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The anatomy of the gastrin cell

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Summary

Changes in the numbers of G cells and the formation and release of gastrin granules have been studied by means of radioimmunoassay and quantitative electron microscopy. The appearance of G-cell granules was affected by the pH and duration of fixation, and after prolonged fixation immature newly formed granules could be identified. In rats fasting up to 3 days first the release and then in turn the maturation and synthesis of granules were depressed. Ultimately the renewal of G cells was inhibited and their numbers declined. During an acute stimulus only a small proportion of total antral gastrin was released and the appearance of G cells was unaltered. In patients treated with cimetidine for 12 months there was no G-cell hyperplasia despite raised stimulated gastrin levels.

Introduction

The hormone gastrin stimulates parietal and pepsin cells, increases gastric mucosal blood flow *(1)*, and has a trophic effect on gastric, duodenal, and probably colonic mucosa *(2)*. The cellular origin of gastrin, the gastrin or G cell, was first determined in 1967 by means of electron microscopy *(3)* and this was later confirmed by immunofluorescence techniques. Eight different individual endocrine cells have now been discovered in the stomach alone and of these the identification of the G cell is the most certain *(4)*.

The greatest concentration of G cells is found in the gastric antrum in the mid and deep zones of the antral glands. There are around half a million G cells/cm^2 and in man this amounts to a total of about 10 million G cells *(5)*. The number of cells decreases along the duodenum and none are usually present in the gastric fundus. A typical G cell (Fig. 1) is flask-shaped, with a broad base and a narrow neck. Within the cytoplasm are numerous secretory granules, particularly in the infranuclear area, some appearing empty while others contain a dense homogeneous material, with a range of forms in between *(6)*. The free surfaces of the narrow apical poles of the cells project into the gut lumen as microvilli which probably act as direct luminal receptors.

During the cephalic phase of digestion vagal branches to the antrum stimulate G cells directly. When food reaches the stomach gastrin is released owing to the co-operative effects of distension, the buffering action of food on antral pH, and specific chemical stimuli within food, mainly polypeptides and amino-acids. After a meal the buffering action of food wanes, the pH of the gastric contents drops, and this, together with the inhibitory effects of small-bowel hormones, completes a negative feedback loop. There is an attractive economy in this scattered population of G cells each with individual luminal receptors. Such an arrangement allows for the amount of gastrin released to be titrated against the volume and concentration of stimuli depending on the number of cells exposed to a meal.

Electron microscopic and immunochemical studies of antral mucosa from patients with duodenal ulcer, Zollinger–Ellison syndrome, antral G-cell hyperplasia, acromegaly, hyperparathyroidism, and pernicious anaemia have demonstrated changes in the numbers and appearance of G cells *(7)*. Pale secretory granules were thought to represent the emptied sacs remaining after the release of gastrin from over-active G cells, implicating them in the causes or effects of these diseases. This inter-

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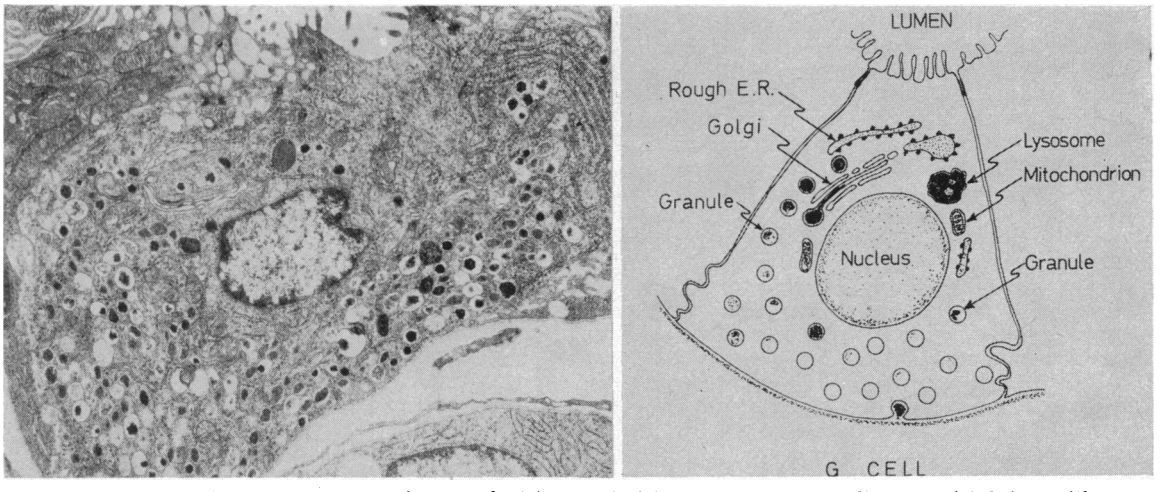

