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The Identification of Gastrin Cells as G Cells

A. G. E. Pearse and G. Bussolati*
Department of Histochemistry, Royal Postgraduate Medical School, Hammersmith Hospital, London

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Summary. In the pyloric antrum of dog and pig it is possible to show, by direct re-staining of formaldehyde-fixed sections after localization of the gastrin cells by immunofluorescence, that these cells are identical with the argyrophilic, lead haematoxylin-positive G cells.

Reports to the contrary must be ascribed to the use of inadequate or erroneous techniques.

A recent paper by Mitschke (1971), employing immunofluorescence and cytochemical techniques on human gastric antral biopsies, concludes that "the argyrophilic G-cells and the Gastrin-cells are probably two different types of endocrine cells". The purpose of the present paper is to show that this view is a misconception, based on inadequate technology. The importance of the misconception, and our reason for refuting it, lies solely in the negative contribution which it makes to the problem of understanding and classifying the various endocrine cells of the gastric mucosa.

A number of "endocrine" cells, clearly distinguishable from enterochromaffin (EC) cells, were detected in the pyloric mucosa by cytochemical, staining, and ultrastructural studies (Solcia *et al.*, 1967; Forssmann *et al.*, 1967). Since the distribution of these cells was precisely that of gastrin Solcia *et al.* (1969a) and Forssmann and Orci (1969) suggested that they were the source of the hormone. The first-named author proposed to call them G cells. It most species these cytochemically identifiable G cells were found to be argyrophilic by the Grimelius (1968) method, but not with the Sevier-Munger (1965) method (Vassallo *et al.*, 1971). They were stained positively by the lead haematoxylin method (Solcia *et al.*, 1969b), were metachromatically stained by toluidine blue at pH 5.0 after mineral acid hydrolysis (Solcia *et al.*, 1969a; Bussolati *et al.*, 1969), and they possessed typical secretory granules at the EM level (Forssmann and Orci, 1969; Solcia *et al.*, 1969a).

Gastrin was localized by McGuigan (1968), using an immunofluorescence technique, in "endocrine" cells in human and porcine pyloric glands. These cells were not argyrophilic (by the Sevier-Munger method) nor could they be identified by any other procedure. Bussolati and Pearse (1970a) were able to re-stain immunofluorescent gastrin-containing cells in the porcine antrum by means of the Grimelius (1968) silver method. An erroneous reference to his (1964) method was corrected in an addendum to the paper (1970b). Immunofluorescent gastrin cells could not be re-stained with silver by McGuigan and Greider (1971) but their results are not surprising, since they used the Grimelius (1964) technique.

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Parallel (sequential) immunofluorescence and cytochemical studies are here presented to show that the cytochemical (and argyrophilic) G cell is identical with the immunofluorescent gastrin-containing G cell.

Material and Methods

The methods used for immunofluorescence demonstration of gastrin have been reported elsewhere in detail (Bussolati and Pearse, 1970; Pearse and Bussolati, 1970; Polak *et al.*, 1971). They need only be summarized here.

Canine and porcine pyloric mucosal samples were fixed, immediately after death, in cold (4°) 4% formaldehyde in 0.1 *M* phosphate buffer at pH 7.4. Alternatively, they were fixed in 3% formaldehyde and 1% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4, 4°) for 2 hours.

The tissues were embedded in paraffin wax and paraffin sections were dewaxed, hydrated, and placed in phosphate-buffered saline. Rabbit anti-gastrin globulins and FITC-labelled goat anti-rabbit γ-globulins (Hyland) were used in an indirect immunofluorescence procedure with a Zeiss (Oberkochen) fluorescence microscope using epi-illumination.

Selected areas were photographed, using Ilford FP4 film, keeping the overall exposure to ultra-violet light to a minimum since long exposures affect silver reactions.

After a short rinse in distilled water, or in 0.01 *M* HCl, the sections were re-fixed for 14 hours in 4% formaldehyde or in Bouin's fluid. They were then re-stained by the Grimelius (1968) procedure or by the lead haematoxylin method (Solcia *et al.*, 1969b).

