Gut and Brain — Endocrine Connections

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At the end of the last century, Pavlov put forward the first rational explanation of how the gastrointestinal tract could respond with such precision to the exact food stimulus presented to it. Noting the fine nerve connections between organs he proposed that the main control was neural in origin. But, in 1889, Brown Sequard declared his rejuvenation by injections of testicular extract in a paper that led to the use of many supposedly active organ extracts[1]. Hence it was not too surprising that when Bayliss and Starling were given the task of repeating some of Pavlov's experiments by Edward Schafer at University College Hospital, they should try the effect in 1902 of an 'organ extract' of the area they were studying, the duodenum. Injection of the extract gave all the results Pavlov had claimed for his nervous reflex but their genius was to conceive a more general theory of chemical messengers acting via the blood stream. In the Croonian lecture[2] of 1905, they unveiled the term hormone.

The new subject of endocrinology was easy to study. Removal of the gland gave the deficiency syndrome and injections of the extract produced symptoms of excess. Although in at the beginning, progress in defining the role of the digestive hormones was slow. In the gut, the endocrine cells (Fig. 1), though very numerous, were dispersed and not gathered in easy to handle glands. The gut is thus an example of the 'diffuse endocrine system' which is certainly larger, and may be more important, than the more traditional glandular endocrine system. It is, however, far more difficult to study and it was not until 1961 that the first gut hormone, secretin, was finally isolated and even then the intestines of 10,000 hogs were required for a few mg of pure hormone[3]. In the last decade, enormous advances in the technology of protein purification have occurred and it has been possible to isolate and put in sequence most of the active peptides in the gut. It has also become easy to prepare synthetic peptides in bulk so that research is no longer hampered by the shortage of natural hormones for testing pharmacology or the development of assays.

Circulating Hormones from the Gut

Eight gut hormones are now generally agreed to be physiologically important (Table 1). Each of these has a distinct distribution pattern and pharmacology. Their precise individual roles in physiology and their importance in human disease is less well defined, though under active investigation. It is clear that for any physiological function of the gut several hormones work together as agonists and antagonists. Thus, a proper

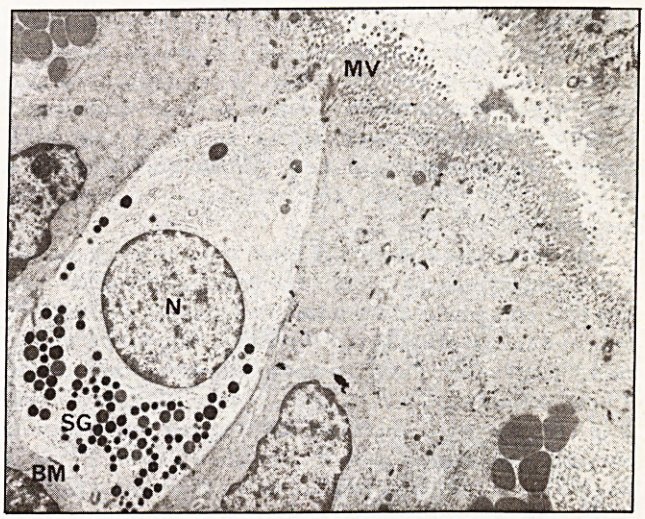


Figure 1. *An endocrine cell at the electron microscopical level showing: SG—Secretory granules; BM—Basal membrane; N—Nucleus; MV—Microvilli reaching into the lumen (x 7500)*

Table 1. Physiologically important gut hormones.

Peptide	Location	Possible action
1 Gastrin	Antrum, upper small intestine	Stim. gastric acid, trophic to mucosa
2 Pancreatic polypeptide	Pancreas	Inhib. panc. enz. and gallbladder contraction
3 Secretin	Duodenum and jejunum	Stim. panc. bicarb.
4 Cholecystokinin-pancreozymin	Small intestine	Stim. panc. enz. and gallbladder contraction
5 Motilin	Small intestine	Stim. upper GI motor
6 Gastric inhibitory peptide	Small intestine	Insulinotropic
7 Neurotensin	Ileum	Inhib. gastric motor
8 Enteroglucagon	Ileum and colon	Trophic to enterocyte

