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EFFECT OF ANTRUM EXCLUSION ON ENDOCRINE CELLS OF RAT STOMACH

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SUMMARY

1. Following antrum exclusion the serum gastrin concentration was raised and independent of the prandial state. The antral gastrin concentration and number of gastrin cells were greatly lowered.

2. The histamine content and the number of histamine-storing endocrine ('enterochromaffin-like') cells in the oxyntic mucosa was almost doubled and the mucosal histidine decarboxylase activity was greatly elevated following antrum exclusion.

3. At the ultrastructural level both types of histamine-storing endocrine cells (ECL and A-like) were found to be enlarged and to have a reduced number of granules per unit cytoplasm. These changes are compatible with an increased secretory activity. The G (gastrin) cells were not increased in size but their granule volume density was lowered.

4. We propose that antrum exclusion results in uninhibited gastrin release causing profound changes in the histamine-storing endocrine cells of the oxyntic mucosa. The cells respond to the hypergastrinemia by an increase in functional activity (activation of histidine decarboxylase and reduction of granule volume density) as well as by an increase in number and size.

INTRODUCTION

Endocrine cells are numerous in the gastric mucosa of the rat. They comprise five major cell populations: gastrin cells and 5-hydroxytryptamine-storing enterochromaffin cells in the pyloric gland area (Håkanson, 1970; Larsson, Håkanson, Rehfeld, Stadil & Sundler, 1974); somatostatin cells which have a fairly uniform distribution in the entire glandular mucosa (cf. Alumets, Sundler & Håkanson, 1977); and histamine-storing 'enterochromaffin-like' cells which are of two different types, ECL and A-like cells, both of which occur in the oxyntic gland area where they are very numerous (Håkanson & Owman, 1967, 1969; Håkanson, Owman, Sporrong & Sundler, 1971; see also Solcia, Capella, Vassallo & Buffa, 1975).

Gastrin stimulates at least one, possibly both of the two histamine-storing endocrine cell types (ECL and A-like cells) of the oxyntic mucosa, causing release of histamine and activation of the histamine-forming enzyme, histidine decarboxylase (Håkanson, Kroesen, Liedberg, Oscarson, Rehfeld & Stadil, 1974). Moreover, antral

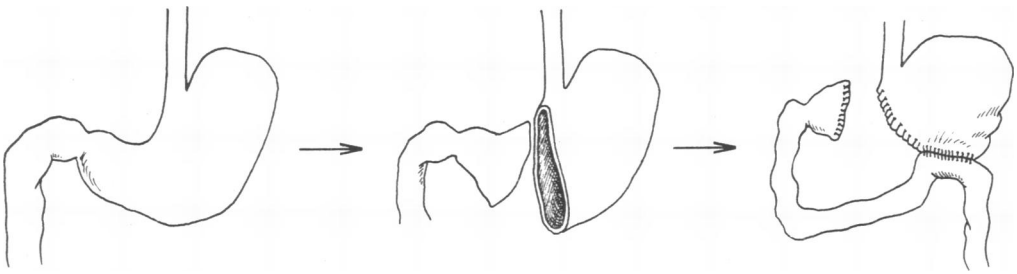
gastrin seems to exert trophic control of the ECL and A-like cells (Håkanson, Larsson, Liedberg, Oscarson, Sundler & Vang, 1976). Thus, antrectomy reduced the number of both cell types and in addition, the ECL cell size was greatly reduced (Håkanson *et al.* 1976).

The present study is concerned with the effects on the various gastric endocrine cells of long-lasting hypergastrinaemia induced by antrum exclusion.

METHODS

Experimental

Adult male Wistar or Sprague-Dawley rats (body weight 150–250 g were used. Antrum exclusion was performed successfully on fifty-eight rats by separating the antrum from the rest of the stomach and closing it blindly. The rest of the stomach was joined to the jejunum end-to-side (Text-fig. 1). Before closing the antrum, remaining oxyntic mucosa was trimmed off in order to prevent acid from reaching the gastrin cells. The effectiveness of this trimming was subsequently verified histologically in each animal. The nervous and vascular



Text-fig. 1. Schematic drawing illustrating the procedure used in excluding the antrum.

supply to both parts of the stomach was spared in all but five rats which were subjected to bilateral subdiaphragmatic vagal denervation. The survival rate following antrum exclusion was about 50 %; several of the rats that died had ulcers in the oxyntic gland area. Surviving rats were killed 6–10 weeks after surgery. If not otherwise stated each rat was fasted for 48 hr before killing (at between 9 and 11 a.m.). Blood was drawn from the abdominal aorta under diethyl ether anaesthesia until exsanguination. Approximately 10 % of the antrally excluded rats had gastric ulcer.

In one series of experiments, L-DOPA (100 mg/kg) was injected I.P. to four antrally excluded rats and seven control rats. The rats were killed 1·5 hr later.

