Framework of the Enteric Nerve Plexuses: 
An Immunocytochemical Study in the Guinea Pig Jejunum Using an Antiserum to S-100 Protein

Shigeru Kobayashi,1 Michiko Suzuki,1 Toyoshi Endo,2 Shigeru Tsuji3 and Edwin E. Daniel4

Departments of Anatomy (Prof. S. Kobayashi)1 and Medicine (Prof. T. Onaya),2 Yamanashi Medical School, Tamaho, Yamanashi, Japan; Laboratory of Cytology (Prof. J. Taxi),3 Faculty of Science, University of Pierre and Marie Curie, Paris, France; and Health Science Center (Prof. E. E. Daniel)4, McMaster University, Hamilton, Ontario, Canada

Received February 10, 1986

Summary. Immunostained sections and whole-mount preparations of the layers of the guinea pig jejunum were investigated by an improved peroxidase-antiperoxidase method using an antiserum to S-100 protein. A delicate latticework of S-100 protein immunopositive glial cells was demonstrated extending in the longitudinal muscle layer, myenteric or Auerbach's plexus, circular muscle layer including the deep muscular plexus, submucous layer including the submucous or Meissner's plexus, lamina muscularis mucosae and lamina propria mucosae. The whole enteric nerve plexuses consisted of two subsystems; nerve plexuses of the muscular coat and those of the submucous and mucous coats. These two subsystems were joined to each other by thick, connecting branches perforating the inner circular muscle layer. Extrinsic nerves entering the myenteric plexus formed a specialized junctional structure containing S-100 protein immunopositive glial cells, whereas those entering the submucous plexus ran along the submucous arteries. We proposed the term enteroglial cells to designate the S-100 protein immunopositive cells which ensheathed the somata and processes of the enteric neurons. The frameworks of all structures in the enteric nerve plexuses from the largest ganglia to the thinnest nerve fasciculi were constructed of these enteroglial cells.

A spectrum of the enteroglial cells was presented. Those in the myenteric and submucous ganglia were found similar to the astroglia of the central nervous system and to the satellite cells in the peripheral ganglia. Those in the primary and secondary fasciculi of the myenteric plexus formed a kind of neuropil together with the neuronal processes. Those in the tertiary fasciculi of the muscular coat formed the framework of the autonomic ground plexus. We tentatively concluded that the interstitial cells of Cajal contain an immunoreactivity for S-100 protein, and thus are glial in nature. The occurrence of specialized enteroglial cells with a neuron-like function was discussed in the autonomic ground plexus of the muscular coat. In the lamina propria mucosae, there was a fine latticework of the S-100 protein immunopositive enteroglial cells. This latticework corresponded to that of the interstitial cells of Cajal in the villous and periglandular plexuses.

* We wish to dedicate this paper to Professor emeritus Shomatsu YokoYAMA of the Fukushima University, a pioneer in enteric neurophysiology, on the occasion of his 73rd birthday.

**This research was supported by a Grant-in-Aid for Cooperative Research from the Ministry of Education, Science and Culture of Japan (No. 60304042).
S. Kobayashi et al.

Moore (1965) isolated an acidic soluble protein apparently characteristic of the nervous system and termed it the S-100 protein. A high immunoreactivity for bovine S-100 protein was detected in brain extracts of a large variety of mammals (Zomzely-Neurath and Walker, 1980). Later it was demonstrated that this protein is not absolutely specific to the nervous system, i.e., S-100 protein-like immunoreactivity was demonstrated in the adenohypophysis (Nakajima et al., 1980), chondrocytes (Stefansson et al., 1982a, b), melanocytes and Langerhans cells of the epidermis (Cocchia et al., 1981) and reticular cells of the lymphatic organs such as the thymus (Cocchia et al., 1983; Ushiki et al., 1984), lymphatic nodules and spleen (Takahashi et al., 1981; Iwanaga et al., 1982). However, it has also been recognized that the predominant location of the S-100 protein is still the nervous system. Immunocytochemical studies in the central nervous system have shown that S-100 protein is primarily localized in the glial elements, especially in the astroglia, whereas no demonstrable S-100 protein is present in either neuronal somata or processes (Matus and Mughal, 1975; Ludwin et al., 1976; Ghandour et al., 1981). A similar situation exists in the peripheral nervous system, where Schwann cells and satellite cells possess abundant S-100 protein (Stefansson et al., 1982a).

Concerning the enteric nervous system, Ferri et al. (1982) demonstrated the occurrence of immunoreactive S-100 protein in non-neuronal elements, i.e., glial cells, in the ganglia of both the myenteric and the submucous plexuses and in the nerve strands in all non-epithelial layers of the gut wall. They proposed that S-100 protein can be regarded as "a common marker for the glial components of the enteric nervous system." However, because of technical difficulties, our knowledge of the morphological features of the S-100 protein-immunopositive glial cells in the gut wall has been rather imprecise. We have recently improved on the immunostaining method for whole-mount preparations of the layers of the gut wall, the method originally invented by Costa et al. (1980) (Kobayashi et al., 1984, 1985; Kobayashi and Nishisaka, 1985). With this method, the immunoreactivity and fine structure of the S-100 protein immunopositive glial elements in the enteric nerve plexuses are well-preserved. Thus, the first purpose of the present paper is to describe the distribution and morphology of the glial cells in the gut wall as visualized by our immunostaining technique. We propose that they form the framework of the enteric nerve plexuses.

Cajal (1893, 1911) described a complex feltwork of interstitial cells associated with the terminal arborizations of the enteric neurons. Many authors, among them Dogiel (1895, 1899), Lawrence (1926), Hill (1927), Oshima (1929), Schabadasch (1930), Boeke (1949), Meyling (1953), Taxi (1952, 1959, 1965), Stöhr (1957), Hillarp (1959), Suzuki (1963), Ottaviani et al. (1964) and Stach (1972) investigated these cells, generally called the "interstitial cells of Cajal" (for review see Thuneberg, 1982). However, there is still controversy as to their nature and significance. Cajal cautiously suggested that the interstitial cells are "cellules (nerveuses) interstitielles," "neurones sympathiques interstitieles," or "vraies cellules nerveuses d'un caractere primitive." Lawrence (1926), Schabadasch (1930), Stöhr (1957) and Hillarp (1959) did not distinguish them from the Schwann cells. Taxi (1952) distinguished between Schwann and interstitial cells. Based on the results of his electron microscopic studies, Taxi (1959, 1965) later proposed that the interstitial cells are homologous to the cells of Henle's sheath enveloping the somatic nerves (Taxi, 1959, 1965). Yamamoto (1960) in his pioneering electron microscope study concluded that the interstitial cells of Cajal are none other than Schwann cells. However, this work was neglected by many later researchers. On the other hand, there are electron microscopists who have stated that the interstitial cells of Cajal represent either modified fibroblasts (Richardson, 1958, 1960; Yamauchi, 1964;
S-100 Protein Immunopositive Framework of Enteric Nerve Plexuses

ROGERS and BURNSTOCK, 1966; GABELLA, 1972; COOK and BURNSTOCK, 1976; KOMURO, 1982) or a kind of smooth muscle cells (IMAIZUMI and HAMA, 1969; YAMAMOTO, 1977). We compared the features of the enteric glial cells with those of the interstitial cells originally described by CAJAL (1893, 1911). Thus, the second purpose of the present paper is to discuss the natural history of the glial elements in the enteric nerve plexuses with particular reference to their identity with and differences from the interstitial cells of Cajal.

MATERIALS AND METHODS

Anti-S-100 protein serum
Bovine S-100 protein was prepared by ENDO et al. (1981). An antiserum to this protein was obtained from rabbits, as previously described (HIDAKA et al., 1983). Immunocytochemical studies using this anti-S-100 protein serum have been published elsewhere (HARA et al., 1983). The characterization of the immunoreactive S-100 protein, which is known to be a heterogeneous group of proteins with similar antigenic sites (ZOMLEY-NEURATH and WALKER, 1980), is beyond the scope of the present study. However, we confirmed that the antiserum selectively reacted with astroglia in the brain, stellate cells in the adenohypophysis and chondrocytes in the trachea. Furthermore, it specifically demonstrated the glial cells in the intestinal wall of the guinea pig.

