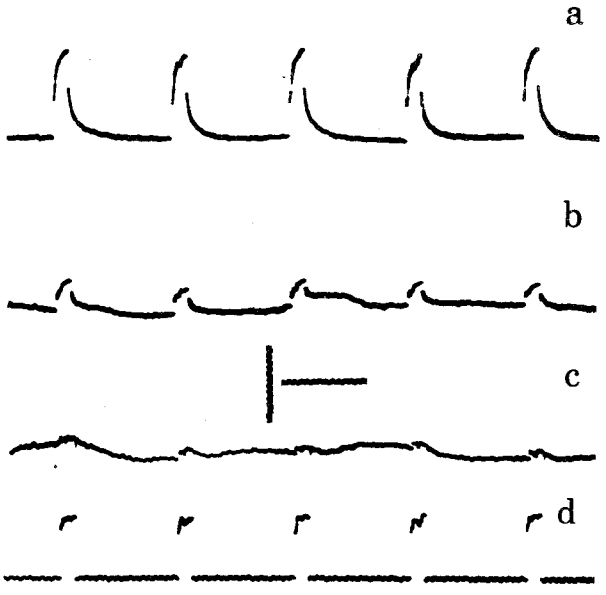

Fig. 2. Electrical uncoupling of myocardial cells produced by intracellular sodium injection. a) control values of electrotonic potentials recorded from a myocardial cell adjacent to the site of sodium injection immediately after the beginning of sodium release; b) and c) after 15 and 30 sec of injection respectively. Resting potential in a) −80 mV and in c) −83 mV. Vertical calibration, 5 mV; horizontal calibration, 200 msec. Lower trace: outward current pulses (2.5×10^{-7} A). Temperature 36 °C.

known that a rise in internal sodium concentration releases calcium from mitochondria, and this can also contribute to the development of the electrical uncoupling.

The present results rise the possibility that the Na-K pump in heart and in other cells has an indirect role on the control of cellular communication. It is reasonable to think that the failure of the sodium in a cell or group of cells can interfere markedly with intercellular communication through a marked change on the intracellular calcium concentration. It is conceivable, indeed, that the block of impulse conduction produced by cardiac glycosides in heart tissues can be related, in part, to changes on junctional conductance, secondary to its blocking effect on sodium extrusion. Experiments are in progress in our laboratory in order to investigate this hypothesis[13].

Résumé. L'influence de l'augmentation de la teneur en Na^+ intracellulaire sur le processus de communication entre les cellules cardiaques du lapin est étudiée. Une suppression de la communication électrique est provoquée par l'injection de Na^+ dans la cellule. Ce résultat est probablement expliqué par une augmentation de la concentration en Ca^{2+} intracellulaire, subséquente à l'injection de Na^+ et par une diminution de la perméabilité des jonctions intercellulaires.

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Quantification of Secretin Release by Acid, Using Immunocytochemistry and Radioimmunoassay

When secretin was successfully purified by JORPES and MUTT[1], sequenced by MUTT and JORPES[2], and its synthesis undertaken by BODANSZKY, ONDETTI, LEVINE, NARAYANAN, VON SALTZA, SHEEHAN, WILLIAMS and SABO[3], and by BODANSZKY and WILLIAMS[4], availability of the hormone in pure form gave rise to numerous studies designed to elucidate its physiological role in digestion. The production of suitable antisera permitted identification of scattered secretin-producing endocrine cells in the upper intestinal mucosa[5–7], and the development of a radioimmunoassay for the hormone in blood and tissue extracts[8]. Doubts remained, however, as to the precise response of the endocrine cells of the upper intestine to instillation of acid into the duodenum, and therefore the primacy and degree of the secretin response to acid remained unknown.

For this reason we proposed to investigate the effect of such acid instillation on the secretin and other endocrine cells by 1. Measurement of secretin release into the blood stream, 2. Assay of the secretin content of the mucosa, 3. Assessment of immunocytochemical changes in the endocrine cells and, 4. Quantification of intracellular secretin levels.

