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Recognition and the Histopathologic Classification of ECL Cell Proliferations

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INTRODUCTION

Among the nineteen functionally distinct endocrine cell types that populate the gastrointestinal mucosa, some (e.g., the serotonin-producing enterochromaffin (EC)[b] cells, and the somatostatin-producing D cells, etc.) are dispersed diffusely all along its length, while others (e.g., the histamine-producing enterochromaffin-like (ECL) cells, the gastrin-producing G cells, the secretin-producing S cells and the cholecystokinin-producing I cells) have a much more restricted distribution and are confined to specific segments or anatomic locations (Figure 1). Since the secretory products of these cells act as chemical messengers to orchestrate the absorptive, secretory and propulsive functions of the gut, both the distributional pattern and the numerical density of the various cell types is preordained by the physiologic needs pertinent to a given anatomic segment or site [1]. Thus the oxyntic (acid-producing) portion of the human gastric mucosa contains at least six ultrastructurally distinct endocrine cells types: the EC cells, the ECL cells, the D cells, the D_1 cells, the P and the X cells. The vast majority of these cells are dispersed randomly in the middle- and lower-third of the mucosal thickness, and only occasional endocrine cells are seen in the superficial third of the oxyntic mucosa. Rare stray endocrine cells have additionally also been described in association with nerve fibers and Schwann cells (neuroendocrine complexes) in the lamina propria [2, 3].

The entire endocrine cell population of the oxyntic mucosa can be visualized by immunohistochemical stains for such markers of neuroendocrine differentiation as chromogranin A, neuron specific enolase, synaptophysin and pancreatostatin, etc., and can be dissected into its component cell types by their specific histochemical or immunohistochemical profiles (Figure 2). Histochemically, while these cells are argyrophil in nature, not all of them can be simultaneously identified by any one argyrophil stain. Thus, the Grimelius stain identifies all oxyntic endocrine cells except the D cells that show argyrophilia only with the Hellerstrom-Hellman technique. The Sevier-Munger technique for argyrophilia, on the other hand, selectively stains the ECL cells and a small subset of the EC and D_1 cells (Figure 2). However, since the relative proportion of the EC and D_1 cells stained by this technique is negligible, the Sevier-Munger stain is currently regarded as more or less specific for ECL cells in formalin-fixed, paraffin-embedded tissues processed for light microscopy. However, since the Sevier-Munger stain is technically difficult and somewhat capricious, most workers rely on the Grimelius stain for visualizing ECL cells, realizing fully well that the number of positively stained cells is not an accurate depiction of their numbers since this technique also stains other argyrophil cells in the oxyntic

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[b]*Abbreviations:* EC, enterochromaffin; ECL, enterochromaffin-like; TGF-α, tumor growth factor alpha; CG-A, chromogranin-A.

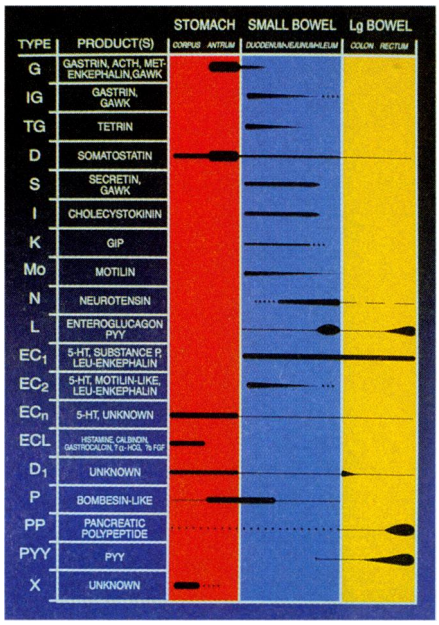


Figure 1. Schematic depiction of the anatomic distribution and the secretory products of the various endocrine cell types in the gastrointestinal mucosa. The extent of the solid lines indicates the presence of the various cell types in the different segments of the gut, while the width depicts their relative population densities. The interrupted lines denote the presence of relatively fewer cells of a given type in that location. (Adapted from Larsson, L.I. Scand. J. Gastroent. 14(suppl 53):128, 1979).

