AN UNUSUAL INTIMATE RELATIONSHIP BETWEEN ENDOCRINE CELLS AND OTHER TYPES OF EPITHELIAL CELLS IN THE HUMAN STOMACH

WALTER RUBIN. From the Departments of Medicine (Division of Gastroenterology) and Anatomy, The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

The fine structure of human (1–4) and other mammalian (3, 5–12) gastric endocrine cells ("argyrophile," "argentaffin," "enterochromaffin," "pale," "clear," "endocrine-like") has been described. This report describes an unusual morphological and cytochemical relationship observed between human gastric endocrine cells and other types of gastric epithelial cells. The endocrine cells are frequently "embedded" within other epithelial cells and the apposing plasmalemmae exhibit an exceptionally high ATPase activity as demonstrated by cytochemistry.

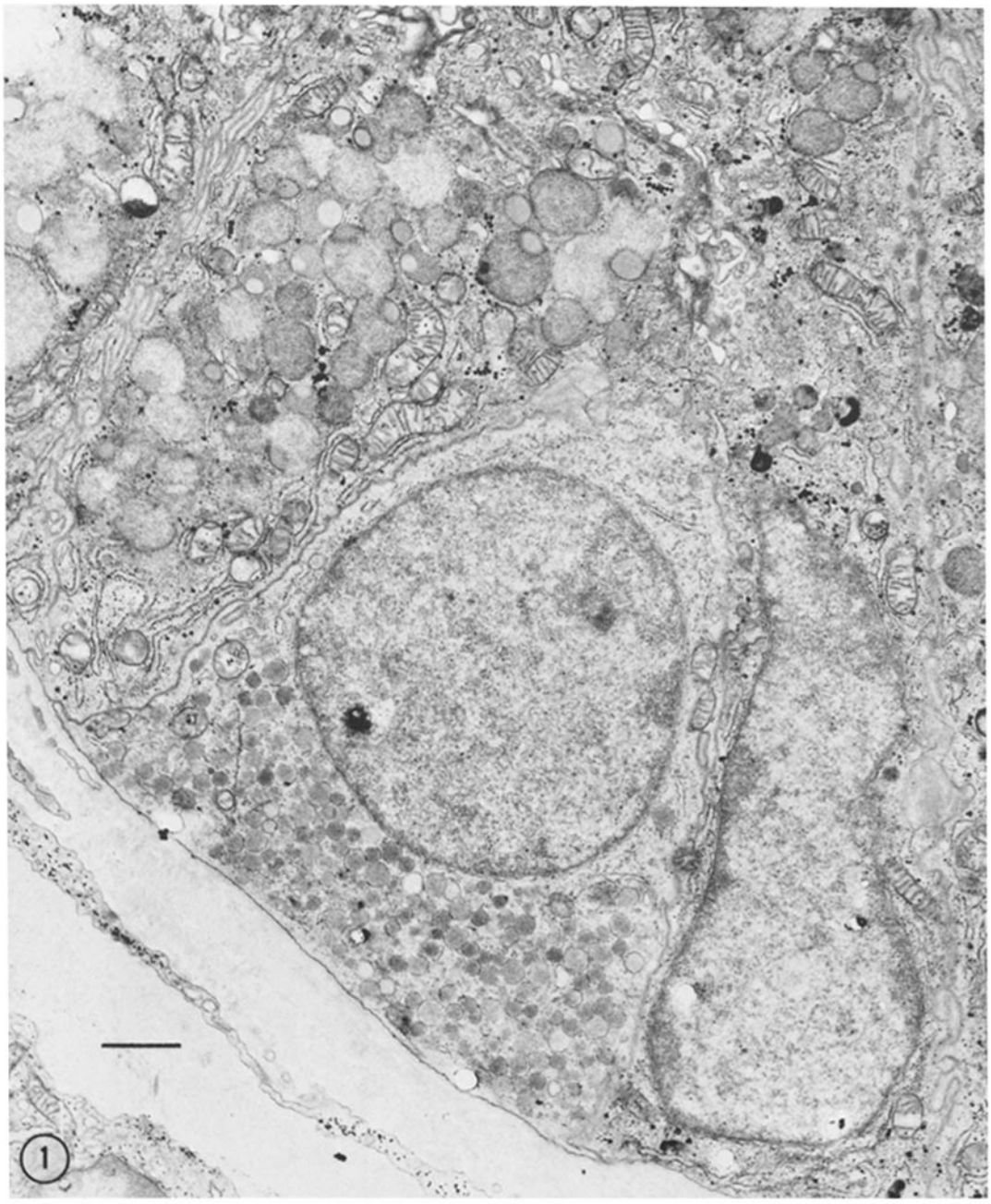

FIGURE 1 This D cell (4), located in a pyloric gland, appears "embedded" in a pyloric gland cell, i.e., its superior and lateral surfaces are surrounded by a single cell. Approximately ×11,000.

