Immunohistochemical and Ultrastructural Detection of the Secretin Cell in the Pig Intestinal mucosa

Recently, it has been shown that a specialized endocrine cell of the dog small intestine reacts to antisera against synthetic [1] or natural [2] porcine secretin. The staining of the secretin cell in immunofluorescence was found to be improved by the use of carbodiimide-fixed tissue [2]. The morphology, distribution and staining patterns of immunofluorescent cells were in keeping with those of ultrastructurally identified S cells [3, 4], whose involvement in secretin secretion and/or storage had already been suggested [4, 5].

In this study antisera against synthetic porcine secretin have been used to detect secretin cells in carbodiimide-fixed pig intestine. Parallel electron microscopy studies have been made in order to gain further information concerning the ultrastructure of the secretin cell.

Materials and methods. As reported elsewhere [1], anti-secretin antibodies were obtained by coupling synthetic porcine secretin (Squibb, lot SQ 18,773-ES-XXXI-14A) to rabbit serum albumin (Pentex) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Ott), and injecting the conjugate – in incomplete FREUND's adjuvant – in 2 New Zealand rabbits. Each rabbit received 6 doses at monthly intervals, each dose corresponding to 0.5 mg of secretin. The serum obtained after the sixth injection was assayed with a conditioned hemagglutination technique [6]. The titre of the antibodies was found to be $^1/_{1024}$ in one rabbit and $^1/_{1536}$ in the other.

Samples from pig duodenum, jejunum, ileum, colon, stomach and pancreas were fixed in carbodiimide according to KENDALL et al. [7] and sectioned in a cyrostat; sections were incubated with the globulin fraction obtained from anti-secretin sera through ammonium sulphate precipitation, washed and treated with fluoresceinated goat anti-rabbit 7 S γ-globulin serum (HYLAND), Controls were made by 1. using normal rabbit globulins, rabbit anti-porcine insulin serum or rabbit anti-human (2–17) gastrin I serum instead of anti-secretin globulins, 2. adding excess synthetic porcine secretin to the anti-secretin globulins, and 3. omitting the first step and staining sections with fluoresceinated goat serum.

Samples from the above tissues were also fixed 3 h at 4 °C with 2% formaldehyde + 2,5% glutaraldehyde in 0.1 *M* phosphate buffer pH 7.4, postfixed with 1% osmium tetroxide, dehydrated in ethanol and embedded in Epon 812; ultramicrotomic sections were stained with uranyl acetate and lead citrate. Other samples were fixed in formaldehyde, formaldehyde-glutaraldehyde mixture or Orth's fluid and embedded in paraffin; sections were stained with diazonium, xanthydrol, argentaffin, HCl-toluidine blue, lead-hematoxylin or Grimelius' silver methods, as selective stains for endocrine cell granules [1, 2, 4].

Results and discussion. In immunofluorescence tests using anti-secretin antibodies, numerous cells displaying selective green fluorescence were found to be scattered in the epithelium lining both the criptae and the villi of the pig duodenum and upper jejunum (Figure 1, A and B). Fluorescent cells showed endocrine-like pattern and failed to appear in control tests. Immunofluorescent cells were not observed in sections from the ileum, colon, stomach and pancreas. Unlike in formaldehyde-fixed tissues, in carbodiimide-fixed specimens enterochromaffin (EC) cells failed to show any spontaneous fluorescence.

With both granule stains and electron microscopy, numerous EC and endocrine non-EC cells were detected in the mucosa of the pig gastrointestinal tract. As in other mammals [1–5], among pig intestinal non-EC endocrine cells S, L, I and D cells were distinguished, according to the ultrastructural pattern of their granules [8]. L cells showed round osmiophilic granules with mean diameter of