Fluorescence microscopy

Pieces from the oxyntic and pyloric portions of the stomach were frozen to the temperature of liquid nitrogen in a propane–propylene mixture. The specimens from the oxyntic gland area were taken from the major curvature about 5 mm from the ridge separating the rumen from the glandular mucosa. The specimens from the pyloric gland area were 3 × 3 mm squares cut from the major curvature about 1 mm from the pyloric sphincter. After freeze-drying, some of the specimens were exposed to gaseous formaldehyde for 1 hr at 80 °C according to the Falck–Hillarp technique (Björklund, Falck & Owman, 1972) for the demonstration of catecholamines and 5-hydroxytryptamine whereas other specimens were heated for 1 hr at 80 °C without formaldehyde. The tissue blocks were then embedded in paraffin *in vacuo* and sectioned at 6 μm thickness. Sections from specimens not treated with formaldehyde were exposed to gaseous *o*-phthaldialdehyde (OPT) for the demonstration of histamine (Brody, Håkanson, Owman & Sundler, 1972). The sections were mounted in xylene or Entellan (Merck) and examined in a fluorescence microscope. Immunohistochemical demonstration of gastrin cells and somatostatin cells was performed as

follows: sections from formaldehyde-fixed specimens were deparaffinized and exposed to anti-gastrin serum (raised against synthetic human gastrin I 2–17 covalently bound to serum albumin; dilution 1:640) or anti-somatostatin serum (diluted 1:40; a kind gift from Dr M. P. Dubois, Nouzilly, France). After incubation for 3 hr in a moist chamber at room temperature the sections were rinsed thoroughly in phosphate-buffered saline. Fluoresceinated anti-rabbit-IgG serum from goat (Statens Bakteriologiska Laboratorium, Stockholm, Sweden), diluted 20-fold, was applied for another 30 min. After rinsing, the sections were mounted in buffered glycerine (pH 7·2). The enterochromaffin cells exhibited a yellow formaldehyde-induced fluorescence (due to the presence of 5-hydroxytryptamine) (cf. Håkanson, 1970) that could easily be distinguished from the green immunofluorescence. Controls were treated identically except that the antisera were inactivated by the addition of synthetic human gastrin I (I.C.I., England) or synthetic ovine somatostatin (Beckman, Switzerland) (10 μg/ml. diluted antiserum).

The fluorescence microscope was equipped with an epi-illumination system. For examination of OPT-induced fluorescence peak excitation was at 365 nm, for formaldehyde-induced fluorescence at 405 nm and for immunofluorescence at 490 nm.

The number of epithelial cells displaying gastrin or somatostatin immunofluorescence or formaldehyde-induced fluorescence was established by examining sections cut transversally to the mucosal surface at a magnification of 125 × (objective 10 ×, eye-piece 12·5 ×, visual field diameter 1·53 mm). Cells in five randomly selected visual fields (entire thickness of mucosa visible) from each section were counted. At least four sections from each animal were examined. Cell counts are expressed as number of cells per visual field.

Electron microscopy

Four antrally excluded and four sham-operated Wistar rats (anaesthetized with diethyl ether) were perfused via the aorta with glutaraldehyde (2·5 % in 0·075 M-sodium phosphate buffer, pH 7·2) for 5–10 min. Small pieces were taken from the gastric mucosa and immersed in the fixative overnight (16–20 hr). The tissue specimens were post-fixed for 1 hr in 1 % osmium tetroxide in 0·075 M-phosphate buffer, dehydrated in graded ethanol solutions, contrasted *en bloc* in a mixture of 1 % phosphotungstic acid and 0·5 % uranyl acetate in ethanol and embedded in Epon. Ultrathin sections (60–80 nm) were stained with uranyl acetate and lead citrate and then examined and photographed in a Philips EM 300 electron microscope.

The material from each experimental group was pooled, antrum and oxyntic gland area separately. At least a hundred sections, randomly selected, were examined for gastrin cells in specimens from the pyloric gland area and for the two predominant endocrine cell types (ECL and A-like) in the oxyntic gland area. The cells, which were identified by their characteristic cytoplasmic granules, were photographed and used for morphometry only if sectioned in such a way that the nucleus was visible. The photographs were reproduced in magnification 15,000 ×. 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 ECL cells from control rats. The standard deviation of a single measurement was calculated according to Eränkö (1955) and expressed as % mean. The method errors were 1·6 % for the cell profile area, 2·2 % for the nucleus and 3·2 % for the cytoplasm. The cytoplasmic granules in each endocrine cell section were counted. Granule profile size was established by measuring the profile diameter of all granules in eight to twelve cells of each type from each experimental group.

Because only those endocrine cells which showed their nuclei were photographed, the nuclear volume density was systematically overestimated. By using the formula of Konwinski & Kozlowski (1972), intended for spherical cells and nuclei, this overestimation can be compensated. Because the sections have a finite thickness, a certain error is included also in the figures for granule volume density (Holmes effect). In addition, volume changes and/or distortion of the tissue during the processing for electron microscopy contribute certain errors. However, as the changes induced by the surgical treatments were of greater interest to us than the values in absolute terms we decided to present the experimental data uncorrected.

Chemical analysis

Determination of gastric histidine decarboxylase activity. The stomachs were cut open along the greater curvature and the mucosal surface was cleaned with 0·9 % saline. The mucosa of the oxyntic gland area was scraped off and homogenized in 0·1 M-phosphate buffer, pH 6·9–7·0, to a final concentration of 100 mg (wet weight) per ml. Aliquots of 0·5 ml. of the homogenates were incubated with [1-^{14}C]L-histidine in the presence of pyridoxal-5-phosphate at 37 °C for 1 hr under nitrogen. Total reaction volume was 0·53 ml. The amount of $^{14}CO_2$ formed during the reaction was measured by liquid scintillation counting (Håkanson *et al.* 1974; Håkanson, Larsson, Liedberg, Rehfeld & Sundler, 1977).