METHODS

Biopsies were obtained from the body and pylorus of normal and atrophic human stomachs as previously described (4, 13, 14), and were fixed for 3–48 hr at 0–4°C in 2.5% glutaraldehyde buffered at pH 7.4 with 0.1 M cacodylate or phosphate (15). Specimens for cytochemistry were fixed for only 3 hr and were buffered only with cacodylate. Approximately 50-μ

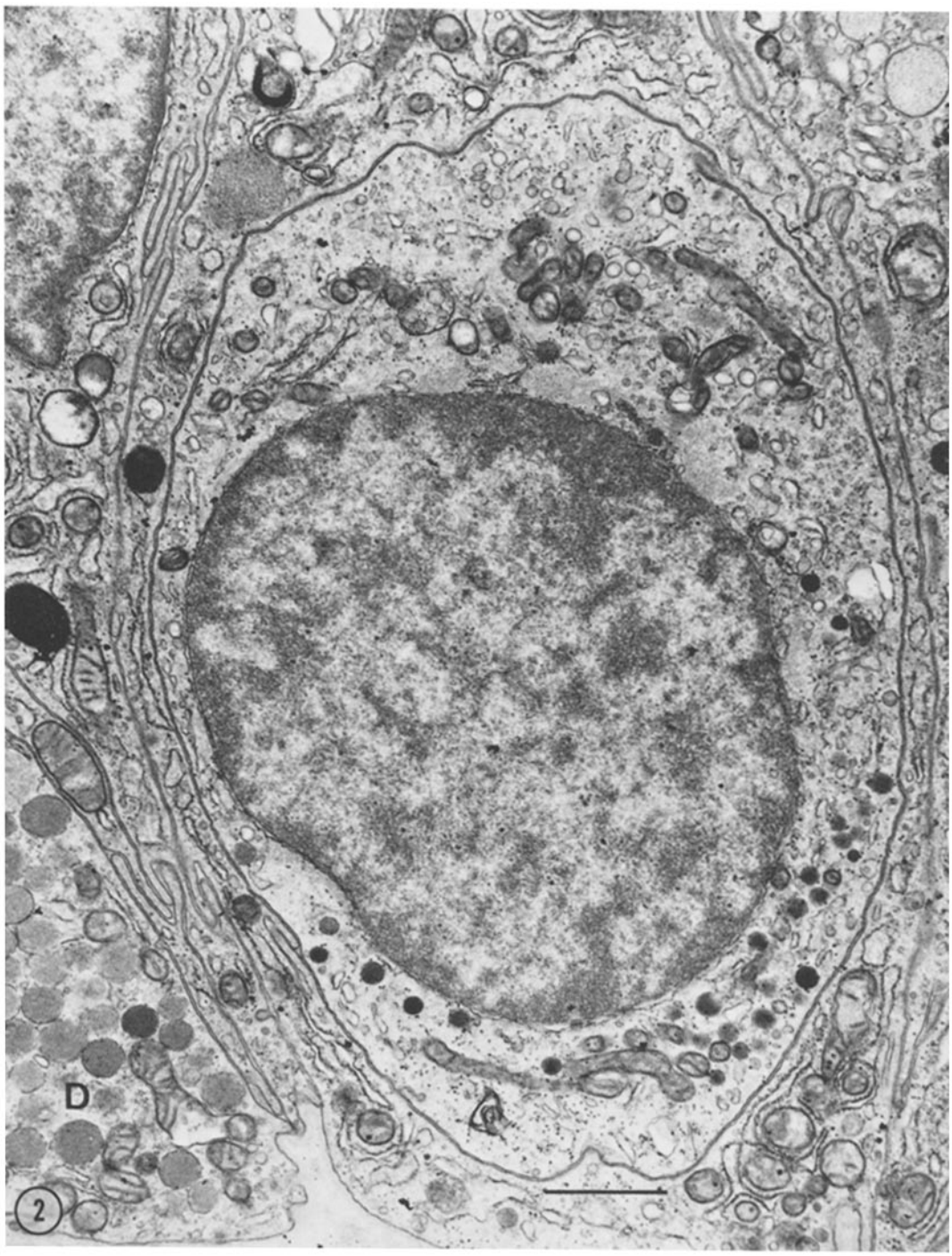

FIGURE 2 This ECL cell (4), located in a gland in the body of the stomach, appears to be completely enclosed by a mucous gland cell (13). Despite such profiles, the inferior surfaces of all endocrine cells are thought to reach the basal lamina, although serial sections were not achieved to prove this hypothesis. *D* denotes a portion of a D cell. Approximately ×18,000.

