**PROCEEDINGS OF THE NUTRITION SOCIETY**

*The Three Hundred and Eighteenth Scientific Meeting was held in the Medical and Biological Sciences Building, University of Southampton, on* 20 *and* 21 *July* 1978

## SYMPOSIUM ON 'HORMONES AND FOOD UTILIZATION'

### Gut hormones

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#### *History*

At the end of the last century Pavlov proposed that the control of alimentary function was by nervous reflex. His explanation was intellectually satisfying and a great advance on previous extremely woolly theories. Histologically fine nerve fibres which could be seen running in the gut wall and sectioning the main nerve trunks undoubtedly affected gastrointestinal function. Meanwhile, however, Brown Sequard, attempting to rejuvenate his ageing body, was able to show that extracts of testes greatly increased his prowess in several directions. His findings, published in 1889, caught the public imagination and by the end of the century extracts of numerous organs were available for the purpose of treating real and imagined ailments. Thus when Bayliss & Starling were investigating the influence of the duodenum on the exocrine pancreas, the idea of making duodenal extracts came readily. They were astonished to find that such an extract had all the effects on the exocrine pancreas that had been previously attributed to Pavlov's nervous reflexes. They proposed that there must be a chemical messenger released from the duodenum which acted via the circulation and proposed the term hormone.

#### *Nature of the gut endocrine system*

Although the first substance to be named hormone, secretin, was from the alimentary tract, progress in the understanding of gut endocrinology was very slow. Histology, and later ultra-structural examination, showed the gut to contain many different types of endocrine cells. These, however, were scattered diffusely through the mucosa and not gathered together to form an endocrine gland. The gastrointestinal tract is thus an example of the diffuse endocrine system. The many different types of endocrine cells are spread widely, and in overlapping configuration, throughout the gastrointestinal mucosa. This is because they are required to respond to a similarly diffuse signal. Thus a representative subject may

have just consumed a meal of caviar washed down with liberal quantities of champagne. The gut is expected to analyse exact quantities of the contained fat, carbohydrate and protein and produce an integrated signal which will result in the release of an appropriate quantity of gastric acid, pancreatic enzymes, bicarbonate and small intestinal juice. In addition appropriate changes in blood flow and motility are required and a suitable signal sent to the endocrine cells of the pancreas to stimulate an appropriate release of insulin. It may also be that more general signals are sent, for example, to the brain to regulate appetite and thus the input of further food. Thus the regulatory task set the diffuse endocrine system of the alimentary tract is indeed considerable.

The first step in analysing such a system is to extract and purify the hormones so that their pharmacology may be studied. This has proved difficult because of the very diffuse nature of the alimentary endocrine system and the fact that a plentiful supply of destructive proteolytic enzymes was always extracted with the hormones. Recent advances in biological chemistry have to a great part overcome these difficulties. It is now possible to purify a few femtomoles of active peptide hormone from a kilogram of gut. Once purified these hormonal peptides could be sequenced. Such are the advances in protein chemistry that once a peptide sequence is known its synthesis, and free availability, rapidly follow.

## *Techniques*

Availability of pure hormonal peptide allows the use of two highly sensitive techniques—radioimmunoassay and immunocytochemistry. Both rely on the availability of highly specific antisera resulting from immunization of animals, usually rabbits, with the pure peptide. Both techniques also have a sensitivity far in excess of any other measurement system. They allow accurate measurement of hormone concentrations in the circulation and also accurate localization of the particular cell producing that hormone in the gastrointestinal mucosa.

Needless to say, there are problems with these techniques. There have been several papers published which contain erroneous results. One such problem arises from the fact that the antibody binds with a particular part of the hormone and therefore does not accurately reflect the biological activity. For example, pro-hormone may be measured by the assay but be quite without any biological importance. Similarly, anything that affects the avidity of antibody antigen reactions will interfere in these systems. Considerable care and expertise is thus required before reliable measurement can be made of hormones in the circulation and their localization in tissues can be achieved.

