

# Gastrin and the ultrastructure of G cells in the fasting rat

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**SUMMARY** The effect of fasting on serum and antral gastrin concentrations and G cell ultrastructure in the rat has been examined using a radioimmunoassay and quantitative electron microscopy. Serum gastrin levels in fasting animals were markedly reduced and there was also a significant decrease in antral gastrin concentrations after 48 hours and 72 hours of fasting. This was associated with a significant fall in the granule content and cytoplasmic volume of individual G cells, at its greatest by 48 hours. A relative absence of electron dense granules in the Golgi zones of cells from animals fasted for 72 hours suggested a paucity of newly formed granules, but fasting produced no detectable change in the electron density of the granule population taken as a whole. The results indicate that, during fasting, release and then synthesis of gastrin is inhibited, so that granule stores and cell size diminish. The correlation between the granule content of G cells and the antral content of gastrin suggests that hormone release occurs by exocytosis, rather than by any change in the content of individual granules.

There are a number of reports of the ultrastructural appearance of gastrin cells (G cells) of the pyloric antrum in a variety of pathological conditions. These conditions include primary G cell hyperplasia (Polak *et al.*, 1972; Cowley *et al.*, 1973; Ganguli *et al.*, 1974); antral G cell hyperplasia secondary to pernicious anaemia (Creutzfeldt *et al.*, 1971; Polak *et al.*, 1971a, 1973); acromegaly (Creutzfeldt *et al.*, 1971), or hyperparathyroidism (Creutzfeldt *et al.*, 1971; Polak *et al.*, 1971b); and duodenal ulcer (Solcia *et al.*, 1970; Creutzfeldt *et al.*, 1975; 1976). Pale or electron lucent granules found within the cytoplasm of G cells in these conditions have been interpreted as the emptied sacs remaining after the release of gastrin from the hormone granules of over-active cells. However, there have been few experimental studies relating structure to function in the antral G cell. Changes in the appearance of G cell granules have been described during a feeding-fasting cycle (Forssmann and Orci, 1969), and in one study (Creutzfeldt *et al.*, 1975) these morphological changes were correlated with differences in the antral content of extractable gastrin measured by radioimmunoassay. When antral gastrin concentrations were low, mainly 'empty' granules were found, and, conversely,

when gastrin concentrations were high, more electron dense granules predominated. By contrast other studies of G cells subjected to hormone releasing stimuli have failed to reveal such dramatic changes in the appearance of G cell granules, and exocytosis has been suggested as the mode of hormone release (Kobayashi and Fujita, 1973; Osaka *et al.*, 1973). Our recent studies on the effect of different fixation conditions on the appearance of G cell granules suggest that 'empty' granules, seen ultrastructurally, represent mature granules which have been disrupted by inappropriate fixation conditions (Mortensen and Morris, 1977). We have thus reinvestigated the effect of fasting for up to three days on serum and antral concentrations of gastrin, and on the ultrastructure of G cells in the rat antrum, in an attempt to correlate structure with function.

## Methods

Thirty-six male Wistar rats weighing 220-275 g were housed individually in cages with a 1 in. mesh floor to minimise coprophagia. Animals were allowed free access to food (Oxoid breeding pellets, H. Styles, London) and tap water, for an acclimatisation period of one week. At the beginning of the experimental period the animals were again weighed, and food, but not water, was withheld from 18 rats, both food and water being available

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to the remaining 'fed' controls. Twenty-four hours, 48 hours, and 72 hours later six animals from each group, taking alternate fasted animals and fed controls, were weighed and then killed by decapitation. All animals were killed between 9 am and 10 am in order to minimise differences resulting from any diurnal fluctuations in G cell activity.

#### SELECTION OF TISSUES

Two to three millilitres of blood were collected from the neck of each decapitated animal. The abdomen was opened, and the distal half of the stomach removed. The lesser curve was incised and the opened stomach laid flat on a sheet of soft modelling wax. Using a slicer consisting of three razor blades held in parallel 1 mm apart, two strips of antrum adjacent to the pylorus were removed so that comparable samples of tissue were taken from each animal.

#### PROCESSING OF TISSUES FOR RADIOIMMUNOASSAY (RIA)

One strip of antrum was weighed and placed in 2 ml boiling distilled water in a sealed bijou bottle immersed in a boiling water bath for 15 minutes. The heat extracted sample was centrifuged at 2650 g, 4000 rpm, for 15 minutes, the supernatant filtered, and a 50  $\mu$ l aliquot diluted in 300 volumes of distilled water. The clotted blood samples were centrifuged and the serum, together with the antral extracts, stored at  $-20^{\circ}\text{C}$  for up to two weeks before radioimmunoassay for gastrin.