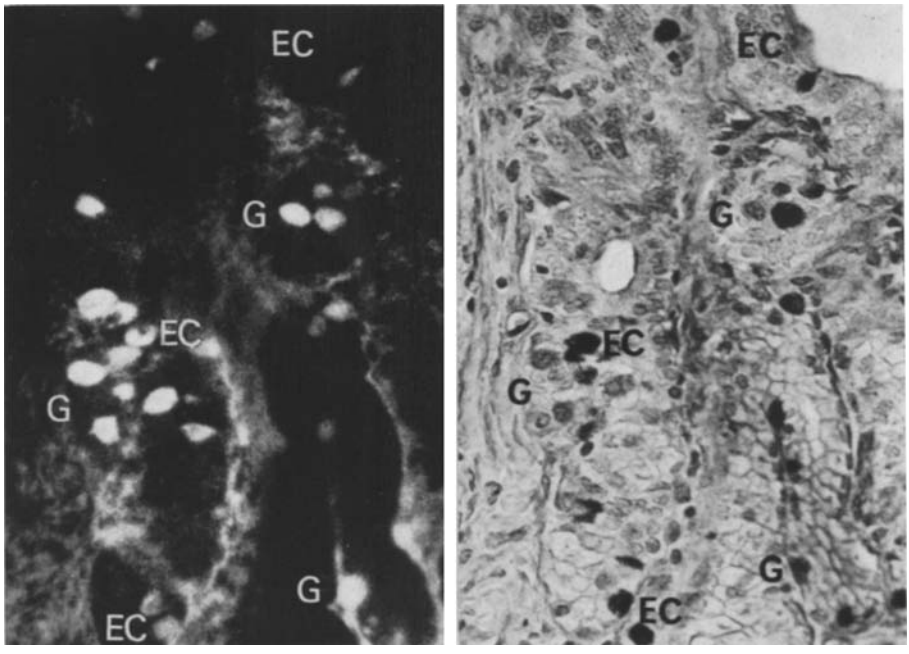

Fig. 1. Dog pyloric mucosa. Formaldehyde fixation (250 ×). Left: Immunofluorescence method for the localization of gastrin. Gastrin (*G*) cells are intensely fluorescent, and localized mainly in the middle layer of the mucosa. Some enterochromaffin (*EC*) cells show a weak formaldehyde-induced fluorescence. Right: The same area, re-stained with the Grimelius (1968) silver method. The gastrin (*G*) cells show a weak, but definite argyrophilia. The *EC* cells are intensely argyrophilic