FIG. 1 *Rat antral G cell, electron micrograph, ×7070 (left). Compare with diagram (right) to illustrate the principal intracellular components and anatomical features of a G cell.*

pretation was based on laboratory experiments in which a feeding–fasting cycle was described. G-cell granules were dense during fasting and pale or 'empty' after feeding, the gastrin, it was suggested, being released from the granules into the cytoplasm before leaving the cell by a process called molecular dispersion *(8)* (Fig. 2). There is increasing evidence, however, that many polypeptides are secreted by exocytosis, in which small numbers of whole granules migrate to and fuse with the basement membrane, releasing their contents, and there is some evidence that this occurs in gut endocrine cells *(6,9)*.

These conflicting reports leave room for further studies on the structure and function of G cells. The work described here was in two parts. In the first, experiments were devised to test the effect of varying preparation techniques on the histological appearance of G cells and to examine the effects of starvation and acute stimulation on gastrin and G cells. In the second, clinical section, biopsy specimens from patients with duodenal ulcer given long-term cimetidine treatment were studied for evidence of G-cell hyperplasia.

Experimental studies

METHODS

Male Wistar rats were used for the first three studies. Immunoreactive gastrin (IRG) in serum and antral extracts was measured using a conventional radioimmunoassay (RIA) with synthetic human gastrin I (MRC 68/439) as standard. The method and characterisation of the assay have been reported in detail

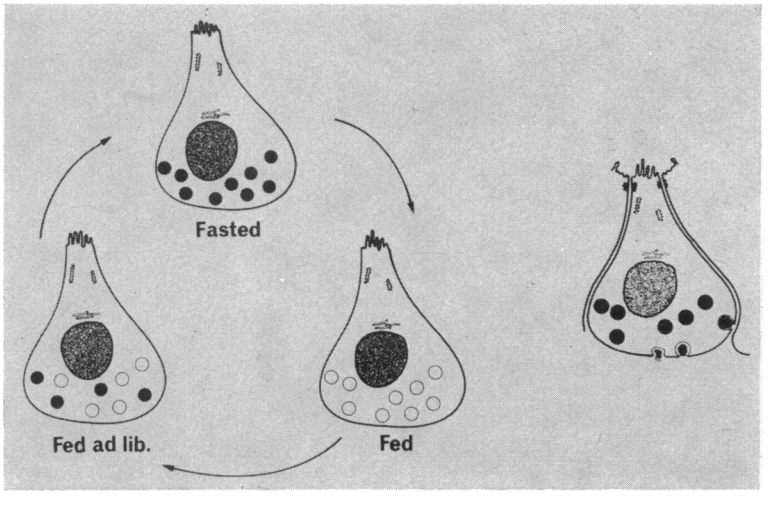

FIG. 2 *Diagram illustrating the two theories on the mechanism of release of gastrin. Left, 'molecular dispersion', in which after gastrin release by a feeding stimulus the hormone passes across the cytoplasm and out of the cell, leaving granules with an empty appearance. Right, 'exocytosis', in which entire granules fuse with the cell membrane and release their contents.*

elsewhere *(7)*. The ultrastructural details of G cells were assessed by quantitative electron microscopy (QEM) and never by simple observation alone *(6)*. Micrographs of G cells were taken and assessed 'blind' in a systematic random manner in order to eliminate observer bias. Four main methods were used:

1) The appearance of individual gastrin granules was assessed with reference to a set of standards arbitrarily dividing them into three groups—electron dense, pale, and empty (see Fig. 3). The proportion of each type was deduced from either an analysis of every granule on the micrograph or a representative selection of granules chosen at random by sampling.

2) When large numbers of cells were studied the cellular components, particularly the granules, were sampled using point counts. A square lattice on transparent film was placed over each micrograph and the number of intersections (points) overlying granules was counted together with the total number of points overlying the cytoplasm. The granule content of G cells could then be calculated and this was directly related to the hormone content of the tissue *(6,10)*.

