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GASTRIN CELL PROLIFERATION AFTER CHRONIC STIMULATION: EFFECT OF VAGAL DENERVATION OR GASTRIC SURGERY IN THE RAT

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SUMMARY

1. Chronic stimulation of the antral gastrin cells by elevated antral pH was achieved by fundectomy, antrum exclusion, fundectomy plus antrum exclusion, antrocolic transposition, and vagal denervation plus pyloroplasty. For comparison we studied also the effects of pyloroplasty alone and of portacaval shunting.

2. All operations that elevated the antral pH resulted in high gastrin concentrations in serum. Particularly high concentrations were observed in fundectomized rats. Vagal denervation of fundectomized or antrum excluded rats reduced the serum gastrin concentration slightly compared with the corresponding innervated animals. Portacaval shunting reduced the gastrin concentration in serum.

3. The antral gastrin concentration was raised or unchanged following fundectomy and vagal denervation, and reduced following antrum exclusion, antrum exclusion plus vagotomy, fundectomy plus antrum exclusion, fundectomy plus vagotomy, antrocolic transposition and portacaval shunt. The gastrin cell density in the antral mucosa was raised following fundectomy, vagotomy, and fundectomy plus vagotomy, unchanged following fundectomy plus antrum exclusion and antrocolic transposition, and reduced following antrum exclusion and portacaval shunting.

4. Ultrastructurally the gastrin (G) cells in the excluded antrum and in the antrum of fundectomized rats showed signs of secretory activity in that the granule volume density or the number of granules per unit cytoplasm was lowered. In the fundectomized rats moreover, the endoplasmic reticulum of the G cells was increased, the Golgi area enlarged and the proportion and volume density of electron dense granules greatly increased. The granule profile diameter was not affected by either antrum exclusion or fundectomy.

5. The results on the excluded antrum indicate that elevated antral pH *per se* is not sufficient to produce gastrin cell proliferation. In the fundectomized rats, where the hyperlasia of antral gastrin cells was considerable, there is the added stimulus of ingested food. In fundectomized plus antrum excluded rats this stimulus is eliminated and no proliferation ensues. The passage of intestinal material, as in the rats subjected to antrocolic transposition, did not elicit gastrin cell proliferation which seems

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to suggest that the character of the luminal material is important. We propose therefore that gastrin cell proliferation is due to the combined stimulation of high antral pH and passage of food. Vagal innervation is not required.

INTRODUCTION

Gastrin cells were demonstrated in the antral mucosa by McGuigan in 1968. Since then our knowledge of their histochemical and ultrastructural properties has increased considerably (Forssman, Orci, Pictet, Renold & Rouiller, 1969; Vassallo, Solcia & Capella, 1969; Pearse & Bussolati, 1972; Larsson, Sundler, Håkanson, Grimelius, Rehfeld & Stadil, 1974).

In man, a high density of antral gastrin cells has been described in achlorhydria (Creutzfeldt, Arnold, Creutzfeldt, Feurle & Ketterer, 1971; Polak, Coulling & Doe, 1971; Pearse, Bussolati & Polak, 1972; Polak, Hoffbrand, Reed, Bloom & Pearse, 1973; Stockbrügger, Larsson, Lundquist & Angervall, 1977). Experimental data from rats support the view that the antral gastrin cell population is susceptible to quantitative variation (Sundler, Håkanson, Larsson, Liedberg, Oscarson, Rehfeld & Stadil, 1974; Lehy, Voillemot, Dubrasquet & Dufougeray, 1975; Delince, Willems & de Graef, 1978).

In the present study we have examined the effects of vagal denervation, gastric surgery or portacaval shunting on the antral gastrin cell population of the rat in an attempt to elucidate the mechanisms that control gastrin cell proliferation.

METHODS

Adult male Wistar rats (weighing 150–200 g at time of surgery if not otherwise stated) were used. Vagal denervation was performed on eighty-five rats by cutting both vagal trunks immediately below the diaphragm. A pyloroplasty was always made at the same time to prevent gastric dilation. Sixty-nine rats received a pyloroplasty alone. In seventy-two rats the entire oxyntic gland area of the stomach was removed by wedge resection (fundectomy). Continuity was re-established by a simple end-to-end anastomosis between the prepyloric part and the rumen. The nervous and vascular supply to the remaining part of the stomach was spared except that in sixteen of these rats vagotomy plus pyloroplasty was added. Antrocolic transposition was performed on fourteen rats as described by Lehy, Bonnefond, Dubrasquet, Nasca, Lewin & Bonfils (1973). The antrum with the pylorus was freed from the rest of the stomach and the duodenum, sectioned and transposed with the innervation and blood supply intact to the transverse colon. The fundus was then joined with the duodenum end-to-end. Antrum exclusion (fifty-seven rats) was achieved by transecting the stomach at the borderline between the fundic (oxyntic) and the antral (pyloric) mucosa. The vagal innervation was spared except in nineteen rats (antrum exclusion plus vagotomy). The antrum was closed by suture, and the fundic part of the stomach anastomosed to a jejunal loop end-to-side. In twenty-six rats fundectomy plus antrum exclusion was carried out. Porta-caval shunt was made on 100 rats (weighing 300–350 g at time of surgery) by anastomosing the peripheral end of the divided portal vein to the inferior caval vein end-to-side. Thus, the liver was deprived of its portal blood supply. Eighty-six age-matched rats were sham-operated by a mid-line incision in the abdomen. All operated rats were allowed to recover for 6–10 weeks, porta-caval shunted rats for at least 8 weeks before being used in the experiments. At sacrifice (9–10.30 a.m.) the rats were either fed freely or fasted for 48 hr with free access to drinking water. Blood was drawn from the aorta under diethyl ether anesthesia, and serum lyophilized until analysis. The stomach was divided into full-thickness antrum and fundus after excision of the rumen. The antrofundic junction is clearly visible in the

