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Partial Gastric Corpectomy Results in Hypergastrinemia and Development of Gastric Enterochromaffinlike-Cell Carcinoids in the Rat

HILLEVI MATTSSON, NIILO HAVU, JOHN BRÄUTIGAM, KERSTIN CARLSSON, LARS LUNDELL, and ENAR CARLSSON

Gastrointestinal Research, AB Hässle, Mölndal; Department of Tumor Pathology, Karolinska Hospital, Stockholm; Safety Assessment, AB Astra, Södertälje; and Department of Surgery, Sahlgren's Hospital, Göteborg, Sweden

Studies in the rat have shown that partial gastric corpectomy, in which about 75% of the acid-producing oxyntic mucosa was removed, leads to markedly reduced acid secretion and a feedback increase in the plasma gastrin levels. Ten weeks after operation, the gastric enterochromaffin (ECL)-like cell density in the remaining part of the oxyntic mucosa had increased significantly. In the present study, the effects on the gastric ECL cells of lifelong persistent hypergastrinemia induced by partial (75%) corpectomy have been investigated. Seventy-five partially corpectomized rats and 40 control rats were investigated for plasma gastrin and oxyntic mucosal changes in a 124-week study. The partially corpectomized rats showed increased plasma gastrin levels after the operation; the mean increase compared with the controls was almost 10-fold during the entire study. The remaining oxyntic mucosa of the partially corpectomized rats differed from that of control rats in two respects, showing first general hypertrophy and second a marked hyperplasia of argyrophil ECL cells. The degree and incidence of these changes increased towards the end of the study, i.e., in the aging rats. An age-related increase in ECL-cell density occurred spontaneously also in the control rats but to a lesser extent than in the partially corpectomized group. ECL-cell carcinoids were found in the oxyntic mucosa of 26 of the 75 partially corpectomized rats. The first carcinoid was found 78 weeks after the beginning of the study. Six rats with carcinoids (23%) were found before week 104 (2 years) and the remainder, 20 (77%), were discovered later. No carcinoid tumor was found in the control rats. It is concluded that lifelong hypergastrinemia induced by partial corpectomy leads to the development of ECL-cell carcinoids in the oxyntic mucosa of some rats towards the end of their life span. This observation strongly supports the hypothesis that the gastric ECL-cell carcinoids found in rats treated with antisecretory drugs are caused by long-standing hypergastrinemia developing secondary to inhibition of gastric acid secretion.

Gastric enterocromaffinlike (ECL)-cell carcinoids in rats have been reported during long-term administration (2 years or more) of various antisecretory compounds (1–8). The ECL-cell carcinoids have developed regardless of the mechanism by which acid secretion is inhibited, i.e., carcinoids were found after treatment with histamine H_2-receptor blockers (ranitidine, sufotidine, loxtidine, SK&F 93479, BL-6341, and ICI 162.846), an acid pump inhibitor (omeprazole), and a hypolipidemic agent with gastric acid antisecretory properties (9,10).

Release of gastrin from the antral G cells is stimulated by various factors, including vagal nerve activity, distension of the antrum, and the presence of amino acids and peptides originating from ingested food. A low pH of the antral content inhibits gastrin release, whereas inhibition of acid secretion, which increases antral pH, results in hypergastrinemia (11).

Numerous studies have shown a relationship between plasma gastrin levels and density of gastric ECL cells in the rat. Administration of increasing doses of acid secretion inhibitors to rats results in increasing

Abbreviation used in this paper: ECL, enterochromaffinlike.

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degree of hypergastrinemia secondary to acid inhibition and in parallel, a hyperplasia of ECL cells (12,13).

Antrectomy, i.e., removal of the gastrin-producing antral mucosa, prevents the development of ECL-cell hyperplasia during profound and sustained inhibition of acid secretion (12). It has also been demonstrated that administration of exogenous gastrin or pentagastrin leads to ECL-cell hyperplasia in the rat stomach (14,15). Thus acid inhibition is not a prerequisite for the hyperplasia.

Based on the results of these studies, it has been hypothesized that marked hypergastrinemia will result in ECL-cell hyperplasia in rats and, eventually, the development of gastric ECL-cell carcinoids if the hypergastrinemia is sustained during most of the life span (1–3,12).

In a previous study, it was shown that surgical removal of about 75% of the acid-producing oxyntic mucosa increased the plasma gastrin to the same extent as during profound inhibition of acid secretion in the rat (16). Ten weeks after the operation, the ECL-cell density was also significantly increased in the remaining part of the oxyntic mucosa (16). This kind of operation has been referred to as a partial fundectomy. However, in the rat it is, strictly speaking, a partial corpectomy.

The aim of the present study was to induce a chronic hypergastrinemia in the rat by means of partial corpectomy, in order to delineate the development of ECL-cell hyperplasia and carcinoids.