## *The known circulating hormones*

There are now eight peptide hormones from the gastrointestinal tract which are generally agreed to have physiological significance. These are listed in the Table below. It can be seen that they fall into four anatomical groups, originating in the stomach, pancreas, upper small intestine and lower small intestine. This division is especially useful when considering the various phases of the digestive process and

Table 1. *Peptide hormones from the gastrointestinal tract*

| Hormonal peptide | Major tissue of origin | Other tissues of origin | Proposed physiological role |
|---|---|---|---|
| Gastrin | Antrum | Duodenum | Acid secretion, parietal cell growth |
| Pancreatic polypeptide | Pancreas | — | Inhibitory modulation of gall bladder and pancreatic secretion |
| Secretin | Duodenum | Jejunum | Pancreatic bicarbonate secretion |
| Cholecystokinin | Jejunum | Duodenum | Gall bladder contraction, pancreatic enzyme secretion and acinar cell growth |
| Motilin | Jejunum | Duodenum | Enhanced motor activity of upper GI tract |
| GIP | Jejunum | Duodenum | Glucose dependent stimulation of insulin release |
| Neurotensin | Ileum | — | Unknown |
| Enteroglucagon | Ileum | Jejunum colon | Inhibition intestinal transit. Growth of enterocytes |

also when investigating human pathology, as many disease processes are confined to a single area of the gut.

## *Stomach*

*Gastrin.* Gastrin is now well recognized to be physiologically important in the control of postprandial release of gastric acid. Reduction in gastric acid output, either as a result of gastric disease or the ingestion of drugs, results in an increased release of gastrin by a feed-back mechanism. Patients with gastrin-producing tumours have excessive acid production which leads to serious complications from duodenal ulcers, which may then perforate or bleed. In addition gastrin has recently been shown to have a role in promoting growth of the mucosa of the stomach. Thus the gastric atrophy produced by starvation can be prevented if gastrin is administered. Similarly in the patients with gastrin-producing tumours, there is usually gross hypertrophy of the mucosa which hangs in redundant folds. This gives rise to a typical radiological picture when such patients are examined by X-ray and is often the first sign that the patient has indeed a gastrin-producing tumour. Gastrin is released by distension of the stomach and also by proteins and peptides. This release is blocked by acid. Great excitement was generated when it was found that there were several forms of gastrin in the circulation, the major forms being little gastrin (seventeen amino acids) and big gastrin (thirty-four amino acids). However, it was subsequently found that these two forms of gastrin have very nearly the same biological actions and it is unclear why it is advantageous to have two hormones released from a single endocrine cell (the G cell) and performing the same function.

*Pancreas*

*Pancreatic Polypeptide.* This thirty-six amino acid polypeptide was discovered by accident as a contaminant of insulin. It was later found to be produced by a small granulated endocrine cell whose function had previously been a puzzle. A significant concentration of pancreatic polypeptide (PP) is found in fasting plasma. In patients who have had a total pancreatectomy, however, PP is totally absent from the circulation showing that its only source of origin is indeed the pancreas. A very considerable rise in plasma PP occurs after eating, Figure 1. The major components producing this are protein and fat. Intravenous infusions of amino acids or fat produced no change in PP however. Thus the presence of an entero-PP axis allowing the intestinal stimulus of food to release the pancreatic hormone PP had to be postulated. It was found that insulin induced hypoglycaemia, a known powerful stimulus to the vagus, produced a large rise in PP (Figure 2). This rise could be obliterated by vagotomy. Thus a vagal reflex appeared to be the main mechanism for postprandial PP release. It was found that in the isolated perfused pancreas gut hormones could release PP and further patients that had had a total vagotomy still had a quite reasonable rise of PP after a meal. Thus the entero-PP axis appears dependent on both hormones and vagal influences.

A recent report suggested that the administration of PP to the *ob/ob* obese mouse completely cured the syndrome. This exciting finding suggested that PP might be important in the control of appetite. Unfortunately such experiments are prone to many errors. As is well known, almost anything that causes an animal to feel unwell will reduce food intake. When we analysed the PP content of the *ob/ob* mouse we found it to be quite normal. What then are the actions of PP? These have been recently investigated by infusing PP into human volunteers. The major findings are that while there is no change in circulating metabolites, insulin or glucagon release, or indeed the rate of gastric emptying or acid production, there is a marked effect on the secretion of pancreatic enzymes juice and also of bile. Release of these latter juices is greatly inhibited. It seems highly paradoxical that PP, a hormone released by food, should act to prevent normal digestive processes. PP has been nicknamed 'the hormone of indigestion'. Its real purpose still eludes us. It is interesting to note that patients with tumours which produce PP appear to be quite normal in their digestive processes.