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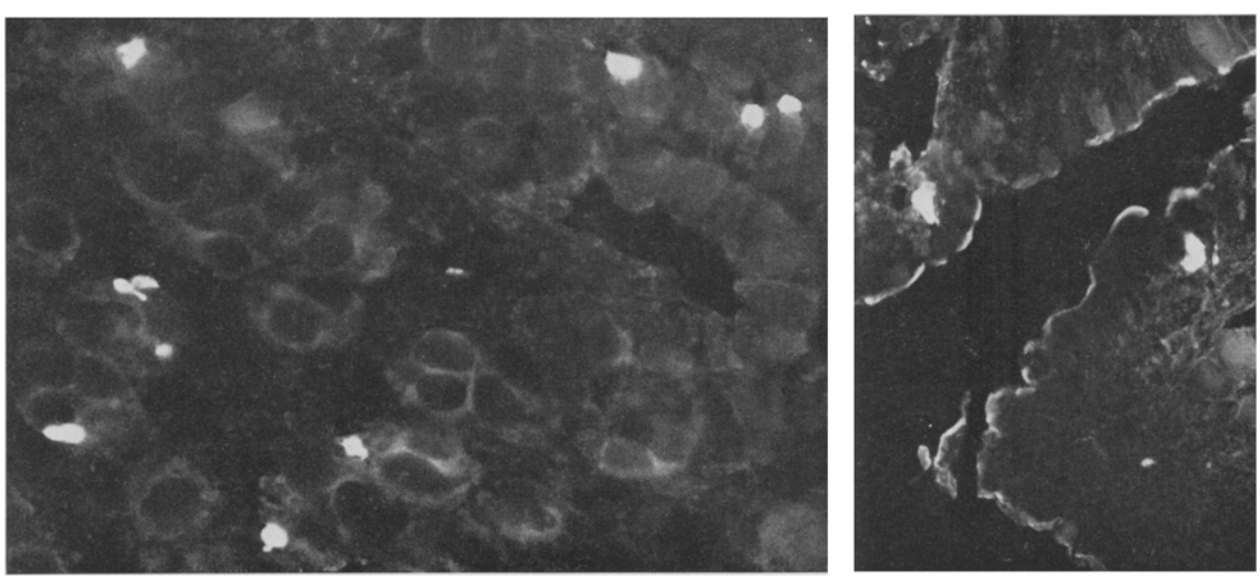

Fig. 1. Secretin cells in the criptae (A) and villi (B) of the pig duodenum stained with indirect immunofluorescent technique. ×650.

