

Appearance of Gastrin and Somatostatin in the Human Fetal Stomach, Duodenum and Pancreas¹

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Abstract. The gestational time of appearance of gastrin and somatostatin in the human fetal stomach, duodenum and pancreas was examined. Immunoreactive gastrin (IRG) is detected in antral, duodenal and pancreatic extracts of a 7.0-cm (crown-heel length) fetus. More IRG is extracted from the duodenum than the antrum. Duodenal IRG concentrations from fetuses of 16.0–26.0 cm are higher than younger fetal and adult concentrations. Antral IRG concentrations are one tenth of the adult contents. Very small IRG concentrations are present in the human fetal pancreas. Gastrin immunohistochemical staining is positive first in duodenal (6.5-cm fetus) and later in antral (12.5-cm fetus) mucosa; pancreatic tissue is negative for gastrin immunohistochemistry. Type IV cells are encountered in antral and duodenal mucosa of 4.0-cm fetuses; other endocrine cells appear with fetal growth. Not until much later in gestation (21.0 cm) do typical G cells appear. These results suggest that early in fetal life gastrin is produced by the type IV cell. Somatostatin immunohistochemical staining is positive in stomach, duodenum and pancreas in 6.5-cm fetuses. Immature D cells are found in antral and duodenal mucosa of 5.0-cm fetuses and mature D cells in 11.0-cm fetuses.

Introduction

Little is known about the presence and physiology of cells producing gastrointestinal hormones in the digestive tract of the human fetus. Immunoreactive gastrin has been measured in antral, duodenal and pancreatic ex-

tracts (1, 2). Immunohistochemical evidence has been presented for antral gastrin (3), duodenal gastrin (3, 4), CCK (4) and somatostatin (5) and pancreatic somatostatin (6). Ultrastructurally, EC cells have been detected in the stomach (7, 8) and a variety of endocrine cells in the small intestine (9). These three approaches have produced some information about the appearance of cells producing gastrointestinal hormones in the human fetus. How-

¹ Dedicated to Prof. Paul F. Glees on his 70th birthday.

ever, the temporal relationship between the ultrastructural identification of a specific endocrine cell type and the ability to detect a hormone by radioimmunoassay or immunohistochemistry has not been examined. Examination of this relationship is important to discover which cells are present with which hormones during fetal gastrointestinal development.

The present investigation examines three aspects of human fetal gastrointestinal development: concentrations of immunoreactive gastrin in antral, duodenal and pancreatic extracts; immunohistochemical demonstration of gastrin and somatostatin-producing cells, and ultrastructural identification of different types of endocrine cells in antral and duodenal mucosa.

Materials and Methods

Tissue Sources

Human fetuses were obtained from legal abortions performed in three hospitals. Fetuses were measured (crown-heel length), the presence of buccal membrane noted and then the fetus was dissected. The crown-heel length was used to assign gestational age. The stomach, duodenum and when possible the pancreas were removed; when size permitted, the stomach was divided into three portions. Adult antral and duodenal mucosal specimens were collected as described previously (10).

Tissue Preparation for Ultrastructural Study

The stomach (antral-pyloric region when possible) and duodenal tissue were diced into 1-mm pieces and fixed for 3 h by immersion in Karnovsky's fixative. The fixed tissue was washed in sodium cacodylate buffer, post-fixed in 1% osmium tetroxide in sodium cacodylate buffer, dehydrated in a graded acetone series, block stained with phosphotungstic acid and lead citrate and finally embedded in Vestopal resin. Ultrathin sections were cut with the LKB microtome, stained with lead citrate and viewed in the Zeiss EM 9S with a built-in condenser. The visual identification of different endocrine cell types was according to

the Lausanne classification (11). Tissue was examined from 60 human fetuses ranging in crown-heel length from 1.5 to 32.0 cm.

Tissue Preparation for Immunohistochemistry

The tissue was immersed in Bouin's fixative for 4 h, washed in 70% ethanol and embedded in paraffin. Cells containing gastrin-like immunoreactivity were stained as described elsewhere (12); 19 antral, 18 duodenal and 6 pancreatic specimens were examined. Basically the same technique was employed to search for cells containing somatostatin-like immunoreactivity. The characteristics of the somatostatin antibody have been described (13).

Sections were incubated with anti-somatostatin serum diluted 1:20 and washed after 45 min. Peroxidase-labelled rabbit anti- γ -globulin was placed onto the sections for an additional 45 min. Following repeated washings the benzidine reaction was performed. 7 corpus, 19 antral, 18 duodenal and 10 pancreatic tissues were examined for somatostatin-like immunoreactivity.

Tissue Preparation for Immunoreactive Gastrin Measurement

Tissue was weighed, homogenized in 10 mM phosphate buffer (pH 7.5), placed in a water bath at 100 °C for 15 min, the homogenate centrifuged and the supernatant stored at -20 °C until assay. Immunoreactive gastrin (IRG) was estimated by radioimmunoassay (10, 14) and protein content by the Lowry method. Tissue IRG concentrations were expressed as nanograms of gastrin per milligram of protein. IRG concentrations in 18 antral, 12 duodenal and 11 pancreatic extracts were measured; adult concentrations from 6 antral and duodenal extracts were employed for comparative purposes (10).

Column Chromatography

Tissue extracts were chromatographed upon a Sephadex G-50 F column (1.0 × 200.0 cm) in 200 mM NH₄HCO₃. IRG was measured in successive 1-ml fractions from the void volume (60 ml) to the excluded volume (150 ml). For comparative purposes, distributions of gastrin components from adult antral and duodenal mucosa were taken from previously published work (10). The column was characterized with natural human G-34 and G-17; both gastrin components were the gifts of Prof. R.A. Gregory, Liverpool.

Results

IRG was detected in human fetal antral, duodenal and pancreatic extracts (fig. 1). In the smallest fetus examined (7.0 cm crown-heel length), an antral concentration of 2 ng IRG/mg protein was detected. Subsequent antral extracts displayed a progressive increase in IRG concentration reaching 70–100 ng/mg. Adult concentrations ranged from 319 to 672 ng/mg. Extracts of proximal duodenum from younger fetuses (<16 cm crown-heel length) contained less IRG (30–60 ng/mg) than those of older fetuses (100–150 ng/mg). Adult concentrations ranged from 13 to 73 ng/mg. Pancreatic extracts contained low IRG concentrations (0.25–4.50 ng/mg) and no increase was noted with gestational age. No IRG was detected in extracts of adult pancreas.

Fetal and adult antral and duodenal mean IRG concentrations are presented in table I. During fetal life significantly less antral IRG was found than in the adult. The duodenal extracts exhibited a different pattern. Duodenal IRG contents from 16- to 28-cm fetuses were higher than younger fetuses and those of the adults.