sections of these latter specimens were prepared by the method of Smith and Farquhar (16) as described previously (17), and were incubated at 37°C for 10–60 min in specific substrate for demonstrating the following enzymes by the respective methods: adenosine triphosphatase (ATPase) by the method of Wachstein and Meisel (18); alkaline and acid phosphatases by the methods of Gomori (19); and inosine diphosphatase and thiamine pyrophosphatase by the methods of Novikoff and Goldfischer (20). Control slices were incubated in media lacking specific substrates. All specimens were postfixed for

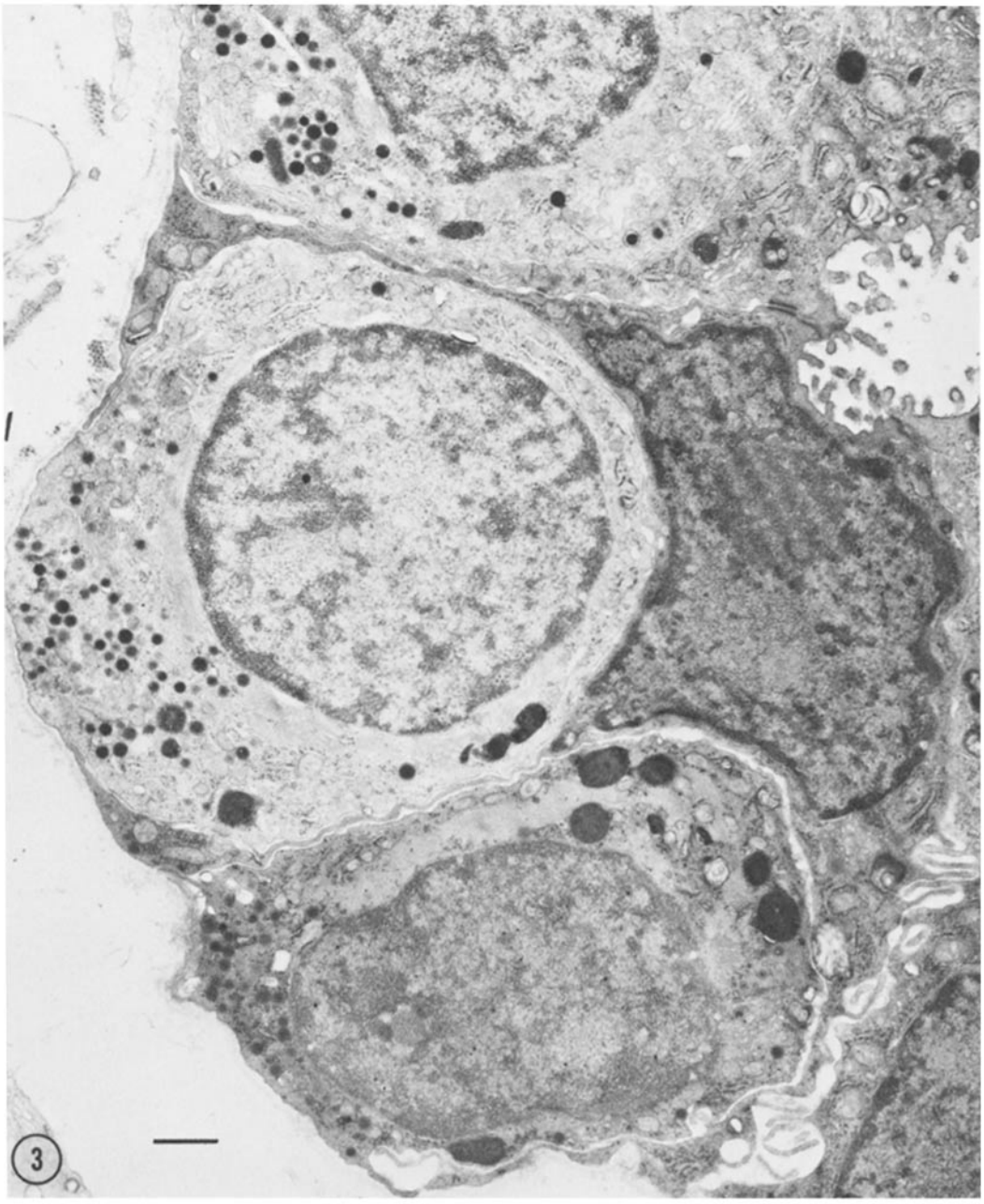

FIGURE 3 In atrophic gastric mucosa, endocrine cells are more abundant, frequently occurring in bunches (14). This section through the lower portions of a gland in the body of an atropic stomach reveals three ECL cells. Note that the two lower ones are "embedded" in a single epithelial cell, whereas the upper endocrine cell is embedded in a different cell. Approximately ×9000.