Animals and methods of perfusion fixation
We used 20 Hartley strain guinea pigs of both sexes, 300–500 g in body weight, and fed ad libitum. Heparin (1,000 units/kg body weight) was intraperitoneally injected to prevent blood coagulation during the procedures of perfusion fixation. Under anesthesia with an intraperitoneal injection of pentobarbital sodium (50 mg/kg), the animals were stunned and bled from severed carotid arteries. Oxygenated Tyrode’s solution was perfused (40–60 ml/min) through a polyethylene tube inserted into the descending aorta for 3 min at room temperature, followed by one of the following fixatives for about 10 min: 1) Bouin’s fluid; 2) 4% formaldehyde in 0.1 M phosphate buffer at pH 7.2; 3) a fixative containing 4% formaldehyde and 0.5% picric acid; 4) a fixative containing 4% formaldehyde and 0.5% glutaraldehyde which was adjusted at pH 7.2 with 0.1 M phosphate buffer; and, 5) 10% formalin. The results described below were mostly obtained in those specimens fixed in Bouin’s fluid or the paraformaldehyde and picric acid mixture. Segments of the upper portion of the jejunum were removed and further fixed by immersion in the same fixative for 2–16 hrs at room temperature.

Immunostaining of whole-mount preparations
Whole-mount preparations of the different intestinal coats were prepared as described previously (KOBAYASHI et al., 1984, 1985; KOBAYASHI and NISHISAKA, 1985). Using the antiserum to S-100 protein, the tissues were immunostained by the peroxidase-antiperoxidase method (STERNBERGER, 1979) which was reformed for the whole-mount preparations.

Immunostaining of cryostat sections
Segments of the fixed jejunum were kept overnight in a phosphate buffer containing 20% sucrose at 4°C. Frozen sections, 10–30 μm thick, were cut on a Sakura CM-41 cryostat and mounted on gelatin coated glass slides. The sections were kept in a 0.3%
Triton X-100 in a 0.1 M phosphate buffer of pH 7.2 for 4 days at 4°C. The conventional peroxidase-antiperoxidase (PAP) method (STERNBERGER, 1979) was used with slight modifications. The tissue sections were treated with 0.3% H₂O₂ in methanol for 30 min to block endogenous peroxidase activity, then washed with a phosphate buffered saline (PBS: 0.01 M, pH 7.2 with 0.14 M NaCl) followed by non-immune bovine serum albumin. The sections were incubated with rabbit anti-bovine S-100 protein antiserum (1:1000) for 48 hrs at 4°C, washed with PBS and incubated with goat anti-rabbit IgG (Miles, Lot. G404), washed with PBS and incubated with rabbit PAP complex (Miles, Lot. E170) for 100 min at room temperature. After washing with PBS, the sections were treated with 3,3'-diamino-benzidine (Wako Chem., 20 mg/100 ml) in a 0.05M Tris-HCl buffer (pH 7.6) containing 0.01% H₂O₂.

Specificity controls
Specificity controls for the immunocytochemical reactions included the use of the antiserum preabsorbed with 100 µg/ml of the bovine S-100b protein (see also HARA et al., 1983). No glial elements were immunostained with the preabsorbed antiserum.

Microscopy and photography
An Olympus light microscope (Vanox AHB-LB) equipped with a green filter (IF550) was used for examination and photography. Photomicrographs were taken with Kodak Panatomic X-film.

The camera lucida drawings of immunostained tissue preparations were made with an Olympus microscope (BHTU) at a magnification of ×1200 using an Olympus drawing tube (BH-DA-LB).

RESULTS

1. Glial cells with immunoreactive S-100 protein (Enteroglial cells)
Figures 1 and 2 show examples of immunostained whole-mount preparations of the guinea pig jejunum examined in the present study.

The wall of the guinea pig jejunum consisted of four coats: 1) serous, 2) muscular, 3) submucous, and 4) mucous (Fig. 2). 1) The serous coat was composed of peritoneum supported by a layer of subserous connective tissue. 2) The muscular coat was formed by a thin longitudinal muscle layer and a thick circular muscle layer. The myenteric plexus or Auerbach’s plexus occupied the narrow space between the longitudinal and circular muscle layers. 3) The submucous coat was constructed of a loose connective tissue containing blood and lymphatic vessels. The submucous plexus or Meissner’s plexus extended in this layer of the gut wall. 4) The mucous coat was provided with intestinal crypts and villi which were lined with or covered by a characteristic epithelium. The lamina muscularis mucosae separated the submucous coat and mucous membrane. The core of the villi and the inter-cryptal space formed the lamina propria mucosae.

Most, if not all (vide infra), of the S-100 protein-immunopositive cells were glial in nature. They occurred in the domain of the enteric nervous system which contained no blood vessels or connective tissue elements. All the enteric neurons, both their somata and processes, were devoid of immunoreactive S-100 protein. We propose a term, enteroglial cells, to designate collectively the S-100 protein immunopositive glial elements in the enteric nerve plexuses.
There were no myelinated elements among glia of enteric nervous system. The enteroglial cells were distributed throughout the non-epithelial portion of the intestinal wall to form a three-dimensional latticework. This enteroglial cell latticework was absent in the peritoneal mesothelium, villous and cryptal epithelium, duodenal gland parenchyme, and vascular endothelium and media. Adipose cells in the subserous and submucous spaces and reticular cells in the lymphatic nodules in the mucous coat were immunoreactive for S-100 protein, but they were excluded from consideration because they did not participate in the formation of the framework of the enteric nerve plexuses. At times a certain degree of immunostaining occurred in the epithelial cells of the villi and crypts and in various connective tissue cells, but these “background” reactions did not seriously hinder the identification of the enteroglial cells.

The enteroglial cells possessed elliptical nuclei; their major axis measured 8–10 μm, whereas their minor axis measured 4–6 μm. They were remarkably shorter in the major axis and longer in the minor axis than those of the smooth muscle cells, and smaller than the vacuolar nuclei of the ganglionic cells. The nuclei of the enteroglial cells were rich in heterochromatin and possessed a conspicuous nucleolus.

The S-100 protein-like immunoreactivity in the nuclei of enteroglial cells was dependent on the kind of fixative. After Bouin fixation, the immuno-coloration of the nuclei was paler than that after fixation in the formaldehyde/picric acid mixture.
The Bouin's fluid, buffered formaldehyde or formaldehyde/glutaraldehyde mixture gave an intermediate degree of immuno-coloration of the nuclei between the reactivity after formalin fixation and that after formaldehyde/picric acid-fixation. The nuclei of non-glial cells, such as ganglionic cells, smooth muscle cells, mucous and serous epithelial cells, contained no S-100 protein-like immunoreactivity. Therefore, it seems reasonable to suggest that the immunocoloration of the enteroglial cell nuclei represents an authentic occurrence of S-100 protein. However, further, studies are needed to exclude the possibility that the immunoreaction might be due to the diffusion of cytoplasmic S-100 protein during the procedures of specimen preparation.

The enteroglial cells possessed long cytoplasmic processes which enveloped the somata and processes of the enteric neurons. It was not possible in the present study to determine the lengths of the cytoplasmic processes, because we could not separate individual enteroglial cells. However, each enterogial cell most likely projected several cytoplasmic processes longer than 25 or 50 μm, because the nuclei of the cells usually lay 50-100 μm apart from each other. It was also probable that their processes were in contact with each other and with ganglionic cells: we confirmed the non-syncytial nature of the enteroglial cells by electron microscopy (Y. ENDO, T. ENDO and S. KOBAYASHI, unpublished).

The cytoplasm of the enteroglial cells was relatively homogeneous and reduced to a narrow rim. In the present study, the immunoreactive S-100 protein was not associated with any particular cell organelles of the enteroglial cells. Mitochondria, a Golgi apparatus, ergastoplasm and lysosomes were not visible as none of these were specifical-
ly immunostained for S-100 protein. No secretory granules occurred in the enteroglial cell cytoplasm.

There was a longitudinally-striped pattern associated with the long cytoplasmic processes which branched at right angles. A single stripe was frequently traced for more than 100-200 μm in length. We understood these longitudinal striations to represent the formation of a kind of neuropil by the conglomerated enteroglial cells and neuronal processes.