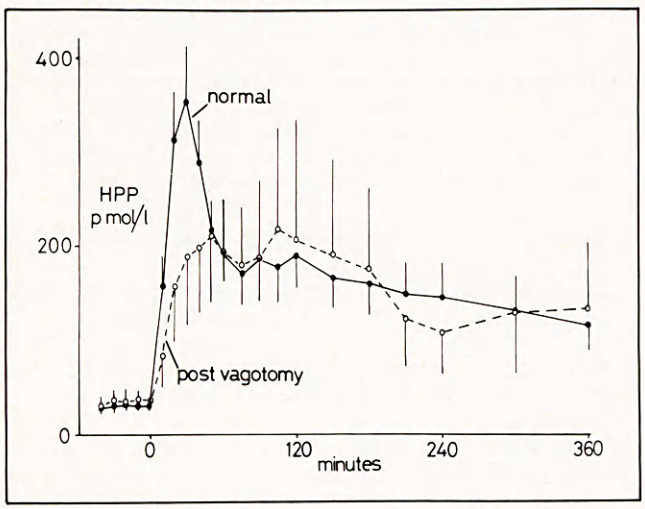
understanding of the gut hormone system depends on looking at the complete gut hormone profile, and study of individual components, particularly in abnormal situations, is likely to be misleading. The details of two representative gut hormones, pancreatic polypeptide and motilin, follow.

Pancreatic Polypeptide (PP)

Pancreatic polypeptide was found as a contaminant of insulin[4] and it is produced by endocrine cells in the islets of Langerhans and also lying between the acinar cells[5]. PP levels in blood rise very rapidly after a meal (Fig. 2). In patients who have had a total pancreatectomy no PP is detectable, either fasting or postprandially, demonstrating that PP originates only from the pancreas. Thus, after a meal, some signal from the gut must stimulate the pancreatic PP cell. The most likely mechanism is the absorption of food itself, raising the concentration of blood metabolites such as glucose. However, infusions of glucose, lipid or amino acids do not affect PP levels. Infusion of hormones such as cholecystokinin greatly stimulate PP release and so also does vagal stimulation[6]. Thus, a dual neural and hormonal signalling system allows the gut to control PP release (enteropancreatic axis). When one segment is deficient the other can compensate, as is seen after vagotomy (Fig. 2)[6].

Pharmacological studies of PP in the dog suggested the most sensitive action was to inhibit gallbladder contraction and pancreatic enzyme secretion[7]. Because PP still contaminates many commercial insulin preparations, and indeed causes diabetics to develop antibodies against PP[8], it was important to find out what PP does in man. Experiments were therefore undertaken in medically qualified volunteers and it was shown that PP at a concentration three times that seen after a meal did not affect the concentration of insulin, glucagon, glucose or other metabolites[9], though such effects had been reported in the bird. In contrast, when even a low dose of PP was given, exactly mimicking the rise seen after a meal, a highly significant inhibition of gallbladder contraction and pancreatic enzyme secretion was noted[10], as had originally been reported in the dog[7]. No effect was seen on other parameters such as gastric acid secretion or the rate of gastric emptying. As the PP cells are located in the pancreas itself, they are presumably acting in some way to prevent excessive pancreatic secretion.

