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Preface

With this volume, Pathology Annual marks the start of a third decade of publication. The concept for these books originated with Dr. Sheldon Charles Sommers at a time when the number of regular journals devoted to subjects specifically of interest to diagnostic pathologists was very limited. Problem-solving required a personal review of the literature or recourse to a relatively small number of specialized text books. In this setting the pathologist's library was composed of volumes detailing the pathology of specific organ systems and much information of interest was scattered among clinical journals to which pathologists would not ordinarily subscribe. The format of Pathology Annual was devised to assist the pathologist in daily practice. The goal has been to provide useful, current information about diverse subjects in a literate, easily readable, and extensively illustrated form. Success of the project has in no small measure been due to Dr. Sommers' extraordinarily broad knowledge and experience, for he is one of the few Renaissance figures in pathology today.

By 1975 the number of articles contributed to Pathology Annual was sufficient to warrant expansion and beginning with Volume 11 in 1976, two parts have been published annually. It has been my pleasure to serve as Co-editor during this second decade of the Annual. The editorial policies set forth by Dr. Sommers in his Preface to the Pathology Annual Cumulative Index (1966–1979) have been continued:

> The philosophy of Pathology Annuals is that the contributors are mature and experienced professionals. They know what information they wish to convey. Editing is confined to assuring that the sentences are clearly understandable and grammatical.

Dr. Sommers also described in the Preface the trials of editorship which I have since come to appreciate. We have applied his principle of the "drunken guest" to "slightly bedraggled manuscripts" or those exhibiting "lamentable, subtotal confusion of subject and verb..." This precept requires that such papers, reminiscent of an inebriated guest, not be turned away. Instead the visitor is firmly encouraged to compose himself before joining the

assemblage. We have been fortunate to have the enthusiastic assistance of contributors whose willingness, in most cases, to assist in manuscript production has made our job easier. Recently, one author reported having reviewed a series of galley proofs "scrutinously", an effort beyond the call of duty.

During the second decade of Pathology Annual, several publication experiments were undertaken. The most successful of these has been the introduction of Symposia as a regular feature of the books. Symposia provide an opportunity for several experts to engage in a written discussion of a controversial topic. The participants in these exercises develop a common set of questions among themselves. Each contributor replies separately to the questions. The complete set of responses is then circulated to each author for a concluding statement, thus permitting an opportunity to comment on replies made by the other authors. Subjects covered in Symposia include *in situ* carcinoma of the breast, dysplastic colonic lesions, malignant melanoma, endometrial carcinoma, and the decline of the autopsy.

A Pathology Annual Monograph dealing with the "Malignant Lymphomas" appeared in 1983. This volume developed from an exceptional number of manuscripts dealing with lymphomas received in one year. To maintain diversity in the subject matter of Pathology Annual volumes, this group of lymphoma papers was published as a separate book. Pathology Annual Monographs will appear on occasion when there are sufficient articles whose common subject warrants this form of presentation.

For a number of years Dr. Sommers has warned that he wished to channel his seemingly inexhaustible energy into projects other than Pathology Annual. It was possible to forestall this decision for a time but as we approached the completion of 20 volumes Dr. Sommers chose, in his characteristically orderly fashion, to leave the Annual in 1986 at the conclusion of two decades. Those who have had the privilege and stimulating experience of working closely with Dr. Sommers know that he will not be idle.

I am very pleased that Dr. Robert E. Fechner has joined me as Co-editor of Pathology Annual. Dr. Fechner is well known to readers of the Annual for his scholarly contributions to past books. His expertise in many areas of diagnostic pathology will continue the tradition established by Dr. Sommers for providing useful and meticulously edited volumes as we embark upon a third decade.

Paul Peter Rosen, M.D.
Tenafly, New Jersey
June 1, 1985

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The Endocrine Cells of the Gastrointestinal Tract

The Normal Endocrine Cells and Their Hyperplasias

PART I

Klaus J. Lewin

In the last few years there has been a veritable explosion of information on the endocrine cells of the gastrointestinal tract. This has been brought about principally by the isolation and chemical characterization of many intestinal polypeptide hormones. As a consequence of this development, specific antibodies to these hormones have been produced allowing for accurate measurements of serum hormone levels by radioimmunoassay, and the localization of these hormones to specific endocrine cells in the gastrointestinal tract (GIT), by immunocytochemistry. The immunohistochemical advances coupled with ultrastructural studies have led to the recognition of endocrine cell hyperplasia and a much better understanding of the carcinoid tumors, particularly in regard to their histologic spectrum, hormonal content, and clinical presentation. They have also lent further support to the elegant allograft studies by LeDouarin[36,37] and others on the embryogenesis of the GI endocrine cells, the histogenesis of carcinoid tumors, and the relationship of these tumors to the other epithelial tumors of the gastrointestinal tract.

