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EFFECTS OF ANTRECTOMY OR PORTA–CAVAL SHUNTING ON THE HISTAMINE-STORING ENDOCRINE-LIKE CELLS IN OXYNTIC MUCOSA OF RAT STOMACH. A FLUORESCENCE HISTOCHEMICAL, ELECTRON MICROSCOPIC AND CHEMICAL STUDY

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SUMMARY

1. The argyrophil (enterochromaffin-like) cells in the oxyntic gland area of the rat stomach contain histamine, which can be demonstrated fluorescence microscopically after exposure to gaseous OPT. After administration of L-dopa (or L-5-hydroxytryptophan), these cells produce and temporarily store dopamine (or 5-hydroxytryptamine), demonstrable by its characteristic formaldehyde-induced fluorescence. Ultrastructurally, the enterochromaffin-like cells, which have the appearance of polypeptide hormone-secreting cells, comprise two main cell types, the most predominant one having vesicular type granules (ECL cells), the second most predominant one having smaller, uniformly electron dense granules (A-like cells).

2. Rats were subjected to the following surgical treatments: antrectomy; porta–caval shunting; antrectomy + porta–caval shunting; or sham-operation. Three to eight weeks after surgery the histamine-storing cells (enterochromaffin-like cells) of the oxyntic mucosa were analysed by fluorescence histochemistry, light and (quantitative) electron microscopy, and fluorometric determination of amines.

3. After antrectomy, fluorescence histochemistry and silver staining revealed a reduced number of enterochromaffin-like cells. The histamine content in the oxyntic mucosa was reduced by about 50 %. As in unoperated rats injection of pentagastrin seemed to mobilize histamine. Feeding or injection of insulin failed to do so in antrectomized as opposed to control rats. Ultrastructurally, the cytoplasmic granules of both endocrine-like cell types were less numerous than in the unoperated rats. The reduction in cell number and granularity was particularly conspicuous with regard to the ECL cells.

4. After porta–caval shunting the number of enterochromaffin-like cells increased markedly. Chemical determination revealed a twofold increase in the histamine concentration of the oxyntic mucosa. Feeding or injection of insulin or pentagastrin lowered the histamine concentration. As judged by electron microscopy, the proliferation of endocrine-like cells induced by porta–caval shunting was restricted to the ECL cell type. Besides occurring in greater number, these cells were larger than those in unoperated controls, and their cytoplasm was densely packed with granules that were increased in size.

5. Following antrectomy of the porta–caval shunted rats the number of enterochromaffin-like cells and the oxyntic histamine concentration was reduced.

6. The results support the idea that gastrin exerts trophic as well as excitatory effects on oxyntic endocrine-like cells.

INTRODUCTION

Non-mast-cell histamine in the rat stomach occurs in argyrophil, so-called 'enterochromaffin-like' cells, which are numerous in the basal part of the oxyntic mucosa (Håkanson, Lilja & Owman, 1967; Håkanson & Owman, 1966, 1967, 1969). The presence of histamine in these cells is reflected by their *o*-phthalaldehyde (OPT)-induced fluorescence (Thunberg, 1967; Håkanson & Owman, 1967, 1969). After injection of L-3,4-dihydroxyphenylalanine (L-dopa), the enterochromaffin-like cells, which now contain dopamine, can be readily detected by their typical formaldehyde-induced catecholamine fluorescence (Håkanson *et al.* 1967). At the electronmicroscopic level, these cells have the appearance of polypeptide hormone-secreting cells (cf. Håkanson, Owman, Sporrong & Sundler, 1971). On the basis of the morphology of their cytoplasmic granules two predominant and distinctly different endocrine-like cell types can be recognized (see Pl. 1). One of these two cell types contains cytoplasmic granules of vesicular type with a wide electron-lucent zone between the irregular dense core and the surrounding membrane. This cell type has been referred to as the ECL cell (Solcia, Pearse, Grube, Kobayashi, Bussolati, Creutzfeldt & Gepts, 1973). The other cell type contains uniformly electron-dense cytoplasmic granules with only a narrow electron-lucent rim between the dense core and the surrounding membrane. This cell type has been referred to as the A-like cell (Solcia *et al.* 1973). Histamine is mobilized from its cellular store in the rat stomach as a result of feeding, vagal excitation or gastrin injection (Kahlson, Rosengren, Svahn & Thunberg, 1964; Kahlson, Rosengren & Thunberg, 1967; Lundell, 1974). This mobilization is reflected in reduced mucosal histamine concentration and increased urinary excretion of histamine.

In the present report we describe the effect of various surgical and pharmacological treatments on the histamine concentration and on the number and ultrastructure of the histamine-storing enterochromaffin-like cells in the oxyntic mucosa.

METHODS

Experimental

Adult male Wistar rats (body weight 150–200 g unless otherwise stated) were used. Antrectomy was performed on sixty-five rats by resection of the distal half of the glandular stomach (the pyloric gland area together with the adjacent portion of the oxyntic gland area) and the duodenal bulb (Håkanson & Liedberg, 1970). Great care was taken to remove the entire lesser curvature including the limiting ridge between the glandular and non-glandular mucosa at the cardia. The nervous and vascular supply to the remaining part of the stomach was spared. Gastrointestinal continuity was re-established by an end-to-end gastroduodenostomy. The completeness of the antrectomy was verified histologically in each animal. Ninety-eight rats (weighing 300 g) were porta–caval-shunted 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. Another group of nine rats (300 g) was first porta–caval shunted and 2–3 weeks later, antrectomized. One hundred and sixty rats (150–200 or 300–350 g) were sham-operated by a mid-line incision in the abdomen. All operated rats were allowed to recover for 3–4 weeks (porta–caval shunted rats were left for 2 months) before being used in experiments. Unless otherwise stated all rats were killed by exsanguination under diethyl ether anaesthesia. Before sacrifice the rats were treated as follows: (1) fasted for 48 hr (with free access to drinking water); (2) fasted (48 hr) and fed during 30 min (ordinary rat food pellets) and sacrificed at various times later; (3) fasted, injected subcutaneously with insulin (2·5 or 10 u/kg, Vitrum, Sweden) and killed 2 hr later; (4) fasted, injected subcutaneously with pentagastrin (500 μg/kg) and killed 1 hr later.

In one series of experiments, DL-dopa (50 mg/kg) or L-5-hydroxytryptophan (50 mg/kg) was injected I.P. to fasted rats which were killed 2 hr later.

Fluorescence and light microscopy

Fasted rats were used. Pieces from the oxyntic portion of the stomach were frozen to the temperature of liquid nitrogen in a propane–propylene mixture. 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 Zeiss fluorescence microscope equipped with an HBO 200 high pressure mercury lamp. For examination of formaldehyde-induced fluorescence we used a Schott BG 12 as excitation filter and a Schott OG 4 as barrier filter. The corresponding filters used for examination of OPT-induced fluorescence were UG 1 and GG 9. Occasionally, after examination in the fluorescence microscope the sections were stained with silver according to the technique of Grimelius (1968) (argyrophil stain).

