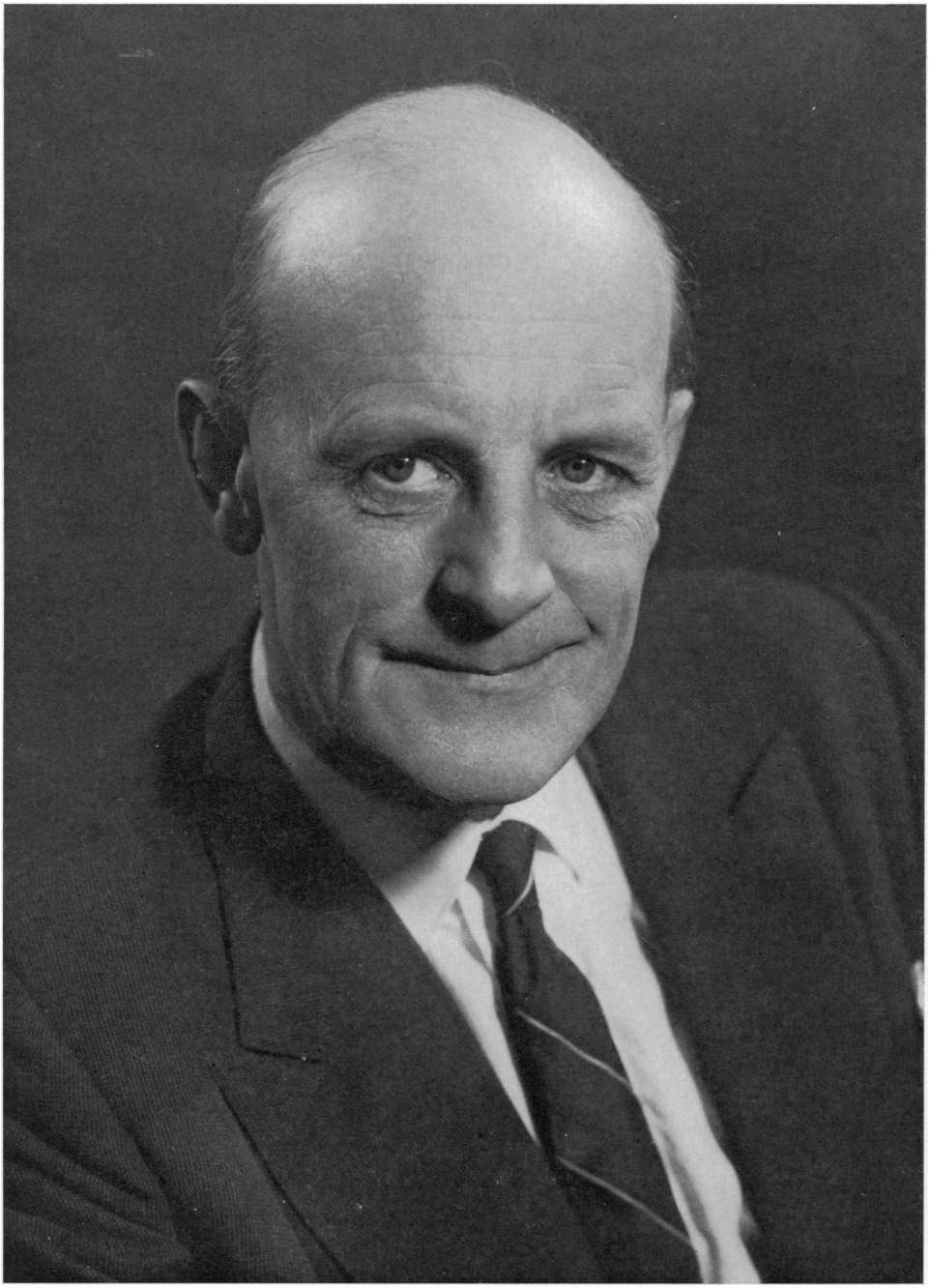

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THE BAYLISS–STARLING LECTURE 1973*

THE GASTROINTESTINAL HORMONES: A REVIEW OF RECENT ADVANCES

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For any physiologist whose research is concerned with the gastrointestinal hormones, there could hardly be a greater compliment from his peers than to be invited to give the Bayliss–Starling Lecture in University College, London, which was for so long the academic home of those two great men. This is an honour of which I am most deeply appreciative, for I was first an undergraduate student here, next Bayliss–Starling Memorial Scholar, and then Sharpey Scholar to Sir Charles Lovatt Evans; and it was he who suggested to me that I should go to Northwestern University, Chicago and learn about gastrointestinal physiology from Andrew Ivy, then at the height of his powers in this field of research.

