# THE GASTROINTESTINAL HORMONES: AN HISTORICAL REVIEW

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## THE DISCOVERY OF SECRETIN

It has been said that the great discoveries of science are those which can be seen – sometimes long afterwards – to have changed our way of thought about natural phenomena and so to have turned the course of discovery in a new and fruitful direction which none had earlier foreseen. By this criterion, the discovery in 1902 by Bayliss and Starling, at University College London, of the duodenal hormone secretin was indeed a signal event in the history of physiology. A simple experiment, the work of a single afternoon, revealed that the functions of the body were normally co-ordinated not only by the nervous system, but also by the mediation of specific chemical agents formed in, and transmitted from, one organ to others by way of the circulation, conveying a message intelligible only to those cells equipped to capture the 'chemical messenger' and decipher the encoded instructions for modification of their activity. By the discovery of what came to be called 'hormones' there was opened a new era of physiology, the beginning of endocrinology as we know it today.

To understand how the discovery of secretin came about, it is necessary to look at the events which led up to it, which took place not in London but elsewhere in Europe and above all in St Petersburg. There, during the last twelve years or so of the nineteenth century, much of the foundations of modern gastroenterology was laid by the studies of Pavlov and his pupils on the work of the digestive glands. The functions of the digestive system had preoccupied Pavlov from his early days. When in 1929 he visited Montreal and was shown round the physiological laboratory of McGill University, he took from a shelf in the library volume I of George Henry Lewes's *The Physiology of the Common Life* (1859) opened it at page 230 and showed to his companions a diagram of the alimentary tract. 'When in my very young days I read this book in a Russian translation,' he said, 'I was greatly intrigued by this picture. I asked myself: how does such a complicated system work? My interest in the digestive system originated at that time' (Babkin, 1949). Early in his career Pavlov came

under the influence of S. P. Botkin, according to whose doctrine of 'nervism' most of the bodily functions were regulated by the nervous system; and in the working of the digestive glands Pavlov saw a new and fruitful field for the study of nervous influences. He realized at the outset that his experiments must be made as far as possible in the conscious animal; and being endowed with great surgical skill he perfected methods for the surgical preparation of his dogs so that the secretions of the various digestive glands could be studied daily in the same conscious healthy animal.

Pavlov was first concerned to establish beyond doubt the secretory innervation to the pancreas and stomach, about which there was still uncertainty. In 1888, using a conscious dog provided with a pancreatic fistula and one vagus nerve divided in the neck some days previously (so as to give time for the cardiac fibres to degenerate) he stimulated the peripheral end of the nerve and obtained from the fistula a free flow of pancreatic juice. The next year, with his life-long assistant Madame Schumov-Simanovskaia, he performed his famous experiment of 'sham-feeding' a conscious dog provided with an oesophageal fistula and a gastric fistula. This resulted in a prompt secretion of gastric juice rich in acid, mucus and pepsin, which was prevented by previous vagotomy or the administration of atropine. These two experiments appeared at the time to have established beyond further question the role of the vagus in causing secretion from the pancreas and stomach and the nature of its effect on the secretory cells of those organs. However, in 1894 Pavlov's pupil Dolinski found that the introduction of dilute hydrochloric acid into the duodenum of a conscious dog provided with a pancreatic fistula caused a profuse and watery secretion of pancreatic juice. As a matter of fact, this discovery had already been made as early as 1825 by Leuret and Lassaigne (Mutt, 1959) but their work had been overlooked. Dolinski was perhaps led to his experiment by the observation of Bekker (1893) that carbonated water introduced into a dog's duodenum stimulated pancreatic secretion. It was at once apparent that in Pavlov's previous experiment on the pancreas, stimulation of the cervical vagus would also have excited gastric secretion, and the acid passing into the duodenum must have been at least partly responsible for the observed flow of pancreatic juice. In 1896 Pavlov again stimulated the cervical vagus, this time in an acute experiment on a dog in which the pyloric canal was occluded by a plug of cotton wool soaked in bicarbonate solution so as to prevent entry of acid into the duodenum but not to damage the vagal fibres which ran across the pyloric sphincter to the duodenum and thence to the pancreas. The result was very different from that previously observed – a slow flow of viscid juice rich in enzyme, which evidently represented the true effect in the dog of direct vagal excitation to the pancreas.



Thus there arose the problem of the nervous pathways followed by 'Dolinski's reflex', as it was called, the totally unexpected solution to which was to come from the hands of Bayliss and Starling six years later. Pavlov nearly stumbled on the answer; in 1897 he considered the possibility that acid absorbed into the circulation from the duodenum might be the active agency, but rejected this because acid introduced into the rectum (from which it was presumed to have been absorbed) did not excite pancreatic secretion. Attempts by others to define the nervous pathways concerned in the supposedly reflex effect proved fruitless; for instance Popielski (1901), a former pupil of Pavlov's, showed that acid in the duodenum still stimulated pancreatic secretion after section of the vagi and splanchnics, destruction of the medulla and spinal cord, removal of the coeliac ganglia and transection of the pylorus; he concluded that short reflex pathways must exist between the duodenum and the ganglion cells present in the pancreas itself. Wertheimer & Le Page (1901) made similar denervation experiments with the same result. They also showed that the intravenous injection of hydrochloric acid did not stimulate pancreatic secretion; and observing that atropine, though it blocked the action of the vagus and of pilocarpine, did not affect the response to acid in the duodenum, concluded that the efferent pathways of the reflex must be sympathetic in character, rather than vagal. They then went to the brink of the great discovery by showing that pancreatic secretion was excited on introduction of acid *into a loop of jejunum resected from the rest of the intestine*; they concluded from this that the centre for the reflex was situated in the ganglia of the solar plexus.

At this point in the story there are no better words than those of Bayliss & Starling (1902*b*)

> but they [*Wertheimer & Le Page*] did not perform the obvious control experiment of injecting acid into an isolated loop of jejunum after extirpation of these ganglia. The apparently local character of this reaction interested us to make further experiments on the subject in the idea that we might have here to do with an extension of the local reflexes whose action upon the movements of the intestines we have already investigated. We soon found, however, that we were dealing with an entirely different order of phenomena and that the secretion of the pancreas is normally called into play not by nervous channels at all but by a chemical substance which is formed in the mucous membrane of the upper parts of the small intestine under the influence of acid and is carried by the blood stream into the gland cells of the pancreas.

The 'local reflexes' already studied by Bayliss and Starling referred to their investigation on the mechanism of the intestinal movements (Bayliss & Starling, 1899) in which they had shown that local stimulation of the intestine, as by a distending bolus, excited a dual response of reflex nature, the pathways of which lay in the myenteric plexuses, resulting in

contraction behind and relaxation before the point of stimulation. This dual response of opposite sign transmitted down the gut in the plexuses they named 'peristalsis'.

The work they were now to recount in detail had been briefly described in a preliminary communication (Bayliss & Starling, 1902*a*) to the Royal Society on 23 January 1902; it was entitled 'On the causation of the so-called peripheral reflex secretion of the pancreas'. The first experiment was made on 16 January, and their friend Sir Charles Martin later described what had taken place (Martin, 1927).