rat stomach. Mucosa was scraped off the pyloric gland area with a scalpel, weighed immediately, homogenized in boiling redistilled water and heated at 100 °C for 20 min. After centrifugation at 10,000*g* for 15 min at 0 °C, the supernatant was lyophilized and stored in the deep-freeze until assayed for gastrin in dilutions from 1:3 to 1:10,000. Measurement of gastrin concentrations in serum and tissue extracts was performed by *radioimmunoassay* (Stadil & Rehfeld, 1971, 1973) using antiserum 2604-8 raised against synthetic human gastrin (2–17) (Rehfeld, Stadil & Rubin, 1972). This antiserum measures gastrin components I–III with equimolar potency (Rehfeld, 1976). For histological examination (fasted rats only), a 2 × 3 mm strip was cut from the major curvature approx. 1 mm from the pyloric sphincter. The strips were frozen to the temperature of liquid nitrogen in a mixture of propane and propylene, and freeze-dried. The freeze-dried specimens were treated with formaldehyde gas at +80 °C for 1 hr and embedded in paraffin. Sections (6 μm) were cut from the long side perpendicular to the mucosal surface, deparaffinized and processed for gastrin *immunohistochemistry* as described in detail elsewhere (Larsson, Sundler, Håkanson, Rehfeld & Stadil, 1973). Gastrin cells were counted at 125 × magnification (objective 10×, eye piece 12·5×, visual field diameter 1·53 mm). Cells in two randomly selected visual fields (entire thickness of mucosa visible) from each of five sections from each of the different animals were counted. Each group comprised at least six rats. For each rat, a mean cell count per unit area was obtained by averaging the counts in each section and then averaging the mean result of the five sections together. Comparisons between the groups with respect to the number of gastrin cells per visual field were made with Student's *t* test. For *electron microscopy* five fasted rats from three of the groups studied (no operation, antrum exclusion, fundectomy) were anaesthetized with diethyl ether and perfused via the heart with glutaraldehyde (2·5% in 0·075 M-sodium phosphate buffer, pH 7.2) for 5–10 min. Small blocks of mucosa were cut from the pyloric gland area and immersed in the fixative for 1–2 hr. (For a discussion of fixation of gastrin cells see Mortensen & Morris, 1977.) The material from each experimental group was pooled. All specimens were post-fixed for 1 hr in 1% osmium tetroxide in buffer, dehydrated in graded ethanol solutions, contrasted *en bloc* in a mixture of 1% phosphotungstic acid and 0·5% uranyl acetate and embedded in Epon 812. Ultrathin sections (60–80 nm) were cut on an LKB Ultrotome, contrasted with uranyl acetate and lead citrate and examined in a Philips EM 300 electron microscope.

At least 100 sections, randomly selected from each experimental group, were examined for gastrin (G) cells. The cells were photographed and used for morphometry only if sectioned in such a way that the nucleus was visible. The photographs were reproduced in magnification × 30,000. Morphometric analysis was performed using the point counting technique (multipurpose test system) described by Weibel & Bolender (1973). The results are expressed as μm^2 cell area or cytoplasm area. The error of this method was determined by repeating after a few months interval the morphometric procedure on ten gastrin cells from control rats. The standard deviation of a single measurement was calculated according to Eränkö (1955) and expressed as per cent of mean. The method errors were 1·7% for the cell profile area, 2·0% for the nucleus and 3·0% for the cytoplasm. The cytoplasmic granules in each gastrin cell section were counted. The number and proportion of granules displaying high electron density in each cell was also recorded. Granules were divided into three arbitrary types: (1) 'electron dense', (2) 'pale' with either a homogenous core of lower electron density or an eccentrically placed core surrounded by an electron-lucent halo, (3) 'electron lucent' without any electron-dense material. Since it was possible to classify 'electron dense' granules with greater confidence than either of the other two forms, only the differences in the proportion of electron dense granules was analysed statistically. Granule profile size was established by measuring the profile diameter of all granules in at least ten cells from each experimental group.

RESULTS

The results of the determinations of serum gastrin concentrations are summarized in Table 1. The highest concentrations were recorded in the fundectomized rats. The serum gastin concentration in these animals was not affected by the prandial state. Less elevated serum gastrin concentrations were measured following fundectomy plus vagal denervation, fundectomy plus antrum exclusion, antrum exclusion alone

and antrocolic transposition. Also in these groups the serum gastrin level was unaffected by fasting or feeding. The serum gastrin concentration was slightly lower following antrum exclusion plus vagotomy than following antrum exclusion alone (not statistically significant). Vagal denervation *per se* raised the serum gastrin concentration when compared to controls (rats with pyloroplasty). The denervated freely fed rats had higher serum gastrin concentration than the fasted ones ($P < 0{\cdot}001$). In porta-caval shunted rats the serum gastrin concentration in the freely fed group was significantly lower ($P < 0{\cdot}001$) than in freely fed sham operated age-matched animals.

TABLE 1. Effect of vagal denervation, gastric surgery or portacaval shunt on gastrin concentrations in serum

	pg SHG equiv/ml.	
	Freely fed	Fasted
No operation	224 ± 17·0 (50)	46 ± 3·3*** (47)
Antrum exclusion	311 ± 34·1† (12)	320 ± 33·9††† (22)
Antrum exclusion plus vagal denervation	234 ± 21·0 (10)	240 ± 40·5††† (5)
Fundectomy	970 ± 85·8††† (11)	865 ± 191·5††† (8)
Fundectomy plus vagal denervation	470 ± 97 (4)	568 ± 108††† (4)
Fundectomy plus antrum exclusion	300 ± 28·0 (9)	349 ± 47††† (6)
Antrocolic transposition	177 ± 22·2 (4)	214 ± 5·3††† (4)
Pyloroplasty	264 ± 23·9 (21)	52 ± 5·2*** (30)
Vagal denervation plus pyloroplasty	345 ± 33·8 (24)	193 ± 12·6***††† (61)
Sham operation	234 ± 16·3 (52)	38 ± 5·1*** (15)
Portacaval shunt	132 ± 9·3††† (55)	45 ± 3·5*** (45)

Mean ± S.E. (n = number of rats). Student's t test was used to establish significant differences: † or * for $0{\cdot}01 < P < 0{\cdot}05$, †† or ** for $0{\cdot}001 < P < 0{\cdot}01$, and ††† or *** for $P < 0{\cdot}001$. The symbol * denotes the difference between fasted rats and freely fed rats within one horizontal row. The symbol † denotes the difference between an operation and its control within one vertical row. The group under the heading no operation is control for pyloroplasty, antrum exclusion, fundectomy, fundectomy plus vagal denervation, fundectomy plus antrum exclusion and antrocolic transposition. Pyloroplasty is control for vagotomy. Sham operation is control for portacaval shunt.

The antral gastrin content and concentration in the various experimental groups is presented in Table 2. In unoperated rats the gastrin content and concentration was significantly lowered by 48 hr fasting. Pyloroplasty reduced the antral gastrin concentration and content in the freely fed state. Irrespective of the prandial state vagotomy plus pyloroplasty resulted in an increased amount of gastrin in the antrum when compared to pyloroplasty alone. The lowest gastrin concentration was observed in the excluded antrum; with or without fundus made no difference. The gastrin concentration was reduced also following antrocolic transposition. In all these animals the gastrin concentration was unaffected by the prandial state. The gastrin concentration in freely fed fundectomized rats was not different from that in the unoperated rats; in fasted fundectomized rats the gastrin concentration was higher than in the unoperated controls. Upon portacaval shunting the gastrin concentration and content was considerably reduced both in fasted animals and in freely fed animals.