I could not know personally either Bayliss or Starling, but in an odd way it was Bayliss who first excited my interest in physiology; for while in the sixth form at school I found in the local public library his famous book *Principles of General Physiology*. It was the 1924 edition, the revision of which A. V. Hill and other friends of Bayliss had completed for him during his last illness. I spent most of a long vacation reading it, fascinated by its unique style and by its revelation to me of the way in which physics and chemistry could be used in the study of biological problems. I determined to become a physiologist – to the consternation of my teachers, who confidently predicted that I would soon find myself on the bread-line if I persisted in so unheard-of an ambition!

Bayliss's self-effacing modesty is perhaps nowhere better revealed in his writings than by his account of the discovery of secretin (Bayliss & Starling, 1902*a*, *b*); I quote from the first edition of the book (Bayliss, 1915) which later became for me a treasured possession. He says (p. 344), 'It was shown by Pavlov and his fellow workers that the presence of various substances in the duodenum, especially acids, causes pancreatic secretion to be poured in. This excitation of the pancreas was looked upon as a reflex through the nervous system until Bayliss and Starling, in investigating the local nervous reflexes connected with the alimentary canal, found that it was still produced by acid in the duodenum after all accessible nervous communications had been divided. This fact suggested that some

* Given at University College London on 23 March 1973

chemical mechanism was at work, set going by the acid. The injection of acid into the blood current has no effect, as would be expected, so that some substance must be produced by the action of the acid on the mucous membrane of the intestine, which substance then diffuses into the blood and, arriving at the pancreas, excites it to action. The next step was to scrape off the mucous membrane and rub it up with sand and dilute hydrochloric acid. After neutralization and filtration, this extract was injected into a vein and we were naturally delighted to find that a copious flow of pancreatic juice was the result.' There is nothing in this account to indicate to the uninitiated reader that these experiments – the work of a single afternoon (Martin, 1927) – and their dramatic conclusion, constituted something of a milestone in the history of physiological ideas. They opened a new era in gastrointestinal physiology and began the study of endocrinology as we know it today. For the first time, a hormonal mechanism in action, integrating the functions of duodenum and pancreas, had been demonstrated in a simple laboratory experiment – easily repeated and utterly convincing.

Of the word 'hormone' Bayliss says (p. 706), 'When we came across the mode by which the pancreas is excited to activity, it became obvious to Starling and myself that the chemical agent concerned is a member of a class of substances of which others were previously known. The group... is characterized 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 emphasize their nature as messengers. Finally, Mr W. B. Hardy proposed the name '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.' The word 'hormone' was introduced by Starling in the first of his Croonian Lectures to the Royal College of Physicians (Starling, 1905); and in the third Lecture, he referred to a recent discovery of a second such mechanism in the digestive tract, concerned with the regulation of gastric secretion.

A few weeks previous to Starling's lecture John Sydney Edkins, formerly and briefly a demonstrator in my own department under Sherrington and by that time lecturer in physiology in St Bartholomew's Medical College, had presented before the Royal Society (Edkins, 1905) a brief communication (Pl. 1) in which he reported that simple aqueous extracts of gastric pyloric mucosa – but not those of the fundic mucosa – stimulated

gastric secretion when injected intravenously into anaesthetized cats. By analogy with the secretin mechanism Edkins suggested that the active principle, which he named 'gastrin', was absorbed into the circulation during gastric digestion and continued to excite the fundic glands after the initial vagal reflex aroused by eating had come to an end. These simple experiments, later described in full (Edkins, 1906), initiated a controversy as to the existence of a gastric antral hormone which was to last for more than forty years.

It was discovered by those who sought to confirm and extend Edkins's observations that an apparently identical gastric secretagogue was widely distributed in the body tissues; and before long it seemed clear that 'gastrin' could not be included in the class of specific 'chemical messengers' defined by Bayliss and Starling. Edkins might be said to have had the unhappy distinction of being the first known victim of that ubiquitous enigma histamine, years before its existence in the body (Barger & Dale, 1910; Dale & Laidlaw, 1910) and its powerful effect on gastric acid secretion (Popielski, 1919) was recognized; for his pyloric extracts contained both histamine and gastrin, while his fundic extracts contained histamine but no gastrin. His manner of testing them – rapid intravenous injection in the anaesthetized cat – happened to distinguish between the two, for in such circumstances gastrin stimulates gastric secretion but histamine does not; but in the experiments of others, made for the most part on conscious dogs, only the histamine was effective and secretion was obtained whatever tissue was used for extraction.

