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The Structure of the Autonomic End Apparatus in the Guinea-pig Small Intestine and the Problem of the Interstitial Cells of Cajal

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Summary. **This review deals with the historical advances of concepts concerning the autonomic innervation in the gut. Discussion is focused on the problem of determining the characteristics of the interstitial cells of Cajal, as this has become the crucial point in understanding the controversies which have surrounded the past studies of the autonomic end-apparatus in the gut.**

The author has gathered morphological data on the nerve plexuses in the guinea-pig small intestine stained by the Champy-Maillet (ZIO) method as investigated by light microscopy and transmission electron microscopy. Scanning electron microscopic examination of the nerve elements in various layers of the intestinal wall has also been performed. The results indicate that the peripheral innervation consists of an autonomic ground plexus in which neuronal processes extend along a glial-cell framework. Projections from all the neurons form a network of separated, non-interconnected bundles, enclosed and supported by rows of independent glial cells.

In the light microscopy of the ZIO stained specimen, amalgamation of glial cells and neuronal projections frequently occurs. The images thus caused appear identical to the structures described by Cajal as interstitial cells. Cajal attempted to apply his neuron theory to the enteric nervous system on the basis of his findings of these "interstitial cells" which he believed to represent primitive neurons. The "interstitial cells of Cajal", as designated by later researchers, were looked for by many histologists in the earlier half of the 20th century; however, they were not true cells but artifact images due to the staining of the autonomic ground plexus and/or nearby cellular elements. The "interstitial cells" defined by authors, including many electron microscopists from the 1960s to the 1980s were other type of cells, i.e., interstitial fibroblast-like cells.

INTRODUCTION

Towards the end of the 19th century when glial cells in the peripheral autonomic nervous system had still not been clearly demonstrated, Cajal reported characteristic triangular, star and spindle-shaped structures projecting bundles of varicose fibers in the mucous coat of the mammalian small intestine (guinea-pig and rat).[1-3] These structures termed by later authors the "interstitial cells of Cajal" actually represented silhouettes of the terminal portion of the autonomic nervous system which could be only partially silver-impregnated by the Golgi's method (*vide infra*). However, Cajal stated that effector cells receive a double innervation, i.e., principal and accessory. The accessory "interstitial cells" controlled by the principal system, modified the function of the effector cells.[2] According to Boeke (p. 88-89 of his 1940 monograph)[4] "Cajal has stuck to his original conception throughout his scientific career, and even in his last book on the structure of the nervous system, he seems still convinced of the truth of his description of the year 1894". This description strongly suggests that Cajal believed in the "interstitial cells" constituting a single type of specialized neuron.

There have been debates on the nature of the interstitial cells of Cajal, including those by Hill,[5] Boeke,[4,6] Clara,[7] Taxi,[8-10] Suzuki,[11] Botar,[12] Kappers,[13] Schofield,[14] Gabella[15] and Thuneberg.[16,17] Lawrentjew,[18,19] Boeke,[4,6] Oshima,[20] Schabadasch,[21] Stöhr,[22,23] Clara,[7] Hillarp,[24,25] Jabonero[26-28] and Mostafa et al.[29] considered the interstitial cells of Cajal to be more or less Schwann cells in their properties. Taxi[8] found two distinct kinds of networks in the smooth muscle coat of the intestine,

namely, a Schwann cell network and a fibroblast-like cell "neuronoid" network. He concluded that the latter corresponded to the interstitial cells of Cajal. Furthermore, in his later light and electron microscopic studies, Taxi[9,10] proposed that the interstitial cells of Cajal were not related to the autonomic terminal plexus (Schwann cell network) but homologous to the cells of Henle's sheath enveloping the somatic nerve.[10] On the other hand, most of the electron microscopists, except a few such as Yamamoto[30] and Honjin et al.,[31] were of the opinion that the interstitial cells of Cajal represented modified fibroblasts[32-44] or specialized cells intercalated between the neuronal terminals and the smooth muscle cells.[45-48] Although Gabella[15] and Thuneberg[16,17] have recently published reviews on the interstitial cells of Cajal, their attention was mainly focused on cells in the smooth muscle coat and they did not fully investigate the interstitial cells of the mucous coat and pancreas where Cajal originally described the morphological structures in question.[1-3] Thus, occurred the misleading statement: as was already stressed by Cajal himself, the interstitial cells have only been found in the smooth muscle coat of the alimentary canal (p. 109 of Ref. 35). This misunderstanding, as well as many others, shows that a new investigation of the history regarding the "interstitial cells of Cajal" must be undertaken before one can begin to disentangle the structure of the autonomic end-apparatus.

In our immunocytochemical study of the framework of the enteric nerve plexuses using an antiserum to S-100 protein, we tentatively concluded that "the interstitial cells of Cajal contain an immunoreactivity for S-100 protein, and thus are glial in nature".[49] The framework of the enteric nerve plexuses never ends freely but is everywhere a continuous network. In the addendum of the paper by Kobayashi et al., it was pointed out that "what Cajal illustrated as the interstitial neurons included silver-impregnated chimera of an enteroglial cell, i.e., S-100 immunopositive glial cells of the enteric nervous system, and fragments of neuronal processes".[49] We confirmed the specific artifices of the Golgi silver-impregnation method to produce the neuron-like silhouette of inseparable complexes constructed by glial cells and long fragments of varicose neuronal processes (axons).[49,50] The results of our previous scanning electron microscopic study on the fine three-dimensional structure of the different layers of the guinea-pig small intestine were consistent with the idea that the terminal autonomic plexus is composed of nerve fiber bundles embedded in a glial (Schwann) cell framework.[49-51] This structure basically corresponded to that predicted by Hillarp in his "autonomic ground plexus theory".[24,25]

In this review, results of recent studies on the histology of the autonomic end-apparatus in the enteric nerve plexuses will first be briefly mentioned, followed by discussion on the problem of the interstitial cells of Cajal. Guinea-pig small intestine served as the material of the present study, because many morphological and most electrophysiological studies of the enteric nervous system have been performed using this animal species.[14,46,52,53] For visualization of the nerve plexuses, Champy-Maillet's (zinc-iodide osmium tetroxide: ZIO) method[42,45,54-58] was used. This methodology presently seems to be most frequently used for the demonstration of the histological structure of the autonomic innervation and the interstitial cells of Cajal (i.e., interstitial fibroblast-like cells).[52,59-62] What previous researchers saw in the ZIO stained specimen were probably not greatly different from those observed in the present study. However, results obtained by transmission and scanning electron microscopic methodologies have provided us with evidence for a new interpretation of both the peripheral autonomic innervation and the interstitial cells of Cajal in the gut.

METHODOLOGICAL COMMENTS

The osmic acid-potassium iodide stain of Champy[54] was modified by Maillet[55,56] by introducing zinc iodide (ZI) instead of potassium iodide.[45,57] The method of staining used in the present study has been described by Taxi[10] (see also Stockinger and Graf[58]).

The staining solution was a mixture of 3 to 4 parts of zinc iodide and 1 part of a 2% solution of osmium tetroxide (OsO_4). ZI was prepared as follows. In a fume cupboard, 1 gm crystalline iodine was put into 40 ml distilled water in a glass beaker and mixed with 3 gm zinc (metal, powder). The mixture was agitated for about 2 min, allowed to stand for about 5 min, and then filtered through Whatman No. 2 filter paper. The zinc iodide was freshly prepared each time.

Whole mount preparations containing a myenteric plexus layer, deep muscular plexus layer, submucous layer with lamina muscularis mucosae and periglandular plexus layers were prepared under the dissection microscope using watch-maker forceps and other ordinary instruments. They were mounted on chrome alum glass slides by a routine slide sandwich method. ZIO stained whole-mount preparations sand-

wiched in the space between the glass slides were dehydrated through an ethanol series, unsandwiched, dipped in 100% ethanol followed by xylene, and then embedded in Enthelan. For visualization of the specimens an Olympus Vanox microscope was used.

For the study of the mucosal plexus, especially the periglandular and villous plexuses sections were sometimes used. Pieces of the intestinal mucosa, block-stained in the ZIO soluion for 12-21 h, were washed in several changes of distilled water, dehydrated through an ethanol series, treated with xylene and embedded in paraffin. Sections (12-16 μm thick) were cut on a microtome, deparaffinized with xylene and mounted in Enthelan.

Small modifications of the ZIO-staining method mentioned above were made as follows:

1) Addition of a small amount of Tris-HCl buffer.
2) Increase of relative osmium concentration in the ZIO solution.
3) Long-term treatment in ZIO solution.

Transmission electron microscopy

The Champy-Maillet method has been applied by many authors including Stockinger and Graf[58] for the demonstration of the ultrastructure of the autonomic nerve terminals in electron microscopy. In the present study, small tissue blocks not exceeding $1\times1\times2$ mm in the side of the ZIO stained intestine were immersed in 1.0% hydroquinone solution for 1 h at room temperature. They were then dehydrated with a graded series of ethanol, treated with propylene oxide and embedded in Epon epoxy resin. Thin sections were made by a diamond knife on a Porter-Blum MT-2 microtome and stained doubly with uranyl acetate and Millonig's lead. Observations were performed using the Hitachi H-700 electron microscope.

Scanning electron microscopy

The NaOH maceration method invented by Takahashi-Iwanaga and Fujita[63] was used with minor modifications. Guinea-pigs were anaesthetized with sodium pentobarbital and perfused through the thoracic aorta with Ringer's solution followed by 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2. Pieces of the small intestine were removed and immersed in the same fixative for at least one week, and rinsed in distilled water.

The tissue pieces were placed in a concentrated NaOH solution (7.2 g NaOH in 30 ml distilled water) for 14-16 min at 60℃. After the NaOH maceration, the tissue pieces were rinsed in a diluted (1 : 10) Ringer's solution for 10 min at 60℃. After several changes of diluted Ringer's solution, the tissue pieces were carefully dipped in a solution containing 2% arginine chloride, 2% glycine, 2% sodium glutamate and 2% sucrose, rinsed in distilled water and immersed for 2 to 14 h in 2% tannic acid in distilled water according to Murakami.[64] They were then treated for 2-12 h in 1% OsO_4 solution, washed thoroughly in distilled water, dehydrated through an ethanol series, transferred to isoamyl acetate and critical-point dried using liquid CO_2. After critical-point drying, the intestinal tissues were carefully dissected under a microscope using watchmaker's forceps. The specimens were evaporation-coated in an Eiko 1B-3 ion coater with gold-palladium and examined and photographed in a JSM T220 scanning electron microscope at an acceleration voltage of 10 or 15 kV.

FINE STRUCTURES OF ENTERIC NEURONS, GLIAL CELLS AND INTERSTITIAL FIBROBLAST-LIKE CELLS

Champy-Maillet (ZIO) positive structures

Staining the tissue pieces containing autonomic innervation apparatuses by the ZIO method usually afforded a clear demonstration of the varicose nerve terminals and of the glial cells. Fibroblast-like cells and vascular elements associated with the autonomic innervation apparatuses were also rendered selectively visible by this staining. The addition of a small amount of Tris-HCl buffer (0.1 M, pH 7.6) greatly changed the stained cellular elements. It was our impression that the addition of Tris-HCl buffer enhanced the staining of the non-neuronal elements, whereas that of neuronal elements was suppressed by this procedure. Excess Tris-HCl buffer was rather unfavorable for the visualization of both neuronal and non-neuronal elements.

An increase in the the relative osmium concentration of the ZIO solution changed the number of visible non-neuronal cells. Although we did not perform any systematic experiment, it was certain that ZIO positive non-neuronal cells were more numerous in the preparations stained in the solution containing 1 part of OsO_4 and 3 parts of ZI than those in the preparation treated in the solution containing 1 part of OsO_4 and 4 parts of ZI. Most of the ZIO positive non-neuronal cells were found to be fibroblasts or fibroblast-like cells (Fig. 1). Their morphology perfectly corresponded to that of the interstitial cells described by Taxi,[8-10] Rogers and Burnstock,[41] Stach,[42] Christensen et al.,[52,59,60] Rumessen and

Thuneberg,[61] and others. Non-nervous cells such as smooth cells and vascular endothelial cells were also stained by ZIO. Basal-granulated cells in the epithelial tissue were constantly stained.