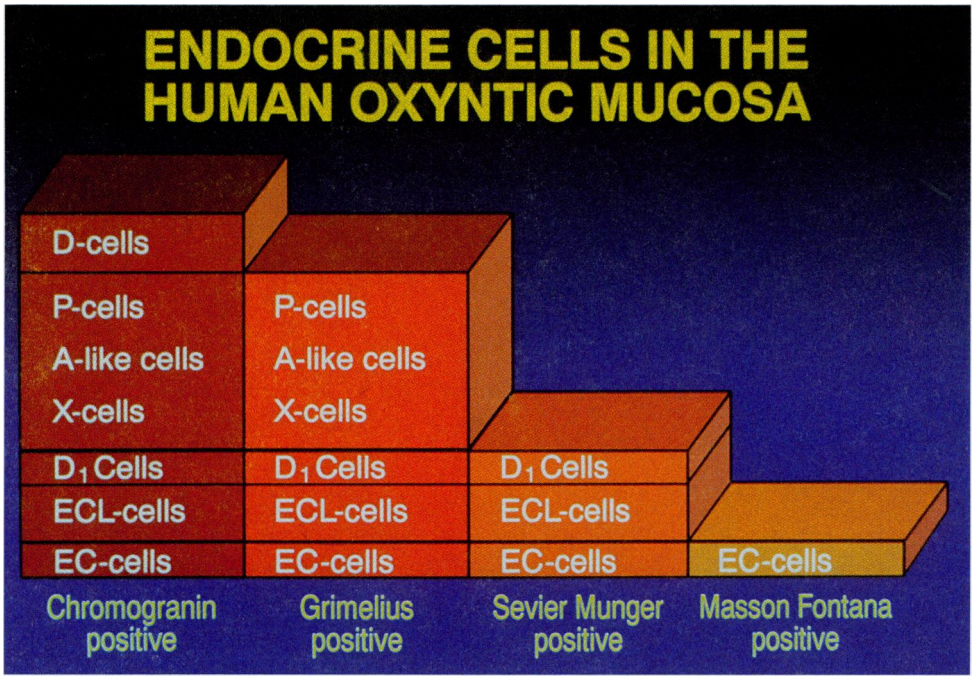


Figure 2. Diagramatic depiction of the endocrine cells of the human oxyntic mucosa, and the immunohistochemical and histochemical stains commonly employed for their identification and visualization.

Table 1. Endocrine cells of the human oxyntic mucosa.

Cell type	Secretory product	Relative (%)	Volume density*	CG-A stain	Grimelius stain	S-M stain	H-H stain	M-F stain
ECL	Histamine Calbindin	30-50	30 ± 9	+	+	+	-	-
D	Somatostatin	10-30	22 ± 4	+	-	-	+	-
EC	Serotonin	5-15	7 ± 5	+	+	±	-	+
D1	Unknown	5-10	9 ± 8	+	+	±	-	-
P	Bombesin?	5-10	24 ± 7	+	(+)	-	-	-
X	Endothelin-1	1-5	0.6 ± 0.7	+	+	-	-	-

CG-A, chromogranin-A; S-M, Sevier-Munger stain for argyrophilia; H-H, Hellerstrom-Hellman stain for argyrophilia; M-F, Masson-Fontana stain for argentaffinity.
*, volume density figures represent mean ± SD and indicate % of the whole endocrine cell mass [66].
+, positive; (+), weakly positive; -, negative; ±, few cells positive.

mucosa as well [4]. The relative numbers, secretory product(s), histochemical, immunohistochemical and ultrastructural characteristics of various endocrine cells in the human oxyntic mucosa are summarized in Table 1.

ENTEROCHROMAFFIN-LIKE (ECL) CELLS - THEIR CURRENT STATUS

In all vertebrate species studied so far, the ECL cells are normally confined to the oxyntic mucosa of the stomach and constitute its dominant endocrine cell type [5]. Scattered randomly in the lower and intermediate thirds of the mucosal thickness, these closed-type endocrine cells lie in close proximity to the parietal and chief cells. Although they lack apical processes that are characteristic of open-type endocrine cells, the ECL cells show prominent neuronal-like cytoplasmic processes that extend to and terminate on the surface of neighboring parietal and chief cells (Figure 3). ECL cells thus release their secretory products locally (to modulate parietal cell function by a paracrine mode of action) and into the capillary network around the glands (to exert their endocrine mode of function). In humans, the ECL cells are essentially identified by their topographic distribution, their ability to stain with the Grimelius and the Sevier-Munger stains for argyrophilia and the ultrastructural features of their neurosecretory granules. The ECL cell granules are morphologically heterogeneous. Most numerous, and regarded as characteristic of ECL cells, are the large (up to 300 nm-sized) membrane-bound vesicles that have an eccentrically located irregular, coarsely textured electron dense argyrophil core (Figure 4). In addition there may also be a variably sized minority population of small round granules with a homogeneous coarsely granular core surrounded by a thin halo bounded by a delicate, wavy limiting membrane. While some ECL cells contain both types of granules, others may show only the small granules in their cytoplasm [6].

The ECL cells have receptors for a number of regulatory substances and other chemical mediators (e.g., gastrin, somatostatin and histamine, etc.) on their surface, and although a variety of products including calbindin, a calcium-binding protein, the alpha subunit of HCG, transforming growth factor-alpha (TGF-α), etc., have been localized in their cytoplasm, histamine has emerged as the most physiologically relevant secretory product of the ECL cells [5, 7-10]. Whereas it was originally thought that gastrin directly stimulated the parietal cells to secrete acid, this action is now believed to be mainly histamine-mediated; the actual sequence of events is that gastrin stimulates the ECL cells to secrete histamine, which then binds to the H_2-receptors on the parietal cells and activates the H^+/K^+ ATPase proton pump to generate acid [11]. One of the most significant biologic characteristics of