3) The preferential distribution of dense granules around the Golgi complex of G cells was measured using an oblong mask placed lengthways over the long axis of the Golgi stacks (Fig. 3). The granules in this sample were compared with either those in a similar area of cytoplasm chosen at random or those from cells under differing physiological conditions.

4) The number and density of G cells within antral glands was measured by counting on low-power micrographs.

The effects of changes in the preparation of material for electron microscopy *(9)* were studied in two groups of 6 animals each. The stomach was removed and opened along the lesser curve; 1-mm cubes of antrum adjacent to the pylorus from the first group were fixed for 24 h at pH 5.0, 6.0, 7.0, and 8.0 in glutaraldehyde and those from the second fixed for 30 min, 1 h, 2 h, and 24 h in glut-

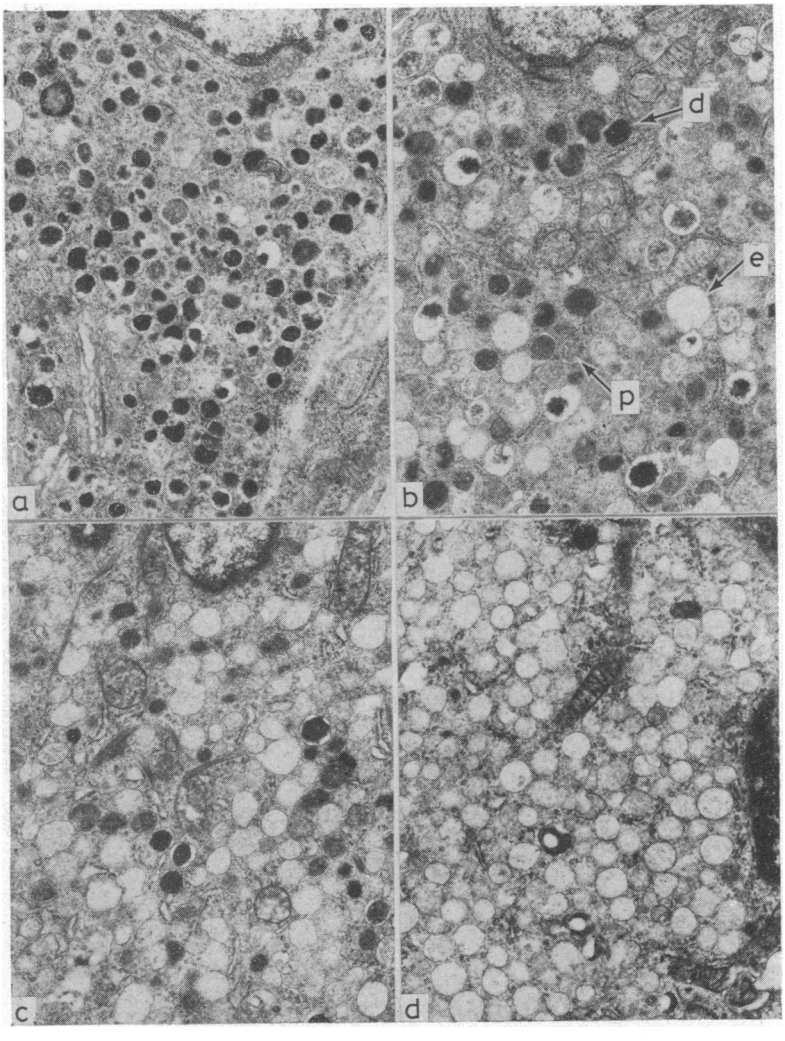

FIG. 3 *Effect of duration of fixation on appearance of G-cell granules,* ×11 170. *Key: d, 'dense' granule; p, 'pale' granule; e, 'empty' granule. Fixation at pH 7.4 in 4% glutaraldehyde for (a) 0.5 h, (b) 1 h, (c) 2 h, (d) 24 h. Note the increased proportion of 'empty' granules as the period of fixation is prolonged.*

araldehyde at pH 7.3. Micrographs of G cells were prepared and all the gastrin granules classified into the three types described above, more than 5000 granules being examined for each experiment.

The effect of fasting on gastrin and G cells was investigated in groups (n=6) of matched fasted animals and fed controls killed after 24 h, 48 h, and 72 h *(7)*. A 2-ml sample of blood was collected for RIA of serum. From the opened stomach two adjacent 1-mm strips of antral mucosa were removed from each animal. One strip was weighed, boiled in distilled water, and the extract stored for RIA. The other strip was divided into 1-mm cubes and fixed for 1 h at pH 7.3. Micrographs of G cells were assessed by QEM.