2. Enteroglial framework in different layers of the jejunum wall

The enteroglial cells, as building blocks, comprised the framework of the ganglia and interconnected nerve fasciculi of the enteric nerve plexuses. Their distribution, arrangement and shape were markedly varied from layer to layer in the jejunum wall as shown in Figures 3-10. Characteristic features of the glial framework in the different jejunum coats will now be described.

a. Serous coat

On the mesenteric border of the jejunum, there were considerable numbers of extrinsic nerves whose Schwann cells contained immunoreactive S-100 protein. The extrinsic nerves were tortuous in course though uniform in caliber for a long distance. Some of them ran through the subserous layer independent of the blood vessels, whereas others penetrated the layer with the blood vessels into the submucous layer. No nerve plexus was formed in the subserous connective tissue.

A few extrinsic nerves directly entered the ganglion (Fig. 5C) or primary fasciculus (Fig. 5D) of the myenteric plexus after penetrating through the longitudinal muscle layer of the muscular coat. There were unique structures at the junction of the extrinsic nerves and the myenteric plexus (Fig. 5C, D). These structures were variable in appearance. The one shown in Figure 5D is fan-shaped with the basis on the side of the primary fasciculus of the myenteric plexus. The S-100 protein immunopositive glial cells in this junctional device exhibited transitional features between the Schwann cells in the extrinsic nerve and the enteroglial cells in the ganglia and primary fasciculi of the myenteric plexus.

No immunopositive ramifying cells were present in the subserous connective tissues.

b. Muscular coat

The enteric nervous system consisted of two subsystems: 1) muscular and 2) submucous plus mucous. For the convenience of description, the nervous system in the muscle coat was divided into: 1) the plexus in the longitudinal muscle layer, 2) myenteric or Auerbach’s plexus, 3) superficial plexus of the circular muscle layer, and 4) deep muscular plexus. Nerve plexuses in the muscular coat are shown in Figures 3-6.

1) Longitudinal muscle layer

All the extrinsic nerves which entered the enteric plexus first passed the subserous layer and then penetrated the longitudinal muscle layer. However, the intramuscular segments of the extrinsic nerves were so short that few or no profiles of the extrinsic nerve bundles were demonstrated in the immunostained sections.

In the whole-mount preparations of the longitudinal muscle layer in which the myenteric ganglia were carefully removed, a delicate meshwork of the enteroglial cells was visible (Fig. 5B). The immunopositive meshwork was relatively uniform in
Fig. 3*. Framework of the myenteric plexus in the guinea pig jejunum. A. Prominent ganglia (g) and interconnecting thick nerve strands (p) constituting the characteristic latticework of the primary plexus. The secondary plexus is composed of medium-sized nerve bundles running in a circular direction (s). Fine meshes of the S-100 protein immunopositive cells represent the tertiary plexus. Lymphatic vessel. x 30. B. Distribution of immunoreactive S-100 protein in and around a myenteric ganglion. Shapes of individual enteroglial cells are fairly visible in the secondary (s) and tertiary fasciculi (t). In the primary fasciculi, nuclear profiles of some enteroglial cells are identified (arrowheads). Somata of the myenteric ganglion cells (asterisks) are apparently immunonegative to S-100 protein. Profiles of empty blood vessels (thin arrows) weave around the ganglion. x 250

*Photomicrographs of the whole-mount preparations are so arranged as to place the top of each picture in the oral direction.
Fig. 4. Enteroglial cells seen in the myenteric plexus of the guinea pig jejunum. A. Myenteric ganglion, nerve strands and autonomic ground plexus framed by enteroglial cells. There are nuclei of enteroglial cells (arrowheads) within the primary (p), secondary (s) and tertiary (t) fasciculi which show a longitudinally-striped pattern. ×390. B. “Solitary” ganglionic cell and “mini-ganglion” containing only three neuronal somata situated in a secondary fasciculus (s). Primary (p) and tertiary fasciculi (t) are also present. bv Blood vessel. ×390
pattern throughout the jejunal tube. It was frequently impossible to separate the nerve plexus in the longitudinal muscle layer and the myenteric tertiary plexus. The nerve plexus of the longitudinal muscle layer consisted of only the tertiary fasciculi. Thus, the longitudinal muscle layer of the guinea pig jejunum possessed only the autonomic ground plexus.

2) Myenteric plexus

We use the term, myenteric plexus, as the equivalent of Auerbach's plexus in a broader sense of the term. This prominent nerve plexus containing large ganglia and thick primary fasciculi extended in the interval between the longitudinal and circular muscle layers. The myenteric plexus consisted of three grades of latticework: primary, secondary and tertiary plexus. All three grades of myenteric plexuses contained the enteroglial cells.

The primary plexus, which corresponds to the nervous network originally described by AUERBAcH (1862, 1864), was a coarse meshwork consisting of large, elongate ganglia oriented transversely, of short primary fasciculi oriented transversely, or of primary fasciculi oriented longitudinally (diameter 30-60 μm) (Fig. 3A, B; 4A, B). The enteroglial cells were distributed throughout the primary plexus. In the primary fasciculi, their elliptical nuclei were oriented parallel to the long axis. In the ganglia, the enteroglial cells surrounded the immunonegative somata of the ganglionic cells. The orientation of the long axis of the enteroglial cell nuclei in the ganglia was apparently random. Concerning the shape of the enteroglial cells in the primary plexus, we carefully examined both the immunostained whole-mount preparations and sections. However, the enteroglial cells were so crowded in both the ganglia and primary fasciculi that the detailed structure of their cytoplasmic processes proved unobtainable (Fig. 4A).

The secondary plexus originated as branches of a relatively uniform caliber 10-15 μm from the ganglia and fasciculi of the primary plexus. Most of the secondary fasciculi were oriented transversely. Their branching and anastomosis were not very frequent. We had the impression that the plexiform secondary fasciculi in the interval between the longitudinal and circular muscle layers represented the outermost portion of the continuous latticework spreading throughout while remaining within the circular muscle layer (Fig 6A). Although no direct observation was possible, it was probable that the enteroglial cells in the secondary plexus had long cytoplasmic processes, because their nuclei were usually located 30-60 μm apart from each other (Fig. 4A). At the junction of the secondary and tertiary plexuses, there were star-shaped enteroglial cells which apparently possessed five or more cytoplasmic processes.

There were occasional ganglionic cell somata in the course of the secondary fasciculi as illustrated in Figures 4B and 5A. These "solitary" ganglionic cells were surrounded by the cytoplasmic processes of the enteroglial cells.

The tertiary plexus, recognized as a fine meshwork of the ramifying enteroglial cells, was the same as the autonomic ground plexus. This plexus was continuous to the nearby secondary plexus. Some of the enteroglial cells in the tertiary plexus originated directly from the secondary plexus. The tertiary myenteric plexus was continuous to the nerve plexus of the longitudinal muscle layer in its outer aspects and to that of the circular muscle layer in its inner aspects (Fig. 4A, 6A).

3) Superficial plexus of the circular muscle layer

The superficial plexus of the circular muscle layer, which is shown in Figures 6A and B, consisted of the intramuscular fasciculi and interconnecting fine nerve fasciculi.
Fig. 5. Cellular structures in and around the myenteric ganglion. A. "Solitary" ganglionic cell (asterisk) associated with a secondary fasciculus (s). Portions of a myenteric ganglion (g) and primary fasciculus (p) are included in the picture. bv Blood vessel. ×360. B. Enteroglial cells in the longitudinal muscle layer. By chance, a myenteric ganglion (g) was partially broken so that the autonomic ground plexus in the longitudinal muscle layer beneath the ganglion (g') can be visualized. ×300. C. Extrinsic nerve (asterisk) entering the myenteric ganglion (g). A unique fan-shaped structure consisting of the S-100 protein immunopositive cells at the junction between the extrinsic nerve and the myenteric ganglion. ×300. D. An extrinsic nerve entering the primary fasciculus of the myenteric plexus. The looping route of the extrinsic nerve is indicated by arrows. g Myenteric ganglion. ×220
Fig. 6. Enteroglial cells seen in the muscular coat of the guinea pig jejunum. A. Outer longitudinal muscle layer containing a lymphatic vessel (lv), myenteric plexus containing primary (p), secondary (s) and tertiary fasciculi (t). Arrows indicate blood vessels. The framework of all the fasciculi are composed of enteroglial cells as building blocks. The enteroglial cell marked by the asterisk is similar in appearance to the interstitial cells illustrated in Figure 573 of Cajal (1911). B. Superficial portion of the circular muscle layer. S-100 protein immunopositive nerve bundles are intermingled with blood vessels (bv) and smooth muscle fibers (sm). s Intramuscular fasciculus, t tertiary fasciculus. C. Deep muscular plexus. The pattern of the latticework of enteroglial cells (their nuclei indicated by the arrowheads) coincides with that of the interstitial cells described by Cajal (1911) and Taxi (1952). A–C: × 400
4) **Deep muscular plexus**

The deep muscular plexus, illustrated in Figure 6C, was localized immediately outside the inner (mucosal) aspect of the circular muscle layer. Its numerous anastomosing nerve bundles were framed by the enteroglial cells which ran transversely in the direction of the smooth muscle bundles. Regularly spaced thick fasciculi with frequent thin interconnections were present. Short obliquely oriented fasciculi with a medium diameter connected the deep muscular plexus to the myenteric plexus (Fig. 7B, D). Direct connections between the deep muscular plexus and the submucous plexus were infrequently seen (Fig. 7A, B).