Determination of histamine. The homogenate of the oxyntic mucosa was rehomogenized in equal volumes of 3 % trichloroacetic acid. After centrifugation, the supernatant was diluted 1:50 with redistilled water and 100 μl. were taken for histamine assay as described by Håkanson & Rönnberg (1974).

Determination of 5-hydroxytryptamine. The mucosa of the pyloric gland area was scraped off, weighed and placed in 2 ml. 80% acetone at +4 °C overnight. This treatment extracts all 5-hydroxytryptamine (cf. Hanson, 1966). The acetone extract was evaporated to dryness *in vacuo* and the dry residue was extracted twice with 2 ml. acidified *n*-butanol (100 ml. *n*-butanol + 100 μl. conc. HCl). 5-Hydroxytryptamine was then returned to an aqueous phase by shaking with 1·5 ml. 0·1 N-HCl and 6 ml. heptane. Aliquots (0·5 ml.) of the HCl extract were taken for fluorometric determination of 5-hydroxytryptamine by condensation with *o*-phthaldialdehyde (Maickel, Cox, Saillant & Miller, 1968).

Determination of gastrin. Serum was freeze-dried and stored at −20 °C until analysed for gastrin using a radioimmunoassay for human gastrin (Stadil & Rehfeld, 1973). The details of this procedure as applied to rat serum have been given in a previous communication (Håkanson *et al.* 1974). The mucosa of the pyloric gland area was scraped off, weighed and boiled in 5 ml. redistilled water for 20 min. The precipitate was spun down and the supernatant was freeze-dried and stored at −20 °C until measurement of gastrin concentrations in dilutions from 1:10 to 1:10,000.

RESULTS

Chemical analysis

The antral gastrin concentration was much lower in the antrally excluded rats than in unoperated rats and moreover, the concentration was not affected by fasting or feeding (Text-fig. 2). In the antrally excluded rats the serum gastrin concentration was not affected by fasting or feeding (Text-fig. 3). In the fasted state the serum gastrin concentration was increased sixfold following antrum exclusion as compared to unoperated controls. The concentration of 5-hydroxytryptamine in the mucosa of the pyloric gland area was 24·6 μg/g ± 1·8 (S.E. of mean, n = 4) in the control group and 26·1 μg/g ± 4·0 (n = 4) in the antrally excluded rats. Oxyntic mucosal histamine content was almost doubled and histidine decarboxylase was greatly activated by antrum exclusion (Text-fig. 4). One group of rats was subjected to antrum exclusion + vagotomy. The serum gastrin concentration in these animals was 240 pg SHG equiv/ml. ± 40 (S.E. of mean, n = 5), the oxyntic mucosal histamine concentration was 110 μg/g ± 11 (S.E. of mean, n = 3), and the histidine decarboxylase activity was 126 p-mole CO_2/mg.hr ± 17·9 (S.E. of mean, n = 4). These values are not different from those of rats subjected to antrum exclusion alone.

Light microscopy

In sections from formaldehyde-treated specimens the enterochromaffin cells, which are numerous in the pyloric gland area, were detected by their yellow fluorescence, reflecting their content of 5-HT. Antrum exclusion reduced the number of these cells

(Table 1). Gastrin and somatostatin cells were demonstrated by immunofluorescence. Their number was lower in the excluded antrum than in control antrum (Table 1). In specimens from L-DOPA-loaded rats endocrine cells throughout the gastric mucosa but most numerous in the oxyntic gland area displayed green formaldehyde-