In one series of experiments, we assessed the number of epithelial cells displaying formaldehyde-induced fluorescence following injection of DL-dopa. Transverse sections were examined at a magnification of × 125 (objective × 10, eyepiece × 12·5). Cells in three randomly selected visual fields (entire thickness of mucosa visible) from each section were counted. At least three sections from each animal were examined. Thus, for each animal the cell count recorded was the mean of at least nine independent counts. Cell counts are expressed as number of cells per visual field.

Electron microscopy

The following experimental groups were examined: unoperated rats; antrectomized rats; and porta–caval shunted rats (at least three animals in each group). Fasted animals (anaesthetized with diethyl ether) were perfused via the ascending aorta with glutaraldehyde (2·5% in 0·2 M sodium cacodylate buffer, pH 7·2) for 7–10 min. Small pieces were taken from the mucosa of the oxyntic gland area and immersed in the fixative for 1–2 hr. The tissue specimens were post-fixed for 1 hr in 1% osmium tetroxide, 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 Vestopal W or Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 300 electron microscope. From each experimental group, at least 200 sections were examined and at least twenty-eight endocrine-like cells of each of the two predominant types were photographed. Cells were used for morphometry only if cross-sectioned in such a way that the nucleus was visible. The photographs were reproduced in magnification × 15,000. All endocrine-like cell profiles and nuclei were traced with a pencil on transparent paper, cut out and weighed. The weight was compared to that of pieces of paper of known surface. The results are expressed as μm^2 cell area or cytoplasm area. The cytoplasmic granules in each endocrine-like cell section were counted. Granule size was established by measuring the diameter of all granules in at least ten cells of each type from each experimental group.

Chemical determination of amines

The stomachs were cut open along the major curvature. After washing the mucosal surface with ice-cold 0·9% saline, the mucosa of the oxyntic gland area was scraped off with a scalpel and weighed.

Histamine. For the determination of histamine, the mucosa was homogenized in 5% trichloroacetic acid to a concentration of 50–100 mg/ml. The homogenate was left in a refrigerator for a few hours and precipitated proteins were spun down at low speed. One ml. portions were diluted with 1 ml. H_2O, whereafter 0·5 ml. 5 N-NaOH and 1 g Na_2SO_4 were added. The mixture was extracted with 15 ml. 3:2 mixture of *n*-butanol and chloroform (Burkhalter, 1962) for about 5 min. The organic phase was first washed with 4 ml. salt-saturated 0·1 N-NaOH by shaking for 1 min and histamine was then back-extracted with 3 ml. 0·1 N-HCl after the addition of 5 ml. *n*-heptane. Two ml. aqueous extract were evaporated to dryness on a steam bath. The dry residue was re-dissolved in 2 ml. H_2O and aliquots of 10–25 μl. were taken for fluorometric assay of histamine as described in detail elsewhere (Håkanson & Rönnberg, 1974).

Dopamine. For the determination of dopamine, the mucosa was homogenized in 0·4 N perchloric acid to a concentration of 100 mg/ml. The deproteinized extract was neutralized (to pH 6) by the addition of potassium carbonate and the resulting potassium perchlorate precipitate was eliminated by centrifugation in the cold. Dopamine was isolated by ion exchange chromatography (Dowex 50) according to

the procedure of Bertler, Carlsson & Rosengren (1958) and determined fluorometrically as described by Anton & Sayre (1964).

5-Hydroxytryptamine. For the determination of 5-hydroxytryptamine the mucosa was extracted in ten volumes of aqueous acetone (80%) overnight and the extract was then evaporated to dryness under reduced pressure. The dry residue was taken up in 3 ml. water to which 1 g NaCl and 0·5 ml. 10% Na_2CO_3 was added. This mixture was extracted with 15 ml. *n*-butanol by shaking for 5 min. After washing the butanol phase for 1–2 min with 4 ml. 0·1 M borate buffer, pH 10, 5-hydroxytryptamine was returned to an aqueous phase by shaking with 2 ml. 0·1 N HCl and 4 ml. *n*-heptane. 5-Hydroxytryptamine was determined fluorometrically as described by Maickel, Cox, Saillant & Miller (1968).

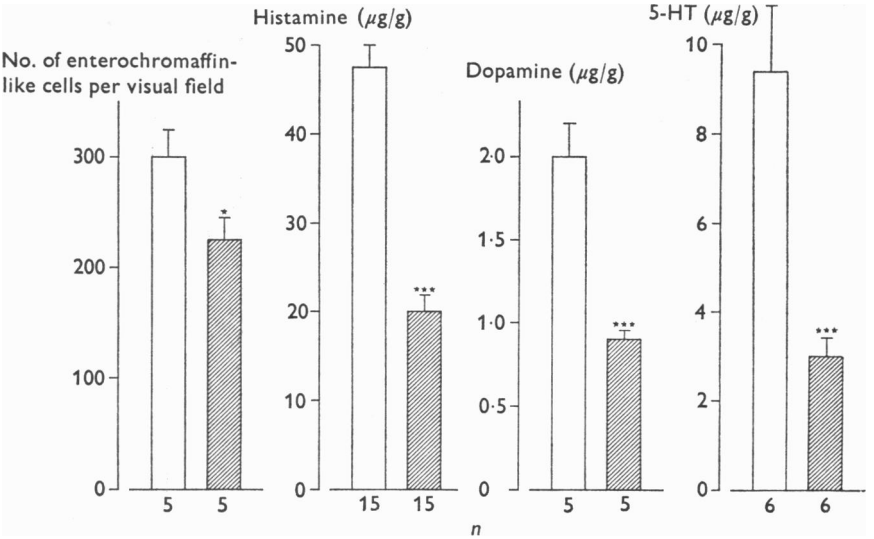
RESULTS

Effect of antrectomy

In the antrectomized rats the number of enterochromaffin-like cells in the oxyntic mucosa was lower than in the sham- or unoperated rats (Text-fig. 1). The reduction in cell number was established by the fluorescence histochemical method for visualizing histamine, by the formaldehyde method for demonstrating dopamine after administration of L-dopa, and by silver impregnation (Pl. 2). With both fluorescence microscopic methods it was apparent that the enterochromaffin-like cells of antrectomized rats displayed reduced fluorescence intensity compared with that in unoperated controls. The concentration of dopamine and 5-hydroxytryptamine in the oxyntic mucosa following injection of DL-dopa or L-5-hydroxytryptophan was lower in antrectomized rats than in unoperated rats (Text-fig. 1). By electron microscopy, the different types of endocrine-like cells in the oxyntic mucosa were found to be equally reduced in number after antrectomy. Of ninety-one endocrine-like cells examined, fifty-eight (64%) were ECL cells, twenty-two (24%) were A-like cells and eleven (12%) belonged to other categories. These proportions were very similar to those observed in unoperated rats. Here, of 116 endocrine-like cells, seventy-six (66%) were ECL cells, twenty-eight (24%) A-like cells and twelve (10%) belonged to other categories. The ECL cell type, but not the A-like cell type, was reduced in size by antrectomy (Table 1). The size reduction involved primarily the cytoplasm. In both cell types antrectomy reduced the number of granules (Pls. 3 and 4) whether expressed per cell section or per square micrometre cytoplasm. The granule size was slightly but significantly reduced in the ECL cells and unaffected in the A-like cells (Table 1 and Text-figs. 2 and 3). As can be seen from Table 2 antrectomized rats had lower histamine concentration in the oxyntic mucosa than unoperated rats. Fasted sham- or unoperated rats responded to feeding and to injection of insulin or pentagastrin with reduction of the oxyntic histamine content. Antrectomized rats responded to pentagastrin only (Table 2).