Antral, duodenal and pancreatic extracts were chromatographed upon Sephadex G-50. Sephadex G-50 IRG elution patterns of fetal

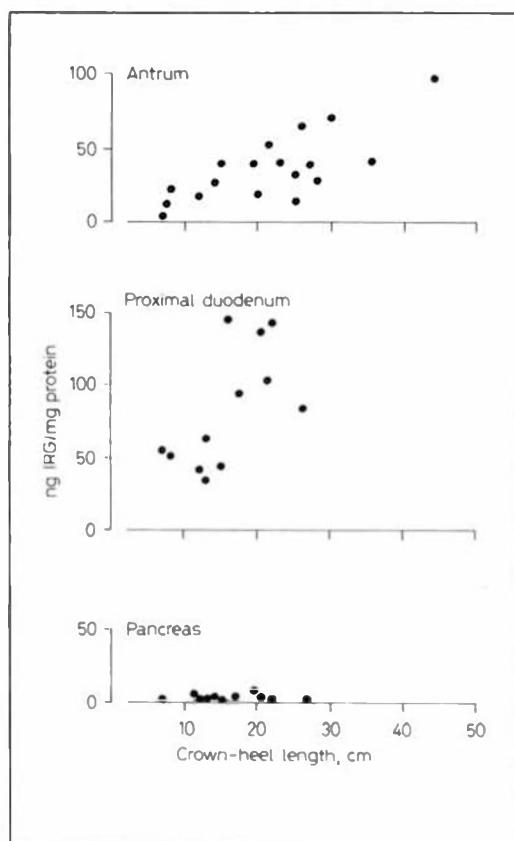


Fig. 1. Immunoreactive gastrin (IRG) concentrations of antral, duodenal and pancreatic extracts from human fetuses. Gastrin concentrations are listed as nanograms of IRG per milligram of protein. The age of the fetus is indicated by its crown-heel length in centimeters.

Table I. Immunoreactive gastrin (IRG) content of human fetal and adult antral and duodenal extracts

Group	Crown-heel length, cm (mean \pm SEM)	n	Antrum ng IRG/mg protein	n	Duodenum ng IRG/mg protein
Fetus	<16	6	21 \pm 5	6	50 \pm 4
	>16	12	37 \pm 6	6	104 \pm 23
Adult		6	304 \pm 47	6	40 \pm 10

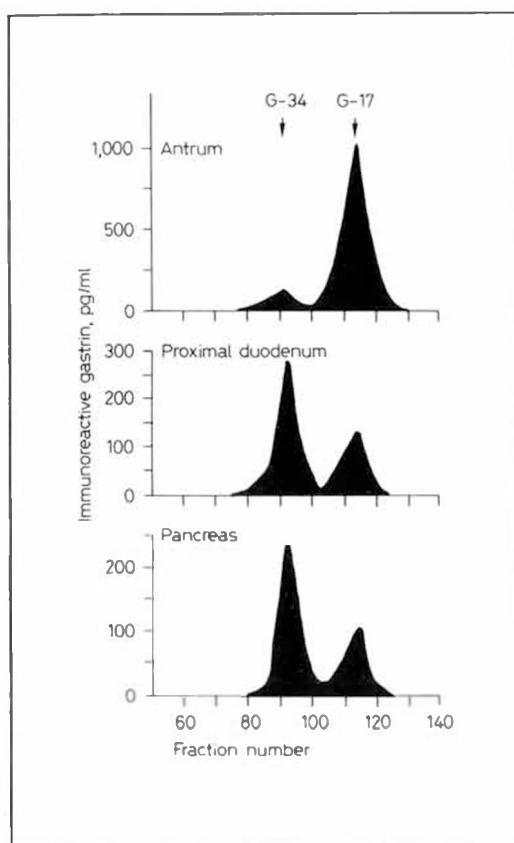


Fig. 2. Sephadex G-50 immunoreactive gastrin (IRG) elution patterns of antral, duodenal and pancreatic extracts of a 27.5-cm (crown-heel length) fetus. The elution volumes of G-34 and G-17 are indicated.

antral, duodenal and pancreatic extracts from a 27.5-cm fetus are illustrated in figure 2. In all three tissue extracts two major IRG components were found corresponding to G-34 and G-17. The effect of increasing gestational age upon the IRG distribution in fetal antral and duodenal extracts is shown in table II. Table II lists the antral and duodenal G-34 and G-17 percentages from four fetal and five adult extracts. With increasing fetal age a dramatic shift in the antral distribution occurred from a proponderance of G-34 to G-17. The largest

Table II. Sephadex G-50 percent distribution of immunoreactive gastrin components in extracts of human fetal and adult antrum and stomach

Sample	Antrum		Duodenum	
	G-34	G-17	G-34	G-17
Fetus				
crown-heel length, cm				
14.5	68	32		
25.0	21	79	100	—
27.5	13	87	64	36
33.5	10	90	66	34
Adult				
n = 5	5 ± 1	92 ± 3	36 ± 4	59 ± 4

fetus (33.5 cm) examined had a distribution comparable to the adult. Fetal duodenal extracts contained high G-34 percentages; the percentages decreased with fetal growth but still remained double that found in the adult.

Immunohistochemistry

The gastrin and somatostatin antibody-staining characteristics of the different tissues in relationship to fetal age (crown-heel length, cm) are shown in table III. No cells with either gastrin or somatostatin immunoreactivity were demonstrated in fetuses of less than 6.0 cm crown-heel length. Beginning with 6.5-cm fetuses, sparsely distributed cells revealing gastrin immunoreactivity appeared in the duodenal but not in the antral mucosa. At the same time few somatostatin immunoreactive cells were demonstrated in the antral, fundic and duodenal mucosa and pancreatic tissue. Positive staining of antral G cells commenced in 12.5-cm fetuses. At this time patchy distributed gastrin-positive cells appeared in the middle part of the antral mucosa and increased slightly

Table III. Relationship between positive gastrin and somatostatin staining of fetal stomach, duodenum and pancreas and fetal age (crown-heel length)

Tissue	n	Hormone						
		Gastrin						
Antrum	19	--	-----	--+	+	+++	++++	+
Duodenum	18	-	+++	++	++	+++	++++	+
Pancreas	6	--	---	-				
		Somatostatin						
Corpus	7	+	++	+	+	+	+	+
Antrum	19	--	++++	+++	+	+++	++++	++
Duodenum	18	-	++++	+++	++	+++	++++	+
Pancreas	10	++	+++	+	++	+		+
Crown-heel length, cm	0	5	10	15	20			25
Age, weeks		6 8	12	16			20	

-- = Negative; + = positive.

in number with fetal growth. No gastrin-positive cells were detected in the pancreatic specimens. Gastrin- and somatostatin-producing cells in the antral and duodenal mucosa of a 12.5-cm fetus are depicted in figure 3. Gastrin- and somatostatin-producing cells in the antral and duodenal mucosa from a 15.3-cm fetus are depicted in figure 4.