> I happened to be present at their discovery. In an anaesthetised dog a loop of jejunum was tied at both ends and the nerves supplying it dissected out and divided so that it was connected with the rest of the body only by its blood vessels. On the introduction of some weak HCl into the duodenum, secretion from the pancreas occurred and continued for some minutes. After this had subsided a few cubic centimetres of acid were introduced into the denervated loop of duodenum. To our surprise a similarly marked secretion was produced. I remember Starling saying 'Then it must be a chemical reflex'. Rapidly cutting off a further piece of jejunum he rubbed its mucous membrane with sand and weak HCl, filtered and injected it into the jugular vein of the animal. After a few moments the pancreas responded by a much greater secretion than had occurred before. It was a great afternoon.

Bayliss & Starling (1902*b*) continued,

> Since Wertheimer and Le Page had shown that the effect of acid in the small intestine diminishes in proportion as the place where it is introduced approaches the lower end, so that from the last six inches or so of the ileum no secretion of the pancreas is excited, it was of interest to see whether the distribution of the substance . . . is similar in extent.

An extract made from the lower ileum in the same way as the jejunal extract had no effect on the pancreas; but since both extracts caused a similar fall in blood pressure it was thus established that the effect on pancreatic secretion was not due merely to vasodilatation, but to an agent located only in that region of the intestinal mucosa from which the pancreatic response to acid could be obtained.

In our supposedly fast-moving times it is salutary to note that by the time Bayliss and Starling came to write the full account of their work only a few months after the preliminary note to the Royal Society, they could refer to several publications by others which had already appeared on the subject. Among them was a demonstration by Wertheimer that the blood coming from a loop of intestine into which essence of mustard had been introduced was capable of exciting pancreatic secretion when infused intravenously into another dog; and also an objection by Pflüger to their interpretation of their findings. He argued that the denervation of the intestinal loop in the original experiment could not have been complete because of nerves running within the walls of the mesenteric vessels. Their reply was,



> We admit that it is difficult to be certain that all nerve channels were absolutely excluded . . . but we submit that since the result of the experiment was such as has been demonstrated it does not in the least matter whether the nerves were all cut or not; the only fact of importance is that it was the *belief* that all the nerves were cut that caused us to try the experiment of making an acid extract from the mucous membrane, and that led to the discovery of secretin. Exit Pflüger!

Bayliss and Starling were unable to repeat successfully Pavlov's demonstration that vagal stimulation would excite pancreatic secretion, and stated, 'In our opinion the chemical mode of excitation, viz. by the production of secretin in the mucous membrane by the action of the acid chyme from the stomach upon it, is the normal one. At all events, this mode of stimulation must take place whether there is a concomitant nervous process or not, so that this latter is superfluous and therefore improbable.' Their view was eventually changed by the visit to University College London in 1912 of G. V. Anrep, a pupil of Pavlov's, who demonstrated the experiment to them; they had failed because their dogs were always given morphine as a preoperative sedative, and the drug causes spasm of the pancreatic duct system. On the other hand Bayliss and Starling's observations were easily confirmed in St Petersburg, where they had a significant outcome. Pavlov asked his assistant Savich to perform the experiment, and Babkin (1949) who was present later recounted the scene. 'The effect of secretin was self-evident. Pavlov and the rest of us watched the experiment in silence. Then without a word Pavlov disappeared into his study. He returned half an hour later and said, "Of course, they are right. It is clear that we did not take out an exclusive patent for the discovery of the truth" '. The doctrine of 'nervism' in the affairs of the digestive system was obviously dead, and Pavlov later remarked to Babkin, 'Of course we may continue to study with success the physiology of digestion, but let other people do it. As for myself, I am getting more and more interested in the conditioned reflexes.' In 1904 when Pavlov received the Nobel Prize for his studies on the work of the digestive glands his acceptance speech was largely concerned with the nature of appetite, the 'psychic' stimulation of secretion, and conditioned reflexes. In 1913 and 1914 the discovery of secretin was declared by the Nobel examiner to be worthy of a prize, but the war intervened; and when Starling was again nominated in 1926 (Bayliss had died in 1924), the work was thought too old to be eligible (Liljestrand, 1952).

The wider significance of the discovery of the 'messenger' role of secretin was discussed by Bayliss & Starling (1904) in their joint Croonian Lecture to the Royal Society and by Starling (1905) in his Croonian Lectures to the Royal College of Physicians, entitled 'The chemical correlation of the functions of the body'. In the first lecture he remarked,

These chemical messengers, however, or 'hormones' from the Greek ὁρμάω (as we might call them) have to be carried from the organ where they are produced to the organ which they affect by means of the blood stream, and the continually recurring physiological needs of the organism must determine their repeated production and circulation throughout the body.

This was the first use of the word 'hormone' and of its choice Bayliss (1915) later said,

When we came across the mode by which the pancreas was excited to activity it became obvious to Starling and myself that the chemical agent concerned was a member of a class of substances of which others were previously known. The group . . . is characterised by the property of serving as *chemical messengers*. They enable a chemical correlation of the functions of the organism to be brought about through the blood side by side with that which is the function of the nervous system. This being so, it seemed desirable and convenient to possess a name to distinguish the group. That of 'internal secretion' already in use did not sufficiently emphasise their nature as messengers. Finally Mr W. B. Hardy proposed the name of 'hormone' derived from ὁρμάω (I arouse to activity) and although the property of messenger was not suggested by it, it was adopted. It has in fact been generally understood as having the meaning intended and not to be applied to any kind of substance which excites activity.

Needham (1936) says that according to local tradition the word was born in the hall of Caius College, Cambridge. 'Schäfer or Starling was brought in to dine by Hardy and the question of nomenclature was raised. W. T. Vesey, an authority on Pindar, suggested ὁρμάω and the thing was done.'

## GASTRIN

The first major consequence of the discovery of secretin was the discovery of gastrin. In the third of his Croonian Lectures (27 June 1905) Starling said,

In the alimentary canal itself the chemical correlation between intestine and pancreas does not stand alone . . . Edkins has shown that a secondary secretion of gastric juice is determined by the production of a hormone in the pyloric part of the mucous membrane (of the stomach) under the influence of the first products of digestion, and that this hormone is absorbed by the blood and carried by it to the gastric glands in the fundus, which are thereby excited to renewed activity.

Five weeks previously J. S. Edkins had read to the Royal Society a preliminary communication entitled 'On the chemical mechanism of gastric secretion' (Edkins, 1905). It began:

It has long been known that the introduction of certain substances into the stomach provokes a secretion of gastric juice . . . On the analogy of what has been thought to be the mechanism at work in the secretion of pancreatic juice by Bayliss and Starling, it is probable that in the process of absorption of digesting food in the stomach a substance may be separated from the cells of the mucous membrane which, passing into the blood or lymph, stimulates the secretory cells of the stomach to functional activity.