Materials and Methods

General

Female Sprague–Dawley rats were selected because they are known to develop ECL-cell carcinoids after lifelong administration of acid secretion inhibitors (1–3). After arrival from Møllegaards Breeding Center, Skensved, Denmark, the animals were allowed at least 1 week's acclimatization before surgery. The rats were 9–10 weeks old when operated on. Partial corpectomy was performed in 101 rats and was successful in 86. Forty unoperated rats of the same age were used as controls. The animals had free access to a pelleted diet (AB Ewos, Södertälje, Sweden). Initially, brand R 3 was used; from experimental week 51, brand R 34. The rats were provided with water in drinking bottles with nozzles. The bottles were replaced every second day. The animal room was illuminated on a 12-hour light-dark cycle, and the room temperature was kept between 18 and 22°C and humidity between 35% and 70%.

The design of the study was approved by the Local Ethics Committee for animal experiments, Gothenburg, Sweden.

Operation: Partial Corpectomy

Before surgery the rats were starved for 24 hours but had free access to water. The partial corpectomy was performed under anesthesia with xylazine (Rompun; Bayer, Leverkusen, Germany; 20 mg/mL) and ketamine (Ketalar; Parke–Davis, Barcelona, Spain; 50 mg/mL) given IP. A stock solution of 1.0 mL xylazine and 8.0 mL ketamine was prepared; the dose given was 2 mL/kg body wt. The animals were placed on a heating pad, and an intrarectal thermometer was applied to ensure constant body temperature. The abdomen was approached through an upper midline incision, and the stomach was mobilized. The blood vessels entering the major curvature of the oxyntic corpus region were divided. The border between the antrum and the oxyntic gland area was divided, and great care was taken to maintain intact vagal innervation to the antrum when resecting the oxyntic gland area at the lesser curvature. A partial corpectomy was accomplished by leaving some (~25%) oxyntic gland mucosa bordering on the forestomach and the lesser curvature (Figure 1). The gastric continuity was reestablished by forming an end-to-end anastomosis between the proximal part of the remaining oxyntic mucosa and the antrum by suturing with 6-0 silk in one layer. After careful examination of the hemostasis, the abdomen was closed in layers with synthetic absorbable sutures (Dexon; Davis & Geck, Gosport, Hampshire, England) and nylon sutures (Dermalon; Davis & Geck) in the skin.

Postoperative Care and Supportive Treatment

Immediately after surgery, the animals received 10 mL of Ringer's solution (Ringer-Glucose; Kabi–Vitrum, Stockholm, Sweden) SC. This injection was repeated twice daily for 3 days. From day 5, they had free access to both animal food in pellets and tap water in bottles. The partially corpectomized animals were administered (IM) vitamin B_{12} (Behepan; Kabi–Vitrum) 0.3 mg per animal. This dose was given 2 weeks after the operation, then every 2 months and, from experimental week 43, every 6 weeks.

Several studies indicate that a low-protein diet prolongs the life span of rats (17). Therefore, the diet was changed to a low-protein brand (R 34; AB Ewos) in experimental week 51.

In some animals, symptoms of partial upper gastrointestinal obstruction and dehydration developed; they were treated according to the clinical demands, e.g., by suction of excess fluid from the stomach and SC rehydration with Ringer's solution with glucose.

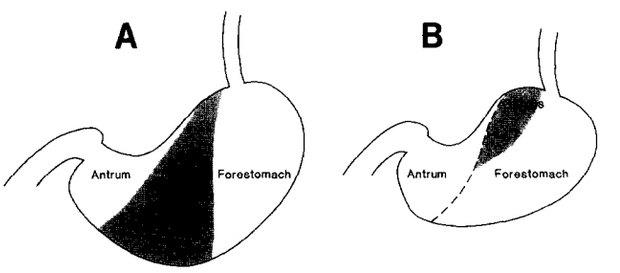

Figure 1. Schematic illustration of the partial corpectomy.

A. Normal stomach.

B. Stomach subjected to partial corpectomy: 75% of the acid-secreting corpus mucosa was removed.

In some animals in both groups, SC tissue masses, clinically appearing as fibromas or mammary fibroadenomas, developed during the latter phase of the study. When they tended to be of clinical significance, they were surgically removed under anesthesia with ketamine and xylazine and, from experimental week 86, under ether anesthesia (Aether; Nobel Industries, Sweden), which was more easily controlled. Animals showing signs of infectious disease (e.g., after surgery) were treated with injections of ampicillin (Doktacillin; Astra, Södertälje, Sweden). Dermatological problems were treated by applying a silicon-based zink oxide ointment (Silon; Pharmacia, Uppsala, Sweden).

Investigations During Lifetime and at Death

Body weight. The body weight of each rat was recorded every 2 weeks for 6 months and thereafter once a month.

Clinical observations. Throughout the study, the general condition of the animals was observed at least twice daily. When a rat showed loss of weight or was considered to be in a moribund state for other reasons, it was killed.