Ultrastructure

In the youngest human fetus examined (1.5 cm) the gastric mucosa contained presumptive type IV cells; these were the only endocrine cells observed (fig. 5). These cells contained small homogeneous electron-dense granules surrounded by a closely fitting limiting membrane; the granules were scattered throughout the cytoplasm. Sometimes, the nuclei seemed mitotic containing clumps of chromatin.

Four distinct types of endocrine cells were seen in the gastric mucosa of 3.5- to 4.0-cm

fetuses. Type IV cells contained numerous homogeneous dense granules encompassed by a tightly fitting membrane. Very rarely was an electron-lucent granule encountered. The second type of endocrine cell found at this time is depicted in figure 6. These cells were found throughout gestation and could be presumptive G cells. The granules were encompassed by a limiting membrane and were of varying electron opacity. Electron-dense granules were rare and smaller than the electron-lucent ones. Occasionally the presence of a small dense granule surrounded by a narrow electron-lucent halo encased in a smooth membrane was seen. Immature EC cells containing pleomorphic granules were the third type of cell encountered (fig. 7). Some granules showed a heterogeneous filamentous content encompassed by a closely fitting membrane. The electron density varied as opposed to mature EC cell granules. Some ovoid and round granules appeared electron

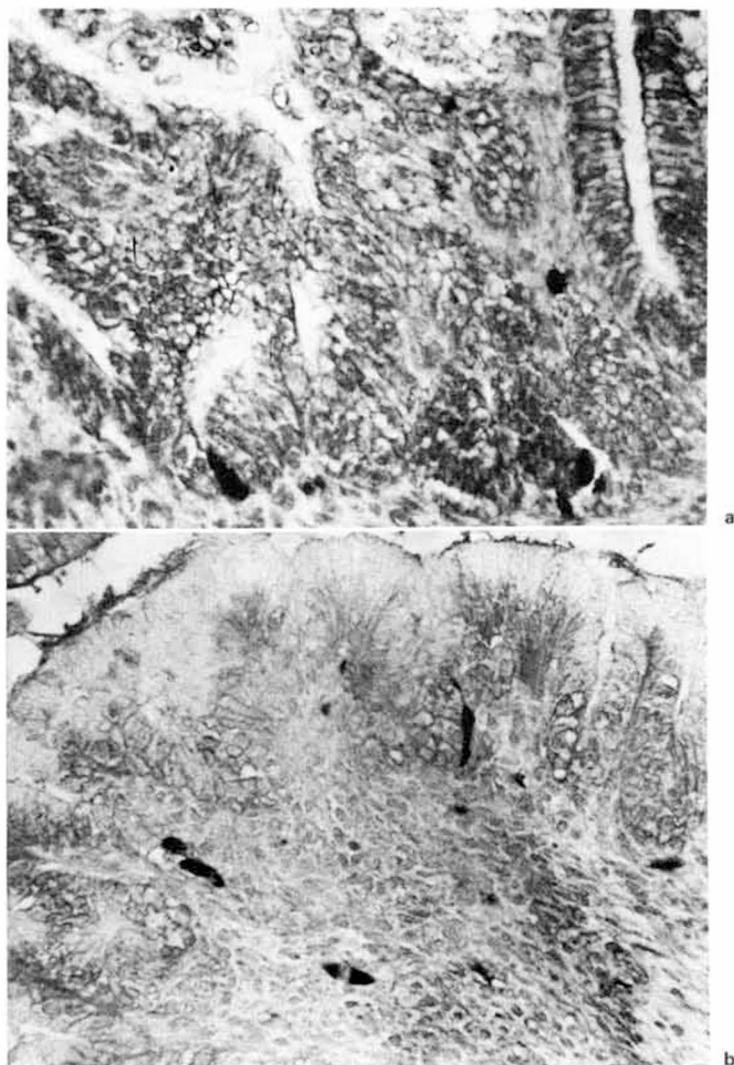


Fig. 3. **a** Immunohistological demonstration of gastrin-producing cells in antral mucosa of a 12.5-cm fetus. Bouin's fixation, paraffin embedding. Incubation with 1:500 diluted antigastrin serum; after washing incubation with 1:20 diluted peroxidase-labelled sheep anti-rabbit γ -globulin. $\times 400$. **b** Immunohistological demonstration of somatostatin-producing cells (neighboring section). Technique as in figure 3a; instead of antigastrin serum, 1:50 diluted antisomatostatin serum was used. $\times 400$.

dense. The fourth cell type observed was a presumptive ECL cell containing few electron-dense granules located eccentrically in a wide halo encompassed by a wavy membrane. Most granules were of varied electron density.

Immature D cells and mature ECL cells were the new cells seen in the gastric mucosa of 5.0-cm fetuses. The granules of the immature D cells were weakly osmophilic with a homoge-

neous core and in some a closely applied limiting membrane was seen. The granules were not as numerous nor was their limiting membrane as distinct as in mature D cells. The cytoplasm contained lysosomes, ribosomes and small mitochondria.

Mature D cells in considerable number were present in addition to the previously observed endocrine cells in the gastric mucosa of 11.0- to

13.0-cm fetuses (fig. 8). The secretory granules of these D cells were round, relatively large, weakly osmophilic, showing slight variability in their densities and encompassed by a well-defined limiting membrane. This membrane sometimes seemed interrupted.

In addition to the aforementioned endocrine cells, a cell type was encountered at 14.0–15.0 cm fetal length intermediate between a

type IV cell and an inactive/active G cell (fig. 9). This cell contained many small dense homogeneous granules often with a closely applied limiting membrane. The limiting membrane became more apparent the less dense the granules appeared. Few larger granules were electron lucent. This cell type was encountered throughout the remaining period of gestation studied.