He went on to describe simple experiments on anaesthetized cats in which the intravenous injection of aqueous extracts of pyloric mucosa stimulated gastric secretion, while similar extracts made from the fundic mucosa did not; the heat-stable active principle he named 'gastrin'.

Edkins was no novice in the study of digestion, and long before the discovery of secretin he was thinking about the problem of how gastric secretion was continued in the later stages of gastric digestion, after the initial vagal reflex demonstrated by Pavlov had presumably come to an end. To volume I of Schäfer's *Textbook of Physiology* (1898) he contributed a chapter entitled 'Mechanism of secretion of gastric, pancreatic and intestinal juices' in which he cited the finding of Heidenhain (1879) that food placed in the main stomach of a conscious dog caused secretion in a vagally denervated pouch of the gastric fundus (the earliest type of gastric pouch, which Heidenhain had invented the year before). This fact is now recognized to constitute powerful evidence that gastric secretion is hormonally stimulated; Heidenhain had concluded that certain products of digestion absorbed from the stomach excited the secretion. Edkins remarked, 'If it is absorbed digestion products that provoke secretion, is it a specific product or products that cause this to occur, or is it a common characteristic of all?' Quoting some experiments of Chishin in Pavlov's laboratory showing that peptone was particularly effective in exciting gastrin section, Edkins went on: 'We may assume that small quantities of peptone may be normally formed in the stomach, and becoming absorbed there in some way influence the epithelium (of the gastric glands) so that secretion results.' Elsewhere in the chapter he had discussed the vague ideas of the day concerning the role of the pyloric glands, and the supposed presence in them of pepsin, concluding, 'It yet remains to be discovered whether the cells of the pyloric glands possess other more important functions.' With the discovery of secretin his ideas crystallized. The function of the pyloric region was to absorb the products of gastric digestion, notably peptone; and there was carried with them into the blood stream a hormone stored in the pyloric glands. This is no doubt why in his later search for gastrin he made his extracts of pyloric mucosa with solutions of peptone, dextrose and maltose.

Edkins's triumph was short-lived. Within a few years it was being shown by others that aqueous extracts made from a variety of organs would stimulate gastric secretion when injected subcutaneously or intramuscularly into conscious dogs provided with gastric fistulae or pouches. The discovery of histamine in intestinal mucosa (Barger & Dale, 1911), the recognition of its presence in every organ in the body, and the demonstration by Popielski (1919) that it was a most potent stimulant of gastric acid secretion, led to the view that Edkins's pyloric extract owed its

power to stimulate gastric secretion to the presence of this substance, which because of its ubiquitous distribution could hardly be included in the select group of 'chemical messengers' as originally defined by Bayliss and Starling. Only Lim (1922) repeated Edkins's experiments exactly as he had performed them and concluded that he had been right; there was a stimulant distinct from histamine in the pyloric mucosa. Lim's work went unheeded, and the explanation of the puzzle was only to come after the isolation of gastrin (Gregory, 1970); meanwhile the negative results of apparently well-conceived physiological tests of the 'gastrin theory' and a failure to find in pyloric mucosal extracts any stimulant of gastric secretion other than histamine fostered the general belief that a gastric hormone did not exist. Even the discovery by Komarov (1938) in Montreal that a gastric secretagogue of protein character separable from histamine was present in pyloric extracts did little to influence contemporary opinion, for 'Komarov's gastrin' as it was called appeared to be effective only in the anaesthetized cat, where its action was totally resistant to atropine, which was well known to inhibit the response to a meal in conscious animals and man. In the end this resistance to atropine in the anaesthetized, though not the conscious, animal proved to be one of the remarkable properties of gastrin; Komarov's extract, like Edkins's, had indeed contained the hormone. After more than forty years of general disbelief, the existence of an antral hormone was proven by physiological experiment (Grossman, Robertson & Ivy, 1948); it was isolated in 1962 (see Gregory, 1962), identified as a heptadecapeptide amide in 1963 and its total synthesis accomplished in 1964 (Gregory, 1970).

Meanwhile, over the years, secretin had become the subject of a vast number of physiological and chemical studies; but the hormone itself had defied all attempts at capture. This was achieved almost exactly sixty years after its discovery by the Swedish chemists Erik Jorpes and Viktor Mutt, who isolated it from porcine duodenum and identified it as a strongly basic peptide of twenty-seven amino acid residues (Jorpes, Mutt, Magnusson & Steele, 1962). Elucidation of the sequence, completely different from that of gastrin, and total synthesis of the hormone, followed in 1966 (Jorpes, 1968). What has been called the 'biochemical era' in the study of the gastrointestinal hormones had begun.

## THE OTHER HORMONES

The discovery of secretin and what is now recognized to have been the discovery of gastrin inevitably prompted attempts to establish the existence of other gastrointestinal hormones. In most cases these efforts followed the pattern laid down by Bayliss and Starling's classical experiment; a physiological response associated with a meal, hitherto regarded as a nervous reflex, was examined for the presence of a hormonal component by looking for the persistence of the effect after the interruption of nervous connexions between the site of origin and the target organ. Attempts were then made to prepare a mucosal extract which would reproduce the physiological effect upon injection and so might be considered to contain the putative hormonal principle. It is of interest to review these studies in order to trace the subsequent history of the ideas involved.



## INCRETIN

In point of time it might be said that the idea of this hormonal action originated perhaps even earlier than the idea of a gastric hormone. In December 1902 Starling was reviewing, in a paper given to the Pathological Society of London, the pathological implications of recent work on the pancreas (see Starling, 1903), and speaking of secretin and its actions, he said,

> Since diabetes appears to be connected in some way with the pancreas, we thought it possible that some effect might be produced on the disease by intravenous injections of solutions of secretin. This hope however proved to be unfounded, Dr. Spriggs (of the London Hospital) having tried the intravenous injection of secretin in a case of diabetes without producing any effect whatever on the course of the disease.

There is no further reference to this idea in the writings of Bayliss or Starling; but at that time, or soon afterwards, Dale began to study in Starling's laboratory the effect of a secretin extract on the islets of Langerhans, which were suspected to be the source of the internal secretion of the pancreas. Dale's histological observations seemed to support the view already expressed by others that the islets were a phase in the life-cycle of the acinar cells, for the number of islets appeared to increase on prolonged stimulation with secretin (Dale, 1904). These ideas did not pass unnoticed; Moore, Edie & Abram (1906) tried the effect of a secretin extract (orally) in cases of diabetes, on 'the hypothesis that the *internal* secretion of the pancreas might be stimulated . . . by a substance of the nature of a hormone or secretin yielded by the duodenal mucous membrane . . .'. From Dale's results, they surmised

> that the pancreas contains but one type of secreting cell which yields both the internal and external secretion, and that the cells of the islets of Langerhans are ordinary pancreatic cells in a phase of exhaustion. If this be the case, the likelihood is increased that anything which stimulates the external secretion will also stimulate the internal secretion.

Others failed to confirm their belief that secretin improved the diabetic state (Bainbridge & Beddard, 1906).