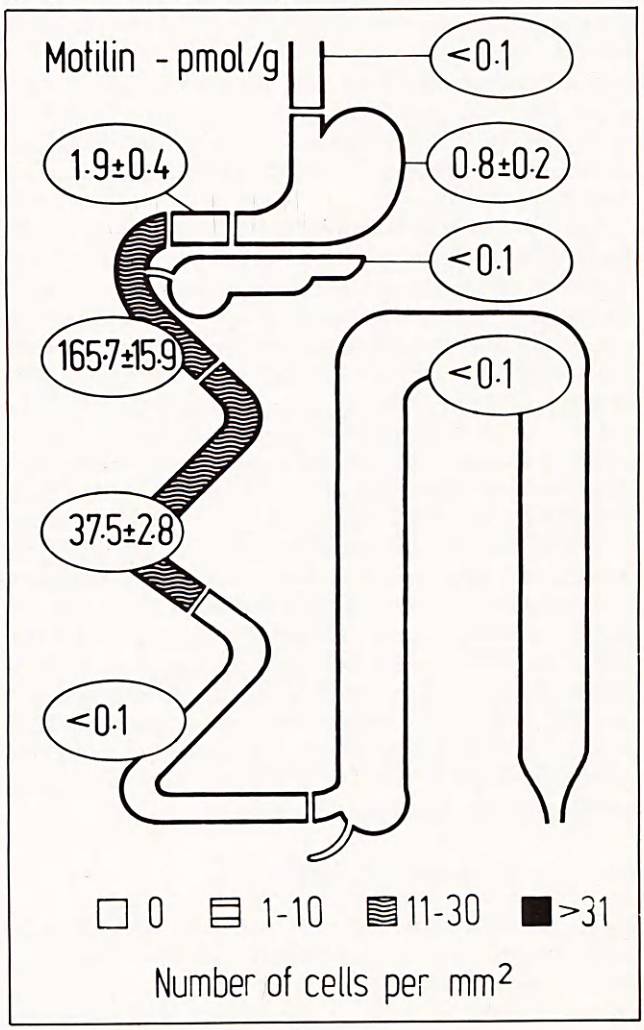
Figure 2. *Plasma PP levels after lunch in normal subjects and patients who previously had a successful truncal vagotomy for duodenal ulcer.*



Motilin

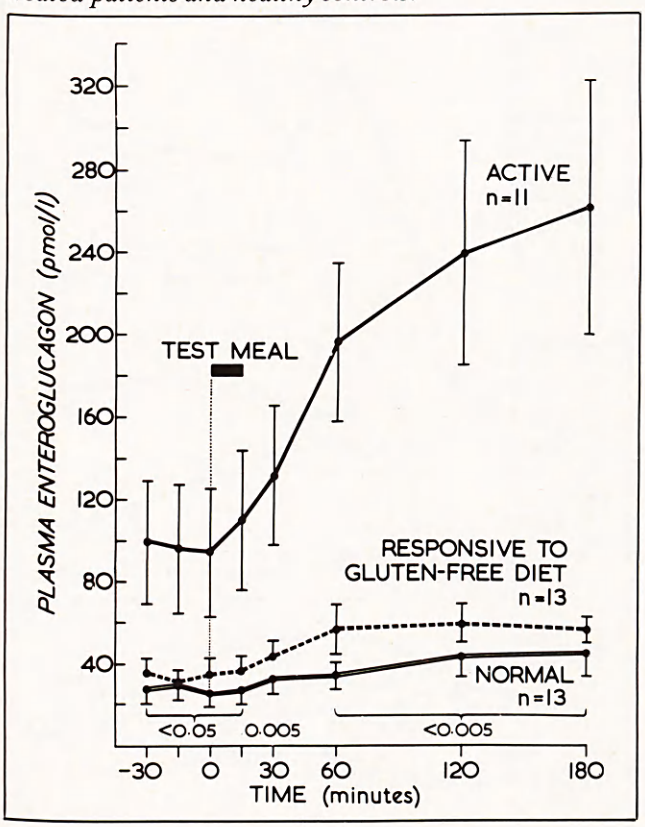
Motilin was discovered when, in 1966, John Brown noted that stimulation of the upper small intestine caused gastric contractions[11]. He was able to purify a 22 amino acid peptide that had powerful pharmacological effects both in the whole animal and on isolated muscle strips[12]. Development of a motilin radioimmunoassay showed that it comes exclusively from endocrine cells in the upper small intestine (Fig. 3) and is present in

Figure 3. *Map of the distribution of motilin in man (prepared using fresh surgical tissue).*



considerable concentrations in fasting human plasma. Eating a normal meal does not have much influence on motilin levels but ingestion of fat alone causes a significant rise, while carbohydrate and protein cause a fall. Interestingly, an almost identical change in plasma motilin occurs when these same nutriments are given by intravenous infusion[13]. Throughout the fasting state in man, waves of contraction, starting in the stomach and ending in the ileum, pass down the gastrointestinal tract approximately every hour and a half. These regular contractions, known as interdigestive myoelectric complexes, are thought to be responsible for removing secretions and debris from the fasting gut lumen and preventing bacterial overgrowth. Motilin levels show regular fluctuations in the fasting state and the peaks occur just before the onset of each new interdigestive myoelectric complex. Infusions of motilin, even immediately after the last complex when the gut is normally quiescent, can induce the onset of new contractions[14]. It has also been shown that a small and physiological increment in motilin considerably enhances the rate of gastric emptying[15], presumably by increasing gastric fundic tone, while a large increment causes inhibition of gastric emptying[16], perhaps by causing an overriding contraction of the pyloric sphincter. Significant concentrations of plasma motilin are thus found in the fasting state but they are little influenced by normal mixed meals; motilin seems likely to regulate the interdigestive motor activity of the gastrointestinal tract and influence the speed of gastric emptying after a meal. Motilin levels vary widely from individual to individual and it may well be that its most important role is in adjusting the particular setting of the gut's basal motor tone.