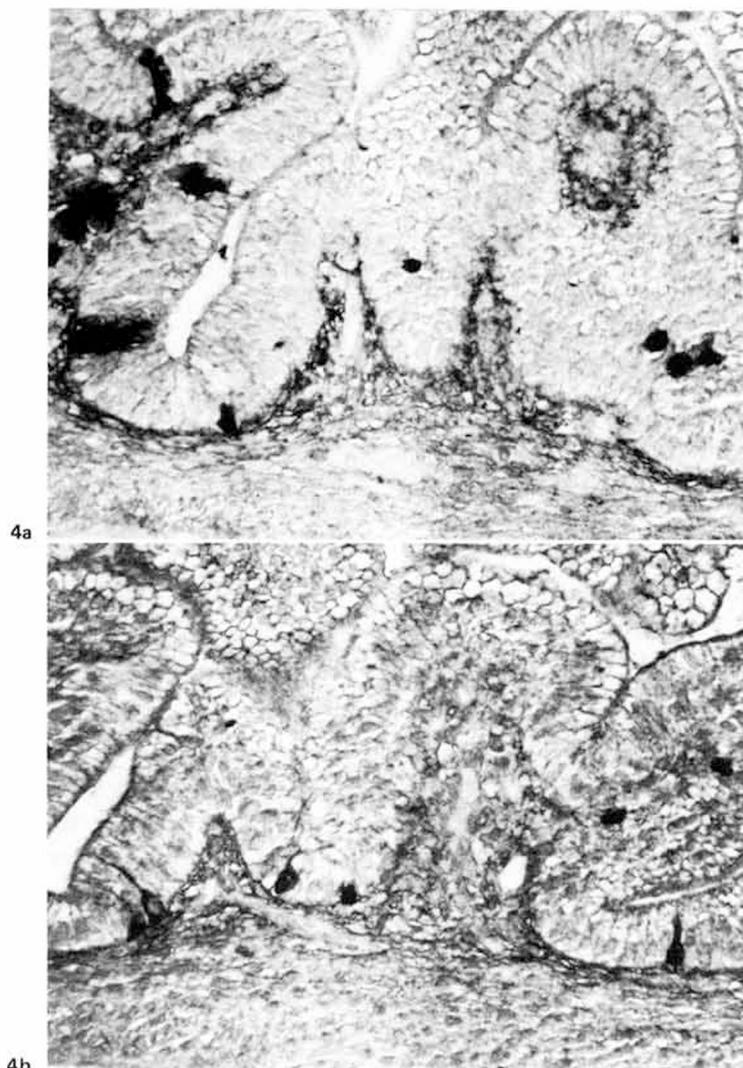
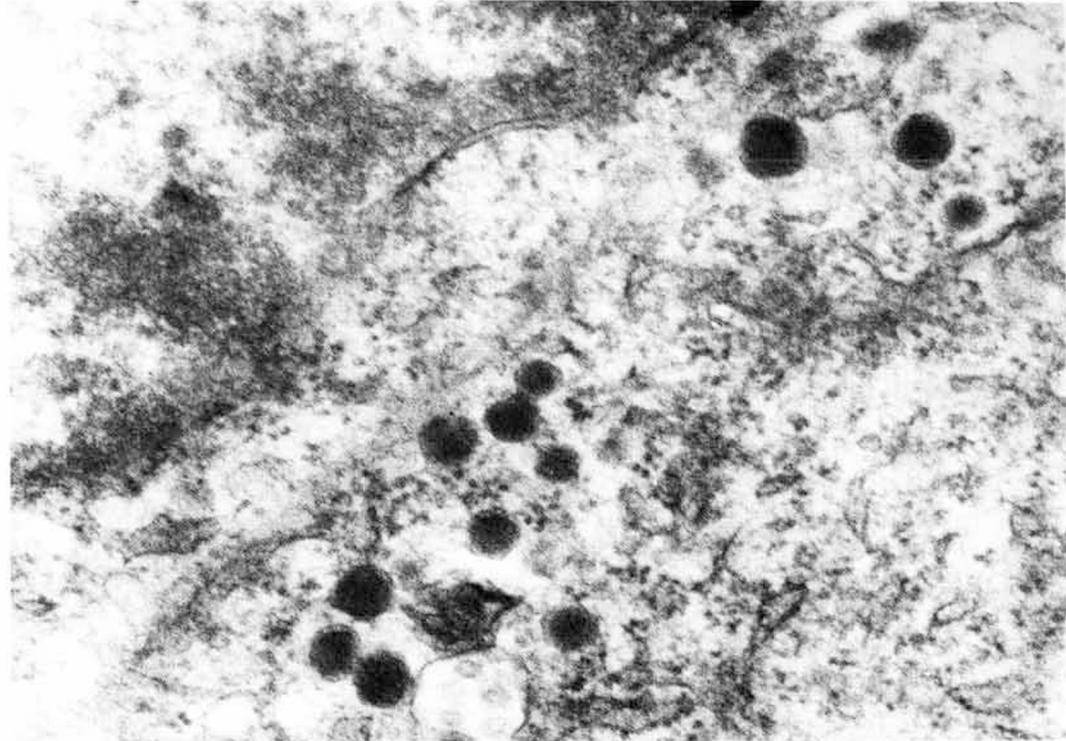


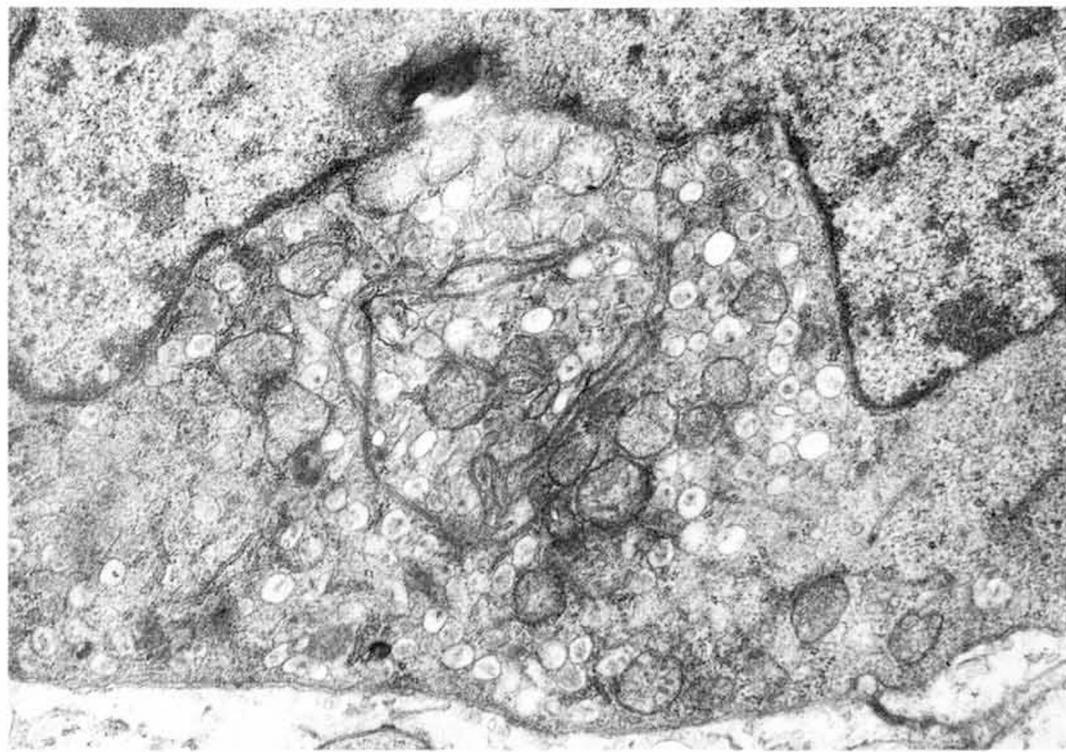
Fig. 4. **a** Immunohistological demonstration of gastrin-producing cells in duodenal mucosa of a 15.3-cm fetus. Technique as in figure 3a. $\times 400$. **b** Immunohistological demonstration of somatostatin-producing cells (neighboring section). Technique as in figure 3b. $\times 400$.