#### RADIOIMMUNOASSAY

Antisera were generated in rabbits against synthetic human gastrin I, residues 2-17, conjugated to bovine serum albumen by the method of McGuigan (1968) (kindly provided by Dr J. C. Thompson). Synthetic human gastrin I (G 17), MRC 68/439, was used as standard. Synthetic human gastrin I (ICI, UK Ltd) was labelled with  $^{125}\text{I}$  (Amersham, UK), purified by column chromatography on G-10 Sephadex, and used as a label in a radioimmunoassay system of the type described by Odell *et al.* (1967). The assay has been used for work previously reported in the literature (Clendinnen and Owens, 1973; Spence *et al.*, 1977), and on a molar basis the assay has equal cross-reactivity with synthetic human gastrin I, natural little and big gastrins I (17 and 34 residues), approximately twice the reactivity with minigastrin, but only half the reactivity with the sulphated little gastrin (II) (17 residues) (Table 1); cross-reactivity with cholecystokinin-pancreozymin was less than 1 in 1000. Prior absorption with charcoal removes 95% of activity from serum and therefore the measured

basal serum gastrin values are not an artefact of non-specific inhibition by plasma proteins. More precise gastrin absorption experiments with bound antisera have, however, not been performed, as these would have required more antiserum than was available (R. F. Harvey, personal communication). Figure 1 illustrates the inhibition curves for aqueous rat antral extract and for human gastrin standard. As they are parallel, the assay appears to be suitable for the estimation of rat gastrin.

Table 1 Characterisation of radioimmunoassay: the inhibitory  $D50s^*$  (f mol/ml incubation mixture) of a set of standards measured in this gastrin assay

Source	Standard	Inhibitory $D50$
MRC†	68/439	36.1
CURE‡	HG 17-I	42.5
CURE	HG 17-II	105
CURE	HG 34-I	42.5
CURE	HG 13-I	18.0

\*Inhibitory  $D50$ —the dose of peptide required to reduce binding of labelled standard to 50% of the initial value, having subtracted blank values.

†The standard usually employed—synthetic human gastrin I (G—17).

‡CURE: Centre for Ulcer Research and Education.

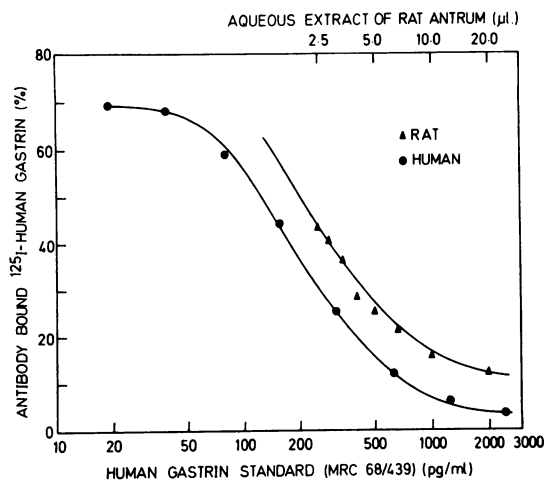


Fig. 1 Comparison of the behaviour of an aqueous extract of rat pyloric antrum and human gastrin standard (MRC 68/439) in the radioimmunoassay. The two substances give parallel displacement of human gastrin I from the antibody-hormone complex.

#### PROCESSING OF TISSUE FOR ELECTRON MICROSCOPY

The other strip of antral tissue was divided into 1 mm cubes which were fixed by immersion for exactly one hour in 4% glutaraldehyde in 0.1 M

sodium cacodylate buffered at pH 7.3 at room temperature. For animals in the 72 hour group only, some cubes of antral tissue were immersed for 24 hours in the glutaraldehyde fixative at the same pH. Fixed tissues were washed in 0.1 M sodium cacodylate buffer (pH 7.3) containing 7.5% sucrose, postfixed in 1% osmium tetroxide in Palade's (1952) buffer, dehydrated in a series of ethanol concentrations and propylene oxide, and embedded in Spurr's (1969) resin. No more than two minutes elapsed between decapitation of an animal and the immersion of tissue samples in either fixative or boiling water.

#### ELECTRON MICROSCOPY

Ultra-thin sections of silver interference colour were doubly stained with 2% methanolic uranyl acetate and Reynolds (1963) lead citrate and examined in a Philips EM 300 microscope operated at 80 kV. Longitudinal sections of antral mucosa were examined for G cells in a systematic random manner (Weibel, 1969) and, from each animal, six micrographs were exposed of profiles of G cells which included the nucleus. G cells were identified by their well-defined ultrastructure (Solcia *et al.*, 1975). Their most characteristic feature is the secretory granule population they contain. Most importantly, the granules vary in electron density (Forssmann *et al.*, 1969; Capella and Solcia, 1972; Mortensen and Morris, 1977; Sato, 1978). This characteristic is not shared with other endocrine cells of the antrum from which the G cells may readily be distinguished. Grids were examined and micrographs exposed and analysed with the observer unaware of the pre-treatment of the animals.