Treatment of the specimen for a long time (24-36 h) increased the contrast of the interstitial fibroblast-like cells that were described by many previous authors,[8-10,21,42,52,59,60] thus facilitating their visualization (Fig. 1). However, extended staining resulted in a heavily contaminated specimen. Furthermore, excess staining hindered the discrimination of individual neuronal processes. Conglomerations of rows of glial cells and bundles of neuronal processes frequently occurred (Fig. 2). They corresponded in appearance to the interstitial cells described by Cajal in the intestinal mucosa and pancreas,[2,3,65,66] to the sympathetic ground plexus of Boeke[4,6] and to the terminal syncytium of Jabonero.[26,27] They were also identical to the structures that Dogiel[67,68] and Kölliker[69] described as a kind of specialized connective tissue cell: their morphology corresponded exactly to that of the "eigenartige, sternförmige, anastomosierende *Bindegewebszellen*, welche die Arterien, Venen und Kapillaren durch die ganze Darmwand hindurch begleiten (p. 12, Müller[70])". We understood that precise ultrastructural discrimination between the nerve fibers and glial cell cytoplasm was practically impossible in the kind of specimens which Cajal used when he first described the interstitial cells.[1-3]

Transmission electron microscopy

Transmission electron microscopy revealed fine structures of all sorts of both cellular and fibrous elements in each layer of the intestinal wall. In the nerve strands of the autonomic ground plexus, two kinds of cellular elements were distinguished: 1) neuronal processes and 2) enteric glial (Schwann) cells (Fig. 3). It was fully evident that there were no such syncytial connections (or cytoplasmic fusions) between different neuronal processes and/or between neuronal processes and glial cells as had been predicted by many light microscopists of the earlier half of this century[4,6,18,19,21,71-77] (see also Ref. 7 and 28).

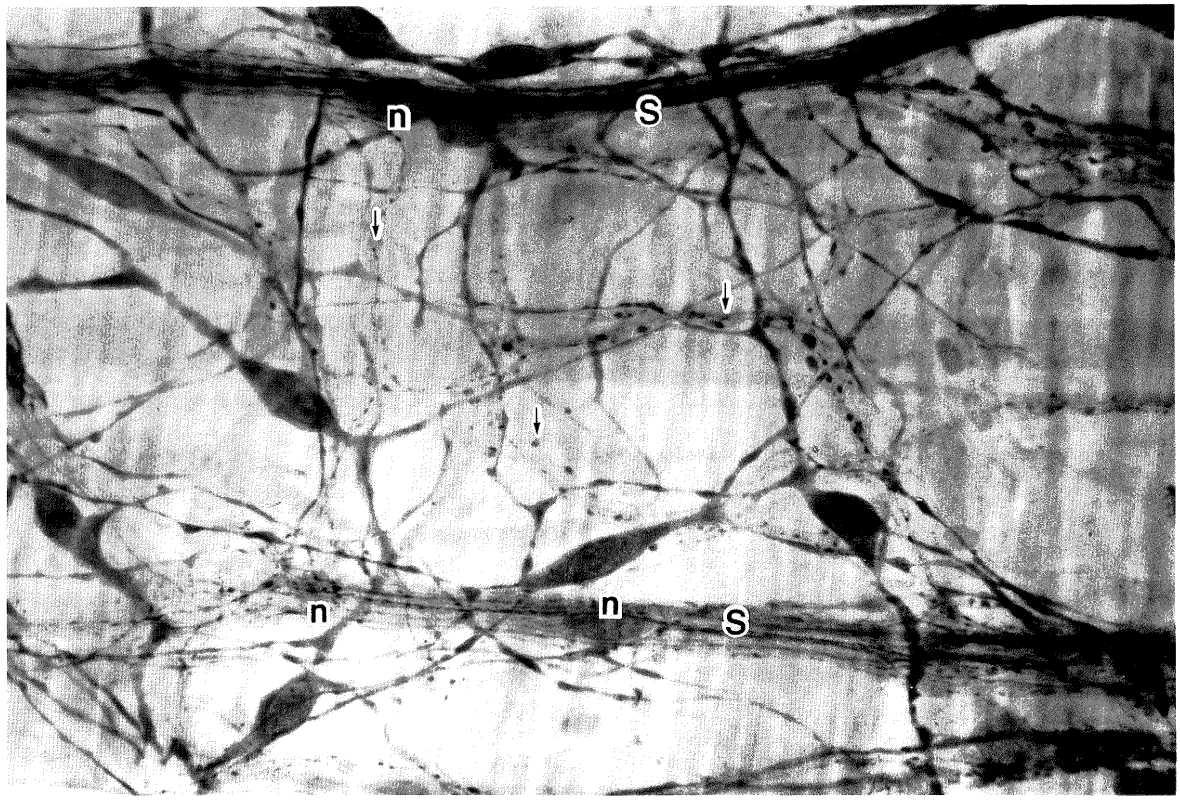

Fig. 1. Fibroblast-like cells with long tapering processes in the myenteric plexus of the guinea-pig jejunum. In this ZIO-stained preparation, the autonomic ground plexus is almost unstained except for a few terminal varicosities (small arrows). Profiles of secondary strands (*S*) are identified by a few ZIO-stained nerve fibers. *n*: Glial cell nucleus in the secondary strands. ×600

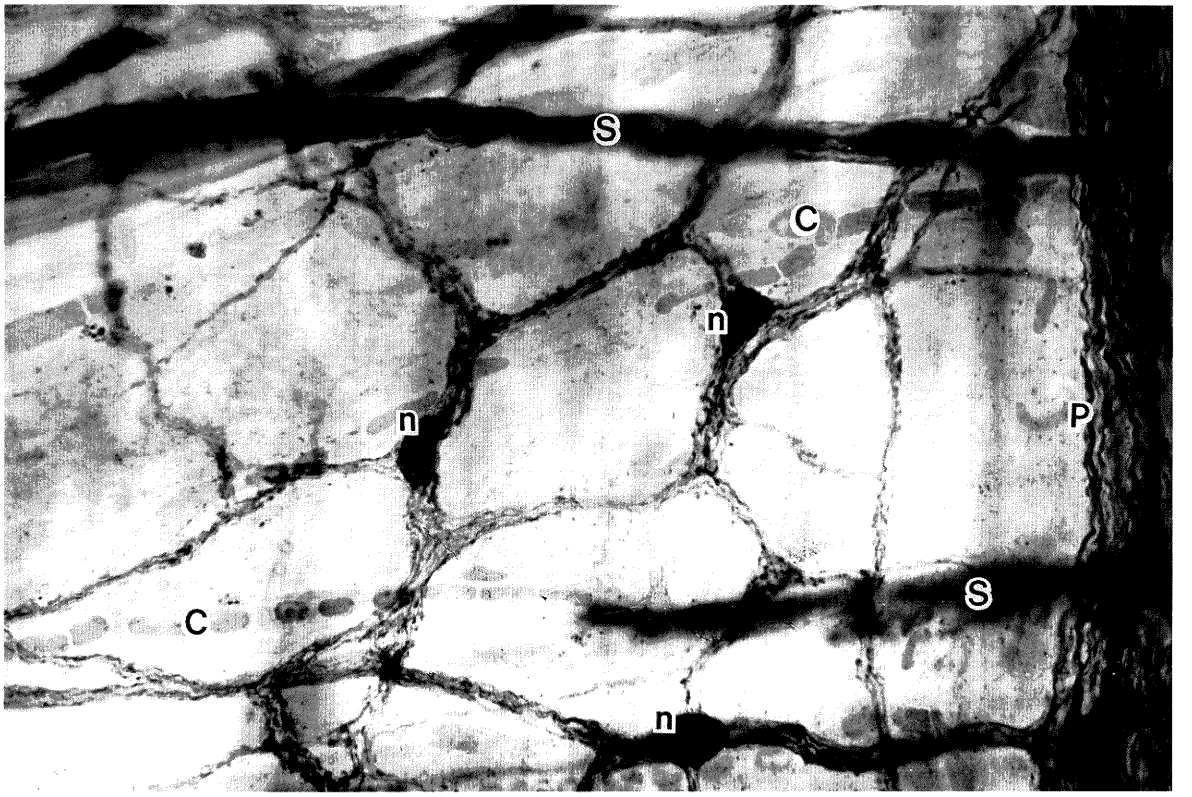

Fig. 2. Autonomic ground plexus of the myenteric plexus in the guinea-pig jejunum. ZIO-staining. The chimeric composition of glial cells (nucleus; *n*) and nerve fiber bundles is illustrated. There are also primary (*P*) and secondary nerve strands (*S*) of the myenteric plexus. Erythrocytes are arranged in a row in a blood capillary (*C*). ×600

Neuronal processes containing mitochondria, smooth-surfaced endoplasmic reticulum, microtubules and neurofilaments were grouped into bundles incompletely enclosed by a glial cell sheath as reported by previous authors.[9,10,30–35,40,41,44–48,78,79] Several nerve fibers were present within a single groove produced by the infolding plasma membrane of the glial cell. Positive ZIO staining was found in various numbers of these nerve fibers as pointed out by Stockinger and Graf[58] and Pellegrino de Iraldi.[57] The problem of ZIO positive and ZIO negative fibers was, however, not further investigated.

Enteric glial cells or enteroglial cells possessed a chromatin-rich nucleus. Their cytoplasm was irregular in shape and enclosed neuronal processes which were either positive or negative to ZIO staining. In the cytoplasm of glial cells, there were centrioles, Golgi complexes, mitochondria, granular endoplasmic reticulum and lysosomes as reported by previous authors. Microtubules and fine filamentous structures were also present.[10,30,36,41,43,48]

In the interstices between the smooth muscle cells and nerve strands and between epithelial cells and blood capillaries, there were profiles of fibroblast-like cells (Fig. 3). These corresponded to the specialized connective tissue cells previously described by the term connective tissue cells,[43] fibroblasts,[39,40,80] fibroblast-like cells[33,41,44] or interstitial cells of Cajal.[37,38,61] They were thus characterized by: (1) highly developed granular endoplasmic reticulum, (2) a lack of basal lamina, (3) frequent apposition with the cell membrane of smooth muscle cells and other fibroblast-like cells, and (4) close topographical relationship to a nerve bundle or a ganglion. In the smooth muscle coat, they corresponded to the four types of interstitial cells reported by Thuneberg (ICC-I, myenteric plexus; ICC-II, subserous plexus; ICC-III, deep muscular plexus; and ICC-IV, circular muscle plexus).[16,17] The cytoplasm and nucleus of some interstitial fibroblast-like cells were positively stained by the ZIO method (Fig. 3).