We determined the number of gastrin cells per visual field and made no attempt to estimate the total gastrin cell mass; changes in local cell density may not always

TABLE 2. Effect of vagal denervation, gastric surgery or portacaval shunt on antral gastrin concentration and content

	μg SHG equiv per g mucosa		ng SHG equiv per antrum	
	Freely fed	Fasted	Freely fed	Fasted
No operation	13·7 ± 1·5 (12)	3·7 ± 0·3*** (43)	1487 ± 147 (12)	319 ± 33*** (43)
Antrum exclusion	0·7††† ± 0·1 (11)	0·7††† ± 0·4 (7)	19††† ± 3 (11)	25††† ± 15 (7)
Antrum exclusion plus vagotomy	1·1††† ± 0·3 (6)	—	31††† ± 9 (6)	—
Fundectomy	11·7 ± 2·3 (12)	8·3† ± 1·5 (27)	1418 ± 270 (12)	823† ± 166 (27)
Fundectomy plus antrum exclusion	0·8††† ± 0·2 (9)	0·3††† ± 0·05 (5)	15††† ± 3 (9)	9††† ± 2 (5)
Antrocolic transposition	1·0††† ± 0·4 (4)	0·5††† ± 0·1 (4)	56††† ± 7 (4)	39††† ± 16 (4)
Pyloroplasty	4·5††† ± 0·6 (12)	4·8 ± 0·6 (29)	338††† ± 49 (12)	294 ± 37 (29)
Vagal denervation plus pyloroplasty	6·2 ± 0·6 (12)	6·1 ± 1·0 (31)	615† ± 55 (12)	499† ± 85 (31)
Sham operation	9·4 ± 0·6 (24)	7·8 ± 1·0 (33)	817 ± 75 (24)	643 ± 117 (34)
Portacaval shunt	4·7††† ± 0·4 (9)	2·7††† ± 0·4** (18)	474†† ± 85 (17)	271††† ± 48* (18)

Mean ± S.E. (n). Student's t test was used to establish significant differences: † or * for $0·01 < P < 0·05$, †† or ** for $0·001 < P < 0·01$, and ††† or *** for $P < 0·001$. The symbol * denotes the difference between fasted rats and freely fed rats (within one horizontal row). The symbol † denotes the difference between an operation and its control (within one vertical row). The group under the heading no operation is control for pyloroplasty, antrum exclusion, antrum exclusion plus vagotomy, fundectomy, fundectomy plus antrum exclusion, and antrocolic transposition. Pyloroplasty is control for vagotomy. Sham operation is control for portacaval shunt.

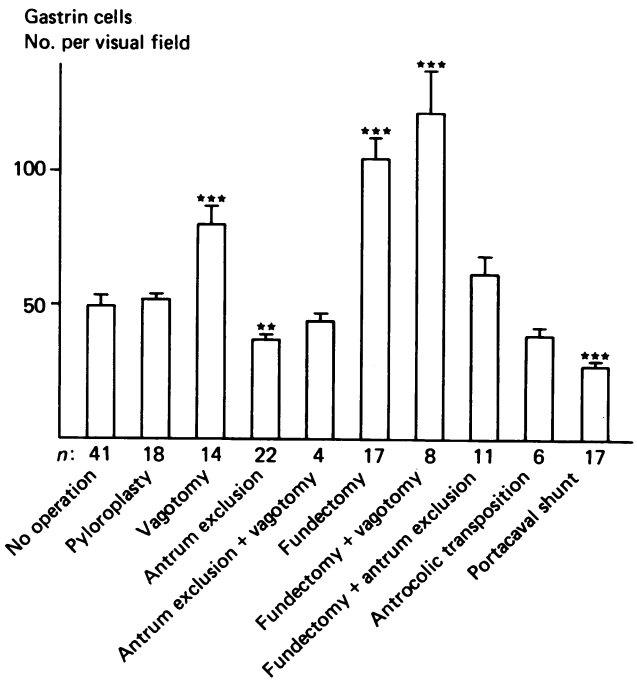

Fig. 1. Antral gastrin cell count following vagal denervation, gastric surgery or portacaval shunting. The rats were fasted for 48 hr. Vertical bars give S.E. (n = number of rats).** for $0{\cdot}001 < P < 0{\cdot}01$ and *** for $P < 0{\cdot}001$ refer to difference between operated and unoperated rats.

TABLE 3. Effects of antrum exclusion or fundectomy on ultrastructural properties of antral G cells

Treatment	No. of cells analysed	Cell size (μm^2)	Nuclear size (μm^2)	Nuclear volume density (% of cell area)	Cytoplasm (μm^2)
No operation	38	57·2 ± 3·8	15·3 ± 1·1	27·9 ± 1·8	41·7 ± 3·4
Antrum exclusion	31	57·2 ± 3·5	16·8 ± 1·0	31·5 ± 2·2	40·4 ± 3·4
Fundectomy	60	63·5 ± 2·2	16·9 ± 0·9	27·5 ± 1·6	46·6 ± 2·1

Treatment	No. of cells analysed	Cell size (μm^2)	Granule volume density (% of cytoplasm)	No. of granules per cell profile	No. of granules per μm^2 cytoplasm
No operation	38	57·2 ± 3·8	26·7 ± 1·7	185 ± 17·8	4·5 ± 0·3
Antrum exclusion	31	57·2 ± 3·5	18·5* ± 1·9	140* ± 19·9	3·3*** ± 0·2
Fundectomy	60	63·5 ± 2·2	21·3* ± 1·3	157 ± 11·5	3·4*** ± 0·2

Mean ± S.E. of mean (n = number of cells). Student's t test was used to establish significant differences between unoperated and operated rats: * for $0{\cdot}01 < P < 0{\cdot}05$, ** for $0{\cdot}001 < P < 0{\cdot}01$, and *** for $P < 0{\cdot}001$.

reflect changes in total cell mass since the size of the antral mucosa may vary. The number of gastrin cells per visual field was raised by fundectomy and by vagotomy, unchanged by antrocolic transposition, and reduced by antrum exclusion and porta-

