The G cells in pernicious anaemia

JULIA M. POLAK, I. COULLING, W. DOE, AND A. G. E. PEARSE

From the Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, London

SUMMARY An indirect immunofluorescence technique, using the globulin fraction of rabbit anti-human gastrin serum, was applied to formalin-fixed material obtained by suction biopsy from the fundic mucosa of nine cases of pernicious anaemia. Cytochemical tests for endocrine polypeptide cells of the APUD series, in which the G cell is included, were carried out in parallel with immunofluorescence and with ultrastructural observations.

G cells were present, in large numbers, in five of the nine cases studied. In the remaining four cases the predominantly intestinalized glands contained only enterochromaffin in cells.

Because of their low gastrin content (immunofluorescence), low secretion granule content (cytochemistry), and the associated ultrastructural findings, it is suggested that the G cells of the fundic mucosa are in a state of high synthetic and high secretory activity.

The first successful application of an immunofluorescence technique for the localization of gastrin in gastric mucosa was made by McGuigan (1968). He found a good correlation between the distribution of immunofluorescent cells and gastrin levels in similar regions, as determined by radioimmunoassay. Using cytochemical and staining reactions Solcia, Vassallo, and Sampietro (1967) identified the predominant endocrine cell of the gastrin-bearing area of the stomach and named it the G cell. Subsequently Bussolati and Pearse (1970), using a double staining technique on single sections, showed that the argyrophil G cells of the porcine pyloric antrum were indeed the gastrin-containing cells, and Pearse and Bussolati (1970) found that the anti-gastrin immunofluorescent cells of the human antrum were identical with the G cells of Solcia *et al* (1967).

The presence of 'endocrine-like epithelial cells' in the atrophic mucosa of patients with treated pernicious anaemia was described by Rubin (1969). McGuigan and Trudeau (1970) found that nine out of 29 patients with pernicious anaemia had serum gastrin levels in the hypergastrinaemic range, characteristic of the Zollinger-Ellison syndrome.

The purpose of this study was to determine the true nature of Rubin's 'endocrine-like' cells and to assess, as far as possible, their functional state and distribution.

Material and Methods

Capsule biopsy samples from the fundic mucosa of nine patients with pernicious anaemia (well established by clinical and laboratory criteria) were studied. Similar biopsies from two normal individuals were used as controls. All biopsies were obtained from the fundus of the stomach by a radioscopically positioned peroral biopsy tube. Each specimen was divided into three portions. The first portion was fixed for 24 hours in 6% glutaraldehyde, buffered at pH 7.4 with 0.1M phosphate, or for a similar period in Bouin's fixative. In both cases the tissues were subsequently washed in water for several hours, dehydrated in alcohols, cleared and embedded in paraffin wax. Sections (5 μ m) from these blocks were stained by haematoxylin and eosin; toluidine blue (pH 5) before and after acid hydrolysis (0.2N HCl, 60°, $\frac{1}{2}$ to 2 hours); McConaill's lead haematoxylin (as modified by Solcia, Capella, and Vassallo, 1969a); and three silver methods for argyrophilia; (1) the Grimelius method (as modified by de Grandi, 1970), (2) the Bodian method, and (3) the Davenport method. Sections were also stained by the Masson-Hamperl method for argentaffinity and the xanthydrol method for indoles (as modified by Solcia, Sampietro, and Capella, 1969b).

A second portion of the gastric mucosa was immediately fixed for 12 to 24 hours in cold 4% form-

aldehyde, freshly prepared from paraformaldehyde (Graham and Karnovsky, 1966). The blocks were subsequently washed for 24 hours in 30% sucrose in 0.01M phosphate buffered saline at pH 7.4. They were then dehydrated, cleared, and embedded in 56° paraffin wax. Sections (5 μ m) were picked up from water and dewaxed in light petroleum for use with immunofluorescence techniques. An indirect method was employed, using antihuman gastrin serum. The globulin fraction was obtained by cold precipitation with ammonium sulphate (Nairn, 1969). The test sections were treated by antigastrin globulin, followed by fluorescein-labelled antirabbit IgG and antigastrin globulin absorbed with bovine serum albumin (Bussolati and Pearse, 1970). The control series were treated by anti-gastrin globulin absorbed with synthetic human gastrin, followed by fluorescein-labelled antirabbit IgG serum and normal rabbit serum, followed by fluorescein-labelled antirabbit IgG serum.