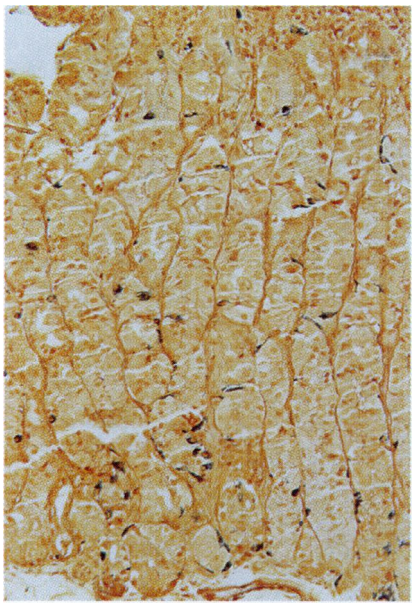


Figure 3. Human oxyntic mucosa showing the normal distributional pattern of the ECL cells. Note that these cells are mostly present in the lower and middle-third of the mucosal thickness and are randomly dispersed in the glandular lining. Lateral cytoplasmic processes of some ECL cells can be seen extending towards the adjacent parietal cells (Sevier Munger stain).

the ECL cells is their exquisite sensitivity to the secretagogue and trophic influence of gastrin. Acute hypergastrinemia causes histamine release from the ECL cells and enhances the synthesis of histidine decarboxylase, which catalyses the formation of histamine from histidine [12, 13]; while chronic hypergastrinemic states, on the other hand, induce an ECL cell hyperplasia that can in some cases progress to neoplasia (ECL cell carcinoids) [13-30]. Although the precise mechanism by which gastrin stimulates ECL cell proliferation

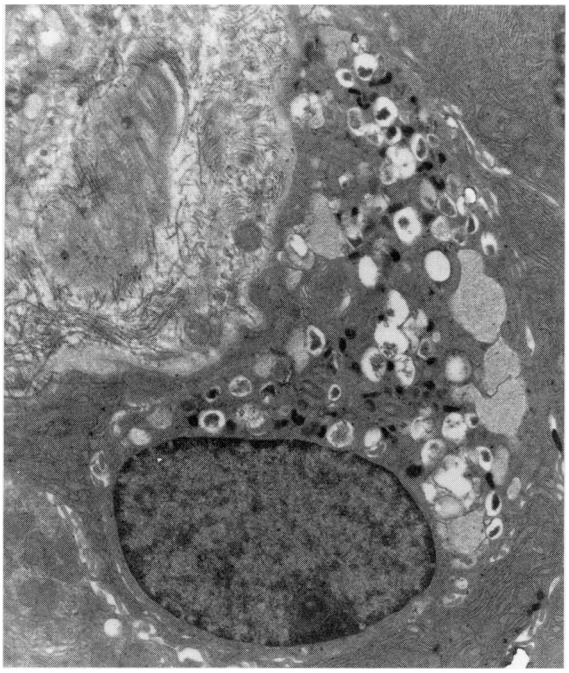


Figure 4. Photomicrograph of the ultrastructural characteristics of a human ECL cell. Note the characteristic large sized vacuolated type of neurosecretory granules with an eccentric electron dense core and wide peripheral halo. (Courtesy of Dr. Herbert Helander).

remains unclear, it has been suggested that this too is perhaps mediated through histamine release from the ECL cells since in addition to being a potent neurotransmitter, histamine can also act as a mitogen [31].

Despite the fact that they are the dominant endocrine cell type in the oxyntic gastric mucosa in all vertebrate species studied so far, the ECL cells remained largely ignored until recently, and whereas knowledge related to the pathobiology of other gastrointestinal endocrine cells advanced rapidly, that related to the ECL cells lagged woefully behind. All this changed rapidly within the last decade or so when ECL cell hyperplasias and ECL cell carcinoids were seen to develop in the glandular stomach of rats after prolonged administration of high doses of antisecretory agents (e.g., cimetidine, ranitidine and omeprazole, etc. [16, 17, 19, 32-36]). A flurry of investigative activity focusing on these cells subsequently showed that both the hyperplastic and neoplastic ECL cell lesions were pathogenetically related to the chronic hypergastrinemia induced by the prolonged suppression of acid secretion in these animals [13, 16, 17, 19, 20, 22, 36].

Attention swiftly shifted towards human ECL cells to see if patients on long-term antisecretory therapy might also be at risk for developing similar lesions. This represented a very genuine and relevant concern, since these agents were being increasingly prescribed not only for the long-term management of duodenal ulcer disease, gastroesophageal reflux disease and the Zollinger-Ellison syndrome but have since then also been recommended as adjuvants for eradicating *Helicobacter pylori* infection as well [37-41].

ECL CELL HYPERPLASIA

Hyperplasia signifies a non-autonomous proliferation of a given cell type that results in an increase in its total cell mass. It is a morphologic phenomenon that implies the presence of increased numbers of cells per unit area and requires morphologic criteria for its recognition and confirmation. Since the population density of a given endocrine cell type (e.g., the G, D, EC or ECL cell) in the gut not only shows considerable normal variation between the different anatomic locations but is also influenced by age and gender [42, 43], a diagnosis of G, D, EC, or ECL cell hyperplasia relies primarily on the identification of increased numbers (beyond twice the standard deviation in age-, gender- and site-matched controls) of cells per unit area of mucosa [4, 44]. This, in the past, entailed a labor-intensive, time-consuming morphometric quantitation of the cells to document an increase in absolute cell numbers before a diagnosis of hyperplasia could be established.

