A Fine Structural Characterization of the Proliferated Endocrine Cells in Atrophic Gastric Mucosa

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This study shows that most of the proliferated endocrine cells in nonintestinalized epithelium in the body of stomachs in patients with pernicious anemia have the fine structure of ECL (enterochromaffin-like) cells, the principal endocrine cell in the body of normal stomachs, and notes an absence of G (gastrin) cells, the principal endocrine cell in the normal pylorus. Since gastrin has been identified in many of these proliferated cells by immunofluorescence, these findings question the position that gastrin synthesis in the stomach is only associated with G cells (Am J Pathol 70:109–118, 1973).

INCREASED NUMBERS OF ENDOCRINE CELLS have been observed in the body of stomachs from patients with pernicious anemia.[1–3] Recent electron microscopic studies have classified the normal human gastric endocrine cells into at least four morphologic types—G (gastrin), ECL (enterochromaffin-like), Ec (enterochromaffin), and D (D-like);[4–11] this nomenclature was adopted in 1969 at the Wiesbaden Conference on the gastrointestinal hormones.[4] Specific and separate endocrine functions have been suggested for each morphologic type;[3–23] gastrin synthesis has generally been attributed to G cells,[3–21] which are located in the pyloric antrum, where they constitute the majority of endocrine cells.[10]

The present study classifies the proliferated endocrine cells in the nonintestinalized epithelium in the body of stomachs from patients with pernicious anemia in accordance with their fine structure. The findings show that most of these cells were ECL cells, the principal endocrine cells observed in the body of normal stomachs [5–10] and that G cells were absent among them. Since gastrin has been identified within many of these proliferated cells by immunofluorescence,[2] the present study also questions the position that gastrin synthesis in the stomach is only associated with G cells.

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Materials and Methods

Biopsies of gastric mucosa were obtained by a fluoroscopically positioned peroral biopsy tube [24] from the body of the stomachs of 11 adult patients with treated pernicious anemia, as previously described.[1] The specimens were fixed for 3 to 48 hours at 0 to 4 C in 2.5% glutaraldehyde buffered at pH 7.4 with 0.1 M phosphate or cacodylate.[25] They were postfixed in 1% osmium tetroxide in the same buffer at 0 to 4 C for 3 hours, dehydrated in graded alcohols and propylene oxide, and embedded in Epon 812.[26] The specimens were reembedded, as previously described,[27] so that serial thick (approximately 1 μ) and thin (silver to pale yellow) sections could be cut perpendicular to the luminal surface and extending to the muscularis mucosae. Thick sections were stained with toluidine blue [28] and examined by light microscopy, while thin sections were stained with uranyl acetate [29] and/or lead citrate [30,31] and examined with an electron microscope. In at least one thin section from each specimen, all endocrine cells observed were photographed and classified in accordance with their fine structural resemblance to the four morphologic types of endocrine cells observed in the body and pylorus of normal gastric mucosae.[10]

Results

As noted previously,[1,27] the numerous endocrine cells within heterotopic intestinal epithelium resembled those of normal small intestine. The present study focuses on the numerous endocrine cells in the nonintestinalized epithelium in the body of these stomachs. The fine structure of these cells resembled that of endocrine cells observed in the body of normal stomachs.[10] Of 260 photographed endocrine cells, 195 (75%) were ECL cells, 26 (10%) were Ec cells, 11 (4%) were D cells and 28 (11%) could not be classified with certainty. No G cells were identified in the body of these atrophic stomachs.

Thus most of these proliferated cells exhibited the fine structural characteristics of ECL cells [10] (Figures 1–3). They contained small (averaging 0.1 to 0.15 μ in diameter), regular, usually dense, round granules (Figures 1–3) and many small, relatively dense mitochondria, often in clusters (Figures 1–3). They frequently exhibited prominent filaments, especially near the nucleus (Figure 1); were not observed to reach the glandular lumen superiorly; and often appeared "embedded" within other single epithelial cells [10,32] (Figures 1 and 2). The clusters of endocrine cells that are frequently observed in these stomachs [1] were usually composed of ECL cells (Figures 1 and 3). Two small clusters of Ec cells were observed in sections from two specimens and accounted for the majority of Ec cells observed in this study.