Edkins sought to defend his hypothesis in some further experiments, and in these he was joined by a young graduate Miss May Tweedy (Edkins & Tweedy, 1909); she was in later life to become Lady May Mellanby. The experiments did not succeed in their objective, but for Edkins there was nevertheless a happy outcome, for through his acquaintance with May he came to know her equally charming sister Nora, who became Mrs Edkins and eventually succeeded her husband in the Chair of Physiology at Bedford College, London. Some belief in the existence of an antral hormone lingered on, but apparently well conceived physiological tests of the idea (Ivy & Whitlow, 1922; Priestley & Mann, 1932) provided no support for it; and attempts to identify in pyloric mucosal extracts a stimulant of gastric secretion other than histamine were unsuccessful (Sacks, Ivy, Burgess & Vandolah, 1932; Gavin, McHenry & Wilson, 1933). Gastrin was consigned to the limbo of unsubstantiated hypotheses.

Secretin and related duodenal peptides

Meanwhile secretin had gone on from strength to strength, after an initial period of doubt as to its physiological specificity. Bayliss and

Starling's original intestinal extract lowered the blood pressure as well as causing pancreatic secretion and it was argued by others that extracts of many tissues, which also contained 'vasodilatin' as it was termed, would cause a flow of pancreatic juice. The objection was disposed of by the preparation of potent, vasodilator-free secretin extracts, for which only the small intestinal mucosa was an effective starting material and it was eventually realized that a major component of 'vasodilatin' was histamine, which is capable of causing a small flow of pancreatic juice. Secretin was found in the small intestinal mucosa of all of the many vertebrate species in which it was sought; the physiological properties of the hormone were

TABLE 1. The amino acid sequences of the porcine hormones VIP, secretin, glucagon and GIP (only twenty-nine residues of GIP are shown) (Bodanszky *et al.* 1973)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
VIP	His	Ser	Asp	Ala	Val	Phe	Thr	Asp	Asn	Tyr	Thr	Arg	Leu	Arg
Secretin	His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Glu	Leu	Ser	Arg	Leu	Arg
Glucagon	His	Ser	Gln	Gly	Thr	Phe	Thr	Ser	Asp	Tyr	Thr	Lys	Tyr	Leu
GIP	Tyr	Ala	Glu	Gly	Thr	Phe	Ile	Ser	Asp	Tyr	Ser	Ile	Ala	Met

	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
VIP	Lys	Gln	Met	Ala	Val	Lys	Lys	Tyr	Leu	Asn	Ser	Ile	Leu	Asn	NH_2
Secretin	Asp	Ser	Ala	Arg	Leu	Gln	Arg	Leu	Leu	Gln	Gly	Leu	Val	NH_2	
Glucagon	Asp	Ser	Arg	Arg	Ala	Gln	Asp	Phe	Val	Gln	Trp	Leu	Met	Asp	Thr
GIP	Asp	Lys	Ile	Arg	Gln	Asp	Phe	Val	Asn	Trp	Leu	Ala	Gln	Gln	

extensively studied and its importance for the stimulation of pancreatic water and bicarbonate and thereby the regulation of duodenal acidity, was established (Grossman, 1958). However, secretin itself successfully eluded its many pursuers; early hopes that it might prove to be a simple base like adrenaline, which had been isolated and identified chemically by Takamine (1901) were soon dashed. A claim to have isolated secretin (Greengard & Ivy, 1938) was followed by disillusionment as much more potent preparations were achieved by others; and with increasing purity came problems of instability and disappearance of activity. Slowly the formidable nature of the task became apparent. It was in fact far beyond the technical resources of the time, and was only to be accomplished with the advent of such sophisticated techniques as Sephadex gel filtration, ion exchange chromatography, electrophoresis and counter-current distribution, together with the application of great and skilful perseverance by those concerned.

The distinguished Swedish chemists Erik Jorpes* and Viktor Mutt began their work on secretin in 1952. Ten years later (Jorpes, Mutt,

* Died 10 July 1973.