#### STEREOLOGICAL AND STATISTICAL TECHNIQUES

The secretory granule population of the cytoplasm was assessed by stereological techniques (Weibel, 1969). A test system comprising a 0.7 cm square lattice was placed over each micrograph and the numbers of test points overlying secretory granules was counted, together with the total points overlying both the cytoplasm and the nucleus. The cytoplasmic, nuclear, and thus complete G cell profile areas were calculated from the number of point intercepts for the compartment and the magnification of the micrographs. The point density (Pp) occupied by the granules in the cytoplasm of a G cell profile was calculated by:

$$\frac{\text{number of points overlying granules}}{\text{number of points overlying cytoplasm}}$$

The Pp for granules gives an estimate of their

volumetric density (volume occupied by granules per unit volume cytoplasm) in the cytoplasm of individual G cells. Since the area of randomly selected G cell profiles in sections is directly related to the volume of the cells, the average area for a group is related to their average volume. Therefore, a value directly related to the total volume occupied by granules in G cells (which we will call the 'secretory granule content' per average G cell profile—SGC) can be calculated by the product:

$$\text{SGC} = \text{Pp granules} \times \text{average area of G cell cytoplasm (for the group)}$$

Calculation of this value compensates for any changes in cell size and should therefore be more directly related to the hormone content of the tissue.

To obtain a quantitative assessment of the electron density of the secretory granule population, for every point overlying a granule, the electron density of the granule was assessed with reference to a set of standard granule images which arbitrarily divides granules into three groups—electron dense; pale; and electron lucent or empty (Mortensen and Morris, 1977). The relative proportion of each form of granule was then estimated from the point counts.

We have previously demonstrated that, in tissues prefixed for 24 hours at pH 7.3, there is a preferential localisation of dense-cored granules around the Golgi complex, while the majority of the granules scattered in the cytoplasm appear electron lucent, and we have suggested that the granules that remain electron dense after this prolonged fixation represent the most newly packaged granules (Mortensen and Morris, 1977). In order to test further whether 72 hours' fasting altered the production of G cell granules, a separate assessment was made of the granules in the Golgi area by placing lengthways over the long axis of the Golgi stacks an oblong mask,  $1 \mu\text{m} \times 3 \mu\text{m}$ . The electron density of granules in this 'Golgi region' was then assessed, and the proportion of dense-cored granules in the region compared between fed and fasted animals.

Much of the data was not normally distributed, and therefore non-parametric statistical tests have been used. Within-treatment effects were analysed using the Kruskal-Wallis one-way analysis of variance; differences between any two group of data by the Mann-Whitney-U test.

#### Results

##### BODY WEIGHT

The two groups of animals were well matched by weight at the beginning of the experimental period.

The fasted animals progressively lost 20 g of body weight per 24 hours, while the weight of fed controls slightly increased at about the rate normally expected for this strain and age of rat (Table 2).

Table 2 *Effect of starvation for 24, 48, or 72 hours on body weight (g) (mean  $\pm$  SEM) of rats*

Duration of experiment (h)	Body weight (g) (mean $\pm$ SEM)		Significance of difference (P)
	Starved (6)	Fed controls (6)	
0	249 $\pm$ 2	247 $\pm$ 3	NS
24	224 $\pm$ 4	262 $\pm$ 3	0.001
48	206 $\pm$ 2	259 $\pm$ 7	0.001
72	181 $\pm$ 4	263 $\pm$ 4	0.001

Number of rats in parentheses.

#### IMMUNOREACTIVE GASTRIN (IRG) IN SERUM AND ANTRUM

The IRG levels in the sera of the fasted animals were significantly lower ( $P < 0.001$ ) than those of fed controls at each time studied. This 'basal' serum IRG level in fasted animals remained constant at around 50 pg/ml. There was no significant difference between serum IRG concentrations on any of the three experimental days within either the fasted animals or the fed controls (Fig. 2). There were no significant differences between antral

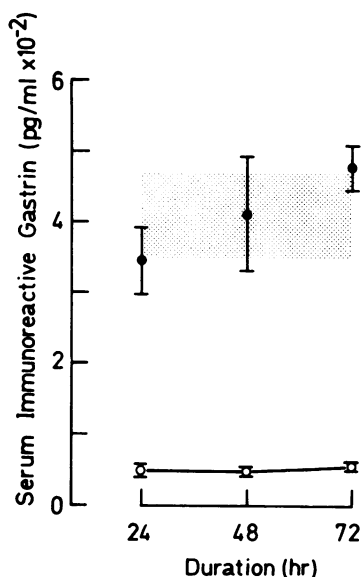


Fig. 2 *The effect of fasting on the immunoreactive gastrin content (pg/ml; mean  $\pm$  SEM) of the serum of rats. The shaded area represents the mean  $\pm$  1 SEM for the serum gastrin data for all the fed controls taken as a single group.*

IRG concentrations at any time in the fed controls (Fig. 3) but, by contrast, IRG levels in the antrum fell progressively over the period of starvation. Antral IRG concentrations were significantly lower in fasted than in fed control animals at 48 hours ( $P < 0.05$ ) and 72 hours ( $P < 0.05$ ).

