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Cytology and Microenvironment of Somatostatin (D) Cells in the Gastric Fundic Mucosa of Rodents

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ABSTRACT

Somatostatin from gastric D-cells exerts an inhibitory effect upon the release of gastric acid, enzymes and gastrin. From previous investigations, a paracrine mode of action of somatostatin has been postulated. However, the exact route of the delivery of gastric somatostatin remains still uncertain and controversial. To obtain a closer view of fundic D-cells, the complete shapes and their microanatomical relationships to neighboring tissue elements were examined in immunostained serial semithin (0.5 μm) sections of the fundic mucosa of rats, mice and golden hamsters, exemplarily by the combined utilization of computer-assisted 3D reconstructions. All the D cells examined in the present study were found to belong to the 'closed-type' of entero-endocrine cells lacking contiguity to the luminal surface. In their shape, the D cells in this region displayed an expressed 'pleomorphism'. A subpopulation of D cells, ovoid in shape, were thoroughly enclosed by single parietal cells. Most of the D cells appeared to be intimately juxtaposed to parietal cells and/or chief cells with their cell bodies or cytoplasmic processes, but simultaneously blood capillaries were regularly located in close vicinity to such D cells. Thus, somatostatin from the fundic D cells may act upon parietal cells and chief cells via both paracrine (direct cell to cell or diffusion) and endocrine (local circulatory system). The morphological heterogeneity of gastric fundic D cells may reflect certain functional states.

Key words: *Somatostatin cell, Endocrine, Paracrine, Gastric fundic mucosa*

Somatostatin is an inhibitory peptide which occurs ubiquitously in the organism[19,24)]. Within the gastroenteropancreatic (GEP) endocrine system, somatostatin is localized in the D cells of the endocrine pancreas and gastrointestinal epithelia[25)]. In the gastric fundic mucosa, the main function of somatostatin is the inhibition of acid, pepsinogen and intrinsic factor secretion[2,3,21,29,30)]. Previous morphological investigations on gastric D cells have shown that these cells possess long cytoplasmic processes terminating on putative effector cells, i.e. gastrin cells, parietal cells and chief cells[1,13,15)]. Therefore, D cells have served as a paradigm for Feyrter's concept of 'paracrinia'[4)]. Hence, mainly paracrine pathways have been considered for the delivery of somatostatin to its target cells in the gastric mucosa, but its exact route has remained uncertain and controversial[7,12,16,23)].

The present study was designed to find certain clues to this question. Whereas former morphological investigations in this field have been performed with paraffin and cryostat sections, with single thin sections or with the isolated mucosa, we investigated immunostained serial semithin sections. This technique, combined with computer-assisted 3D reconstructions, is appropriate to give a closer view and a detailed morphological image of endocrine cells and of their microanatomical relationships to neighboring structures[8)].

MATERIALS AND METHODS

Tissue preparation

Small specimens from the fundic mucosa of addult rats (n=3), mice (n=2) and golden hamsters (n=2) were snap-frozen in melting Freon 22 at –150°C. After freeze-drying for 72 hr, the tissues were fixed with vapor-phase paraformaldehyde, and embedded in epoxy resin (Araldite). Serial semithin sections cut at 0.5 μm were mounted on microscopic slides[8)].

Immunohistochemistry

After removal of the resin by sodium methoxide, the sections were immunostained by the per-

oxidase antiperoxidase (PAP) technique[32] as modified for semithin sections[8]. Antisomatostatin serum (the gift of Dr. S. Ito, Akita, Japan) was used at a dilution of 1:4000. The other steps of the immunohisto-chemical protocol and specificity controls were performed as described previously[8]. The immunostained sections were viewed by phase contrast optics. Completely sectioned D-cells were examined. About 20 sections pass through the same D-cells.

Analysis of serial sections

A series of 50 semithin sections were immunostained by antisomatostatin. In the series, the shape of single D cells, the intracellular distribution of immunoreactive somatostatin, and the microanatomical relationships of the D cells to neighboring structures (capillary, parietal cells and chief cells) were carefully examined. Fifty randomly selected D cells (20 from rats, 15 from mice, and 15 from golden hamsters) were analyzed. The topographical relationship between D cells was also examined.