Effect of porta–caval shunting

In porta–caval shunted rats the number of enterochromaffin-like cells in the oxyntic mucosa was much higher than in sham-operated rats (Text-fig. 4). This was documented by the OPT and formaldehyde methods and by silver staining (Pl. 2). The enterochromaffin-like cells displayed enhanced fluorescence intensity following porta–caval shunting. The oxyntic dopamine and 5-hydroxytryptamine concentration following administration of DL-dopa or L-5-hydroxytryptophan was higher in the porta–caval shunted



Text-fig. 1. Effect of antrectomy on the number of enterochromaffin-like cells in oxyntic mucosa and on the gastric mucosal content of histamine and of dopamine and 5-hydroxytryptamine (5-HT) following pre-treatment with DL-dopa or L-5-hydroxytryptophan (50 mg/kg I.P.): □, controls; ▨, antrectomy. The rats (200 g body weight) were fasted for 48 hr and sacrificed 2 hr after injection of the amine precursor. Controls were unoperated. Enterochromaffin-like cells were counted in sections from DL-dopa-injected rats. The number of cells is expressed per visual field (magnification × 125). *n* denotes the number of rats tested. Student's *t* test was used to establish significant differences between unoperated and antrectomized rats: * for $0{\cdot}01 < P < 0{\cdot}05$; ** for $0{\cdot}005 < P < 0{\cdot}01$; *** for $P < 0{\cdot}005$.

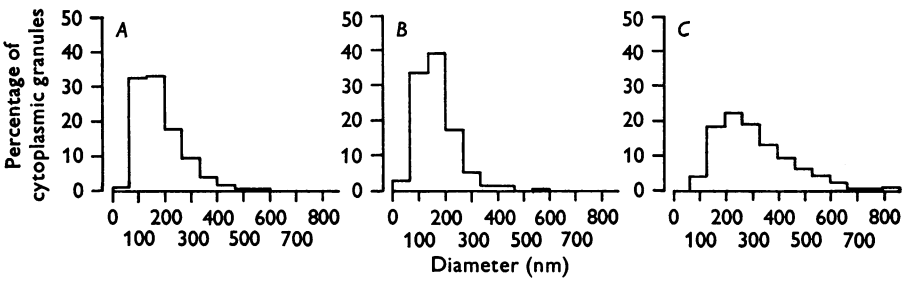
rats than in the sham-operated animals (Text-fig. 4). Electron microscopy showed a clear increase in the number of endocrine-like cells of the ECL cell type. Of 111 endocrine-like cells examined, ninety-two (83 %) were ECL cells, fifteen (14 %) A-like cells and four (4 %) belonged to other categories. If we assume that the number of A-like cells is unchanged

TABLE 1. Effects of antrectomy or porta–caval shunting on ultrastructural properties of ECL and A-like cells

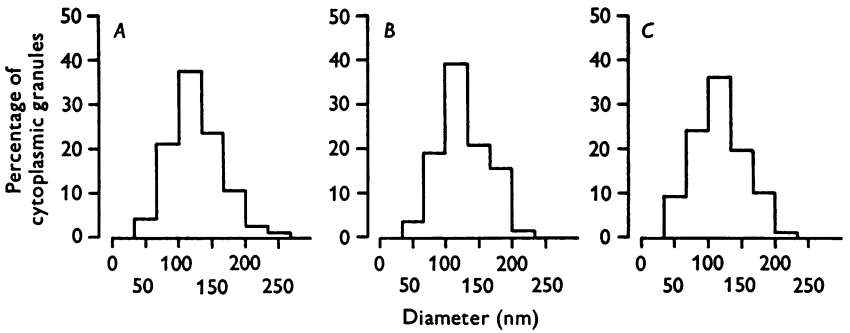
Treatment	Granule diameter (nm)	No. of granules per sectioned cell	Cell size (μm^2)	Nuclear size (μm^2)	Cytoplasm (μm^2)	No. of granules/μm^2 cytoplasm
ECL cells						
No operation	206 ± 3·2 (786)	95 ± 11·6 (32)	27·2 ± 1·7 (32)	9·1 ± 0·8 (32)	18·1 ± 1·3 (32)	5·1 ± 0·3 (32)
Antrectomy	192 ± 3·5 (432)**	41 ± 4·6 (28)***	17·9 ± 1·2 (28)***	8·4 ± 0·7 (28)	9·5 ± 0·7 (28)***	4·2 ± 0·3 (28)*
Porta–caval shunt	341 ± 6·3 (752)***	124 ± 10·8 (33)	49·1 ± 2·9 (34)***	13·3 ± 0·9 (34)**	35·8 ± 2·5 (34)***	3·6 ± 0·2 (33)**
A-like cells						
No operation	137 ± 1·2 (1057)	143 ± 11·4 (39)	31·9 ± 1·8 (39)	11·5 ± 0·9 (39)	20·6 ± 1·3 (39)	7·3 ± 0·5 (39)
Antrectomy	140 ± 1·5 (651)	77 ± 8·8 (28)***	27·5 ± 1·4 (28)	11·0 ± 0·8 (28)	16·6 ± 1·0 (28)*	4·6 ± 0·4 (28)***
Porta–caval shunt	127 ± 1·2 (946)	147 ± 12·0 (31)	30·7 ± 1·5 (31)	9·4 ± 0·8 (31)	21·3 ± 1·2 (31)	7·2 ± 0·5 (31)

Mean ± S.E. of mean (n). 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 $P < 0{\cdot}001$.

then these figures indicate almost a doubling of the number of ECL cells. The ECL cells sometimes occurred in clusters of three or four cells (Pl. 5), an arrangement never seen in control rats. The ECL cells, but not the A-like cells, were increased in size by porta–caval shunting (Table 1). The size increase involved primarily the cytoplasm. In the ECL cells the



Text-fig. 2. Size distribution histogram (diameter in nm) of cytoplasmic granules in the ECL cell of: *A*, unoperated; *B*, antrectomized; and *C*, porta–caval shunted rats. Note the increase in granule diameter following porta–caval shunting. The decrease following antrectomy is less conspicuous but statistically significant (see Table 1).