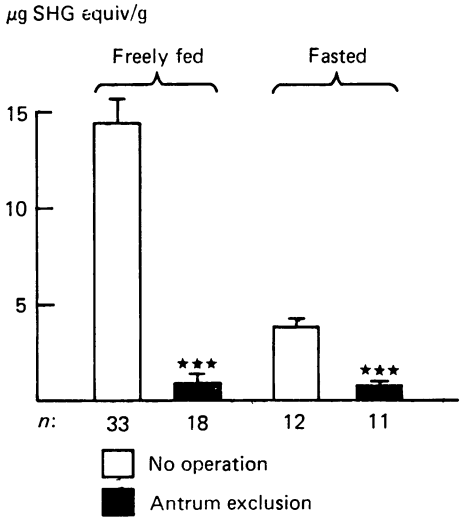


Fig. 2

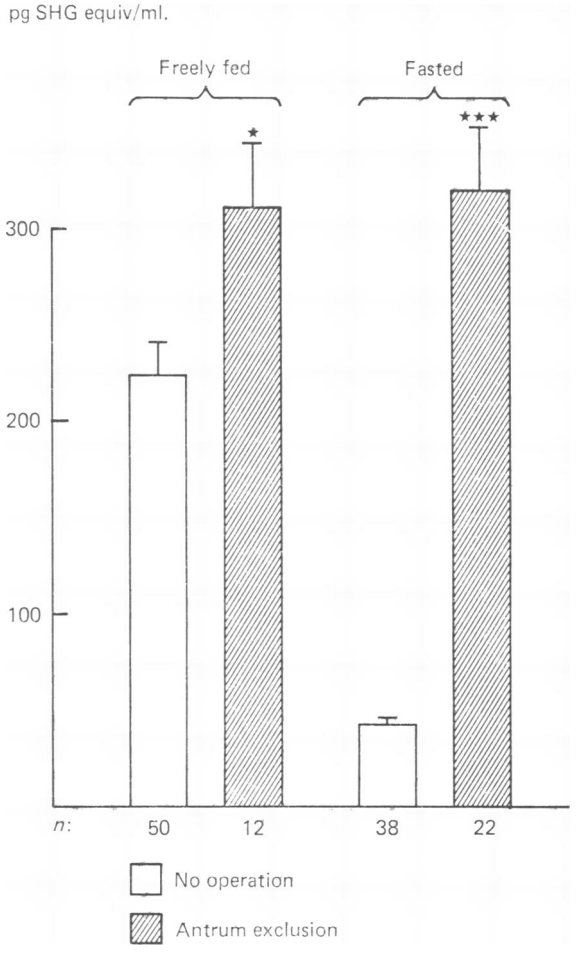


Fig. 3

Text-fig. 2. Concentration of gastrin in antral mucosa in control rats and in antrally excluded rats. The animals were either fasted (for 48 hr) or freely fed. Mean ± S.E. of mean. Statistical difference between antrally excluded rats and control rats was assessed by Student's t test. $P < 0{\cdot}001$.

Text-fig. 3. Effect of antrum exclusion on the serum gastrin concentration. The animals were either fasted (for 48 hr) or freely fed. Vertical bars give S.E. of mean.

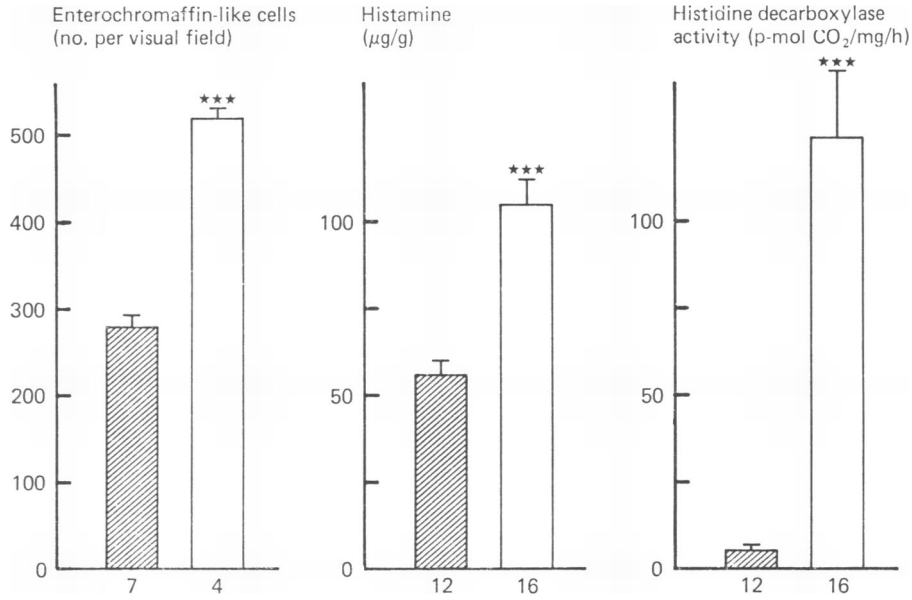
* $0{\cdot}01 < P < 0{\cdot}05$, ** $0{\cdot}001 < P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

induced fluorescence, reflecting their content of dopamine. These cells have been referred to as 'enterochromaffin-like' (see Håkanson, 1970). Antrum exclusion greatly increased the number of such cells in the oxyntic mucosa (Text-fig. 4). The endocrine cells in the oxyntic mucosa can also be demonstrated by their OPT-induced fluores-

cence, which reflects their content of histamine (cf. Håkanson & Owman, 1967). After antrum exclusion the number of endocrine cells that displayed OPT-induced fluorescence was much increased, from visual estimation by more than 50%.

Electron microscopy

The histamine-storing endocrine cells in the oxyntic mucosa are of two types: one with granules of vesicular appearance (ECL cells) and another with round, electron dense cytoplasmic granules (A-like cells) (Pls. 1 and 2). Both types of cells were affected by antrum exclusion. Of ninety endocrine cells examined fifty-three (59 %) were ECL cells, thirty (33 %) were A-like and seven (8 %) belonged to other categories.



Text-fig. 4. Effect of antrum exclusion on the number of enterochromaffin-like cells, histamine content and histidine decarboxylase activity in the oxyntic mucosa (fasted rats). The enterochromaffin-like cells were demonstrated by virtue of their dopamine content following administration of L-DOPA. Antrum exclusion (open bars), no operation (hatched bars). S.E. of mean is indicated. *** $P < 0{\cdot}005$.