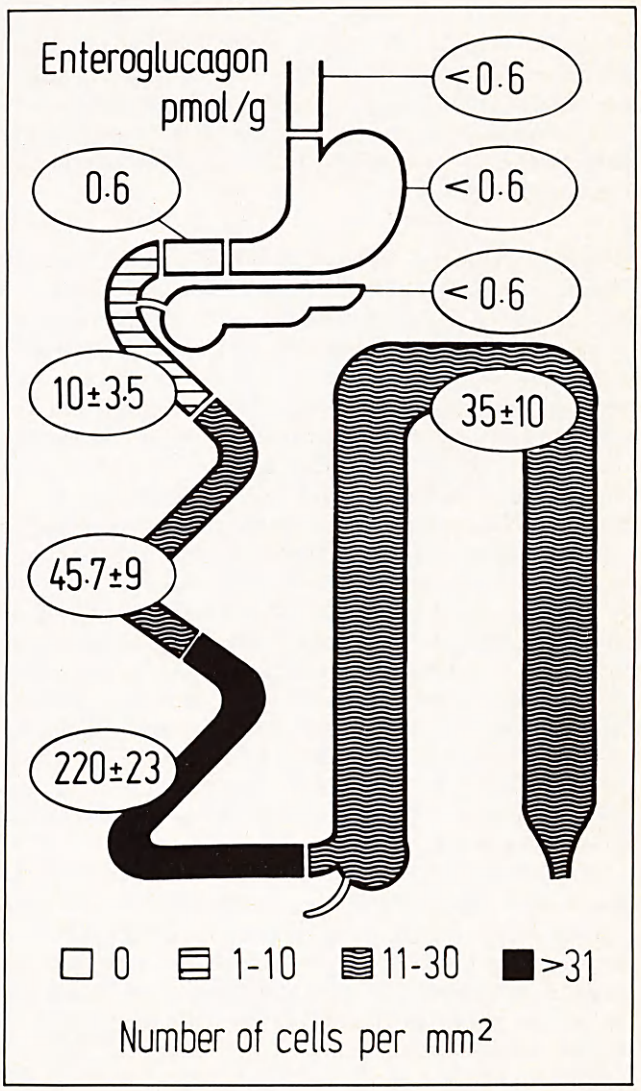
Figure 4. *Plasma enteroglucagon concentrations after a test breakfast in patients with active coeliac disease, treated patients and healthy controls.*



Influence of Disease on the Gut Hormone Profile

A standard breakfast of two eggs, toast, marmalade and orange juice (530 calories) has been administered to defined groups of patients with different gastrointestinal diseases. The gut hormone response to this everyday stimulus has been compared with matched groups of healthy subjects. In coeliac disease the most striking feature was an increase of enteroglucagon (Fig. 4), a hormone localised to the ileum and colon (Fig. 5). As can be seen in the total profile shown in Fig. 6, there was also a marked reduction in glucose-dependent insulinotrophic

Figure 5. *Map of the distribution of enteroglucagon from the same tissues as Figure 3.*