*Upper small intestine*

*Cholecystokinin.* Cholecystokinin, like gastrin, exists in several forms. Indeed its amino acid sequence is similar to gastrin and in high doses it stimulates gastric acid production. Cholecystokinin is so named because of its potent action in causing gall bladder contraction. Another proposed hormone, pancreozymin, important as the main stimulant for pancreatic enzyme secretion, was found on purification to be identical to cholecystokinin. Cholecystokinin-pancreozymin is thought to be released by intraduodenal fat and protein. It has proven very difficult to develop reliable radioimmunoassays to measure the plasma levels however. Thus its exact role in physiology is still somewhat uncertain. A surprising finding

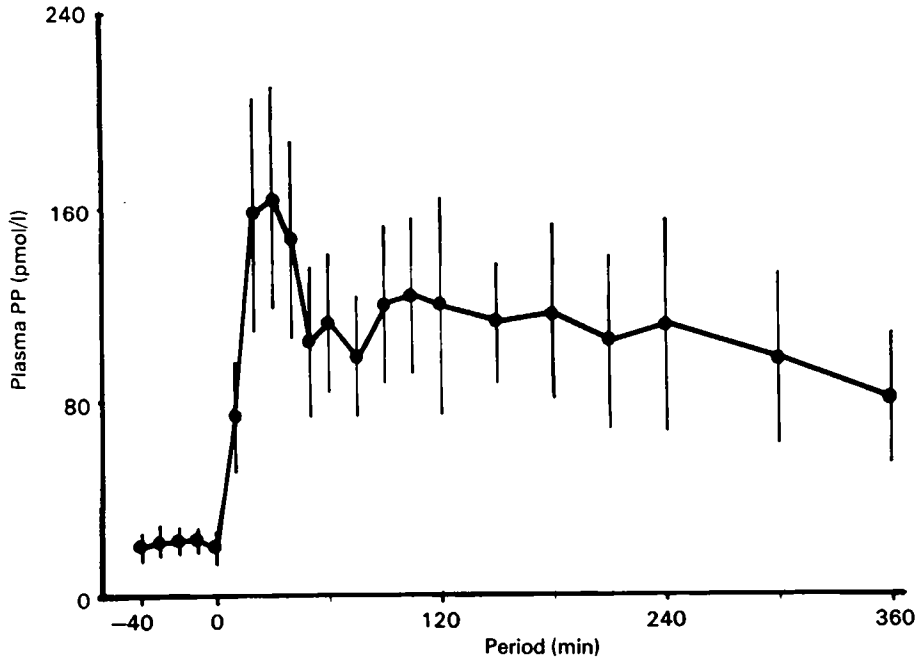


Fig. 1. Plasma pancreatic polypeptide (PP) concentrations in seven healthy individuals following a meal at time zero.

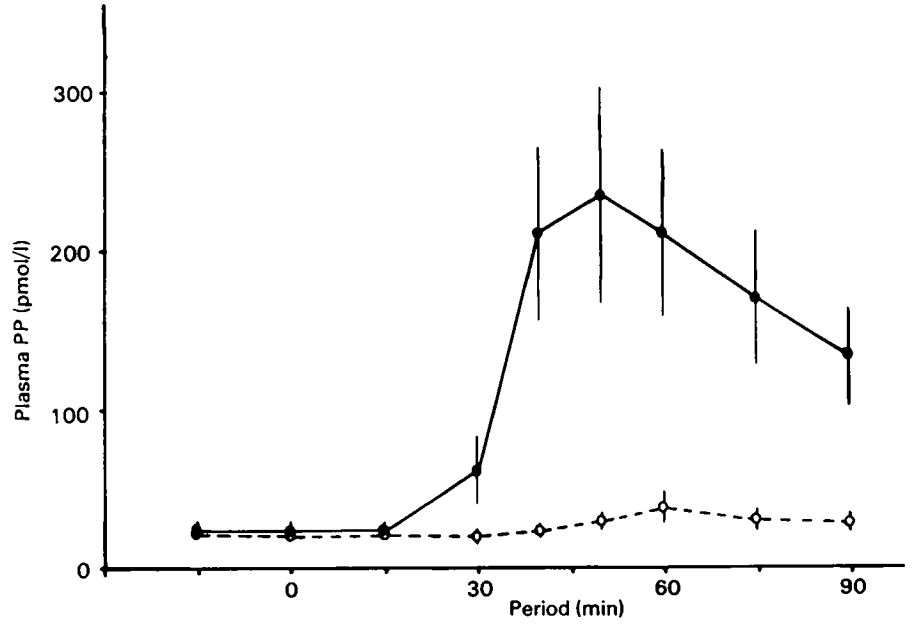


Fig. 2. Plasma pancreatic polypeptide (PP) concentrations following 0·2 μ kg insulin at time zero in eight normal subjects (———) and seventeen patients following a complete truncal vagotomy (— — —).