Plasma gastrin. Three and 7 weeks after the operation and then every 3 months, a blood sample (300 μL) was taken from freely fed animals by tail tip bleeding for determination of plasma gastrin. At death, blood was collected from the neck. All blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-treated plastic tubes and then put on ice. Immediately after blood sampling, the tubes were centrifuged at 1000 × *g*, and the plasma was transferred to plastic tubes and stored in a freezer (−18°C) until analysis. Gastrin was determined in 100 μL plasma by means of a double-antibody liquid phase [^{125}]-radioimmunoassay (Diagnostic Corp., Los Angeles, CA). Results are expressed as picogram equivalents of synthetic human gastrin-17 per milliliter of plasma. With gastrin levels above 3000 pg/mL, the radioimmunologic method allowed only an approximate estimate of the gastrin levels.

Histopathologic evaluation. The stomachs were removed from both partially corpectomized and control rats and opened along the major curvature. They were rapidly washed in NaCl 0.9% and pinned flat on a paraffin bed or a cork plate and preserved in a neutral (pH 7.2) buffered 3.7% formaldehyde solution. In the partially corpectomized rats, all the remaining oxyntic mucosa was taken for histological analysis by cutting it up into 2-3–mm specimen strips, 7–8 strips on average (variation range, 2–15) from each rat. An average of 4 strips (variation range, 2–5) was taken from each control rat.

After dehydration, all specimens were embedded in Paraplast (Monoject Scientific, Inc., Athy, Ireland), sectioned at 4–5 μm, and stained with H&E. Both the Grimelius and Sevier–Munger silver staining methods were used on specimens from all rats to visualize the argyrophil cells (18,19).

The argyrophil cell changes were evaluated in the same way as in the studies with gastric secretion inhibitors (2,3): argyrophil cell hyperplasia was graded slight when there was a diffuse increase in the number of these cells, predominantly within the lower half of the oxyntic mucosa (Figure 2*C*). The hyperplasia was considered moderate when the increased argyrophil cell population expanded up to the foveolar level (Figure 2*C*). With a further increase in number and together with a more frequent tendency toward aggregation of the argyrophil cells, the term moderately severe was used (Figure 2*D*). The term severe was used when the increased argyrophil cells formed microfocal coalescing strands within the glands (Figure 3*A*). When three or more such changes occurred grouped together forming nodules not wider than 500 μm, they were classified as micronodular hyperplasia (Figure 3*B*). Larger nodules (> 500 μm) of such changes or more solid ones that were microinvasive to lamina propria were classified as intramucosal carcinoids. The carcinoids were regarded as microinvasive carcinoids when the argyrophil cell clusters passed beyond the lamina muscularis propria into the submucosal compartment.

Results

Survival

During the first 6 months of the study, the survival rate was lower in the partially corpectomized animals than in the controls, probably because some of the operated animals were still in the postoperative phase when they entered the study. Thus, 11 rats were killed during this period of the study because of postoperative problems; no histological samples were taken from these animals. Seventy-five partially corpectomized rats and all 40 control rats survived 6 months or more after the start of the study, and the oxyntic mucosa from these rats was analyzed. The survival rates were roughly comparable in the two groups (Figure 4).

During the study (survival > 6 months) five partially corpectomized rats were found dead in their cages. All the other rats were killed when they were in a moribund state. At termination of the study (124 weeks after the start of the study), the remaining rats, 13 partially corpectomized rats and 6 controls, were killed.

Body Weight

The increase in body weight followed a similar pattern in the two groups (Figure 5), although the control rats had higher mean body weights than the rats operated on up to about 2 years (104 weeks) after surgery.

Plasma Gastrin

An early increase in plasma gastrin was observed after partial corpectomy, and the hypergastrinemia was sustained throughout the study. The gastrin levels in the corpectomized rats increased during the first month of the study to a level approximately 10-fold higher than in the controls, after which no further progressive increase was observed (Figure 6).