3/4 hr (the cytochemical ones) or 3 hr at 0–4°C in 1% osmium tetroxide in the same buffers used for the glutaraldehyde fixation; were dehydrated in graded alcohols and propylene oxide; and were embedded in Epon 812 (21). Both unstained and uranyl- (22) and/or lead-stained (23, 24) thin sections were examined with a Philips 200 or 300 electron microscope at original magnifications of 2800–50,000.

RESULTS

In random sections of normal human gastric mucosa, the endocrine cells frequently appeared embedded within individual epithelial cells of all varieties, such as chief, parietal, undifferentiated neck (13), mucous gland (13), pyloric gland, and pit cells (Figs. 1–5). The basal surfaces of the endocrine cells extended along the basal lamina (Figs. 1, 3), but superiorly these cone- or dome-shaped cells frequently extended into the basal surfaces of neighboring epithelial cells, thereby producing concave configurations of these basal surfaces (Figs. 1, 3). This morphological relationship was most readily appreciated in sections perpendicular to the basal lamina; in more tangential sections which failed to include their basal surfaces, the endocrine cells often appeared to be completely surrounded by single epithelial cells (Figs. 2, 4, 5).

In contrast, other types of gastric epithelial cells exhibited no such morphological relationships with each other. Similarly, cells infiltrating the gastric epithelium, such as lymphocytes, mast cells, and neutrophiles, extended within the intercellular spaces between adjoining epithelial cells, but did not extend into single epithelial cells.

Of 298 endocrine cells identified in random sections of normal gastric mucosa, 97 (33%) appeared embedded (Table I); i.e., they were surrounded at least on their lateral and superior surfaces by single epithelial cells. Sections of these 97 cells included a large portion of the

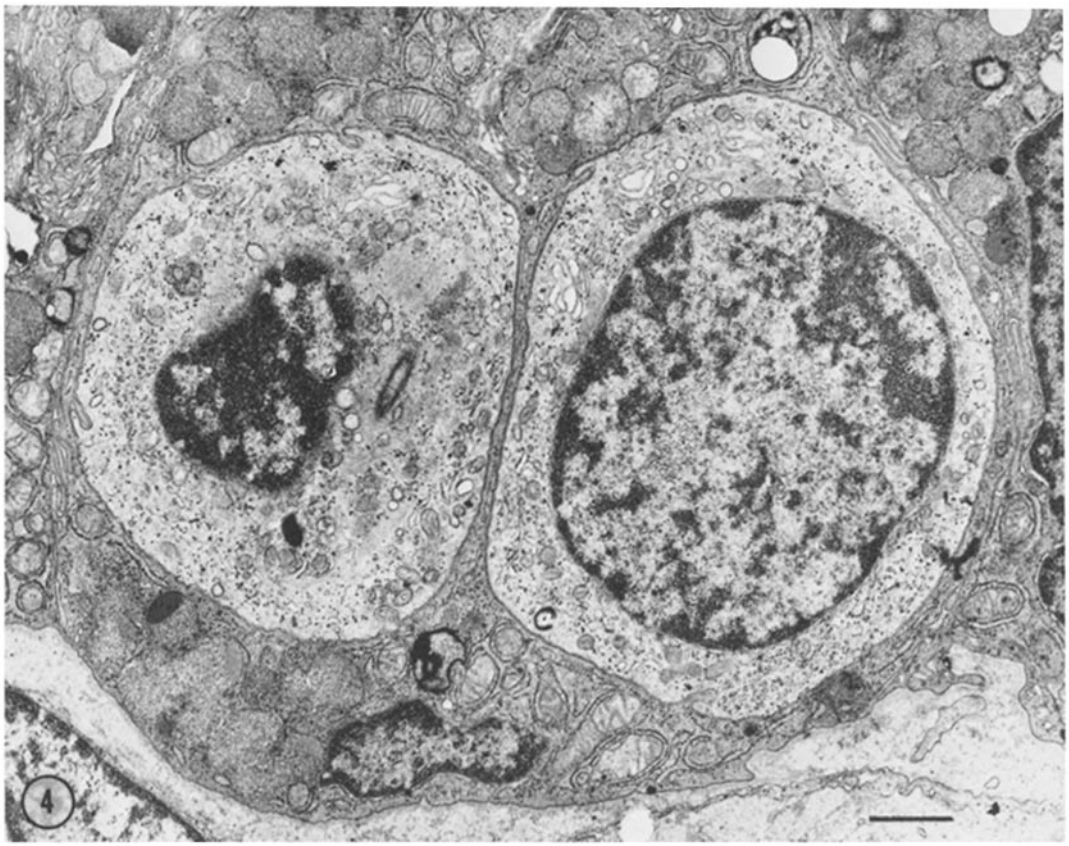

FIGURE 4 This section from the body of another atrophic stomach shows two endocrine cells "embedded" within the same mucous cell. The cells in this figure are thought to have the same morphological relationship as those in Fig. 3, but to have been sectioned more tangentially. Approximately ×11,000.