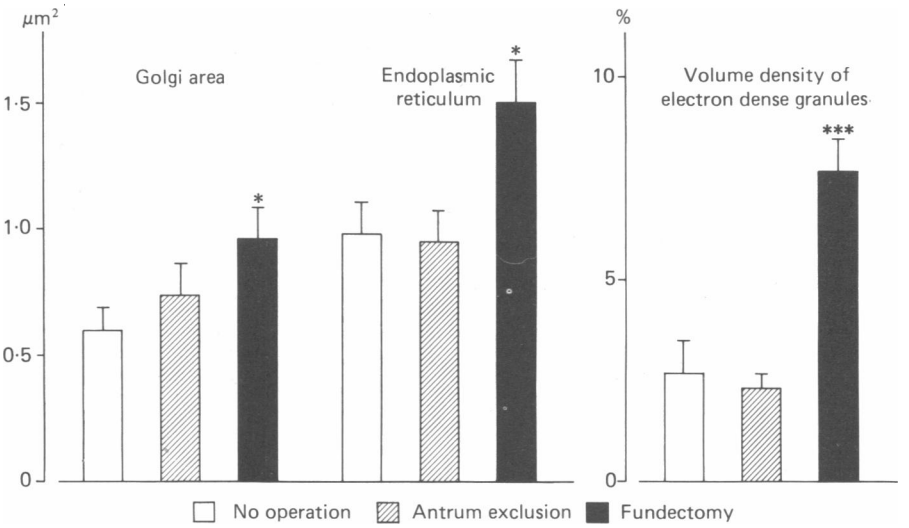

Fig. 2. The Golgi area and the area occupied by endoplasmic reticulum together with the percentage of cytoplasm occupied by electron dense secretory granules in gastrin cells from unoperated, antrum excluded or fundectomized rats. The material from which these results were collected is the same as in Table 3. Vertical bars give S.E.* for $0{\cdot}01 < P < 0{\cdot}05$,*** for $P < 0{\cdot}001$.

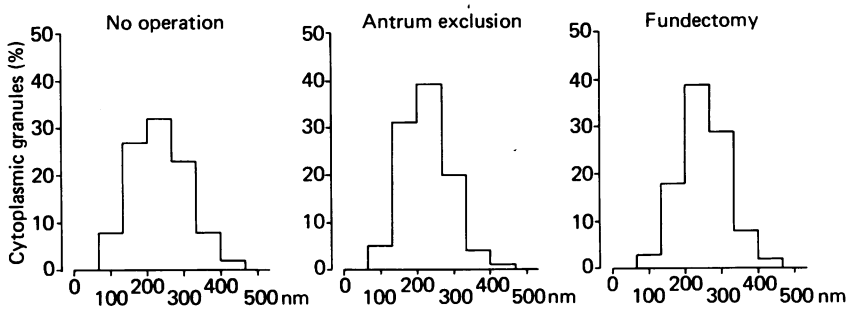

Fig. 3. Size distribution histograms (diameter in nm) of secretory granules in gastrin cells from unoperated, antrum excluded or fundectomized rats. See also Table 4.

caval shunting (Fig. 1 and Pl. 1). In the fundectomized rats the gastrin cells regularly formed clusters of 4–8 cells (Pls. 1 and 2). Following antrum exclusion plus fundectomy there was no change in the gastrin cell number compared with unoperated controls and there was no clustering of cells. Vagal denervation of fundectomized or antrum excluded rats raised the gastrin cell density somewhat compared with the corresponding innervated animals (not statistically significant) (Fig. 1).

Electron microscopic examination revealed that the G cells in the excluded antrum had a reduced granule volume density compared to G cells in the antrum of unoperated

controls ($P < 0.05$) (Pl. 3). G cells in fundectomized rats were slightly enlarged (not statistically significant) with a reduced granule volume density and a reduced number of granules per unit cytoplasm ($P < 0.05$) (Table 3 and Pl. 4). Highly electron-dense granules were more numerous in the gastrin cells of the fundectomized rats than in those of the unoperated rats (Pls. 4 and 5). This was reflected in a higher proportion

TABLE 4. G cell morphometry: mean granule profile diameter (nm)

Operation	Granule profile diameter
Control	250 ± 1·9 (1687)
Antrum exclusion	240 ± 1·6 (1591)
Fundectomy	268 ± 1·7 (1618)

Mean ± S.E. of mean (n = number of granules). Each group comprised granules from ten to fifteen cells. Analysis of variance was performed. The F value for the difference between unoperated and fundectomized rats was 1·122, not significant.

of electron dense granules in fundectomized rats than in unoperated rats or antrum excluded rats: 33·5 ± 2·5 % (S.E.) of the G cell granules in fundectomized rats were electron dense; the corresponding figure in the control rats was 10·3 ± 3·1 % and in the antrum excluded rats 26·9 ± 6·7 %. Also the volume density of electron-dense granules was higher in the G cells of fundectomized rats than in those of unoperated rats (Fig. 2). Moreover, the endoplasmic reticulum was greatly increased and the Golgi area enlarged upon fundectomy (Fig. 2 and Pl. 5). The granule profile diameter was not affected by either antrum exclusion or fundectomy (Table 4 and Fig. 3).

DISCUSSION

Antral pH controls gastrin release by a sensitive feed-back mechanism. Accordingly the serum gastrin concentration was greatly elevated in all experimental situations involving high antral pH. The highest concentration was observed upon fundectomy. By comparison, antrum exclusion and vagal denervation were much less effective. Failure of mechanisms for elimination and degradation of gastrin may also result in a high serum gastrin level. The roles of the kidneys (El Munshid, Håkanson, Liedberg, Rehfeld & Sundler, 1980) and small intestine (Becker, Reeder & Thompson, 1973*b*) in the degradation of endogenous gastrin have received much attention. The gastric fundus is also considered to be an important site of inactivation of circulating gastrin (Evans, Reeder, Becker & Thompson, 1974). Therefore it seemed likely that fundectomy by removing one important site of gastrin degradation exaggerated the hypergastrinaemia.

In the fundectomized rat the number of gastrin cells per visual field was greatly increased. Lehy *et al.* (1975) described gastrin cell hyperplasia following antrocolic transposition, a finding which we have not been able to confirm. Since the gastrin cell number was lowered following antrum exclusion and unchanged following antrocolic transposition, prevention of acid from reaching the pyloric glands cannot explain the hyperplasia following fundectomy. Possibly, ingested food provides a stimulus that together with the elevated pH will promote gastrin cell hyperplasia. Alternatively, the acid-secreting region of the stomach is the source of an agent which

serves to suppress gastrin cell proliferation and possibly gastrin release. Removal of this agent by fundectomy would elicit gastrin cell hyperplasia. However, the antral gastrin concentration in rats which had been subjected to antrum exclusion plus fundectomy was even lower than in those with antrum exclusion alone which seems to rule out any involvement of a fundic agent. Moreover, the serum gastrin concentration in the fundectomized plus antrum excluded animals was not different from that upon antrum exclusion alone. The results favour the view that the passage of food supplies the stimulus which serves to maintain a normal gastrin cell population and that the passage of food plus chronic antral stimulation, e.g. in the form of elevated pH will result in gastrin cell hyperplasia. However, complicating factors seem to exist. Following fundectomy or antrocolic transposition the antrum is in continuity with the digestive tract and consequently in both cases trophic stimulation by food might be expected. But while fundectomy resulted in gastrin cell hyperplasia, antrocolic transposition did not. On the contrary, the gastrin concentration was reduced. Perhaps the character of the luminal material that passes the gastrin cells is important; saliva, for instance, may contain agents which are stimulatory to the gastrin cells (see Levine, 1965).