Acetylcholine is the most potent topical stimulant of antral gastrin release *(1)*. It was used to study the effects of a short maximal stimulation on gastrin and G cells and was coupled with a bicarbonate buffer to minimise any inhibition of gastrin by the concomitant secretion of gastric acid. The method has already been described in detail *(10)*. Briefly, in matched animals fasted for 24 h and anaesthetised with urethane a cannula was passed from a duodenotomy through the pylorus and secured while a clip occluded the oesophagus. The isolated stomach was washed out and instilled with 2 ml of warm 2% acetylcholine in bicarbonate or with distilled water. In two groups of animals (n=26) the experiment ran for 30 min and in a third (n=13) for 120 min. A further group of controls (n=8) were sham-operated. Serum and antral mucosal samples were taken for gastrin RIA and QEM as in the previous experiment.

RESULTS

1) Preparation of antral mucosa for electron microscopy (9) Increasing the pH of fixation resulted in a decline in dense granules. At pH 5.0 roughly one-half were dense, but at pH 8.0 the majority were empty ($p<0.001$). With prolonged fixation there was a progressive fall in the proportion of dense-cored granules (Fig. 3) from 50% after $\frac{1}{2}$ h to 15% at 24 h ($p<0.001$). A similar effect has been observed in human G cells. In G cells fixed for 24 h at pH 7.3, when most of the granules appeared empty, there was a greater proportion of dense granules around the Golgi zone ($p<0.001$). These, we suggest, are newly formed granules (Fig. 4).

2) Fasting (7) Fasted animals progressively lost weight. Serum IRG levels in fasting animals were one-eighth of those in fed controls ($p<0.001$), but there was no difference on any of the three experimental days within either the fasted animals or fed controls (Fig. 5). Antral IRG was unchanged in fed controls, while in fasted animals there was a slight increase after 24 h and a progressive fall after 48 h to one-half of control levels ($p<0.05$).

At QEM there was a decrease in the numbers of G cells after 72 h fasting. There were constant numbers of granules in controls throughout the experiment (Fig. 6). In fasted animals there was an increase in granule content at 24 h ($p<0.001$). After 48 h and 72 h, however, the situation was reversed and the granule content was reduced ($p<0.001$) without any change in the proportion of dense granules. The correlation coefficient (r) between average antral IRG and granule content during the period of fasting was 0.98 ($p<0.001$). There was a decrease in the proportion of dense granules from fasted animals in the special population of granules around the Golgi zone ($p<0.001$).

3) Stimulation with acetylcholine (10). Sham-operated and control animals had similar serum IRG levels at 30 min and 120 min, while in

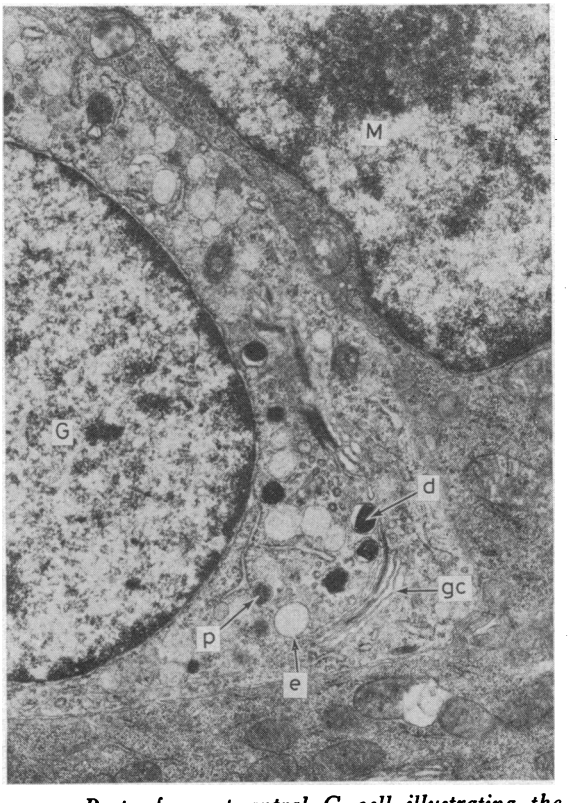

FIG. 4 *Part of a rat antral G cell illustrating the preferential relationship of dense cored granules with the Golgi zone,* ×11 370. *Fixation in 4% glutaraldehyde for 24 h. Key: d, 'dense' granule; p, 'pale' granule; e, 'empty' granule: gc, Golgi cisternae; G, G cell; M, mucus cell.*