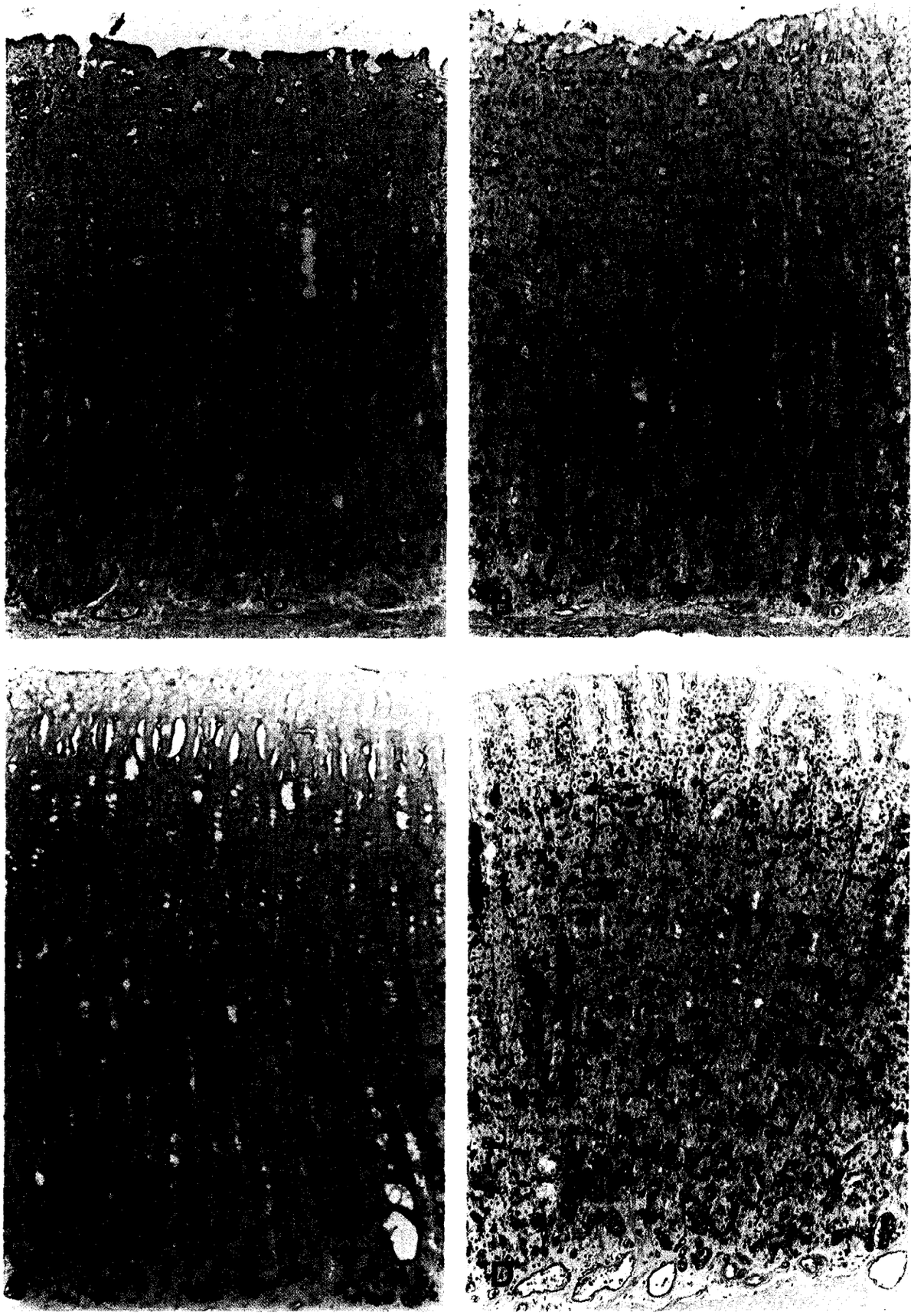

Figure 2. Classification of the argyrophil cell changes: normal pattern in aging rat (*A*) and slight hyperplasia (*B*). For this degree, it is important that the upper third of the mucosa is mainly unaffected by the hyperplasia. With the moderate hyperplasia degree, the increase in number of argyrophil cells is accompanied by extension of the hyperplasia up to the foveolar level of the mucosa (*C*). When further increase in argyrophil cell number together with a general tendency to aggregation of argyrophil cells occurred, the term *moderately severe hyperplasia* was applied (*D*) (×100).

The gastrin level (mean ± SEM) during the whole study period was 2100 ± 160 pg/mL (n = 75) in the partially corpectomized rats and 240 ± 6 pg/mL (n = 40) in the controls. The mean plasma gastrin levels analyzed at death were 1900 ± 170 pg/mL (n = 69) in the partially corpectomized rats and 150 ± 10 pg/mL (n = 40) in the controls.

Argyrophil Cells

As a result of surgery, the stomachs of all partially corpectomized rats had a minor portion of oxyntic mucosa which was bulging out because of thickened foldings (Figure 7).

Microscopically, there was a variety of postsurgical cicatricial changes in the stomachs of the partially corpectomized rats. In accordance with the known disposition of rodents to cicatricial neoplasia (20–23), four cases each of squamous papilloma and carcinoma and fibrosarcoma occurred within the areas operated on. No case of primary adenocarcinoma occurred. Sixty-one of 75 partially corpectomized rats had small, erosive gastric ulcers within the anastomosis area in the oxyntic mucosa or immediately adjacent in antral mucosa. One third of the rats operated on, 26 of 75, were noted for focal gastritis; all except one were of slight degrees related to cicatricial or ulcer changes in the anastomosis area.