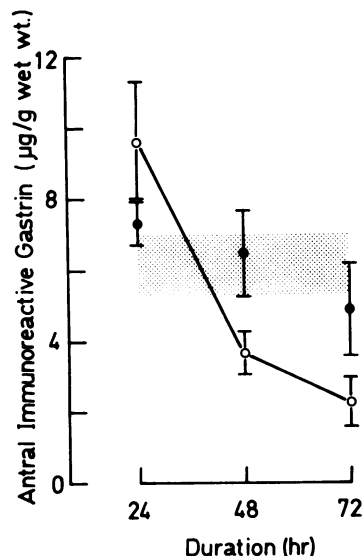
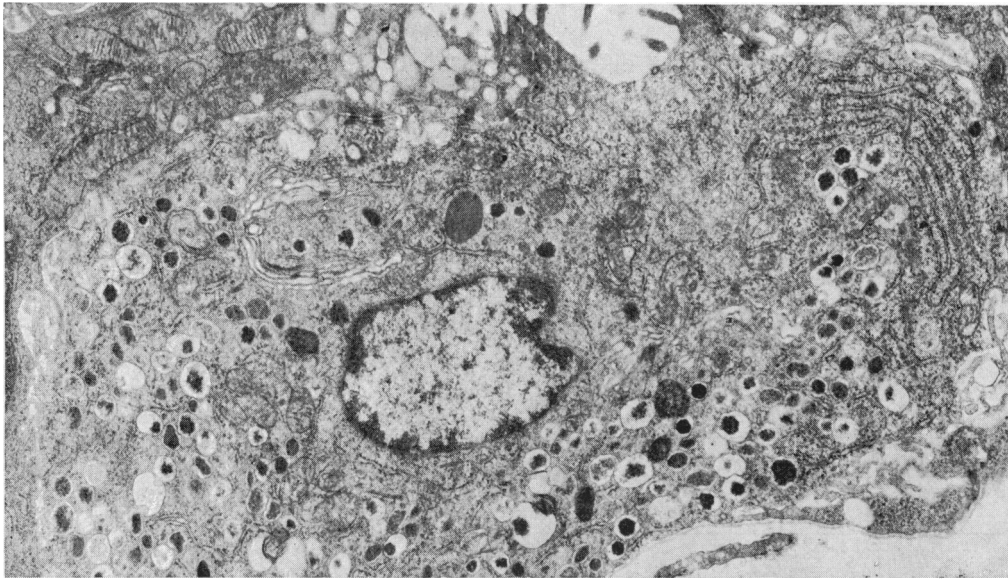


Fig. 3 *The effect of fasting on the immunoreactive gastrin content ( $\mu$ g/g wet tissue) of the pyloric antrum of rats. The shaded area represents the mean  $\pm$  1 SEM of the antral gastrin data for all the fed controls taken as a single group.*

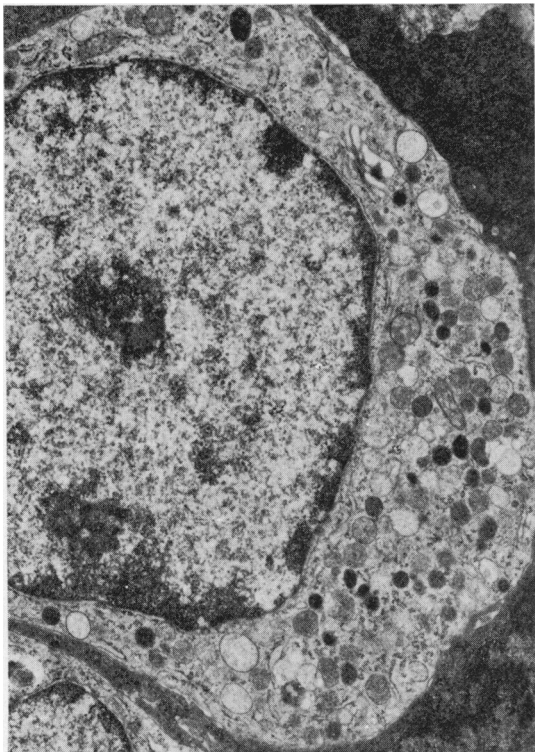
#### ULTRASTRUCTURE OF G CELLS

The general ultrastructural features of the antrum were not greatly changed during the period of fasting, though there appeared to be many fewer G cells in tissue from animals fasted for 72 hours. An attempt was made to measure this impression by counting G cell profiles in the electron microscope, but the degree of variation from one section to another from the same tissue precluded statistical analysis. However, it is noteworthy that, by comparison with the study of fed controls and animals fasted for 24 or 48 hours (1) many more sections had to be cut from tissue from animals fasted for 72 hours and (2) much more time was needed to search those sections for G cells, in order to collect the number of micrographs required for quantitative analysis.