Text-fig. 3. Size distribution histogram (diameter in nm) of cytoplasmic granules in the A-like cell: *A*, unoperated; *B*, antrectomized; and *C*, porta–caval shunting. Antrectomy and porta–caval shunting are without effect.

number of granules per cell section was not significantly affected by the shunting but the number of granules per square micrometre cytoplasm was reduced. This is explained partly by the fact that the cells increased in size and partly by the fact that the granules were greatly enlarged (Table 1 and Pl. 3). In the A-like cells the number of granules was unaffected (Table 1 and Pl. 4), whether expressed per cell section or per square micrometre. The size of the A-like cell granules was unaffected by porta–caval shunting (Text-figs. 2 and 3). Porta–caval shunted rats which had markedly

TABLE 2. Effects of re-feeding or injection of insulin or pentagastrin on the gastric histamine content

Treatment	Body weight (g)	Mucosal histamine concentration ($\mu g/g$), means ± S.E. of mean (n)			
		A, fasted	*B*, re-fed†	*C*, insulin‡	*D*, pentagastrin§
1. No operation	150–200	47·9 ± 2·2 (15)	33·6 ± 3·3 (14)**	30·5 ± 2·7 (15)***	37·5 ± 2·4 (23)**
2. Sham-operation	150–200	49·8 ± 2·5 (16)n.s.	37·9 ± 3·0 (13)**	40·4 ± 3·3 (15)*	41·0 ± 2·9 (17)*
3. Antrectomy	150–200	20·1 ± 1·7 (15)***	19·9 ± 1·6 (6)n.s.	21·3 ± 1·6 (12)n.s.	13·4 ± 0·9 (6)**
4. Sham-operation	300–350	60·0 ± 3·3 (28)	49·0 ± 2·8 (18)*	45·0 ± 3·3 (19)**	41·4 ± 2·8 (5)***
5. Porta–caval shunt	300–350	119·0 ± 2·3 (19)***	84·2 ± 6·0 (14)***	95·0 ± 4·4 (13)***	65·5 ± 3·3 (10)***
6. Porta–caval shunt + antrectomy	300–350	42·3 ± 2·7 (4)***	—	—	32·0 ± 3·4 (5)*

Student's t test was used to establish significant differences between: *A*, fasted; and *B*, *C* or *D*, treated rats; and between fasted unoperated (1) or sham-operated (4) rats on one hand and operated (2, 3 and 5, 6) rats on the other.

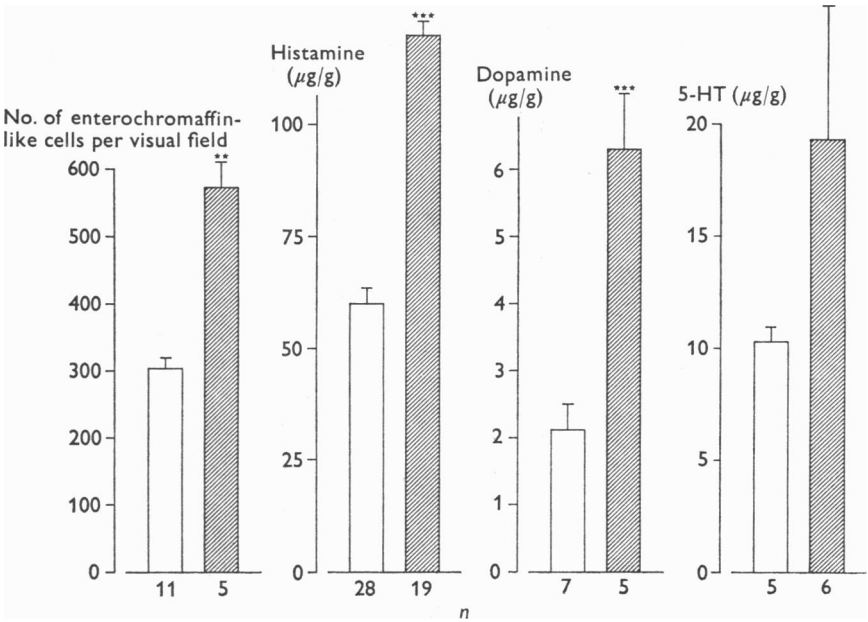
* for $0{\cdot}01 < P < 0{\cdot}05$; ** for $0{\cdot}001 < P < 0{\cdot}01$; and *** for $P < 0{\cdot}001$.

† Fed during 30 min and killed 2 hr later.

‡ Injected subcutaneously with insulin, 10 u./kg (1, 2 and 3) or 2·5 u./kg (4, 5 and 6), and killed 2 hr later.

§ Injected subcutaneously with pentagastrin, 500 μg/kg, and killed 1 hr later.

elevated levels of histamine in the oxyntic mucosa (Table 2) responded to feeding and to injection of insulin or pentagastrin with reduction of the histamine content. The time course of the reduction of the histamine content in unoperated and porta–caval shunted rats following feeding after a period of fasting is illustrated in Text-fig. 5.



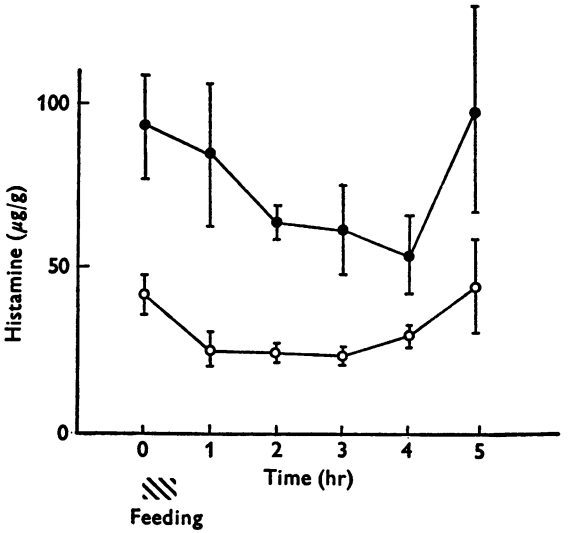
Text-fig. 4. Effect of porta–caval shunting on the number of enterochromaffin-like cells in oxyntic mucosa and on the mucosal content of histamine and of dopamine and 5-hydroxytryptamine (5-HT) following pre-treatment with DL-dopa or L-5-hydroxytryptophan (50 mg/kg I.P.): □, controls; ▨, porta–caval shunt. The rats (300 g body weight) were fasted for 48 hr and sacrificed 2 hr after injection of the amine precursor. Controls were sham-operated. Enterochromaffin-like cells were counted in sections from DL-dopa-injected rats. For experimental and statistical details see Text-fig. 1.

Effect of antrectomy + porta–caval shunting

Following antrectomy of porta–caval shunted rats the number of enterochromaffin-like cells was about the same as in control rats (not shown in Figure) and the histamine concentration in the oxyntic mucosa was reduced compared with age-matched sham-operated rats (Table 2).

Sham-operation *per se* had no effect on the enterochromaffin-like cells. The mucosal histamine concentration was the same as in unoperated rats (150–200 g body weight), while a significantly higher content ($0{\cdot}001 < P < 0{\cdot}005$) was found in the larger (300–350 g) rats which were used as

controls for the shunted rats. This emphasizes the importance of using properly age-matched controls in studies on the histamine content of rat gastric mucosa. After sham-operation, the animals responded to feeding or to injection of insulin or pentagastrin with lowering of oxyntic histamine just as did the unoperated rats (Table 2).