Material and methods. Healthy English White pigs were obtained from the same breeder. Their weight was between 24 and 30 kg. For 1 week before each experiment they were kept in an animal house, washed, dewormed and then starved for 18 h before experimentation. The pigs were anaesthetized with halothane induction, and received no barbiturates. They were then intubated with an endotracheal tube and given continuous nitrous oxide and oxygen. The mean arterial blood pressure was continually monitored and maintained at normal levels by i.v. saline infusion. The bile duct, pancreatic duct and stomach were canulated and catheters placed in the portal vein and femoral artery. All blood and juice samples were collected at 5 min intervals.

The duodenum and proximal 20 cm of jejunum were isolated and canulated for acid perfusion. After a 15 min basal period, biopsies (approximately 4×2 cm) of the duodenal mucosa, 5 cm distal to the pylorus, were taken for histology, immunocytochemistry and tissue extract assays. 0.1 *N* HCl was then perfused at a constant rate of 11 ml/min for 30 min, and a second mucosal biopsy taken.

Secretin levels in blood and tissue extracts were carried out by radioimmunoassay[9]. The biopsy samples, taken before and after acid instillation, were subjected to the

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following procedures: 1. Immediately after removal, the mucosa was stripped off, flattened, and then quenched in Arcton (Freon) 22 at −158 °C. It was freeze-dried overnight in a thermoelectric freeze-dryer. From each large piece of the dried tissue 8 identical samples were taken, using a selector punch designed for the purpose (Figure 1). With this equipment it is possible to punch out manually a set of 8 numbered samples, each 3 mm^2 and separated from each other by 1 mm (Figure 2). The cutting blades are mounted in a free swinging block

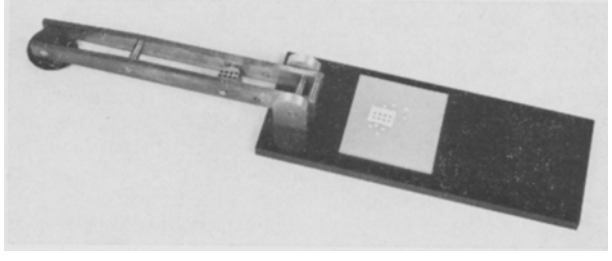

Fig. 1. General view of tissue sampling punch.

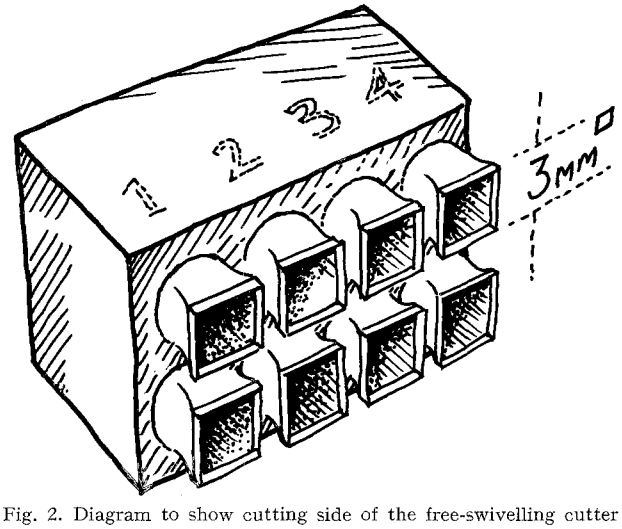

Fig. 2. Diagram to show cutting side of the free-swivelling cutter block.

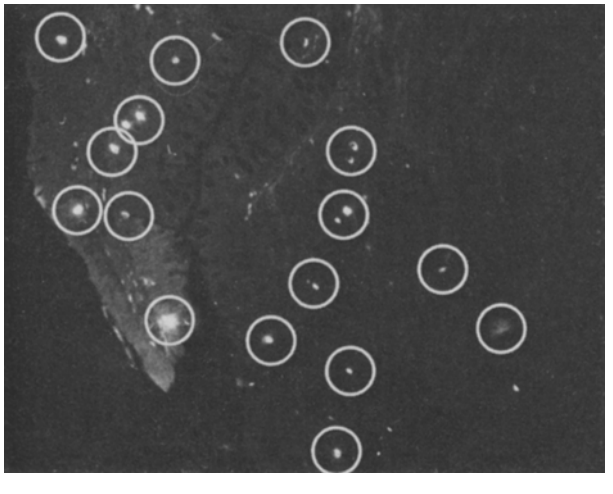

Fig. 3. Indirect immunofluorescence preparation shows secretin cell content of a single representative block from duodenal mucosa prior to instillation of acid. 16 S cells can be seen and the majority are brightly fluorescent. ×220.

suspended by a pivot from a lever. All cutting blades are thus positioned perpendicular to the mucosa while pressure is exerted.