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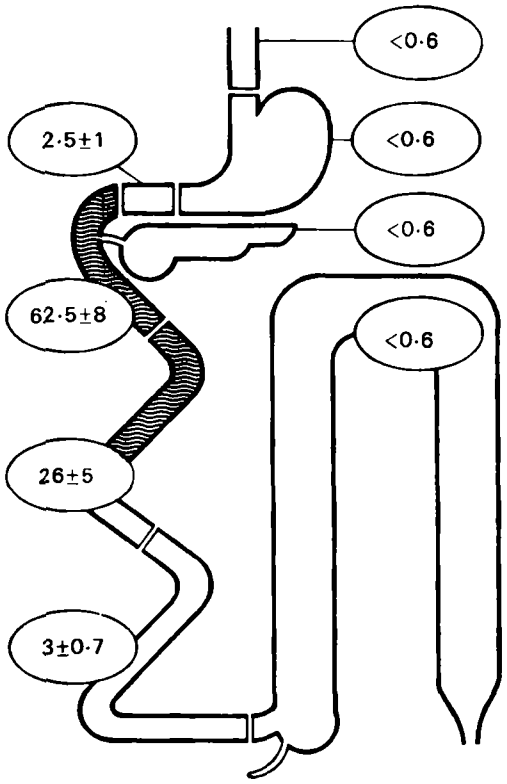


Fig. 3. The distribution in fresh human specimens of apparently normal bowel, obtained at surgery, of cholecystokinin, in pmol/g wet weight whole bowel (circled). Number of CCK cells/mm$^2$ of mucosa indicated as shading: ○, □; 11–30, ■.

is that it is also present in the brain. The major molecular form here is the eight amino acid octapeptide which is somewhat smaller than the main form found in the gut containing thirty-three amino acids. Like gastrin, cholecystokinin has recently also been found to have a role in controlling growth, in this case of the pancreas. Thus daily injections of cholecystokinin cause pancreatic hypertrophy, and also dimunition of appetite. This latter interesting point, particularly relevant as cholecystokinin is found in the hypothalamus, the presumed physiological regulatory centre for appetite, is still under active investigation to assess its physiological importance.

*Secretin.* Secretin appears to be released physiologically only by acid and its sole physiological function seems to be in control of the pancreatic output of bicarbonate. It is thus the guardian of duodenal pH. The rise after a meal is very small and only the most sensitive radioimmunoassays can detect it. Nonetheless, in the postprandial situation, the pancreas is very sensitive to these low levels of secretin and responds with a considerable increase in bicarbonate secretion. As duodenal ulcer is the result of duodenal pH being chronically too acid, a failure of secretin release is suggested. Secretin release is certainly blocked by inflammation

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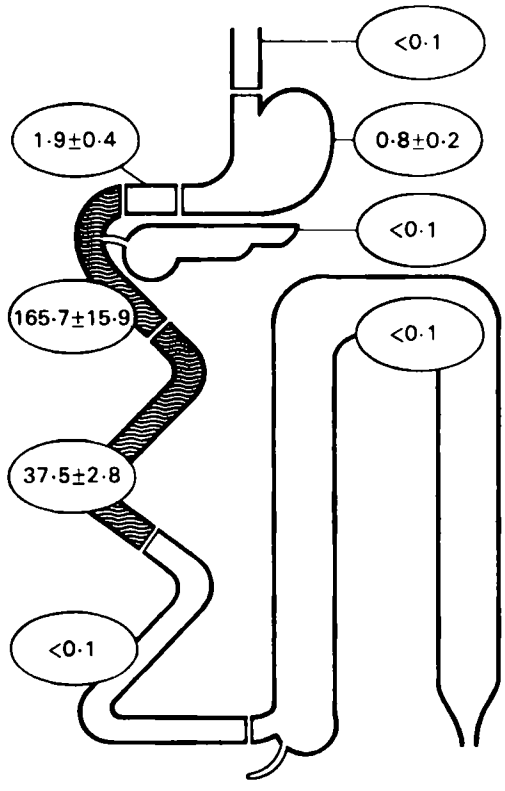


Fig. 4. The distribution in fresh human specimens of apparently normal bowel, obtained at surgery, of motilin, in pmol/g wet weight whole bowel (circled). Number of M cells/mm$^2$ of mucosa indicated as shading: ○, □; 11–30, ■.

of the duodenum and this is commonly found with duodenal ulceration. A negative feedback perhaps exists with a duodenal ulcer preventing the normal acid neutralization in the duodenum and thus causing the ulcer to worsen.