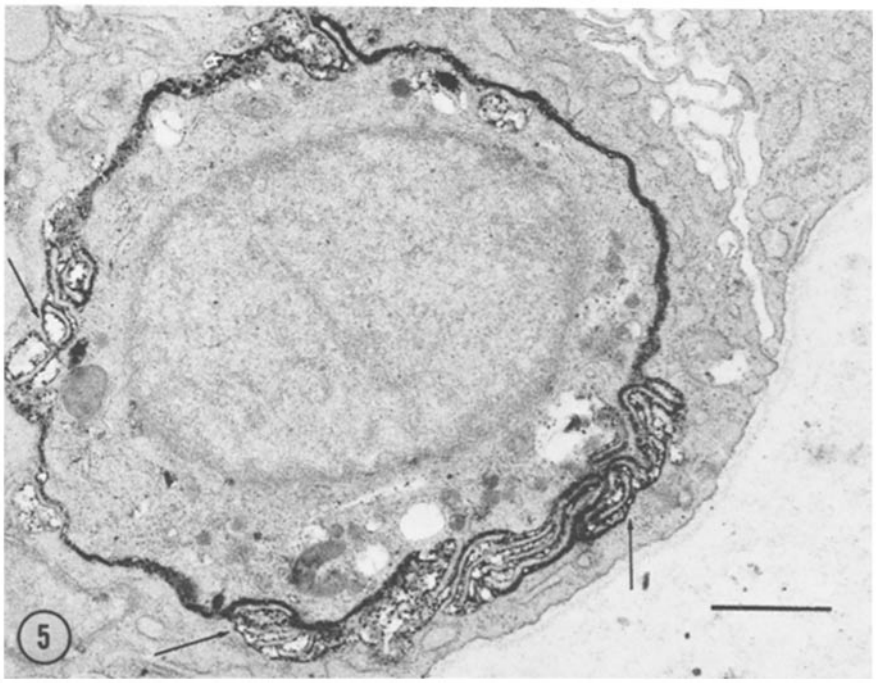

FIGURE 5 The apposing plasmalemmae of gastric endocrine cells (of all types) and of the surrounding epithelial cells (arrows) exhibit a strong ATPase activity by the Wachstein-Meisel reaction. This endocrine cell (an ECL) is embedded in a mucous gland cell. Note that the nonapposing plasmalemmae of the surrounding mucous gland cell show no reaction, but would do so after longer incubations. This section is unstained. Approximately ×16,000.

TABLE I

Per Cents of Endocrine Cells Appearing "Embedded"

Cell type (4)	Number observed	Number embedded	Embedded
			%
ECL	110	46	42
Ec	31	12	39
D	36	11	31
G	85	14	16
Uncertain	36	14	39
Totals	298	97	33

cytoplasm and usually the nucleus, and were thought to be reasonably perpendicular to the basal lamina, as illustrated by Figs. 1 and 3. Of the 85 G cells (4) included in these totals, only 14 (16%) appeared embedded (Table I); G cells, which are located only in the pylorus (4), are frequently observed to reach the lumen of the gland superiorly. However, of the other 213 gastric endocrine cells, the varieties of which are rarely, if ever, observed to reach the lumen (4), 83 (39%) appeared embedded (Table I). Sections of most of the other 130 of these 213 cells were generally inadequate to indicate whether they were embedded or not; some of these cells, however, seemed to extend between several adjoining epithelial cells, as illustrated in Fig. 6.

In atrophic gastric mucosa where endocrine cells are more abundant (14) this morphological relationship between endocrine and other epithelial cells was even more apparent; single epithelial cells frequently enclosed more than one endocrine cell (Figs. 3, 4).