The aim of this chapter is to update the fascinating and evolving story of the GI endocrine cells and their proliferative lesions. It deals with the exciting new data on the GI endocrine cells and their hyperplasias. The endocrine cell tumors will be discussed in Part II.

Before we describe recent data on the endocrine cells, a brief historic review is included to provide a better perspective of recent advances.

This is the first of two sections. Part II appears in Pathology Annual 1986, Part 2.

ENDOCRINE CELLS OF THE GASTROINTESTINAL TRACT

Historic Review

The endocrine cells of the intestinal tract consist of a group of widely dispersed single cells found in all vertebrates. They were originally discovered in the intestinal tract of the lizard by Nicholas[46] in 1891 and subsequently rediscovered by Kultschitzky[31] in 1896. Kultschitzky described a basigranulated cell situated in the crypts of Lieberkuhn, which contained small infranuclear eosinophilic granules. Subsequently, other investigators discovered another cell, the "clear cell," which was characterized by a diffusely vacuolated cytoplasm. A variety of names have been given to these two cell types, and reflect their histologic or histochemical properties; for example, "clear cells" to describe their vacuolated cytoplasm,[15] "yellow or enterochromaffin cells,"[10,65] attributable to the yellow staining of the granules with chromium salts, and argentaffin and argyrophil cells[41] to describe their silver-reducing power. Soon after the discovery of the argentaffin reaction of the GI endocrine cells,[40] some endocrine cells were discovered which lacked argentaffinity,[27,30] but would reduce silver if a reducing agent was first added to the sections, the argyrophil reaction.[27] Initially, it was thought that the argentaffin and argyrophil reactions represented different stages of development of one endocrine cell. The finding of carcinoid tumors with atypical clinical manifestations, however, gave rise to the suspicion that more than one cell type was involved, and this was subsequently confirmed in early electron microscopic studies, which revealed a number of distinct cells with characteristic granules.[67,73]

The full impact of the endocrine cell nature of the argentaffin cell was only realized about 15 years ago. Although secretin and gastrin were known to be produced by the gastrointestinal tract since 1902,[3] it was thought that they were produced by the interstitial tissues in some undefined way. In 1952, Erspamer[13] demonstrated serotonin in the argentaffin cell but further work on the gastrointestinal hormones then shifted to studies on the islets of Langerhans, where it was shown that glucagon was produced in the A-cells and insulin in the B-cells. It was only after these studies and the realization that some hormones, such as glucagon, had a common origin in the pancreas and gut that a search for endocrine cells in the gastrointestinal tract was undertaken, culminating in the demonstration of the gastrin cell in 1969.[72] Since then, histochemical, immunocytochemical, and electron microscopic studies have demonstrated the presence of a large number of distinct endocrine cells in the gastrointestinal tract.

Origin of GI Endocrine Cells

The origin of the GI endocrine cells is still uncertain and a matter of some controversy. As early as 1924, Masson[40] postulated a neuroectodermal origin for argentaffin cells, based on his observation of the association of appendiceal carcinoids with submucosal nerve fiber hyperplasia. Then in 1938, Feyrter[15] suggested that the GI endocrine cells formed an integral part of a diffuse

endocrine system, which differed from the typical endocrine system in that its constituent cells were diffusely scattered throughout the tissues of the body either singly or in small groups, and had a paracrine function, namely exerted local hormonal control. It was Feyrter's contention that the constituent cells of the diffuse endocrine system arose from the epithelia in which they were found, which in the case of the GI tract would be the endoderm.