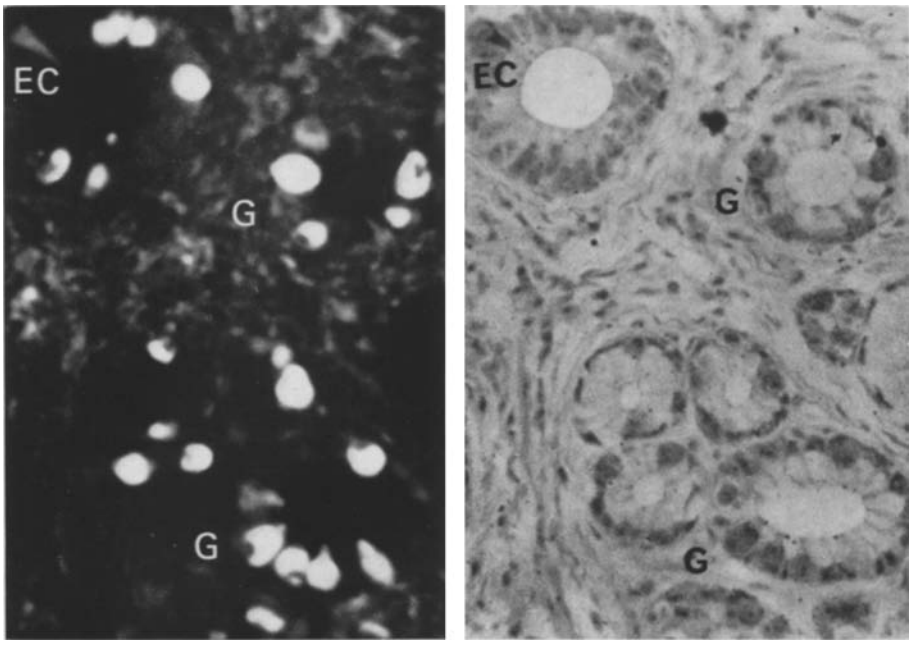

Fig. 2. Dog pyloric mucosa. Formaldehyde fixation (250 ×). Left: Immunofluorescence staining for gastrin. The gastrin (*G*) cells are intensely fluorescent, and lining the pyloric glands, which are cut transversely. A weakly flurescent enterochromaffin (*EC*) cell can be seen in the upper left corner. Right: The same section stained with the lead-haematoxylin method. The *G* cells, as well as the *EC* cells, are positive with this method

Results

Following the immunofluorescence procedure numerous gastrin-containing cells could be seen in the pyloric glands, mainly occupying the middle third of the mucosa. In control sections, and in positive immunofluorescence preparations, the orange-yellow fluorescence of the enterochromaffin (EC) cells was easily distinguished. These cells were less numerous than the gastrin cell, which could still be demonstrated after a period of 2 months' fixation in cold buffered formaldehyde. Gastrin cells could be demonstrated also in tissues fixed briefly in the formaldehyde-glutaraldehyde mixture.

Immunofluorescence sections were re-stained either by the Grimelius (1968) method or with the lead haematoxylin procedure. The former, especially when a double impregnation procedure was employed, showed a weak but definitely positive argyrophilia of the G cells (Fig. 1a and b). The EC cells displayed, as is well known, a strong degree of argyrophilia. Gastrin cells were also stained positively by the lead haematoxylin method. This is shown for canine pylorus in Figs. 2a and b, and for porcine pyloric antrum in Figs. 3a and b.

Discussion

There are essentially three ways in which a given type of immunofluorescent cell can be identified. These are: 1. immunofluorescence followed by a second