5) **Perforating or connecting nerve branches**

In the whole-mount preparations containing the myenteric plexus layer, many stumps of nerve strands of a caliber comparable to those of the primary and secondary fasciculi
Enteroglial cells seen in the submucous coat of the guinea pig jejunum. 

A. Latticework of the enteroglial cell in the submucous plexus. * Submucous ganglion. bv Blood vessel with perivascular plexus. ×80.

B. Submucous ganglia and interconnecting nerve strands. Ganglionic cell somata forming submucous ganglia are fewer than those of the myenteric ganglia. "Solitary" ganglionic cells frequently occur in the submucous plexus (arrowhead). bv Arteries with perivascular plexus. ×200.

C. Submucous ganglion. Immunonegative ganglionic cell somata (asterisks) are surrounded by enteroglial cells whose immunostained nuclei are indicated by arrowheads. ×800.

D. Duodenal gland. Glandular acini are placed in a basketwork of fine processes of enteroglial cells (arrows). There are nerve strands of the submucous plexus (asterisks). ×500.
projected from the ganglia and primary fasciculi (Fig. 3A, 4A, B). In the immunostained sections, thick nerve branches frequently bridged between the myenteric and submucous ganglia (Fig. 7C, D). These perforating nerve branches through the circular muscle layer were connecting nerve strands between the myenteric and submucous plexuses. Enteroglia cells in the perforating nerve branches were identical in morphological features with those of the primary fasciculi of the myenteric plexus.

c. Submucous coat
Figures 8–10 show nerve plexuses in the submucous and mucous coats. The nerve plexuses in these two coats were integrated into a subsystem in the enteric nervous system. They communicated with the nerve plexus in the muscular coat mainly through the perforating or connecting nerve branches mentioned above. There were extrinsic nerves which entered the submucous plexus via the perivascular plexus (Fig. 8A, 9A, C).

1) Submucous plexus
The submucous plexus, i.e., Meissner’s plexus (MEISSNER, 1857) consisted of ganglia and interconnecting nerve strands, both of which were framed by the enteroglial cells as building blocks (Fig. 8, 9). It lay within the connective tissue containing numerous profiles of blood and lymphatic vessels.

The submucous plexus was different from the myenteric plexus in many respects. For one, the ganglia were smaller in size than those of the myenteric plexus. The ganglionic cell somata seen as immunonegative spots in the whole-mount preparations were also remarkably smaller than those in the myenteric ganglia. The interconnecting nerve strands showed a gradation in thickness from 4–10 μm. They were oriented obliquely to the axis of the intestinal tube. The development of the autonomic ground plexus in the submucous coat was poor.

In the immunostained sections, direct connections between the submucous and myenteric plexuses through the perforating nerve branches were encountered (Fig. 7A, B). However, the nerves in the submucous plexus appeared independent from those of the circular muscle layer, and rarely communicated with the adjacent deep muscular plexus.

2) Glial framework around the duodenal glands
There were occasional profiles of duodenal glands in the submucous coat of the jejunum. A characteristic basketwork of branching enteroglial cells surrounded the glandular parenchyme as shown in Figure 8D. This fine cellular basket was continuous to the submucous plexus.

3) Perivascular plexus
Arteries supplying the jejunum entered the serous coat on the mesenteric border, divided into branches which penetrated the muscular coat, and entered the submucous coat where they formed the submucosal arterial network. The mucous coat was supplied by mucosal arterioles, whereas the muscular coat was supplied by another arteriolar system. Thus the vascular bed of the mucous coat and that of the muscular coat were independent of each other as shown by SPANNER (1932), OHASHI et al. (1976) and OHTANI et al. (1983).

The submucosal arteries were surrounded by a well-developed nerve plexus called the perivascular plexus (Fig. 8A, 9A, C). The framework of the perivascular plexus was formed by the S-100 protein immunopositive glial cells. Although there were no
Fig. 9. Enteroglial cells seen in the submucous plexus, in the lamina muscularis mucosae and in the periglandular plexus of the mucous coat of the guinea pig jejunum. **A.** Periglandular plexus (pg) around the intestinal crypts and submucous plexus (smg). Enteroglial cells constitute a delicate latticework extending to both the submucous and mucous coats. The arrow indicates the nerve plexus in the lamina muscularis mucosae. bv Blood vessel. ×200. **B.** Lamina muscularis mucosae. There is a latticework of enteroglial cells with fine cytoplasmic processes. ×200. **C.** Perivascular plexus. There is a distinct perivascular plexus whose S-100 protein immunopositive cell processes are continuous to the neighboring submucous plexus. The three cut ends of Y-shaped artery are indicated by the arrows. ×300
morphological differences between the S-100 protein immunopositive cells in the extrinsic nerve and the enteroglial cells of the submucous plexus, we will restrict the use of the term enteroglial cells to designate cells in the proper enteric nervous system, because the perivascular plexus provided pathways for the extrinsic nerves. Many S-100 protein immunopositive glial cells in the perivascular plexus ran along the axis of the artery without forming a network. Connections between the perivascular and submucous plexuses were frequently seen (Fig. 8A, 9A, C). Thus, it was frequently impossible to distinguish the extrinsic nerve from the nerve strands of the submucous plexus.

There were occasional immunostained nerve bundles around the thick veins and lymphatic vessels.

d. Mucous coat

Figure 10 shows the framework of the mucous plexus. For investigation of the enteroglial framework in the mucous coat, the usefulness of the whole-mount preparations was limited. Examination of the sections was essential, especially for the study of the framework of the nerve plexus in the villi.

1) Lamina muscularis mucosae

Figure 9B shows the characteristic nerve plexuses in the lamina muscularis mucosae. The lamina muscularis mucosae was the outermost layer of the mucous coat. It lay on the inner aspect of the submucous coat, and consisted mainly of smooth muscle fibers which were continuous to those in the lamina propria mucosae. Many nerve fasciculi passed through the lamina muscularis mucosae between the submucous and mucous coats.

2) Periglandular plexus

Figures 9A and 10C show the enteroglial framework of the periglandular plexus in the immunostained whole-mount preparation and section respectively. There was a fine latticework of ramifying enteroglial cells in the honeycomb-like connective tissue space between the intestinal crypt, where many gut endocrine cells (basal-granulated cells) were present. The endocrine cells possessed no immunoreactive S-100 protein.

3) Villous plexus

The villous plexus represented an extension of the submucous and periglandular plexuses. The framework of the periglandular plexus swelled into the core of the villi forming the villous plexus (Fig. 10A). The branching and anastomosing cytoplasmic processes of the enteroglial cells were the elementary component of the three-dimensional latticework of this plexus. The cytoplasmic processes were thin in caliber, measuring 2-3 μm.