The APUD System. Between 1966 and 1968, Pearse[48,49] described a group of widely dispersed endocrine cells, whose principal products appeared to be amine and peptide hormones, that were linked together by their possession of a common set of cytochemical characteristics. He coined the term APUD system for this group of dispersed cells, an acronym for their common ability to take up amine precursors and decarboxylate them. He subsequently concluded that it corresponded to Feyrter's diffuse endocrine system.[15,55] Initially, the APUD system included the GI endocrine cells, the pancreatic islet cells, the C-cells of the thyroid, adrenal chromaffin cells, and the ACTH- and MSH-producing cells of the pituitary. Later, it was expanded to include most endocrine cells producing polypeptide hormones, carotid body type I cells, melanoblasts, and the clear cells of the urogenital tract.[35,51,53–55]

The common cytochemical features of the APUD cells were interpreted by Pearse[54] to reflect a common embryologic origin, which he postulated was of neural crest derivation,[49,55] based on the following observations:

1. Avian allograft studies which showed that C-cells of the thyroid and the carotid body type I cells migrated from the neural crest.
2. The discovery that many of the hormonal peptides present in the gut, such as VIP, substance P, somatostatin, cholecystokinin (CCK), gastrin, bombesin, neurotensin, and enkephalin, also served as neurotransmitter substances in the brain, ganglia, and nerves of the autonomic nervous system, suggesting that the nervous system and the endocrine system were part of an integrated functional unit, the so-called brain-gut axis.[55]

Pearse's hypothesis has not generally held up in recent experimental studies. Thus, while allograft studies on the neural crest in quail-chick chimeras (in which quail cells can be identified by their prominent nucleoli, in contrast to chick cells which lack them) have conclusively shown the C-cells of the thyroid to be derived from the neural crest, they have been equally conclusive in showing that the GI and pancreatic endocrine cells originate from endoderm.[36,37] Other studies by Andrew[1] and by Pictet[56] have shown that removing the neural crest from developing rabbit embryo does not prevent the development of insulin-producing B-cells, which appear to have a common endodermal precursor with pancreatic exocrine cells. Furthermore, anatomic studies by Leblond and Cheng,[34] on the turnover of normal intestinal mucosal cells, point to the crypt base cell as the cell of origin for all epithelial cell types including the endocrine cells. Recent studies on GI tumors have demonstrated an increasing number with mixed epithelial and endocrine cell

components and rare tumors in which dense core granules and zymogenic granules are found within the same cells.[77] These findings lend additional support to the concept of a common precursor cell for gut epithelial and endocrine cells.

How then can one reconcile the above discrepancies and come up with a unifying concept regarding the origin and function of the GI endocrine cells? Pearse in response to the elegant work of LeDouarin and others contends that the APUD cells are derived from neuroendocrine-programmed cells originating in embryonic ectoblast that migrated from neuroectoderm at a stage in embryonal development too early to be detected by the experimental procedures described to date.[50] Furthermore, he argues that recent work demonstrating the presence of neuron-specific enolase (a recognized marker for neuronal tissue) within the APUD cells, supports this hypothesis.[4,50] In his view, the APUD system makes up the third arm of the nervous system, functioning in part to modulate actions of the autonomic division of the nervous system. An alternative explanation for the discrepant findings, and one favored by us, is that the chemical messengers of the body, consisting of biogenic amines and peptides, are limited in number and shared by the nervous and endocrine systems, and that possession of these hormones does not necessarily denote a common embryologic origin. If we accept this premise, then the endodermal origin of the GI endocrine cells appears logical, and the anatomic, experimental, and pathologic findings fall into place.

TABLE 1. DISTRIBUTION OF DIGESTIVE HORMONES IN THE GASTROINTESTINAL TRACT

	Substance	Cell Type	Localization
1.	Cholecystokinin (CCK)	I	Duodenum and jejunum
2.	Gastric inhibitory polypeptide (GIP)	K	Duodenum and jejunum
3.	Gastrin	G	Antrum and duodenum
4.	Glucagon	L	Small and large intestine
5.	Motilin	Mo	Duodenum and jejunum
6.	Neurotensin	N	Small intestine—colon
7.	ACTH-like peptide	G	Antrum
8.	Somatostatin	D	Fundus, antrum, duodenum, and jejunum
9.	Secretin	S	Duodenum and jejunum
10.	Substance P	EC	Small intestine
11.	Serotonin	EC	Stomach, small and large bowel
12.	Vasoactive intestinal polypeptide (VIP)	?D	Stomach and duodenum
13.	Pancreatic polypeptide	?PP	? Duodenum
14.	Enkephalin	?	Stomach and duodenum
15.	Thyrotropin-releasing hormone	?	Small intestine
16.	Bombesin	?	Small and large intestine