PROBLEMS RELATED TO THE STUDY OF ECL CELL HYPERPLASIA

One of the major problems in the study of ECL cell hyperplasias relates to the technical limitations in our ability to specifically identify ECL cells at the light microscopic level in routinely processed tissues. The histochemical techniques currently used for their visualization lack specificity and provide only a rough approximation of their numbers. While other endocrine cell types are easily identified immunohistochemically by antibodies directed towards their secretory products, this methodology cannot be applied to ECL cells since histamine is either leached out, destroyed or otherwise rendered nonimmunoreactive in formalin-fixed, paraffin embedded tissues. Thus, the special procedural modifications (freeze-drying, vapor-fixation and cryostat sectioning, etc.), so essential for their specific immunohistochemical localization, have not only impaired our ability to visualize ECL cells in routinely processed surgical pathology material, but have also precluded any retrospective studies on archival material as well [5, 7]. Until quite recently, therefore, a

specific identification of ECL cells had traditionally depended on a combination of their argyrophil and ultrastructural characteristics [45]. Secondly, ECL cell hyperplasias have traditionally been difficult to recognize clinically because these cells release most of their histamine locally into the tissues and do not release enough of it into the blood to give rise to a specific biochemical abnormality or clinical syndrome. In fact, the very entity of ECL cell hyperplasia had remained suspect until recently when its association with chronic hypergastrinemia was first recognized. Thirdly, even though we now know that such hyperplasias are commonly associated with certain clinical conditions, they are hard to detect since they do not produce any distinctive recognizable lesions that can be biopsied endoscopically or sampled selectively in resected specimens. Histologically too, they can be easily missed on routine hematoxylin and eosin stained specimens unless they are specifically sought for by special argyrophil stains such as the Grimelius or Sevier-Munger. Fourthly, in certain conditions (e.g., chronic atrophic gastritis and pernicious anemia), commonly associated with such hyperplasias, the involved gastric mucosa is significantly atrophied, and it is difficult to establish if the increased population density of ECL cells represents a genuine hyperplasia or a mere overcrowding of involuting cells brought about by mucosal atrophy and decreased mucosal volume, or a combination of the two. Similarly, in conditions such as the Zollinger-Ellison syndrome, the presence of increased numbers of ECL cells dispersed throughout the mucosal thickness may be masked by the generalized oxyntic mucosal hypertrophy. Lastly, confusion related to documentation of endocrine cell hyperplasia had also resulted from differences in the morphometric methodologies and the frames of references employed by various workers. Thus, while some workers counted individual cells, others used computerized point-counting techniques, while yet others quantitated the hyperplastic population by measuring absorbance of light and the fractional surface area of the tissue section in which such absorbance occurred [46, 47]. Results, too, were expressed in such variable terms of reference as absolute cell counts per square mm of mucosa, cells per unit length of mucosa, high power field, gland or crypt and even in terms of volume density (proportion of mucosal volume occupied by endocrine cells) [23, 48, 49].

MECHANISMS OF ECL CELL HYPERPLASIA

Despite the sudden spurt in our interest in the pathobiology of these cells, we are still unclear about the basic mechanisms responsible for ECL cell hyperplasia. Cumulative evidence indicates that hyperplasias of ECL cells can theoretically result from a number of different mechanisms such as prolongation of their half-life, differentiation of a larger

Table 2. Conditions associated with increased risk for ECL cell hyperplasia and multicentric gastric carcinoids.

- Chronic atrophic gastritis (Type A)
- Pernicious anemia
- Zollinger-Ellison syndrome (familial and sporadic)
- Patients on pharmacologic gastric acid blockade (H_2 receptors; proton pump inhibitors)
- Chronic renal failure (± hemodialysis)
- Autoimmune disorders:
  - Polyglandular autoimmune syndrome
  - Diabetes mellitus - Type 1 (insulin-dependent diabetes mellitus)
  - Rheumatoid arthritis
  - Sjogren's syndrome
  - Addison's disease
  - Myxedema

Table 3. Classification of ECL cell proliferations.

- Hyperplasia:
  - Simple (diffuse)
  - Linear
  - Micronodular
  - Adenomatoid
- Dysplasia (pre-neoplastic stage):
  - Enlarging micronodule
  - Fusing micronodule
  - Microinvasive lesion
  - Nodule with newly formed stroma
- Carcinoid (neoplastic stage):
  - Intramucosal carcinoid
  - Invasive carcinoid

fraction of pluripotent stem cells into ECL cells, enhanced self-replication of functionally mature ECL cells or even a modulation of their apoptotic destruction. Each of these mechanisms could be triggered by a release from the normal restraining influence of an inhibitor of ECL cell proliferation, an interruption of the normal physiologic negative feedback mechanism governing their status, or the trophic stimulation by a secretagogue and/or regulatory substance (e.g., a peptide such as gastrin, or an amine such as serotonin or histamine)[16, 17, 50-53]. More recently, with the recognition that ECL cells not only secrete histamine but also have H_3-receptors on their surface, an autocrine mechanism whereby histamine liberated from the ECL cells can bind to these receptors and modulate ECL cell proliferation has also been implicated [31]. Since these mechanisms are not mutually exclusive, ECL cell hyperplasias can result from a combination of these mechanisms. Furthermore, the local tissue and intraluminal microenvironment (e.g., gastric motility, distension, *H. pylori* infection, mucosal atrophy, epithelial metaplasia with a consequent rise in intraluminal pH and over expression of their gastrin receptors, etc.) could, by influencing any one or more of these triggering mechanisms, also play an important role not only in the initiation and maintenance of ECL cell hyperplasias but also in their progression to ECL cell carcinoids [49, 54].