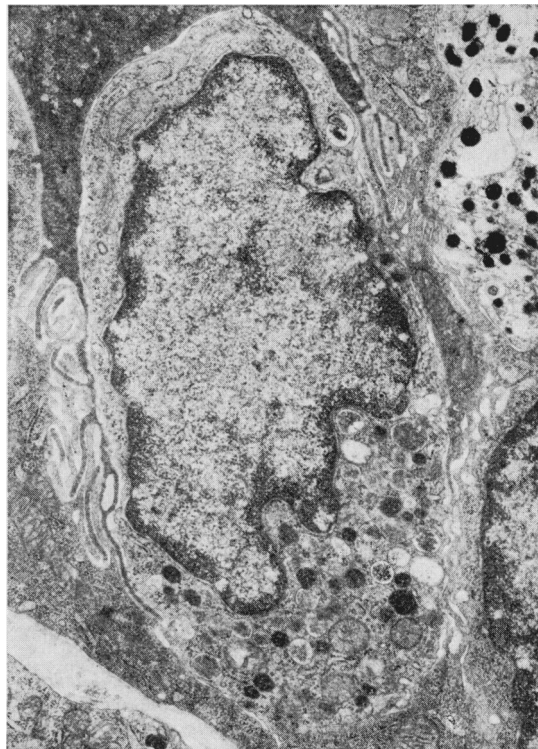
On visual examination, the ultrastructure of G cells did not appear to differ between fasted and fed animals, though G cells in animals fasted for 72 hours were apparently smaller and contained fewer granules (Figs 4, 5, 6). The other changes presented here were revealed only by morphometry.



**Fig. 4** *G cell from the pyloric antrum of a rat fasting during the preceding 24 hours. Note the large numbers of granules in the basal part of the cell, which is of normal morphological appearance. Electron micrograph,  $\times 10\ 600$ .*



**Fig. 5** *Normal ultrastructural morphology of a G cell from the pyloric antrum of a control rat feeding normally during the preceding 72 hours. Granules are plentiful and of pleomorphic appearance. Electron micrograph,  $\times 10\ 600$ .*



**Fig. 6** *G cell from the pyloric antrum of a rat fasting for the preceding 72 hours. The micrograph illustrates the reduction in the size of G cells and in the number of granules found in their cytoplasm. Electron micrograph  $\times 10\ 600$ .*

With fasting, there was no significant difference ( $P = 0.1$ ) between cytoplasmic areas of animals fasted for 24 hours and fed controls. There was, however, a highly significant ( $P < 0.001$ ) decrease in the cytoplasmic areas of G cell profiles between 24 hours and 48 hours fasting, with little further change between 48 and 72 hours. The fed controls were a homogeneous group with respect to cytoplasmic area ( $P = 0.3$ ) (Table 3).

Table 3 *Effect of starvation for 24, 48, or 72 hours on cytoplasmic profile area ( $\mu\text{m}^2$ ) (mean  $\pm$  SEM) of rat antral G cells, from tissue fixed for one hour at pH 7.3*

Duration of experiment (h)	Cytoplasmic profile area ( $\mu\text{m}^2$ ) (mean $\pm$ SEM)		
	Starved (36)	Fed controls (36)	Significance of difference (P)
24	33.0 $\pm$ 2.2	28.4 $\pm$ 2.2	NS
48	23.7 $\pm$ 2.0	29.6 $\pm$ 2.3	0.02
72	22.1 $\pm$ 2.0	25.1 $\pm$ 1.8	NS

Number of cells assessed in parentheses.

Effect of starving (Kruskal-Wallis analysis of variance)  $P < 0.001$ .

Effect of feeding (Kruskal-Wallis analysis of variance)  $P = 0.3$ .

The secretory granule content per average G cell profile (SGC) is illustrated in Fig. 7. Compared with fed controls, the SGC was significantly ( $P < 0.001$ ) increased after 24 hours' fasting.

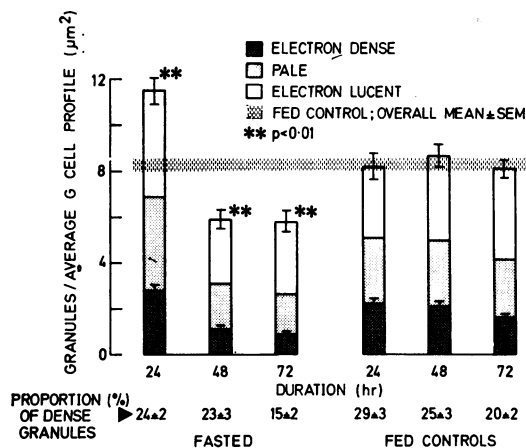


Fig. 7 *The effect of fasting on the amount and appearance of secretory granules in G cells of the pyloric antrum of rats. The amount of secretory granules is expressed as the area ( $\mu\text{m}^2$ ; mean  $\pm$  1 SEM) occupied by secretory granules per average G cell profile; this total is divided into the parts contributed by dense-cored, pale and empty granules. The amount of dense-cored granules as a proportion of the total granule content (%: mean  $\pm$  1 SEM) is given numerically.*

After both 48 and 72 hours' fasting the situation was reversed, and the SGC was significantly reduced ( $P < 0.001$ ). The fed controls form a homogeneous group both in terms of the SGC and the volumetric density (Pp) of granules.