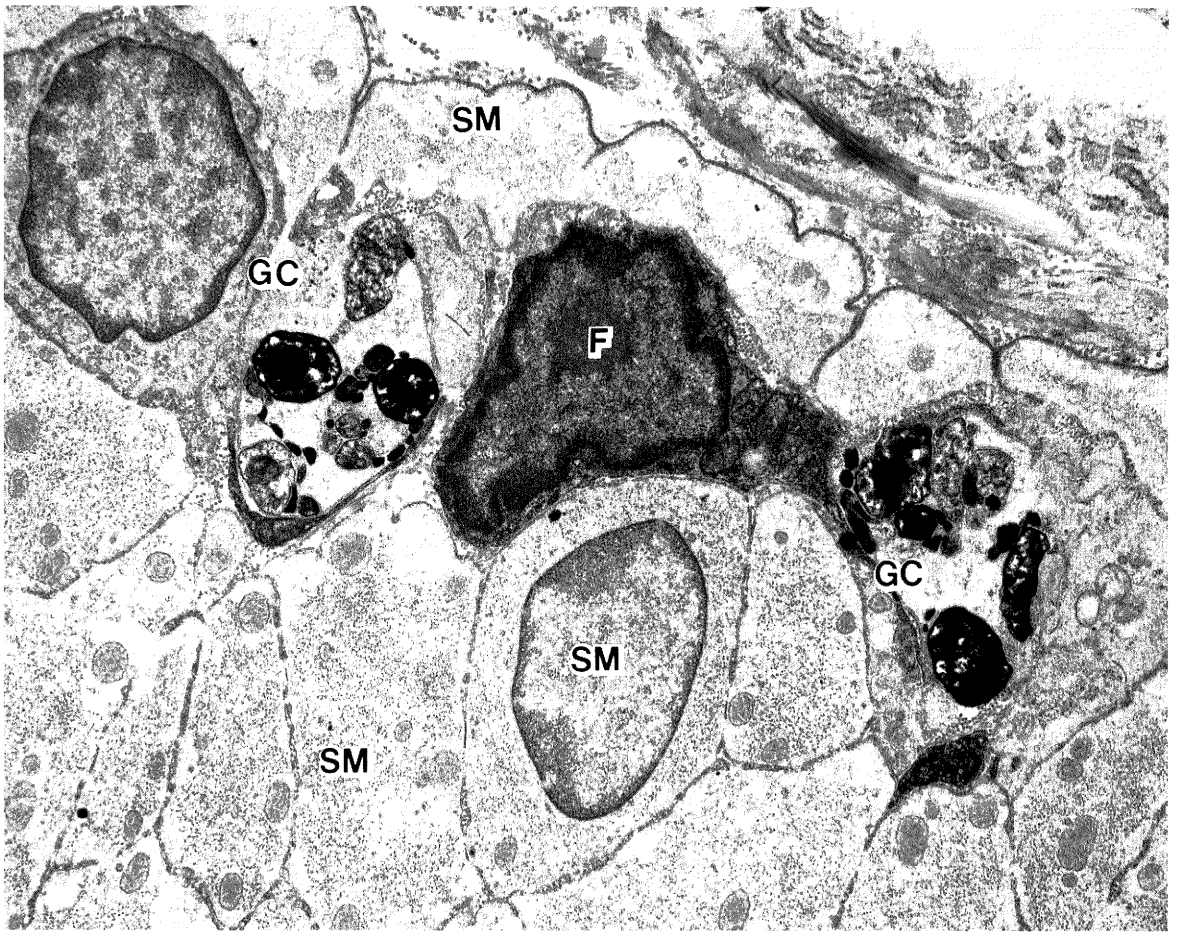

Fig. 3. Transmission electron micrograph showing the innermost portion of the circular muscle layer of the guinea-pig jejunum. A few nerve fibers ensheathed by glial cells (*GC*) and fibroblast-like cells (*F*) of the deep muscular plexus are positively stained by ZIO. Smooth muscle cells (*SM*), glial cells and elements of the submucous layer are not stained by ZIO in this micrograph. ×11,000

Scanning electron microscopy

Scanning electron microscopy of the enteric nerve plexuses was performed by previous authors.[17,50,81–83] Aided by scanning electron microscopy with an NaOH maceration method developed by Takahashi-Iwanaga and Fujita,[63] we were able to visualize the entire shape of various cells such as smooth muscle cells, fibroblasts, fibroblast-like cells, capillary endothelial cells and glandular epithelial cells. As this technique facilitated the removal of collagenous and elastic fibers resulting in the exposure of these cellular elements, we obtained a clear view of nerve strands forming an irregular network. Although there were severance-ends apparently caused during tissue preparation procedures, no true blind ends were found. Small nerve strands swelled at the sites where the enteric glial cell bodies were located. Some of them ran along the blood vessels, while others coursed beneath the network of flattened fibroblast-like cells. A considerable number of them were inserted into the space between the fibroblast-like cells and blood capillaries. Neuronal and glial elements could be identified at a higher magnification (Fig. 4).

Under the scanning electron microscope, the distinction between the terminal autonomic plexuses and the interstitial fibroblast-like cells was convincing. Networks of the fine nerve fiber bundles associated with the rows of glial cells fully corresponded to the structural images of Hillarp's autonomic ground plexus.[24,25] Glial cell nuclei occurred in the same general positions as those of the interstitial cells described not only by Cajal[1,2,65] but also by Lawrentjew,[18,19] Schabadasch[21] and others.

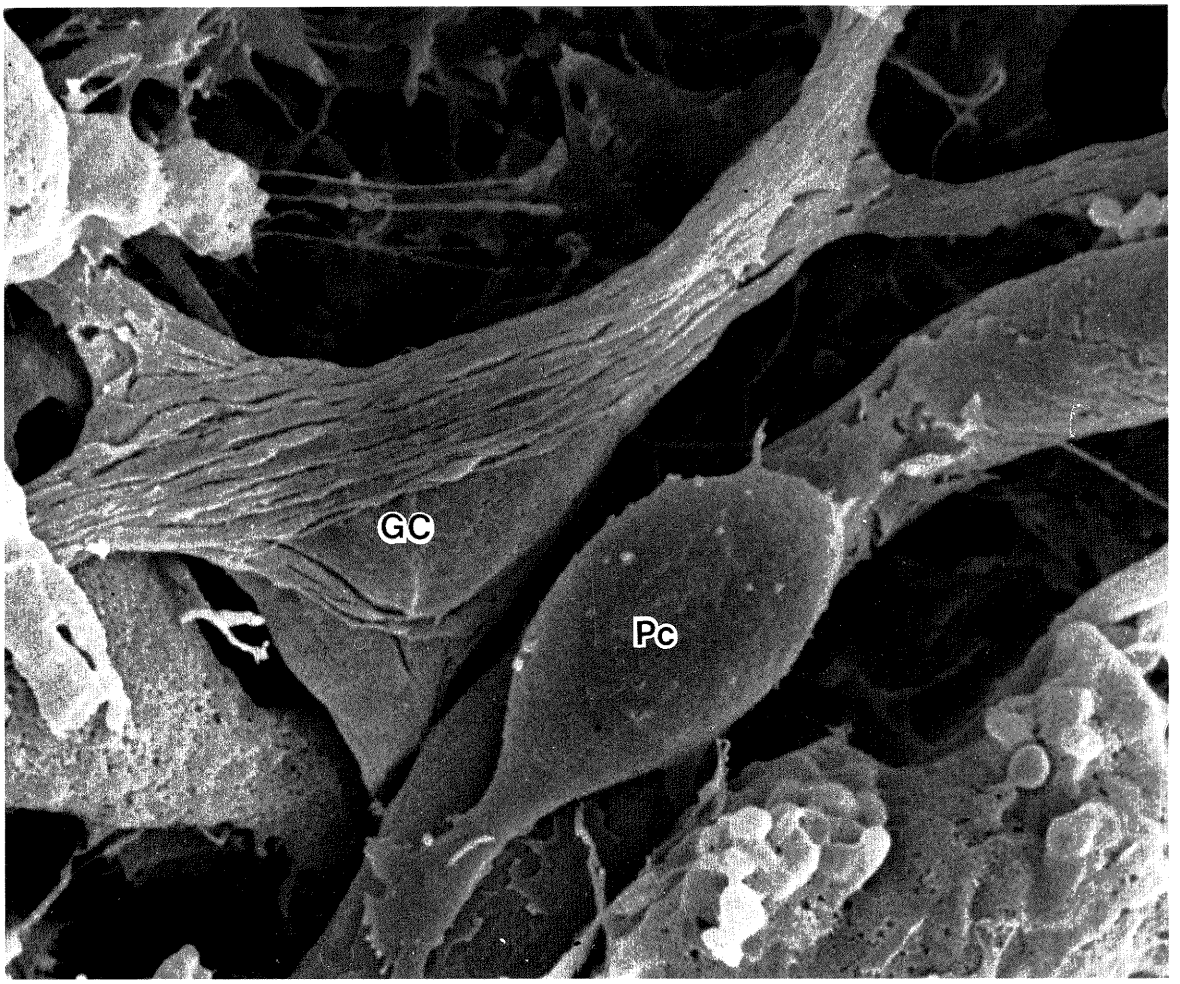

Fig. 4. Scanning electron micrograph showing a node of an autonomic ground plexus. This portion contains glial cell nucleus (*GC*). The star-shaped figure of the conglomeration of glial cell and neurites gives a neuron-like image in silhouette. A pericyte around a blood capillary is indicated by *Pc*. From the lamina propria mucosae of guinea-pig small intestine. ×13,000

HISTOLOGICAL STRUCTURE OF EACH ENTERIC NERVE PLEXUS

The enteric nerve plexuses were basically a three-dimensional network consisting of ganglia and nerve strands as described by Meissner,[84] Billroth,[85] Drasch,[86] Cajal,[65] La Villa,[87,88] Schabadasch,[21] Oshima,[20] Stöhr[22,76,89] and others.[90,91] There were, however, considerable variations of the histological structure in different layers of the intestinal wall. Cajal[65] reported interstitial cells not only in the mucosal coat but also in the smooth muscle coat. Therefore, we examined various layers of the enteric nerve plexuses.

Serous coat

The serous coat consisted of a layer of mesothelial cells and subserous layers. Squamous mesothelial cells were occassionally stained by the ZIO method, thereby displaying a typical mosaic pattern. The subserous layer consisted mainly of connective tissue intercalated between the serosal mesothelium and the external longitudinal muscles. There were subserous plexuses which connected the extrinsic nerves in the mesentery with the intrinsic nerve plexuses of the intestinal wall. The subserous plexuses were well-developed near the mesenteric attachment; here small ganglia were occasionally encountered.

In the ZIO-stained preparations, nerve elements in the subserous plexus were constantly demonstrated. Furthermore, fibroblasts were visualized between zig-zag collagenous fibers. These fibroblasts were identical to the interstitial cells of Cajal (ICC-II) of Thuneberg.[16,17]

Smooth muscle coat

Outer longitudinal muscle layer

Nerve plexus in the outer longitudinal muscle layer was poorly developed. It consisted of fine nerve bundles which ran parallel to the smooth muscle fibers. When the longitudinal muscle layer was thin, it was frequently difficult to decide whether the independent longitudinal muscle plexus was present. However, where the external longitudinal muscles formed thicker bundles, the longitudinal muscle plexus was distinct.

In the ZIO stained preparations fibroblast-like cells with blunt cytoplasmic processes existed. Both transmission and scanning electron microscopy showed that the histological features of these cells were identical to those of Thuneberg's ICC-II[16,17] of the subserous layer except for their localization.

Myenteric plexus

This nerve plexus was discovered by Auerbach.[92,93] As already noted by previous authors such as Taxi,[8-10] Stach,[42,62] Rumessen and Thuneberg,[61] and Christensen et al.,[52,59,60] both the glial and neuronal elements of the myenteric plexus are well-stained by the ZIO-method (Fig. 5). The outline of the tertiary plexus, i.e., the autonomic ground plexus, was identical to that reported by Kobayashi et al.[49] However,

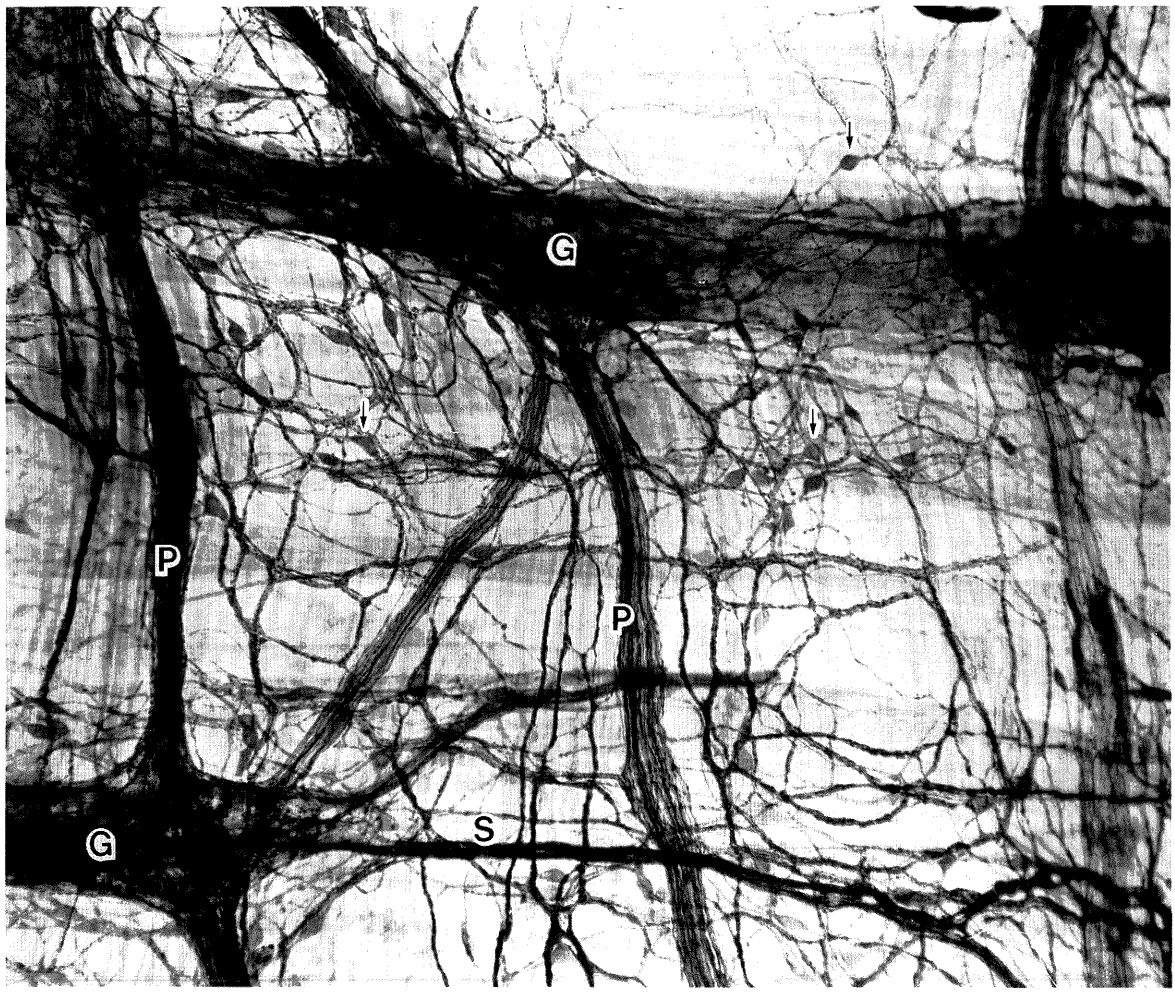

Fig. 5. Myenteric plexus of guinea-pig jejunum stained by ZIO. There are myenteric ganglia (*G*) and primary (*P*) and secondary nerve strands (*S*). Fibroblast-like cells (small arrows) are associated with the autonomic ground plexus. ×190

immunocytochemistry for S-100 protein showed only glial elements, whereas by the ZIO method both the glial and neuronal elements were demonstrated, although it was difficult to obtain a consistent results.