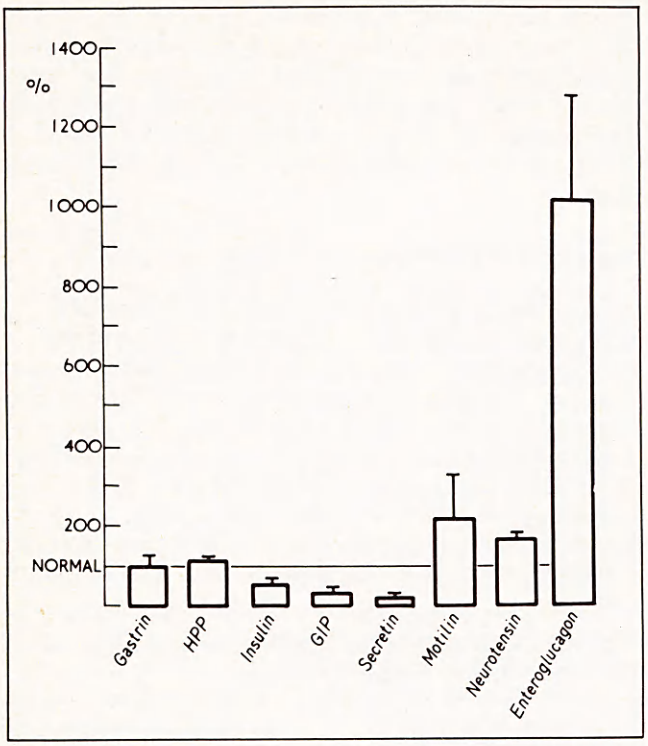


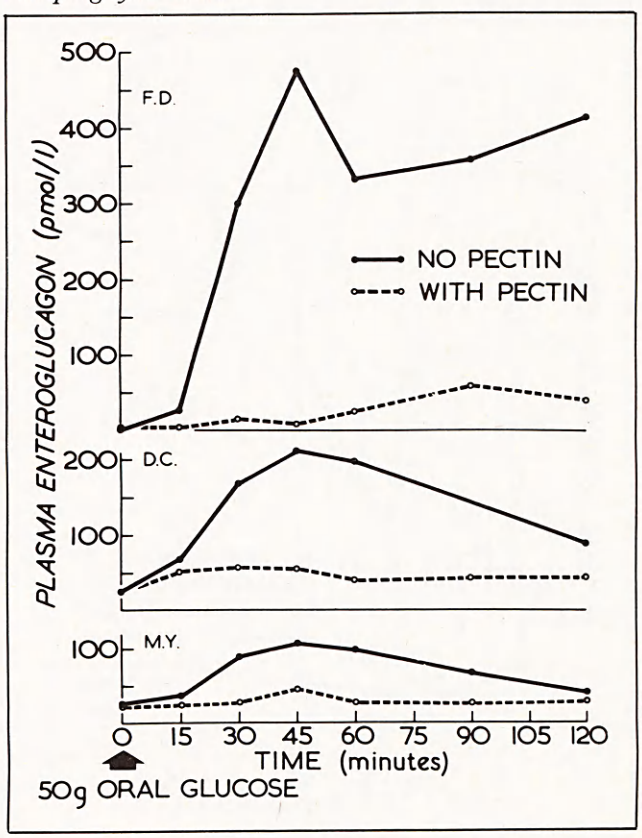
Figure 6. *The gut hormone profile of patients with active coeliac disease (as shown for enteroglucagon in Figure 4). The integrated hormone values after a test breakfast are expressed as a percentage of the totals found in the normal control subjects.*

peptide (GIP) and secretin release[17]. The lesion in coeliac disease is confined to the upper small intestinal mucosa, so it is not surprising to find that hormone release in this area is diminished, or that hormones, such as enteroglucagon, released from the intestinal tract beyond the lesions are increased. Hormones from the stomach and pancreas (gastrin and PP) are unaffected. Thus, the hormone profile may prove useful in indicating the anatomical distribution of the disease process. It also helps explain certain aspects of the pathophysiology. In coeliac disease it has been known for some time that there is a functional failure of pancreatic exocrine secretion (e.g. to a meal) but this appears normal when tested by infusion of secretin and cholecystokinin. This apparent discrepancy can now be explained by the failure of endogenous hormone release. Similarly, there is an increase in enterocyte turnover in the intestinal mucosa and increased absorption in the distal small bowel, which may be explained by the high concentrations of enteroglucagon, a hormone thought to be trophic to the intestinal mucosa.