It must, however, be mentioned that although ECL cell hyperplasia can either be a primary event or be secondarily induced, only secondary hyperplasias of the ECL cells have been described so far. Such hyperplasias are most often diagnosed because of a high index of clinical suspicion when such predisposing conditions as chronic atrophic gastritis, pernicious anemia, Zollinger-Ellison syndrome or chronic hypergastrinemia from other causes are encountered (Table 2). While each of these conditions is known to have a strong association with ECL cell hyperplasia, these hyperplasias are now also being increasingly observed in patients receiving long-term gastric acid suppressive therapy with H_2-blockers or proton pump inhibitors [23, 55-60]. The ECL cell hyperplasia seen in these patients is also due to the hypergastrinemia that is secondarily induced by these therapeutic agents [55, 59, 60]. In the oxyntic mucosa of chronically hypergastrinemic patients (with gastrin levels greater than three times normal), argyrophil stains show a morphologic spectrum of hyperplastic lesions ranging from a diffuse increase in ECL cell numbers at one end, through linear and nodular aggregates to frank ECL cell carcinoids [24, 25, 61, 62]. Such a progression from diffuse hyperplasia through linear and micronodular hyperplasia to carcinoid tumors was formalized into a hyperplasia–neoplasia sequence by an international committee headed by Enrico Solcia. The resultant classification, published in 1988, has, therefore, come to be known as the "Solcia Classification" [4] and is reproduced in Table 3.

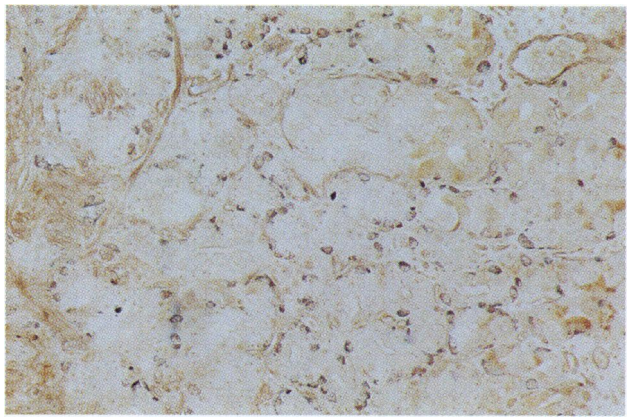


Figure 5. Simple (Diffuse) hyperplasia of ECL cells in the oxyntic mucosa of a patient with chronic atrophic gastritis type A. Note that despite the rather diffuse distributional pattern of these cells throughout the mucosal thickness, some preferential clustering is present in the lower third. The argyrophil ECL cells are hypertrophied and dispersed singly or in small clusters of three or four cells in the closely packed glands (Sevier-Munger stain).

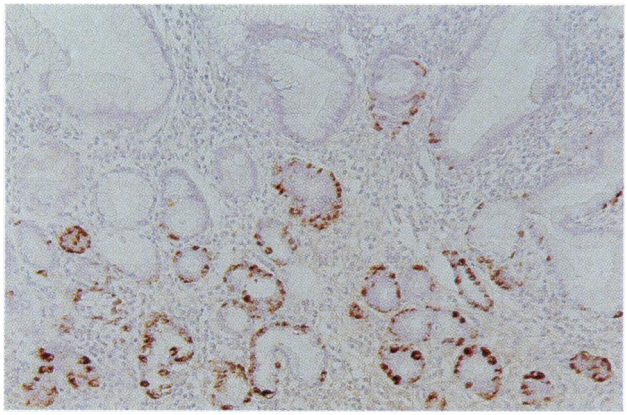


Figure 6. Linear hyperplasia of ECL cells in the oxyntic mucosa of a patient with severe chronic atrophic gastritis type A. Note the mucosal atrophy, architectural distortion and the markedly increased numbers of ECL cells arranged in a linear fashion along the basement membrane of the gastric glands. A couple of small compact micronodular aggregates of positively stained endocrine cells are also present. (Chromogranin stain with hematoxylin counterstain).

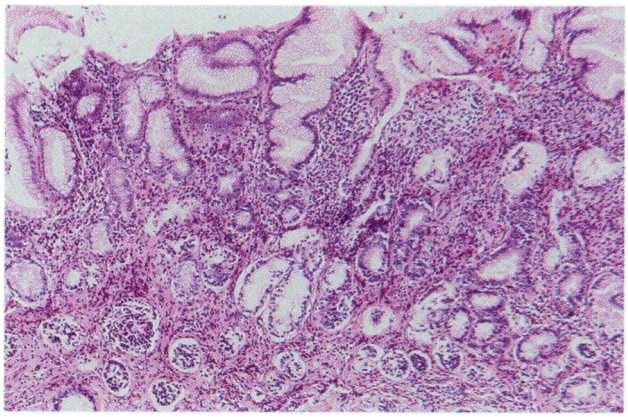


Figure 7. Oxyntic mucosa of a patient with pernicious anemia showing marked chronic atrophic gastritis and endocrine cell micronodular hyperplasia. Note the mucosal atrophy, architectural distortion and the micronodular clusters of endocrine (ECL) cells deep in the mucosa abutting the muscularis mucosae. These clusters ranging up to 100 pm in size approximate the diameter of the normal oxyntic glands. (Hematoxylin and eosin stain).