Magnusson & Steele, 1962) they announced the isolation of secretin from hog duodenal mucosa in the form of a strongly basic peptide having twenty-seven amino acid residues (Table 1). The heroic scale of their efforts is well illustrated by the fact that in order to obtain 1 mg of pure secretin it is necessary to commence by extracting one thousand pieces of cooked hog duodenum each one metre long (Mutt & Jorpes, 1971). The total synthesis of secretin, identical in potency with the natural product, was accomplished by Bodanszky, Ondetti, Levine, Narayanan, von Saltza, Sheehan, Williams & Sabo (1966).

TABLE 2. Actions of secretin

I. *The following are produced by low doses*

1. Stimulates secretion of water and electrolytes from pancreas
2. Stimulates secretion of water and electrolytes by liver (bile)
3. Inhibits lower oesophageal (cardiac) sphincter
4. Inhibits gastric emptying
5. Inhibits gastric acid secretion
6. Stimulates gastric pepsin secretion
7. Decreases duodenal motility

II. *The following are produced by high doses*

1. Releases insulin
2. Stimulates Brunner's glands (duodenum) in cats and dogs
3. Stimulates renal excretion of water, Na and K in dogs
4. Stimulates cardiac output and splanchnic blood flow in cats and dogs
5. Stimulates lipolysis in fat cells

The availability of the hormone in a pure state made possible for the first time meaningful studies of its physiological properties; and these, like those of gastrin, have proved to be of remarkable range (Tables 2 and 4). Besides the action on pancreatic secretion, by which its existence was discovered and which until its isolation was generally assumed to be its only effect, the hormone not only stimulates hepatic bile-flow and gastric pepsin secretion, inhibits gastrointestinal motility and gastric acid secretion, but also has effects on insulin release, fat cell lipolysis, renal function and the cardiovascular system (Hubel, 1972; Grossman, 1973*a*). As with gastrin, some of the actions of secretin vary greatly in magnitude in different species or experimental conditions. Again, a distinction can be drawn between those actions which are produced by blood concentrations of the hormone thought to lie within physiological limits and those which are only evoked by much higher levels; this presumably reflects the existence of more than one order of receptor sites on the target cells, as have been identified in respect of other hormones.

Secretin has not been isolated from any mammalian source other than hog, so nothing is known of possible species differences in its structure; but pancreatic glucagon, which closely resembles secretin (Table 1) is identical in man, hog and cow (Bromer, Boucher & Koffenberger, 1971) so it may be that secretin also is invariant.

Jorpes & Mutt's crude duodenal extract has proved to be an immensely valuable source of other biologically active substances; and there has emerged a remarkable picture of a 'secretin family' of peptides, closely related in structure and having many physiological actions in common. The other members so far identified are pancreatic glucagon, 'gastric inhibitory peptide' (GIP) and 'vasoactive intestinal peptide' (VIP) (Table 1). GIP was extracted from duodenal mucosa by Brown, Pederson, Jorpes & Mutt (1969) and isolated by Brown, Mutt & Pederson (1970). It has not yet been synthesized and its properties are not fully documented, but it is known that it inhibits gastric acid secretion (Pederson & Brown, 1972) and stimulates intestinal secretion (Barbezat, 1973); it is considerably larger than other members of the family (Brown, 1971). VIP was discovered and isolated by Said & Mutt (1970, 1972) and synthesized by Bodanszky, Klausner & Said (1973). It is a weak stimulant of pancreatic volume-flow but not enzyme secretion; its other actions include inhibition of gastric acid secretion, stimulation of intestinal secretion, vasodilatation, increase of cardiac output, enhancement of cardiac contractility, glycogenolysis, and relaxation of tracheal, gastric and gallbladder musculature. Its 'messenger' role in normal circumstances is doubtful, since it is largely inactivated in passing through the lungs (Said, 1973). Another member of the family seems likely to be 'gut glucagon' the hyperglycaemic, glycogenolytic agent in intestinal extracts which cross-reacts with antibodies to pancreatic glucagon (Unger, 1973).

Information as to structure-function relations for members of the secretin family is fragmentary, but it appears that no partial sequence of secretin has significant biological activity; even removal of the N-terminal histidine residue virtually inactivates the molecule (Ondetti, Sheehan & Bodanszky, 1968); the same is true of glucagon. C-terminal sequences of VIP show some activity (Bodanszky *et al.* 1973) and a C-terminal fragment of GIP is said to inhibit gastric secretion (Brown & Pederson, 1970).