Text-fig. 5. Histamine concentration in oxyntic mucosa at various times after feeding for 30 min: ○, unoperated rats (200 g); ●, porta–caval shunted rats (300 g). Each value is the mean of four to six determinations. Vertical bars give S.E. of mean.

DISCUSSION

The mucosal histamine concentration of the rat stomach can be greatly affected by various experimental manipulations. Short-term reduction of gastric histamine is observed following feeding or injection of gastrin or insulin (Kahlson *et al.* 1964). As shown in the present study, long-lasting changes can be induced by for instance antrectomy which reduces the mucosal histamine concentration by half (see also Håkanson & Liedberg, 1971; Lundell, 1974), or by porta–caval shunting which almost doubles the histamine concentration (see also Fischer & Snyder, 1965). The bulk of gastric histamine in the rat is contained in the enterochromaffin-like cells of the oxyntic mucosa (Håkanson & Owman, 1967; Aures, Håkanson & Schauer, 1968); mast cells are few in this location (Aures *et al.* 1968). For this reason, changes in the gastric histamine level are thought to reflect changes in the histamine content of the enterochromaffin-like cells. This view is supported by the finding (Thunberg, 1967) that the reduction in histamine that follows from feeding or injection of insulin is only observed

in the basal part of the mucosa where enterochromaffin-like cells are numerous and not in the superficial part of the mucosa where mast cells are found. The histamine-storing enterochromaffin-like cells have previously been shown to have the capacity to take up and decarboxylate administered L-dopa or L-5-hydroxytryptophan and to retain the product, dopamine or 5-hydroxytryptamine, in the cytoplasm for a few hours (Håkanson, 1965; Håkanson & Owman, 1967, 1969; Håkanson *et al.* 1967). The concentrations of dopamine and 5-hydroxytryptamine in the oxyntic mucosa after injection of DL-dopa or L-5-hydroxytryptophan were affected by antrectomy and porta–caval shunting much like the gastric histamine concentration suggesting that the monoamines and histamine are stored in the same cell compartment. From fluorescence and light microscopy it could be concluded that the changes in amine concentrations following antrectomy and porta–caval shunting were associated with, and probably reflected, alterations in the number of enterochromaffin-like cells in the oxyntic mucosa. In addition, the fluorescence intensity of the cells was reduced by antrectomy and enhanced by porta–caval shunting, probably reflecting alterations in the cellular content of the amines. Ultrastructurally, the enterochromaffin-like cells comprise at least two distinctly different endocrine-like cell populations, distinguishable on the basis of the morphology of their cytoplasmic granules (Håkanson *et al.* 1971). These two cell types have been referred to as ECL cells and A-like cells, respectively (Solcia *et al.* 1973). From electron microscopic observations the changes in gastric histamine concentration were associated predominantly with alterations in the number, size and granularity of one of the two enterochromaffin-like cell types, namely the one with vesicular-type granules (ECL cells). Antrectomy reduced the number and size of these cells, whereas they were very numerous, and enlarged, after porta–caval shunting. The A-like cells were less conspicuously affected by antrectomy and seemed unaffected by porta–caval shunting.

The mechanism behind the change in enterochromaffin-like cell mass following antrectomy and porta–caval shunting is obscure. Possibly, the reduced enterochromaffin-like cell mass in antrectomized rats is a consequence of antral gastrin deprivation, related to the well-known trophic effect of the hormone (Johnson, Aures & Håkanson, 1969; Johnson, Aures & Yuen, 1969; Johnson & Chandler, 1973). Following antrectomy, parietal cell mass as well as peptic cell mass is reduced (Martin, Macleod & Sircus, 1970; Capoferro & Nygaard, 1973). Under conditions of increased serum gastrin or as a result of administration of pentagastrin in depot form the parietal cells proliferate (Crean, Marshall & Rumsey, 1969). The increase in number of enterochromaffin-like cells following porta–caval shunt engaged mainly the ECL cells whereas the A-like cells seemed unaffected.

Proliferation of ECL cells has previously been observed in gastric disorders associated with raised serum gastrin levels (Rubin, 1969, 1973; Bordi, Cocconi, Togni, Vezzadini & Missale, 1974; Bordi, Costa & Missale, 1975). The histamine content in antrectomized porta–caval shunted rats was lower than in rats that were porta–caval shunted only, but not as low as in non-shunted antrectomized rats. This observation seems to support the contention that antral gastrin is instrumental in bringing about the ECL cell hyperplasia following porta–caval shunt but suggests also that gastrin is not solely responsible.

Short-term reduction of gastric histamine is elicited by gastrin and by feeding or injection of insulin. Upon removal of the endogenous gastrin store through antrectomy, gastrin retains its capacity to lower gastric mucosal histamine but those stimuli which are dependent upon gastrin release are no longer effective. This is of particular interest with regard to vagal excitation which is claimed to act directly on the histamine cell to release histamine (Kahlson *et al.* 1967). This hypothesis is based on studies with insulin. The present study shows that the capacity of insulin to reduce the gastric histamine concentration requires an intact antrum, which suggests that an antral agent, most probably gastrin, is the mediator of the insulin-induced reduction of gastric histamine. This is the more likely as pentagastrin retained its capacity to reduce the histamine content following antrectomy (see also Lundell, 1974).

In conclusion, antrectomy reduced the oxyntic histamine level and the number of enterochromaffin-like cells. Porta–caval shunting raised the oxyntic histamine level as well as the number of enterochromaffin-like cells. Short-term reduction of gastric mucosal histamine after feeding or insulin injection seems to be mediated by antral gastrin, as antrectomized rats failed to respond to these stimuli, while responding to pentagastrin. Thus, gastrin seems to exert trophic as well as excitatory effects on oxyntic endocrine-like cells.

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REFERENCES

ANTON, A. H. & SAYRE, D. F. (1964). The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. *J. Pharmac. exp. Ther.* **145**, 326–336.

AURES, D., HÅKANSON, R. & SCHAUER, A. (1968). Histidine decarboxylase and DOPA decarboxylase in rat stomach: properties and cellular localization. *Eur. Jnl Pharmac.* **3**, 217–234.

BERTLER, Å., CARLSSON, A. & ROSENGREN, E. (1958). A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta physiol. scand.* **44**, 273–292.

BJÖRKLUND, A., FALCK, B. & OWMAN, CH. (1972). Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In *Methods of Investigative and Diagnostic Endocrinology*, ed. BERSON, S. A., vol. 1, *The Thyroid and Biogenic Amines*, ed. RALL, J. E. & KOPIN, I. J., pp. 318–368. Amsterdam: North-Holland Publishing.

BORDI, C., COCCONI, G., TOGNI, R., VEZZADINI, P. & MISSALE, G. (1974). Gastric endocrine cell proliferation associated with Zollinger–Ellison syndrome. *Archs Path.* **98**, 274–279.

