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Electronimmunocytochemical Evidence for the K Cell Localization of Gastric Inhibitory Polypeptide (GIP) in Man

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Summary. Application of the semithin-thin section technique indicates that the previously proposed identification of the ultrastructurally-defined K cell with the immunocytochemically-defined GIP cell is essentially correct.

The K cell is established as a distinct entity and the way is open for an explanation of its role in the physiology and pathology of the gastroenteropancreatic system.

Introduction

Gastric Inhibitory Polypeptide (GIP) was first discovered as a contaminant during the purification of cholecystokinin-pancreozymin (CCK) (Brown et al., 1969). The peptide was further purified and finally sequenced by Brown and Dryburgh (Brown et al., 1971). It is a 43-amino acid peptide with considerable structural similarities to secretin, glucagon and Vasoactive Intestinal Polypeptide (VIP).

Physiological studies on the metabolic role of GIP have shown an inhibition of gastric acid production in the fundic mucosa (Pederson and Brown, 1972) and an increase in secretion of insulin from the pancreatic β cells when administered in the presence of glucose (Dupre et al., 1973). GIP may be considered as a major component of the enteroinsular axis and one of the important mechanisms by which the gut influences carbohydrate metabolism.

The distribution of GIP in the gastrointestinal tract has been assessed by immunocytochemical and radioimmunoassay techniques (Bloom et al., 1974). The highest concentration of GIP, as measured by radioimmunoassay, is found in the jejunum but there are significant amounts in the duodenum and ileum (Bloom, 1974a). Immunocytochemistry has shown that GIP cells are confined to the mucosa (Polak et al., 1973) and that their distribution parallels that of the total extractable GIP as measured by radioimmunoassay (Bloom, 1974b). There is no evidence for GIP-like immunoreactivity in the nerves of the intestinal tract (Polak, 1977).

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The sequential application of the Sevier-Munger silver technique to immunocytochemical preparations, correlated with the same technique applied at the ultrastructural level, has implicated the K cell defined by Solcia et al. (1975a), as the site of GIP storage (Buffa et al., 1975).

Materials and Methods

Tissue Preparation

Surgical samples of human duodenum, jejunum, pancreas and colon were obtained at operations. Adjacent samples from each area were fixed by the following methods

1. 2.5% vacuum distilled glutaraldehyde in 0.05 M phosphate buffer pH 7.3 for 5–10 min.
2. 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 for 3 h followed by postfixation in 1.5% osmium tetroxide.
3. 2.5% glutaraldehyde + 2% paraformaldehyde in 0.1 M phosphate buffer pH 7.3 for 3 h followed by postfixation in 1.5% osmium tetroxide.

Material fixed by the first method was dehydrated and embedded in araldite for use in the semithin-thin technique (Polak et al., 1975). Material fixed by methods 2 and 3 was dehydrated and embedded in an epon-araldite mixture for conventional ultrastructural studies. Sections (60 nm) prepared by all three methods were counterstained with uranyl acetate and lead citrate.

Semithin-thin Technique

Sections were cut in serial pairs of 800 nm (semithin) and 60 nm (thin) thickness. The 800 nm (semithin) sections were mounted on glass slides, and araldite was removed by immersion in saturated NaOH in methanol. The sections were stained by the indirect immunofluorescence technique of Coons, Leduc and Connolly (1955). 60 nm (thin) sections were floated on to copper grids and counterstained as above.

Antibodies

Production. New Zealand white rabbits were used for the raising of antisera to porcine GIP (99.9% pure, from Dr. J.C. Brown, Vancouver) which was coupled to bovine serum albumin or keyhole limpet haemocyanin (Sigma) by the carbodiimide method (Goodfriend et al., 1964). The antigen was emulsified in Freund's complete adjuvant and injected into multiple subcutaneous sites at three monthly intervals. The total amount of GIP per injection was 125 µg. Sera were harvested after two or three months.

Characterization. Evaluation of the antisera was carried out by the *E*nzyme *L*inked *I*mmuno*s*orbent *A*ssay (ELISA) (Voller et al., 1976) and by radioimmunoassay. Radioimmunoassay of the sera showed that some had titres ranging from 1/4000 to 1/120,000 (final dilution) tested by their ability to bind 50% of labelled antigen. Serum 12 showing a titre of 1/4000 was used for immunocytochemical work. The antisera showed no cross-reactivity with CCK, VIP, Secretin, Somatostatin or Gastrin.