In the 1920s, after the discovery of insulin, there appeared many papers suggesting that a hypoglycaemic agent was present in duodenal mucosa. A leading figure in this field was La Barre (1936), who suggested that the secretin molecule was a complex consisting of 'excretin', which stimulated pancreatic exocrine secretion, and 'incretin', which stimulated the internal secretion, i.e. insulin. The claims of a duodenal hypoglycaemic hormone failed to survive critical examination (Best, Jephcott & Scott, 1932; Loew, Gray & Ivy, 1940); but the careful studies of Laughton & Macallum (1932) can now be seen to have provided substantial support for what would be regarded today as an 'incretin' effect. They prepared a secretin-free duodenal extract which did not lower the fasting blood sugar in a normal dog, or in a totally pancreatectomized dog, so that it did not contain an agent which was itself hypoglycaemic, i.e. insulin-like. However, it did markedly diminish the hyperglycaemia produced in normal animals by the intravenous injection of glucose or the hyperglycaemia produced by feeding glucose to partially pancreatectomized animals. They concluded,

> As to the mode of action of our preparation it would appear that the most probable explanation lies in the assumption that the preparation stimulates the islets of Langerhans to secrete insulin. If this is correct there must be a very delicate balance or the insulin if in excess would tend to produce a hypoglycaemia. Our observations gave no evidence that this occurs.

After a long interval, interest in the existence of a duodenal factor in insulin release was revived by suggestions that the oral administration of glucose accelerated the rate of disappearance of a subsequent intravenous load, and that for a given rise in blood glucose, blood insulin-like activity was higher when the glucose was given intraduodenally than when it was injected intravenously (Arnould, Bellens, Franckson & Conard, 1963).

Finally McIntyre, Holdsworth & Turner (1964, 1965), who measured plasma insulin levels by the specific and sensitive method of radioimmunoassay, demonstrated unequivocally in human subjects that for the same glucose load the blood sugar curve was lower, and the plasma insulin curve higher, when it was given intrajejunally than when it was given intravenously (Fig. 1); and in human subjects also, Dupré (1964) showed that a crude commercial secretin extract accelerated the disappearance of an intravenous glucose load. There was thus established the existence of a duodenal agent of hormonal character which controlled insulin release. The problem that remained was the identity of 'incretin'.



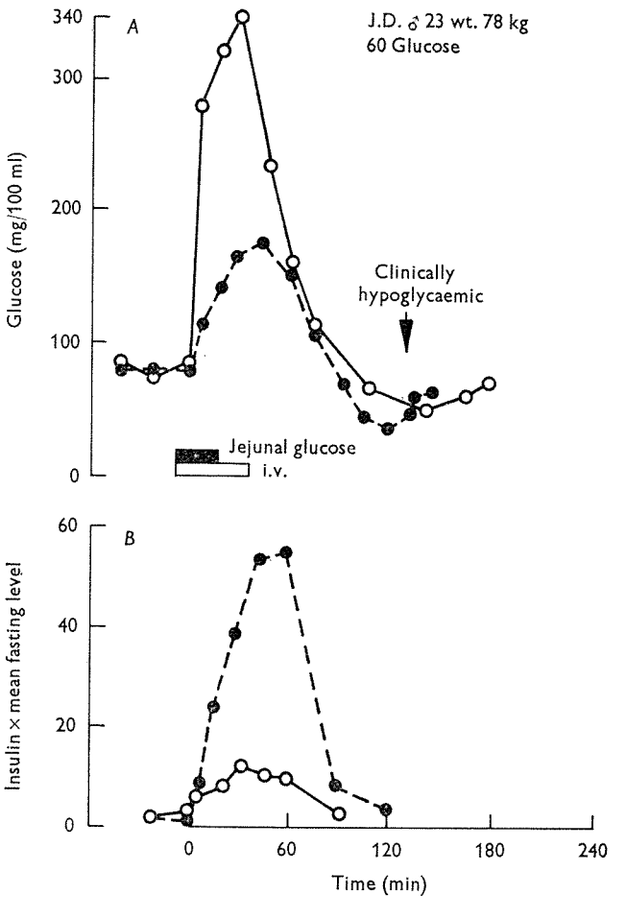


Fig. 1. The effects on (*A*) blood glucose and (*B*) plasma insulin levels of intrajejunal (●) and intravenous (○) glucose in a human subject. For the same glucose load (60 g) the blood glucose curve is lower and the plasma insulin curve higher for intrajejunal administration than for intravenous infusion. (From McIntyre *et al.*, 1965.)

## ENTEROGASTRONE

Ewald & Boas (1886), introducing to clinical gastroenterology the idea of a test-meal for the study of gastric secretion using the newly invented stomach tube, discovered that olive oil added to a meal of starch paste given to human subjects inhibited gastric secretion and delayed gastric emptying. Pavlov and his pupils studied the effect (considered to be a reflex) and showed that it resulted from the presence of fat not in the stomach itself, but in the duodenum. Farrell & Ivy (1926) discovered by accident that in a conscious dog provided with a completely transplanted

gastric pouch (a preparation invented by Ivy) a meal containing fat inhibited tone and motility; they had in fact anticipated a stimulation of motility claimed by Le Heux to result from the liberation of an intestinal 'motor hormone'. Their observation led Feng, Hou & Lim (1929) to show inhibition of gastric acid secretion, by fat in the duodenum in a similar preparation; and Kosaka & Lim (1930) prepared a crude intestinal extract which inhibited gastric secretion. They named the active principle 'enterogastrone' and there were speculations later that there might be two enterogastrones, one for inhibition of gastric secretion and one for inhibition of gastric motility. Efforts to purify enterogastrone, notably by Gray, Bradley & Ivy (1937) and by others after them proved fruitless and interest in the hormone gradually waned despite the great attraction of its possible therapeutic value in the treatment of peptic ulcer, which had been the main stimulus to their studies.

### CHOLECYSTOKININ

Ivy & Oldberg (1928) established by cross-circulation experiments in dogs that the effect of fat in the duodenum in causing contraction of the gall bladder involved a humoral mechanism. They made active extracts from porcine duodenal mucosa and distinguished the principle, which they named 'cholecystokinin', from secretin; but while their findings were generally accepted little further interest was taken in the hormone for many years.

### ENTEROCRININ

Nasset (1938) detected a small secretory response in denervated jejunal loops in conscious dogs after feeding and proposed the existence of a hormonal mechanism. He made extracts which had a similar action and named the active principle 'enterocrinin'.

### VILLIKININ

The observation that motor activity of the intestinal villi is stimulated by a meal led Kokas & Ludany (1934) to demonstrate that hydrochloric acid introduced into the duodenum of a dog increased villus activity in a jejunal loop temporarily transplanted into the neck of the animal. They made extracts of intestinal mucosa which stimulated villus motility and were considered to contain a hormone 'villikinin'.

