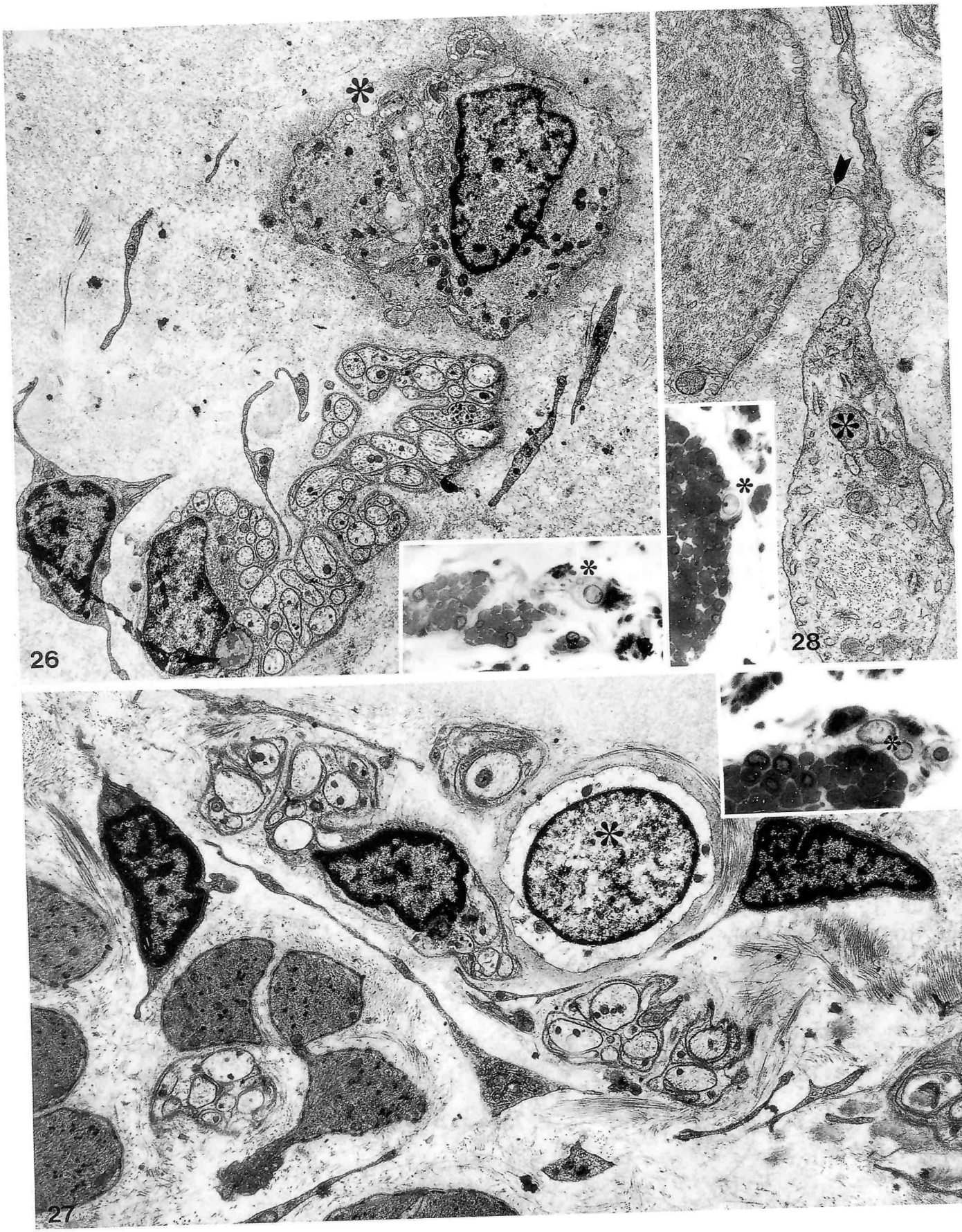
Figures 22 to 25 Human stomach. Greater curvature.

FIGURE 22 Two S-ICC (white stars) located outside a group of smooth muscle cells of the circular muscle layer and contacting them. NF: nerve fibres. × 7,500.