2. Each of the small blocks, carefully labelled, was then fixed in diethylpyrocarbonate vapour[10], for 3 h at 45 °C, and subsequently embedded in paraffin wax at 56 °C.

3. Biopsies from a consecutive series of 5 pigs, all of which showed satisfactory cytological preservation, constituted the material on which our report is based. Immunohistochemical techniques for 4 upper intestinal polypeptides (gastrin, gastric inhibitory peptide, motilin and secretin) were then carried out as follows:

4. 150 5 µm serial sections were cut from each block selected and from these 2 (serial) sections from each run of 30 were mounted on separate albuminized slides. Indirect (sandwich) techniques were then applied, using antisera to pure porcine secretin for the first layer and either FITC- (Hyland) or peroxidase- (Dakopats) labelled anti-rabbit globulin sera for the second layer.

5. Quantitative studies were carried out using 2 methods: a) Using immunofluorescence preparations, the number of secretin cells per low power field was counted. The results were then treated statistically. b) Immunoperoxidase preparations were analyzed with a Television Image Conversion Analyser (M.R.C. Cyclotron Unit, Hammersmith Hospital). The measuring area was selected so as to fit each cell or group of cells, a suitable colour having been chosen to represent their particular density range. 16 readings per cell were made and all areas of the section which contained cells were measured. Data from the average of each reading was transferred to paper tape on a teletype machine and calculation of the ratio between cell content and background was made with an Elliott 4100 computer. The final figures gave an indication of cellular hormone content.

Results. When acid was introduced into the duodenum a dramatic pancreatic response was seen. This commenced a few min after the start of the experiment and reached a peak after about 15 min. Secretin levels in the blood also began to rise a few min after the administration of acid and the curve of secretin release was observed closely to parallel the curve of pancreatic response[9].

There was no change in immunofluorescence (hormone content) in the GIP, motilin or immunoreactive gastrin cells after the 30-min acid stimulation. On the contrary, however, the number of fluorescent secretin cells was considerably reduced, as was their intensity of fluorescence (Figures 3 and 4).

Quantitative studies carried out by cell counting showed that, although the number of secretin cells observed in the post-stimulation samples was smaller than in pre-stimulation samples, the results were not statistically significant. Analysis of the hormone content of the cells showed an average decrease of 52% post-stimulation. This result is highly significant (p 0.001).

The results obtained by Television Image Conversion Analysis were compared with those obtained by parallel radioimmunoassays on duodenal mucosal extracts. The comparison is shown in Figure 5, where dotted lines represent the results obtained in biopsies from each of the 5 pigs and the solid line the average result from all the animals. The correlation between the results of the 2 assay procedures (52% and 72%) is as close as could reasonably be expected.

Discussion. We have shown here that introduction of acid into the duodenum produces immunocytochemically detectable release of secretin from the cells containing it

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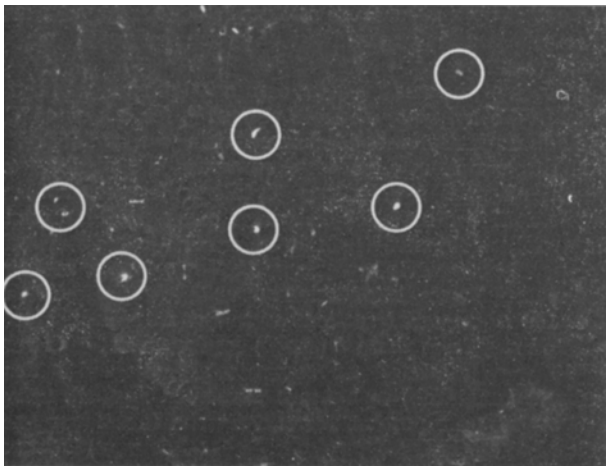

Fig. 4. For comparison with Figure 3. Block from duodenal mucosa after 30 min perfusion with acid. 7 S cells are visible and some exhibit very little fluorescence. ×220.