In addition to the above-mentioned nerve elements, non-neuronal as well as non-glial cells were stained by the ZIO method. These fibroblast-like cells projected dendritic processes which apparently formed a network. However, these fibroblast-like cell processes were devoid of the regular varicosities character-

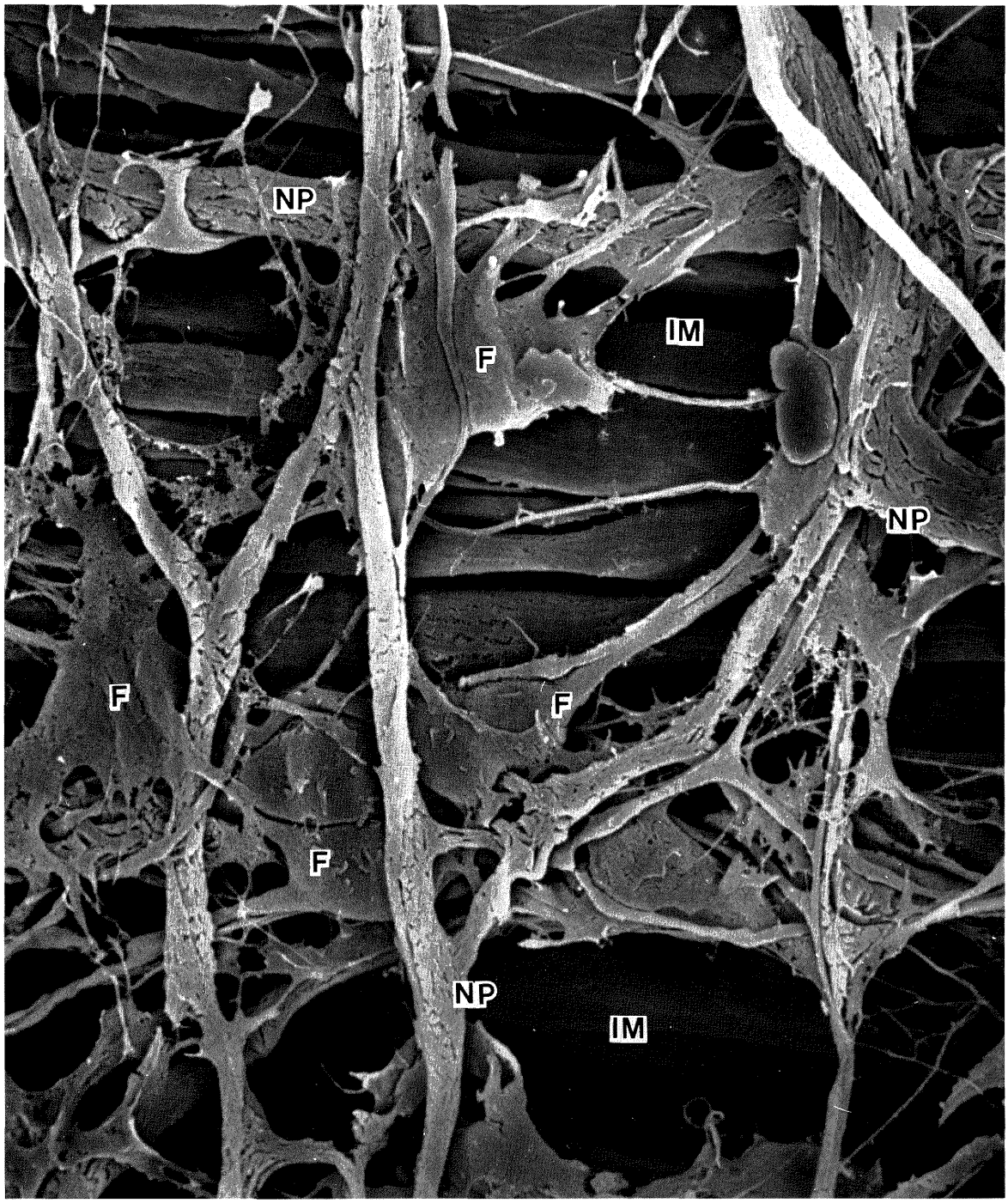

Fig. 6. Scanning electron micrograph of the myenteric plexus of guinea-pig jejunum. Note two kinds of networks: autonomic ground plexus (*NP*) and fibroblast-like cell network (*F*). Inner circular muscle layer is in the background (*IM*). ×6,000

istic to nerve terminals. Thus they corresponded to the interstitial cells of Cajal depicted by Taxi,[8-10] Stach,[42] Rumessen and Thuneberg[61] and Christensen et al.[52,59,60] The network of the fibroblast-like cells basically differed from the tertiary plexus (Fig. 6). Although a detailed description of these cells is unnecessary, the following points deserve mention:

1) ZIO-overstained tertiary plexus in the myenteric plexus layer was identical in appearance to the "interstitial cell network" of Cajal which he thought to be independent of "Auerbach's plexus".[65] Rintoul,[94,95] like Taxi[8-10] and Richardson[39,40] distinguished two kinds of cellular networks in the myenteric plexus layer: an autonomic ground plexus and a fibroblast-like cell network. Comparison of our results with the interpretation by Rintoul showed the following. The autonomic ground plexus represented what Rintoul called interstitial cells of Stöhr, whereas the fibroblast-like cells were the interstitial cells of Cajal. Our results demonstrated that La Villa's illustration (Fig. 2 of his paper[88]) of these structures in the myenteric plexus layer (Fig. 572 of Cajal's 1911 textbook[65]: see also Fig. 2 of Thuneberg[17]) was ambiguous as already pointed out by Schofield.[14] However, La Villa's "interstitial cells of Cajal"[87,88] must be nothing more than the structures described by Cajal[1,2] and Cajal and Sala[3] which are definitely a glial cell/neurite chimera.

2) The interstitial fibroblast-like cells were identical to the "interstitial cells of Cajal (ICCs-I)" of Thuneberg.[16,17] They probably corresponded to some of the structures which Dogiel illustrated as interstitial cells of Cajal in Fig. 5 of his 1895 paper.[67] However, Dogiel also included a chimeric composition of glial cells and neuronal processes in his Cajal'sche Zellen.[67] A typical example is found in Fig. 19 of his 1899 paper[68]; notice that Dogiel described his "sternförmige (bindegewebige) Zellen (i.e., Cajal'sche Zellen)" in the myenteric ganglion where, to the present-day knowledge, no fibroblast-like cells exist.

Superficial plexus of the circular muscle layer

The superficial plexus of the circular muscle layer is localized in the superficial (outer) portion of this layer.[14,21,49,53,96] It consisted of thicker intramuscular strands and interconnecting fine nerve strands. The intramuscular strands were mostly oriented transversely and similar in size and structure to the secondary strands of the myenteric plexus. In fact there were no sharp distinctions between these two strands: the secondary strands of the myenteric plexus gradually turned into the intramuscular strands of the circular muscle layer.[14,96]

In the ZIO stained preparations many profiles of fibroblast-like cells occurred. They were identical to the interstitial cells of Cajal (ICC-IV) of Thuneberg.[16,17] The interstitial cells reported by Lawrentjew,[18,19] Schabadasch[21] and Boeke[4,6] were not identical in profile to the fibroblast-like cells but were similar in shape to the autonomic ground plexus associated with fragmented nerve fiber bundles. Cajal (Fig. 573)[65] illustrated La Villa's drawing (Fig. 3)[88] of the interstitial cells of the circular muscle layer. Although a little ambiguity remains, we are of the opinion that these interstitial cells are different from the ICC-IV of Thuneberg.[16,17]

Deep muscular plexus

The deep muscular plexus was first described by Cajal[65] as the plexus muscularis profundus.[16] Li[97] demonstrated that this nerve plexus with no ganglionic cells (occasional ganglionic cells are known today to occur here) is sandwiched between two sublayers of the inner circular muscle, i.e., an inner, narrow layer consisting of specific thin, short smooth muscle cells, and an outer thick layer consisting of ordinary smooth muscle cells.

The deep muscular plexus has been examined by many authors using the ZIO technique.[8-10,50,61,62,98] The results of the present investigation were mostly in accordance with those of the previous authors (Fig. 7). However, the following points must be mentioned:

1) Both the autonomic ground plexus and fibroblast-like cells are demonstrated by scanning electron microscopy (Fig. 8).[99] The fibroblast-like cells corresponded to the interstitial cells of Cajal (ICC-III) described by Thuneberg and his coworkers.[16,17,61]

2) Cajal illustrated interstitial cells of the deep muscular plexus in Fig. 575 of his 1911 textbook.[65] On the right hand side of this figure, only neuronal processes were illustrated; the glial cell nuclei were hollowed out as blank spaces. The glial cell/neurite chimerae or fibroblast-like cell/neurite chimerae observed in the present study were identical to at least some of the interstitial cells illustrated on the left hand side of Fig. 575 of Cajal's 1911 textbook.[65]

3) Taxi[9,10] pointed out that his interstitial cells were devoid of varicose projections, whereas Cajal's interstitial cell possess these projections[1-3,65]. This difference may be a crucial point in resolving the cause of the debates.