FIGURE 23 Two S-ICC (black stars) rich in smooth endoplasmic reticulum and filaments. Nerve: one nerve bundle containing a large number of axons. × 15,000.

FIGURE 24 A detail at high magnification of the filaments of one S-ICC. In the square, a bundle of the filaments 4-5 nm thick. The others have the same diameter as the 'thin' filaments of the neighbouring smooth muscle cells (upper left side). × 50,000.

FIGURE 25 Detail of the 'close' contact areas between one S-ICC (black star) and both one nerve ending (arrowhead) and one smooth muscle cell (arrow). × 20,000.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have been found also in the antrum. 3) We can also confirm that the gastric ICC have two preferential localizations, one at the myenteric plexus level (the so-called S-MP-ICC) and the other throughout the circular muscle layer (the so-called S-ICC). 4) The gastric S-ICC and S-MP-ICC have some similarities, but also some differences, in structure. 5) We have also recognized structural differences among the S-ICC according to the various gastric areas (fundus, corpus and antrum). 6) According to the level examined (fundus, antrum, greater curvature and other parts of the corpus), we were also able to observe a different number and distribution of the S-ICC compared to the muscle cell groups. 7) We also noted that the different pattern in the S-ICC distribution inside the thickness of the circular muscle layer exactly reflects a similar pattern of the nerve fibres. 8) No substantial differences, on the contrary, have been found in the relationships between all types of ICC or between them and smooth muscle cells and nerve endings. 9) We have also found a peculiar aspect and arrangement of the connective tissue enveloping the ICC.

In summary, the most significant findings of the present study concerning S-MP-ICC are the following: 1) These cells are identical at all levels; 2) these cells are filled in endoplasmic reticulum (especially smooth) and filaments (two types of filaments, 6-7 nm and 4-5 nm in diameter); 3) these cells are long and highly ramified and are connected to each other making up a three-dimensional network apposed to the myenteric surface of the circular muscle layer, the cells of which are also contacting them; 4) a filamentous material, constituting a thick capsular-like structure, incompletely envelops these ICC and elastic fibres bridge them together with each other and with both smooth muscle cells and nerve endings.

On the contrary, as this research revealed, a correlation exists between the S-ICC morphology and their location in the fundus, corpus and antrum. There are even differences between the various corporal and antral areas. The most significant findings concerning the S-ICC present in the *fundus* are the following: 1) these ICC are particularly few and possess scarce branches and filaments; 2) these cells are located in the wide intermuscular connective spaces, strictly apposed to the smooth muscle cells, with which they have extended contact areas; 3) elastic fibres bridge them together and with the smooth muscle cells; 4) the few nerve endings present in the fundic area are nearer to the ICC (but not in 'close' contact with them) than to the smooth muscle cells.

The S-ICC present in the *corpus* are 1) particularly frequently found (about 8.3%), i.e. five-six times more than in the fundus and several times more than in the dog (1/900, Daniel *et al.*, 1984); 2) are extremely long (70 μm or more) and variably ramified (see Table 1); 3) are filled in filaments (two types of filaments) and smooth endoplasmic reticulum; 4) all the S-ICC located in the largest connective spaces peripheral to the smooth muscle cell groups are enveloped by a thick capsular-like material identical to that already described for S-MP-ICC. Elastic fibres bridge all these ICC together with each other and with the smooth muscle cells; 5) those present in the *greater curvature* are mainly located at the periphery of large groups of smooth muscle cells, have a lesser number of branches than those of the other parts of the corpus and only some of these branches enter the smooth muscle cell groups. Therefore, this S-ICC network has ample meshes and runs together with an analogous nerve network. The nerve endings present in this gastric area are nearer or in 'close' contact with the ICC than with

Figures 26 to 28 Human stomach. Antrum. Submucosal border of the circular muscle layer.

FIGURE 26 A group of interstitial cells of Cajal (S-ICC) (asterisk) enveloped by a capsule-like material, near a nerve bundle and in proximity of the circular muscle layer. The perineural sheath is discontinuous and lacks on the side facing the ICC. Inset: semithin section, toluidine blue. × 7,500; inset: × 700.