BORDI, C., COSTA, A. & MISSALE, G. (1975). ECL cell proliferation and gastrin levels. *Gastroenterology* **68**, 205–207.

BRODY, M. J., HÅKANSON, R., OWMAN, CH. & SUNDLER, F. (1972). An improved method for the histochemical demonstration of histamine and other compounds producing fluorophores with OPT. *J. Histochem. Cytochem.* **20**, 945–948.

BURKHALTER, A. (1962). The formation of histamine by fetal rat liver. *Biochem. Pharmac.* **11**, 315–322.

CAPOFERRO, R. & NYGAARD, K. (1973). Effects of antrectomy on the gastric mucosa of the rat. *Scand. Jnl Gastroenterol.* **8**, 347–352.

CREAN, G. P., MARSHALL, M. W. & RUMSEY, R. D. E. (1969). Parietal cell hyperplasia induced by the administration of pentagastrin (ICI 50, 123) to rats. *Gastroenterology* **57**, 147–155.

FISCHER, J. E. & SNYDER, S. H. (1965). Increased gastric synthesis of histamine: a possible mechanism for the gastric acid hypersecretion following porta-caval shunt. *Fedn Proc.* **24**, 1334–1340.

GRIMELIUS, L. (1968). A silver nitrate stain for α_2 cells in human pancreatic islets. *Acta Soc. Med. upsal.* **73**, 243–270.

HÅKANSON, R. (1965). Formation of amines in the gastric mucosa of the rat. *Nature, Lond.* **208**, 793–794.

HÅKANSON, R. & LIEDBERG, G. (1970). The role of endogenous gastrin in the activation of gastric histidine decarboxylase in the rat. Effect of antrectomy and vagal denervation. *Eur. Jnl. Pharmac.* **12**, 94–103.

HÅKANSON, R. & LIEDBERG, G. (1971). Evidence against histamine as the final chemostimulator of gastric acid secretion. *Am. J. Physiol.* **221**, 641–643.

HÅKANSON, R., LILJA, B. & OWMAN, CH. (1967). Properties of a new amine-storing cell system in the gastric mucosa of the rat. *Eur. Jnl Pharmac.* **1**, 188–199.

HÅKANSON, R. & OWMAN, CH. (1966). Distribution and properties of amino acid decarboxylases in gastric mucosa. *Biochem. Pharmac.* **15**, 589–599.

HÅKANSON, R. & OWMAN, CH. (1967). Concomitant histochemical demonstration of histamine and catecholamines in enterochromaffin-like cells of gastric mucosa. *Life Sci. Oxford* **6**, 759–766.

HÅKANSON, R. & OWMAN, CH. (1969). Argyrophilic reaction of histamine-containing cells of murine gastric mucosa. *Experientia* **25**, 625–626.

HÅKANSON, R., OWMAN, CH., SPORRONG, B. & SUNDLER, F. (1971). Electron microscopic identification of the histamine-storing argyrophil (enterochromaffin-like) cells in the rat stomach. *Z. Zellforsch. mikrosk. Anat.* **122**, 460–466.

HÅKANSON, R. & RÖNNBERG, A. L. (1974). Improved fluorometric assay of histamine: condensation at $-20°$ C. *Analyt. Biochem.* **60**, 560–567.

JOHNSON, L. R., AURES, D. & HÅKANSON, R. (1969). Effect of gastrin on the *in vivo* incorporation of ^{14}C-leucine into protein of the digestive tract. *Proc. Soc. exp. Biol. Med.* **132**, 996–998.

JOHNSON, L. R., AURES, D. & YUEN, L. (1969). Pentagastrin-induced stimulation of protein synthesis in the gastrointestinal tract. *Am. J. Physiol.* **217**, 251–254.

JOHNSON, L. R. & CHANDLER, A. M. (1973). RNA and DNA of gastric and duodenal mucosa in antrectomized and gastrin-treated rats. *Am. J. Physiol.* **224**, 937–940.

KAHLSON, G., ROSENGREN, E., SVAHN, D. & THUNBERG, R. (1964). Mobilization and formation of histamine in the gastric mucosa as related to acid secretion. *J. Physiol.* **174**, 400–416.

KAHLSON, G., ROSENGREN, E. & THUNBERG, R. (1967). Accelerated mobilization and formation of histamine in the gastric mucosa evoked by vagal excitation. *J. Physiol.* **190**, 435–463.

LUNDELL, L. (1974). Histamine metabolism of the gastric mucosa following antrectomy. *J. Physiol.* **241**, 437–451.

MAICKEL, R. P., COX, R. H., SAILLANT, J. & MILLER, F. P. (1968). A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. *Int. Jnl Neuro-pharmacol.* **7**, 275–281.

MARTIN, F., MACLEOD, I. B. & SIRCUS, W. (1970). Effects of antrectomy on the fundic mucosa of the rat. *Gastroenterology* **59**, 437–444.

RUBIN, W. (1969). Proliferation of endocrine-like (enterochromaffin) cells in atrophic gastric mucosa. *Gastroenterology* **57**, 641–648.

RUBIN, W. (1973). A fine structural characterization of the proliferated endocrine cells in atrophic gastric mucosa. *Am. J. Path.* **70**, 109-118.

SOLCIA, E., PEARSE, A. G. E., GRUBE, D., KOBAYASHI, S., BUSSOLATI, G., CREUTZFELDT, W. & GEPTS, W. (1973). Revised Wiesbaden classification of gut endocrine cells. *Rend. Gastro-enterol.* **5**, 13–16.

THUNBERG, R. (1967). Localization of cells containing and forming histamine in the gastric mucosa of the rat. *Expl Cell Res.* **47**, 108–115.

EXPLANATION OF PLATES

PLATE 1

Low magnification electron micrograph of endocrine-like cells in oxyntic mucosa of an unoperated rat. ECL cell to the left, A-like cell to the right. × 9200. According to the revised Wiesbaden terminology (Solcia *et al.* 1973) the cells having vesicular-type granules are termed ECL cells and the cells having round, electron dense cytoplasmic granules are termed A-like cells. Both cell types are enterochromaffin-like according to our definition (see Håkanson *et al.* 1971).

PLATE 2

Enterochromaffin-like cells demonstrated by various techniques in transverse sections of oxyntic mucosa. × 150. *A*, unoperated rat; *B*, antrectomized rat; *C*, porta–caval shunted rat. *a*, OPT-induced fluorescence; *b*, silver staining according to Grimelius (1968); *c*, formaldehyde-induced fluorescence following DL-dopa injection. Note the low number of enterochromaffin-like cells in the antrectomized rat and the high number in the porta–caval shunted rat.