Submucous coat

The submucous coat consisted of a highly-

Fig. 7. ZIO-stained deep muscular plexus of guinea-pig jejunum. **A:** General view. At this magnification identification of fibroblast-like cells is difficult. There are no ganglia in the deep muscular plexus. ×150. **B:** Autonomic ground plexus and fibroblast-like cells (small arrows) in the deep muscular plexus. The autonomic ground plexus consists of glial cells (*n*, their nuclei) and nerve fiber bundles. ×600

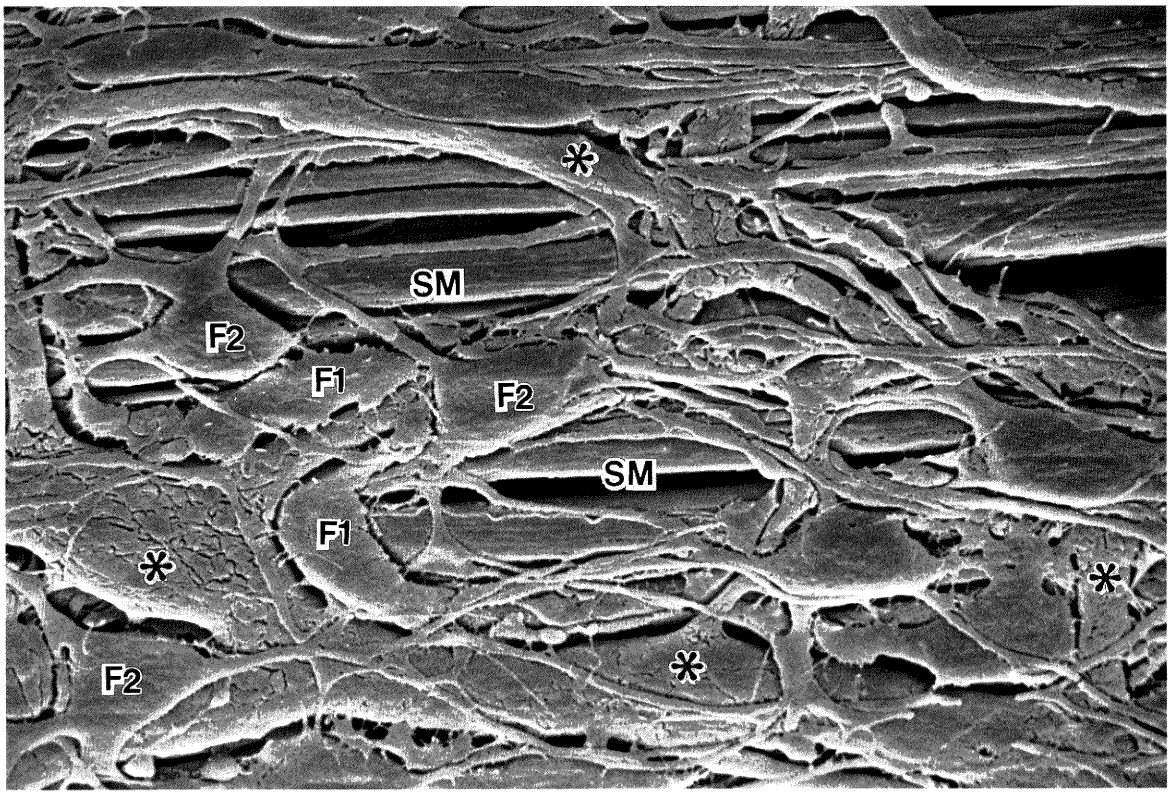

Fig. 8. Scanning electron micrograph of deep muscular plexus in the guinea-pig jejunum. There are autonomic ground plexus (asterisks), smooth muscle cells (*SM*) and two types of fibroblast-like cells (*F1:* cells with blunt cytoplasmic processes; *F2:* cell with long tapering processes). ×2,200

vascularized connective tissue located between the mucous coat and smooth muscle coat (Fig. 9A). It contained a richly branched nerve network called the submucous plexus of Meissner. The ganglia in this plexus were remarkably smaller in size than those in the myenteric plexus. There were no such thick nerve bundles as the primary strands of the myenteric plexus. Occurrence of an autonomic ground plexus was rare.

A considerable number of duodenal glands (Brunner's glands) constantly occurred in the proximal portion of the guinea-pig jejunum. Light microscopy of ZIO stained preparations showed baskets of the autonomic ground plexus ensheathing the aggregated alveoli of the duodenal glands.

Perivascular nerve plexus

The perivascular nerve plexuses[53,96] in the submucous coat are of particular importance, since Cajal and Sala[3] left illustrations of "the interstitial cells" associated with an artery (Fig. 5E of their 1891 paper). Similar structures were described by Dogiel[67,68], Bethe,[71] Schabadasch,[21] and many other researchers.[100]

The submucous coat of the guinea-pig small intestine contained thick branches of blood vessels (Fig. 9A). Relatively thick branches of arteries possessed dense perivascular and paravascular nerve networks as shown in Fig. 9A, B. In the ZIO stained preparations, fibroblasts and/or fibroblast-like cells were visible (Fig. 9B). However, differences in size and morphology between these cells and "Zellen Ramón y Cajal's" or "sternförmige Zellen" of Dogiel[67,68] were obvious. The ZIO-overstained chimeric compositions of the perivascular rows of glial (Schwann) cells and bundles of neuronal processes (Fig. 9B) fully corresponded to the structures illustrated by Cajal and Sala (Fig. 5E of their 1891 paper[3]) and Dogiel (Fig. 6 of his 1895 paper[67]; Fig. 20A–C of his 1899 paper[68]). Thus the occurrence of the glial cell / neurite chimerae in the perivascular plexus provides evidence that to what Dogiel[67,68] and Müller[101] described as the interstitial cells of Cajal truely corresponded (at least partly) what Cajal[1,2] originally described as interstitial cells. Furthermore, Bethe[71] and

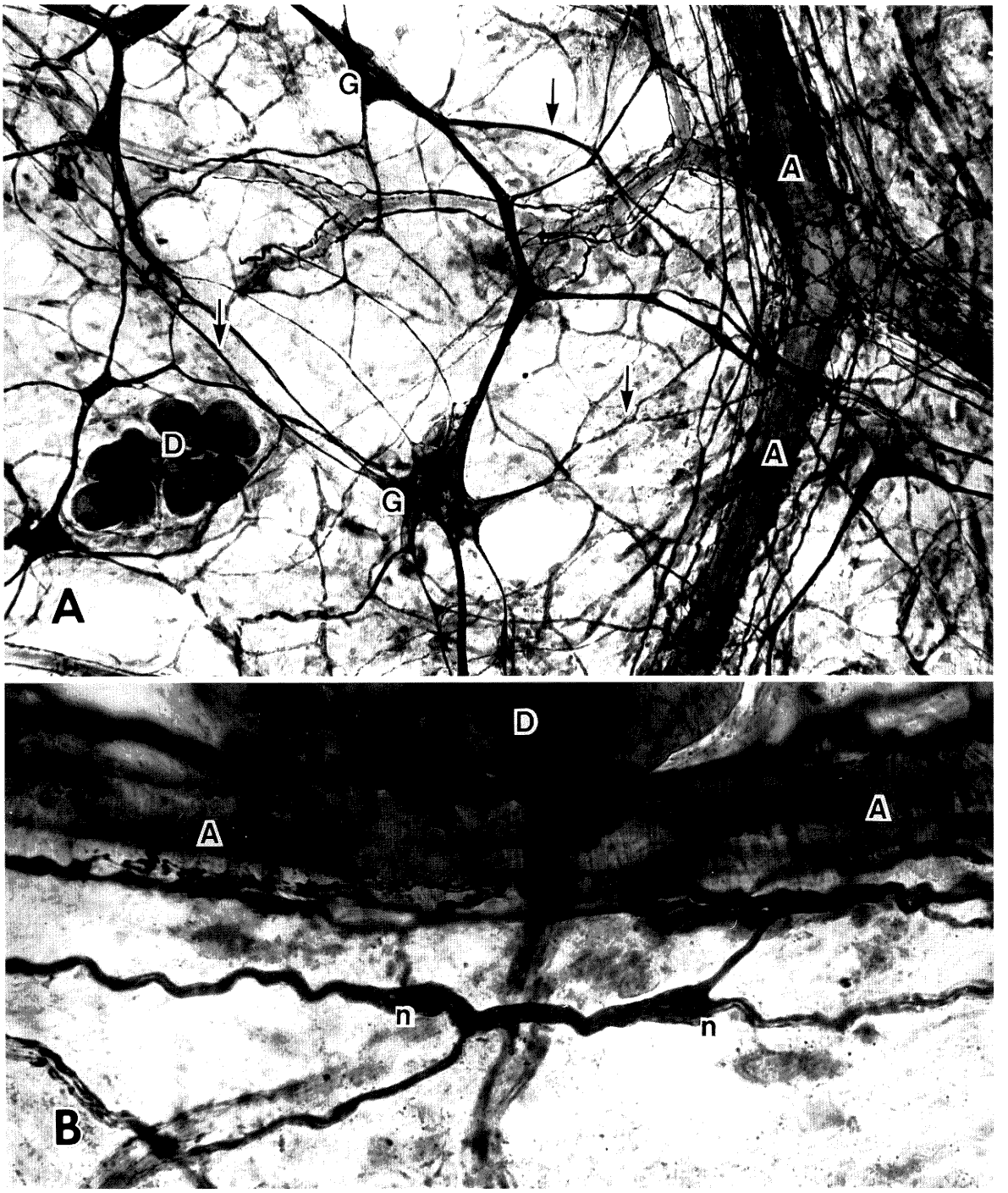

Fig. 9. ZIO-stained submucous layer of guinea-pig jejunum. **A:** General view. There are arterial system with perivascular plexus (*A*), submucous plexus with ganglia (*G*) and nerve strands (arrows) and duodenal gland (*D*). ×150. **B:** Nerve plexuses associated with an arteriole (*A*). *n:* Glial cell nucleus. Over-stained image of the autonomic ground plexus shown in this micrograph is identical to the illustrations of interstitial cells by Cajal and Sala (Fig. 5E of their 1891 paper[3]), Dogiel (Fig. 6 of his 1895 paper[67]; Fig. 20A-C of his 1899 paper[68]), and Schabadasch (Fig. 19-20 of his 1930 paper[21]). *D:* Duodenal gland. ×600

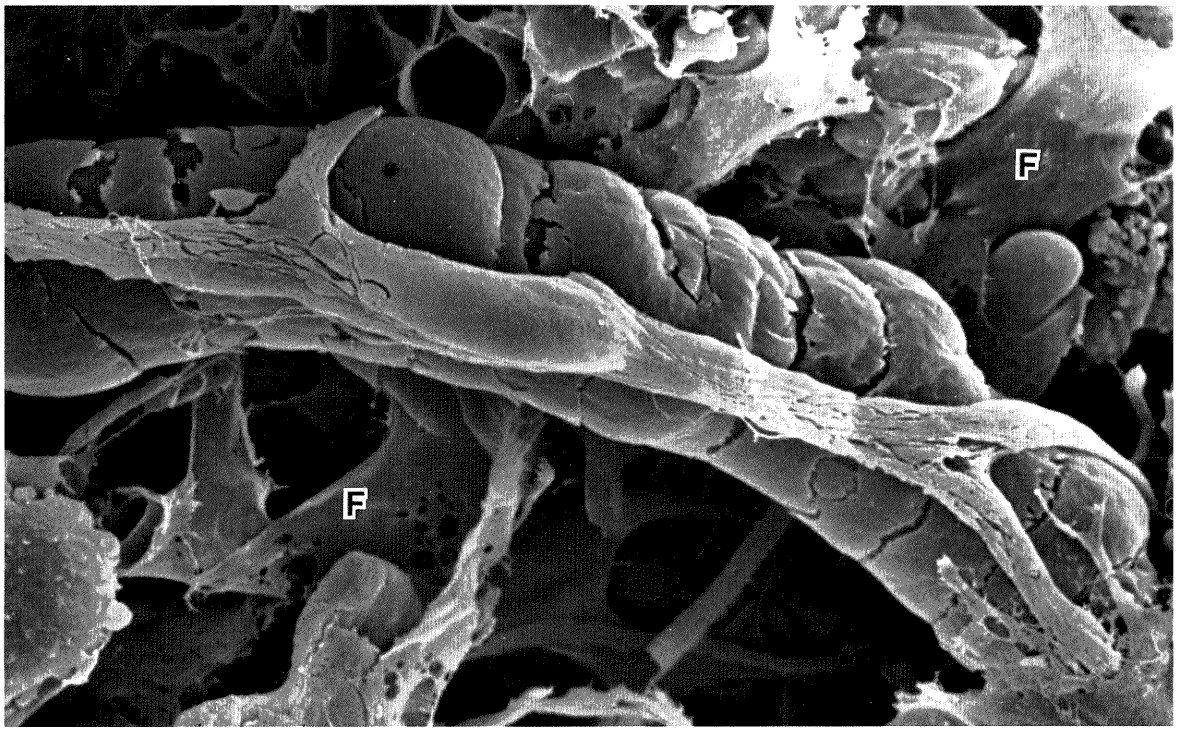

Fig. 10. Scanning electron micrograph showing an arteriole associated with perivascular nerve plexus. This arteriole was localized at the bottom of an intestinal villus in the guinea-pig jejunum. A helix of smooth muscle cells envelops the arteriolar wall. *F* indicates fibroblast with a blunt cytoplasmic process. ×2,900

Schabadasch[21] did not investigate the fibroblast-like cells or nerve cells but rather the same structures as Cajal[1,2,65] and Dogiel[66,67] (disintegrated perivascular terminal autonomic plexuses). Under the scanning electron microscope, the fine structure of the glial cell/neurite chimera was visualized (Fig. 10).