TABLE 1. Effect of antrum exclusion on number of endocrine cells in gastric mucosa

	No. of cells/visual field	
	Controls	Antrum exclusion
Pyloric gland area		
Enterochromaffin cells	50 ± 3·9 (7)	34* ± 3·4 (5)
Gastrin cells	60 ± 4·5 (7)	25*** ± 3·0 (5)
Somatostatin cells	45 ± 4·0 (7)	17*** ± 2·6 (4)
Oxyntic gland area		
Somatostatin cells	30 ± 3·5 (7)	22 ± 1·7 (4)

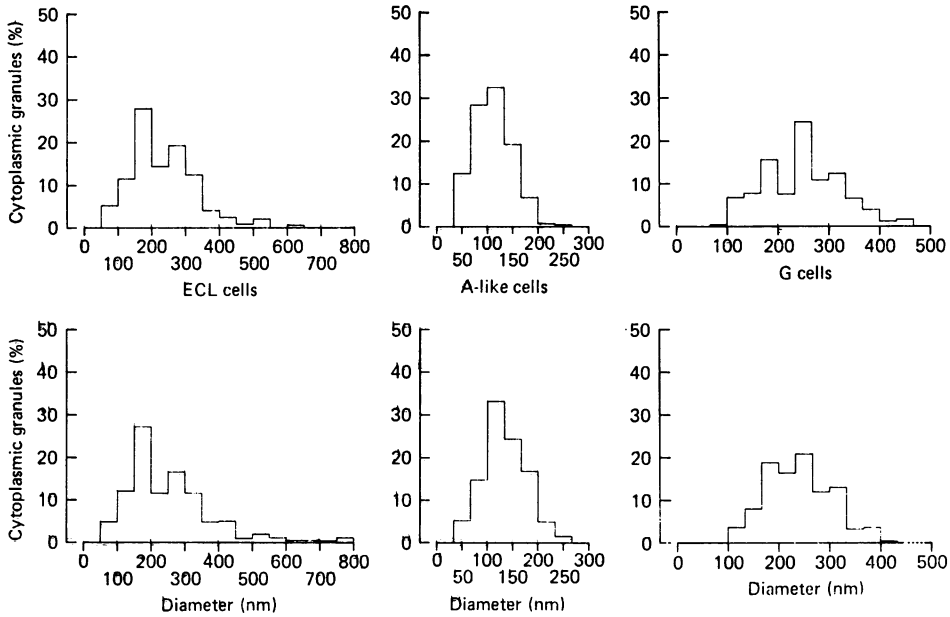
Mean ± S.E. of mean (n = number of animals) Student's t test was used to establish significant differences between unoperated and operated rats: * for $0{\cdot}01 < P < 0{\cdot}05$, ** for $0{\cdot}001 < P < 0{\cdot}01$, and *** for $P < 0{\cdot}001$.

TABLE 2. Effects of antrum exclusion on ultrastructural properties of ECL, A-like and G cells

Cell type	Treatment	No. of cells analysed	Cell size (μm^2)	Nuclear size (μm^2)	Nuclear volume density (% of cell area)	Cytoplasm (μm^2)	Granule volume density (% of cytoplasm)	No. of granules per cell profile	No. of granules per μm^2 cytoplasm
ECL cells	Controls	55	26·1 ± 1·4	9·3 ± 0·7	37·0 ± 2·0	16·8 ± 1·2	28·5 ± 1·5	86 ± 7·3	5·3 ± 0·2
	Antrum exclusion	58	34·6*** ± 2·0	14·7*** ± 1·0	42·7 ± 1·8	19·8 ± 1·3	20·1*** ± 1·7	42*** ± 3·4	2·1*** ± 0·1
A-like cells	Controls	50	30·9 ± 1·5	11·1 ± 0·8	35·7 ± 2·1	19·7 ± 1·1	20·0 ± 1·1	143 ± 10·1	7·4 ± 0·4
	Antrum exclusion	39	44·3*** ± 2·5	14·7* ± 1·2	32·7 ± 2·3	29·6*** ± 1·8	17·5 ± 1·4	151 ± 15·0	5·0*** ± 0·3
G cells	Controls	36	58·4 ± 3·7	15·9 ± 1·1	28·4 ± 1·8	42·6 ± 3·4	26·6 ± 1·6	189 ± 17·2	4·3 ± 0·3
	Antrum exclusion	26	60·8 ± 4·1	18·1 ± 1·4	31·3 ± 2·4	42·8 ± 3·8	20·3* ± 2·0	133* ± 14·2	2·9*** ± 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 $P < 0{\cdot}001$.

These proportions were not very different from those observed in unoperated rats. Here, of 134 endocrine cells, eighty-seven (65 %) were ECL cells, thirty-four (25 %) A-like cells and thirteen (10 %) belonged to other categories. Following antrum exclusion both A-like cells and the ECL cells were enlarged. For the ECL cell the increase in size reflected predominantly an increase in the size of the nucleus. For the A-like cell the increase was in the cytoplasm area. Both cell types had a reduced number of granules per unit cytoplasm (Table 2). The size of the G cells (Pl. 3) was not affected by antrum exclusion but they had a reduced granule volume density (i.e. the proportion of cytoplasm occupied by granules) (Table 2). The granule profile diameter in the A-like cells was increased, in the other two types of endocrine cells there was no effect of antrum exclusion on the granule size (Table 3 and Text-fig. 5).



Text-fig. 5. Size distribution histogram (diameter in nm) of cytoplasmic granules in ECL cells, A-like cells and G cells. No operation (top) and antrum exclusion (below).