PLATE 3

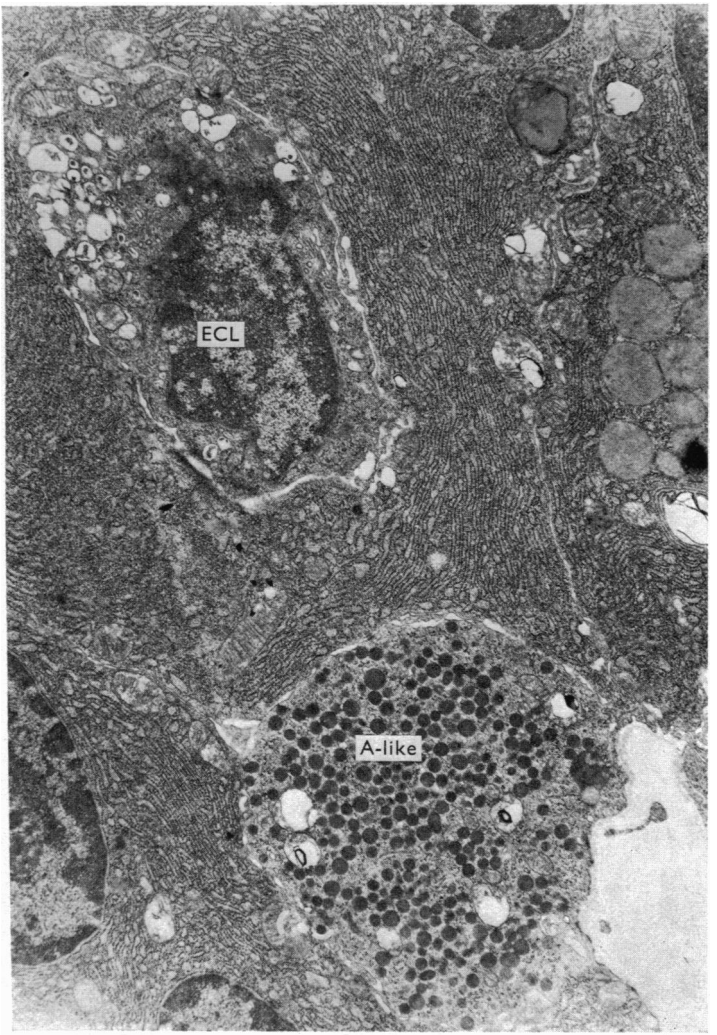
Electron micrographs of ECL cells. *A*, unoperated rat. *B*, antrectomized rat. Cell size is reduced. Cytoplasmic granules are few. *C*, porta–caval shunted rat. Cell size is increased. Cytoplasm densely packed with large electron-lucent granules. × 11,200.

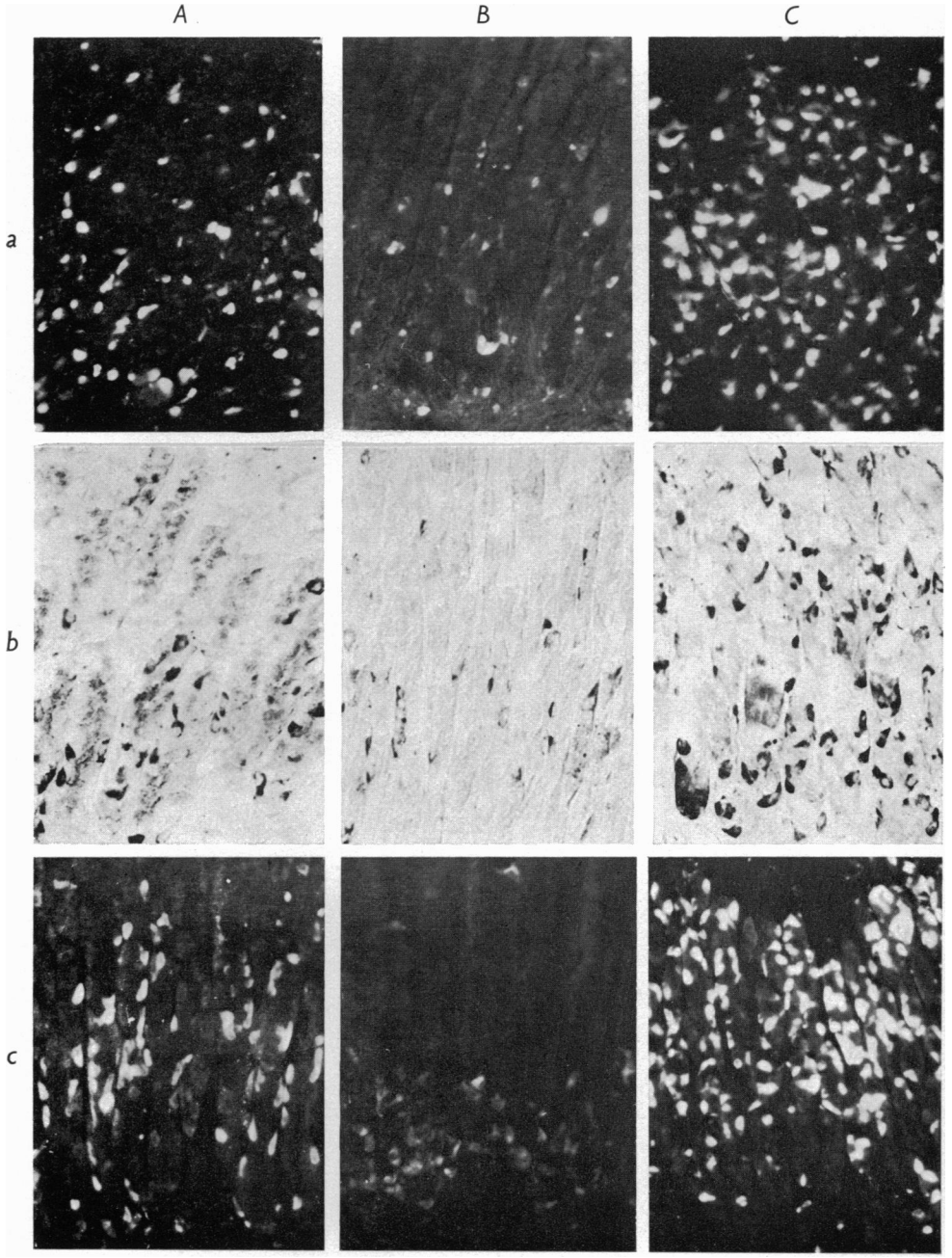
PLATE 4

Electron micrographs of A-like cells. *A*, unoperated rat. *B*, antrectomized rat. Number of granules reduced as compared with unoperated animals. *C*, porta–caval shunted rat. No overt effects. × 11,200.

PLATE 5

Electron micrograph showing ECL cell cluster, characteristic of porta–caval shunted rats, at the base of a gastric gland. × 9200.