Mucous Coat

Lamina muscularis mucosae

The lamina muscularis mucosae was a thin sheet consisting mainly of smooth muscle cells and separating the mucous coat from the submucous coat. Light microscopy of the ZIO stained preparations showed a fine nerve network extending between tangled smooth muscle cells. Scanning electron microscopy revealed that bundles of nerve fibers were associated with a glial cell framework (Fig. 11). No ganglion cell body was recognizable. Abundant profiles of fibroblast-like cells occurred on and in the lamina muscularis mucosae. However, their cytoplasmic projections were apparently shorter than those in the deep muscular plexus and myenteric plexus.

Periglandular and villous plexuses

As described by Billroth[85] and Drasch,[86] there was a well-developed nerve network in the lamina propria mucosae in the villi and periglandular space. This was a three-dimensional latticework consisting of nerve strands embedded in the connective tissue space between the epithelial tissue and central lacteal and intermeshed with networks of blood vessels (Fig. 12-14).[49,102] No ganglia were found. Neither were recognized nerve cell bodies.

It was not always possible to discriminate the two cellular elements: glial cells and neuronal processes. However, many features such as the outline, localization, size, branching and anastomosis of the strands indicated that the ZIO positive nerve network represented the autonomic ground plexus proposed by Hillarp.[24,25] This was confirmed by transmission electron microscopy of the ZIO stained materials. Scanning electron microscopy showed that neuronal processes were varicose in appearance (Fig. 14). Fibroblast-like cells were present as reported by Desaki et al.[33] and Takahashi-Iwanaga and Fujita[103] (Fig. 12-14). However, they were different from the interstitial cells described by Cajal.[1,2,65]

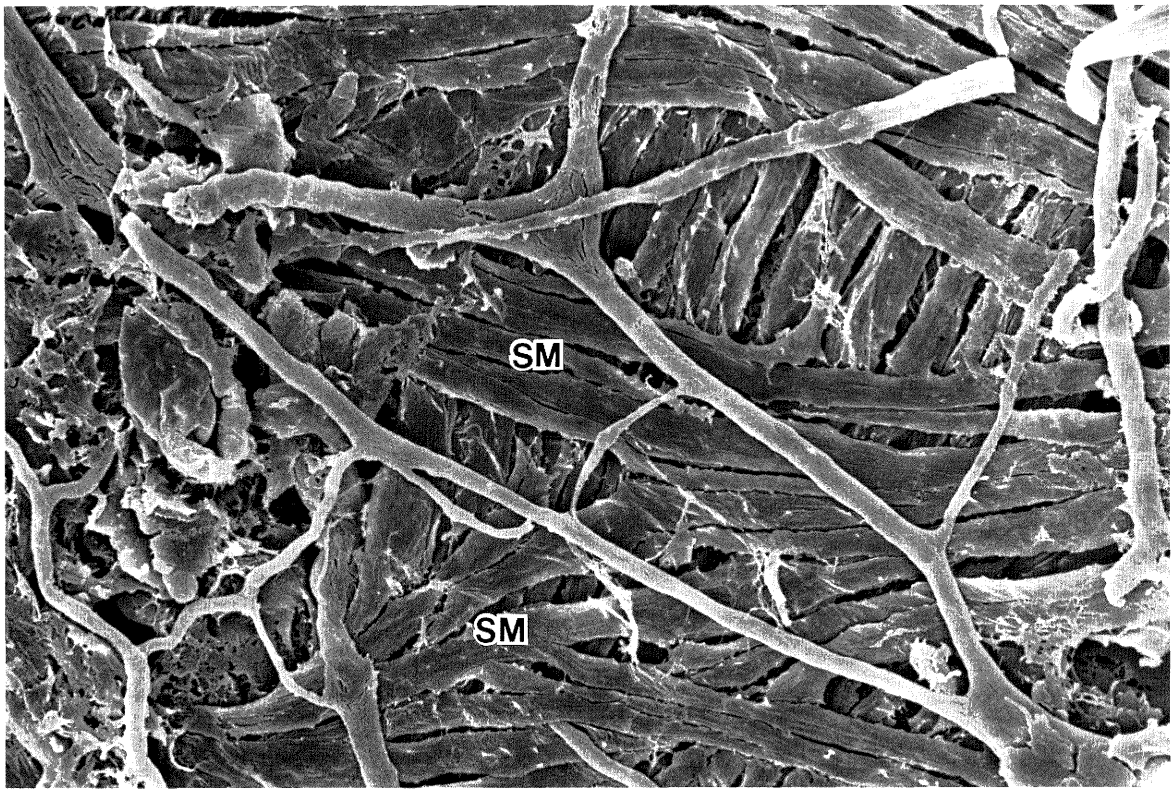

Fig. 11. Scanning electron micrograph of the lamina muscularis mucosae of the guinea-pig jejunum. A network of the autonomic ground plexus intermeshes with bundles of smooth muscle cells (*SM*). ×2,800

The network of the nerve plexus demonstrated in the present study was identical to the structure that Cajal referred to as interstitial cells except for the following points:

1) In the Cajal's preparation only a part of the villous plexus was demonstrated. The ZIO method demonstrated almost the whole length of the nerve network in the villus. The outline of the nerve plexuses was similar to that reported by Stach[102] using the acetylcholinesterase method, and Kobayashi et al.[49] using S-100 protein immunocytochemistry. The acetylcholinesterase method demonstrated both glial and neuronal elements, whereas the S-100 protein immunocytochemistry showed a specificity for glial cells.

2) Varicose, thin twig-like processes which projected from the tip of the thicker cytoplasmic process of the interstitial cells in Cajal's drawings[1,2,65] (see also Fig. 1 of Jabonero's 1965 paper[26]) were also seen in the specimen prepared by the ZIO-method. However, our scanning electron-microscope studies revealed that they were nerve strands of the autonomic ground plexus torn during specimen preparation.

PREVIOUS AND NEW CONCEPTS

Our light and transmission-electron microscope observations of Champy-Maillet (ZIO) stained preparations of the guinea-pig small intestine and the scanning electron microscope findings support the "autonomic ground plexus theory" proposed by Hillarp,[24,25] which he showed to be compatible with the independency of neurons and the network-formation of the autonomic end-apparatus. Concerning the historical development of concepts on the autonomic neurons,[104] glial cells and the so-called "interstitial cells of Cajal", we hold the following view. Among the vast literature particular attention was paid to the articles published by Cajal[1-3,65], Dogiel[66,67], Lawrentjew,[18,19] Stöhr,[22,76,89] Feyrter,[72-74] Hillarp,[24,25] and Thuneberg.[16,17] The last author gave a comprehensive survey of the past literature of the "interstitial cells of Cajal (1860-1980)".[16] We further reexamined discrepancies between the descriptions and figures in the works of these previous authors.

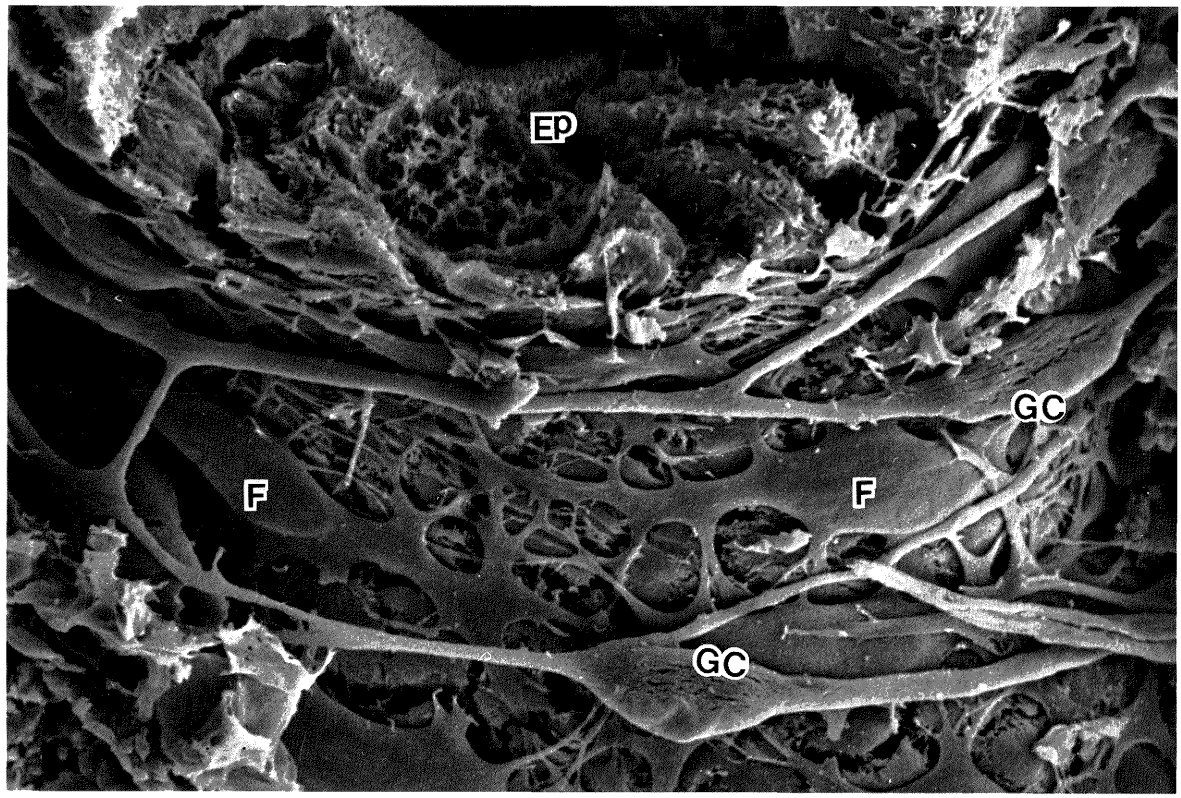

Fig. 12. Scanning electron micrograph of the periglandular nerve plexus in the guinea-pig jejunum. *Ep:* Epithelial cells of the intestinal gland. *GC:* Glial cell nucleus. *F:* Fibroblast-like cells forming a network which surrounds the intestinal gland. ×2,200

1. Neuronal theory: what Cajal described as interstitial cells

Schofield,[14] Rogers and Burnstock[41] and Gabella[15] discussed the possibility that the term interstitial cells of Cajal covers a number of different cell types. However, there is no sound evidence that Cajal described different structures under the same term. According to Rogers and Burnstock,[41] "Cajal's original description of sympathetic interstitial cells was actually a description of connective tissue cells (p. 255 of their 1966 paper).[41] We cannot accept this view so far as the guinea-pig small intestine is concerned. It is even doubtful whether these electron microscopists compared their micrographs with Cajal's original description.[1-3]

Cajal[65] outlined the enteric nerve plexuses in the mucosa, submucosa, muscular layer and serosa and suggested that the interstitial cells are terminal neurons. In some of his specimens prepared by the Golgi silver-impregnation method, he could not separate the bundles of neuronal processes from the glial cells which ensheathed them (Figs. 15, 16). Cajal originally used the term "interstitial cells" to describe the Golgi-impregnated nerve network in the mucous coat of the intestine and in the pancreas[1-3] (see also Ref. 28): hence the interstitial cells are, in fact, a chimera (or cellular amalgamation) consisting of glial cell bodies and extremities of neuronal processes. What led Cajal to mistakenly conceive that the interstitial cell was a neuron must have been that silver-impregnated varicose nerve terminals extending from the silhouette of the glial cell/neurite chimera.