*Motilin.* Motilin, as its name suggests, is a powerful pharmacological stimulant of upper small intestinal motor activity. Infusions in man, designed to achieve physiological blood levels, result in a considerable enhancement of the rate of gastric emptying of meals. In addition motilin has been shown to cause contraction of the lower oesophageal sphincter. Sufferers from heart burn may reflect on the possibility that they have deficient motilin levels. In the fasting state an infusion of motilin stimulates the onset of interdigestive myoelectric complexes. These contractions which sweep down the small intestine at intervals of an hour or so during the fasting state have been termed by Code 'house-keeper contractions'. He speculates that they act to free the intestine of unwanted secretions and debris which otherwise would encourage the growth of bacteria and lead to considerable functional disruption. We have found that motilin is released by oral fat, but that oral glucose and amino acids depress the plasma concentration. As a control experiment the same ingredients were administered intravenously and found to be

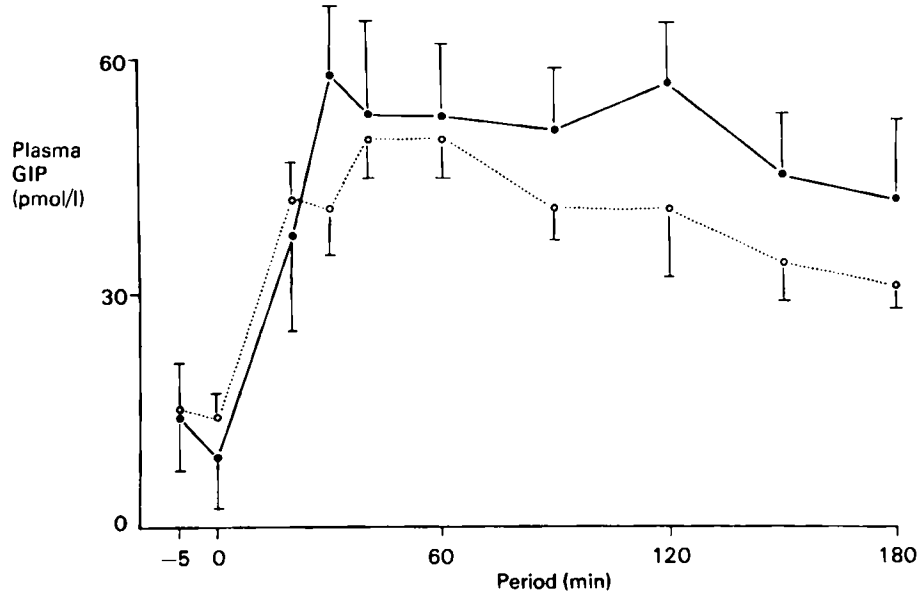


Fig. 5. The plasma GIP concentrations in seven normal subjects (●——●) and seven diabetics (○···○) (diet-treated) following 50 g glucose orally at time zero.

every bit as effective. The control of release of a gut hormone by circulating nutriments is of considerable interest. Motilin may well provide a mechanism whereby metabolism regulates the input of food by controlling motor activity.

*Gastric inhibitory peptide.* Gastric inhibitory peptide (GIP) was isolated as an intestinal factor which was capable of inhibiting even histamine-stimulated gastric acid. Later experiments showed that at smaller, more physiological, doses its main action was in stimulating the release of insulin from the β cell. This action was dependent on the ambient level of glucose and was only seen at concentrations above about 120 mg/100 ml (6·5 mmol/l). The hormone has thus been renamed Glucose-dependent Insulin-releasing Peptide. It is now thought to be the main hormone responsible for the much greater insulin release when a stimulus is taken by mouth than if that same stimulus were to be administered intravenously. If GIP is really the hormone of the entero-insular axis are there any conditions when its release is deficient or excessive and what are their effects on general metabolism? We have looked at several such possible conditions, including maturity onset diabetes (Figure 5), but failed to find any significant abnormality of GIP release. The only circumstance where GIP release is reduced appears to be in the grossly obese patient who has undergone a jejunal ileal bypass, thus effectively bypassing the GIP bearing area of the intestine. Nonetheless GIP is probably an important mechanism whereby the gut influences general metabolism. In Claude Bernard's concept of the 'milieu interieur', it was proposed that any changes in the body constituents would be minimized. Thus after a meal a significant rise in plasma glucose might be considered a physiological failure. GIP release, by enhancing the