ECL
A-like


A
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a
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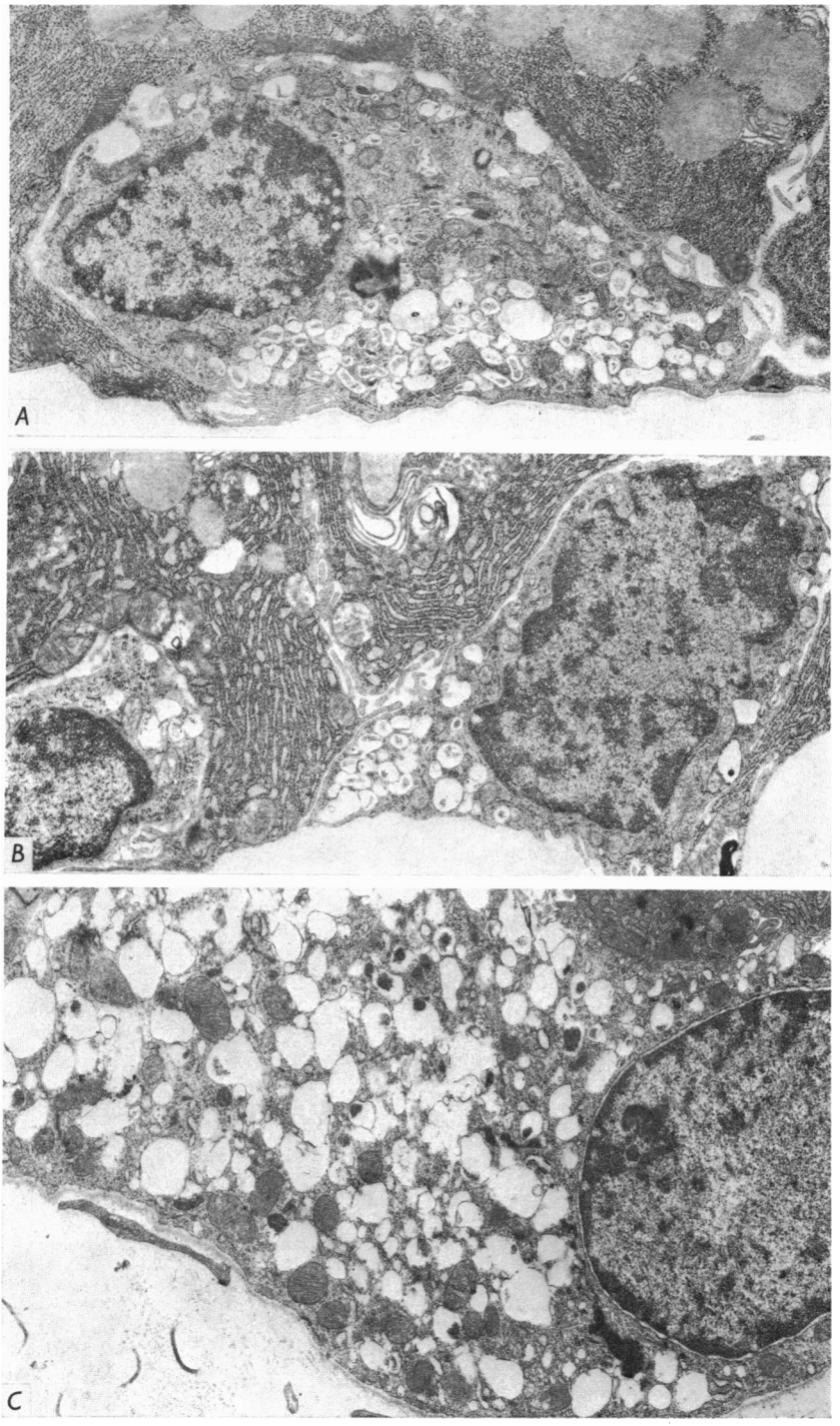
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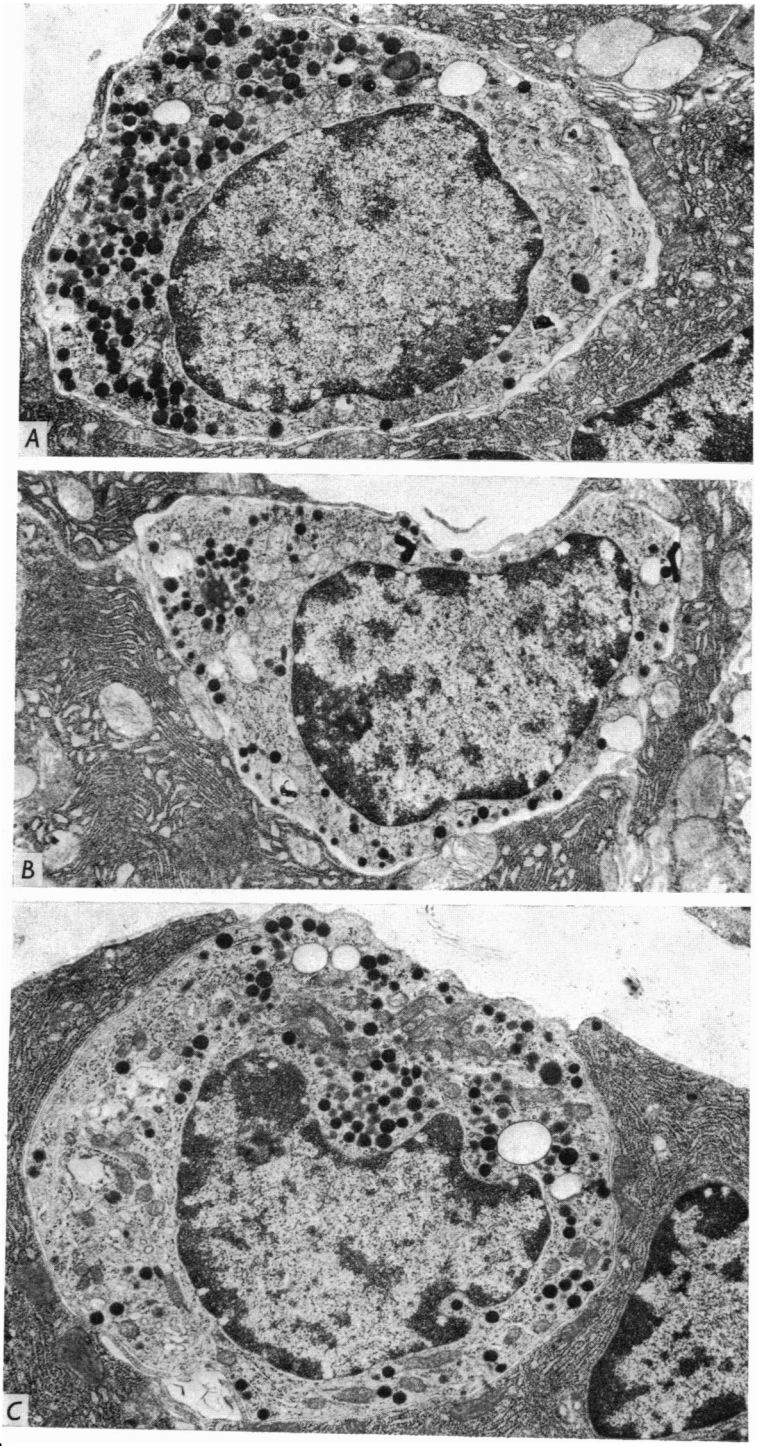
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