In all the rats operated on, the hypertrophy of the remaining oxyntic mucosa was observed macroscopically and was characterized by increased mucosal folding and thickened rugae (Figure 7). In about half

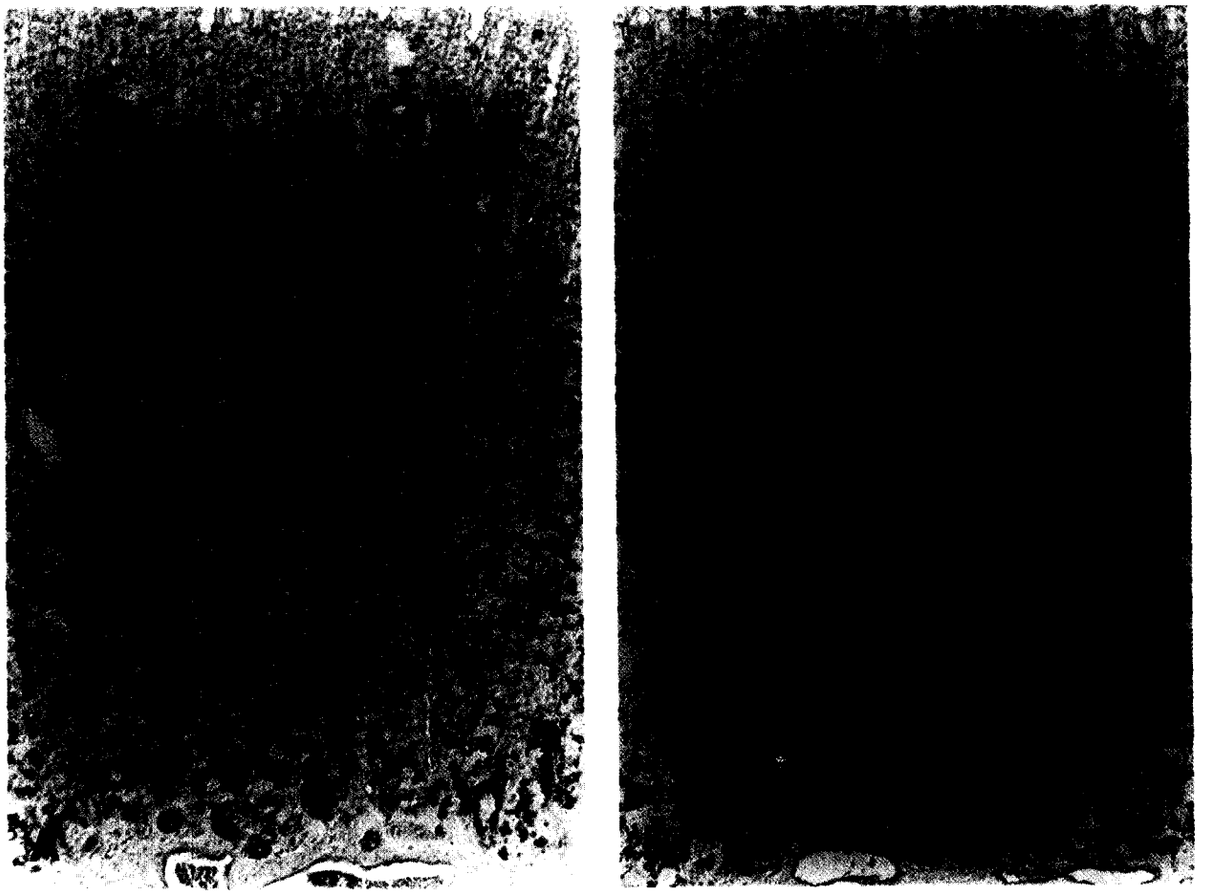

Figure 3. Classification of the argyrophil cell changes. (*A*) There is a severe hyperplasia of argyrophil cells with several glands showing strands of coalescing argyrophil cells that sometimes may be pairs of fused glandular argyrophil cell strands. Micronodular hyperplasia was used when three or more such changes occurred grouped together forming nodules < 500 μm (*B*) (×110).

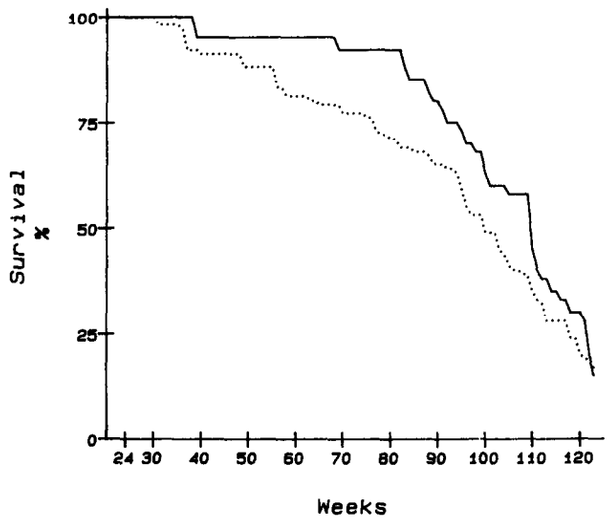

Figure 4. Cumulative survival rate in rats that survived 6 months and more. (–), Controls; (.....) partially corpectomized rats.

of the animals operated on, an increased mucosal thickness was also apparent microscopically. These changes were more frequently seen in the older rats.