Three-dimensional reconstructions

Some D-cells and their neighboring structures

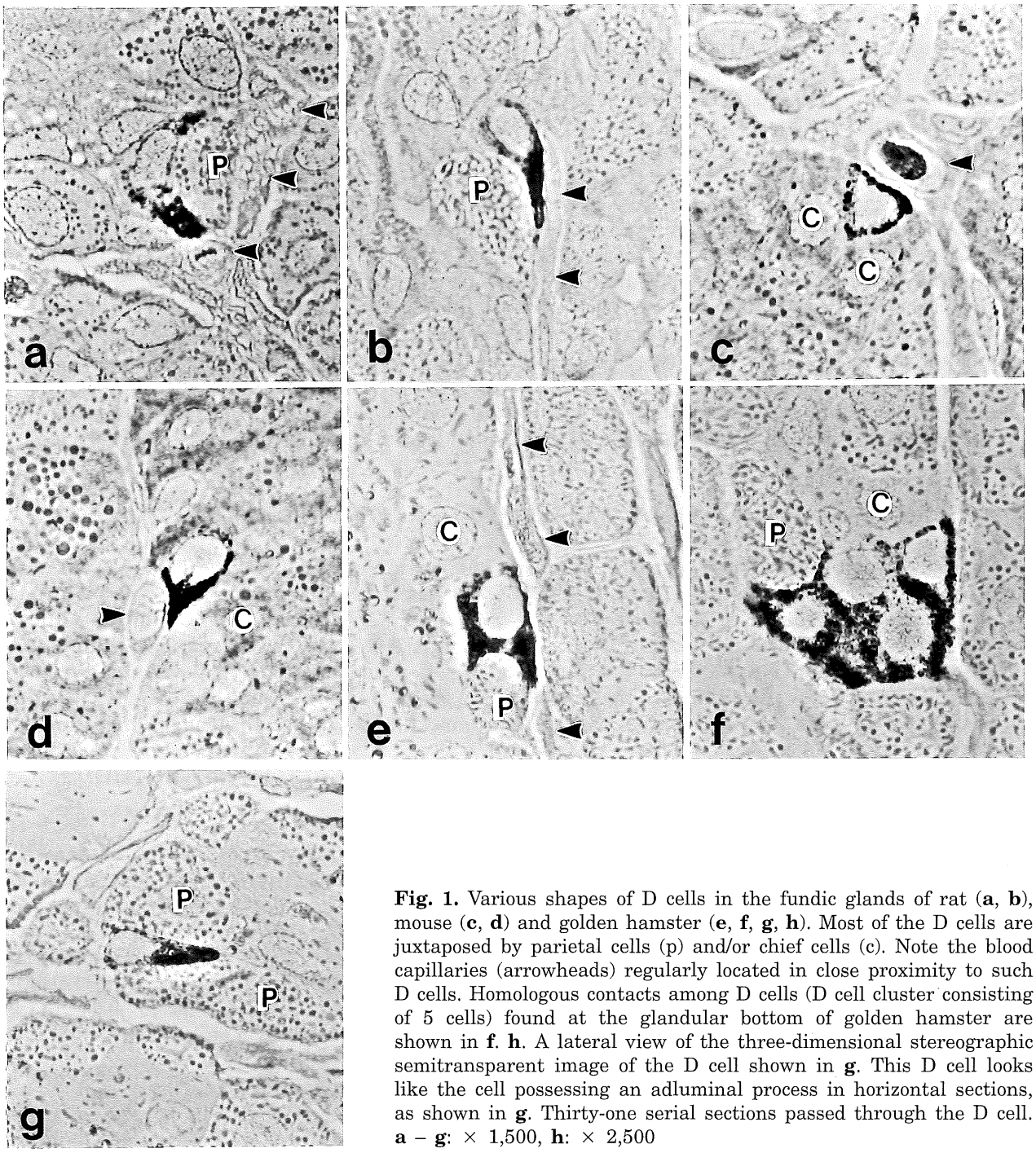

Fig. 1. Various shapes of D cells in the fundic glands of rat (**a**, **b**), mouse (**c**, **d**) and golden hamster (**e**, **f**, **g**, **h**). Most of the D cells are juxtaposed by parietal cells (p) and/or chief cells (c). Note the blood capillaries (arrowheads) regularly located in close proximity to such D cells. Homologous contacts among D cells (D cell cluster consisting of 5 cells) found at the glandular bottom of golden hamster are shown in **f**. **h**. A lateral view of the three-dimensional stereographic semitransparent image of the D cell shown in **g**. This D cell looks like the cell possessing an adluminal process in horizontal sections, as shown in **g**. Thirty-one serial sections passed through the D cell. **a** – **g**: × 1,500, **h**: × 2,500

were three-dimensionally reconstructed with computer-assisted methods (TOSBAC, DS-600, Japan), according to the "3D-Reconstruction of Serial Sections" program (version 1.00: April 1982) commercially available, and "3D-Reconstruction of Serial Sections" program devised by NAKAMAE et al[11,21,28].