The ELISA system showed the antibodies were free of cross-reactivity with the same range of peptide hormones. However a minimal binding to glucagon (10 units/10 mg, Eli Lilly) was detected. This was quantified further by radioimmunoassay and shown to be at the 1% level.

Immunocytochemistry

The GIP antibodies were used at a dilution of 1:600 for 24 h at 4° C. Controls consisted of preabsorption with the antigens VIP, Secretin, Gastrin, Somatostatin, CCK and Pancreatic Polypeptide (PP). None of these had any effect on the staining achieved. Serial sections incubated wi normal rabbit serum did not show any positive cells.

To investigate whether the 1% cross-reactivity with glucagon was significant, tests were carried

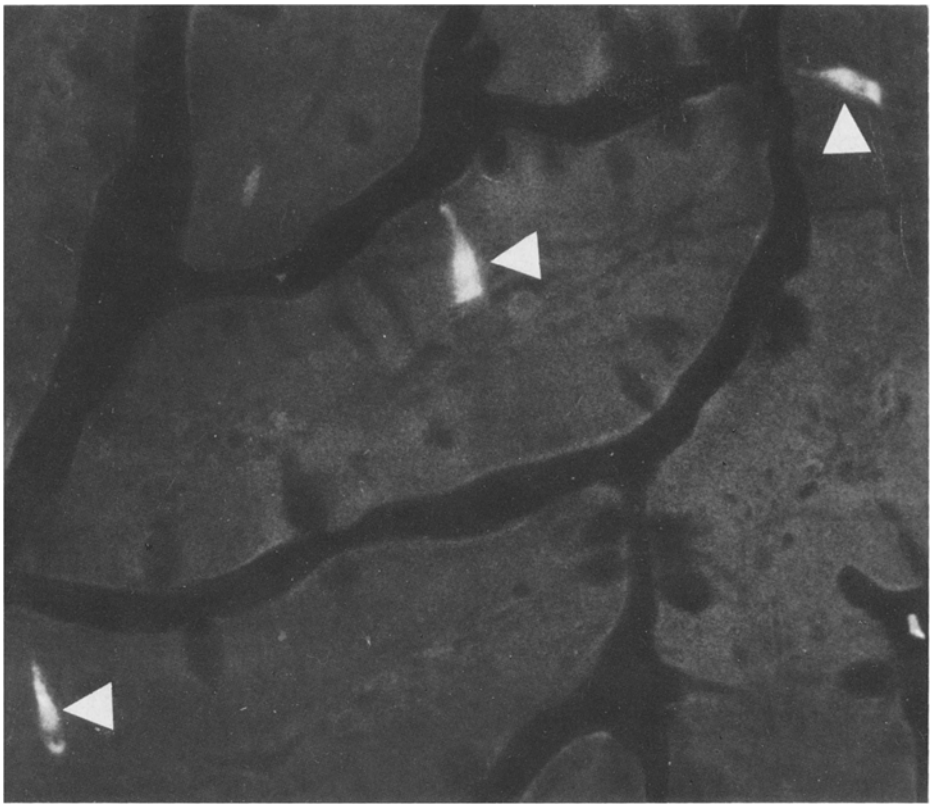


Fig. 1. 5 μ section fixed in Benzoquinone vapour stained with antibodies to GIP; arrows indicate positive cells. × 1350

out on samples of pancreas and colon as well as on the duodenal and jejunal tissue. In the pancreas GIP antiserum stained weakly a reduced number of A (glucagon) cells, this staining was removed by the addition of *both* GIP and glucagon. In the colon, only an extremely faint staining of 'gut glucagon' cells was observed which was removed by the addition of *both* GIP and glucagon. In the duodenum and jejunum numerous bright GIP positive cells were found whose staining was quenched by the addition of GIP but not of glucagon. Serial sections of duodenum and upper jejunum incubated with antiglucagon serum showed only staining of one or two cells. Sections of pancreas and colon incubated with the same serum showed numerous bright positive A and EG cells respectively. The staining of these was only removed by the addition of glucagon being unaffected by the addition of GIP. It can be concluded from these results that the numerous cells in the duodenum and jejunum stained after application of anti-GIP serum are solely GIP-containing cells and not glucagon cells and that the addition of glucagon to anti-GIP serum should prevent interference of cells storing glucagon and/or GLI in the staining of GIP cells. Therefore, 3 μg/ml glucagon was added to the diluted anti-GIP serum used in this work.