The apposing cell membranes of both endocrine cells and the surrounding epithelial cells exhibited a strong ATPase activity as measured by the Wachstein-Meisel reaction (Figs. 5, 6). With short incubations, precipitate was restricted to these apposing cell membranes (Figs. 5 and 6). The basal plasmalemmae of endocrine cells and nonapposing plasmalemmae of the surrounding epithelial cells exhibited a reaction only after longer incubations. The apposing plasmalemmae exhibited no alkaline phosphatase, acid phospha-

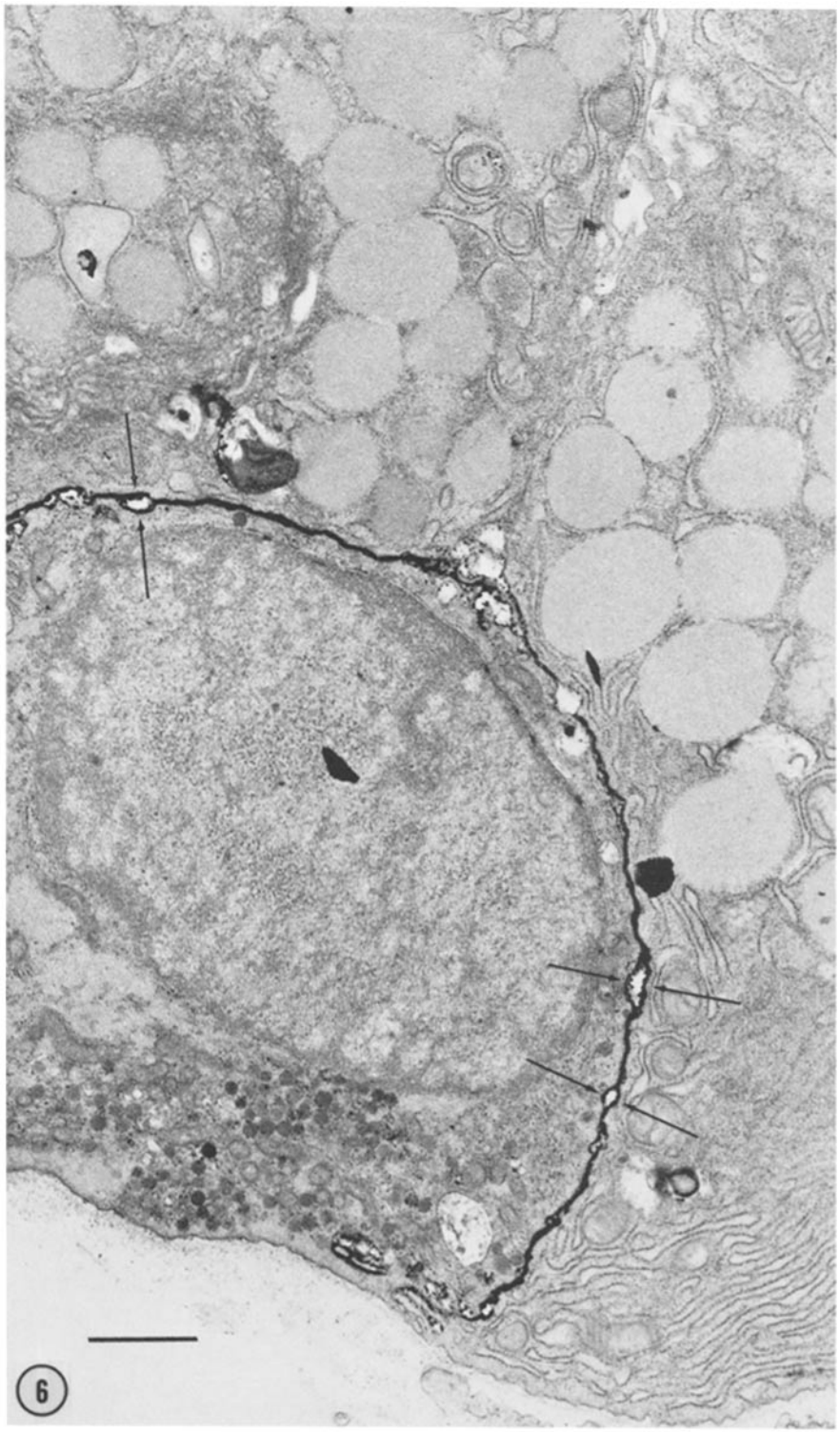

FIGURE 6 This ECL cell does not seem to be "embedded"; rather it seems to extend between two adjoining chief cells. Note again that the Wachstein-Meisel reaction seems to be on both apposing plasmalemmae (arrows), i.e., that of the endocrine cell and that of the surrounding cells. Note that the basal plasmalemma of the endocrine cell and the nonapposing plasmalemmae of the chief cells show no reaction, but would do so after longer incubations. The section is unstained. Approximately ×15,000.

tase, or thiamine pyrophosphatase activities, but did exhibit inosine diphosphatase activity which was similar to that of ATPase in distribution, but which generally required longer incubations and generally produced a lighter and more variable precipitate.