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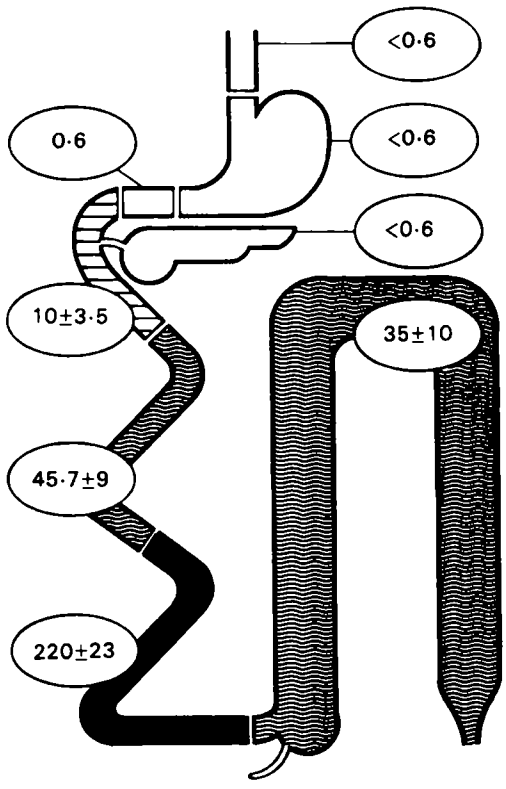


Fig. 6. The distribution in fresh human specimens of apparently normal bowel, obtained at surgery, of enteroglucagon, in pmol/g wet weight whole bowel (circled). Number of EG cells/mm$^2$ of mucosa indicated as shading: ○, □; 1–10 ▤; 11–30, ▩; ⩾31, ■.

release of insulin, acts to prevent such an excessive rise of glucose and helps to maintain the constancy of plasma metabolites.

### *Lower small intestine*

***Neurotensin.*** Neurotensin is the newest member of the group of circulating gut hormones. It was first discovered in the brain as a vasoactive peptide but subsequently found in large concentrations in the ileum. It appears to be released by glucose and fat and circulates in reasonably high concentrations in the plasma of normal man. Pharmacologically it releases glucagon and thus causes hyperglycaemia. It also inhibits gastric acid and results in a rise in plasma gastrin. In addition it releases insulin and may therefore help in the assimilation of glucose. Its release is found to be excessive in the dumping syndrome where its vasoactivity may play some part in the symptomatology. No reports of its administration in man have yet appeared and thus its role in physiology is still a matter of speculation.

***Enteroglucagon.*** Enteroglucagon was first discovered as a crossreacting substance in the early pancreatic glucagon assays. It is produced by the EG cell of

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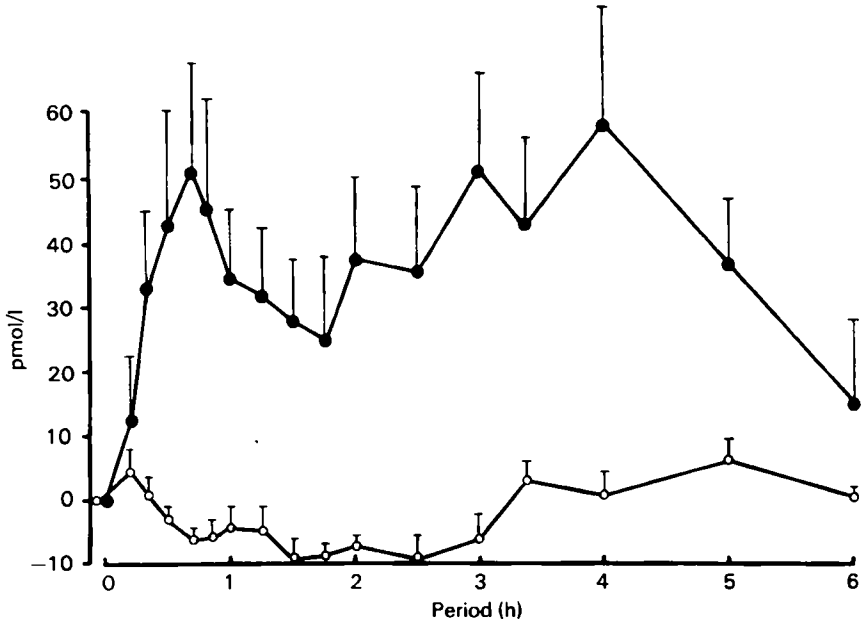


Fig. 7. The incremental change in plasma enteroglucagon, (●——●) and plasma pancreatic glucagon (○——○), following a normal lunch in six volunteers.