FIGURE 27 Encapsulated S-ICC (asterisks) and relative nerve bundles close to the circular muscle layer. The perineural sheath is opened on the side facing the interstitial cells, but seems to partially envelop them also. Inset: semithin section, toluidine blue. × 7,500; inset: × 700.

FIGURE 28 S-ICC (asterisks) intermingled with the most superficial smooth muscle cells and contacting them (arrow). Inset: semithin section, toluidine blue. × 25,000; inset: × 1,000.

the smooth muscle cells; 6) the S-ICC present in the *lesser curvature* and *corpus* are mainly located inside the smooth muscle cell groups, forming, by means of their more numerous branches, a dense three-dimensional network, accompanied by an analogous nerve network. The nerve endings present in these areas are near and in 'close' contact with both ICC and smooth muscle cells. Moreover, peculiar structures, or 'complexes', have been found in these areas. These 'complexes' are made up of one-three S-ICC (contacting each other), one-five smooth muscle cells (contacting each other and the ICC), several elastic fibres (bridging the ICC together and with the smooth muscle cells), all surrounded by nerve fibres (the nerve endings of which can directly contact the ICC) and by a capsular-like material.

The S-ICC present in the *antrum* are identical to the corporal ones (cytoplasmic features, capsule, contacts with the other cells, etc.) except in their distribution and ramifications. In fact, very few of them, less than in the fundus (about 1.0%), are located inside the whole thickness of the circular muscle layer making with their numerous branches (see Table 1) a network with ample meshes. Most of them, on the contrary, make, by means of their long and poorly ramified bodies, groups of interconnected cells forming a dense network (minimum diameter of the meshes 20 μm) superficially located (all along the submucosal border).

Extending the examination to the various areas of the human stomach and completing the description of their ICC it results that the cytoplasmic features of the human S-MP-ICC differ from those of the S-MP-ICC described in the guinea-pig (Cook and Burnstock, 1976) and to the corresponding ones located in the area of the myenteric plexus of the small intestine of man (Faussone-Pellegrini and Cortesini, 1983) and other mammals (Taylor *et al.*, 1977; Yamamoto, 1977; Thuneberg, 1982; Faussone-Pellegrini, 1985*b*) for their richness in filaments and in possessing a specialized connective tissue envelope and peculiar relationships with the smooth muscle cells of the circular muscle layer only. For these same reasons and also for their richness in smooth endoplasmic reticulum, the S-MP-ICC differ from the ICC located in the colonic myenteric plexus area of man (Faussone-Pellegrini, 1987) and of other mammals (Gabella, 1979; Komuro, 1982).

Even more than among the ICC related to the myenteric plexus, several important differences have been found comparing all the gastric ICC related to the circular muscle layer (S-ICC) with the corresponding ones located in the esophageal (E-ICC, Faussone-Pellegrini, 1987) and intestinal (Faussone-Pellegrini, 1987) circular muscle layer. These differences, however, are less consistent compared to the human esophageal-ICC (Faussone-Pellegrini and Cortesini, 1985) than to the others. In fact, both esophageal and gastric S-ICC are rich in smooth endoplasmic reticulum, possess a capsular-like material and are located throughout the circular muscle layer. However, notwithstanding these similarities, there are also some important differences in their distribution inside the circular muscle layer and in their relationships with the nerve endings. In fact, in the stomach only the S-ICC of the lesser curvature and corpus are distributed throughout among the smooth muscle cells, while those of the greater curvature are mainly outside the muscle cell groups, those of the fundus only outside and those of the antrum mainly on the submucosal border of the circular muscle layer. Finally, all the S-ICC have fewer 'close' contacts with the nerve endings compared to E-ICC, even if they are the cell type which the nerve endings preferentially innervate also in the stomach.