At the base and trunk of the villi, the enteroglial framework extended in the connective tissue between the epithelium and the central lacteal. Elliptical nuclei of the enteroglial cells were occasionally seen at the knot of the latticework (Fig. 10C). At the top of the villi, processes of the enteroglial cells formed a fine mesh cap in the space between the epithelium and the closed end of the central lacteal. The densely immunostained nuclei of the enteroglial cells here were smaller than those in the base and trunk of the villi. Granulated connective tissue cells were colored by the immunostaining. However, they did not greatly hinder the identification of fine cytoplasmic processes of the enteroglial cells (Fig. 10B).
Fig. 10. Enteroglial cells seen in a section through the mucous coat of the guinea pig jejunum.  
A. Panoramic view of the mucous coat. Distribution and shape of enteroglial cells are demonstrated. In the core of the villus there is a central lacteal (asterisk). ×300.  
B. Enteroglial cell network in the tip of the villus. Their branching and anastomosing latticework makes a cap-like structure intercalated between the epithelium and the central lacteal. ×450.  
C. Periglandular plexus around the neck of intestinal crypts. ×450
DISCUSSION

I. The spectrum of enteroglial cells

Since Meissner (1857) and Auerbach (1862, 1864) found elaborate nerve plexuses in the submucous and muscular coats of the gut, a large number of studies have been performed on the architecture of the enteric nervous system (Hill, 1927; Oshima, 1929; Matsuo, 1933; Okamura, 1935; Ohkubo, 1936a, b, c; Ito and Nagahiro, 1937; Toyota, 1955, Sugamata, 1955; Yamamoto, 1957: for reviews see Schofield, 1968; Gabella, 1979; Furness and Costa, 1980; Gershon and Erde, 1981). Silver impregnation by Golgi's method and methylene-blue staining (Ehrlich, 1886) have been the most successfully applied methodologies in demonstrating both the neuronal and glial elements. However, with these classic methodologies, there have been difficulties in the constant demonstration of the specified cells (Dogiel, 1899; Cajal, 1911). Both glial and neuronal elements, especially the former, could be stained only capriciously. Furthermore, entire populations of a single, neuronal or glial cell type distributed in different positions of the gut wall have never been stained.

By the immunocytochemical method recently improved by us (Kobayashi et al., 1984, 1985; Kobayashi and Nishisaka, 1985), the demonstration of virtually any immunopositive element in whole-mount preparations of the layers of the gut wall is possible, provided that an adequate antiserum be available. In the present study, our improved method was combined with the immunocytochemical technique for S-100 protein in the guinea pig jejunum. As a result, a series of glial cells were constantly and selectively demonstrated along the entire extent of the enteric nervous system. Accordingly, we were able to visualize how the S-100 protein immunopositive cells as building blocks constitute the framework of the ganglia and nerve fasciculi of the enteric nerve plexuses. Thus, we propose to call them generically the "enteroglial cells."

Figure 11 shows camera lucida drawings of the nerve plexuses in different layers of the guinea pig jejunum. Figure 12 is a schematic representation of the framework of the enteric nerve plexuses as revealed in the present study.

The enteroglial cells exhibited considerable variations concerning their morphological features. A spectrum of their different positions in the gut wall is summarized as follows:

1. Enteroglial cells in the muscular coat
   a. Enteroglial cells in the myenteric ganglia: These surrounded the somata and proximal portions of the cytoplasmic processes of the ganglionic cells and nerve terminal varicosities which made synapses with the ganglionic cells. They are homologous to the astroglia in the central nervous system and to satellite cells in the peripheral ganglia.
   b. Enteroglial cells in the primary fasciculi: These ensheathed bundles of neuronal processes forming a neuropil which frequently extended into the ganglia and finally penetrated through them.
   c. Enteroglial cells in the secondary fasciculi including those in the intramuscular fasciculi: These projected multiple cytoplasmic processes. Some of these processes were collected into the secondary fasciculi, whereas others extended into the tertiary fasciculi.

Small ganglia and solitary ganglionic cells were frequently found in association
with the secondary fasciculi. The enteroglial cells surrounding the ganglionic cells in the secondary plexus were identical to those in the myenteric ganglia. They likely correspond to satellite cells of the peripheral ganglia in function.

d. Enteroglial cells in the tertiary fasciculi: Tertiary fasciculi formed the tertiary plexus, i.e., plexus terminaux (Cajal, 1911) or autonomic ground plexus (Hillarp, 1959). In our opinion, the enteroglial cells in the tertiary plexus included the interstitial cells described by Cajal (1893, 1911) (vide infra).

e. Enteroglial cells in the perforating or connecting branches: Thick, short
branches consisting of enteroglial cells and of bundles of neuronal processes originated mainly from the myenteric ganglia, perforated through the circular muscle layer, and entered the submucous ganglia. These nervous branches connected the myenteric plexus with the submucous and mucous plexuses. The enteroglial cells in these perforating branches are believed to be identical with those of the primary fasciculi of the myenteric plexus.
2. Enteroglia in the submucous and mucous coats

f. Enteroglia in the submucous ganglia: These surrounded the somata and cytoplasmic processes of the ganglionic cells. They were similar to the astroglia of the central nervous system and satellite cells of the peripheral ganglia.

g. Enteroglia in the interconnecting nerve strands: No thick interconnecting nerve strands were present in the submucous plexus. The enteroglia cells here possessed two or more flattened cytoplasmic projections which extended into the submucous ganglia, interconnecting nerve strands, the perivascular plexus, and the nervous basketwork around the duodenal glands. However, in the layer beneath the glandular tissues, any formation of the autonomic ground plexus was rare.

h. Enteroglia around the duodenal gland: Their cytoplasmic processes were small in caliber, and formed a basketwork around the parenchyme of the duodenal gland. This basketwork may represent the framework of the autonomic ground plexus.

i. Enteroglia in the lamina muscularis mucosae: These formed a characteristic network, providing support for the enteric neuronal processes which control the motility of the smooth muscle cells.

j. Enteroglia in the periglandular plexus: They formed a honeycomb-like network around the crypts. This network corresponded to the autonomic ground plexus which was continuous to the submucous plexus in the outer aspect and to the villous plexus in the inner aspect.
k. Enteroglial cells in the villous plexus: They were continuous to the submucous and periglandular plexuses. Their delicate network corresponded to that of the interstitial cells described by CAJAL (1893, 1911) (vide infra).

In the present study, the presence of immunoreactive S-100 protein was also demonstrated in the glial cells of the extrinsic nerves. There were two groups of extrinsic nerves: 1) extrinsic nerves entering the myenteric plexus, and 2) perivascular plexuses entering the submucous plexus.

The glial cells in the extrinsic nerves entering the myenteric plexus are nothing but Schwann cells. We demonstrated in the present study that, at the junction between the extrinsic nerve and the myenteric plexus, there were characteristic fan-like structures containing S-100 protein immunopositive glial cells. This junctional structure between the extrinsic nerve and myenteric plexus was described by CAJAL (1911, see Fig. 570). We wonder whether CAJAL himself saw the "interstitial cells" at this junction.

The glial cells in the perivascular plexus constituted a densely packed network around the arteries in the submucous coat. In every aspect, they were identical to the "sternförmige Zellen" of DOGIEL (1895, 1899) and to the perivascular interstitial cells of MEYLING (1953). The perivascular plexuses were continuous to the adjacent submucous plexus which was intrinsic to the gut. No distinctive features were found between the extrinsic nerves and the intrinsic nerve strands concerning the S-100 protein immunopositive elements.

II. Interstitial cells of Cajal as enteroglial cells

a. Serous and muscular coats

A comprehensive treatise on the interstitial cells of Cajal in the muscular coat of the gut has recently been provided by THUNEBERG (1982). Based on the bibliographical survey and on the results of his own light and electron microscopic studies in the mouse gut, THUNEBERG (1982) proposed a classification in which four types of the interstitial cells of CAJAL (ICCs I-IV) were characterized:

1) The ICCs-I were present in the interstices between the Auerbach’s plexus (myenteric plexus of a narrower sense) and the adjacent muscle cells of the longitudinal and circular muscle layers, and were innervated by the “tertiary fasciculi of the Auerbach’s plexus.”

2) The ICCs-II occurred in the subserous connective tissue as a single layered cellular network, were not associated with neural elements, and showed ultrastructural features similar to those of the fibroblasts.

3) The ICCs-III lay in the deep muscular plexus, made apparent synaptic contacts with varicose nerve terminals, and formed numerous gap junctions with smooth muscle cells of the innermost layer of the muscular coat.

4) The ICCs-IV existed in the superficial portion of the circular muscle layer. They were scattered along the nerve fasciculi, and exhibited fibroblast-like features.