Discussion

In 1969 [1] we described a proliferation of endocrine cells in the body of stomachs of patients with pernicious anemia, an observation

subsequently confirmed by Polak and co-workers.[2,3] The present study shows that these proliferated cells in the nonintestinalized epithelium resemble the endocrine cells observed in the body of normal stomachs [10]—*ie*, most of them are ECL cells, and G cells are absent. These findings are at odds with those of Polak and co-workers,[2] who considered most of the proliferated cells, both in their own cases and in our previously published illustrations,[1] to be G cells, and who wondered how the fundic mucosal glands, normally devoid of G cells, acquires them in relatively large numbers.

The discrepancy between these two studies probably results from discrepancies among the recent electron microscopic descriptions of the normal human gastric endocrine cells.[5–10] Our own observations of the endocrine cells in normal stomachs [10] are essentially in agreement with those of Vassallo and co-workers,[7] who have also interpreted our previously published illustrations of some of these proliferated cells[1] as representing ECL cells.[6] Kobayashi and co-workers,[8] however, have classified endocrine cells with small dense granules and prominent perinuclear filaments as a separate type of endocrine cell which they have termed their "third type" and have noted that most of the proliferated endocrine cells previously illustrated by us[1] correspond to this type, which we[10] and Vassallo and co-workers[7] consider to be ECL cells. At any rate, none of the proliferated endocrine cells in the body of stomachs of patients with pernicious anemia resembled the G cells observed by us[10] or described by these Italian[6,7] and Japanese[9] investigators; most of the proliferated cells resemble endocrine cells observed in the body, not the pylorus, of normal stomachs.[10] Pearse and co-workers,[5] on the other hand, described the ECL cells of the normal human stomach as containing "vacuolated" granules similar to the granules observed in the corresponding cells of other species[15–17,19–21] and unlike the granules in the proliferated endocrine cells that we previously illustrated [1] or that they observed in the stomachs of their patients with pernicious anemia.

Many recent studies have endeavored to associate the different morphologic types of gastric endocrine cells with the production of known peptides and amines.[3–23] Mainly because of their location in the pyloric mucosa and some cytochemical properties, G (gastrin) cells have generally been thought to be the source of gastrin,[3–21] an hypothesis which led to the nomenclature adopted in 1969 at the Wiesbaden conference on the "origin, chemistry, physiology and pathophysiology of the gastrointestinal hormones."[4] Indeed, in a recent abstract,[33] McGuigan and Greider have allegedly localized gastrin in G cells in the human antrum by electron microscopic immunocytochemis-

try. However, if many of the endocrine cells in the body of stomachs from patients with pernicious anemia are producing gastrin, as Polak and co-workers have recently indicated,[2] then gastrin synthesis within the stomach may not be restricted to cells with G-cell morphology. To what extent the morphologic differences among gastric endocrine cells reflect differences in their metabolism and function has not yet been established.[10,21]

The metabolic and physiologic roles played by the numerous endocrine cells in the stomachs of patients with pernicious anemia have yet to be defined. The immunofluorescent demonstration of gastrin in many of these cells by Polak and co-workers [2] suggests that these cells are responsible, at least in part, for the elevated serum gastrin levels which are observed in patients with pernicious anemia,[34-37] and which are only partially suppressed by the introduction of acid into the stomach.[35-37] Possibly, these cells produce other physiologically active peptides and amines as well.