The differences between the human S-ICC and all the corresponding ones related to the circular muscle layer of the small intestine in humans (Faussone-Pellegrini and Cortesini, 1983) or other mammals (Duchon *et al.*, 1974; Yamamoto, 1977; Thuneberg, 1982; Faussone-Pellegrini, 1983*a,b*) and to those related to the circular muscle layer of the colon of mammals (Faussone-Pellegrini, 1983*a*, 1985*a*) are more notable. In fact, these cells differ: 1) in their location (throughout the circular muscle layer and not confined to a unique (innermost) area); 2) for the absence of relationships with a specific nerve plexus (the plexus muscularis profundus in the small intestine and the outermost subdivision of the plexus submucosus in the colon); 3) for some of their peculiar structural features (filaments, endoplasmic reticulum, caveolae) and 4) for their enveloping connective tissue (basal lamina, capsular-like material, elastic fibres).

In conclusion, all the afore-mentioned correlations demonstrate that the human gastric ICC differ in several respects from the corresponding esophageal and intestinal ICC of both the same and different animal species. We can claim, therefore, that the gastric ICC have structural features so peculiar as to be considered as characteristic of the stomach. Because the many common features we found in the present study, the structural distinction between the ICC located in the myenteric plexus area and those located in the circular muscle layer (as reported for the ileal and colonic ones; see Faussone-Pellegrini, 1987, for review of the literature) is not so clear in the human stomach. Furthermore, the ICC located in the myenteric plexus area of the stomach, at variance with the other parts of the gut, are so much closer to the circular muscle layer (and in contact with its cells) than to the longitudinal muscle layer, as to suggest that they too could be considered as 'belonging' to the circular muscle layer. Consequently, we have no difficulties in seeing the S-ICC network as a continuation, inside the thickness of the cir-

cular muscle layer, of the S-MP-ICC network.

The nature of the ICC has not yet been defined. If we consider the structural data we have obtained for all gastric ICC 1) an elongated shape; 2) richness in filament content (all or many thin filaments?); 3) richness in a smooth endoplasmic reticulum (with an arrangement similar to that of the smooth muscle cells); 4) presence of caveolae (as in the smooth muscle cells); 5) mitochondria with size, shape and intracellular distribution identical to those of the smooth muscle cells; 6) a nucleus with the same size, shape, features and localization as that of the smooth muscle cells; 7) occasional presence of a basal lamina (even if discontinuous) and 8) of a specific connective envelope; we are very strongly induced to consider them as belonging to the smooth muscle tissue. The presence of lateral ramifications is not in contrast with such a nature, whereas we can claim that they surely do not have the characteristics of cells belonging to either connective or nerve tissues. Moreover, a smooth muscle nature was first suggested by Imaizumi and Hama (1969) for the ICC present in the gizzard of a bird. Such a nature for all esophageal and intestinal ICC was also suggested by us in man (Faussone-Pellegrini *et al.*, 1977; Faussone-Pellegrini and Cortesini, 1983, 1985) and by us and others for the ileal ICC of mouse (Yamamoto, 1977; Thuneberg, 1982; Rumessen *et al.*, 1982; Faussone-Pellegrini, 1985*a*), bat (Yamamoto, 1977), hedgehog (Faussone-Pellegrini, 1983*a*), rat (Faussone-Pellegrini, 1983*b*) and for the colonic ones of mouse (Faussone-Pellegrini, 1985*b*, 1987) and rat (Stach, 1972; Faussone-Pellegrini, 1983*b*). We decided, therefore, to continue considering that all ICC belong to the smooth muscle tissue since the structural features revealed by the present research are all in favour of a muscle nature.

It has also not yet been demonstrated whether all the afore-mentioned cells correspond to the 'interstitial cells' described in the small intestine by Cajal. Even if at the moment there is no certainty concerning their nature and no unequivocal proof that the cells identified as ICC both at electron and light microscopy are the same, we prefer to continue to call this peculiar cell type 'interstitial cell of Cajal'.