RESULTS

Cytologic characteristics of D cells

The majority of the D cells was located in the middle and basal parts of the fundic glands. In contrast to their heterogeneity as shown in the pyloric glands of the rat stomach[12], the D cells in the fundic mucosa were found to exclusively belong to the 'closed-type' endocrine cell which showed no contact with the luminal surface and displayed manifold shaped (Figs. 1,2,3,4). Most D cells gave off longer or shorter cytoplasmic processes which extended from the basal parts of the cell. These processes were regularly densely immunostained, often being equipped with a terminal swelling. They varied in thickness and length, and were not observed to bifurcate. A subpopulation of D cells were ovoid in shape and lacking in the proper cytoplasmic processes (Fig. 3). Furthermore, the flat and round shape of the D cell in the three-dimensional image looked like the cell possessing a slender process on some planes of the horizontal section (Figs. lg,h).

The immunoreactive materials were distributed unevenly within the cytoplasm; cell portions or cytoplasmic processes facing blood capillaries in the lamina propria mucosae showed strong immunoreactivities, whereas other cell portions showed only moderate or no staining (Figs. 1,2,3,4).

The D cells in the fundic mucosa of golden hamster were more pleomorphic than those of other animals.

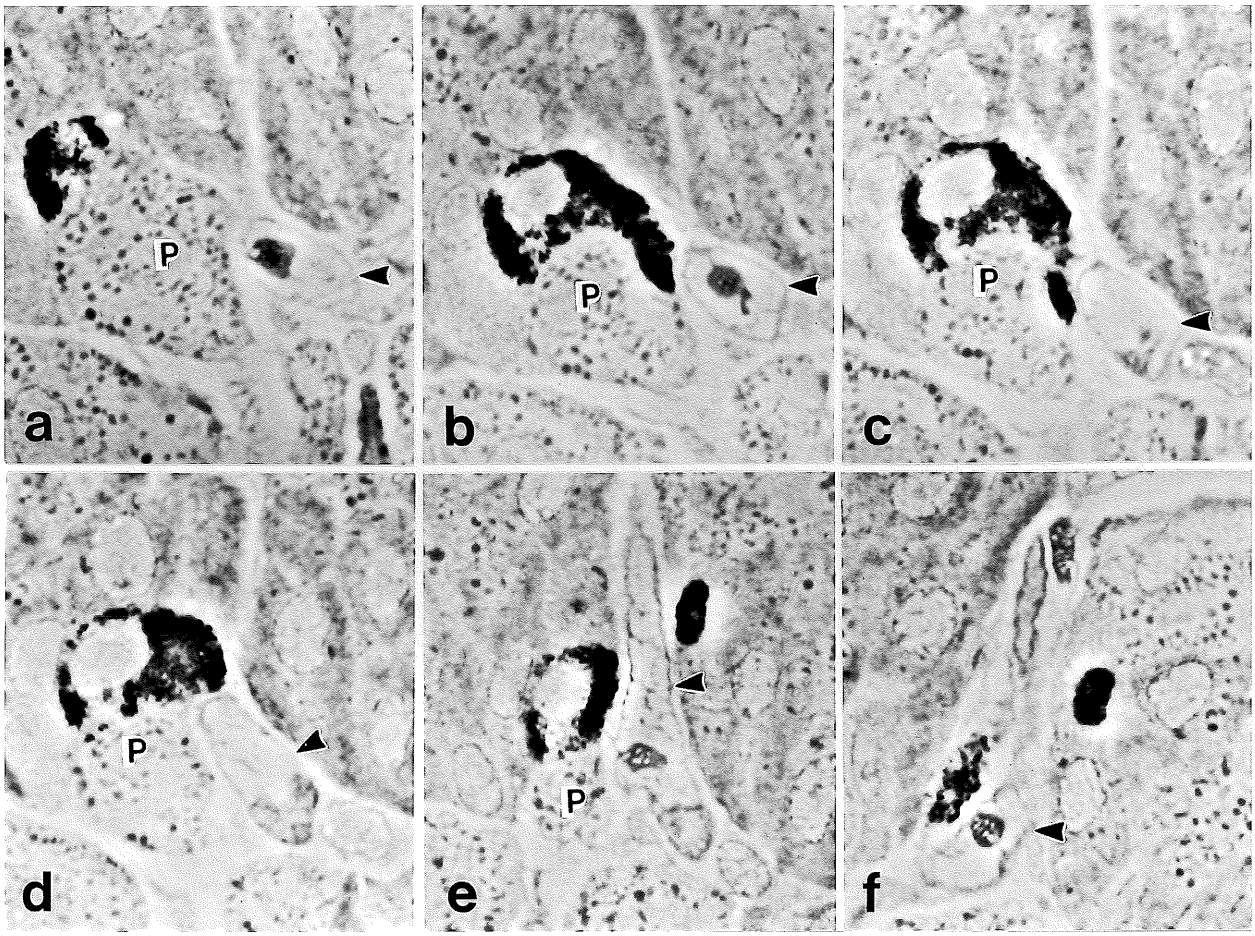

Fig. 2. Three-dimensional stereographic semitransparent images of a D cell (**g**) in the rat fundic mucosa. Eighteen serial sections passed through the D cell. Section Nos 2,5,6,8,14 and 18 are shown in **a** – **f**. The D cell embraces partly the parietal cell, interposing the process with a terminal swelling between the basal portion of the parietal cell (p) and the blood capillary (arrowheads).