### PANCREOZYMIN

The discovery of pancreozymin may be fairly regarded as the third major advance made in knowledge of the control of pancreatic secretion after the discovery of the effect of the vagus by Pavlov and of secretin by Bayliss and Starling. From the time of Pavlov there had accumulated a number of



observations on the stimulation of pancreatic enzyme secretion by foodstuffs, particularly protein and fat placed in the duodenum, which were not satisfactorily explained in terms of the reflex activity supposed to be involved; and during the many unsuccessful attempts to purify secretin there arose a controversy as to its action on pancreatic enzyme secretion. The preparation made by Mellanby did not, and he attributed enzyme secretion entirely to the action of the vagus. On the other hand the secretin made by American workers according to a different procedure undoubtedly did stimulate enzyme production. There the matter rested until Harper & Vass (1941), who were examining in anaesthetized cats the effect on pancreatic secretion of vagal and splanchnic stimulation and of introducing protein into the small intestine using a background flow of enzyme-poor juice produced by Mellanby's secretin extract, discovered that the stimulation of enzyme output produced by casein in the intestine persisted after section of the extrinsic nerves. They recognized the implications of this observation; and Harper & Raper (1943) found a potent stimulant of pancreatic enzyme secretion, which they named 'pancreozymin', in a side-fraction discarded during the preparation of secretin by Mellanby's method. They separated the principle from secretin and demonstrated that its action, unlike that of the vagus, was resistant to atropine in the anaesthetized animal. Later the elegant experiments of Wang & Grossman (1951) provided physiological evidence for the existence of a duodenal hormone stimulating pancreatic enzyme secretion using conscious dogs provided with a portion of the pancreas completely transplanted to the mammary region.

The chemical identity of pancreozymin remained unknown for more than twenty years after its discovery until Jorpes and Mutt in 1964, having completed their work on secretin, turned their attention to the cholecystokinin and pancreozymin activities present in their crude duodenal extract. During purification the two activities remained inseparable and there was eventually isolated a single basic peptide of thirty-three amino acids which proved to be a powerful stimulant both of gall bladder contraction and of pancreatic enzyme secretion (Jorpes, 1968). The same hormone had been discovered twice on the basis of what are now recognized to be two of its principal actions, and it has come to be generally agreed that it should be known as 'cholecystokinin' (CCK) after the action by which its existence was first recognized.

### *Gastric inhibitory polypeptide (GIP)*

Brown & Pederson (1970) noted that two partially purified preparations of CCK provided by Jorpes and Mutt had inhibitory effects on gastric acid secretion which were quantitatively different from their potency for gall

bladder contraction. The inhibitor was isolated by Brown, Mutt & Pederson (1970) and proved to be a peptide of forty-three amino acids (Brown & Dryburgh, 1971). GIP is a potent inhibitor of gastric acid secretion and motility; radioimmunoassay shows that it is released from the duodenum by feeding, particularly of glucose and fat. GIP has also been found to possess 'incretin' activity (Brown, Dryburgh, Ross & Dupré, 1975) and to stimulate intestinal secretion (Barbezat, 1973).

## THE PHYSIOLOGICAL ACTIONS OF THE GASTROINTESTINAL HORMONES

In all of the searches made for new gastrointestinal hormones after the discoveries of secretin and gastrin, there was a general assumption that the hormone sought had a single physiological action, that by which its existence had been first recognized. Although Bayliss and Starling (Starling, 1906) had noted that their secretin preparation also stimulated the flow of hepatic bile and had attributed this to the action of secretin (Fig. 2), and Edkins regarded gastrin as a stimulant of gastric pepsin as well as of acid, there was never seriously considered thereafter the likelihood that one hormone might have more than one physiological action. Neither was there considered the less obvious possibility that more than one hormone might prove to have the same physiological action. It came as something of a surprise when pure gastrin was shown to have several actions besides that of stimulating gastric acid secretion (Gregory & Tracy, 1964); it also stimulated the secretion of gastric pepsin and pancreatic enzyme, it contracted the gall bladder (weakly) and it stimulated gastrointestinal tone and motility. Subsequent studies with the pure natural or synthetic hormone extended this list to include several other actions, including the growth of gastric mucosa and the stimulation of insulin release.

When pure secretin became available the story was repeated; it not only stimulated the secretion of water and bicarbonate from the pancreas and from the liver (as Bayliss and Starling had supposed) but it had a number of other actions also; for instance it inhibited gastric secretion and motility, it stimulated pepsin secretion, the release of insulin, the secretion of Brunner's glands and lipolysis of fat cells, and it antagonized the trophic actions of gastrin. The isolation of CCK and elucidation of its structure established it as a member of the 'gastrin family', for the C-terminal tetrapeptide amide was identical with that of gastrin, already shown to be the minimal totipotent active fragment of the molecule; and as was expected the range of actions of CCK proved to be closely similar to that of gastrin, although certain differences in the structure of the C-terminal heptapeptide of CCK compared with gastrin conferred upon it



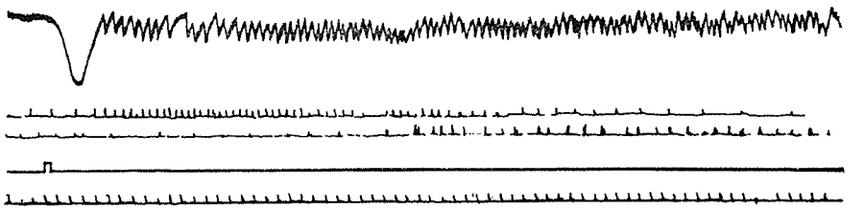

Fig. 2. Effect of intravenous injection of a bile-salt-free secretin extract on the flow of pancreatic juice and hepatic bile in an anaesthetized dog. Tracings from above downwards: (1) blood pressure; (2) drops of pancreatic juice; (3) drops of bile; (4) signal marking injection of secretin extract; (5) 10-sec intervals. (From Starling, 1906.)

the characteristic activity of a most powerful action upon the gall bladder. The resemblance of GIP to secretin identified it as a member of the 'secretin family'; it shared with secretin the power to inhibit gastric acid secretion and motility and to release insulin, and in addition it stimulated intestinal secretion.

As these multiple actions of pure gastrin, secretin, CCK and GIP were revealed in turn, it became increasingly clear that there were candidates among them for the physiological roles attributed to 'incretin', 'enterogastrone', and 'enterocrinin'. They could all be shown to cause insulin release in experiments *in vitro* or *in vivo*; secretin and GIP inhibited gastric secretion and motility, while GIP stimulated intestinal secretion. However, all these actions had been discovered and studied in the first place by the use of the pure peptides in doses which had no known relation to 'physiological limits'. It was obvious from the outset that some actions could be elicited at very low dosage rates, whereas others seemed to require much greater amounts for their demonstration; but what was quite uncertain was how the blood levels produced in such experiments compared with those which occurred in natural circumstances following a meal; which of the many actions found for each hormone could be regarded as of physiological significance? The advent of radioimmunoassay offered the prospect of a sensitive and specific method for determining the circulating levels of the hormones, and so promised an approach to this fundamental problem by (1) measuring the postprandial circulating level of a hormone and then (2) reproducing it in the same animal on another occasion by infusion of the pure hormone, with observation of the actions which might follow on the various target organs. As will be seen, this apparently simple procedure turns out to be far from straightforward in practice. Some information of this nature has appeared in respect of gastrin and GIP but the radioimmunoassays for secretin and CCK are not yet sufficiently refined to enable them to be used for this purpose.