Also, the role played by these cells is still unknown, despite the fact that it has intrigued all Authors from Cajal's time until now. A *mechanical role* has been proposed, suggesting that the ICC should make up the skeletal framework of the circular muscle layer, which, according to Gabella (1979) and Komuro (1982), has to avoid deformations of the nerve bundles and to maintain the arrangement of the muscle cell groups during the contractile activity of the gastrointestinal wall. However, at least for the ICC we described in the human stomach, this role does not seem possible for two reasons. These cells, in fact, 1) possess filaments which seem to be contractile rather than skeletal filaments, and 2) are linked together with one another and with both smooth muscle cells and nerve endings by elastic fibres. It seems to us unlikely that motile and dynamic structures, such as the smooth muscle cells and the nerve endings (varicosities), would be linked by highly deformable structures (the elastic fibres) to cells which must create a rigid network and which, on the contrary, seem to be also deformable (contractile) cells. It seems to us more reasonable to see these cells as motile cells, which, however, are obliged to maintain well defined locations inside the muscle wall and determined relationships with other cells (smooth muscle cells, nerve endings and with other ICC also), as suggested by the presence of specialized contact areas between them, the capsular-like material (enveloping them), and the elastic fibres (bridging them). Indeed, the presence of these elastic bridges and of a capsular-like material is intriguing since, as far as we know, they have been described only here in the human stomach. One possible explanation could be that the elastic fibres contribute to obtain the maximal distension of the gastric wall and the capsular-like material is an envelope which maintains the relationships between ICC, smooth muscle cells and nerve endings during the relaxation period. A proof in favour of this hypothesis is that the capsular-like material is present in the largest connective spaces and absent inside the muscle parenchyma, where this loss is almost impossible.

Another role suggested for the ICC, if we want to respect the historical background of this cell type, was perceived by Cajal himself, even if he considered this cell type as a peculiar neuronal cell. In his textbook on nerve tissue, in fact, in 1911, he wrote on page 928: 'ICC could have a role in the excitation of the smooth muscle cells .... perhaps influenced by the nerve endings (varicosities) terminating on them'. Nowadays, in modern times, denying the neuronal nature of the ICC and giving them a muscular nature, this role has been defined as a *'pacemaker'* role (Faussone-Pellegrini *et al.*, 1977; Thuneberg, 1982). Several studies have been recently attempted to demonstrate this role. Some of them seem to confirm this possibility. In fact, it has been reported that the slow waves can be recorded in the ileum only when and where ICC are present (Suzuki *et al.*, 1986; Hara *et al.*, 1986) and that they can be abolished by elective damaging of the ICC (Thuneberg *et al.*, 1983). But these data are until now limited to the ileal ICC only (in particular to those located in the myenteric plexus area). However, physiological data obtained in the canine colon (Smith *et al.*, 1987*a,b*) allowed the identification of the sites where the slow waves are generated, which are the same as where the ICC have been found at light and electron microsco-

py (Stach, 1972; Faussone-Pellegrini, 1983*a*, 1985*b*).

Trying a morpho-functional correlation in order to see if it is possible to find clues in favour for a pacemaker role for the gastric ICC, we must first of all point out that in the human stomach these cells have different locations, near the circular muscle layer (submucosal border and myenteric area) and inside it, whereas the gastric slow waves can be recorded only inside the circular muscle layer (as El-Sharkaway *et al.*, 1978, suggested for dog and man and as Bauer *et al.*, 1985, confirmed for the canine antrum). These Authors specified also that the slow waves are generated by 'multiple and discrete foci' present inside this layer. The different ICC locations we found in respect of the circular muscle layer of the various areas of the human stomach might not be a problem, considering the exclusive relationships the human gastric ICC in one way or another have with this layer. Considering also that the structural features they have suggest a smooth muscle nature for all the gastric ICC, it seems that there is a real possibility that these cells perform a pacemaking function also in the stomach. We must consider, however, that we found in man for each gastric area, which performs different electrical and mechanical activities, a circular muscle layer which possesses different arrangements and morphology of not only its ICC but also of its smooth muscle cells and nerve endings. In the *greater curvature*, the driving pacemaker area (where the slow waves with the highest intrinsic frequency have been recorded; Kelly and Code, 1971), we found numerous ICC, richly and 'closely' innervated, with their bodies closely applied at the periphery of large groups of smooth muscle cells and with some of their branches entering among the smooth muscle cells of each group and, possibly, of neighbouring groups. The smooth muscle cells, in their turn, are less innervated, but, on the contrary, are connected to each other by numerous gap junctions (Faussone-Pellegrini *et al.*, 1989). Therefore, it results that anatomical and possibly functional units are present in the greater curvature, the ICC of which are mainly contacted (controlled) by nerves. These ICC, in their turn, contact (control) the smooth muscle cells of these units.