Fig. 5. Section of a stomach from a 1.5-cm (crown-heel length) human fetus showing presumptive type IV cells containing small electron dense granules encompassed by a limiting membrane. $\times 60,000$.

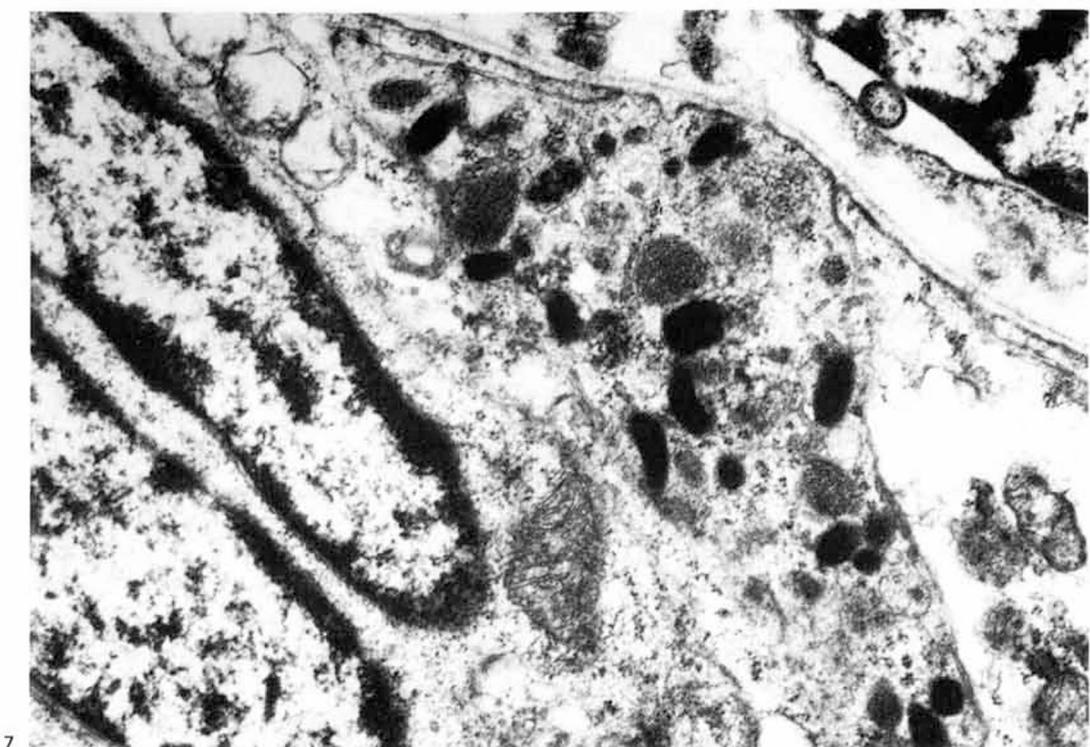
Fig. 6. Section of a stomach from a 4.0-cm fetus containing presumptive G cells with granules of varying electron opacity encompassed by a limiting membrane. Some membranous sacs contain filamentous material while others are electron lucent. $\times 24,000$.