There was an increase in both the incidence and the degree of severity of argyrophil cell hyperplasia in older rats in both groups (Table 1), but this hyperplasia was more pronounced in the partially corpectomized rats. Micronodular hyperplasia was seen in 60% of the partially corpectomized rats during the first 2 years after the operation, with marked increase in the incidence during the last months, whereas none was seen in the control group (Table 1). After 2 years, micronodular hyperplasia occurred in 21% of the controls; in the partially corpectomized rats the incidence increased to 94%.

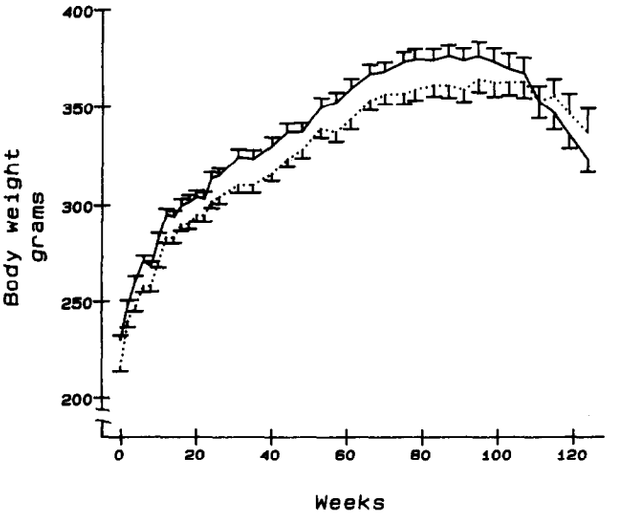

Figure 5. Body weight of the rats during the study (mean ± SEM). (–), Controls; (.....), partially corpectomized rats.

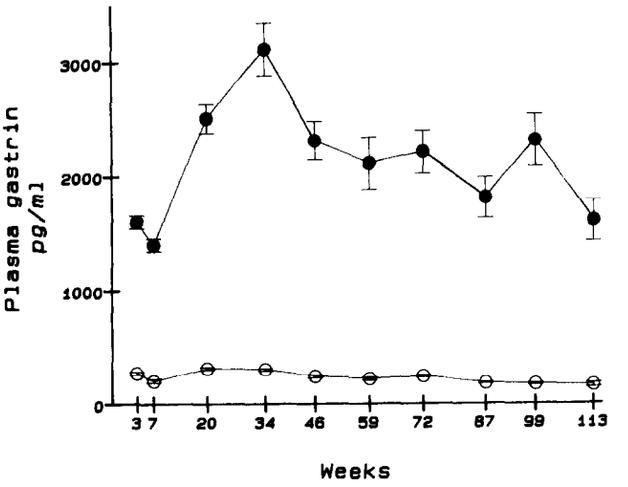

Figure 6. Plasma gastrin levels during the study (mean ± SEM). (○), Controls; (●), partially corpectomized rats.

No carcinoids were observed in the control rats, whereas carcinoids were seen in a total of 26 partially corpectomized rats. Because all of them were argyrophil with the Sevier–Munger staining, they were

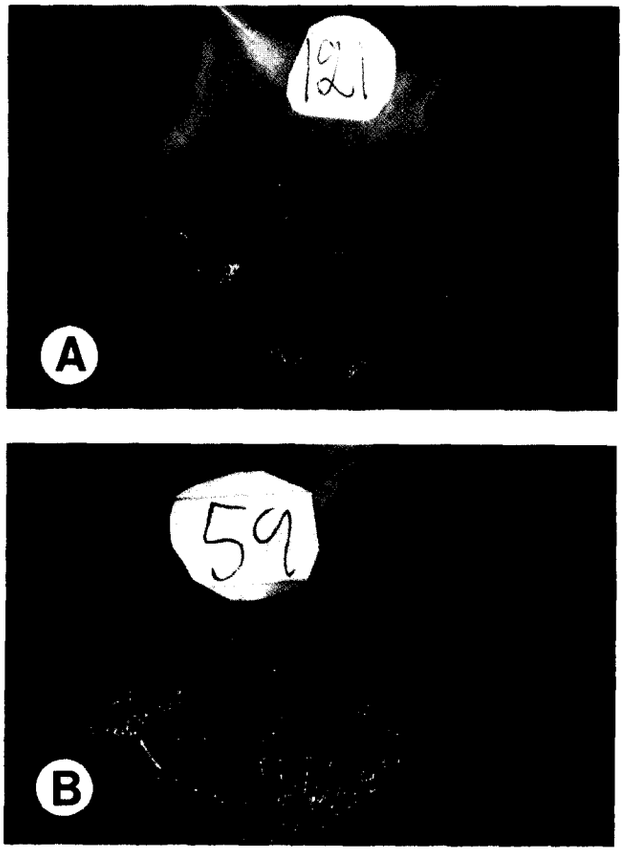

Figure 7. Stomachs from a control rat (*A*) and from a partially corpectomized rat (*B*). These rats were killed on experimental week 124, and the stomachs were opened along the greater curvature.