On the contrary, *in the other parts of the corpus*, where the slow waves recorded are peculiar compared to those recorded in the greater curvature, the ICC are more ramified, the innervation is richer and the gap junctions less numerous. It is also difficult to delineate the afore-mentioned anatomical units, as the ICC and nerve fibres form dense networks scattered everywhere inside the muscle bundles. However, in these parts of the corpus, more in the myenteric part of the circular muscle layer than in its submucosal part, we have identified small 'complexes' made up of nerve endings, ICC and smooth muscle cells kept together by a capsular-like material and interconnected by contact areas and elastic fibres. These 'complexes' can also be considered as anatomical units, even if they are very small when compared with the 'large' units characterizing the greater curvature. The highly rhythmic *antrum* has inside the thickness of its circular muscle layer, similarly to the greater curvature, ICC and nerve networks with ample meshes and smooth muscle cells poorly innervated but connected each other by numerous gap junctions. However, this gastric area has a supplementary and dense ICC and nerve network distributed all along the submucosal border of the circular muscle layer. This second network is connected with the former and has, at variance with that, small meshes with a minimum diameter of 20 μm. To identify anatomical units is practically impossible in the antrum, even if it is easy to conceive their existence also here. The starter point of the ICC function, however, seems to be located at the submucosal border rather than inside the thickness of the circular muscle layer.

In conclusion, notwithstanding the different distribution of both ICC and nerve networks, it seems that everywhere in the rhythmic muscle of the human stomach (corpus and antrum) the ICC are under the 'control' of the nerve endings (containing an inhibitory neurotransmitter; see Furness and Costa, 1987, for review of the literature) and may provide an easy and wide diffusion of own or received information in every direction inside each muscle unit and from one muscle unit to another. We think, therefore, that the present findings may indicate that these ICC, because of their structure and location, can be considered as the starter points of the slow waves from which they will spread on through the muscle parenchyma.

However, we must consider the possibility that all the structural differences we found between antrum and corpus and, in the latter, between the greater curvature and the other parts of the corpus, might be related and/or to other functional requirements peculiar of the gastric wall. For example, the different kind of contacts between the nerve endings and the smooth muscle cells, the different number of gap junctions (Faussone-Pellegrini *et al.*, 1989) and the different distribution and morphology of the ICC among the various gastric areas might be related to the modality of the propagation of the slow waves, which have to spread inside the circular muscle layer in three different directions: 1) from its outermost to its innermost area or viceversa; 2) circumferentially (see the differences between the greater and the lesser curvature) and 3) aborally but with different velocity from each gastric area in order to reach the pylorus at the same time (see Meyer, 1987, for review of the literature).

To reconcile the presence of ICC with their possible

pacemaker role might be a serious problem in the tonic fundus. In fact, it was usually assumed that the fundus is not able to generate slow waves. Recently, however, Schirmer *et al.* (1987) have, in a preliminary paper, reported that pacesetter potentials can be recorded from the human fundus by means of gastric seromuscular stainless steel recording electrodes. If they are correct, hence, it is not surprising to find ICC also in the fundus. In this area, however, these cells differ from the other gastric areas in their number and ramifications.

At the moment, we must conclude that this research furnishes more clues in favour of the pacemaker role than of others. Furthermore, our findings clearly indicate that any morpho-functional correlation between gastric structure and motility must consider a lot of morphological data: those regarding the ICC (their structure, distribution, relationships between them and both smooth muscle cells and nerve endings), those regarding the smooth muscle cells (their possible peculiar morphology and contact areas) and those regarding the nerve endings (their distribution, content and relationships with both ICC and smooth muscle cells). Possible specific connective tissue specializations (elastic fibres, capsule) must also not be misregarded.

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