A rather similar condition, produced artificially, is the jejuno-ileal bypass, performed in the treatment of gross obesity. In these patients the upper 7 inches of jejunum is anastomosed to the last 7 inches of ileum, with resulting rapid initial weight loss. However, after about a year the weight loss ceases and eventually the patients may start to regain weight significantly. It is apparent that an adaptive change has allowed hypertrophy of the bowel remaining in contact with the food which, together with gross slowing of unit transit time, compensates for the loss of intestinal length. The gut hormone profile shows a considerable reduction of release of GIP, matching the reduced insulin secretion. There is an eightfold increase in neurotensin and a sixteenfold increase in enteroglucagon. The gross changes in release of these hormones, which act to slow transit and increase mucosal growth, help to explain the functional changes observed following the bypass operation[18]. Similar changes have been observed following small intestinal resection[19], contrasting with a group of patients following large bowel resection where enteroglucagon levels were actually lower than those observed in matched healthy subjects[20].

In a series of patients with acute infective diarrhoea the most striking finding was an elevation of motilin, which returned to normal after recovery[21]. Motilin was also high in other patients with severe diarrhoea of whatever cause, for example Crohn's disease and acute tropical malabsorption or sprue. In the latter condition, as might be expected from the findings in coeliac sprue, enteroglucagon levels were high and correlated closely with the appearance of breath hydrogen, suggesting a relation to intestinal transit time. Enteroglucagon was also elevated after food in patients with the dumping syndrome but this elevation was reduced if transit speed was artificially slowed by addition to the meal of a viscous material such as pectin (Fig. 7)[22]. A similar increase is

Figure 7. *The enteroglucagon response to oral glucose with or without added pectin in three patients with the dumping syndrome.*



also seen with the ileal hormone neurotensin. The neurotensin cells are confined to the ileum and are overstimulated in the dumping syndrome by the unusual appearance of food components in the terminal ileum[23]. In patients with severe pancreatic destruction and consequent exocrine deficiency the release of PP is greatly obtunded[24], whereas levels are normal in malabsorption due to small bowel disease. Thus, gross alterations of gut hormone release are seen in various gastrointestinal diseases and their measurement promises to be useful both in diagnosis and in monitoring treatment. In some situations gut hormones may be important in aetiology. Much further work, however, is required before these interesting possibilities become a practical clinical reality.

Tumours

The first clinical use of gut hormone measurement was in the diagnosis of pancreatic endocrine tumours. For some reason this gland seems particularly predisposed to developing both endocrine (Table 2) and non-endocrine tumours. The classical gastrinomas and insulinomas have since been supplemented by tumours producing glucagon (glucagonomas), vasoactive intestinal peptide (VIP) (VIPomas), PP (PPomas) and somatostatin (somatostatinomas)[25]. These tumours are nature's experiments. Gastrinomas, for example, have demonstrated the potent effect of a high gastrin concentration on acid production and formation of duodenal ulcers but perhaps more importantly have shown up the long-term effects of gastrin on growth of the gastric mucosa. This led directly to the finding that gastrin is important in the maintenance of normal mucosal thickness. About half the glucagonomas were unexpectedly associated with a characteristic necrolytic migratory erythema[26], which led to a considerable upsurge in the diagnosis of new cases. It has previously been suggested that escape occurred from the long-term effects of glucagon but these patients had uniformly low amino acids and a high hepatic gluconeogenic rate with gross peripheral wasting and showed no evidence at all of 'escape'. Somatostatin has many different effects but the patients with somatostatinomas have four main features, hypochlorhydria, diabetes, malabsorption and gallstones[27, 28]. VIP was initially considered to be a potent vasodilator that also released hepatic glycogen and inhibited gastric acid secretion. The finding that patients with VIPomas had severe watery diarrhoea was unexpected[29]. The measurement of VIP is very useful in detecting these tumours, which are peculiarly sensitive to the cytotoxic drug streptozotocin, allowing patients with unresectable tumours many years of active life. All the hormones produced by these pancreatic endocrine tumours are present in normal fetal and adult pancreatic tissue. Thus, when neural tumours outside the pancreas were found to produce VIP, VIP was sought for, and found, in the central nervous system[30].

Table 2. The pancreatic endocrine tumours (and main clinical features).