Morphology, Identification, and Distribution of the GI Endocrine Cells

The endocrine cells of the gut are widely distributed throughout the gastrointestinal tract from cardia to the anus (Table 1).[20,21] They occur singly or in discontinuous clusters throughout the gastric and intestinal glands. In the pyloric antrum, they are especially prominent at the junction of the gastric foveolae and glands, and in the intestines at the base of the crypts of Lieberkuhn. They are sparse in the intestinal villi. They are found wedged between the epithelial cells and the underlying basement membrane and morphologically consist of two types, the so-called open cells which communicate with the glandular lumen, and the "closed cells" which lack a luminal connection (Fig. 1).[19,21] The "open cells," exemplified by the gastrin cell, are pyramidal shaped and have apical microvilli which project into the glandular lumen. The "closed cells," which are found predominantly in the gastric body and fundus, are spindle shaped and lie in a longitudinal axis between the basement membranes and overlying epithelial cells.[18] The endocrine cells may be recognized in routine histologic sections stained with hematoxylin and eosin (H&E), as basal granulated cells, in which small eosinophilic granules are present beneath the nucleus (Fig. 2), (the majority of these are probably enterochromaffin cells containing serotonin), and as "clear cells" which are typically rounded and have a centrally located vesicular nucleus surrounded by clear cytoplasm (Fig. 3).

Not all endocrine cells or their neoplastic counterparts are stained by H&E in routine sections, and to date no universal marker for GI endocrine

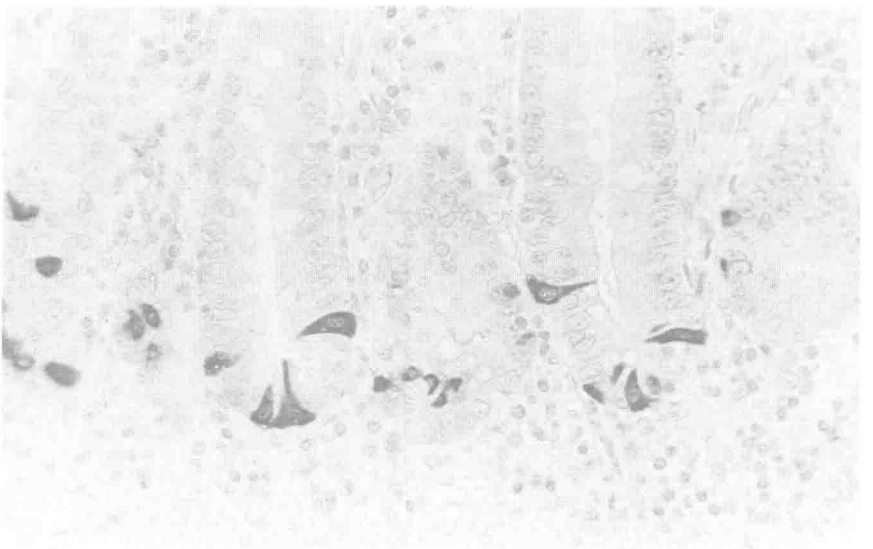

Figure 1. Medium-power view of crypt of Lieberkuhn, stained by immunoperoxidase for serotonin, showing "open cells" which extend from the basement membrane, to the lumen (PAP for serotonin, ×100).

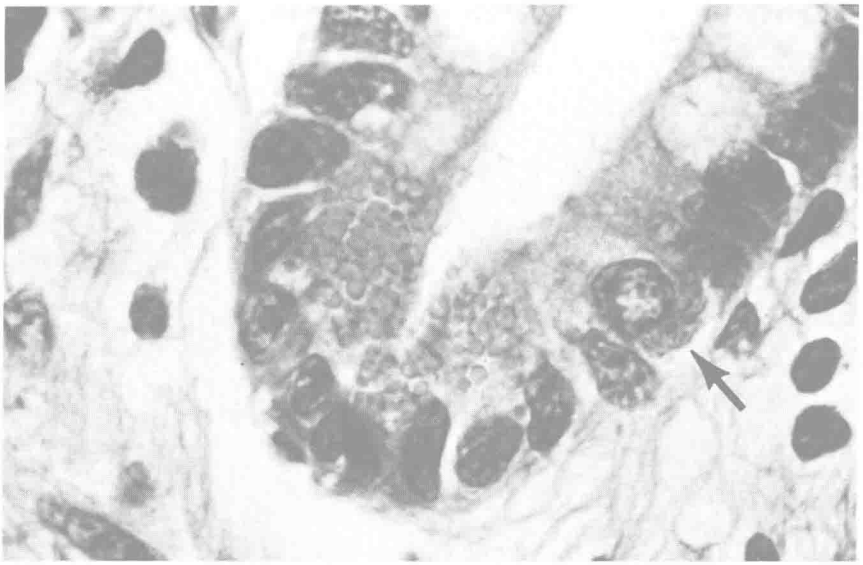

Figure 2. High-power magnification of crypt of Lieberkuhn, showing a basigranular cell (*arrowed*) characterized by infranucler granules, in contrast to the Paneth cells with their typical apical eosinophilic granules (H&E, ×160).