In addition to those in the mucous coat which he studied in 1889 and 1893 Cajal later described interstitial cells in the smooth muscle coat of the intestine.[65] Since he left only a limited number of illustrations, identification of the structure which he actually described in the smooth muscle coat is difficult. However, those in the deep muscular plexus (Fig. 575A of his 1911 textbook[65]) are accounted for by the glial cell/neurite chimera (or fibroblast-like cell/neurite chimera). The nature of the interstitial cells in the myenteric plexus (La Villa's drawing cited as Fig. 572 of Cajal's 1911 textbook[65]) unintentionally left a little ambiguity. Thus the long

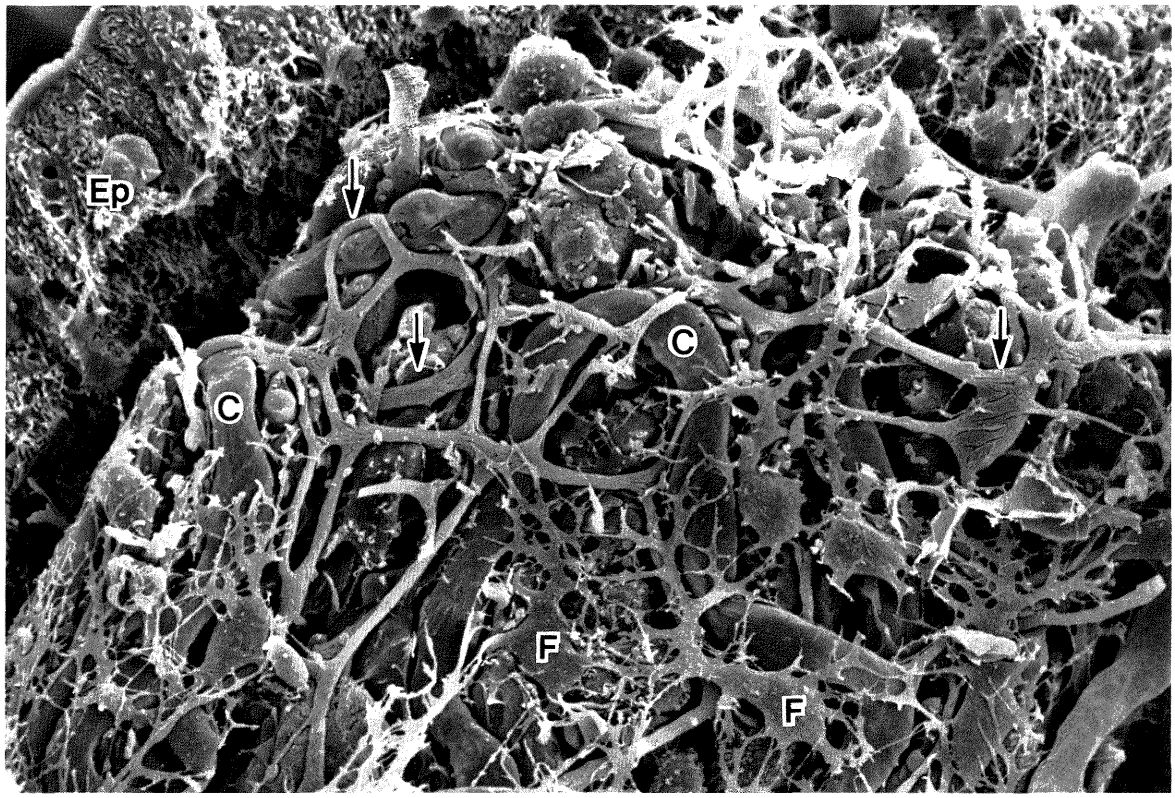

Fig. 13. Scanning electron micrograph showing a nerve network in the lamina propria mucosae at the tip of the intestinal villus as seen from the luminal side. The dome epithelium (*Ep*) was dislocated during specimen preparation procedures. The nerve network (arrows) intermeshes with vascular network (*C*) and fibroblast-like cells (*F*). ×2,800

disagreements on the nature of the interstitial cells of Cajal in the myenteric plexus are at least partly due to the equivocal illustration and description by Cajal himself especially in his textbook of 1911.[65] It is certain, however, that Cajal always believed the interstitial cells in the smooth muscle coat to be homologous to those in the mucous coat, and that all of Cajal's drawings of the mucosal interstitial cells we could examine showed glial cell/neurite chimerae. The drawings by Müller[101] also support our interpretation. It is furthermore worthy to note that Cajal did not refer to the existence of the special network of fibroblast-like cells in the smooth muscle coat which Taxi[8-10] and Richardson[39,40] described. Cajal[65] questioned Dogiel[67,68] who illustrated equivocal structures in Fig. 5 of his 1895 paper.[67] Another crucial point is that Cajal[65] did not deny the continuity of the neurofibrils of the neurons with those of the interstitial cells. Furthermore, Cajal[1,2,65] believed that the interstitial cell network was independent of Auerbach's plexus (primary and secondary strands in the present sense: see also Ref. 88). If Cajal described the fibroblast-like cells as interstitial cells in the myenteric plexus, what did he demonstrate for the terminal autonomic plexus in this layer? Paradoxically speaking, the interstitial cells illustrated in Fig. 572 of his 1911 textbook[65] (Fig. 2 of La Villa[88]) are the one and only structure that corresponds to his description of the tertiary elements in the myenteric plexus.

2. Sympathetic ground plexus theory: interstitial cells of Cajal as neurons

Cajal[65,66] was of the opinion that the interstitial cells were discontinuous to the autonomic post-ganglionic axons. However, we have pointed out that the projections of the glial cell/neurite chimera which Cajal saw in the Golgi-impregnated specimens[1-3] could only be accounted for by fragmented nerve fiber terminals. It is certain that Cajal could not fully demonstrate that axons of different neurons converge into a bundle in the terminal part of the autonomic nervous system (see Ref. 50).

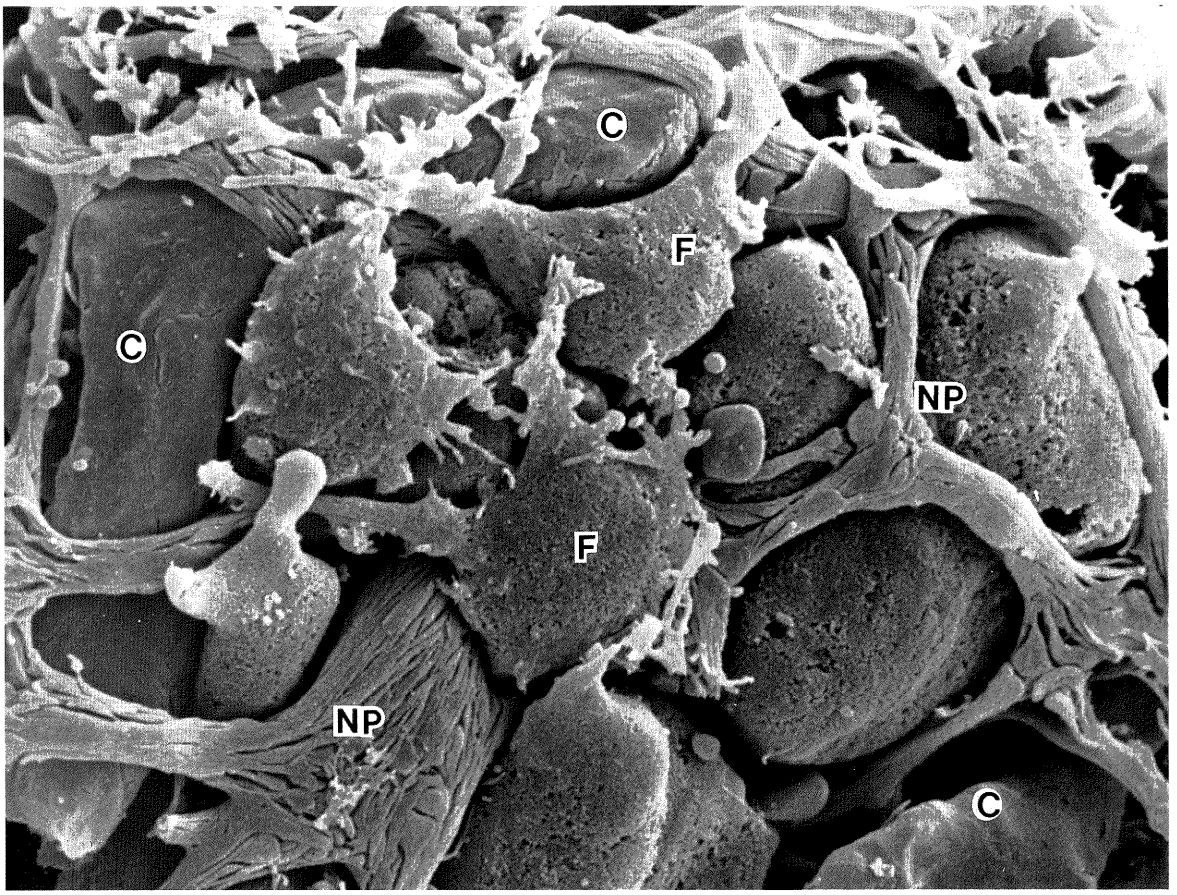

Fig. 14. Scanning electron micrograph showing lamina propria mucosae at the tip of an intestinal villus. View from the luminal side. The dome epithelial cells have been removed. Meshes of the autonomic ground plexus (*NP*) in this portion are smaller than those in the trunk of the villus as illustrated by Cajal (Fig. 568 of his 1911 textbook[65]). *C:* Blood capillary. *F:* Fibroblast-like cell. ×10,000

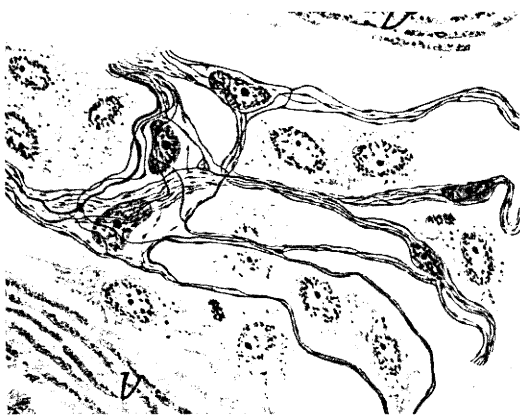

Fig. 15. Interstitial cells illustrated by Jabonero (Fig. 6 of his 1953 paper[26]). Jabonero[28] observed Cajal's original preparations 1889–1894. It is evident that Jabonero[26–28] described the glial cell/neurite chimera as an interstitial cell "syncytium".

Lawrentjew[18,19] who investigated the peripheral innervation apparatus, including that in the smooth muscle coat of the intestine, and proposed that the interstitial cells of Cajal were a kind of "nerve cell" intercalated between the post-ganglionic axons[104] and the effector cells such as smooth muscle cells. According to him, the peripheral autonomic network consisted of anastomosing interstitial "neurons". Thus the interstitial cells of Cajal were "Schwann cells with intracellular neurofibrils". The interstitial cells described by Lawrentjew[18,19] were similar in structure to those described by Cajal (Fig. 17). However, he could not demonstrate the exact relationship between the neuronal processes and glial sheath. Furthermore, Lawrentjew[18,19] could not account for the fact that the post-ganglionic axons continue into the autonomic end apparatus. Thus Lawrentjew considered that autonomic post-ganglionic axons might end either on or inside the interstitial cells of

Cajal. Lawrentjew seems to have understood the neuronal processes ensheathed by a glial cell as the neurofibrillar strands being enclosed in a protoplasmic sheath with dispersed nuclei of Schwann, but without definite cellular sheaths. In much the same way as Lawrentjew, Bethe,[71] Schabadasch,[21] Okamura,[105] Meijling[75] and Jabonero[26,27] regarded

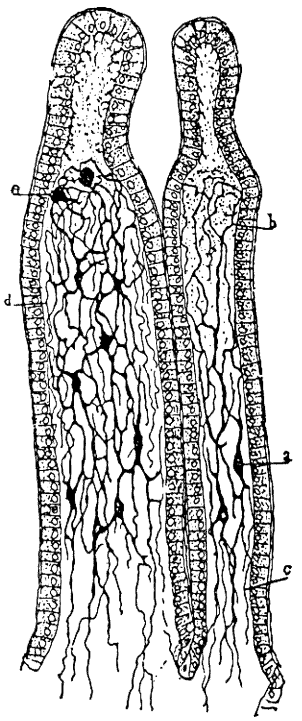

Fig. 16. Interstitial cells of the intestinal villi illustrated by Cajal.[1] Cajal could not differentiate nerve fiber bundles from the glial cell framework of the autonomic ground plexus. Thus, Cajal's interstitial cells were originally glial cell/neurite chimerae (i.e., autonomic ground plexus itself).

the glial cell/neurite chimera as a nerve cell which means the interstitial cell of Cajal. It must be mentioned that Cajal himself, however, was reluctant to accept the opinion of Lawrentjew (see p. 973 of Cajal's 1935 paper[66]). Cajal was not convinced of Lawrentjew's explanation of the terminal autonomic plexus, which was contradictory to the idea that no fusion of cytoplasm exists between different nerve cells (neurons).