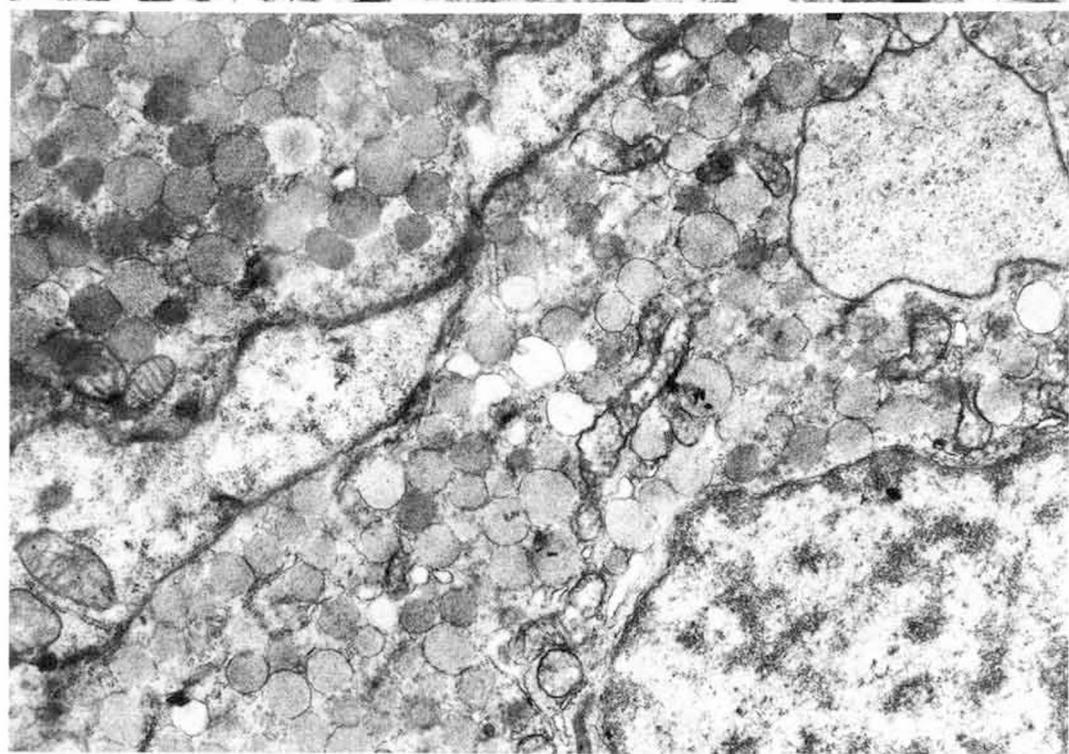
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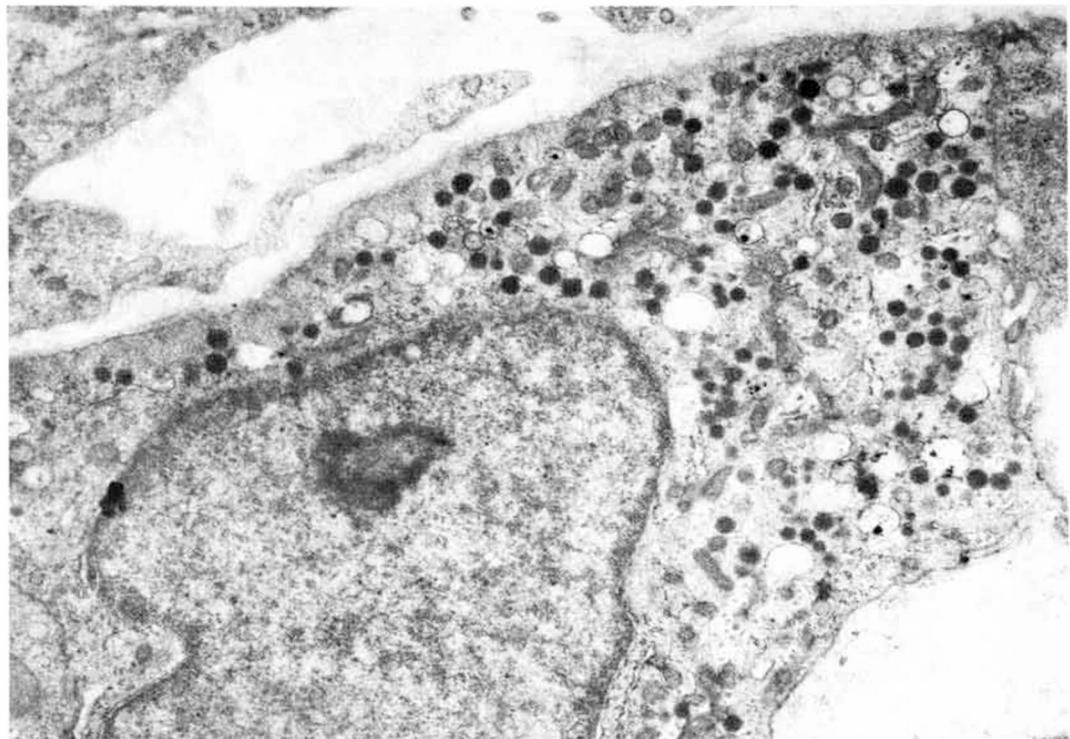
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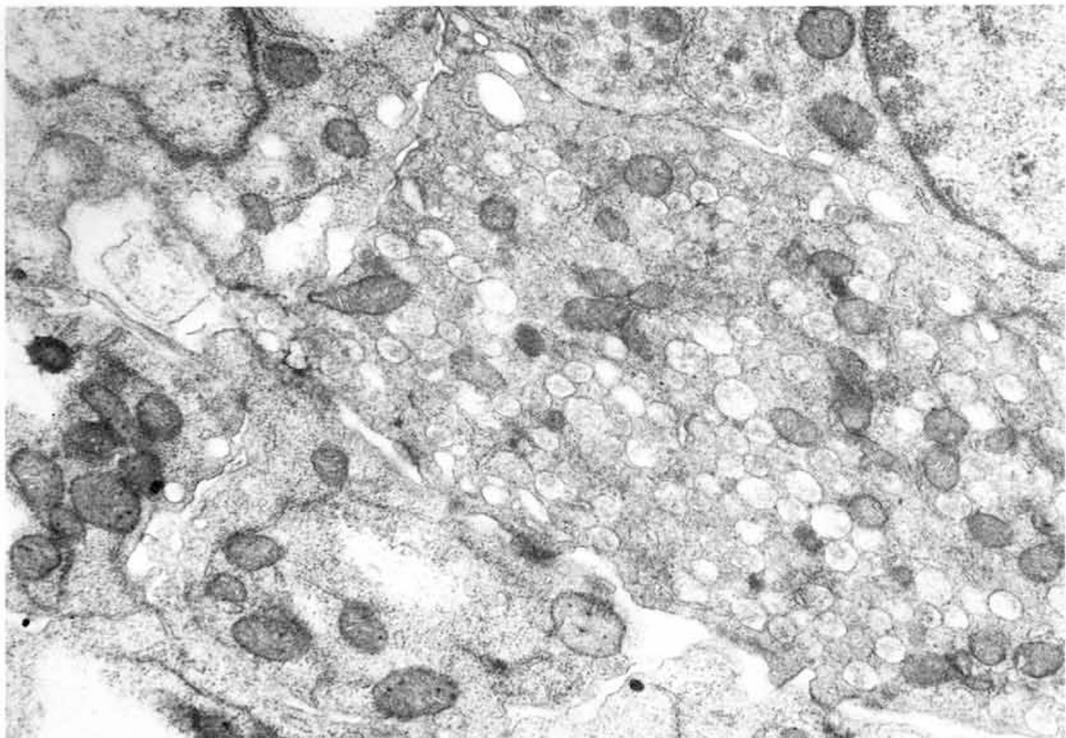
Fig. 7. Section of a stomach from a 4.0-cm fetus showing immature EC cells with pleomorphic granules. Electron-dense granules encompassed by a limiting membrane are intermingled with membranes containing coarse flocculent and other paler filamentous material. Few small dense granules are visible. $\times 24,000$.

Fig. 8. Section of an antrum from a 13.0-cm fetus demonstrating portions of two mature D cells. The abundant secretory granules are round, relatively large and weakly osmophilic. They show slight variability in their densities and are encompassed by a well-defined limiting membrane which sometimes seems interrupted. $\times 24,000$.