TABLE 3. Endocrine cell morphometry: Mean granule profile diameter (nm) in ECL and A-like cells of the oxyntic mucosa and in G cells of the antrum

Operation	ECL cell	A-like cell	G cell
Control	225 ± 3·5 (740)	122 ± 1·1 (1057)	254 ± 2·4 (1053)
Antrum exclusion	239 ± 4·4 (783)	144* ± 0·9 (2001)	246 ± 2·0 (1043)

Mean ± S.E. of mean (n = number of granules). Each group comprised granules from eight to twelve cells. Analysis of variance was performed. For A-like cells $F = 9{\cdot}3$, $P < 0{\cdot}01$ (*).

DISCUSSION

Following antrum exclusion the serum gastrin concentration was high and independent of the prandial state. The high fasting level is probably best explained by the fact that acid gastric juice is prevented from reaching the pyloric mucosa. The

operation lowered the number per unit area of detectable gastrin cells, somatostatin cells and enterochromaffin cells in the pyloric mucosa. The number of somatostatin cells in the oxyntic mucosa was not significantly lowered. The concentration of gastrin in the antrum was much reduced. The apparent reduction in number of endocrine cells in the antral mucosa may therefore be artifactual, due to a low content of hormone per cell, rendering some of them undetectable by immunofluorescence. Against this interpretation speaks the fact that in the recognized gastrin cells the granule volume density was reduced by less than 20 %.

In the unoperated rats the antral gastrin concentration was higher in the freely fed group than in the fasted group (cf. Lichtenberger, Lechago & Johnson, 1975; Mortensen, Morris & Owens, 1978). In antrally excluded rats fasting did not reduce the already low antral gastrin concentration. This may be due to the fact that ingested food is prevented from stimulating the gastrin cells upon exclusion of the antrum. Conceivably, the gastrin cells in the excluded antrum contain little gastrin because they are actively secreting due to lack of acid inhibition. This interpretation is in agreement with the high serum gastrin concentration and with the reduced granule volume density of the gastrin cells.

The elevated serum gastrin concentration following antrum exclusion was associated with greatly activated histidine decarboxylase in the oxyntic mucosa (cf. Håkanson *et al.* 1974). The histamine-forming enzyme is thought to be localized in two different populations of endocrine cells referred to as ECL cells and A-like cells respectively (Håkanson *et al.* 1976). Following antrum exclusion these cells were increased in number, the A-like cells in particular. The mucosal histamine concentration was increased (cf. Lundell, 1974). At the ultrastructural level both cell types exhibited signs of activation in that the mean cell size was increased and the number of granules per unit cytoplasm was lowered. If, as appears likely, histamine resides in the granules, then the observed increase in mucosal histamine concentration reflects the increased cell number and not an increased cellular content of histamine. This interpretation accords with the observation that the cell number increased in proportion to the histamine content. The results suggest that the hypergastrinemia ensuing upon antrum exclusion affects the histamine-storing endocrine cells in the oxyntic mucosa in two ways: by increasing the functional activity (reflected in activated histidine decarboxylase and reduced number of granules per unit cytoplasm) and by exerting a trophic influence (reflected in an increased number and size of both ECL and A-like cells). It may be noted that the trophic influence of gastrin is selective in that the somatostatin cell population in the oxyntic mucosa was not affected.

The chemical changes observed following antrum exclusion + vagal denervation were indistinguishable from those after antrum exclusion alone (analysis by electron microscopy was not performed on material from these rats). Hence it seems that the vagi do not have a decisive influence on these events.