a – **f**: × 1,500, **g**: × 3,700

Microtopography of D cells

Examination of serially sectioned D cells revealed that their cell bodies or cytoplasmic processes were regularly localized in close vicinity to blood capillaries (Figs. 1,2,3). Considering the juxtaposition of D cells and parietal cells, we found that a subpopulation of D cells, ovoid in shape and lacking in the cytoplasmic process, were almost completely enclosed (Fig. 3) or partly enveloped by single parietal cells. Preliminary investigation on the mutual topographic relationship between D cells and chief cells also gave similar results (Fig. 1c).

With regard to homologous contacts between D cells, such contacts were observed in the fundic glands of mouse and golden hamster. D cell clusters consisting of 3–5 cells were sometimes encountered in golden hamsters (Fig. 1f), especially in the bottom of fundic glands.

DISCUSSION

The present morphological observation through the use of serial semithin sections immunostained for somatostatin and computer-assisted methods showed that the somatostatin producing D cells in the gastric fundic mucosa may exert inhibitory effects on their target cells via paracrine (direct cell to cell contacts or diffusion) and endocrine mode (local circulatory system).

In the GEP endocrine system, D cells have attracted special attention as a typical cell type exerting effects on epithelial cells in their immediate neighborhood via a paracrine mode of direct cell to cell interaction with long cytoplasmic processes, which were interpreted as the morphological counterparts of a paracrinous mode of action[1,13,15]. However, some investigations have revealed that pancreatic D cells[7] and antropyloric D cells[12] are morphologically typical endocrine elements whose cytoplasmic processes regularly extend to intra-insular capillaries or to