DISCUSSION

The unusual morphological relationship between human gastric endocrine cells and other types of epithelial cells, which effects an apposition of large areas of cell membranes exceptionally active in enzymatic activity, is probably of physiological importance. This morphological relationship is somewhat similar to that between neurons and their satellite cells. It is interesting that gastric endocrine cells, especially the Ec and ECL varieties (4), contain small membrane-enclosed granules and numerous small mitochondria, often in bunches (4), and synthesize and release physiologically-active amines (12); in these respects, too, they resemble some neurons, their synaptic endings in particular.

The gastric endocrine cells still remain an enigmatic group of cells. An understanding of their ontogenesis, relationships, metabolism, and physiology is yet incomplete. Despite their similarities to neural cells, as suggested above, it is not established whether they are derived from the neural crest or, like other gastric epithelial cells, from endoderm (25, 26). Morphologically and cytochemically they resemble endocrine cells that synthesize amines and peptide hormones (12), and in various species they have been thought to be able to produce such physiologically active substances as serotonin (5-hydroxytryptamine) (11, 27–30), histamine (31–33), catecholamines (12, 29, 30, 33, 34), gastrin (7, 12, 35–39), and enteroglucagon (glucagon-like immunoreactive substance) (8, 40, 41). In recent years several morphological types have been described in various species (1–12)–four in man (1, 2, 4)–but the association of different morphological types with differences in metabolism and function (1–3, 7–12, 36, 40, 41) has yet to be established (4, 11, 38). When the physiology and biochemistry of the gastric endocrine cells has been more adequately defined, the significance of the unusual morphological and cytochemical relationships described in the present study should become more apparent.

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REFERENCES

1. Pearse, A. G. E., I. Coulling, B. Weavers, and S. Friesen. 1970. The endocrine polypeptide cells of the human stomach, duodenum, and jejunum. *Gut.* **11**:649.
2. Solcia, E., C. Capella, and G. Vassallo. 1970. Endocrine cells of the stomach and pancreas in states of gastric hypersecretion. *Rend. R. Gastroenterol.* **2**:147.
3. Solcia, E., G. Vassallo, and C. Capella. 1969. Studies on the G cells of the pyloric mucosa, the probable site of gastrin secretion. *Gut.* **10**: 379.
4. Rubin, W. The endocrine cells in the normal human stomach. A fine structural study. Submitted for publication.
5. Ito, S., and R. J. Winchester. 1963. The fine structure of the gastric mucosa in the bat. *J. Cell Biol.* **16**:541.
6. Toner, P. G. 1964. Fine structure of argyrophil and argentaffin cells in the gastro-intestinal tract of the fowl. *Z. Zellforsch. Mikrosk. Anat.* **63**:830.
7. Solcia, E., G. Vassallo, and R. Sampietro. 1967. Endocrine cells in the antro-pyloric mucosa of the stomach. *Z. Zellforsch. Mikrosk. Anat.* **81**:474.
8. Forssmann, W. G., L. Orci, L. Pictet, A. E. Renold, and C. Rouiller. 1969. The endocrine cells in the epithelium of the gastrointestinal mucosa of the rat. An electron-microscope study. *J. Cell Biol.* **40**:692.
9. Vassallo, G., E. Solcia, and C. Capella. 1969. Light and electron microscopic identification of several types of endocrine cells in the gastrointestinal mucosa of the cat. *Z. Zellforsch Mikrosk. Anat.* **98**:333.
10. Capella, C., E. Solcia, and G. Vassallo. 1969. Identification of six types of endocrine cells in the gastrointestinal mucosa of the rabbit. *Arch. Histol. Jap.* **30**:479.
11. Rubin, W., M. D. Gershon, and L. L. Ross. 1971. Electron microscope radioautographic identification of serotonin-synthesizing cells in the mouse gastric mucosa. *J. Cell Biol.* **50**: 399.
12. Carvalheira, A. F., U. Welsch, and A. G. E. Pearse. 1968. Cytochemical and ultrastructural observations on the argentaffin and argyrophil cells of the gastro-intestinal tract in mammals, and their place in the APUD

series of polypeptide-secreting cells. *Histochemie.* **14**:33.