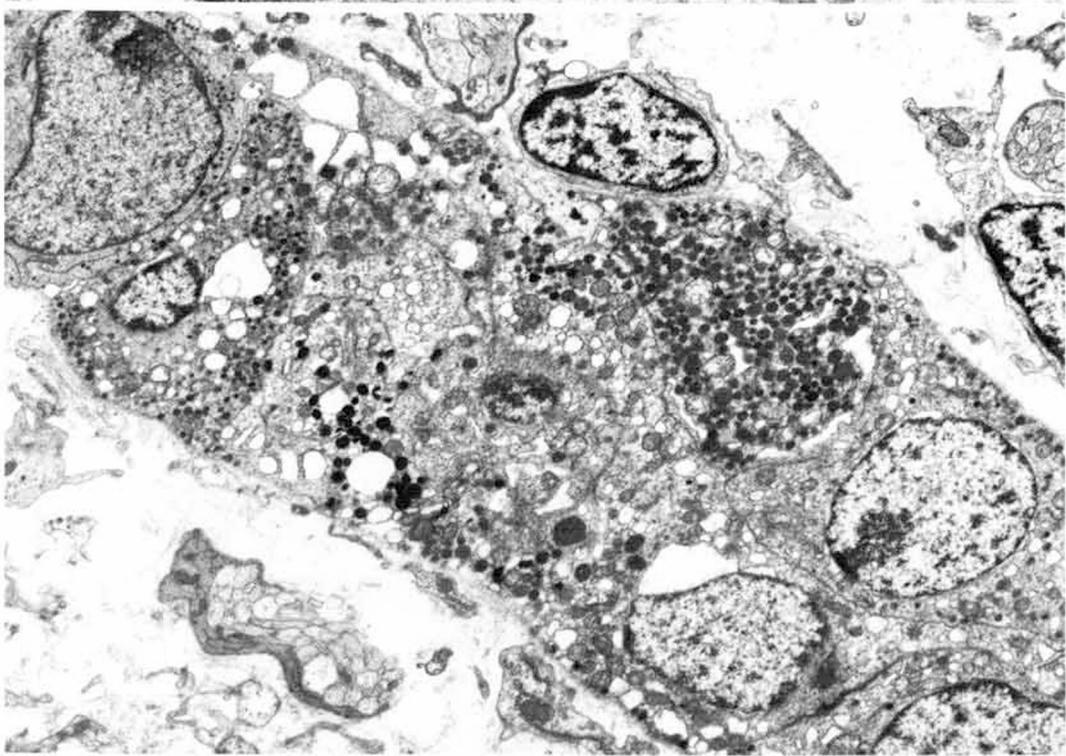
Fig. 9. Section of an antrum from a 15.0-cm fetus showing intermediate type IV and G cells containing numerous small dense granules with a closely applied limiting membrane. The membrane becomes more obvious the less dense the granules are. Few larger granules appear to be electron lucent. Both the diameter and the electron density of the granules vary. $\times 24,000$.

The gastric mucosa of 20.0- to 21.0-cm fetuses contained an increase in the number of type IV and presumptive G cells. The intermediate cell type, between type IV and G cell, occurred also more frequently. The first typical G cells in the antral mucosa were seen in 21.6-cm fetuses (fig. 10). The secretory granules had variable diameters and electron densities. The variety of endocrine cells in the antral mucosa of a 21.0-cm fetus is depicted in figure 11.

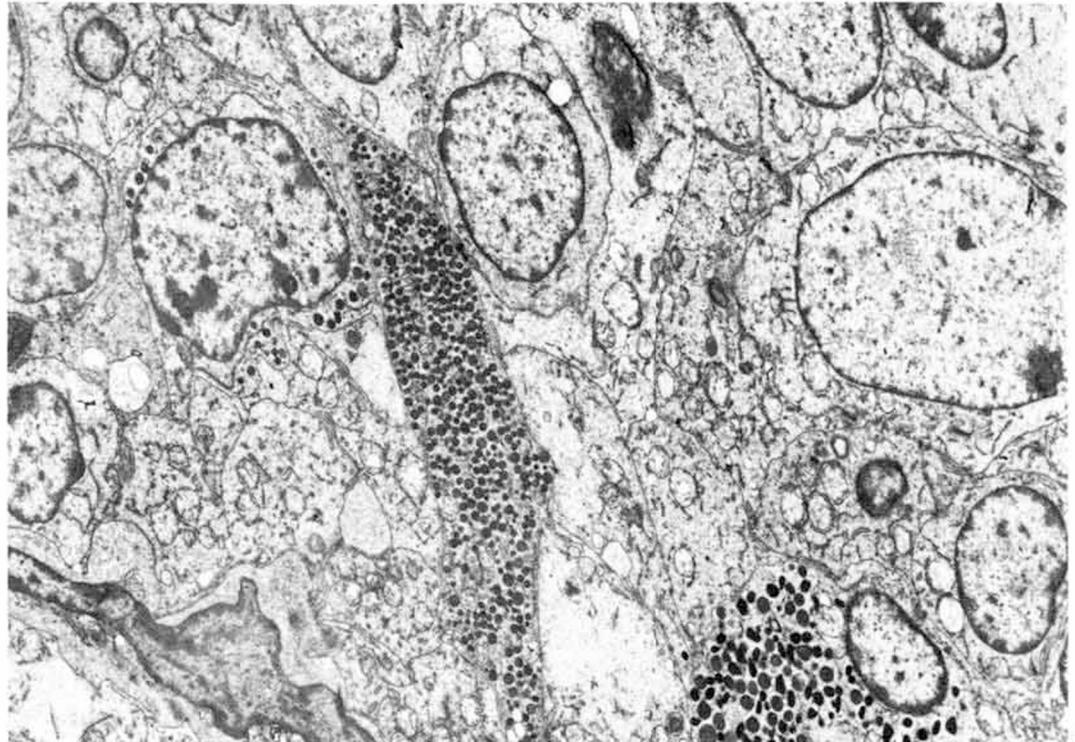
The ultrastructural appearance of endocrine cells observed in the fetal duodenal mucosa was comparable to that of the fetal antral endocrine cells. The duodenum from a 4.5-cm fetus was the youngest sample available for ultrastructural examination. EC, ECL, D and type IV cells and other endocrine cells types not clearly identifiable with characterized gastrointestinal



10



11



12

Fig. 10. Section of an antrum from a 21.6-cm fetus demonstrating portions of two adjacent G cells. The granules show variable diameters and electron densities. High electron density in small granules; larger granules appear electron lucent. Most limiting membranes contain varying degrees of filamentous material. Intercellular spaces with interdigitations with neighboring cells present. This cell shows high activity. $\times 24,000$.

Fig. 11. Section of an antrum from a 21.0-cm fetus showing eight portions of adjacent endocrine cells. Type IV cells, G cells in different activity phases, EC cells and a D cell. $\times 5,400$.

endocrine cells were observed in the duodenal mucosa (fig. 12). These cell types were observed for the remainder of gestation examined. Presumptive G cells and the typical G cells were encountered initially in the fetal duodenal mucosa of a 22.0-cm fetus (fig. 13).

Fig. 12. Section of a duodenum from a 7.0-cm fetus showing a type IV, D and EC cell. $\times 5,400$.

Discussion

Before considering what physiological roles gastrointestinal hormones may play during human fetal development, their gestational time of appearance must be established. Evidence for the presence of a hormone can be obtained by radioimmunoassay, immunohistochemical and ultrastructural examination. Radioimmunoassay and immunohistochemical techniques rely upon antibody recognition of the hormone; immunohistochemistry is dependent also upon the presence of hormone stored in secretory granules. Ultrastructural identification is deduced from the size, shape and inner texture of the cells' secretory granules.