Electronmicroscopy

Immunofluorescent cells were identified ultrastructurally in the 60 nm sections and photographed. The photographs were used to quantify the size of GIP storage granules. The mean diameter of the granules was measured and then corrected for sectioning artefact with the formula

$D = (4/\pi \cdot d$ Baetens et al., 1976).

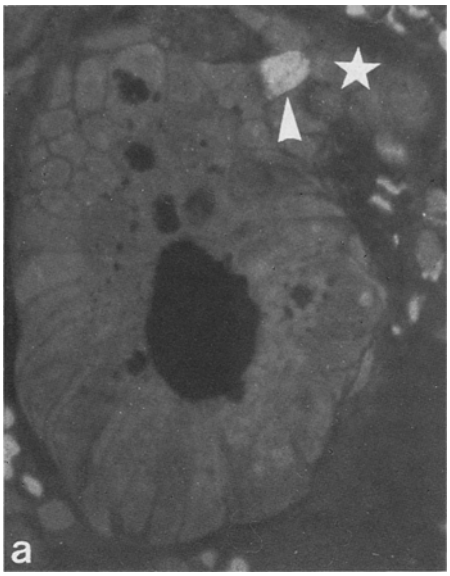
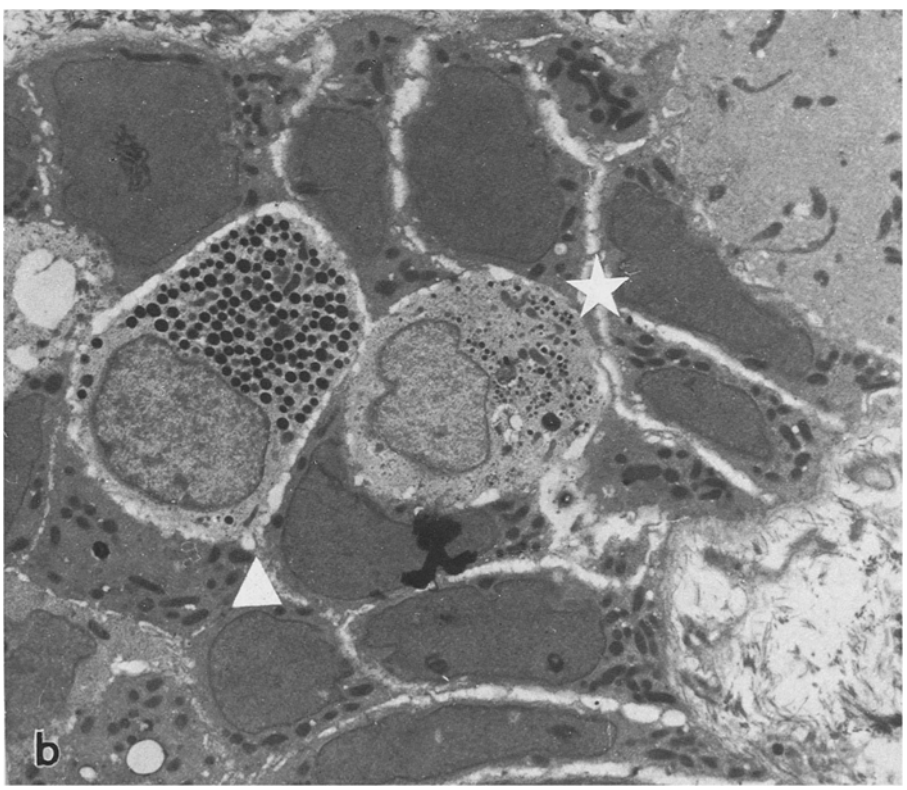


Fig. 2. a Semithin (800 nm) section stained with GIP antibodies, the arrow indicates the positive endocrine (GIP) cell and the asterix indicates a negative adjacent endocrine cell. ×2000.
b Thin (60 nm) section (serial to above); arrow indicates positive K type cell with large granules, asterix indicates the adjacent negative endocrine cell with smaller granules. ×4000