Insulinoma	Hypoglycaemia
Gastrinoma	Peptic ulcer
Glucagonoma	Diabetes, rash, wasting
VIPoma	Severe watery diarrhoea
Somatostatinoma	Diabetes, malabsorption, gallstones
PPoma	None

Locally Active Peptides

The finding of VIP in both gut and brain lent emphasis to the original discovery that substance P was present in both organs[31, 32]. Further peptides were then found to have this dual distribution (Table 3) including cholecystokinin[33, 34] and neurotensin [35] thought to function as normal gut hormones in the periphery. In the brain the peptides are found both in nerve cell bodies and, in larger amounts, in the synaptosomes. Many have been shown to have potent neural effects and they probably act as important neurotransmitters or neuromodulators. Recent work has demonstrated that they are localised to particular pathways and have specialised functions (for example substance P in pain transmission). Considerable interest has been engendered by the discovery of the endorphin family, the smallest members, the enkephalins, being found in the gastrointestinal tract[36]. These substances affect mood and reflect the general observation that the peptide neurotransmitters are important in the central nervous system in the control of longer-term functions. Thus, the cerebellum, which is concerned primarily with rapid postural adjustments, is virtually free of these peptides, while the hypothalamus contains very large amounts. It seems likely that a given peptide has a quite different role in different anatomic locations. Substance P may effect the formation of saliva in the salivary glands, yet be a pain transmitter in the spinal cord. It is nonetheless tempting to speculate that the finding of a common mechanism affecting both brain and gut function may in some way explain the frequent clinical association of mood abnormality with functional bowel disease.

Table 3. Brain gut peptides.

Peptide	Mode in gut
Vasoactive intestinal peptide	Neurotransmitter
Substance P	Neurotransmitter
Enkephalin	Neurotransmitter
Bombesin	Neurotransmitter
Somatostatin	Paracrine
Cholecystokinin	Hormonal
Neurotensin	Hormonal

Somatostatin

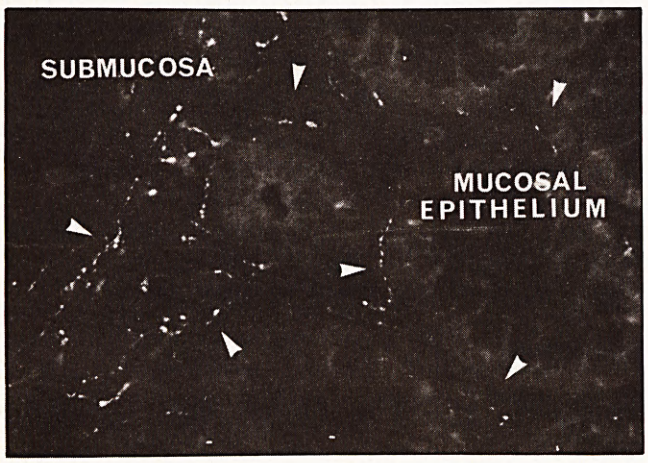
Somatostatin was first isolated from the ovine hypothalamus because it inhibited growth hormone

release[37]. It was later found to have numerous actions on the pancreas and gastrointestinal tract[38] and to be present in these organs in far larger quantities than in the hypothalamus[39]. In the gut and the islets of Langerhans it is localised to the previously described D cell [40], an endocrine cell whose function was hitherto unknown. Somatostatin totally inhibits gastric secretions, pancreatic exocrine and endocrine secretions, and small intestinal activity as well as pituitary function; all these effects occur at about the same dose. It is very difficult to believe that a circulating hormone would have such a wide range of disparate actions and it has therefore been proposed that somatostatin acts mostly as a local hormone (paracrine), influencing only the surrounding cells. Thus, the D cell in the islets of Langerhans will have a different role from the D cell in the gastric antrum. Such a system is particularly difficult to study, being by definition a local tissue phenomenon. It is clearly likely to be of importance both in controlling gastric acid and insulin secretion. Work is therefore under way to develop specific antagonists to allow direct investigation of somatostatin's role.