THUNEBERG (1982) stated that the cells which CAJAL (1893, 1911) originally termed as interstitial cells included only the ICCs-I and ICCs-III. We are in agreement with THUNEBERG (1982) that the ICCs-II and ICCs-IV, i.e., fibroblast-like cells, greatly differ from the interstitial cells described by CAJAL (1893, 1911). However, in our opinion, THUNEBERG (1982) was still prejudiced by the idea that the ICCs were independent of glial or Schwann cells. We feel that whether the ICCs-I and ICCs-III might cover all
the interstitial cells described by Cajal (1893, 1911) remains a matter for future discussion. Thuneberg's opinion on the nature of the interstitial cells of Cajal in the mucous coat may provide a step towards solving this question.

We would like to propose that the interstitial cells described by Cajal (1893, 1911) contain immunoreactive S-100 protein. The reasons why we regard the Cajal's interstitial cells as a kind of the enteroglial cells are as follows: 1) In the longitudinal muscle layer and myenteric plexus, the distribution and shape of the enteroglial cells seen in the present specimens were identical to those of the interstitial cells illustrated by Cajal (1911). Compare Figures 3B; 4A, B; 5B; 11A of the present paper with Figure 572 of Cajal (1911). 2) The unique network of the interstitial cells in the deep muscular plexus which was illustrated in Figures 568 and 575 of Cajal (1911) coincides well with that seen in the present study (Fig. 6C, 11D). 3) Cajal (1893, 1911) described interstitial cells in the mucosal coat (see Fig. 3 of his 1893 paper, and Fig. 568 and 579 of his 1911 book). The enteroglial cells formed the characteristic periglandular and villous latticeworks in the mucous coat. Similarities between the enteroglial cell latticework seen in the present study and that of the interstitial cells illustrated by Cajal (1893, 1911) are striking.

Cajal (1893, 1911) described the interstitial cells based on observations using tissue preparations silver-impregnated by Golgi's method and methylene blue-stained specimens. Using the same methodologies, Taxi (1952, 1959, 1965) further investigated the interstitial cells of Cajal and distinguished them from both the ganglionic and Schwann cells. However, we are of the opinion that the interstitial cells described by Cajal (1893, 1911) include the "Schwann cells" of Taxi (1952, 1959, 1965). The "interstitial cells" shown in Figures 1-8 of Taxi (1952) probably belong to the S-100 protein immunopositive enteroglial cells in the present study. However, we could not clarify whether all the "interstitial cells" especially those shown in his electron micrographs (Taxi, 1959, 1965) contained S-100 protein. Immuno-electron microscopic studies are essential to solve this problem. Our preliminary studies in the guinea pig small intestine (Y. Endo, T. Endo and S. Kobayashi: unpublished) have shown that the Schwann cells contain immunoreactive S-100 protein. Further studies are in progress concerning the "interstitial cells."

In the present study, no immunoreactive S-100 protein was demonstrated in the connective tissue cells in the subserous layer where Thuneberg (1982) described the ICCs-II. We admit that the latter are fibroblasts or fibroblast-like cells.

Thuneberg (1982) pointed out that the ultrastructure of the ICCs-IV was equal to that of the interstitial cells of Cajal provided by a number of electron microscopic studies (Richardson, 1958, 1960; Yamauchi, 1964; Rogers and Burnstock, 1966; Cook and Burnstock, 1976; Komuro, 1982). It is evident that the cells investigated by many electron microscopists under the name of the interstitial cells of Cajal are not the "true" interstitial cells. In the present study, we demonstrated S-100 protein immunopositive ramifying cells in the superficial portion of the circular muscle layer. We believe these "Schwann cells" (see Fig. 6A, B) represent the interstitial cells illustrated in Figure 573 of Cajal (1911). A comparison of the scanning electron micrographs by Komuro (1982, Fig. 1-3) with the drawings by Cajal (1911, Fig. 572, 573) may make clear some electron microscopist's erroneous interpretation of the interstitial cells of Cajal. It should be stated here that we have identified by scanning electron microscopy the enteroglial cells which corresponded to the "true" interstitial cells of Cajal in the superficial portion of the circular muscle layer (Y. Endo, T. Okubo and S. Kobayashi: unpublished).
b. Submucous and mucous coats

Cajal (1893, 1911) illustrated and described interstitial cells in the villous and periglandular plexuses of the mucous coat. We believe that the enteroglial cell lattice-work seen in the present study is the same as that of the interstitial cells. On the other hand, the assumption of Desaki et al. (1984) that the “plexus of Cajal” corresponded to the reticulum of the fibroblast-like cells is unacceptable. Comparison of Figure 1 of Desaki et al. (1984) with Figures 568 and 579 of Cajal (1911) serves to clarify the essential difference between the two kinds of cellular networks. The interstitial cells described by Cajal (1893, 1911) strictly represent not the connective tissue reticulum but the nerve plexus. We suppose that the use of the term fibroblast by Guldner et al. (1972) exacerbated the confusion on the identity of the interstitial cells of Cajal among the electron microscopists. The cells shown in the electron micrographs by Honjin et al. (1965) coincide with the interstitial cells described by Cajal (1893, 1911).

Dogiel (1895, 1899) found many “sternfärmige Zellen” using a methylene-blue technique. Although he used the term “bindegeweibige Zellen” for these ramifying cells, he did not use this term in the sense of fibroblasts. Here we have to correct the distorted statement of some electronmicroscopists who maintain that Dogiel (1895, 1899) regarded the interstitial cells of Cajal as modified fibroblasts (see Gabella, 1979; Thuneberg, 1982). Re-examination of Dogiel’s illustrations (Fig. 5, 6, 1895; Fig. 20A–C, 1899) gave us the impression that the “sternfärmige Zellen” represent the S-100 protein immunopositive glial cells.

The reason why Cajal (1893, 1911) described no interstitial cells in the submucous plexus is unknown. The absence of the autonomic ground plexus here (Ohkubo, 1936a) may provide one of the explanations. Another explanation may be based on the technical situation. Cajal (1911) pointed out that the interstitial cells stained only infrequently by the Golgi method. They never appeared at the same time as the processes of neurons of both the myenteric and submucous ganglia. It is conceivable that slight differences in the environment between the mucosal and submucosal enteroglial cells, if present, may result in their all-or-none stainability in Golgi preparations.

III. Significance of the enteroglial framework

In the ganglia and thick nerve fasciculi, the enteric neurons play a leading part in the function of the enteric nervous system, whereas the enteroglial cells may be ancillary in nature. Thus, the enteroglial cells may play their role in the mechanical support, isolation and insulation, and nutrition and metabolism of somata and processes of the enteric neurons. Furthermore, the enteroglial cells, as the main replenishment, fill the space between the neuronal elements. The domain of the enteric nervous system, except its terminal portions such as those in the deep muscular plexus, is defined by a continuous basement membrane, and contains neither blood vessels nor connective tissue elements (for reviews see Gabella, 1972, 1979; Furness and Costa, 1980; Gershon and Erde, 1981).

In order to clarify the significance of the enteroglial cells in the thin nerve fasciculi, we must refer to past interpretations on the nature of the “interstitial cells.” Cajal (1893, 1911) regarded these cells as interstitial neurons or as interstitial sympathetic neurons. Many authors compared these cells with the primitive ganglionic cells of the invertebrates such as the Hydra (Cajal, 1911; Boeke, 1949). Imaizumi and
Hama (1969), based on the results of their study in the love-bird gizzard, considered the interstitial cells of Cajal to be neither Schwann cells nor fibroblasts. These authors suggested that the interstitial cells of Cajal may correspond to the “transmittal cells (Suzuki, 1963)” which transmit stimuli from nerve terminals to smooth muscle cells. It is also possible that the transmission takes place through the interstitial cells of Cajal in the opposite direction, i.e., from smooth muscle cells to nerve terminals (Daniel, 1977; Gabella, 1979).