Table 1. Microscopic Findings of Argyrophil Cell Hyperplasia and Carcinoids During the First 2 Years of the Study and Thereafter

	Control rats	Partially corpectomized rats
Weeks 31–104		
No. examined	16	42
Argyrophil cell hyperplasia (*total* %)	56.3	97.6
Slight (%)	56.3	14.3
Moderate (%)	0.0	35.7
Moderately severe (%)	0.0	42.9
Severe (%)	0.0	4.8
Micronodular argyrophil cell hyperplasia (%)	0.0	59.5
Carcinoids (%)	0.0	14.3
Weeks 105–124		
No. examined	24	33
Argyrophil cell hyperplasia (*total* %)	83.3	100.0
Slight (%)	41.7	3.0
Moderate (%)	37.5	9.1
Moderately severe (%)	4.2	21.2
Severe (%)	0.0	66.7
Micronodular argyrophil cell hyperplasia (%)	20.8	93.9
Carcinoids (%)	0.0	60.6

Note. The incidence of findings is given as the percentage of animals examined within each period.

classified as gastric ECL-cell carcinoids (Figures 8–10). Ten of the rats with carcinoids had multiple carcinoids, of which all except one appeared in rats killed after 112 weeks. The first carcinoid was observed 78 weeks after the start of the study. One rat with carcinoid (4%) was found during experimental week 78, 5 (19%) during weeks 92–104, the remaining 20 rats with carcinoids (77%) were found during week 105 and later. Thus, the carcinoids occurred during the end of the normal life span of the rats. Most of the carcinoids, 21 of 26 rats, were intramucosal ones. Five of the rats had microinvasive carcinoids with extension locally to the submucosal layer. In no case was there any infiltration of muscularis externa, metastasis to regional lymph nodes, or any other spread outside the submucosa.

Discussion

There is a relationship between the proportion of the oxyntic gland mucosa removed and the ensuing plasma gastrin concentrations. Thus, in a previous study stepwise increase in plasma gastrin was found with the highest levels obtained in rats subjected to 90% or 100% corpectomy (24). These earlier findings suggest that the degree of hypergastrinemia is inversely correlated to the amount of acid draining into the antrum.

In the present investigation, the plasma gastrin levels increased soon after the partial corpectomy had been performed and remained high throughout the study. The mean increase in plasma gastrin levels compared with the controls was about 10-fold. In a few rats, the hypergastrinemia was less marked during the latter part of the study, probably because of some restoration of acid secretion following a gastrin-

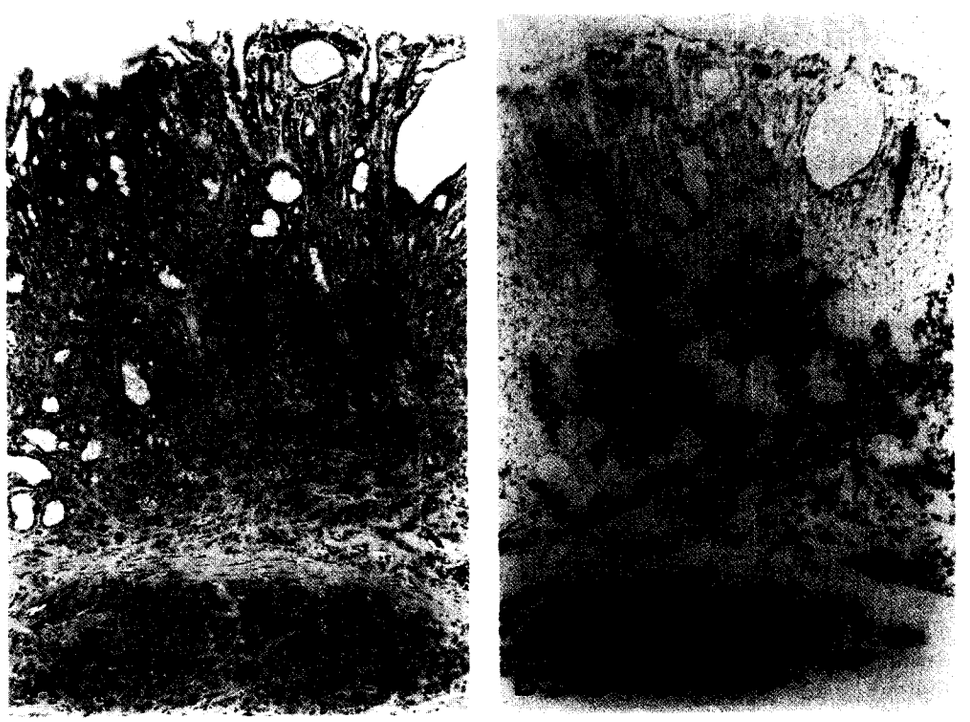

Figure 8. Adjacent sections of an ECL-cell carcinoid with invasion of the submucosa, stained with H&E (*A*) and Grimelius' silver staining (*B*). This carcinoid was found in a rat killed on week 118 with a mean gastrin value of 1400 pg/mL during the study (×55).