In this scheme, the earliest stage, called ***simple or diffuse hyperplasia***, is characterized by a diffuse increase in ECL cell numbers as visualized by the Grimelius or Sevier-Munger stain. The cells are scattered diffusely throughout the mucosal thickness either singly or in clusters of up to three or four cells per gland. The positively-stained argyrophil cells are somewhat hypertrophied, and although they are diffusely distributed throughout the mucosal thickness, are somewhat more numerous and prominent in the lower third. When quantitated morphometrically, the number of positively-stained cells per unit area exceeds 2 standard deviations over the normal range in age- and gender-matched controls, and stand out in sharp contrast to the normal (Figure 5). When the hyperplasia is more severe, the endocrine cells are seen arranged in a linear, semilunar or a daisy-chain-like configuration along the basement membrane of the gland and this stage is, therefore, designated as ***linear hyperplasia*** (Figure 6). These linear chains are most often seen in the lower one-third of the mucosal thickness and involve the basal portion of the glands that abut the muscularis mucosae. They may, however, occasionally also be seen superficially in the region of the glandular necks. These locations tend to reflect the different modes of their histogenesis. Thus, while the hyperplastic ECL cells deep within the mucosa are presumably derived either from replication or longevity of functionally mature ECL cells, modulation of their apoptotic demise or autocrine mechanisms, those in the superficial mucus-neck cell region (the proliferative compartment of the gastric mucosa) [14] are believed to result from a preferential differentiation of the stem cells along an ECL cell phenotype. Because these linear lesions are most often deep within the mucosa and may be focal or diffuse, they frequently require multiple, full-thickness mucosal biopsies for their detection. A diagnosis of linear hyperplasia requires a minimum of five chains of five contiguous ECL cells each in one specimen or a minimum of two such chains per linear millimeter of mucosa in multiple biopsies. This arrangement of argyrophil cells signifies a definite hyperplasia since ECL cells do not normally show this morphologic arrangement. However, the numerical stipulation given above is important since it distinguishes genuine linear hyperplasias from the randomly scattered, discontinuous linear arrangements of three to four argyrophil cells occasionally seen in mild cases of chronic atrophic gastritis.

The next recognizable stage in the sequence is characterized by the presence of solid micronodular ECL cell clusters and is designated as ***micronodular hyperplasia***. These clusters, made up of five argyrophil cells or more, measure 100 to 150 μm in size and do not exceed the diameter of a gastric gland. Micronodular ECL cell hyperplasia is the earliest stage that can be visualized with the hematoxylin and eosin stain (Figure 7) and has been variously referred to as argyrophil cell clusters, argyrophil cell micronodules, argyrophil cell nests, endocrine cell micronests and microcarcinoids by other workers [63-65]. For a diagnosis of micronodular ECL cell hyperplasia a minimum of one such nodule per linear millimeter of mucosa should ideally be present in a background of simple and/or linear hyperplasia. Although such micronodular clusters, bounded by an intact basement membrane contiguous with that of the rest of the gland, are characteristically located in the basiglandular portion of the mucosa, similar but smaller nodules may be seen dissociated from the glands and lying free in the lamina propria abutting the muscularis mucosae (Figure 8). While such a dissociation could in some cases be due to tangential sectioning, tortuousity of the gland or the micronodule itself, or even a budding of the micronodule from the gland, such nodules actually represent aggregates of residual endocrine cells left behind after the glands have atrophied and disappeared. Such dissociated aggregates are, therefore, called micronodular pseudohyperplasia. In contrast to the 100–150 μm sized nodules of genuine ECL cell hyperplasia that occur in a background of simple- and linear hyperplasia, the pseudohyperplastic nodules are much smaller in diameter (< 50 μm), and are neither associated with any increase in absolute ECL cell numbers, nor with any simple or linear hyperplasia. Furthermore, the pseudohyperplastic nodules have a heterogeneous

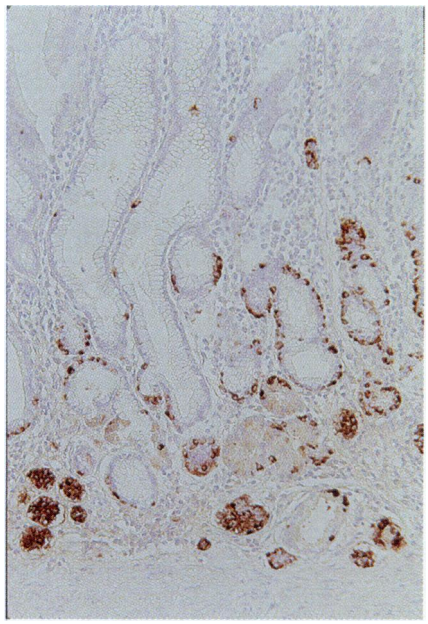


Figure 8. Oxyntic mucosa of the same patient showing several micronodular clusters of endocrine (ECL) cells dispersed in the mucosa in a background of linear hyperplasia. Most of the clusters have between 20-25 positively stained cells in them. The smallest clusters represent pseudohyperplastic micronodules of endocrine cells, from atrophied glands (chromogranin stain).

