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An immunoperoxidase technique for the identification of gastrin-producing cells

J. PIRIS AND R. WHITEHEAD

From the Department of Pathology, Radcliffe Infirmary, Oxford

SYNOPSIS A technique for the identification of gastrin-producing cells (G cells) is described. This is applicable to formalin-fixed, paraffin-embedded material. It is based on an immunohistochemical method using peroxidase-labelled antibodies.

Although several authors (Solcia, Capella, and Vassallo, 1969; Vassallo, Capella, and Solcia, 1971; Pearse and Bussolati, 1972) have claimed that specific identification of the so-called G cells was possible using histochemical methods, this view has not been universally accepted (McGuigan, 1968a; McGuigan and Greider, 1971; McGuigan, Greider, and Grawe, 1972).

Antibodies to gastrin can be raised in rabbits. These have been used in different immunological methods; immunofluorescence (McGuigan, 1968a), a direct technique using rabbit gastrin antibody labelled with horseradish peroxidase (McGuigan and Greider, 1971), and an indirect procedure using another peroxidase-labelled antibody to rabbit IgG (Creutzfeldt, Arnold, Creutzfeldt, Feure, and Ketterer, 1971).

We have applied the last method, with some modifications, to formalin-fixed, paraffin-embedded tissue.

Material and Methods

ANTIBODIES TO GASTRIN

Following the method described by McGuigan (1968b) we used synthetic human gastrin I (SHG) (residues 2 to 17) purchased from Imperial Chemical Industries Ltd to immunize a New Zealand white rabbit.

SWINE ANTIBODIES TO RABBIT IgG

These, already labelled to horseradish peroxidase, were purchased from DAKOPATTS A/S Denmark.

STAINING TECHNIQUE

Sections (4μ thick) were obtained from routinely formalin-fixed, paraffin-embedded blocks of human gastric and duodenal tissue. They were dewaxed, taken to water, and covered with a 1 in 5 solution of antigastrin serum in phosphate-buffered saline (PBS) pH 7·2 for one hour in a moist chamber at room temperature. Subsequently they were immersed in a bath of PBS for an hour, changing the buffer twice.

The swine-antirabbit IgG peroxidase-conjugated serum, also diluted 1 in 5 in PBS, was then allowed to react with the sections for 60 minutes. This was followed by another wash in phosphate-buffered saline.

A mixture of 4 mg of diaminobenzine tetrahydrochloride in 10 ml of Tris buffer pH 7·6 with a few drops of a 0·15% solution of hydrogen peroxide in distilled water was used to develop the peroxidase activity and left on the slides for 15 to 20 minutes.

At this stage the positive cells were easily identifiable by their dark brown cytoplasmic granules. The use of osmium tetroxide to accentuate the result was found unnecessary and it had the drawback of producing background staining.

A counterstain helped in the identification of the different components of the tissues as well as masking the background staining of red cells, inflammatory cells, and connective tissue. In our experience Light Green (0·5% in 0·1 N acetic acid) gave the best results and contrasts well with the granules (see fig). It was enough to submerge the slides in the counterstain for 30 seconds before rinsing them in water, differentiating in graded alcohols and xylol, and mounting in DPX.

Controls

These were set up by omitting in turn each of the two antisera and also substituting the peroxidase-

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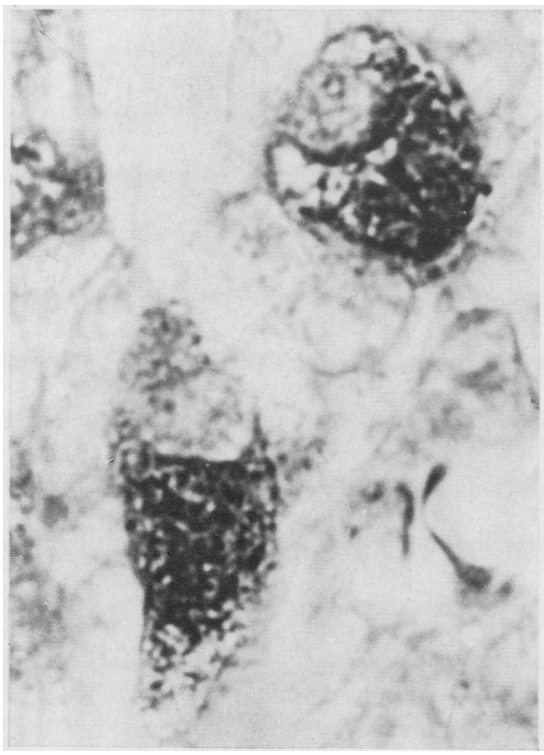

Fig *Gastrin-producing cells which in the final preparation appear filled with brown granules. The background is green (oil immersion × 2400).*

labelled antiserum by an unlabelled one (swine anti-rabbit-IgG). These gave negative results.

The intensity of the reaction could be diminished by diluting either or both antisera.

Tissues other than stomach and duodenum consistently produced negative results.

Discussion

In our hands the technique has consistently been reliable but certain points which may give rise to difficulty need further stress.

One of these is the background staining. The main factors which can give excessive non-specific staining of the tissues are insufficient washing after the exposure to the antisera and excessive exposure to or the use of too concentrated a solution of diamino-benzidine or hydrogen peroxide.

In previous reports (Creutzfeldt *et al*, 1971) varying methods of washing out the excessive antiserum have been advocated. After trial and error we have shown that placing the slides in a bath of PBS for an hour with two changes of the buffer gave best results. Likewise we found that the optimum concentration of diaminobenzidine was 4 mg in 10 ml of Tris buffer and of hydrogen peroxide 0·15%. This method has advantages over the more commonly used immunofluorescent one. The degree of specificity is similar but difficulty arises in differentiating specifically fluorescent cells from autofluorescent cells. This enzyme-labelled technique gives a permanent preparation and the result does not fade with time or exposure to light. This obviates the necessity to photograph the results immediately after the slides have been prepared when a fluorescent method is used and thus it is easier to make comparisons between different batches of slides tested. It also more easily lends itself to techniques for quantitative studies of the positive cells and the quality of the preparations allows a more detailed morphological assessment of their location and characteristics. A light microscope is all that is necessary and the method allows retrospective studies on previously fixed and embedded material.

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