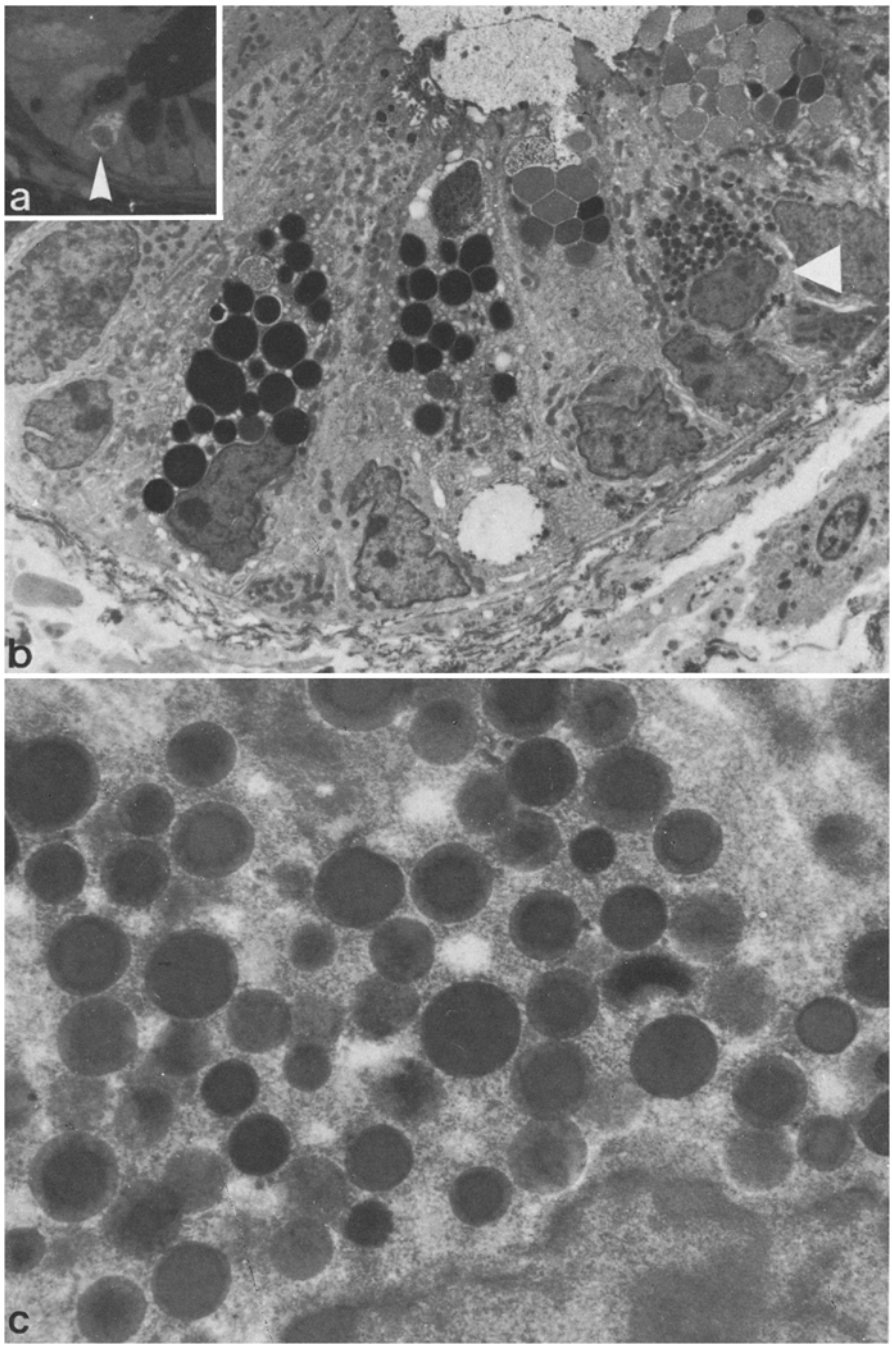


Fig. 3. **a** Semithin (800 nm) section stained with GIP antibodies, the arrow indicates the positive endocrine (GIP) cell. × 1000. **b** Thin (60 nm) section (Serial to above); arrow indicates the positive endocrine (K) cell. Note the goblet cell on either side of the GIP cell and the two paneth cells on the left side. × 3000. **c** Same endocrine (K) cell at high magnification showing details of the secretory granules. Note the double structure of some granules. × 25,000