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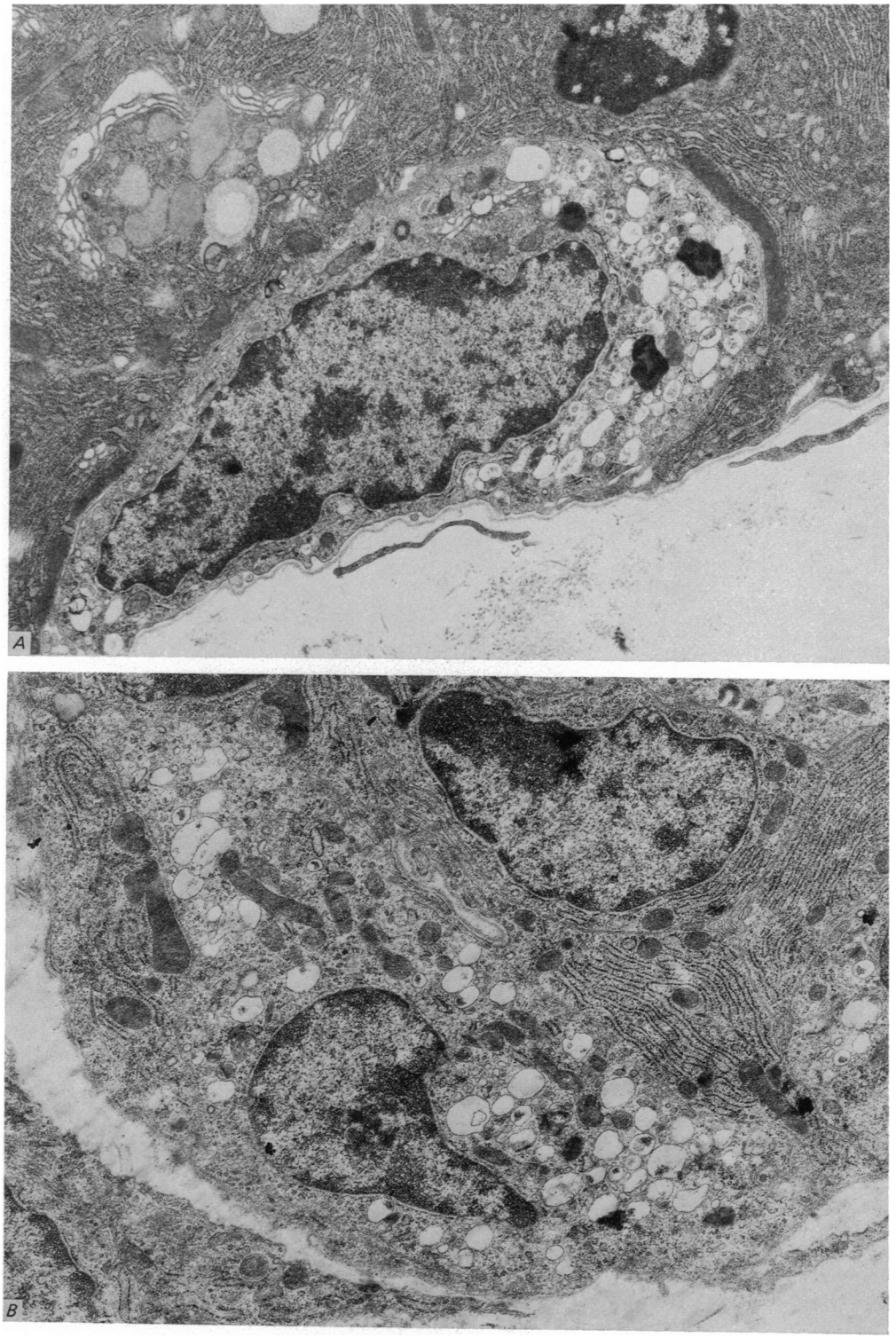
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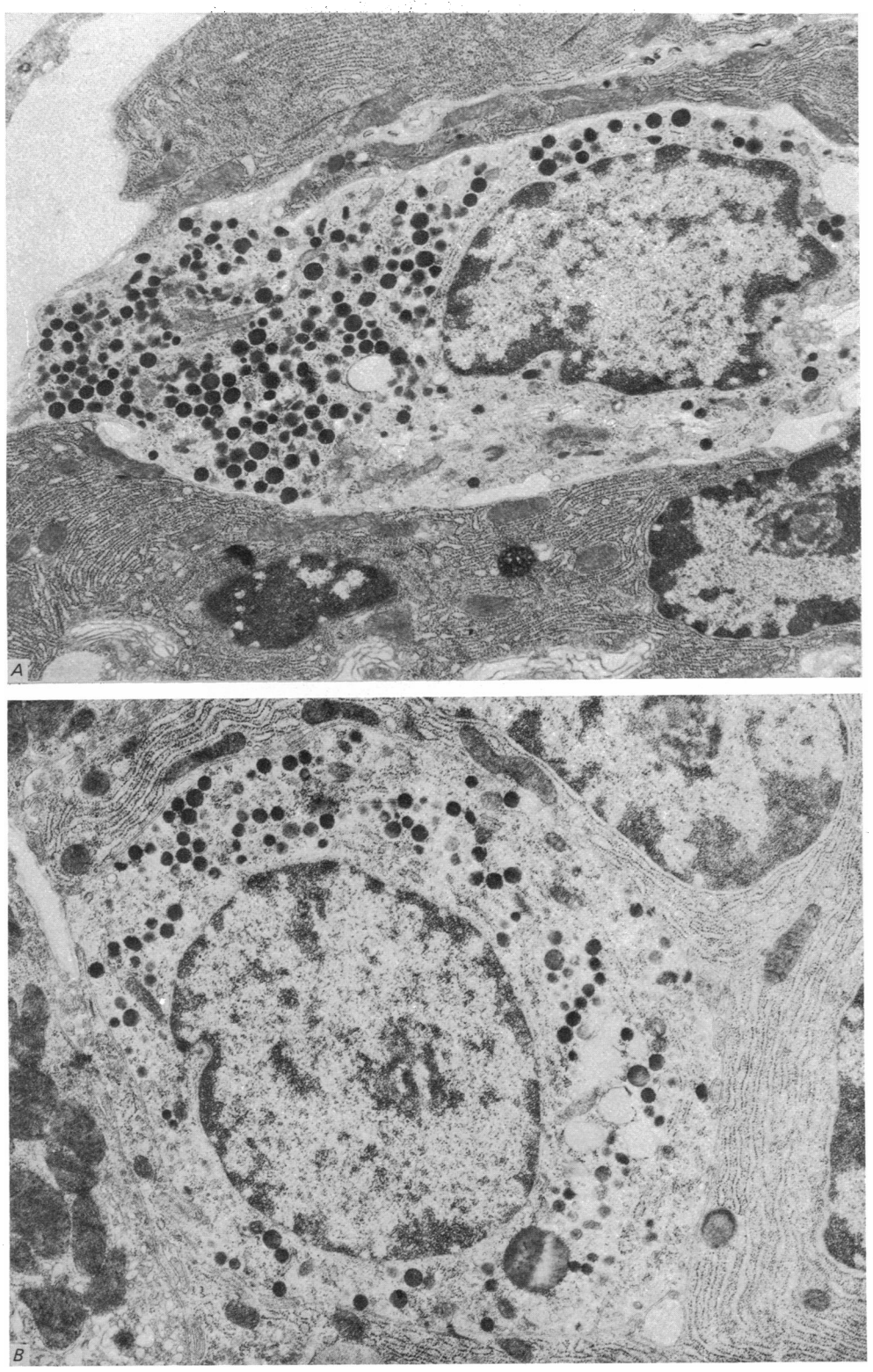
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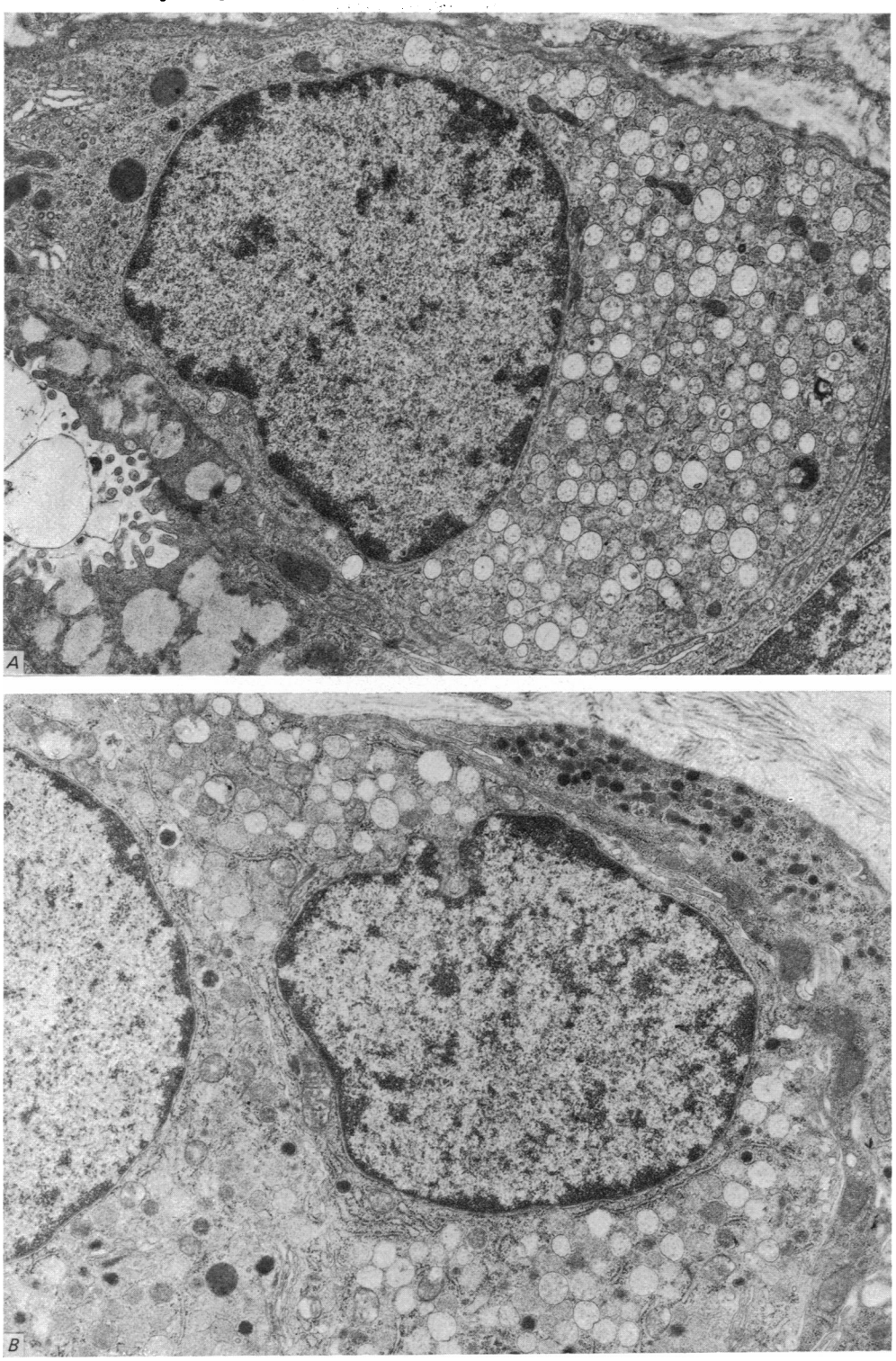

J. ALUMETS AND OTHERS

Explanation of plates

Plate 1

Electron micrographs of ECL cells. *A*, unoperated rat. Cytoplasm contains many large electron-lucent granules. *B*, antrum excluded rat. Cell size is increased (from 26·0 μm^2 in *A* to 37·0 μm^2 in *B*). The granule volume density is lowered (from 32·6 to 14·3 %). × 12,000.

Plate 2

Electron micrographs of A-like cells. *A*, unoperated rat. Numerous round, electron dense cytoplasmic granules. *B*, antrum excluded rat. Cell size is increased and the number of cytoplasmic granules (per unit area) is reduced. × 12,000.

Plate 3

Electron micrographs of G cells, *A*, unoperated rat. The cytoplasm is packed with round granules displaying a varying electron density. *B*, antrum excluded rat. Cell size is unaffected, but the granule volume density is reduced from 30 % in *A* to 23·7 % in *B*. × 12,000.