13. RUBIN, W., L. L. ROSS, M. H. SLEISENGER, and G. H. JEFFRIES. 1968. The normal human gastric epithelia. A fine structural study. *Lab. Invest.* **19**:598.
14. RUBIN, W. 1969. Proliferation of endocrine-like (enterochromaffin) cells in atrophic gastric mucosa. *Gastroenterology.* **57**:641.
15. SABATINI, D. D., K. BENSCH, and R. J. BARRNETT. 1963. Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* **17**:19.
16. SMITH, R. E., and M. G. FARQUHAR. 1963. Preparation of thick sections for cytochemistry and electron microscopy by a nonfreezing technique. *Nature (London)* **200**:691.
17. Rubin, W. 1969. Enzyme cytochemistry of gastric parietal cells at a fine structural level. Cytochemical separation of the endoplasmic reticulum from the "tubulovesicles". *J. Cell Biol.* **42**:332.
18. Wachstein, M., and E. Meisel. 1957. Histochemistry of hepatic phosphatases at a physiologic pH with special reference to the demonstration of bile canaliculi. *Amer. J. Clin. Pathol.* **27**:13.
19. GOMORI, G. 1952. Microscopic histochemistry; principles and practice. University of Chicago Press, Chicago.
20. NOVIKOFF, A. B., and S. GOLDFISCHER. 1961. Nucleoside diphosphatase activity in the golgi apparatus and its usefulness for cytological studies. *Proc. Nat. Acad. Sci. U.S.A.* **47**:802.
21. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:409.
22. Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* **4**:727.
23. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.
24. VENABLE, J. H., and R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**:407.
25. MACKLIN, C. C., and M. T. MACKLIN. 1932. The intestinal epithelium, in Special Cytology. E. V. Cowdry, editor. Paul Hoeber, Inc., NewYork. 2nd edition. 233.
26. PEARSE, A. G. E. 1969. The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic, and pathologic implications of the concept. *J. Histochem. Cytochem.* **17**:303.
27. ERSPAMER, V., and B. ASERO. 1952. Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature (London).* **169**:800.
28. GERSHON, M. D., and L. L. ROSS. 1966. Location of sites of 5-hydroxytryptamine storage and metabolism by radioautography. *J. Physiol. (London).* **186**:477.
29. HÅKANSON, R., and C. OWMAN. 1966. Distribution and properties of amino acid decarboxylases in gastric mucosa. *Biochem. Pharmacol.* **15**:489.
30. HAMMARSTRÖM, L., M. RITZÉN, and S. ULLBERG. 1966. Combined autoradiography and fluorescence microscopy. Localization of labelled 5-hydroxytryptophan in relation to endogenous 5-hydroxytryptamine in the gastro-intestinal tract. *Experientia (Basel).* **22**:213.
31. WEINSHELBAUM, E. I., and D. J. FERGUSON. 1966. Localization of carbon-14 from histamine in Enterochromaffin cells. *Gastroenterology.* **51**: 1028.
32. THUNBERG, R. 1967. Localization of cells containing and forming histamine in the gastric mucosa of the rat. *Exp. Cell Res.* **47**:108.
33. HÅKANSON, R., and C. OWMAN. 1967. Concomitant histochemical demonstration of histamine and catecholamines in enterochromaffin-like cells of gastric mucosa. *Life Sci.* **6**:759.
34. HÅKANSON, R., B. LILJA, and C. OWMAN. 1967. Properties of a new system of amine-storing cells in the gastric mucosa of the rat. *Eur. J. Pharmacol.* **1**:188.
35. MCGUIGAN, J. E. 1968. Gastric mucosal intracellular localization of gastrin by immunofluorescence. *Gastroenterology.* **55**:315.
36. BUSSOLATI, G., and A. G. E. PEARSE. 1970. Immunofluorescent localization of the gastrin-secreting G cells in the pyloric antrum of the pig. *Histochemie.* **21**:1.
37. PEARSE, A. G. E., and G. BUSSOLATI. 1970. Immunofluorescence studies of the distribution of gastrin cells in different clinical states. *Gut.* **11**:646.
38. MCGUIGAN, J. E., and M. H. GREIDER. 1971. Correlative immunochemical and light microscopic studies of the gastrin cell of the antral mucosa. *Gastroenterology.* **60**:223.
39. SOLCIA, E., and R. SAMPIETRO. 1965. Cytologic observations on the pancreatic islets with reference to some endocrine-like cells of the gastrointestinal mucosa. *Z. Zellforsch. Mikrosk. Anat.* **68**:689.
40. ORCI, L., R. PICTET, W. G. FORSSMANN, A. E. RENOLD, and C. ROUILLER. 1968. Structural evidence for glucagon producing cells in the intestinal mucosa of the rat. *Diabetologia.* **4**:56.
41. POLAK, J. M., S. BLOOM, I. COULLING, and A. G. E. PEARSE. 1971. Immunofluorescent localization of enteroglucagon cells in the gastrointestinal tract of the dog. *Gut.* **12**:311.