cells has been found. (Recently it has been reported that neuron-specific enolase allows detection of all endocrine cells, although this remains to be confirmed.[4,64]) Consequently, a number of histochemical stains have been developed to demonstrate various components of the secretion granules. Of these the silver stains and the immunoperoxidase methods are the most useful.

The silver stains are the most useful and widely used histochemical methods currently employed for the demonstration of the GI endocrine cells, and in many instances will give an indication of the number of endocrine cells present. It is clear from the literature, however, that a few endocrine cells fail to stain up with the silver methods. Some cells, such as the G cell, stain inconsistently, in part because of differences in fixative used, perhaps by allowing the hormones to leach out, or due to the different silver-staining methods, which have recently been shown to vary in their ability to stain endocrine cells.[66] The silver stains will not give any information as to which type of endocrine cell one is seeing, and this deficiency is overcome by the specific immunologic techniques.

Specific Immunologic Techniques

The most definitive way to characterize the endocrine cells is to demonstrate their hormonal products immunocytochemically. To date, at least 11 peptides and biogenic amines have been identified and these are listed in Table 1.[26] A number of other peptides also known to occur in the gastrointestinal tract,

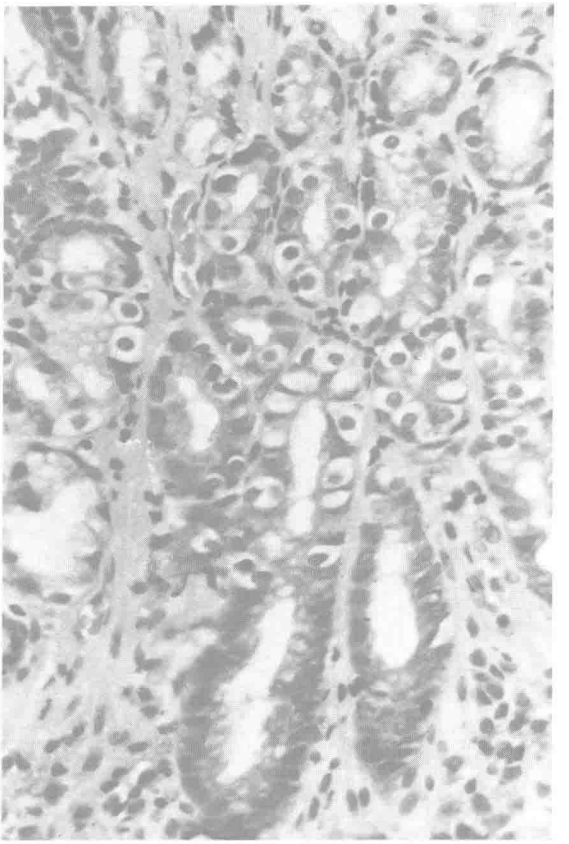

Figure 3. High-power magnification of the pyloric antrum, to illustrate the clear-staining endocrine cells, characterized by a centrally located nucleus and surrounding clear cytoplasm (H&E, ×160).

such as pancreatic polypeptide, vasoactive intestinal polypeptide (VIP), bombesin, enkephalin, and thyrotropin-releasing hormone, have yet to be localized to specific endocrine cells. In general there appears to be only one peptide in each endocrine cell type but a few exceptions have been reported. For example, antral gastrin cells have been reported to contain immunoreactive adrenocorticotropic hormone (ACTH).[32,33,79] By contrast, there are a number of instances of an amine and a peptide being present in one endocrine cell. For example, substance P has been localized to a subpopulation of the serotonin-containing enterochromaffin cells. It is not known how common such amine-peptide pairing is.[26]

The immunocytochemistry of GI endocrine cells generally conforms to the general principles of immunohistochemistry. The commonly employed methods for the demonstration of regulatory peptides are listed in Table 2.

Methods and Controls. Fixatives employed in immunohistochemistry must render antigens insoluble and at the same time preserve their tertiary