The S-100 protein immunopositive enteroglial cells seen in the present study were greater in number than the interstitial cells of Cajal which were reported by Imaizumi and Hama (1969). We suppose that the interstitial cells of Cajal investigated by Imaizumi and Hama (1969) represent a limited population of the interstitial cells originally described by Cajal (1893, 1911). We feel that the immuno-electron microscopic studies using anti-S-100 protein sera of the love-bird’s gizzard are necessary for an understanding of the function of the enteroglial cell framework. This is because the interstitial cells of Cajal in the sense of Imaizumi and Hama (1969), Daniel and Posey-Daniel (1984) and Daniel et al. (1984) may represent a specialized form of the enteroglial cells associated with the terminal varicosities of the enteric neurons. Although we have shown in the guinea pig jejunum that the interstitial cells described by Cajal (1893, 1911) contain “glia-specific” S-100 protein, we do not deny the neuron-like function of these cells. It is possible that, within the autonomic ground plexus, there are specialized enteroglial cells with the function of mediating between the nerve terminal varicosities and smooth muscle cells.

The interstitial cells of Cajal in the mucous coat are peculiar as compared with the satellite and Schwann cells of the peripheral nervous system. They form a characteristic latticework in the core of the intestinal villi and in the periglandular spaces of the mucous membrane. There are basal-granulated cells in the epithelial tissue of the intestine. They are rezepto-secretory in function, and release gut hormones into the connective tissue in response to adequate stimuli in the intestinal lumen (Kobayashi et al., 1970; Fujita and Kobayashi, 1977). It is possible that the basal-granulated cells in the epithelium and the nerve plexuses in the lamina propria mucosae constitute a functional unit, and perform a regulatory role in the gut function. We surmise that there are functional differences between the interstitial cells of Cajal in the mucous coat and those in muscular coat.

Addendum: After submitting this paper, we performed a Golgi study in the guinea pig small intestine. We demonstrated that what Cajal (1893, 1911) illustrated as the interstitial neurons included silver-impregnated chimera of an enteroglial cell and fragments of neuronal processes.

REFERENCES

Auerbach, L.: Über einen Plexus myentericus. Morgenstern, Breslau, 1862. (cited from Auerbach, 1864).
———: Fernere vorläufige Mittheilung über den Nervenapparat des Darmes. Virchows Arch. 30: 457-460 (1864).
Boeke, J.: The sympathetic endformation, its synaptology, the interstitial cells, the periterminal network, and its bearing on neurone theory. Discussion and critique. Acta anat. 8: 18-61 (1949).
Cajal, S. R.: Sur les ganglions et plexus nerveux d’intestin. C. r. Soc. Biol. Paris 45: 217–223 (1893).
Cocchia, D., F. Michetti and R. Donato: Immunochemical and immunocytochemical localization of S-100 antigen in normal human skin. Nature 294: 85–87 (1981).
Cocchia, L., G. Tiberio, R. Santarelli and F. Michetti: S-100 protein in “follicular dendritic” cells of rat lymphoid organs. An immunohistochemical and immunocytochemical study. Cell Tiss. Res. 230: 95–103 (1983).
Cook, R. D. and G. Burnstock: The ultrastructure of Auerbach’s plexus in the guinea-pig. II. Non-neuronal elements. J. Neurocytol. 5: 195–206 (1976).
Costa, M., R. Buffa, J. B. Furness and E. Solcia: Immunohistochemical localization of polypeptides in peripheral autonomic nerves using whole mount preparations. Histochemistry 65: 157–165 (1980).
Daniel, E. E.: Nerves and motor activity of the gut. In: (ed. by) F. P. Brooks and P. W. Evers: Nerves and the gut. CB Slack, New York, 1977 (p. 154–196).
Daniel, E. E. and V. Posey-Daniel: Effects of scorpion venom on structure and function of esophageal lower sphincter (LES) and body circular muscle (BCM) from opossum. Can. J. Physiol. Pharmacol. 62: 360–373 (1984).
Daniel, E. E., L. P. Jager, J. Jury, A. Helmy-Elkholy, M. S. Kannan and V. Posey-Daniel: The mediators and mechanisms causing the non-adrenergic, non-cholinergic nerve responses in opossum esophagus: role of interstitial cells of Cajal. Biomed. Res. 5, Suppl.: 67–84 (1984).
Desaki, J., T. Fujiwara and T. Komuro: A cellular reticulum of fibroblast-like cells in the rat intestine: scanning and transmission electron microscopy. Arch. histol. jap. 47: 179–186 (1984).
Dogiel, A. S.: Zur Frage über die Ganglien der Darmgefäße bei den Säugethiern. Anat. Anz. 10: 517–528 (1895).
Ehrlich, P.: Über die Methylenblau-reaction der lebenden Nervensubstanz. Deut. med. Wochenschr. 12: 49–52 (1886).
Endo, T., T. Tanaka, T. Isobe, H. Kasai, T. Okuyama and H. Hidaka: Calcium-dependent affinity chromatography of S-100 and calmodulin on calmodulin antagonist-coupled Sepharose. J. biol. Chem. 256: 12485–12489 (1981).
Ferri, G.-L., L. Probert, D. Cocchia, F. Michetti, P. J. Marangos and J. M. Polak: Evidence for the presence of S-100 protein in the glial component of the human enteric nervous system. Nature 297: 409–410 (1982).
Fujita, T. and S. Kobayashi: Structure and function of gut endocrine cells. Int. Rev. Cytol. Suppl. 6: 187–233 (1977).
Furness, J. B. and M. Costa: Types of nerves in the enteric nervous system. Neuroscience 5: 1–20 (1980).
Gabella, G.: Fine structure of the myenteric plexus in the guinea-pig ileum. J. Anat. 111: 69–97 (1972).
Gershon, M. D. and S. M. Erde: The nervous system of the gut. Gastroenterology 80: 1571–1594 (1981).
Ghandour, M. S., O. K. Langley, G. Labourdette, G. Vincendon and G. Gombos: Specific and artefactual cellular localization of S-100 protein: an astrocyte marker in rat cerebellum. Devel. Neurosci. 4: 66–78 (1981).
Güldner, F.-H., J. R. Wolf and D. G. Keyserlingk: Fibroblasts as a part of the contractile system in duodenal villi of rat. Z. Zellforsch. 135: 349–360 (1972).
Hara, K., M. Ito, J. Takeuchi, S. Ijima, T. Endo and H. Hidaka: Distribution of S-100b protein in normal salivary glands and salivary gland tumors. Virchows Arch. A. Pathol. Anat. 401: 237–249 (1983).
Hidaka, H., T. Endo, S. Kawamoto, E. Yamada, H. Umekawa, K. Tanabe and K. Hara: Purification and characterization of adipose tissue S-100b protein. J. biol. Chem. 258: 2705–2709 (1983).
Hillarp, N.-Å.: The construction and functional organization of the autonomic innervation apparatus. Acta physiol. scand., Suppl. 157: 1-38 (1959).

Hill, C. J.: A contribution to our knowledge of the enteric plexuses. Phil. Trans. Roy. Soc. B 215: 355-387 (1927).

Honjin, R., A. Takahashi and Y. Tasaki: Electron microscopic studies of nerve endings in the mucous membrane of the human intestine. Okajimas Fol. anat. jap. 40: 409-427 (1965).

Imaizumi, M. and K. Hama: An electron microscopic study on the interstitial cells of the gizzard in the love bird (Urolophus domesica). Z. Zellforsch. 97: 351-357 (1969).

Ito, T. and K. Nagahiro: Zytologische Untersuchungen über die intramuralen Ganglienzellen des Verdauungstraktes. Okajimas Fol. anat. jap. 15: 609-634 (1937).

Iwanaga, T., T. Fujita, T. Masuda and Y. Takahashi: S-100 protein-immunoreactive cells in the lymph node and spleen of the rat. Arch. histol. jap. 45: 393-397 (1982).

Kobayashi, S., T. Fujita and T. Sasagawa: The endocrine cells of human duodenal mucosa. An electron microscope study. Arch. histol. jap. 31: 477-494 (1970).

Kobayashi, S. and T. Nishisaka: Myenteric enkephalin neurons around the laser-photoacoagulation necrosis: an immunocytochemical investigation in the guinea pig jejunum and proximal colon. Arch. histol. jap. 48: 239-254 (1985).

Kobayashi, S., M. Suzuki, T. Uchida and N. Yanaihara: Enkephalin neurons in the guinea pig duodenum: a light and electron microscopic immunocytochemical study using an antiserum to methionine-enkephalin-Arg⁴-Gly⁴-Leu⁴. Biomed. Res. 5: 489-506 (1984).