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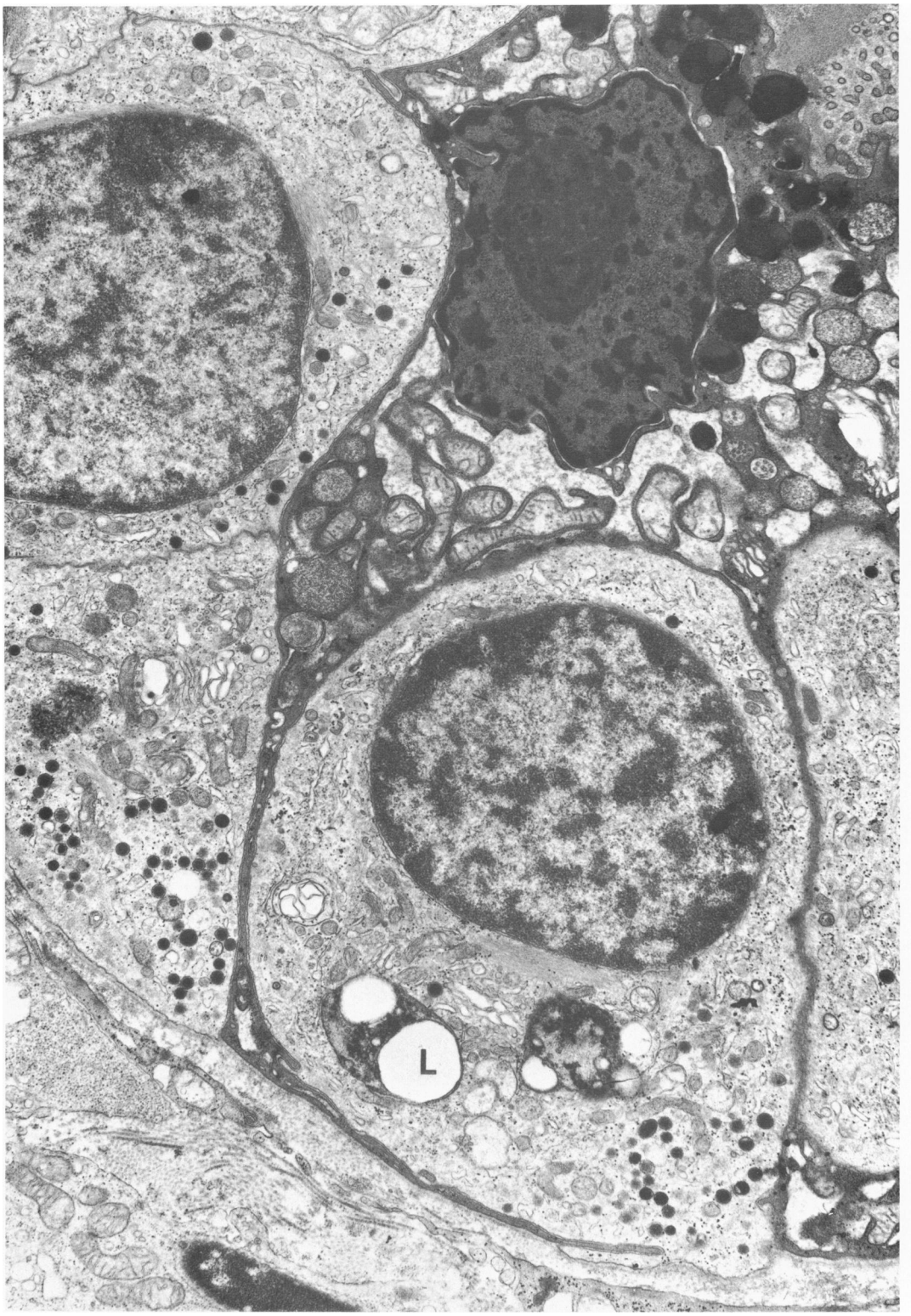
Fig 1—These endocrine cells, located in a gland in the body of an atrophic stomach, exhibit fine structural features of ECL cells.[10] Note their small, regular, round, dense granules; their numerous, small, relatively dense mitochondria, which tend to occur in clusters; the prominent filaments, especially near the nucleus; and a large proportion of agranular reticulum. The two large granules (*L*) in the lower cell probably represent lipofuscin or residual bodies. This lower endocrine cell appears embedded[32] within the dark mucous cell above, which is either degenerative or poorly fixed (Approximately × 12,000).