For instance infusions of gastrin which produce increases in plasma levels considered to be within the physiological range have been shown to stimulate acid secretion and also to increase the plasma insulin response provoked by an intravenous glucose load – the 'incretin' effect (Rehfeld & Stadil, 1973). GIP inhibits gastrin-stimulated acid secretion when infused so as to produce circulatory increases which are within the range observed after feeding a meal. Infusion of GIP plus glucose (in man) increases the rise in plasma insulin observed after glucose alone; the plasma level of GIP is within the range observed after oral administration of glucose (Dupré, Ross, Watson & Brown, 1973).

Assuming for the moment that the radioimmunoassay used gives a true picture of the plasma hormone content, studies of this simple pattern clearly provide vital information; but there will remain uncertainty about those actions of a given hormone which may not be demonstrable by this means. The reasons for failure may include the following: (1) The response of the digestive tract to a meal involves a component of vagal activity as well as the effects of the various hormones and the action of a hormone may be dependent upon concurrent vagal activity. An obvious example of such dependency is that of gastrin on the oxyntic cell, which is greatly potentiated by concurrent vagal excitation. On the other hand it appears from present evidence that vagal excitation to the pancreas is not an important factor in the response of that organ to secretin and CCK. (2) A co-operative interaction at a given target organ may exist between the various hormones liberated by a meal so that giving only one of them in the amount observed postprandially has little or no effect on the target organ. A notable example of this is the interaction of secretin and CCK on pancreatic secretion. Acid in the duodenum is the only known effective releaser of secretin, but acidification of the duodenum to the same degree and extent as is observed following a normal meal produces a volume-response from the pancreas that is far less than that observed in the same animal after a normal meal. Clearly there is a missing factor in the response and this appears to be CCK, which is normally released at the same time as secretin by components such as fat and protein rather than by acid. It can be shown by infusion of the two hormones separately or together into a dog provided with a pancreatic fistula that the presence of CCK greatly potentiates the volume-response produced by secretin. Conversely, it can be similarly shown that secretin increases the stimulation of pancreatic enzyme secretion by CCK. It may thus be necessary to infuse two or more hormones so as to produce for each plasma levels which are within postprandial limits. There is, however, a more formidable problem to be faced that derives from the heterogeneity of the hormone measured by the radioimmunoassay. The nature of the problem and how it may be dealt with are well exemplified by gastrin (Gregory, 1974).



The hormone was first isolated from antral mucosa in the form of a peptide having seventeen amino acids (G17) which appeared to account for virtually all of the gastrin-like activity present in the tissue. The C-terminal tetrapeptide was the active region of the molecule; antibodies raised against conjugated G17 reacted with the C-terminal region and were made the basis of a radioimmunoassay for the hormone. However, in 1970 the use of such a radioimmunoassay in conjunction with the fractionation of plasma by molecular sieving on Sephadex columns showed that although material corresponding to G17 was present, the predominant amount of immunoreactivity corresponded to a larger form of the hormone ('Big' gastrin). On brief digestion with trypsin BG disappeared and its place was taken by an equivalent amount of G17, from which it was surmised that BG might consist of G17 covalently linked through an arginine or lysine residue (the points of attack of trypsin) to a further peptide chain. In 1972 there were isolated from porcine antral mucosa and from human gastrinomas peptides having thirty-four amino acids (G34) which corresponded in immunological and chromatographic behaviour to plasma BG; the C-terminal portion of the molecule was G17, joined by two lysine residues to a further peptide chain.

In antral tissue G34 accounts for no more than about 5 % of the total gastrin present, almost all of the remainder being G17. G34 predominates in plasma chiefly because it has a half-life several times longer than G17, but its potency for stimulation of gastric acid secretion (based on acid responses to equal plasma levels) is several times less than that of G17, and it is the latter which makes the major contribution to postprandial gastric acid responses. Clearly an accurate description of the relationship between postprandial acid secretion and plasma gastrin level measured by radioimmunoassay requires the measurement of both forms of the hormone simultaneously, and this cannot easily be done by the use of a single antibody, which is likely to react with both forms of the hormone. It can, however, be accomplished by the use of two antibodies, which show different reactivities to the two forms (Dockray & Taylor, 1976). The possible presence in the circulation of active forms of the hormone too small to react significantly with the antibodies used for radioimmunoassay cannot be excluded; but there is as yet no strong evidence that such exist.

So far as the presence in circulation of biologically inactive but immunologically reactive forms or fragments of the hormone is concerned there has already been discovered by the use of a double antibody assay, material which corresponds to the inactive N-terminal tridecapeptide of G17,

and it is possible that the inactive fragment which distinguishes G34 from G17 may also circulate; but it is not now difficult to take account of this because the availability of many fragments and forms of gastrin make it possible to characterize with a high degree of precision the reactivity of an antibody before it is used for radioimmunoassay.

For various reasons, the assays for the other hormones have not yet attained the status of that for gastrin in sensitivity, reliability and validation; and it is therefore not yet clear what may be the nature and degree of the heterogeneity of them in circulation, although there have been reports of more than one circulating form of all of them. In the tissue of origin (intestinal mucosa) there is only one report so far of heterogeneity; Jorpes and Mutt have isolated CCK-Variant, six residues longer at the N-terminus than CCK. From what has been said it seems clear that there is a long road to be travelled before the circulating active forms and amounts of the other hormones besides gastrin can be described precisely in terms of postprandial plasma levels and this knowledge applied to define the full range of their truly physiological actions.

## THE CANDIDATE HORMONES

Few would take serious issue today with the view that secretin, gastrin, cholecystokinin and gastric inhibitory peptide can be regarded on the basis of the available evidence as 'established' hormones. For each there is at least one physiological action by which their existence was first recognized and which has been shown to be humorally transmitted; they have each been isolated and chemically characterized, and the pure peptide has been shown to be capable of reproducing the physiological effect attributed to the hormone. Their location in the gastrointestinal mucosa is confined to those regions from which the effects attributed to them can be obtained by application of an appropriate stimulus associated with a meal. Thus, gastrin is confined to the pyloric and upper intestinal mucosa, from which regions the hormonal components of the 'gastric' and 'intestinal' phases of a meal response take their origin; the others are located in the duodenal and jejunal regions and similar physiological evidence associates their distribution with their hormonal role. Radioimmunoassay shows that after feeding, immunoreactivity corresponding to gastrin, GIP and CCK appears in the peripheral circulation; this demonstration has not yet been achieved for secretin, most probably owing to inadequate sensitivity of the assay in its present form. However, there is powerful indirect evidence that the hormone does appear in the peripheral circulation following an ordinary meal (Fig. 3) and it has been demonstrated even with present assays that immunoreactivity appears there following acidification of the