A third portion of the specimen was prepared for ultrastructural evaluation. The tissue was fixed, immediately after removal from the capsule, in 3% glutaraldehyde in 0.1M phosphate buffer at pH 7.6 for 2 hours at 4°. Excess fixative was removed from the block by repeated washing in 0.1M phosphate buffer containing 0.1M sucrose. They were then dehydrated in ethanol and epoxypropane, and finally embedded in Araldite CY 212. With some blocks a double fixation procedure was carried out, using post-fixation with osmium tetroxide at 4° for two hours (Millonig, 1962). Sections were stained by lead citrate and uranyl acetate, and viewed in an AEI 6B electron microscope.

Results

All our cases of pernicious anaemia showed classical changes in the fundic mucosa, described as an atrophic gastritis with varying degrees of intestinal metaplasia (Fig. 1). Four out of the nine cases studied showed predominantly intestinal metaplasia, and in these cases the endocrine polypeptide (APUD) cell series was represented almost exclusively by argentaffin (enterochromaffin, EC, cells). In the remaining five cases the picture was that of an atrophic gastritis with very numerous endocrine polypeptide cells, precisely similar to the 'endocrine-like' cells described by Rubin (1969). These cells appeared in the middle and deeper portions of the fundic glands and they were mostly large, with clear cytoplasm and round nuclei, clearly visible in thick (1 μ m) resin-embedded sections stained with toluidine blue (Fig. 2). They were not stained with toluidine blue at pH 5 except after acid hydrolysis when they appeared violet red (masked meta-

Fig. 1 Paraffin embedded section (5 μ m) of fundus showing classical features of atrophic gastritis with varying degrees of intestinal metaplasia. (H & E $\times$ 140.)

Fig. 2 Resin embedded section (1 μ m) of fundus showing numerous large, clear endocrine polypeptide cells with round nuclei. (Toluidine blue $\times$ 430.)

Fig. 3

Fig. 3 Glutaraldehyde fixed paraffin section (5 μ) of fundus showing numerous large, round cells with some reactive material in their cytoplasm. (Lead haematoxylin $\times 700$.)

Fig. 4

Fig. 4 Paraffin section (5 μ). Indirect immunofluorescence technique for gastrin. A relatively large number of G cells can be seen, although only a small number of them show strong fluorescence. In most cells only weak specific fluorescence can be observed (upper left). ($\times 360$.)

Fig. 5 Electron micrograph showing two endocrine polypeptide cells and part of a third one. These cells, almost degranulated, show signs of high activity (rough endoplasmic reticulum and free ribosomes) and numerous autophagosomes. (Lead citrate and uranyl acetate $\times 5,000$.)

chromasia). Lead haematoxylin staining revealed that most of the cells contained some reactive material in their cytoplasm but that they were clearly degranulated by comparison with normal endocrine polypeptide cells (Fig. 3). Grimelius and Davenport silver impregnations (argyrophilia) were positive after Bouin fixation. The Bodian technique (argyrophilia) and the Masson-Hamperl method (argentaffinity) were negative. The xanthydrol method gave a weak positive reaction with most of the cells, occasional examples being more strongly stained.

Our immunofluorescence studies showed, in the non-intestinalized fundic mucosa, a relatively large number of G cells in the middle and deeper parts of the fundic glands. Although occasional cells showing strong fluorescence (full granulation) were seen (Fig. 4), in most of the G cells only weak specific fluorescence was observed (Fig. 4, upper left). Control sections gave uniformly negative results.

In our ultrastructural studies the predominant (almost the only) endocrine polypeptide cell was a degranulated or poorly granulated G cell (Fig. 5), showing signs of high activity (high levels of rough endoplasmic reticulum and free ribosomes, and numerous autophagosomes). When present the specific granules were invariably round, of varying electron density (mostly moderate), and of a size ranging between 100 and 200 nm.

Discussion

Our results, in the five cases not suffering from gastrointestinal diseases, support the observation of McGuigan and Trudeau (1970) on the mean serum gastrin levels in pernicious anaemia. They found mean fasting levels of 997 (± 182 SE) pg gastrin per ml compared with levels in control patients of 165 (± 28 SE). In nine of their 29 cases serum gastrin levels were in the range characteristic of the Zollinger-Ellison syndrome. It is clear that our knowledge of G cell function in pernicious anaemia would be increased by parallel studies using radioimmunoassay and cytochemical and immunofluorescence techniques on the same cases.