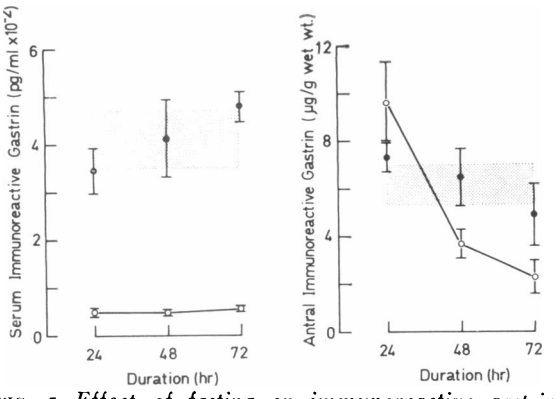

FIG. 5 *Effect of fasting on immunoreactive gastrin content of the serum (pg/ml : mean ±1 SEM) and antrum (μg/g wet tissue) of rats. Shaded area represents the mean ±1 SEM of the gastrin data for all the fed controls taken as a single group.*

acetylcholine-stimulated animals serum IRG was 4 times higher ($p<0.001$). There was no difference in antral IRG at either 30 min or 120 min.

QEM paralleled antral IRG results at an ultrastructural level. In tissue fixed for a short period there was no change after 30 min or 120 min stimulation in either the granule content of G cells or the proportion of dense granules, despite the elevated serum IRG levels. The expected decrease in the proportion of dense granules when tissues were fixed for a longer period was observed, but this change affected control and acetylcholine-stimulated G cells alike.

DISCUSSION

Maturation of gastrin granules. Any method of tissue preparation for electron microscopy has a degree of artefact. These experiments demonstrate that both the pH and duration of fixation profoundly affect the appearance of hormone granules in gastrin cells. The gastrin granule probably contains a carrier protein and an amine in addition to gastrin. An enkephalin has been located in human antral G cells *(11)*, but its significance is not known. These constituents are in the form of an osmotically inactive core in vivo to facilitate secretion, and this is 'etched' by fixation procedures. Not all the granules are the same, however. The dense granules found near the Golgi zone are probably immature, recently synthesised granules and there is further evidence to support this suggestion from the fasting experiment, in which as fasting became prolonged a decline in these granules was observed. During synthesis the hormone and its carrier protein appear to be formed as parts of a larger precursor protein molecule which is subsequently split enzymatically to yield the active hormone. The various molecular forms of gastrin probably arise in this way. Ultrastructurally a dense granule containing the precursor is observed near the Golgi zone, a pale granule with a mixture of components, or an empty granule with only gastrin and cleavage peptide in the basal part of the cell. A second type of G cell has been identified in the duodenum which contains only big gastrin, the predominant form found in the duodenum. These endocrine cells have small, dense granules similar to the dense granules found around the Golgi zone of antral G cells. Perhaps these duodenal G cells do not have the enzyme which splits big gastrin to yield little gastrin *(12)*.

Control of gastrin synthesis and G-cell kinetics The results of the fasting experiment suggest the following sequence of events. After 24 h fasting, while gastrin release has decreased the production of hormone continues, resulting in an increase in intracellular stores. By 48 h the production of new hormone and the granules near the Golgi zone has ceased, but a steady basal secretion of gastrin persists and this may be due to extragastric, perhaps duodenal, secretion of gastrin *(1)*. After 72 h