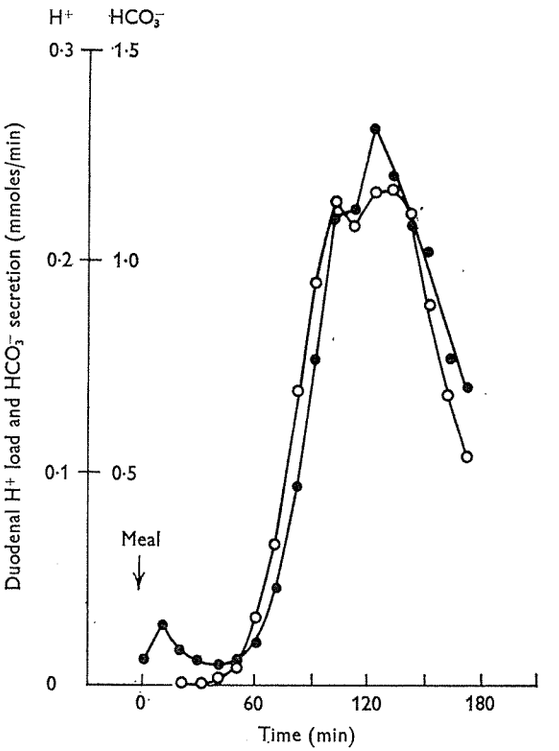


Fig. 3. Conscious pancreatic fistula dog; a test-meal is emptying into the duodenum. Pancreatic secretion (bicarbonate output (●)) runs closely parallel to the quantity of $H^+$ (○) entering from the stomach. (From Moore, Verine & Grossman, 1976.)

duodenum, albeit in somewhat greater extent than normally occurs after feeding.

The great and increasing interest in this field of gastrointestinal endocrinology during the past few years has brought forth a growing number of what have been aptly termed 'candidate hormones' (Grossman *et al.*, 1974; Grossman, 1975); they range from physiologically active pure peptides (some of them already well known from elsewhere in the body) whose possible hormonal role in the affairs of the gastrointestinal tract is uncertain or altogether problematical, to physiological actions of demonstrably hormonal character for which no corresponding mucosal principle has yet been identified. Some members of this group deserve brief discussion here, if only to indicate the many growing points in this area.

### *Vasoactive intestinal peptide (VIP)*

This peptide was isolated from porcine duodenal mucosa by Said & Mutt (1972) on the basis of its vasodilator and hypotensive activity; a similar peptide has since been isolated from avian duodenum (Nilsson, 1974). Structurally it belongs to the 'secretin' family and has several actions similar to those of the other members of the group, though it also has distinctive effects of its own, notably the vascular effects which led to its identification.

It inhibits acid secretion, and has a weak secretin-like effect on pancreatic secretion (the only member of the group to show this). Like secretin, it increases hepatic bile flow, and relaxes the gall bladder. It is a potent stimulant of intestinal secretion in dogs and of adenylate cyclase in rabbit ileal mucosa. Like glucagon, it stimulates both lipolysis and glycogenolysis; but an insulinotropic action appears not to have been demonstrated. What argues against a peripheral hormonal role for VIP are the facts that (1) it is inactivated in the liver and (2) it is widely distributed throughout the gastrointestinal tract from stomach to colon, and is also found in the central nervous system (Bryant *et al.*, 1976); it may thus have some local role, possibly as a neurotransmitter. A radioimmunoassay for VIP is in use, but is not yet sufficiently sensitive to show whether it appears in the peripheral circulation in normal circumstances, although elevated levels have been reported in hepatic cirrhosis, suggesting that it may be released into the portal blood. This peptide has been implicated in the causation of some clinical conditions of watery diarrhoea, notably the Vernier–Morrison syndrome, in which severe watery diarrhoea, hypokalaemia and achlorhydria is associated with a pancreatic tumour which apparently secretes large amounts of the peptide.

### *Enteroglucagon*

Glucagon-like biological activity was discovered in extracts of dog gastrointestinal mucosa by Sutherland & De Duve (1948) and glucagon-like immunological activity (GLI) was identified there by Unger *et al.* (1961). It was virtually confined to the mucosal layer and its distribution was wide, with high concentrations in the gastric fundus, jejunum and ileum. GLI can be differentiated from pancreatic glucagon by site-specific antibodies raised against the latter, and this has been made the basis of a radioimmunoassay for it. Using such an assay, attempts have been made at isolation but without complete success so far; there is evidence that part of the material is identical with pancreatic glucagon and is distributed differently from the remainder, which is chromatographically highly heterogeneous. By radioimmunoassay, GLI has been shown to be released into the circulation after feeding, particularly of carbohydrate and fat, and its most firmly established activity at present appears to be glycogenolysis.



### *Pancreatic polypeptide (PP)*

This candidate for hormonal status is of particular interest because its discovery exemplifies a new turn in the searches for further gastrointestinal hormones. With so many gastrointestinal hormones now known to influence so many of the gastrointestinal functions associated with digestion, it obviously becomes increasingly difficult to identify a new hormone on the basis of its physiological effect; but instances are now appearing in which, as Grossman (1975) has put it, the traditional course of events has been stood on its head; a peptide is isolated first and its possible hormonal role examined by radioimmunoassay afterwards.

During the purification of glucagon and insulin, there were isolated from pancreatic extracts of cow, hog, sheep, man and chicken, new homologous peptides (PP) containing thirty-six amino acids (Lin & Chance, 1974; Kimmel, Hayden & Pollock, 1975). These have been shown to have a wide variety of effects on gastrointestinal secretory and motor functions in experimental animals. Cells apparently containing the peptide have been demonstrated by immunofluorescence not only in the pancreatic islets but also in small groups between the acinar cells; and following a meal in man there is a prompt release of PP immunoreactivity into the circulation lasting several hours (Adrian *et al.*, 1976). This release appears to be dependent upon vagal excitation, since it is greatly decreased in vagotomized patients (Schwartz *et al.*, 1976). Reproducing the postprandial plasma levels by infusion of the pure peptide should throw some light on the problem of its possible hormonal status; the functional significance of PP is at present totally unknown.

## THE HORMONE RECEPTORS

At the heart of that great afternoon's work in 1902, when Bayliss and Starling began it all with their discovery of secretin, was their recognition of the 'messenger' role of hormones, with its implication of the 'recognition' of the circulating hormone molecule by a specific structure, the 'receptor' possessed by the 'target' cell. It has become a fundamental aim of endocrinology to understand at the molecular level the nature of the interaction which takes place between the hormone and its receptor; and for its full achievement this requires ultimately the isolation and physicochemical characterization of the receptor 'molecule', using that term to describe the complex involved in the translation of 'recognition' into cellular response. The primary event in recognition is the

reversible binding of the hormone molecule to the receptor site, which in the case of peptide hormones is generally agreed to be located on the cell surface. In recent years, this process has been increasingly studied in several favourable situations, where two essential prerequisites can be satisfied. These are (1) radiolabelling of the hormone without loss of physiological activity, and (2) the preparation of viable populations of target cells, or of their plasma membranes (Cuatrecasas, 1974). For instance, the reactions of glucagon and insulin with their receptors on liver or fat-cell membranes have been studied by Rodbell, Birnbaumer, Pohl & Sundby (1971), insulin and growth hormone receptors on cultured human lymphocytes have been examined by Gavin, Gorden, Roth, Archer & Buell (1973) and Lesniak, Roth, Gorden & Gavin (1973), and the interactions of glucagon, enteroglucagon, VIP, and secretin at their receptors on liver and fat-cell membranes have been described by Bataille, Freychet & Rosselin (1974).