Also, the vagus nerve plays an important role in the regulation of gastrin release from the antrum. Electrical stimulation of the antral vagus releases large amounts of gastrin (Becker, Reeder & Thompson, 1973*c*, *d*). The elevated serum gastrin level after vagotomy (Becker *et al.* 1973*d*; Håkanson Kroesen, Liedberg, Oscarson, Rehfeld & Stadil, 1974; Schrumpf, Roland & Liavåg, 1974) is probably brought about by loss of acid inhibition. Concomitantly, there was an increase in antral gastrin content and concentration (see also Becker, Reeder & Thompson, 1973*e*; Hughes & Hernandez, 1976; Becker, Arnold, Börger, Creutzfeldt, Schafmayer & Creutzfeldt, 1977) and in gastrin cell number (see also Becker *et al.* 1977; Delince *et al.* 1978). Conceivably, this is the result of the concomitant action of ingested food and high pH. Vagal denervation of fundectomized or antrum excluded rats seems to impair gastrin release since in both groups the serum gastrin concentrations were slightly lower than in the corresponding innervated rats although the gastrin concentration in the antrum was the same and the gastrin cell number somewhat increased compared with the corresponding innervated animals. Hence, vagal denervation does not prevent gastrin cell hyperplasia, on the contrary, vagotomy seems to facilitate the proliferation.

Porta-caval shunting is known to cause gastric hypersecretion in many species (Lebendinskaja, 1932; Gregory, 1958). One possible explanation may be that bypassing the liver elevates the serum gastrin level. This hypothesis could not be confirmed in the present study. On the contrary, porta-caval shunting lowered the serum gastrin concentration in freely fed rats. This may be a reflexion of the reduced number of gastrin cells in the antrum. There is nothing in these observations that explains the hypersecretion following porta-caval shunting. Instead, the reduced number of gastrin cells may be secondary to the acid hypersection. On the whole, the rate of secretion of gastic acid seems to correlate inversely with the gastin cell mass in that hypoacidity is associated with a higher number and normo- and hyperacidity with a normal or reduced number of gastrin cells.

Thus, the results of the present study confirm that the serum gastrin concentration depends on an interplay of a number of factors such as the type of release signals to

the gastrin cells and the rate of removal of circulating gastrin by the sites of degradation. In addition, however, the amount of available tissue gastrin may play a role.

Ultrastructurally, the gastrin (G) cells in the excluded antrum and in the antrum of fundectomized rats exhibited slightly reduced granule volume density (i.e. the proportion of cytoplasm occupied by granules), increased endoplasmic reticulum and enlarged Golgi area. We interpret these changes as signs of stimulated secretory activity. In fasted control rats we found the majority of the secretory granules in the G cells to be electron lucent (for contrasting reports see Creutzfeldt, Track, Creutzfeldt & Arnold, 1975; Track, Creutzfeldt, Arnold & Creutzfeldt, 1978). In the fundectomized rats the proportion and volume density of electron-dense granules was greatly increased.

The gastrin release mechanism is still poorly understood (for a recent discussion see Sato, 1978). Exocytosis is the process commonly associated with peptide hormone release. Forssmann & Orci (1969), however, proposed that gastrin was released into the cytoplasm from otherwise intact granules to leak out from the cell. As a result of this intracytoplasmatic release the gastrin granules were said to become less electron dense (see also Creutzfeldt *et al.* 1975). Other workers have speculated that the electron-density of cytoplasmic granules reflects, among many things, the age of the granules, 'young' granules being more electron dense than 'old' ones; Golgi-associated (newly formed, 'immature') granules generally displayed a higher electron density than more peripherally located (older) granules (see also Solcia, Vassallo & Sampietro, 1967; Fujita & Kobayashi, 1973; Mortensen & Morris, 1977). The G cells of unoperated fasted rats contained a mixture of electron-lucent and electron-dense granules, the electron-lucent ones being in majority. The granule volume density was reduced and the proportion of electron dense granules was increased following antrum exclusion as well as fundectomy, an observation which seems to be in agreement with the view that electron-dense granules are 'young'. The volume density of electron-dense granules (as percentage of cytoplasm) was greatly increased following fundectomy. Although we have not been able to demonstrate signs of exocytosis such as granules opening directly into the extracellular space (omega figures), a phenomenon which is generally thought to accompany exocytosis, our observations are clearly compatible with the exocytosis hypothesis.

In the fundectomized, but not in the antrum excluded, rats, the G cells seem to be under the influence not only of excitatory stimulation but also of trophic stimulation, causing a marked hyperplasia. It is evident from the present study that chronic excitation of gastrin cells in the form of high antral pH, as in the antrum excluded rats, does not invariably lead to gastrin cell proliferation. It appears that a combination of stimuli is required, such as the passage of food past the antrum (the passage of intestinal content is evidently not sufficient stimulus) and high antral pH. The precise mechanisms behind gastrin cell hyperplasia remain to be identified.