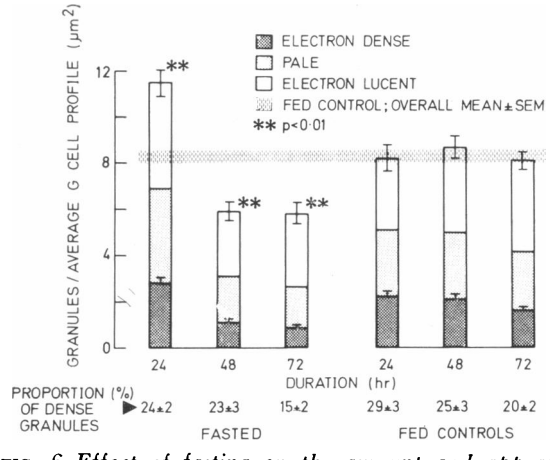

FIG. 6 *Effect of fasting on the amount and appearance of secretory granules in rat antral G cells. In each case the total is divided into the parts contributed by the three types of granule, with the proportion of dense granules (% mean ± 1 SEM) given numerically.*

there is a further fall in antral gastrin levels owing to a decline in the numbers of G cells. Starvation leads to a decline in the intestinal cell population as a result of a reduction in cell renewal and cell migration *(2)*. Mouse intestinal endocrine cells have a turnover time of 4 days *(13)*, but mouse gastrin cells may have a turnover time of 2–4 months *(14)*. A 60% reduction in G cells after 72 h fasting reported by Lichtenburger *et al.* *(15)* is similar to these results and consistent with the shorter turnover time. It is not known, however, whether G cells simply divide, half of them constantly moving up the antral glands to be shed by exfoliation, or whether they arise from a precursor. Small endocrine cells with few granules have been observed which might fulfil this role *(6)*.

The physical presence of food and its chemical constituents in the gut lumen are necessary for normal gastrointestinal function. G-cell renewal and gastrin synthesis also depend upon these factors since parenteral nutrition does not prevent a decline in G-cell activity with starvation *(2)*. The trophic action of food on the upper gut is in turn partly mediated by gastrin. In addition to food growth hormone, serum calcium levels, chronic changes in the gastric pH, and possibly other local endocrine and neural reflexes combine to control the overall numbers of G cells and the general level of G-cell function.

That the gastrin mechanism is resistant to fatigue in the short term is confirmed by the observation that even after 120 min maximal stimulation with acetylcholine no changes in either antral IRG or G-cell ultrastructure were found. We have estimated that between 2 and 10% of antral gastrin stores would be released during this period *(6,10)*, not allowing for synthesis of fresh hormone, and such small changes would not be detectable by current techniques. These calculations do not take into account the possible loss of gastrin into gastric juice *(16)*, but the physiological significance of this discovery is not clear and there is no ultrastructural evidence to support the secretion of gastrin from the luminal surface of G cells.

Mechanism of gastrin release Forssman and Orci *(8)* based their original hypothesis of gastrin release by molecular dispersion on striking changes in gastrin granule electron density during a feeding cycle. On the contrary we have not found changes in granule density with either a fasting-induced inhibition of gastrin or an acute stimulus to gastrin release. The appearance of granules is also very sensitive to fixation conditions. The empty granules thought to signify active G cells found in clinical biopsy specimens *(17)* are probably the result of prolonged fixation and have no physiological significance. In subcellular fractionation studies gastrin is located within granules and not free in the cytoplasm, and the correlation between antral IRG and G-cell numbers shown here suggests that entire granules are lost by exocytosis. This process takes only milliseconds and the chances of observing it in a scattered population of G cells are remote. Many polypeptide hormones are now thought to be released by exocytosis. Conclusive proof for the release of gastrin in this way will not come from electron microscopy but must await the demonstration of the simultaneous release of the other, as yet unknown, components of the gastrin granule core.

Clinical studies

CIMETIDINE AND G CELLS

The histamine H_2 receptor antagonist cimetidine reduces gastric acid secretion to levels found after vagotomy. Metiamide, a forerunner of cimetidine, induces increases in serum gastrin and parietal cell mass in rats. It has been suggested that similar changes in man may lead to increased ulcer recurrence after cimetidine treatment. Small changes in serum and meal-stimulated gastrin levels and acid secretion after 6 months' treatment have been reported *(18)*, but they revert to normal on stopping treatment and G-cell hyperplasia has not been detected. Therefore we have studied 17 patients with duodenal ulcer treated with cimetidine 1.6 g daily for 12 months, looking for evidence of G-cell hyperplasia.