Figure 8 compares the SGC with the IRG content of the serum. A composite control level, derived from the 24, 48, and 72 hours' control data was compared with data for the three fasted groups. At 24 hours, both IRG and SGC were raised above control levels but at 48 hours both fell below control levels to a comparable extent. At 72 hours' fasting, the IRG level fell to 25% of control, whereas the SGC was only slightly less than that observed at 48 hours. The correlation coefficient ( $r$ ) between the average antral IRG concentration and the SGC was  $r = 0.98$  ( $P < 0.01$ ).

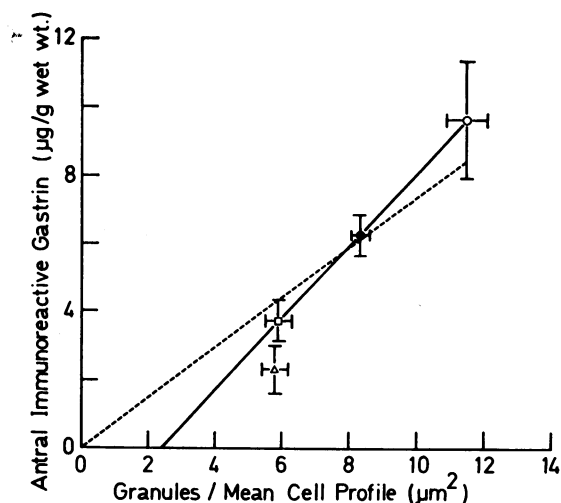


Fig. 8 *The relationship between antral immunoreactive gastrin content ( $\mu\text{g/g}$  wet tissue; mean  $\pm$  SEM) in fed controls ( $\bullet$ ), and animals fasted for 24 hours ( $\circ$ ), 48 hours ( $\square$ ), and 72 hours ( $\triangle$ ). The continuous line is the line of best fit through the mean data for fed controls and 24 hours and 48 hours starved animals. The broken line indicates that a line can be drawn through the origin and also through the standard errors of the mean data.*

When the whole granule population was considered, the relative proportion of dense-cored, pale, and empty granules in fasted animals did not differ significantly from that in fed animals at 24 hours, 48 hours, or 72 hours (Fig. 7). It should be noted, however, that the mean proportion of dense-cored granules was consistently smaller in the fasted animals. In the  $3 \mu\text{m}^2$  cytoplasm of the 'Golgi region' the relative proportion of dense-cored granules was significantly ( $P < 0.001$ ) lower

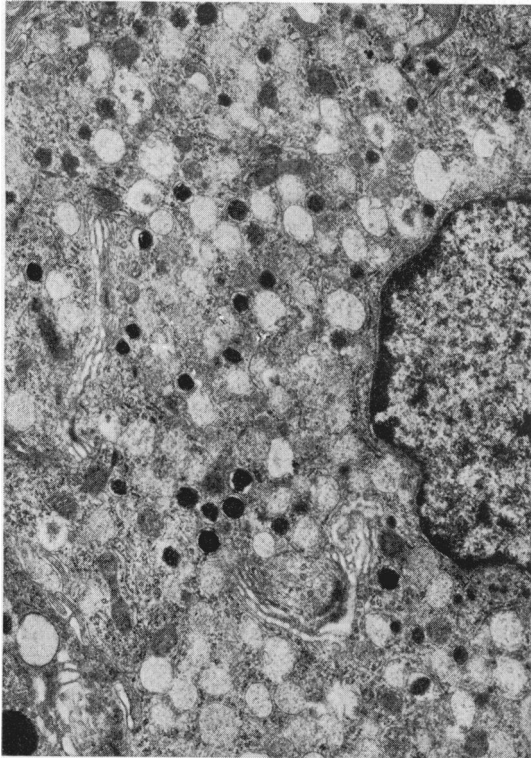


Fig. 9 Part of a G cell from the pyloric antrum of a control rat feeding normally for the preceding 72 hours. Fixation for 24 hours in 4% glutaraldehyde. Numerous electron dense-cored granules can be seen, particularly in the region of the two prominent Golgi zones. Compare the appearance of the granules with that in Fig. 10. Electron micrograph,  $\times 10\ 600$ .