the ileum and colon which has a rather different histological appearance from the α cell of the pancreas which produces pancreatic glucagon. Further, whereas enteroglucagon is released by fat and carbohydrate, these substances suppress the release of pancreatic glucagon. Thus the physiology of pancreatic glucagon and enteroglucagon would appear to be quite dissimilar. A single patient with an enteroglucagon-producing tumour showed considerable hypertrophy of the gastrointestinal mucosa and a very prolonged intestinal transit time. As enteroglucagon has not yet been purified, these abnormalities are our best clue as to the actions of enteroglucagon. Like neurotensin, the release of enteroglucagon is increased when food passes unusually far down the intestine. Thus in states of malabsorption or intestinal hurry (for example the Dumping syndrome) a very large postprandial rise of enteroglucagon occurs (Figure 8). In animals with small intestinal resection and also in states of hyperphagia, e.g. confined to cold environments or with thyrotoxicosis, enteroglucagon levels are found to be extremely high. Thus the situations where enteroglucagon is elevated would appear to agree well with the theory that enteroglucagon is a trophic hormone to the intestinal mucosa.

### *Gut hormone profile*

There may well be several, as yet undiscovered, gut hormones in addition to the eight already mentioned. Following a meal a complex pattern of hormone release is seen. For each intestinal function there are both agonists and antagonists present in the circulation and the resulting physiological function is the result of a delicate

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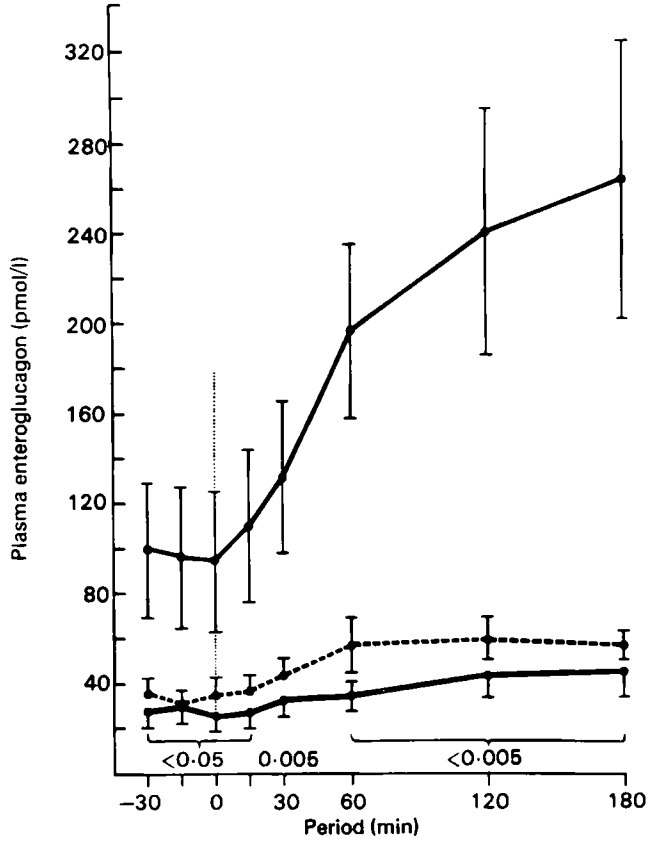


Fig. 8. The plasma enteroglucagon levels following a test breakfast at time zero in eleven patients with active coeliac disease (sub-total villous atrophy) (———) and thirteen patients who had returned to normality on a gluten-free diet (normal mucosal histology) (— — —). In addition the postprandial enteroglucagon responses of thirteen age and weight matched normal subjects are shown (═══).

biological balance. Some information as to the importance of such a balance is gained by looking at gastrointestinal diseases. We have particularly studied the effect of coeliac disease on the hormone responses. It can be seen from Figure 9 that the gastric and pancreatic hormones, gastrin and PP, are relatively little affected by the disease process, which is almost entirely confined to the upper small intestine. In contrast, both GIP and secretin are much obtunded by the intestinal inflammation. In contrast, neurotensin, motilin and enteroglucagon, released from the distal small intestine and effectively beyond the disease process, are much increased. The failure of GIP release may well be responsible for the reduction in insulin release and the larger postprandial rise of glucose. It is certainly recognized that diabetes occurs more commonly in patients with coeliac disease. The failure of secretin release, and probably also cholecystokinin release, is responsible for the relative pancreatic exocrine failure. It has been recognized for some time that whereas the pancreas of coeliac patients responds well to exogenous secretin and