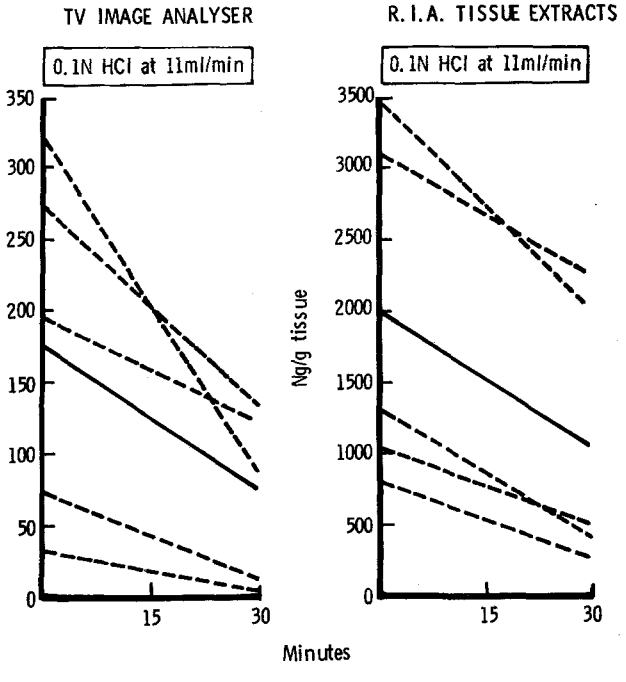

Fig. 5. Comparison of results obtained in the test series.

which correlates well with the fall in tissue secretin as estimated by radioimmunoassay. There was also a rise in blood secretin levels with a peak at 15 min and a pancreatic bicarbonate response which correlated in time well with the peak plasma secretin levels.

Total immunofluorescent cell count is not a sensible way of quantifying hormone secretion because, of course, one can record the same number of cells whether full or partially discharged. We propose, therefore, that quantification of variation in endocrine cell hormone content, under physiological or pathological conditions, should be carried out by procedures of the type described here, that is to say, by quantitative immunocytochemistry.

We conclude that secretin can be released in response to acid in the duodenum and that it probably is so released when the acidity falls below a threshold level, shown by MAYER, WAY and GROSSMAN [11] to be pH 4.5. Furthermore, acid appears not to stimulate discharge in the case of other endocrine cells in the upper intestine, in so far as these were covered by the antisera we employed.

Very little work has been done on cellular aspects of hormone secretion following an appropriate stimulus. Studies of the kind we report here should provide greater understanding of gut hormone physiology and also of the nature of disorders where increased hormone secretion or abnormal storage consequent on failure of release is suspected.

Zusammenfassung. Die Reaktion auf das Einträufeln einer 0,1 *N* HCl (11 ml/min) in das Duodenum des Schweines wurde parallel mit dem Radioimmuntest zur Bestimmung des Sekretingehaltes der Mucosa und mit quantitativer immunhistochemischer Analyse der duodenalen Sekretionzellen untersucht. Nach einer 30minütigen Einwirkung der Säure betrug der durchschnittliche Abfall des zellulären Sekretingehaltes in 5 Schweinen 52%. Der entsprechende Wert, der mit der Radioimmuntestmethode ermittelt wurde, betrug 72%.

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Departments of Histochemistry, Surgery and Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 OHS (England), 25 November 1974.

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A Comparison of the Composition of Epididymal Plasma from the Cauda Epididymidis of the Rat, Hamster and Guinea-Pig

The composition of epididymal plasma obtained from the intact cauda epididymidis of the anaesthetized rabbit and rat has recently been described [1,2] The present paper reports on the chemical composition of epididymal plasma from the intact cauda epididymidis of the hamster and the guinea-pig and a comparison is made with previous [2] and present findings in the rat. Earlier observations in the hamster [3] were on fluid collected by puncturing the epididymal tubules in post-mortem material.

Methods. 7 adult male golden hamsters (110 to 140 g) and 7 adult male guinea-pigs (700 to 800 g) were anaesthetized with sodium pentobarbitone (Nembutal, Abbott

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