Kobayashi, S., M. Suzuki and N. Yanaihara: Enkephalin neurons in the guinea pig proximal colon: an immunocytochemical study using an antiserum to methionine-enkephalin-Arg⁴-Gly⁴-Leu⁴. Arch. histol. jap. 48: 27-44 (1985).

Komuro, T.: The interstitial cells in the colon of the rabbit: scanning and transmission electron microscopy. Cell Tiss. Res. 222: 41-51 (1982).

Lawrentjew, B. J.: Über die Verbreitung der nervösen Elemente (einschließlich der “interstitiellen Zellen” Cajals) in der glatten Musculatur, ihre Endigungsweise in den glatten Muskelzellen. Z. mikrosk.-anat. Forsch. 6: 467-488 (1926).

Ludwin, S. K., J. C. Kosek and L. F. Eng: The topographical distribution of S-100 and GFA proteins in the adult rat brain: an immunohistochemical study using horseradish peroxidase-labelled antibodies. J. comp. Neurol. 165: 197-208 (1976).

Matsuo, H.: A contribution on the anatomy of Auerbach’s plexus. Jap. J. med. Sci. I. Anat. 4: 417-428 (1933).

Matus, A. and S. Mughal: Immunohistochemical localisation of S-100 protein in brain. Nature 258: 746-748 (1975).

Meissner, G.: Über die Nerven der Darmwand. Z. ration. Med. 8: 364-368 (1857).

Meyling, H. A.: Structure and significance of the peripheral extension of the autonomic nervous system. J. comp. Neurol. 99: 495-543 (1953).

Moore, B. W.: A soluble protein characteristic of the nervous system. Biochem. biophys. Res. Comm. 19: 739-744 (1965).

Nakajima, T., H. Yamaguchi and K. Takahashi: S-100 protein in folliculostellate cells of the rat pituitary anterior lobe. Brain Res. 191: 523-531 (1980).

Ohashi, Y., S. Kita and T. Murakami: Microcirculation of the rat small intestine as studied by the injection replica scanning electron microscope method. Arch. histol. jap. 39: 271-282 (1976).

Ohkubo, K.: Studies on the intrinsic nervous system of the digestive tract. I. The submucous plexus of guinea-pig. II. The Plexus myentericus and Plexus subserosus des Meerschweinchens. III. Affe und Mensch. Jap. J. med. Sci. I. Anat. 6: 1-20, 21-37, 219-247 (1936a-c).

Ohtani, O., A. Kikuta, A. Ohtsuka, T. Taguchi and T. Murakami: Microvasculature as studied by the microvascular corrosion casting/scanning electron microscope method. I. Endocrine and digestive system. Arch. histol. jap. 46: 1-42 (1983).

Okamura, C.: Über die Darstellung des Nervenapparates in der Speiseröhrenwand mittels der Vergoldungsmethode. Z. mikrosk.-anat. Forsch. 37: 128-150 (1935).

Oshima: Über die Innervation des Darms. Z. Anat. Entw.-Gesch 90: 725-767 (1929).

Ottaviani, G., C. A. Castelli and M. Satta: Osservazioni sopra le cellule interstiziali di Cajal.
Acta neuroveg. 26: 172-183 (1964).

Richardson, K. C.: Electron microscope observations on Auerbach’s plexus in the rabbit, with special reference to the problem of smooth muscle innervation. Amer. J. Anat. 103: 99-136 (1958).

———: Studies on the structure of autonomic nerves in the small intestine, correlating the silver-impregnated image in light microscopy with the permanganate-fixed ultrastructure in electron microscopy. J. Anat. 94: 457-472 (1960).

Rogers, D. C. and G. Burnstock: The interstitial cell and its place in the concept of autonomic ground plexus. J. comp. Neurol. 126: 255-284 (1966).

Schabadasch, A.: Intramurale Nervengeflechte des Darmrohrs. Z. Zellforsch. 10: 320-385 (1930).

Schofield, G. C.: Anatomy of muscular and neural tissues in the alimentary canal. In: (ed. by) C. F. Code: Handbook of physiology. Section 6. Vol. IV. American Physiological Society, Washington, D. C., 1968 (p. 1579-1627).

Spanner, R.: Neue Befunde über die Blutwege der Darmwand und ihre funktionelle Bedeutung. Morphol. Jahrb. 69: 394-434 (1932).

Stach, W.: Der Plexus entericus extremus des Dickdarmes und seine Beziehungen zu den interstitiellen Zellen (Cajal). Z. mikrosk.-anat. Forsch. 65: 245-272 (1972).

Stefansson, K., R. L. Wollmann and B. W. Moore: Distribution of S-100 protein outside the central nervous system. Brain Res. 234: 309-317 (1982a).

Stefansson, K., R. L. Wollmann, B. W. Moore and B. G. W. Arnason: S-100 protein in human chondrocytes. Nature 295: 63-64 (1982b).

Sternberger, L. A.: Immunocytochemistry. 2nd Ed. John Wiley and Sons, New York, 1979.

Stöhr, P.: Mikroskopische Anatomie des vegetativen Nervensystems. In: Möllendorff-Bargmanns Handbuch der mikroskopischen Anatomie des Menschen. IV/5. Springer-Verlag, Berlin-Göttingen-Heidelberg, 1957.

Sugamata, G.: Innervation of inferior esophagus and pars cardiaca ventriculi in dog. Arch. histol. jap. 7: 585-596 (1955).

Suzuki, K.: The end apparatus of the vegetative nervous system (In Japanese). In: Proc. 16th Gen. Assem. Jap. Med. Congr., April 1963, Osaka, 1963 (Vol. 4, p. 13-28).

Takahashi, K., H. Yamaguchi, J. Ishizeki, T. Nakajima and Y. Nakazato: Immunohistochemical and immunoelectron microscopic localization of S-100 protein in the interdigitating reticulum cells of the human lymph node. Virchows Arch. B. Cell Pathol. 37: 125-135 (1981).

Taxi, J.: Cellules de Schwann et “cellules interstitielles de Cajal” au niveau des plexus nerveux de la musculuse intestinale de cobaye: retour aux définitions. Arch. Anat. microsc. Morphol. exp. 41: 281-304 (1952).

———: Sur la structure des travées du plexus d’Auerbach: confrontation des données fournies par le microscope ordinaire et par le microscope électronique. Ann. Sci. nat. Zool. 1: 571-594 (1959).

———: Contribution a l’étude des connexions des neurones moteurs du système nerveux autonome. Ann. Sci. nat. Zool. 7: 413-674 (1965).

Thuneberg, L.: Interstitial cells of Cajal: intestinal pacemaker cells? Adv. Anat. Embriol. Cell Biol. 71: 1-130 (1982).

Toyota, T.: On innervation of stomach of hedgehog. Arch. histol. jap. 7: 573-584 (1955).

Ushiki, T., T. Iwanaga, T. Masuda, Y. Takahashi and T. Fujita: Distribution and ultrastructure of S-100-immunoreactive cells in the human thymus. Cell Tiss. Res. 235: 509-514 (1984).

Yamamoto, M.: Electron microscopic studies on the innervation of the smooth muscle and the interstitial cell of Cajal in the small intestine of the mouse and bat. Arch. histol. jap. 40: 171-201 (1977).

Yamamoto, T.: Electron microscope investigation on the relationship between the smooth muscle cell of the proc. vermiformis and the autonomic peripheral nerves. Acta neuroveg. 21: 406-425 (1960).

Yamamoto, T. Y.: Histological studies of the rectum of monkey, with special reference to its innervation. Arch. histol. jap. 11: 581-610 (1957).

Yamauchi, A.: Electron microscopic studies on the autonomic neuro-muscular junction in the tenia coli of the guinea pig. Acta anat. nippon. 39: 22-38 (1964).
Zomzely-Neurath, C. E. and W. A. Walker: Nervous system-specific proteins. 14-3-2 protein, neuron-specific enolase, and S-100 protein. In: (ed. by) R. A. Bradshaw and D. M. Schneider: Proteins of the nervous system. 2nd Ed. Raven Press, New York, 1980 (p. 1-57).

Prof. Shigeru KOBAYASHI
Department of Anatomy
Yamanashi Medical School
Tamaho, Yamanashi
409-38 Japan