In a few instances, highly purified preparations have been made of hormone-binding macromolecules which are believed to represent the receptor. Thus, Cuatrecasas (1972) purified by procedures involving affinity chromatography the insulin receptor of liver-cell membranes to a point which was considered to approach theoretical purity; this involved a concentration of nearly 500000-fold, so minute was the amount of receptor in the starting material.

Such fundamental approaches to the problem of the complex hormone–receptor interactions at the major gastrointestinal target cells, e.g. the oxyntic and pancreatic acinar cells, would be of the greatest value; and this area of study will no doubt develop rapidly as the present problems associated with hormone radiolabelling and preparation of viable homogeneous cell populations are surmounted. Two studies of great future promise have recently been reported: (1) Amsterdam & Jamieson (1972) succeeded in isolating guinea-pig pancreatic acinar cells in a state of excellent viability; they were capable of incorporating radiolabelled amino acids into enzyme protein and of releasing this in response to secretagogues such as carbaminoycholine, CCK or caerulein added to the incubation medium. This preparation was used by Klaeveman, Conlon & Gardner (1975) to obtain plasma membranes, which they used to study the interactions at their receptor sites of CCK-octapeptide, VIP, secretin, gastrin and glucagon, as indicated by the changes in activity of membrane-bound adenylate cyclase, which mediates the action of many hormones. As the authors recognized, it would have been ideal to have examined the interactions of these hormones directly, by studying the binding of them in a radiolabelled form, but they were unable at that time to achieve labelling without inactivation. Nevertheless, it was shown for instance that the receptor for CCK-octapeptide, with which gastrin also interacted, was



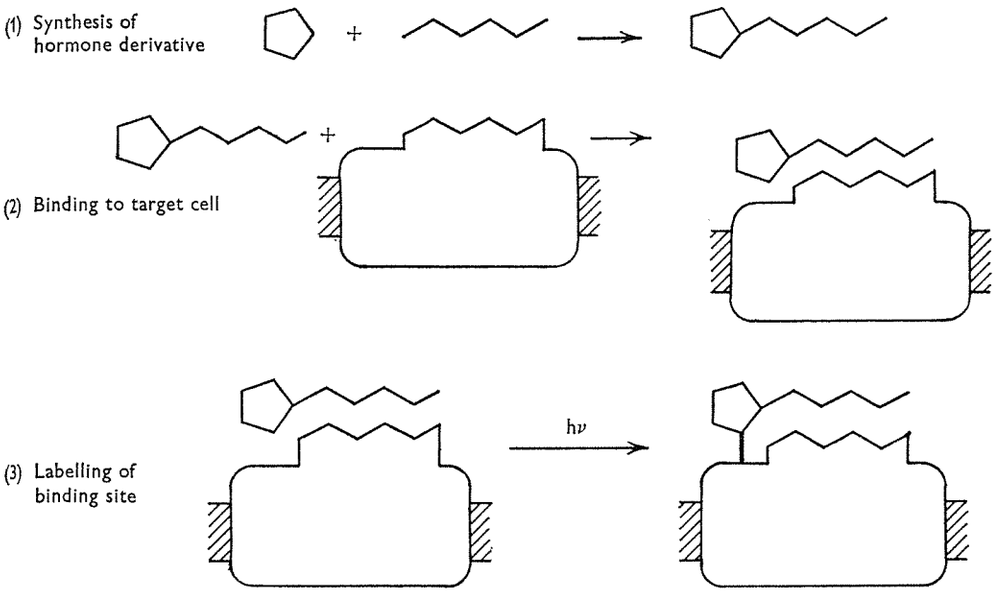


Fig. 4. Photoaffinity labelling of a peptide hormone-binding site: (1) a peptide hormone derivative is prepared which upon photoactivation will covalently bond to a protein. (2) The hormone derivative interacts with its receptor site on the cell plasma membrane. (3) Photolysis results in the formation of a covalent bond between the hormone derivative and protein in the receptor site. (From Galardy & Jamieson, 1975.)

functionally distinct from the receptor with which VIP and secretin interacted. (2) A novel and potentially fruitful approach to the problem of characterizing morphologically and functionally, and perhaps of isolating, the receptor sites for gastrointestinal hormones, is that of photoaffinity labelling.

A peptide hormone derivative is prepared which retains its physiological activity, but which upon photoactivation will covalently bond to protein. The hormone derivative is then allowed to interact with, and so bind to, its receptor site by addition in the dark to a preparation of viable target cells or their plasma membranes. Finally, exposure of the system to light results in photolysis of the hormone derivative with the formation of a covalent bond between the hormone derivative and a receptor site protein (Fig. 4). Galardy & Jamieson (1975) prepared a photoactivatable derivative of pentagastrin (an active analogue of the C-terminal pentapeptide amide of gastrin), namely 2-nitro-5-azidobenzoyl pentagastrin, and added this to a preparation of surviving pig pancreatic lobules (small clusters of acini) which was capable of discharging radiolabelled secretory protein in response to stimulation by CCK or pentagastrin. Incubation of

the lobules in the dark with unphotolysed (or with previously photolysed) pentagastrin derivative gave maximal secretory responses which were abolished by washing the lobules. Exposure of the lobules to light in the presence of photoactivatable pentagastrin derivative resulted in irreversible maximal stimulation of secretion; the response could not be abolished by extensive washing and was blocked only by metabolic inhibition, indicating that the receptor site for pentagastrin on the acinar cells had been successfully labelled.

## CONCLUSION

Endocrinology as we know it today began nearly seventy-five years ago with the discovery of secretin and recognition of the messenger function of hormones; but the gastrointestinal system did not share in the great advances which rapidly followed in other branches of the subject. In retrospect, this can be seen to have been largely due to two difficulties: (1) The cells of origin of the gastrointestinal hormones are not gathered into discrete aggregations but are widely dispersed among the exocrine glands of the stomach and small intestine; this made it impossible to apply the classical approach of studying an endocrine function by removing the gland of origin, and it also made more difficult the attempts to identify an endocrine principle by extraction of it from its site of origin, because of the great amounts of extraneous material present. (2) The hormones themselves proved to be peptides of small or moderate size, present in low concentration, and of such nature as to be easily lost or inactivated by the extraction procedures of the time. These problems eventually found their solutions; and since 1962 the isolation and identification of the major gastrointestinal hormones, together with the application of radioimmunoassay which this made possible, has resulted in a remarkable advance of knowledge in every aspect of this field of study. It has been rightly said (A. G. E. Pearse) that the gastrointestinal tract is proving to be the largest and most complex endocrine gland in the body; and there would seem to be a certain justice in this outcome to the many barren years, since, after all, it was there that it all began.

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