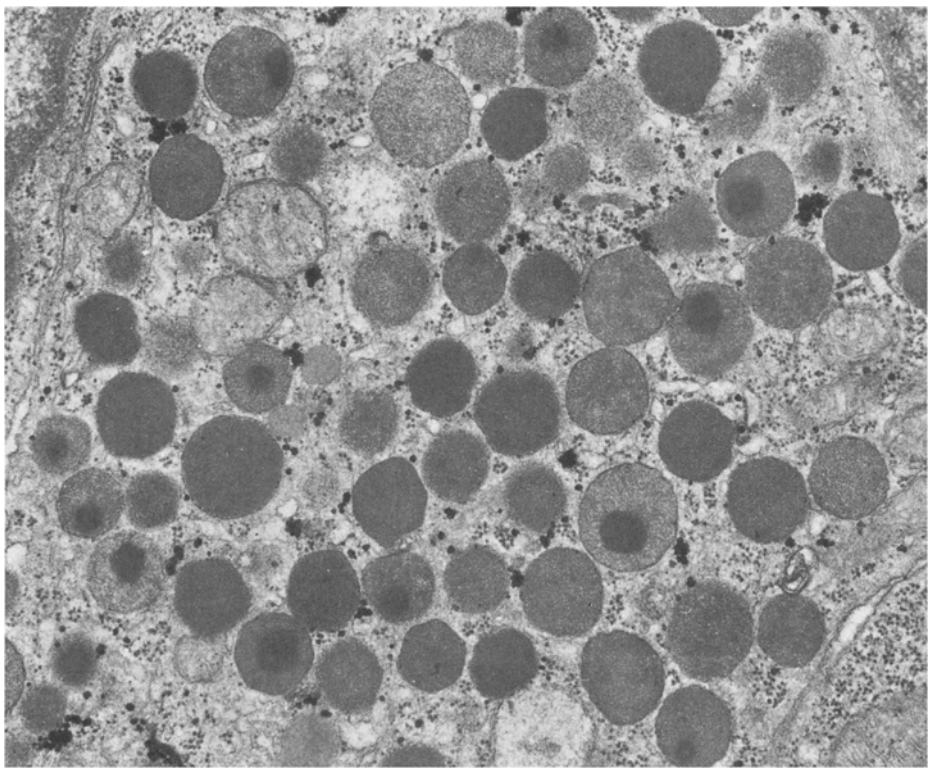


Fig. 4. Conventional electronmicrograph of a K cell showing detail of the secretory granules. Compare with Figure 3c. ×25,000

A total of 500 granules from seven cells were measured. The mean diameter of the granules of K cells identified in conventional electron microscope sections were measured in the same way.

Results

As previously described (Polak et al., 1973) immunofluorescent GIP cells were scattered as single elements in the intestinal villi and upper crypts (Fig. 1). Specific staining was abolished by prior incubation of the diluted antibodies with 3 μg/ml GIP antigen but not by the addition of the other antigens.

Comparison of the semithin sections (800 nm) with their serial ultrathin (60 nm) preparations located the positive GIP cells (Fig. 2a, b; Fig. 3a–c). These cells contained large round, or sometimes irregular, electron-dense, secretory granules (Fig. 2b), many of these possessed the characteristic dense central cores, surrounded by a lighter matrix (Fig. 3c). Cells with comparable granules were seen in conventional electron-microscopical sections (Fig. 4). On the basis of the characteristic appearance of the granules, the cells were identified as

K cells, in agreement with the findings of Solcia et al. (1975a) and Capella et al. (1976).

The mean diameter of granules in immunocytochemically identified GIP cells was 349 nm ∓ 57 nm (corrected value 444 nm). Granules of K cells in conventional electronmicroscopy sections measured 371 nm ∓ 94 nm (corrected value 473 nm). The two measurements do not differ significantly.

Discussion

The K cell was first identified ultrastructurally in the human and canine small intestine as a cell with large, round to irregular, osmiophilic and argyrophil granules showing inner, heavily osmiophilic, argyrophobe bodies (Solcia et al., 1974, 1975a). Similar cells had been reported by Cavallero et al. (1972) as 'pre-enterochromaffin' cells and by Osaka et al. (1973) as 'A cells'. Although some EC cells with poor 5 HT content and a few, glucagon-producing A cells may exist in the upper small intestine, K cells appeared to represent an individual cell population (Capella et al., 1976). By comparing the morphology, distribution and silver staining of the ultrastructurally identified K cells and GIP immunoreactive cells, the K cell was tentatively identified with the GIP cell (Buffa et al., 1975; Solcia et al., 1975b).

The present demonstration of GIP immunoreactivity, in cells showing the ultrastructural features of K cells, by a procedure which does not demonstrate the GLI (glucagon-like immunoreactivity) of the L cells, supports the existence of the K cells as a separate entity and conclusively identifies it as the GIP cell. The complete identification of the GIP cell has opened up new possibilities for the study of GIP release in health and disease. This may be particularly important in pathological conditions such as diabetes, coeliac disease and chronic pancreatitis where an increased release of GIP has been reported (Botha et al., 1976; Creutzfeldt et al., 1976; Crockett et al., 1976).

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