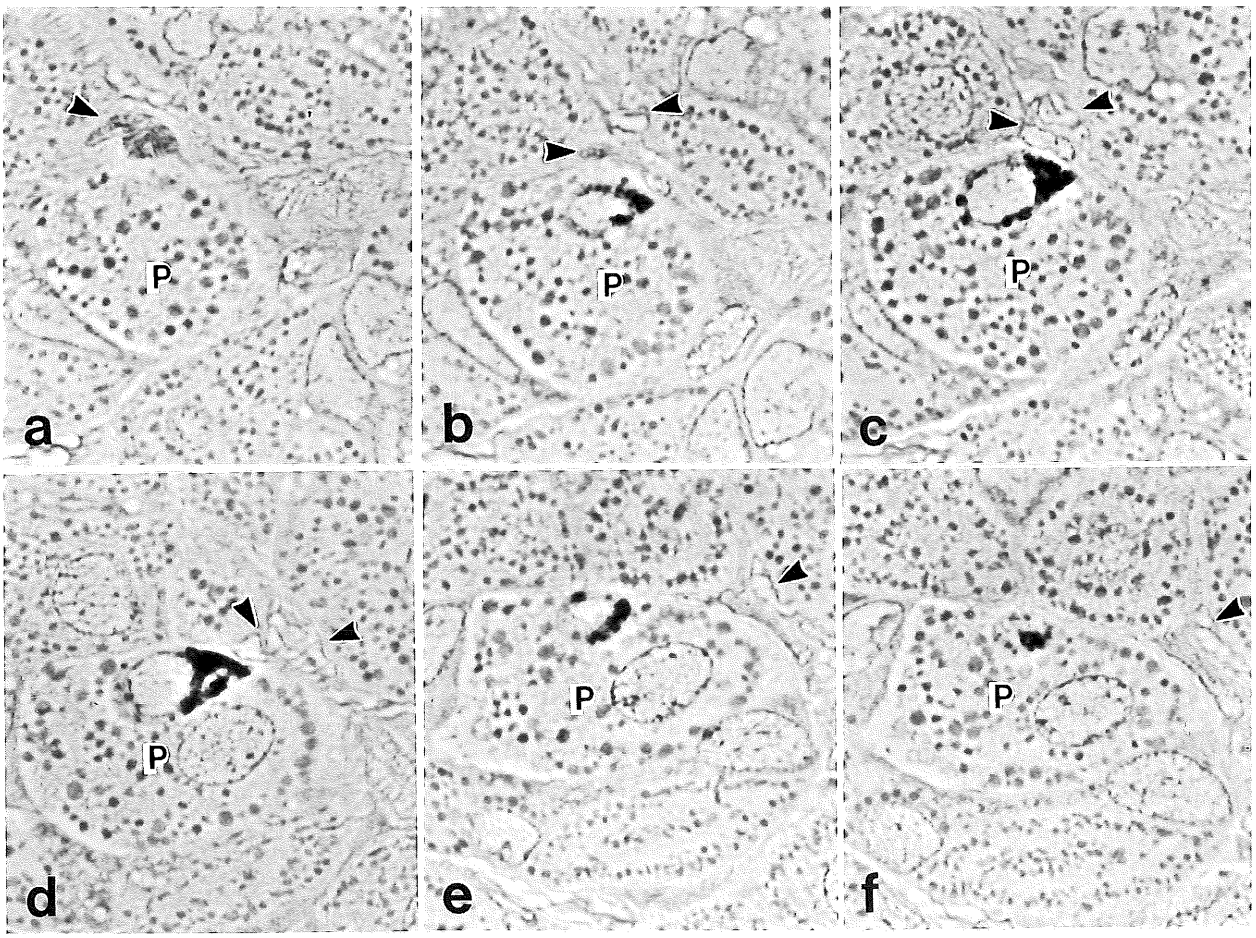

Fig. 3. Three-dimensional stereographic semitransparent image of another type of juxtaposition between a D cell (light blue) and a parietal cell (blue) in the mouse fundic mucosa. A blood capillary (arrowheads) is shown in red. Fourteen serial sections passed through the same D cell. Section Nos. 1,3,5,11,13 and 14 are shown in **a** – **f**. Note that the D cell is almost completely enclosed by the parietal cell (p). The D cell is ovoid in shape and lacking cytoplasmic extension.
a – **f**: × 1,500, **g**: × 2,200

capillaries in the lamina propria mucosae. Therefore, somatostatin from pancreatic D cells and antropyloric D cells may act via a local circulatory system rather than via a paracrine mode of direct cell to cell.

Likewise, D cells in the fundic mucosa of the stomach have hitherto been regarded as typical paracrine cells whose cytoplasmic processes terminate directly on adjacent "target" cells. In the fundic mucosa, the mutual topographic relationship between D cells and parietal cells or chief cells has been particularly noted[1,13,15]. Both parietal and chief cells are endowed with somatostatin receptors[3,17,26,33]. Here also, the inhibitory effect of somatostatin upon gastric acid secretion and pepsinogen secretion was seemingly mediated by direct cell to cell interactions. Indeed, the present study also showed direct contacts between D cells and parietal cells and/or chief cells with their cytoplasmic processes or cell bodies. However, simultaneously, almost all basally located cytoplasmic processes or cell bodies, when examined in serial sections, were found to be closely juxtaposed to blood capillaries. The study of mucosal microvascular architecture in the stomach[6,22] also revealed that blood capillaries in the lamina propria were fenestrated and intimately associated with individual fundic glands. Further, it was concluded that blood flow traveled from the bottom of the glands toward the luminal surface[22]. The fundic D cells are distributed predominantly in the lower half of the glands and a small part of each fundic gland cell is directly contacted by D cells. Therefore it seems likely that only selected parietal cells receive a direct transmission from D cells due to unknown reasons, while others may be subjected to an indirect control of somatostatin by diffusion and by microcirculation which must locally distribute the substance. No decisive morphological data at the ultrastructural level have so far been available to support the concept of a paracrine