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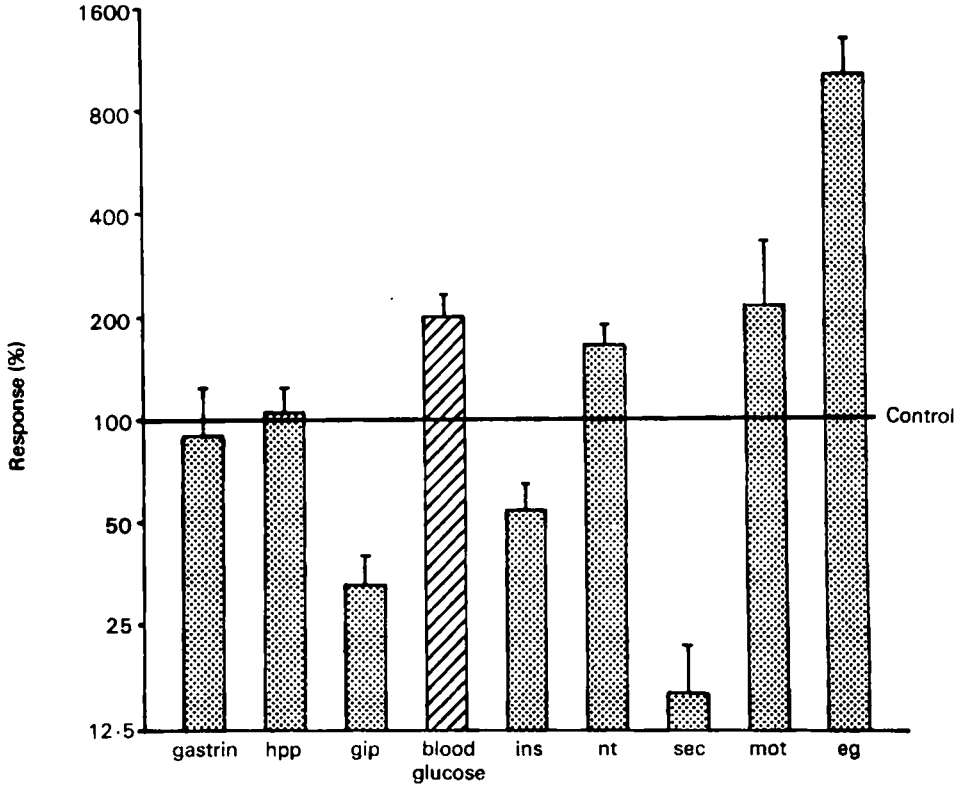


Fig. 9. The mean incremental gut hormone response and blood glucose level, after a test breakfast in eleven subjects with active coeliac disease expressed as a percentage of a normal response derived from thirteen healthy matched controls: Hpp, pancreatic polypeptide; gip, GIP; ins, insulin; nt, neurotensin; sec, secretin; mot, motilin; eg, enteroglucagon.

cholecystokinin, the response to a test meal (for example a Lundh test meal) is very greatly reduced. This can now be seen to be due to the failure of intestinal hormone release. In coeliac disease the enterocyte turnover rate is much increased, as if the mucosa was attempting to overcome the disease process. Indeed absorption from the distal small intestine is much increased. This increased enterocyte turnover is probably the result of the elevated enteroglucagon levels (Figure 8). Thus a knowledge of the gut hormone profile gives considerable insight into the pathophysiology of coeliac disease.

### *Peptidergic innervation*

Several hormonal peptides have been found to be present in both brain and gut. The first to be discovered was substance P but later vascoactive intestinal peptide (VIP), somatostatin, bombesin and enkephalin were found in both locations. In the gut these substances are mostly found in fine nerve fibres where they appear to act as peptidergic neurotransmitter agents. VIP is the substance present in greatest quantity and fine VIPergic nerves are present throughout the

gastrointestinal tract and pancreas. VIP is a twenty-eight amino acid polypeptide with considerable sequence similarities with secretin and GIP. It appears to act locally and, for example, in the pancreas may well, like secretin, stimulate pancreatic bicarbonate production. The exact role of the peptidergic innervation in control of gut function is still unclear but the considerable number of peptidergic nerves, occurring in greater frequency than the more classical adrenergic and cholinergic system, does suggest a significant role in normal physiological control. Some of these hormonal peptides are also found in endocrine cells, particularly somatostatin. Here they may have a local hormonal or paracrine function. The unity of the body's control system is greatly emphasized by finding that peptides can act as neurotransmitters, local hormones or circulating hormones. Indeed sometimes the same peptide acts in different locations in each of these three roles. It is now clear that measurement solely of the circulating gut hormones gives inadequate information on the control gut function. Thus the old argument between Pavlov and Bayliss & Starling over whether control was by nervous reflex or circulating hormones is now seen to be dead; control is by both. An era of new understanding has opened based on these potent hormonal peptides. In the next decade it should be possible to unravel many of the current mysteries of the gut. Hopefully better control of alimentary function and metabolism will soon be within our grasp.

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*Printed in Great Britain*