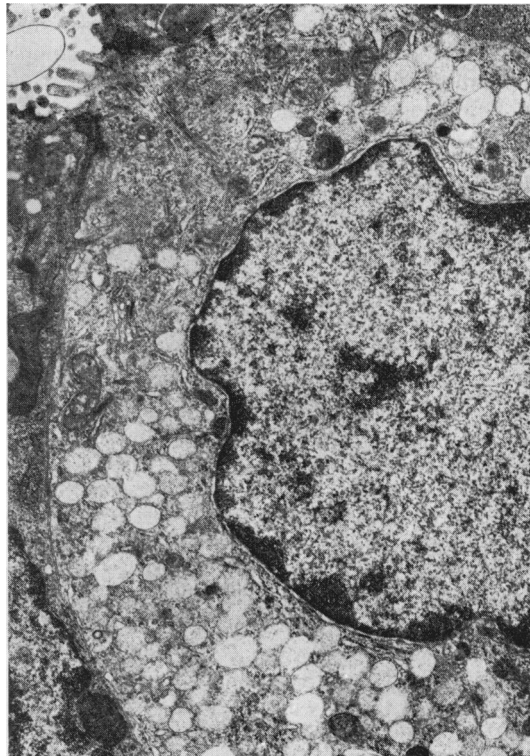


Fig. 10 Part of a G cell from the pyloric antrum of a rat fasting for the preceding 72 hours. Fixation for 24 hours in 4% glutaraldehyde. The micrograph illustrates the lack of electron dense (immature) granules. Electron micrograph,  $\times 10\ 600$ .

in animals fasted for 72 hours ( $3.3 \pm 1.1$  SEM) than in fed controls ( $22.3 \pm 2.7$  SEM) (Figs 9, 10), indicating the presence of far fewer newly formed granules.

A within-treatment analysis of the data for the entire granule population revealed a significant decline in the proportion of dense-cored granule forms in both fed controls ( $P < 0.04$ ) and fasted animals ( $P < 0.01$ ) from 24 hours to 72 hours. The change in fed controls could be ascribed only to day-to-day variations in fixation conditions and highlights the extreme sensitivity of the granules during fixation and the importance of matched controls.

### Discussion

These experiments suggest that, during fasting in rats, the following sequence of events occurs. By 24 hours serum IRG has fallen to a basal plateau

level but antral IRG and granules are increased. At 48 hours serum IRG levels are basal but now both the antral and G cell granule stores have decreased. At 72 hours serum IRG remains basal and antral IRG has fallen still further; individual G cells do not have a proportionally reduced granule content, but there is evidence of greatly reduced formation. Lichtenberger *et al.* (1975) have demonstrated that, after this period of fasting, there is a 60% decrease in the number of G cells. Our observations would be consistent with this, and such a decrease would explain the apparent discrepancy between antral IRG and granules at 72 hours.

Bearing in mind the problems associated with measurement of basal hormone levels in serum, the basal serum IRG in fasted animals in this experiment (50 pg/ml) is almost exactly that found by Lichtenberger *et al.* (1975) on days 2, 4, and 6 of fasting. Our results indicate that serum gastrin has reached a basal level by 24 hours of fasting.

The plateau basal serum IRG suggests a steady background secretion, rather than a complete inhibition of hormone release during fasting.

Reductions of antral gastrin concentrations to 55%, 30%, and 30% of control levels on days 2, 4, and 6 of starvation have been demonstrated in rats (Lichtenberger *et al.*, 1975). Our results indicate a very similar proportional reduction in antral gastrin on day 2 and, by interpolation, on day 3 of fasting, although in absolute terms our values for antral gastrin are about one-third of those reported by Lichtenberger *et al.* (1975) who used 300-400 g rats. The absolute values which we have found in 230 g rats ( $\sim 6.2 \mu\text{g/g}$  wet antrum) are, however, almost exactly those reported by Becker *et al.* (1973) who used a similar extraction procedure and 180-200 g rats ( $6.4 \mu\text{g/g}$ ), and about one-half to two-thirds of those reported by Creutzfeldt *et al.* (1976). Interlaboratory variation in radioimmunoassay of gastrin is known to be wide (Rayford *et al.*, 1975; Harvey, 1976) and, while it is possible that weight and age play a part in the differences between reported values, other technical factors may be equally important. The radioimmunoassay that we have used reacts equally on a molar basis with 'little' and 'big' gastrins, but the relative proportions of the different molecular forms in either serum or antrum may have changed during the course of fasting. Such changes could contribute to the different values for total gastrin achieved in different laboratories.

By contrast with the fall in antral IRG on and after day 2 of fasting there appears to be a small increase after 24 hours' fasting. Lichtenberger *et al.* (1975) predicted that this should occur, though they did not study animals fasted for 24 hours.