The endocrine polypeptide cells of human gastric mucosa were described by Pearse, Coulling, Weavers, and Friesen (1970), using optical microscopical, cytochemical, and electron microscopical techniques. They belong to the APUD series of cells (Pearse, 1968) and are of four types, EC, G, EC-like, and D. The endocrine polypeptide cells of the fundic mucosa in pernicious anaemia can be identified with confidence as the G cells, as shown by the details given in the Table. From the average level of specific fluorescence, and from their ultrastructural characteristics one can postulate a very high rate of

Method	Normal G Cell	Fundic G Cell in Pernicious Anaemia
Masked metachromasia	+	+
Lead haematoxylin	+	+
Argyrophilia	Grimelius + Davenport + Bodian -	Grimelius + Davenport + Bodian -
Argentaffinity	Masson-Hamperl -	Masson-Hamperl +
Indole reaction	Weak +	Weak +
Immunofluorescence (anti-gastrin)	+	+
Electron microscopy (average granule size)	160 nm	180 nm

Table Characteristics of normal pyloric cells in pernicious anaemia

gastrin production and secretion.

The mechanism by which the fundic mucosal glands, normally devoid of G cells (Pearse *et al*, 1970), come to acquire them in relatively large numbers requires explanation. The new G cells could arise from isolated, single, preexisting G cells, unidentified by the usual scanning techniques. Alternatively, they could arise by modification of those endocrine polypeptide cells which are normally present in the region. These, according to the Wiesbaden terminology are enterochromaffin (EC), enterochromaffin-like (ECL), and D. While according to Rubin (1969) the intestinalized portions of the atrophic gastric mucosa in pernicious anaemia contain large numbers of argentaffin (EC) cells his, and our own, observations show that in the non-intestinalized glands EC cells are not present. This leaves the EC-like and the D cell as possible precursors of the fundic gastrin-secreting cell. The function and specific product of both cells are unknown. Transformation of one type of intestinal endocrine polypeptide cell into another type has not been observed, though it is not inherently impossible. At the present time there is no solution to the problem of the derivation of the G cells in the fundic mucosal glands in pernicious anaemia.

This work was carried out with the help of grants from the Wellcome Trust and the Medical Research Council. We are grateful to Professor K. W. Rees (University College Hospital, London) for preparing the antigastrin serum and to Dr V. Hoffbrand for his very helpful collaboration in providing us with material from patients with pernicious anaemia.

References

Bussolati, G., and Pearse, A. G. E. (1970). Immunofluorescent localization of the gastrin-secreting G cells in the pyloric antrum of the pig. *Histochemie*, 21, 1-4.
Graham, R. C., and Karnovsky, M. J. (1966). The early stages of absorption of injected horseradish peroxidase in the proximal

- tubules of mouse kidney; ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.*, **14**, 291-302.
- de Grandi, P. (1970). The routine demonstration of G cells in human and animal thyroid glands. *Virchows Arch. Zellpath.*, **6**, 137-150.
- Grimelius, L. (1968). A silver nitrate stain for α_2 cells in human pancreatic islets. *Acta Soc. Med. upsalian*, **73**, 243-270.
- McGuigan, J. E. (1968). Gastric mucosal intracellular localization of gastrin by immunofluorescence. *Gastroenterology*, **55**, 315-327.
- McGuigan, J. E., and Trudeau, W. L. (1970). Serum gastrin concentrations in pernicious anemia. *New Engl. J. Med.*, **282**, 358-361.
- Millonig, G. (1962). Further observations on a phosphate buffer for osmium solutions in fixation. In *Proceedings of 5th International Congress for Electronmicroscopy*, Philadelphia. Edited by Sydney S. Breese, Jr. Academic Press, New York.
- Nairn, R. C. (1969). *Fluorescent Protein Tracing*. Livingstone, Edinburgh and London.
- Pearse, A. G. E. (1968). Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (The APUD series) and their relevance to thyroid and ultimobranchial G cells and calcitonin. *Proc. Roy. Soc. B.*, **170**, 71-80.
- Pearse, A. G. E., and Bussolati, G. (1970). Immunofluorescence studies of the distribution of gastrin cells in different clinical states. *Gut*, **11**, 646-648.
- Pearse, A. G. E., Coulling, I., Weavers, B., and Friesen, S. (1970). The endocrine polypeptide cells of the human stomach, duodenum, and jejunum. *Gut*, **11**, 649-658.
- Rubin, W. (1969). Proliferation of endocrine-like (enterochromaffin) cells in atrophic gastric mucosa. *Gastroenterology*, **57**, 641-648.
- Solcia, E., Capella, C., and Vassallo, G. (1969a). Lead-haematoxylin as a stain for endocrine cells. *Histochemie*, **20**, 116-126.
- Solcia, E., Sampietro, R., and Capella, C. (1969b). Differential staining of catecholamines, 5-hydroxytryptamine and related compounds in aldehyde-fixed tissues. *Histochemie*, **17**, 273-283.
- Solcia, E., Vassallo, G., and Sampietro, R. (1967). Endocrine cells in the antro-pyloric mucosa of the stomach. *Z. Zellforsch.*, **81**, 474-486.