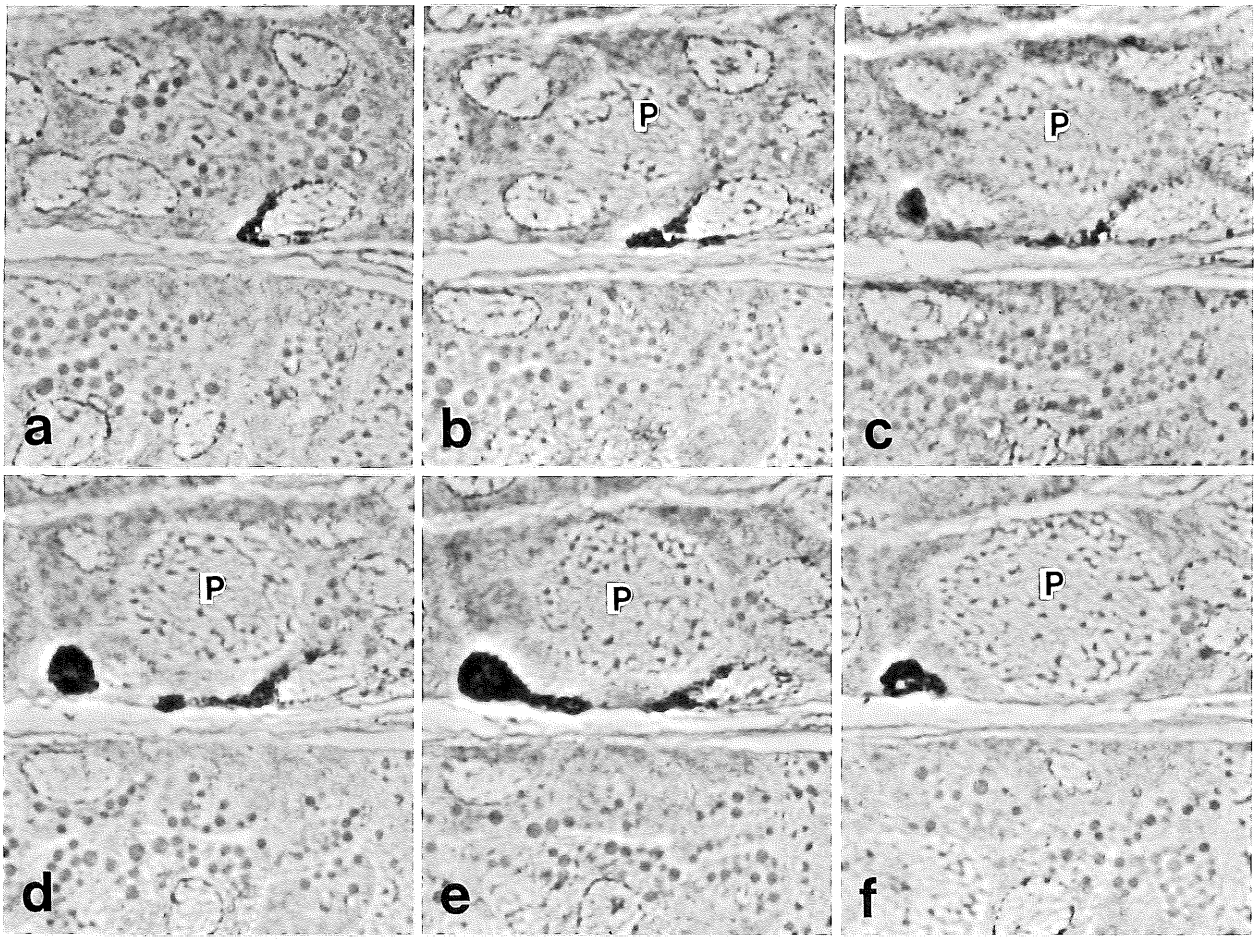

Fig. 4. Three-dimensional stereographic semitransparent image of another type of juxtaposition of a D cell (green) and a parietal cell (blue) in the rat stomach. Fifteen serial sections passed through the D cell. Section Nos. 4,7,9,11 and 14 are shown in **a** – **f**. The lower half of a pariental cell (p) is reconstructed. The D cell extends a slender process with a terminal swelling, without contacting the parietal cell, towards the chief cell.
a – **f**: × 1,500, **g**: × 2200