If all gastrin is intragranular—and there are good reasons for believing that this is so (Mortensen and Morris, 1977)—then the antral IRG content should be reflected by the product:

$$\text{Average volumetric density of granules in G cells} \times \text{average G cell volume} \times \text{number G cells}$$

Figure 8, where the changes in antral IRG are compared with the secretory granule content of average G cell profiles (SGC) demonstrates a very good correlation up to 48 hours' fasting. The line of best fit through the data of control, 24 hours, and 48 hours intercepts the abscissa at  $2 \mu\text{m}^2$  granules/average G cell profile, which could suggest that some G cell granules contain material that is not detected by our radioimmunoassay—possibly a larger molecular weight precursor in immature granules. However, the apparent discrepancy could also represent either experimental error (as a line can be drawn through the origin and the standard errors of these data) or a small,

but undetected decrease in the number of gastrin cells after 48 hours' fasting.

After 24 hours' fasting, the raised SGC in the G cells corresponds with a raised mean antral IRG concentration. The increase in granules within the G cells ( $+ 3.3 \mu\text{m}^2$ /average cell profile) largely accounts for the increase in average cell profile size ( $+ 4.6 \mu\text{m}^2$ ) of G cells at this time. This strongly suggests that the increased cell size results from increased storage of granules rather than from a cellular hypertrophy associated with hyperfunction. The low serum gastrin levels, combined with the raised antral gastrin levels thus indicates that, on day 1, net formation of granules proceeds at a faster rate than their release.

If granule formation does exceed release for a short period, this suggests that the immediate stimuli which result in decreased hormone release do not act in the same manner as those which result in decreased synthesis. At 48 hours' and 72 hours' fasting, both synthesis and release appear to be inhibited, but the depletion in antral gastrin stores suggests that the secretion which sustains the basal serum gastrin level is greater than the production of gastrin granules by the G cells. A nearly complete cessation of gastrin production is suggested by the marked reduction in the dense-cored granules—the putative immature granules (Mortensen and Morris, 1977)—in the Golgi zones of 72 hour fasted animals. We did not fix any material from 24 and 48 hour fasted animals for 24 hours and so could not make the same measurement for these periods. In 24 hour and 48 hour fasted animals, however, there was a small reduction (compared with fed controls) in the overall proportion of dense granules which is similar to that seen in the 72 hour fasted animals (Fig. 7). This could indicate that packaging of granules is already inhibited by 24 hours and 48 hours of fasting. Lysosomal degradation would be another possible cause of the decreased number of granules in G cells but we have found no evidence for this. The decline in the granule population between 24 and 48 hours thus appears to represent primarily a failure of synthesis to keep pace with even the demands of basal secretion.

It is known that starvation leads to a decline in the total intestinal cell population (Steiner *et al.*, 1968) and that this probably results from a reduction of both cell renewal (Brown *et al.*, 1963; Altmann, 1972) and cell migration (McNeill and Hamilton, 1971). The turnover time for mouse intestinal endocrine cells is about four days (Ferreira and Leblond, 1971). If this were true for G cells of the rat antrum and starvation had an immediate effect, then, by 72 hours fasting, a reduction in the



G cell population would be apparent. There is, however, a suggestion that gastrin cells in mice are renewed by replication of other gastrin cells with a turnover time of two to four months (Lehy and Willems, 1976). Our data would favour the four day turnover time unless there were other factors causing G cell loss during fasting.

Starvation for as little as three days is known to cause striking changes in intestinal structure and function (Steiner *et al.*, 1968; Altmann, 1972; McNeil and Hamilton, 1971; Johnson *et al.*, 1975a) and there is evidence that the physical presence of food, especially its chemical constituents (Johnson *et al.*, 1975a; Willems *et al.*, 1971), and adequate circulating gastrin levels (Johnson *et al.*, 1975b; Willems *et al.*, 1972) are necessary for the maintenance of normal gastrointestinal function. These factors could also act on the G cells stimulating G cell renewal and growth as well as gastrin synthesis and release. While 70% of the observed reduction in G cell cytoplasmic area can be attributed to loss of granules, more trophic effects of starvation cannot be ruled out.

The antrum is not the only source of gastrin in the serum (Walsh and Grossmann, 1975). Evidence from other species indicates that only very low levels of either little or big gastrin are to be found in the sera of fasted animals (Straus and Yalow, 1974; Dockray *et al.*, 1975; Stadil and Rehfeld, 1975), and it is not clear from the radioimmunoassay data whether antral G cells in the rat continue to secrete during the first 48 hours of fasting.

Concerning the subcellular mechanism of gastrin release, although our experimental protocols were not exactly comparable, we did not see the striking changes in gastrin granule electron density reported to occur on fasting and refeeding rats which could suggest a release of gastrin from the granules into the cytoplasm (Creutzfeldt *et al.*, 1975, 1976). This demonstration of a correlation between the immunoreactive gastrin content of the rat antrum and the number of G cell granules suggests that entire granules are lost, possibly by exocytosis. The combination of radioimmunoassay and quantitative ultrastructural analysis offers a promising tool for future investigation of gastrin production, turnover, and release.

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