Boeke[4,6] emphasized the observation that anastomoses between different neurofibrils were present in the living body, and proposed the "sympathetic ground plexus theory". Against the "neuronal theory", he considered that the autonomic end-formation consisted of delicate interwoven neurofibrils with scattered nuclei. Boeke stated that "even in a very primitive enteric plexus such as that in *Amphioxus lanceolatus*, the anastomoses of different cell processes may be studied with the utmost clearness" (p. 51 of his 1940 monograph[4]). Boeke considered that the cellular elements of the enteric nerve plexus of *Amphioxus* corresponded to Cajal's interstitial cells. These cellular elements of *Amphioxus,* to our view, probably included true neurons. Anastomoses of these cells can no longer be accepted. Furthermore, we noticed that what Boeke called "interstitial cells of Cajal" includes a wide range structures such as fibroblasts, although he stated that the sympathetic ground plexus consists of "plasmatic nucleated bands with neurofibrils" and that the interstitial cells only occur at the distal end of his ground plexus.

3. Terminal reticulum theory: interstitial cells of Cajal as Schwann syncytium

In the "terminal reticulum theory" ganglionic cells

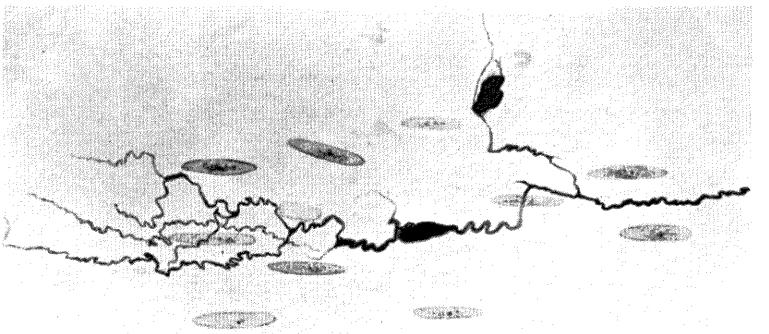

Fig. 17. Interstitial cells in the smooth muscles of the cat intestine illustrated by Lawrentjew (1926, Fig. 5[19]; also Boeke, 1940, Fig. 18[4]). According to Lawrentjew[18,19] the interstitial cells of Cajal were Schwann cells with intracellular nerve fibers (neurofibrils).

are not to be regarded as individuals.[77,106] According to this theory terminal portions of different ganglionic "cells" form a large syncytical mass throughout the whole body. The standard approach to this theory is that the fine argyrophile reticulum demonstrated by Reiser[106] and Stöhr[77] using Bielschowsky's method is a distorted composition of nerves and connective tissue elements caused by the tissue fixation and silver-impregnation procedures. By the reticularist's techniques, it was impossible to preserve the integrity of Schwann cells and the individual nerve fibers.[4,6,11,18,19,21–23,70,72–74,76,77,89,91,105] Furthermore, the optic systems of that time rendered it impossible to discriminate individual nerve fibers and Schwann cell sheath. Our conclusion on the different interpretations between Cajal[1–3,65,66] and Stöhr[22,23,76,77,89] is as follows. In Cajal's preparation silver-impregnated by the Golgi's method, the glial elements were so darkly stained that no neuronal processes ensheathed by them were visible. On the other hand, in the preparations by Stöhr who used Bielschowsky's silver-impregnation method, the cytoplasm of the Schwann cells was transparent. In Stöhr's preparation, the neuronal processes appeared long and smooth and were more numerous, whereas in Cajal's preparation they were short and varicose in appearance and fewer in number. This may have puzzled Stöhr, who at first felt that the cell had a connective tissue nature,[89] in identifying the varicose processes illustrated by Cajal as fragments of the neuronal processes. Thus Stöhr, in his later publications, concluded that the interstitial cells of Cajal were Schwann "syncytium".[23,76]

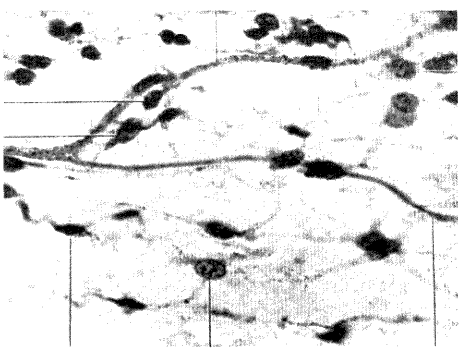

Fig. 18. Interstitial (or intercalated) cells illustrated by Feyrter (Fig. 19 of his 1951 monograph[73]). Section of the human stomach stained with Ehrlich's haematoxylin. Feyrter's intercalated cells are localized in the space between the autonomic ground plexus and effector cells. It is evident that Feyrter's intercalated cells are fibroblasts and/or fibroblast-like cells.

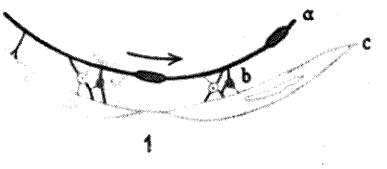

Fig. 19. Intercalated cells illustrated by Feyrter (Fig. 22-1 of his 1951 monograph[73]). Intercalated cells (*b*) bridge a space between the autonomic ground plexus (*a*) and the effector cells (*c*) such as smooth muscle cells. These intercalated cells (*b*) were called "interstitial cells of Cajal" by Thuneberg.[16,17]

4. Intercalation hypothesis of Feyrter

Feyrter,[72–74] basing on his study using Bielschowsky-Gros's silver impregnation method, was of the opinion that both afferent and efferent fibers of the extrinsic nerves, together with processes of intramural ganglionic cells, converge into a "vegetatives nervöses Endnetz" (Fig. 18, 19). Feyrter[72–74] claimed that his theory was similar to the sympathetic ground plexus theory proposed by Boeke.[4,6] However, it is certain that Feyrter too, regarded disintegrated bundles of neuronal and glial cell processes as a fiber network.[72–74] Thus, his vegetative nervous end-net containing neurofibrils was nothing but the autonomic ground plexus consisting of neuronal processes and glial (Schwann) cells. Feyrter did not notice that the interstitial cells described by Cajal directly related to this structure.

Thuneberg[16,17] stated that both Feyrter[72–74] and Cajal[1,2,65] are of the "intercalation hypothesis". However, it must be noted that the "interkalären Zellen" of Feyrter are not equal to the interstitial cells reported by Cajal. We conclude that Feyrter's intercalated cells coinside with Thuneberg's ICC-I and ICC-III (and also ICC-II and IV). Thuneberg did not point out the important fact that Feyrter haphazardly used the same term, "interstitial cells" for true connective tissue cells (see Knoche[107]). Furthermore, Thuneberg did not mention the fact that Dogiel[67,68] and Kölliker,[69] against Cajal's opinion, suggested a connective tissue nature for Cajal's structures, meaning the Golgi-impregnated terminal part of the autonomic nervous system.

5. Autonomic ground plexus theory: interstitial cells of Cajal as Schwann plasmodium

The description of Hillarp[24,25] concerning the structure of the terminal autonomic plexus is quite similar to that established by immunocytochemistry[49,50] and scanning electron microscopy.[108–111] In the "autonomic ground plexus theory", Hillarp explained networks of the autonomic end-apparatus in a way consistent with the independency of the neurons (Fig. 20).

Use of the term "Schwann plasmodium" instead of Schwann cells by Hillarp[24,25] is both of importance and interest. He seems to have used the term "plasmodium" rather than cells, to express the idea that the glial elements in the autonomic ground plexus form a continuous network rather than a row of independent cells. With the methylene-blue technique used by Hillarp, the individuality of glial (Schwann) cells was impossible to demonstrate. Separation of individual Schwann cells is now established by the application of transmission electron microscopes,[9,10] tissue culture and Golgi's silver impregnation method (Kobayashi, unpublished observation). Furthermore, although Hillarp[24,25] described the independency of neurons, he did not illustrate the neuronal terminals (but read p. 164 of Kappers[13]). We now have evidence suggesting that individual nerve fibers have definite end terminals, i.e., motor end plate-like terminals in the esophagus[14,112] and intraganglionic laminar endings in the myenteric ganglia of the esophagus and stomach.[52,113]

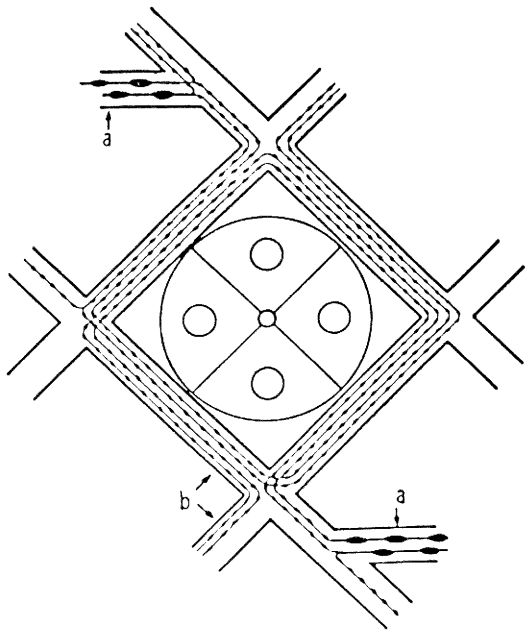

Fig. 20. Autonomic ground plexus illustrated by Hillarp (Textfig. 1 of his 1959 paper[25]). Hillarp[24,25] could explain the network formation of the autonomic innervation without conflicting with the neuron doctorine. Hillarp, however, regarded the interstitial cells of Cajal as "Schwann *plasmodium*". *a:* Axons from the preterminal nerve bundles. *b:* Autonomic ground plexus whose strands consist of glial cells and several axons derived from different neurons.

6. Electron microscopists' view: interstitial cells of Cajal as fibroblast-like cells

At the beginning of the electron microscopic era, Richardson[39,40] published a paper on the fine structure of autonomic nerves in the small intestine. He wrote that "the interstitial cells may be a fibroblast".[40] A similar interpretation had formerly been proposed by Taxi.[8,9] These works stimulated many electron microscopists to examine the "fibroblast-like cells" which they had predicted to be interstitial cells of Cajal.[7,33,35,37–44,51,53] Electron microscopy clearly showed that the axons are enveloped by glial (Schwann) cells and not by "interstitial cells". These electron microscopists did not reconsider what Cajal had actually found. Thus they described only fibroblast-like features such as highly developed granular endoplasmic reticulum, the occurrence of caveolae intracellulares and lack of basal laminae and the occurrence of gap junctions. However, the important point may be that both Taxi[8–10] and Richardson[39,40] denied the presence of long varicose processes of the interstitial cells which had caused Cajal to believe in the neuronal nature of these structures. Thus, many electron microscopists overlooked the point where both Taxi and Richardson described interstitial fibroblast-like cells whereas Cajal described glial cell/neurite chimerae or fibroblast-like cell/neurite chimerae under the same term interstitial cells. It is significant that Kappers[13] in his review of 1964 stated that "their real nature is still questionable (p. 167)".

It must be pointed out that electron microscopists mainly studied the smooth muscle coat, ignoring the interstitial cells of Cajal in the mucous coat. If electron microscopists had compared their micrographs with Cajal's illustrations of the cells in the mucous coat or if they had carefully read one out of several reviews written by Clara,[7] Kappers,[13] Botar[12] and others, they might have noticed that the interstitial cells originally described by Cajal (even those of the smooth muscle coat shown in Fig. 573 of the 1911 textbook)[65] were more closely related to their Schwann cells than to their fibroblasts or fibroblast-like cells.

7. Pacemaker hypothesis

Previous authors among them Taylor,[43] actually suggested that the specialized connective tissue cells are pacemakers for intestinal motility. Regrettably, however, the problem of the interstitial cells is currently separate from that of the pacemakers as the former has been investigated by morphologists while the latter by physiologists.

Thuneberg classified the interstitial cells of Cajal of the smooth muscle coat of mammalian intestine into four groups: ICCs I-IV.[16,17] He suggested that the ICC-I (myenteric plexus) and ICC-III (associated with the deep muscular plexus) as pacemakers for intestinal motility. However, we must point out that although his ICCs truly exist and form an independent cell category, they do not cover what Cajal[1,2,65] described as interstitial cells.

The ICCs I and III of Thuneberg[16,17] are the same cells described by Daniel et al.,[46,78] Fausonne-Pellegrini[34,35] and Christensen et al.[52,59] Since the characteristic localization and morphology of these cells suggest their probable significance in the intestinal function, many physiological and pharmacological studies have been performed.[43,114-117] However, the morphological basis of these studies must be carefully examined. Careless use of the term "interstitial cells of Cajal" gives the erroneous impression that Cajal first described and depicted these interstitial fibroblast-like cells.

Futhermore, the pacemaker "ICCs" reported by Benezin et al.[114] and Sanders et al.[116] are a special type of thin smooth muscle cells in the submucosal border of the circular muscle layer (Kobayashi, unpublished).

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