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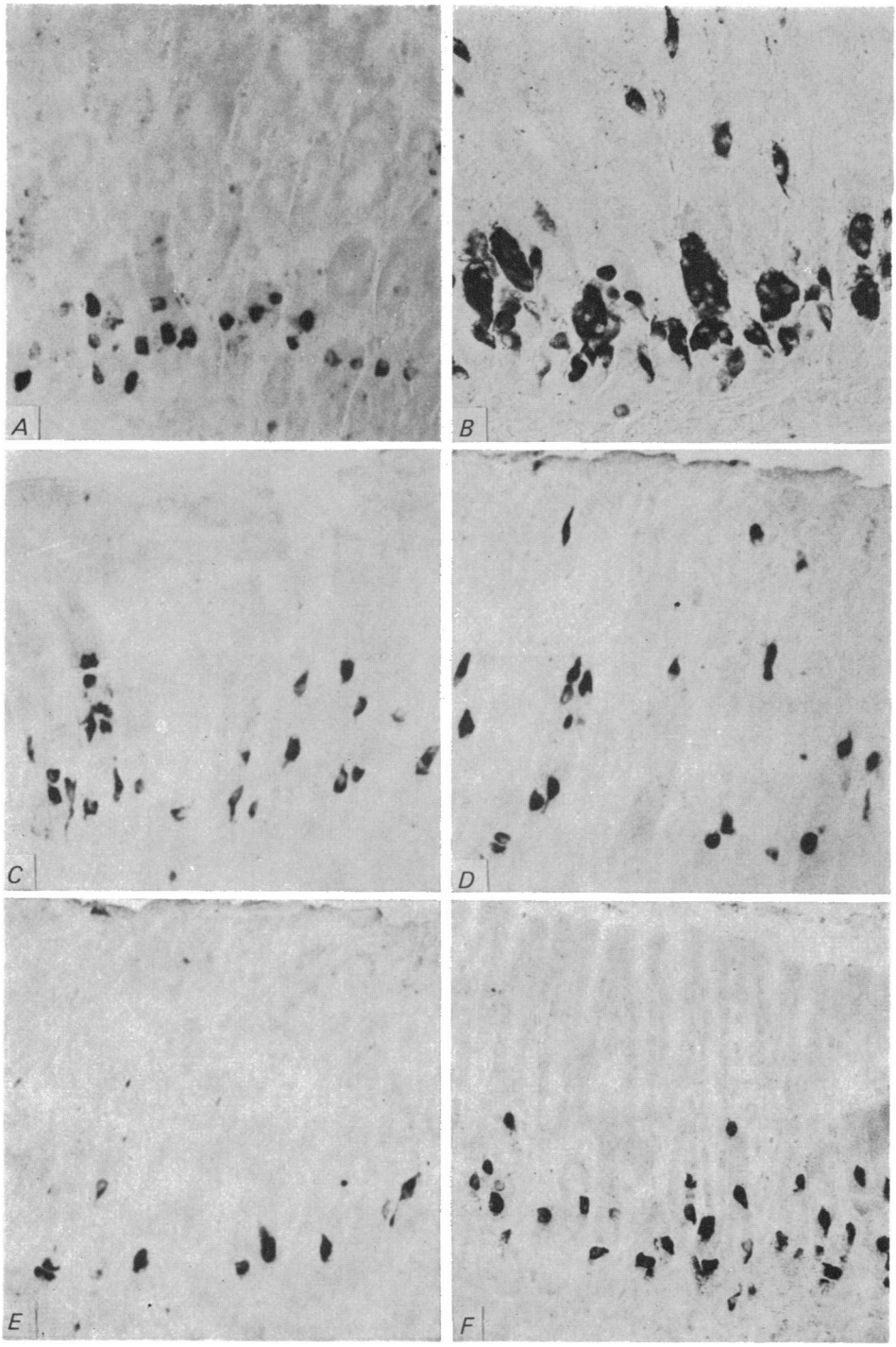
EXPLANATION OF PLATES

PLATE 1

Gastrin cells in the antrum demonstrated by the immunoperoxidase (PAP) technique. *A*, no operation; *B*, fundectomy (note the gastrin cell clusters); *C*, antrum exclusion; *D*, antrocolic transposition; *E*, portacaval shunt; and *F*, vagal denervation (× 200). The gastrin cell density was increased following fundectomy and vagal denervation, unchanged following antrocolic transposition and reduced following antrum exclusion and portacaval shunt. Note that gastrin cells were found not only at the base of the pyloric glands but also higher up following vagal denervation, fundectomy, antrum exclusion, and in particular following antrocolic transposition (see also Lehy *et al.* 1975).