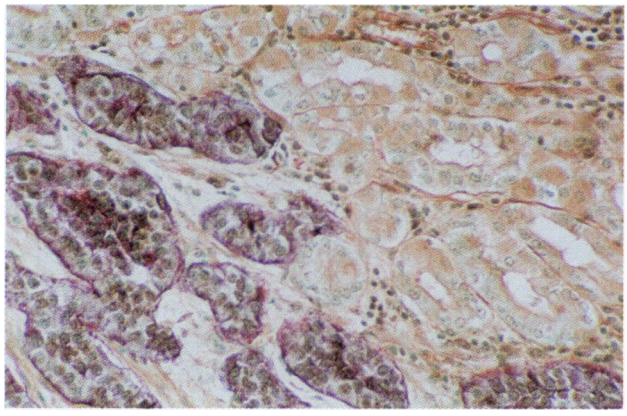


Figure 9. Adenomatoid hyperplasia of ECL cells in the oxyntic mucosa of a patient with chronic atrophic gastritis type A. Note the compact "adenoma-like" clustering of several micronodules, each with its basement membrane still intact (Grimelius stain with methyl green counterstain).

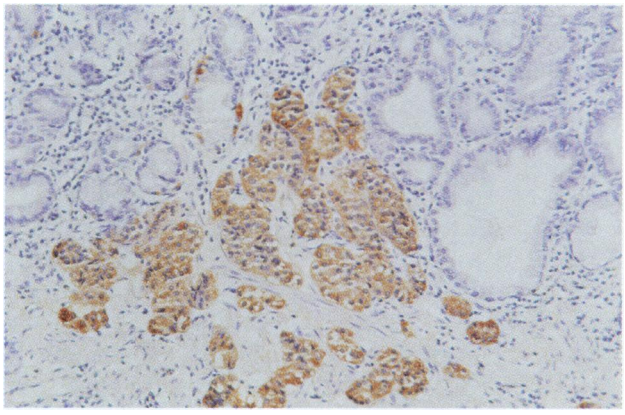


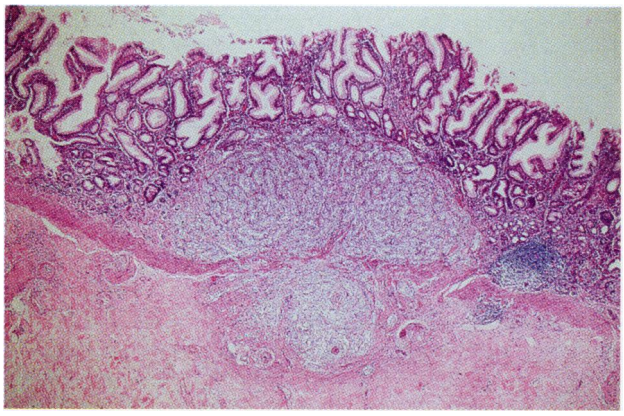
Figure 10. Dysplastic lesion showing a large cluster of endocrine (ECL) cells deep within the oxyntic mucosa of a patient with chronic atrophic gastritis type A. Note that the clusters lack an identifiable basement membrane, and that the dysplastic cells infiltrate both the lamina propria between the glands and the muscularis mucosae. (Chromogranin stain).

cellular composition and, often contain some non-argyrophil and chromogranin-immunonegative residual epithelial cells trapped within them. Ultrastructurally too, the endocrine cells in such clusters contain increased numbers of intracytoplasmic lipid droplets, swollen mitochondria, excessively dilated endoplasmic reticulum, a dense cytoplasmic matrix, decreased numbers of small, dense, non-diagnostic neurosecretory granules and pyknotic nuclei, all of which are features of cytologic involution rather than stimulatory activation. Hyperplastic ECL cells, on the other hand, show such features of active protein synthesis as abundant rough endoplasmic reticulum and a prominent Golgi apparatus. Interestingly, however, these hyperplastic cells show a marked reduction in the number of vacuolated/vesicular granules (which are the hallmark of resting ECL cells) and a marked increase in the coarsely textured granules. Additionally, a punctate type of neurosecretory granule has also been occasionally observed in hyperplastic ECL cells [66]. It should, however, be pointed out that not only do these two types of micronodular lesions frequently co-exist in the gastric mucosa of hypergastrinemic patients with CAG-A and pernicious anemia, but that the pseudohyperplastic clusters often occur alone (and without coexistent simple and linear hyperplasia) in such diverse conditions as *H. pylori* infection, gastric ulcer and gastric cancer, etc., that show significant gastritis. Thus, because of their heterogeneous composition and their involutional character, such extraglandular clusters are clearly different from the monotypic hyperplastic ECL cell micronodules and need to be differentiated from them because they are not influenced by hypergastrinemia and do not have any significant proliferative potential. Such a differentiation is chiefly based on a combination of their small size (50 μm vs. 150 μm), cytological heterogeneity and their presence exclusively in areas of gland atrophy. Because ECL cell hyperplasias occur diffusely in the oxyntic mucosa, the Solcia classification insists on finding a minimum of one 10 to 150 μm-sized micronodule per linear millimeter of mucosa with or without a background of simple and/or linear hyperplasia.