Results from this study demonstrate gastrin

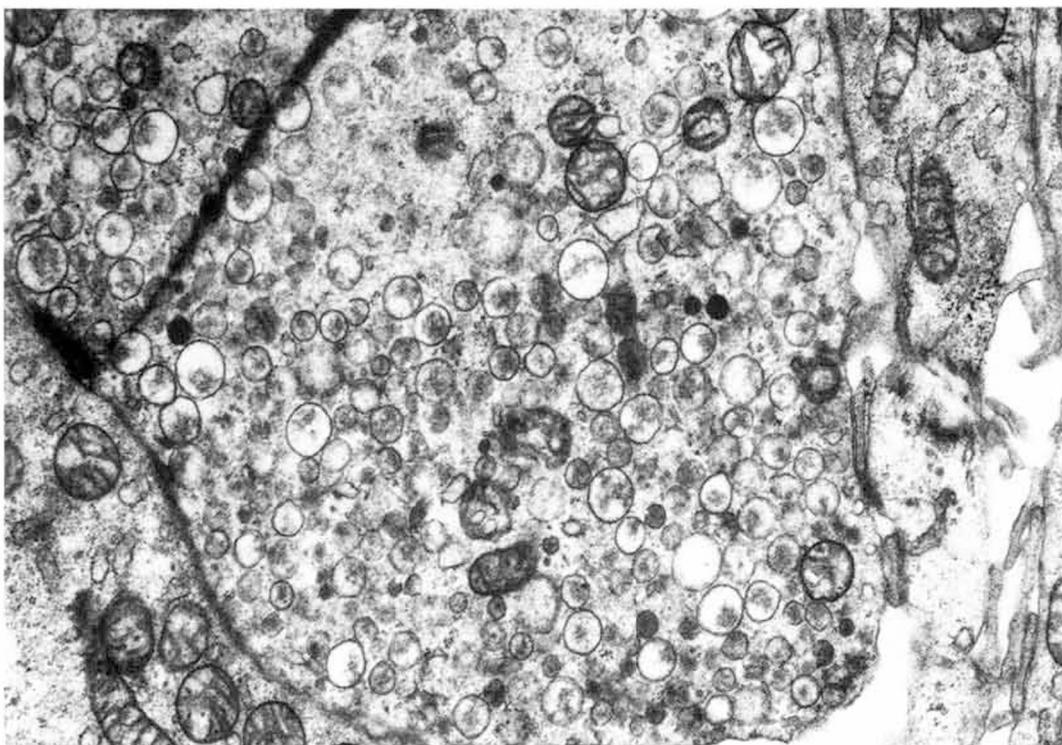


Fig. 13. Section of a duodenum from a 22.0-cm fetus demonstrating an active G cell containing numerous granules of variable densities and diameters. High electron density in small granules, larger granules

appearing electron lucent. The majority of the limiting membranes contain filamentous material. Interdigitations with neighboring cell apparent. $\times 24,000$.

and somatostatin in stomach, duodenum and pancreas of human fetuses of different gestational age.

Gastrin is detected initially in antral, duodenal and pancreatic extracts by radioimmunoassay; duodenal gastrin concentrations are the highest. Elevated duodenal gastrin concentrations and the predominance of G-34 in the extracts have been described (2). In the antrum, a shift from G-34 to G-17 is seen during gestation. G-34 predominates in pancreatic gastrin extracts. The positive immunohistochemical staining of gastrin cells in the duodenum and not the antrum or pancreas of the younger fetuses may be related to higher gastrin concen-

trations and consequent greater number of secretory granules in the duodenal G cells. This early appearance of duodenal G cells by immunohistochemical staining has been described (3, 4). Gastrin immunohistochemical staining is negative for pancreatic tissue. At this stage in gestation no typical antral or duodenal G cells are seen: in the antrum, type IV cells, presumptive G, EC, ECL and immature D cells are encountered; in the duodenum, type IV, EC, ECL and D cells are observed. Not until much later in gestation do typical antral and duodenal G cells appear.

Why is there such a late appearance ultrastructurally of typical antral G cells? Two cell

types are observed in this study which are candidates for premature G cells: the type IV cell and the presumptive G cell. Type IV cells are seen in the early stages of fetal development both in the antrum and duodenum. Presumptive G cells occur early in the antral mucosa while in the duodenal mucosa they are seen simultaneously with the appearance of typical G cells. The type IV cell as a definite entity independent from other endocrine cells was first described by Deconinck *et al.* (15, 16).

Type IV cells are observed in adult human gastric mucosa, duodenal mucosa and pancreas as well as the neonatal pancreas. Type IV cells are frequently found in human insulomas (17), gastrinomas (18) and in the pancreas of an infant with hyperinsulinism (19). Therefore, Creutzfeldt (20) suggested that the type IV cell is an immature stem cell. Early in gestation, long before typical G cells are seen, antral and duodenal type IV cells are present when gastrin is extracted from antral and duodenal mucosa and when gastrin-producing cells are demonstrated by immunohistochemistry. This observation supports the hypothesis that type IV cells are capable of producing different hormones including gastrin (21).

Larsson and Jørgensen (22), in their recent study on the cytodifferentiation of human duodenal endocrine cells, conceive that early endocrine cell types may store different biologically active molecules and that these cells continue their differentiation into various mature endocrine cell types of the gut by restriction in the biosynthetic repertoire of the cell.

Early in gestation somatostatin-producing cells are stained in the stomach, duodenum and pancreas. Concomitantly, mature duodenal D cells and immature antral D cells are observed. Very shortly thereafter, the antral D cells develop a mature appearance. Immunoreactive somatostatin has been detected in the human fetal

pancreas (6) as early as the present finding of morphological evidence for somatostatin-producing cells.

The results from this study emphasize the difficulties in establishing precise gestational appearance times for gastrointestinal hormones. The antral gastrin-producing G cell exemplifies the difficulties. The first endocrine cells seen in the antral mucosa are presumptive type IV cells, theoretically, pluripotent stem cells. At this time in gestation, gastrin radioimmunoassay, immunohistochemistry and ultrastructure are all negative. Immunoreactive gastrin is detected first in the antrum together with the appearance of presumptive G cells. The immunoreactive gastrin is detectable without positive gastrin immunohistochemical staining or typical G cell identification. The first positive gastrin immunohistochemical staining is detected with the appearance of a transitional phase of type IV/G cells. Still no typical G cells are observed. These studies reveal that descriptive morphological studies on their own are inadequate to search for gastroenteropancreatic endocrine cells in the human fetus.

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