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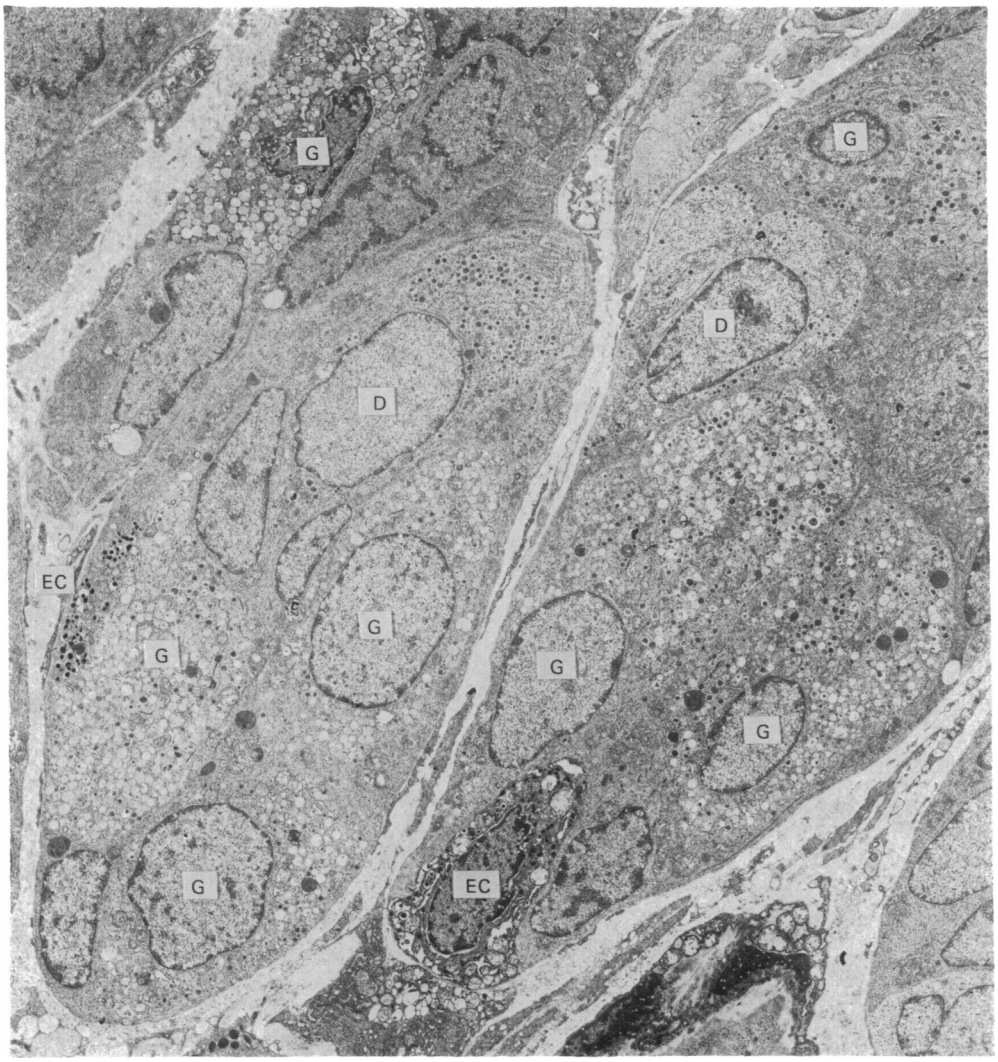
(*Facing p.* 568)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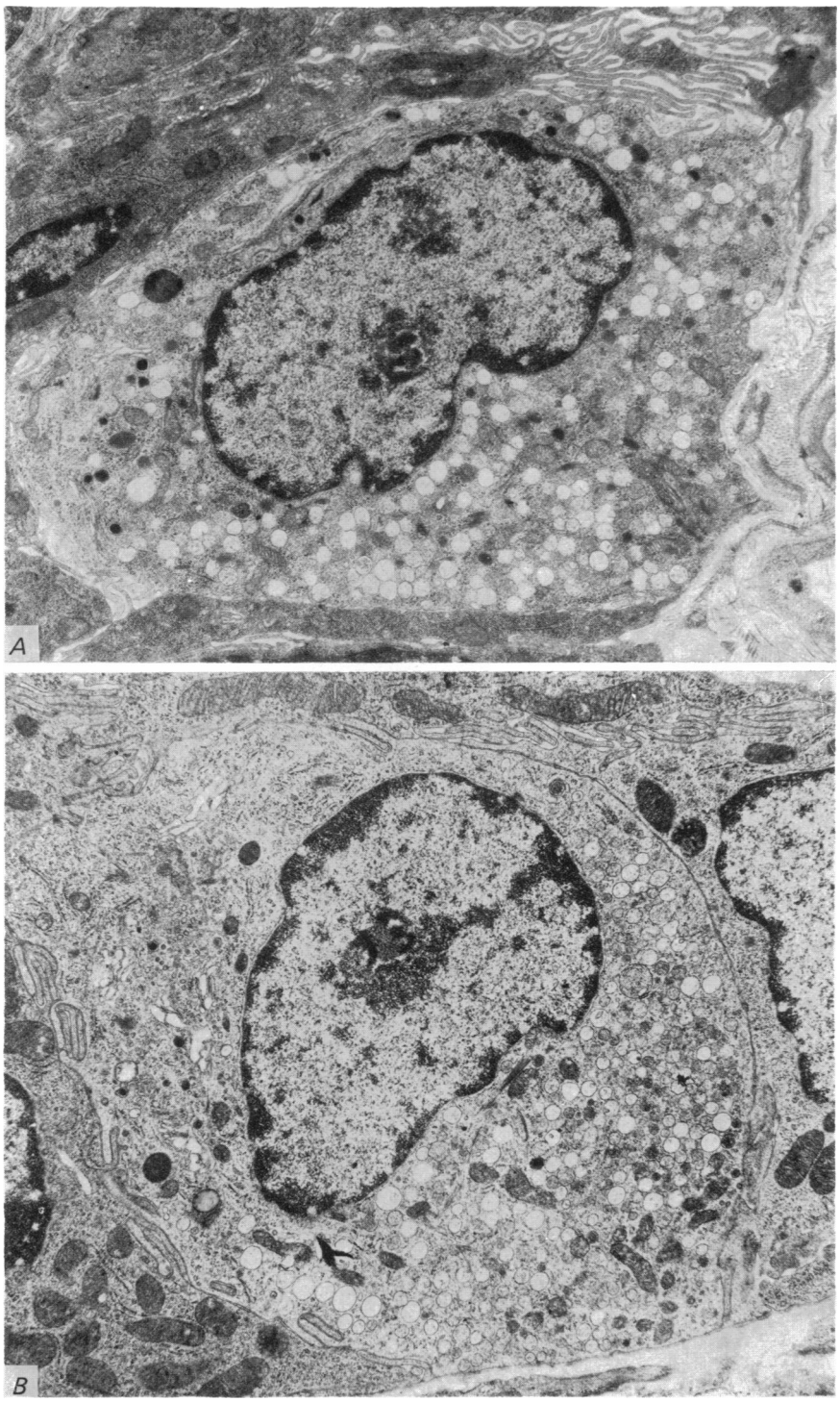
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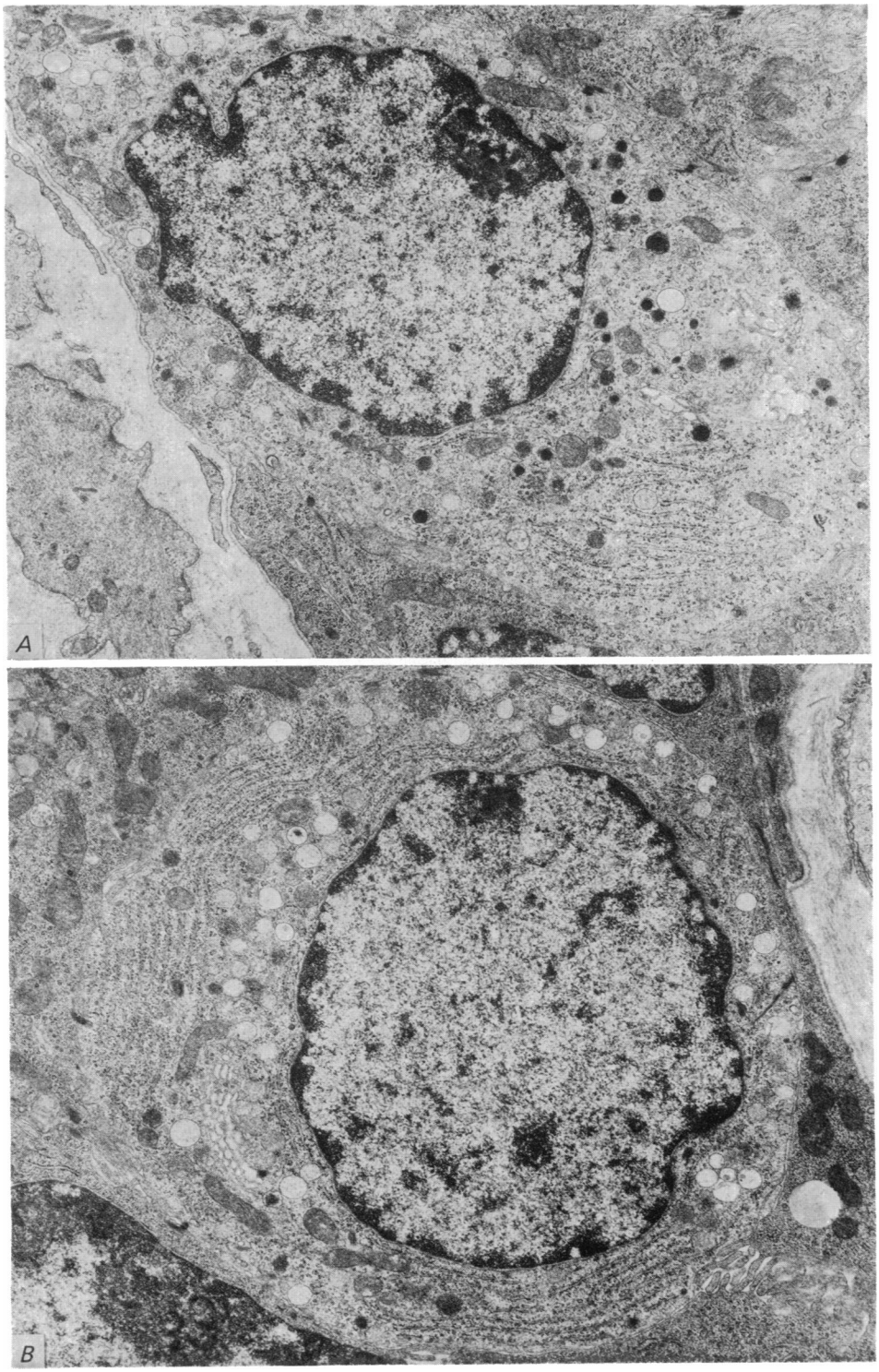