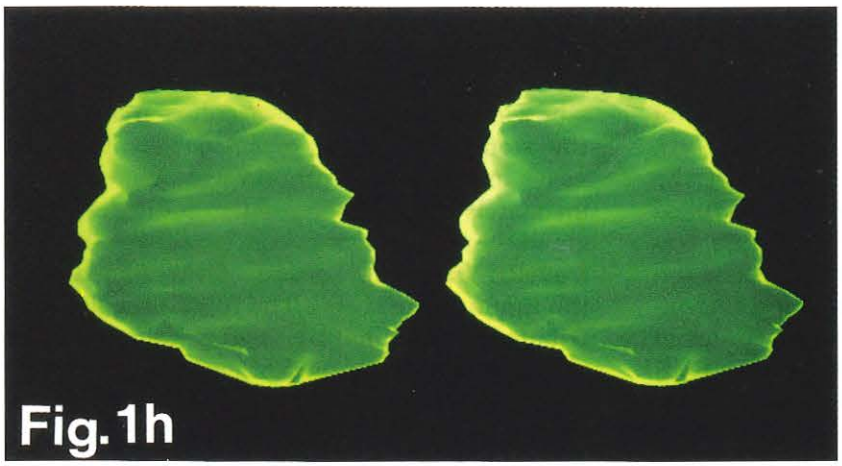

Fig. 1h

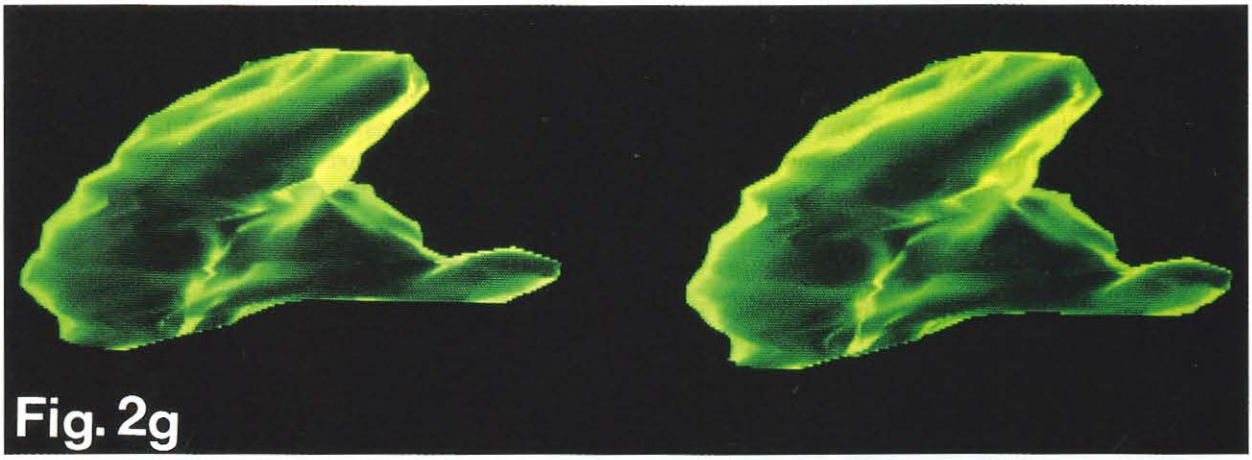

Fig. 2g

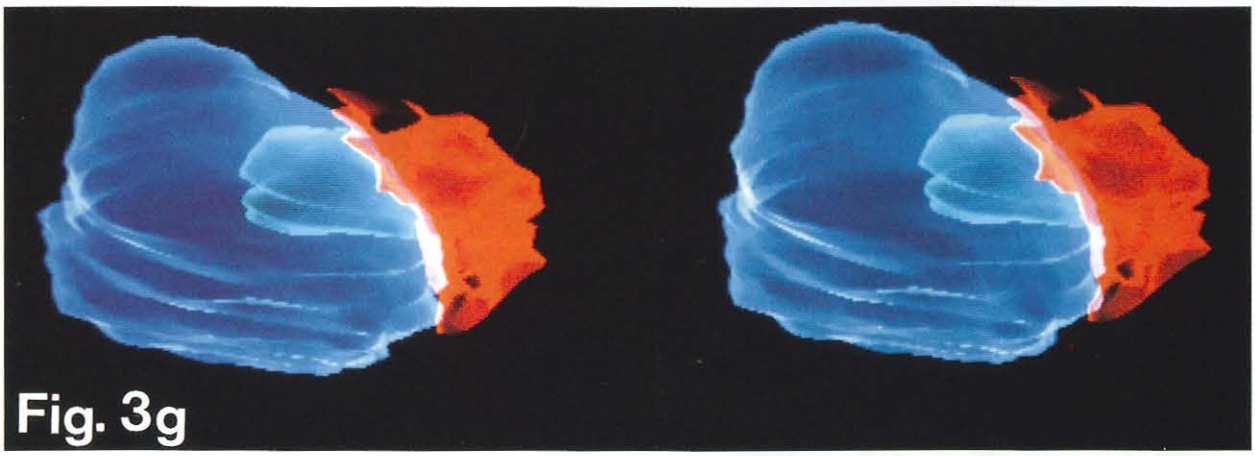

Fig. 3g

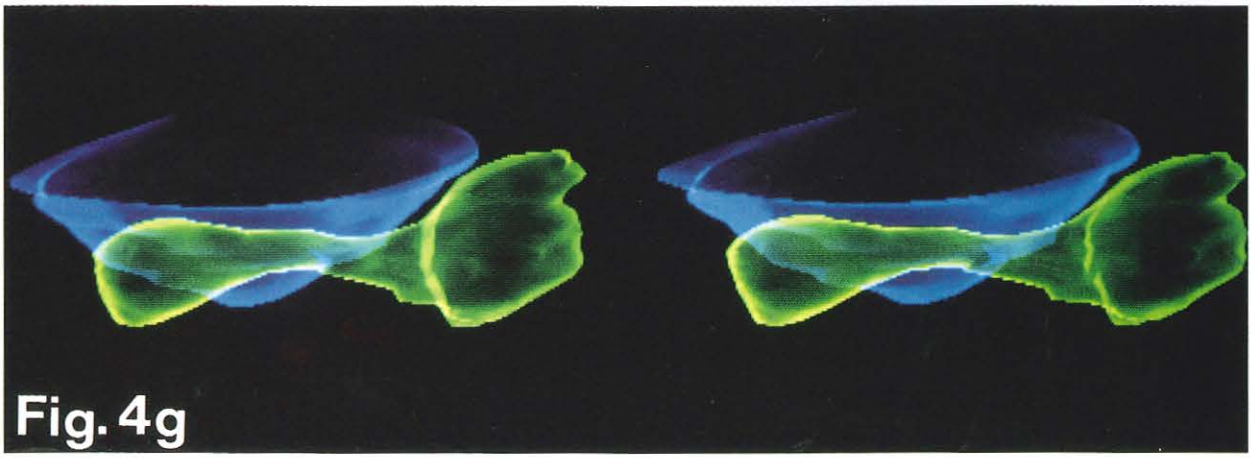

Fig. 4g

mode of action via direct cell to cell contact, i.e. certain membranous specializations between both given cells as represented in synapses[14]. It is not possible to discriminate precisely from the present findings, whether the fundic D cells induce the inhibition of gastric acid, pepsinogen and intrinsic factor secretion via an endocrine mode or a paracrine mode. Thus, to avoid dogmatic hypotheses, it may be valid to assume that the D cells in the fundic glands exert the inhibitory effects on the target cells mainly via microhemocrinia and partly via paracrinia and synaptocrinia[5], possibly to respond adequately to various physiological demands applied to the stomach, although an assessment of the relative importance of the paracrine and endocrine action of D cells is difficult. Further investigations are necessary to obtain a better understanding of the D cell action modes.

With regard to the heterogeneity of D cell shapes in the gastric fundic mucosa, it is worthwhile to mention the studies in which immunohistochemistry was applied to isolated fundic glands[28] and cultured D cells of the fundic mucosa[31] or the pancreatic islet[18]. Satoh et al (1988) examined the whole shapes of D cells by the use of the isolated fundic mucosa stained immunohistochemically and ascertained how the population of D cells possesses basal processes[28]. Consequently, the fundic D cells exhibited manifold shapes and about 20% of the D cells showed the cytoplasmic processes. Soll et al (1984) also reported that 20% of the cultured fundic D cells demonstrated prominent cellular processes[31]. Moreover, according to Matsuba et al (1982), the cultured D cells of the human pancreas varied markedly in size and shape[18]. They were nerve-like in shape, varying from a unipolar to a multipolar type, but the proliferative D cells were relatively spherical in shape and weakly immunostained. These findings on the morphology of gastropancreatic D cells are basically in agreement with the present results which showed three-dimensionally a variety of fundic D cell shapes. What is the physiological significance of the remarkable variations in the fundic D cell morphology? It may suggest that the fundic D cells can move actively with a certain plasticity, probably reacting to various stimuli coming from the blood stream or mechanical stimuli. The mode of cell migration in the fundic mucosa seems to have an influence on the D cell shapes[9,10]. However, our views on the versatility of D cell shape do not go beyond the present range of speculation. Future studies on the ontogenesis and the cellular kinetics of the fundic D cell may contribute to elucidating the significance of heterogeneity in the fundic D cell shape.

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