Gut Neural Peptides

There are four peptides seen to be present in the autonomic nerves of the gastrointestinal tract, namely VIP, substance P, enkephalin and bombesin. Other peptides, including somatostatin, TRH, tetragastrin and angiotensin may also be present in a much smaller number of nerves. It has been known for a long time by morphologists that many of the peripheral autonomic nerve fibres did not have the characteristics of adrenergic or cholinergic nerves. Further, a number of neural functions could not be blocked by adrenergic or cholinergic blocking agents. It now seems that the majority of the fine autonomic nerve fibres in the peripheral tissues of the body contain these active (Fig.8) peptides and may be considered to form a large peripheral peptidergic nervous system. A great deal of further work is required to ascertain its importance but it may well be of greater significance than the more traditional cholinergic and adrenergic autonomic divisions. The actions of each of the four main peptides in the gut is known only in outline. VIP causes vasodilatation, muscle relaxation and intestinal secretion. Substance P causes smooth muscle contraction and may be involved with local pain sensation. Enkephalin inhibits secretions and motor action and may also be involved in pain sensation in an opposite sense to substance P. The fourth peptide, bombesin, was originally isolated from the skin of the *Bombina bombina* but a very similar peptide has now been extracted from the porcine stomach[41]. It stimulates secretions of the stomach and pancreas and releases several gastrointestinal hormones. It thus acts in an opposite manner to somatostatin, and it may be that the two peptides form an agonist—antagonist system.

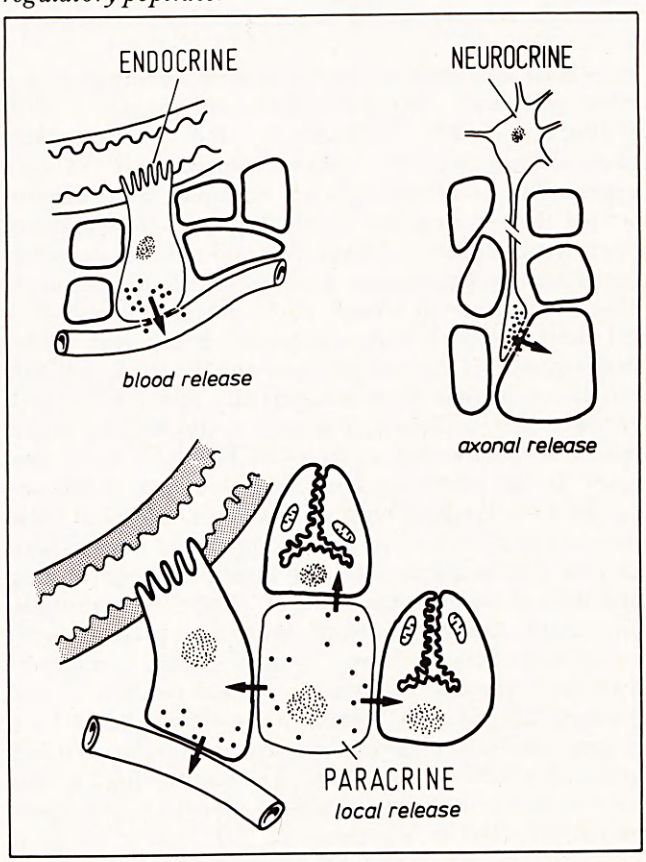
Figure 8. *Immunostained VIP nerves (indicated by arrow heads) in the mucosa of normal human colon (× 450).*



Conclusions

The old fight between Pavlov and Bayliss and Starling, as to whether hormones or nerves controlled the gastrointestinal tract, is now resolved. Both were right. Indeed, the distinction between the hormone and the neurotransmitter has virtually disappeared, as several peptides appear able to fulfil both functions. The body seems to be highly conservative in its use of these regulatory peptides, employing the same mediator for

Figure 9. *Diagram of the three main modes of action of regulatory peptides.*



quite different functions in different areas. Figure 9 depicts the tripartite theory of control, in which a single peptide (for example cholecystokinin) may act as a local regulator, a neurotransmitter or a circulating hormone.

The investigation of control of gastrointestinal function led directly to the discovery of the peptidergic nervous system whose function is still being investigated. It is interesting to note that the quantities of peptides in the nerves of the gut, for example VIP, are very considerably greater than the total quantity of hormone present in the endocrine cells. Thus, if quantity is any indication of importance, the discovery of this system may repair a major gap in our understanding of gut control. The peptidergic nervous system is, of course, not confined to the gut but is present throughout the body in lung, heart, skin, urogenital tract, etc. We know little enough about the actions of the various neural peptides in the gut but even less of their importance in these other organs. Investigating the brain gut connections may have led us in some very fruitful new directions.

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