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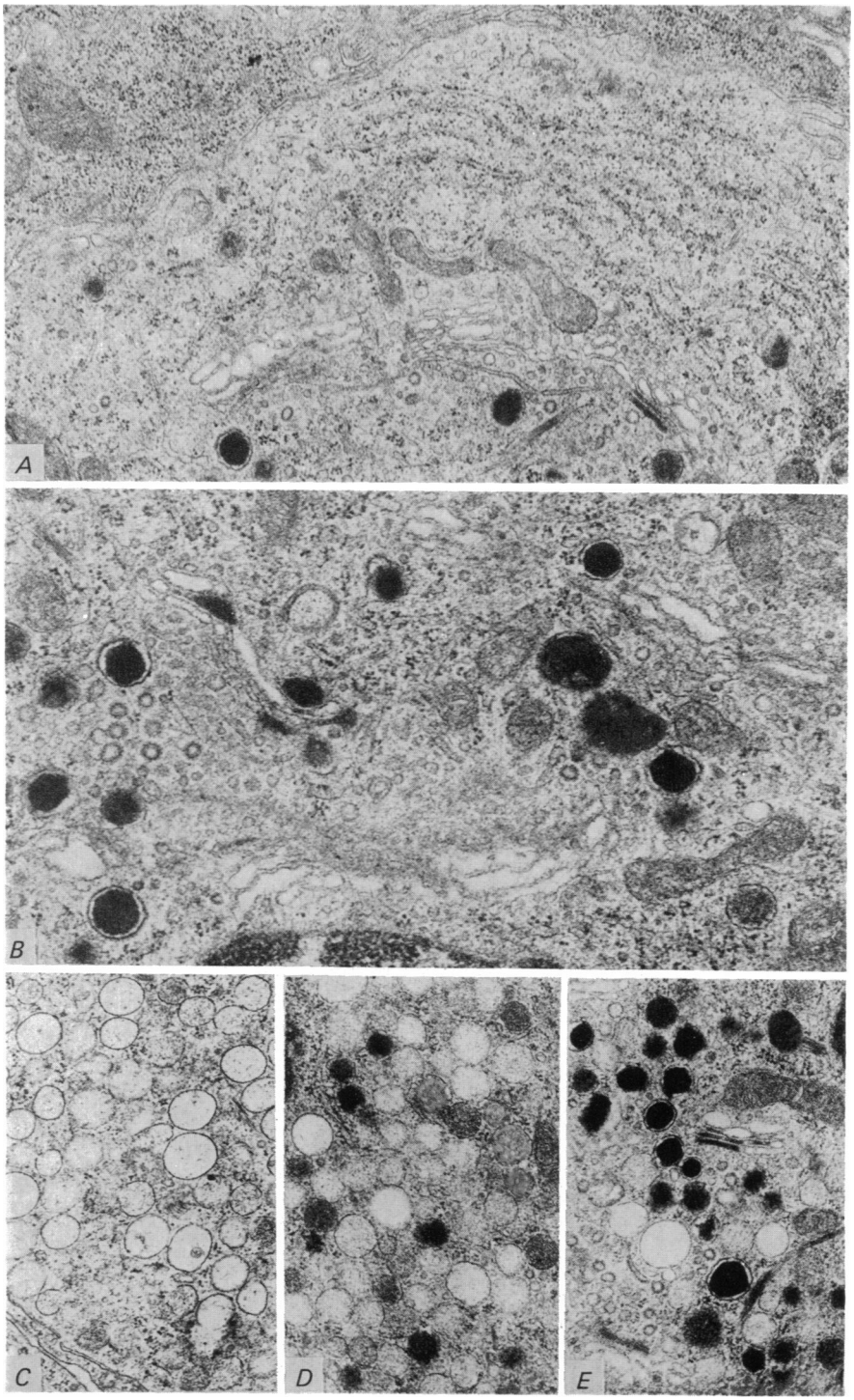

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PLATE 2

Low-magnification electron micrograph showing the base of a pyloric gland in a fundectomized rat. Endocrine cells are numerous. Gastrin cells are labelled G, enterochromaffin cells EC, and somatostatin cells D. × 4000.

PLATE 3

Electron micrographs of gastrin (G) cells, selected to illustrate the effects of antrum exclusion. *A*, no operation, *B*, antrum exclusion. × 12,000.

PLATE 4

Electron micrographs of two gastrin (G) cells (*A* and *B*), both selected to illustrate the effects of fundectomy. Note the reduced granule volume density. Note also that highly electron-dense granules occur in greater proportion following fundectomy and that the endoplasmic reticulum is increased and the Golgi area enlarged. × 12,000.

PLATE 5

High-magnification electron micrographs of highly active gastrin (G) cells from fundectomized rats to show details of (*A*) the well-developed endoplasmic reticulum, (*B*) the enlarged Golgi area, and (*C–E*) cytoplasmic granules of varying electron density: 'electron lucent' (*C*), 'pale' (*D*), and 'electron dense' (*E*). × 20,000.