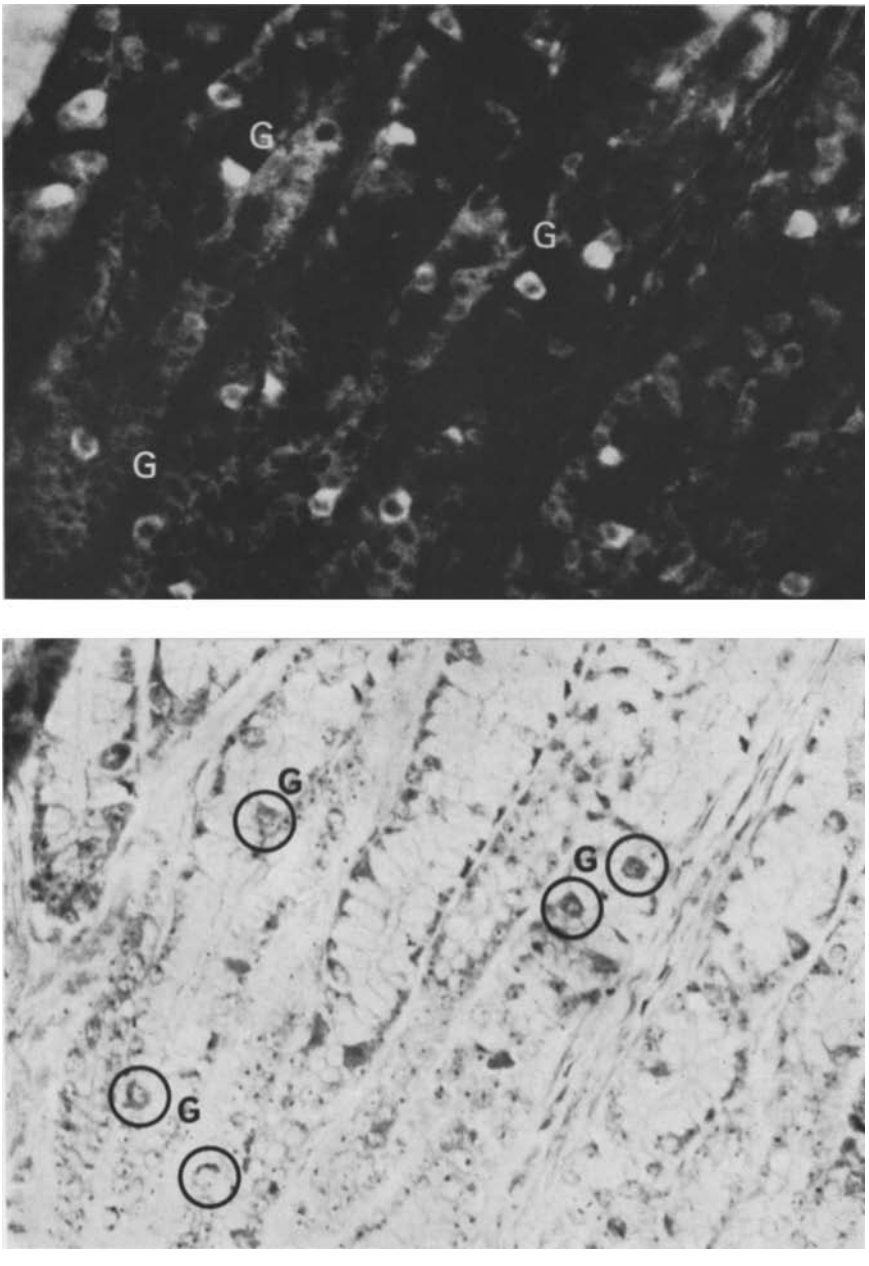

Fig. 3. Pig pyloric mucosa. Formaldehyde-glutaraldehyde fixation (250 ×). Above: Immunofluorescence staining of gastrin. The cells are intensely fluorescent. There is a higher degree of non-specific staining with this fixative than after formaldehyde fixation. Below: The gastrin (*G*) cells can be re-stained with the lead haematoxylin method

(sequential) staining or cytochemical procedure, carried out on the same section 2. immunofluorescence, carried out on one section, and a staining procedure performed on a serial section from a single block; or 3. immunofluorescence on a section from material processed for this purpose, compared with a section, stained for argyrophilia or by lead haematoxylin, from material processed by the optimal method for preservation and demonstration of the two qualities.

In order to use the first method, which is the only direct one, tissues must be fixed and embedded in such a way that both immunofluorescence and the chosen sequential technique can be carried out successfully. Fixation in formal dehyde is excellent for gastrin immunofluorescence and, though far from ideal, it allows both the Grimelius (1968) silver and lead haematoxylin methods to be carried out successfully. In the majority of species a weak results can be achieved with regularity. The fixatives of choice for the last two methods are Bouin and Helly, respectively, but these do not permit successful immunofluorescence studies to be performed.

Mitschke (1971) used human material, from cases of chronic atrophic gastritis, fixed for 3 hours in formalin. It is virtually impossible to demonstrate argyrophilia in human G cells processed in this way. The Grimelius (1968) technique produces negative results even in normal material. Our results presented here are virtually similar to those obtained in gastrin cell hyperplasias by Polak *et al.* (1971), and both confirm the G cell as the gastrin-containing cell of the pylorus.

Immunofluorescence remains the method of choice for the demonstration of cells containing gastrin (and other known polypeptide hormones) and it is the only method by which intracellular gastrin levels can be estimated. It is not only possible but reasonable, however, to use alternative procedures, under optimal conditions, once their specificity has been confirmed by direct comparison with immunofluorescence. Conditions for one species will not be the same as those for another but, where immunofluorescence is ruled out by lack of cross-reactivity, cytochemical and parallel ultrastructural studies will often allow a reasonably secure identification of the G cell. The assumption that it contains gastrin is completely warranted.

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Prof. Dr. A. G. Everson Pearse
Department of Histochemistry
Royal Postgraduate Medical School
Hammersmith Hospital
London W 12 OHD, England