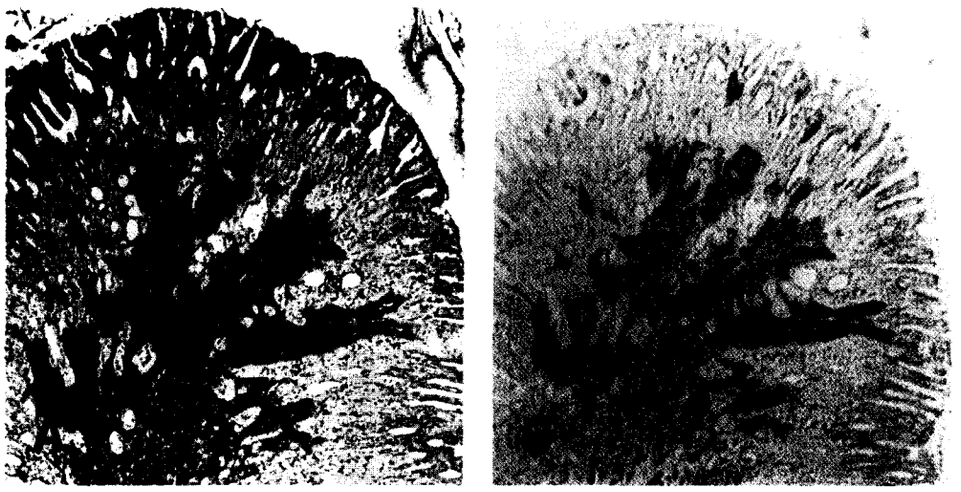

Figure 9. Adjacent sections of an ECL-cell carcinoid silver stained according to Sevier–Munger (*A*) and Grimelius (*B*). The ECL-cell carcinoids stain similarly with the two methods. From a rat killed on week 118 with a mean gastrin level of 3800 pg/mL during the study (×30).

induced increase in the oxyntic mucosal mass (including the parietal cells). This increase in acid-producing capacity results in a lower antral pH, which in turn leads to reduced gastrin release from the antral gastrin cells.

The partially corpectomized, hypergastrinemic rats showed a development pattern of gastric ECL-cell hyperplasia and carcinoids that is analogous to that in rats in which the hypergastrinemia had been induced by treatment with inhibitors of gastric acid secretion (2,3). All the rats with ECL-cell carcinoids had markedly elevated serum gastrin values (2100 ± 210 pg/mL; n = 26) over time compared with the controls (240 ± 6 pg/mL; n = 40). The plasma gastrin levels in corpectomized rats with carcinoids did not differ from those of the other corpectomized rats without carcinoids. The mean gastrin level in the latter rats was 2100 ± 190 pg/mL (in rats killed on week 75 and later; n = 32).

Macroscopically, the gastric ECL-cell carcinoids were difficult to find in the hyperplastic and irregular mucosal foldings, and only one was noted with the naked eye. This is in accordance with previous experience of these lesions. The morphological pattern of the carcinoids did not differ from that described in the previous studies with omeprazole and ranitidine (2,3). In addition, the time lapse between the inducement of hypergastrinemia and the development of hyperplasia and carcinoids was quite similar. The degree of argyrophil cell hyperplasia increased both in controls and in partially corpectomized animals toward the end of the study, but it was considerably more pronounced in the corpectomized rats. The spontaneous increase in the number of gastric ECL cells in aging rats has been reported previously (2,3,25). The carcinoids occurred late in the present study and the incidence and multiplicity increased with age in a manner similar to that previously observed when pharmacological acid inhibition was attained (2,3).

In conclusion, chronic and persistent hypergastrinemia induced by partial corpectomy has been found to be associated with ECL-cell hyperplasia and the development of gastric ECL-cell carcinoids in rats. The carcinoids appeared late in the natural life span of the animals, suggesting the prerequisite of sustained lifelong hypergastrinemia. These results strongly support the hypothesis that the gastric ECL-cell carcinoids found in rats subjected to lifelong administration of antisecretory drugs are caused by hypergastrinemia secondary to inhibition of acid secretion.

Figure 10. Large ECL-cell carcinoid in a rat killed on week 124 with a mean gastrin value of 4700 pg/mL during the study. (Sevier–Munger silver; ×16).

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Address requests for reprints to: Hillevi Mattsson, Ph.D., Associate Professor, Gastrointestinal Research, Department of Biology, AB Hässle, S-431 83, Mölndal, Sweden.

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