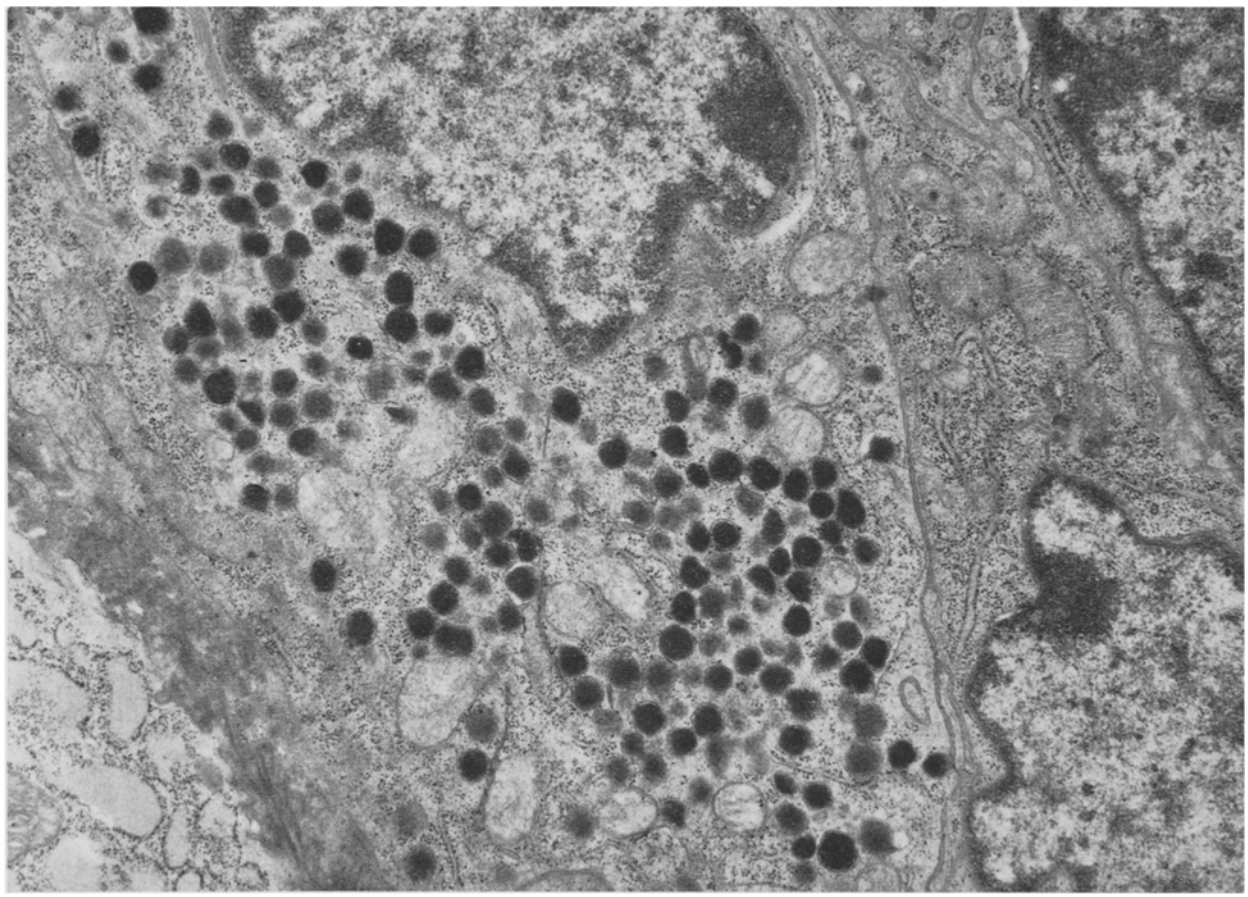

Fig. 2. Ultrastructural pattern of an S cell in the pig duodenum. ×18,000.

about 360 nm; they were found along the whole intestine including the colon. D cells had round poorly osmiophilic granules of about 350 nm; they were few in the intestine and practically restricted to the duodenum, while being well represented in both pyloric and fundic mucosa of the stomach. Clearly, the distribution of L and D cells was quite different from that of immunofluorescent cells. L cells have been suggested recently to produce enteroglucagon[9].

Both S and I cells showed granules with a diameter of about 250 nm; however, S cell granules showed a relatively irregular core surrounded by a clear halo (Figure 2), while I cell granules had a round core with closely applied membrane. Both cells were peculiar to the upper small intestine. In fact, no S cell and very few I cells were identified in the ileum; both cells were more numerous in the duodenal mucosa than in the jejunal mucosa, although S cells more sharply than I cells. On the villi, S cells largely overwhelmed I cells, while in the fundus of the criptae the reverse occurred. The distribution of both S and I cells along different longitudinal tracts of the pig gut resulted to be roughly similar to that of immunofluorescent cells. However, in the duodenal mucosa immunofluorescent cells were mostly located on the villi and in the superficial half of the criptae, while being relatively scarce in the fundus of the criptae; therefore, they reproduced closely the distribution of S cells, while behaving differently from I cells. Thus, the suggested identity of the S cell with the secretin cell seems confirmed. A possible relationship between the I cell and cholecystokinin is presently under study[10].

Riassunto. Le cellule a secretina vengono identificate con l'immunofluorescenza nella mucosa duodenale e digiunale di maiale facendo uso di antisieri anti-secretina sintetica porcina. Parallele ricerche ultrastrutturali consentono di identificare in un tipo peculiare di cellula endocrina l'equivalente ultrastrutturale della cellula a secretina.

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[10] We are much indebted to Dr. M. A. ONDETTI (Squibb, New Brunswick) for his generous supply of synthetic secretin and to Dr. S. GHIELMI (Richter, Milano) for his valuable help in immunizing rabbits.