Continued growth of the intraglandular pure ECL cell micronodules, each with its intact basement membrane, on the other hand, leads to the formation of small aggregates of several micronodules closely packed together in a back-to-back arrangement. Because of this adenoma-like arrangement of micronodules, this stage is referred to as ***adenomatoid hyperplasia*** (Figure 9). This lesion has been regarded as a focal exaggeration of micronodular hyperplasia and is, therefore, closely similar to it both in morphology and biological behavior. It is, however, important to distinguish it from the ***dysplastic*** (precarcinoid) ***lesions*** that are characterized by an enlargement of adenomatoid micronodules, breakdown of the basement membrane surrounding each individual micronodule in the cluster, presence of cytologic atypia, increased nuclear-cytoplasmic ratio and a reduction in the intensity of argyrophilia. Dysplastic lesions, thus, appear as nodular or irregular 150 μm to 0.5 mm sized clusters of argyrophil cells infiltrating the lamina propria (Figure 10). These lesions show a number of morphologic variations that are designated as ***enlarging micronodules*** (nodular aggregates more than 150 μm in diameter), ***fusing micronodules*** (from fusion of several adjacent micronodules), ***microinvasive lesions*** (clusters of atypical cells infiltrating the lamina propria between the glands) and ***nodules with newly formed stroma*** (when the nodules acquire a lobular or trabecular pattern).

Since dysplastic lesions too are multifocal in distribution, any one or more of these variant subtypes may be encountered in a biopsy specimen(s) at any time.

Following the publication of the Solcia classification, several studies have conclusively shown that whereas the simple, linear, micronodular and adenomatoid types of ECL cell hyperplasias have a low or negligible potential for carcinoidogenesis, the dysplastic lesions have a strong association either with the presence of co-existent ECL cell carcinoid(s) or of an increased risk of developing them in the future [67]. Conceptually, therefore, the dysplastic stage marks the borderline between the clearly hyperplastic stages preceding it



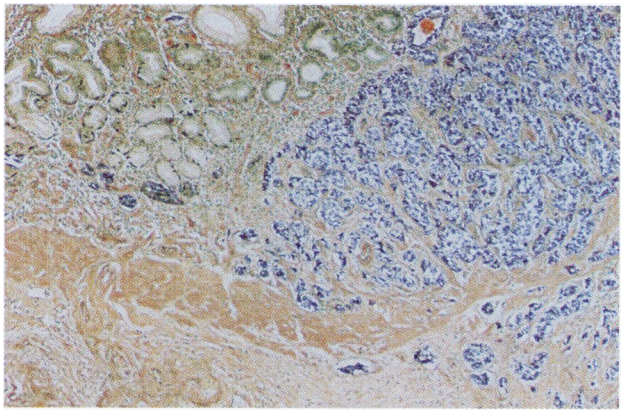


Figure 11a (top). Oxyntic mucosa of a patient with chronic atrophic gastritis type A and multiple gastric carcinoids. The tumor shows invasion into the underlying submucosa. **Figure 11b (bottom). Grimelius stain of the tumor showing argyrophilia of the tumor cells and prominent foci of linear and micronodular ECL cell hyperplasia in the adjacent non-tumorous mucosa.**

and the neoplastic (carcinoid) stage following it in sequence and represents the first "point-of-no-return" in the hyperplasia–neoplasia sequence of ECL cell proliferations. Because dysplastic lesions are less than 0.5 mm in size, they are not visible endoscopically, and are invariably first detected during microscopy. In clinical terms, since the detection of a dysplastic ECL cell lesion in a gastric biopsy indicates the presence of a coexistent carcinoid(s) or a significant risk of developing them, it should prompt a careful re-endoscopy with extensive sampling of the oxyntic mucosa for any ECL cell carcinoid that may have been missed. The ***carcinoid stage*** in this scheme, is characterized by nodular infiltrating growths greater than 0.5 mm in diameter (Figure 11). These lesions are completely intramucosal at first, but gradually extend intramurally, and may even invade vascular or lymphatic channels to produce nodal and distant metastases.

Because the Solcia classification is based on a combination of quantitative and morphological criteria with emphasis on the morphologic pattern of ECL cell hyperplasia, it represents a significant improvement over the labor-intensive, time-consuming morphometric methods that were used before. Based on a visualization of ECL cells by a commonly employed argyrophil stain (Grimelius), this classification clearly identifies the various stages in the hyperplasia-neoplasia sequence of ECL cell proliferation and clearly defines the morphologic criteria for each. The classification is easy to use and has a high degree of inter-observer reproducibility. The ascending grades of hyperplasia in this scheme correlate well with morphologic results [44]. The Solcia classification has, therefore, achieved a high

degree of acceptance amongst practicing pathologists. By calling attention to the biologic characteristics of the various stages of ECL cell hyperplasia and the pseudohyperplastic nodules, the Solcia classification has not only rationalized the current recommendations for antrectomy as treatment in select cases of ECL cell carcinoids, but has also helped explain why antrectomy sometimes results in regression of only some but not all micronodular lesions [68-71]. Furthermore, by establishing both the histologic criteria and the precursor lesions associated with ECL cell carcinoids, the Solcia classification has helped separate the relative benign, slow-growing, gastrin-dependent ECL cell carcinoids that require a conservative therapeutic approach from the more aggressive spontaneously occurring gastric carcinoids where a more radical surgical approach needs to be adopted.

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