1

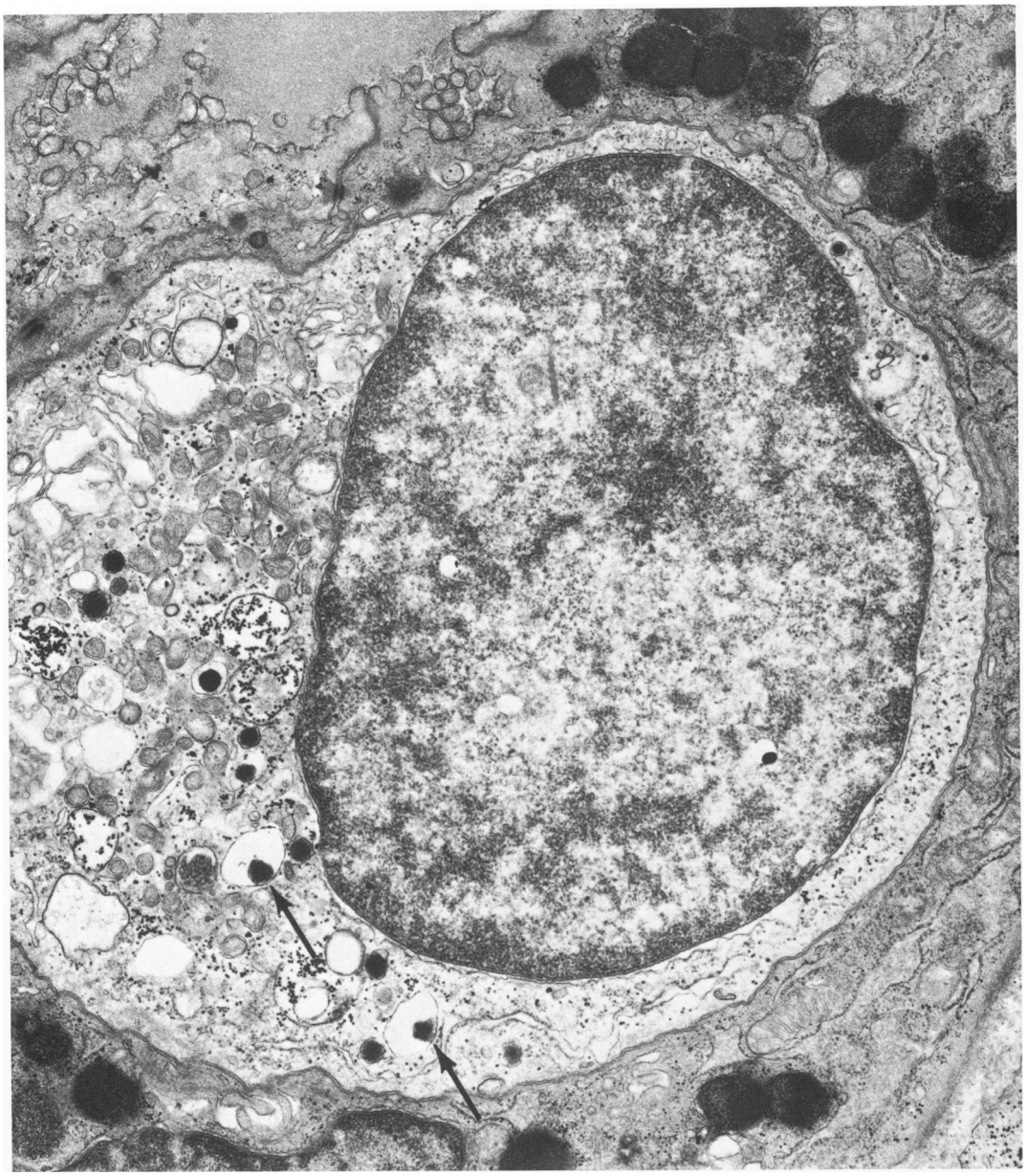


Fig 2—The granules in ECL cells in atrophic as well as in normal stomachs[10] are sometimes dilated and thus resemble vacuoles which appear empty or contain a small amount of eccentric matrix (*arrows*). This appearance is thought to represent a preparative artifact,[10] although some investigators have considered such an appearance to be characteristic of ECL granules.[5] Note again the cluster of many small mitochondria near the nucleus and the apparent enclosure of this endocrine cell by the adjoining mucous cell (Approximately × 16,000).