Gastric function tests and estimations of Oxo-stimulated integrated serum gastrin levels (IGR) were repeated at intervals and have been reported in detail elsewhere *(18)*. Endoscopic antral biopsy specimens, where suitable, were oriented and examined 'blind'. G-cell density—the number of G cells per grid space

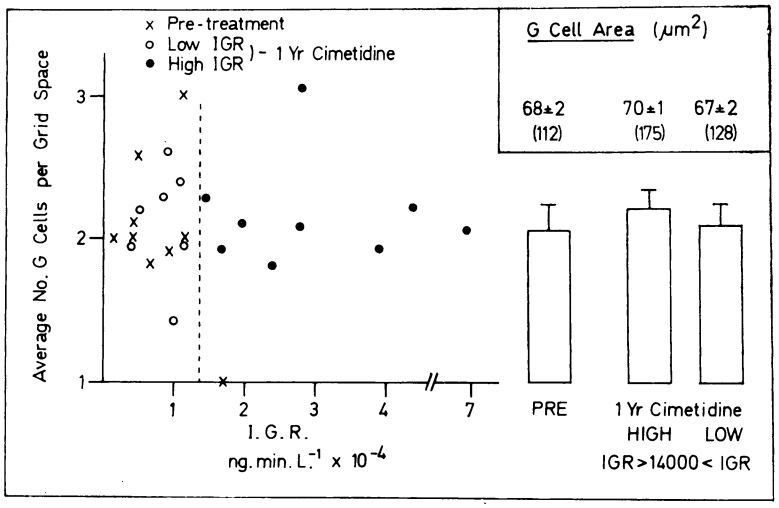

FIG. 7 *Effect of treatment with cimetidine on numbers of G cells (average number per grid space), G-cell area (μm² ± 1 SEM, numbers of cells studied in parentheses), and integrated gastrin responses to an Oxo test meal (ng/min/l × 10⁻⁴). The histograms show the number of G cells (mean ± 1 SEM) before treatment and in the two groups of patients, those with an IGR less than 14 000 and those with a greater IGR, after treatment.*

—was measured in low-power micrographs of all the antral glands in each biopsy section.

Pretreatment IGR were all, with one exception, below 14 000 ng/min/l and G-cell density in the same patients was 2–3 cells per grid space (Fig. 7). After treatment there was a significant overall increase and in half the patients (n=9) the IGR was greater than 14 000 ng/min/l, suggesting that this subgroup might have developed G-cell hyperplasia, but there was no change in G-cell density or cell size in either this subgroup or the treated patients as a whole. At high-power electron microscopy G cells from this group with high IGR exhibited dilated endoplasmic reticulum, suggesting increased gastrin synthesis by individual cells, and this is being investigated. After stopping treatment gastric function returned to pretreatment levels within 6 weeks.

We therefore could not demonstrate G-cell hyperplasia after 12 months' treatment with cimetidine. In man long-term changes in gastric pH—for example, after a vagotomy or in pernicious anaemia—may cause G-cell hyperplasia. These results suggest that this occurs after a period greater than 12 months and that until then at least the normal homoeostatic mechanisms which control gastric acid and gastrin secretion are still intact.

G CELLS AND DUODENAL ULCER

While interest in gastrin as a factor in the aetiology of duodenal ulceration has waned, the complicated relationship between gastrin secretion, G-cell numbers, parietal cell mass, and gastric acid output is becoming clearer. In patients with gastrinomas high serum concentrations are responsible for the acid hypersecretion which produces peptic ulcers. In patients with ordinary duodenal ulcer the fasting serum gastrin level is normal or slightly elevated, but there is an increased response to feeding, an impairment of the mechanism by which acid inhibits gastrin, and an increased sensitivity to gastrin. Patients with duodenal ulcer also have higher basal and maximal acid outputs than normal controls and this might be explained by the higher stimulated gastrin level's trophic effects giving rise to a greater parietal cell mass *(19)*. It is now thought that patients with duodenal ulcer and hypergastrinaemia due to overt antral G-cell hyperplasia are rare. G-cell hyperplasia may represent the extreme end of the normal range of G-cell numbers in the antrum. In one study the G-cell population ranged from 8 to 15 (mean 10) million in control patients, and from 3 to 43 (mean 18) million in those with duodenal ulcer *(5)*. It is possible that increased gastrin levels are a result of hypersecretion from a normal G-cell population. Combined radioimmunoassay and electron microscopic studies such as those presented here could be used to investigate this possibility.

These studies were carried out in the Departments of Anatomy and Surgery, University of Bristol, and the Department of Human Anatomy, University of Oxford. I am especially grateful to Dr J F Morris, with whom I have collaborated, and to Professor B Pickering and Dr R F Harvey, who encouraged and

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