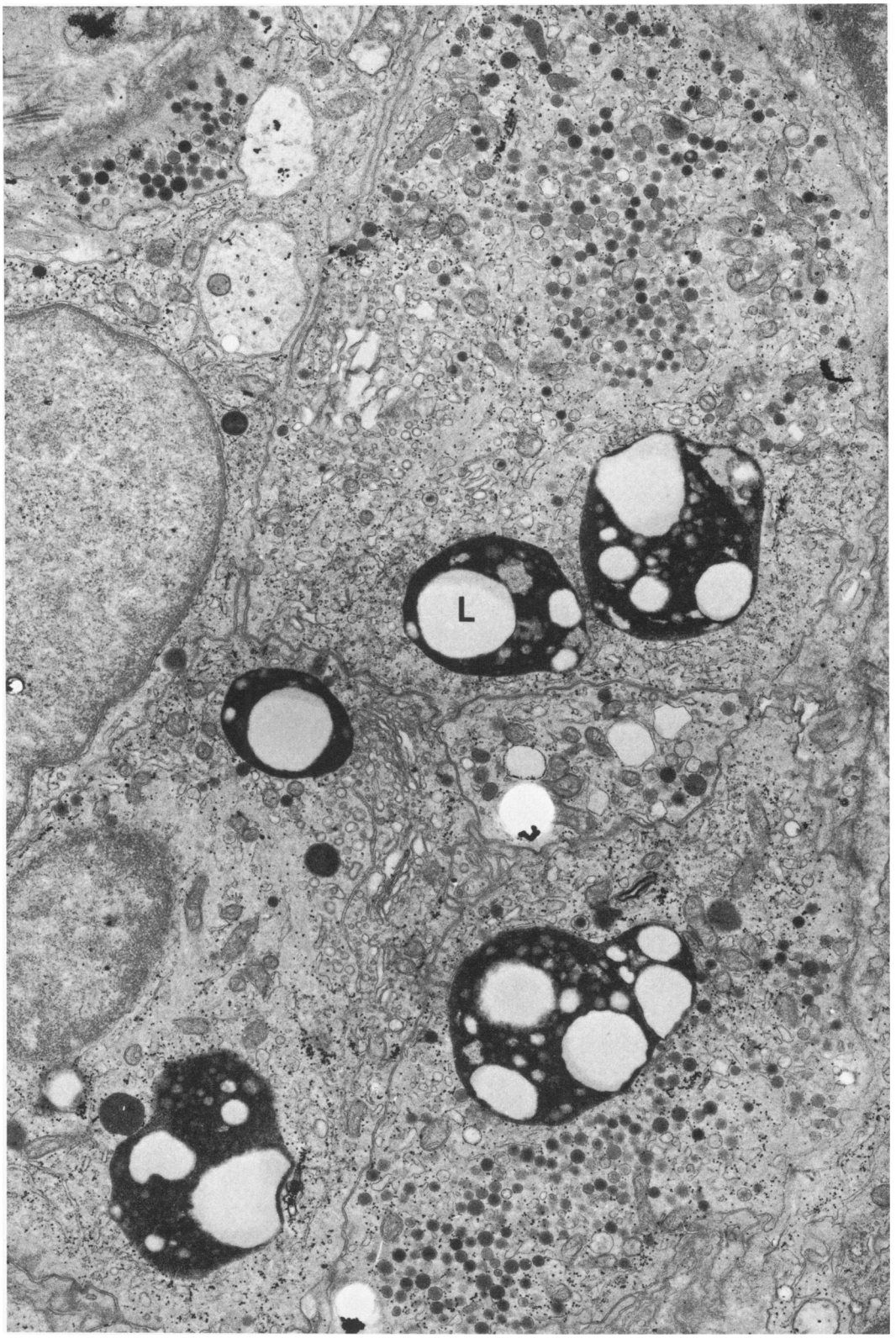


Fig 3—This tangential section through a gland in the body of an atrophic stomach reveals several ECL cells with their characteristic granules and mitochondria. The prominent large granules (*L*) are thought to represent lipofuscin or residual bodies (Approximately × 14,000).

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