Gastrin

In 1938, gastrin was rediscovered. Simon Komarov, working in Babkin's laboratory in Montreal, realized that if gastrin did, after all, really exist and was a protein, it would have been thrown away at the outset by all those who had commenced their study of antral extracts by deproteinizing them; histamine would remain in the supernatant to be duly identified as

the only gastric secretagogue present. He treated an aqueous extract of antral mucosa with trichloracetic acid and found that the histamine-free precipitate stimulated gastric acid secretion when injected intravenously into anaesthetized cats – Edkins' method of testing his extract. Komarov could find no activity in fundic mucosal extracts; a trace was present in the upper small intestine (Komarov, 1938, 1942*a*, *b*). However, although the distribution of the activity was consistent with that to be expected for the postulated hormone, the extract had only a slight effect when injected into conscious dogs, and in the anaesthetized cat its action was totally unaffected by atropine, which was well known to inhibit the gastric secretory response to a meal in conscious dogs and man. For these reasons it was for a long time doubted whether 'Komarov's gastrin' as it was called represented a preparation of the antral hormone. It is now known (1) that atropine does indeed fail to influence a response to gastrin in anaesthetized cats (Blair, Harper, Lake & Reed, 1961) although it is highly effective in conscious dogs (Gregory & Tracy, 1961), (2) that crude histamine-free gastrin extracts may be poorly absorbed if given subcutaneously or even intramuscularly, and (3) in the conscious dog (and only in this species) a rapid intravenous injection of gastrin *inhibits* a gastric secretory response, even to gastrin itself (Gillespie & Grossman, 1963; Gregory & Tracy, 1964).

Following Komarov's work, several attempts were made to purify his preparation or to extract the hormone by other means, but with little success (Munch-Peterson, Ronnow & Uvnäs, 1944; Uvnäs, 1943*a*, *b*, 1945*a*, *b*; Harper, 1946; Jorpes, Jalling & Mutt, 1952). Eventually conclusive proof of the existence of the antral hormone was provided by the physiological experiments of Grossman, Robertson & Ivy (1948) and Dragstedt, Woodward, Oberhelman, Storer & Smith (1951); the important influence of cholinergic activity on the release and action of the hormone, first recognized by Uvnäs (1942), became established (Gregory, 1970) and the inhibitory effect of intragastric acidity on the release of antral gastrin was discovered (Oberhelman, Woodward, Zubiran & Dragstedt, 1952).

By 1959, when Hilda Tracy and I began to work on gastrin, there existed the unusual situation that much was known about the physiology of gastrin but nothing was known of its chemical nature and there was not available even a crude preparation which was reliably active in conscious dogs and which might serve as the basis for further physiological and biochemical studies. It was fortunate for our continued interest in the problem that after an unsuccessful trial of Komarov's method (Gregory, 1970) we hit almost immediately upon a new method of preparing from hog antral mucosa a histamine-free extract which was fully active on subcutaneous injection in conscious dogs (Gregory & Tracy, 1959); further

refinement of it gave a product which was active without significant side-effects in a human subject (Gregory & Tracy, 1960, 1961). However, the method proved to be too laborious and expensive for the large-scale efforts at isolation we soon had in mind; using the product as a model, we completely redesigned the extraction along more sophisticated lines and by this means isolated the first gastrin peptide in April 1962. A further change in the method led to the isolation of a second almost identical peptide in December of the same year – on Christmas morning, to be exact. Our 'gastrin factory' now went into full production to provide ample material for structural studies; and during the next 2 years we were to process more than 50,000 hog antrums – a modest effort, however, compared with that of Jorpes & Mutt! The gastrin we did not need for our own purposes we distributed to the many friends who soon were anxious to use it in their own experiments.

TABLE 3. The amino acid composition of the little gastrins (heptadecapeptides) isolated from gastric antral mucosa. Gastrins type I are shown. In each species a gastrin type II also occurs (tyrosine-SO_3H)

Man

Pyr-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2

(Tyr: (SO_3H))

Hog	-Met-			
Cat			-Ala-	
Dog	-Met-	-Ala-		
Cow	-Val-		-Ala-	
Sheep	-Val-		-Ala-	

Structural analysis of the peptides, made by Professor G. W. Kenner and his colleagues in the Department of Organic Chemistry of Liverpool University, revealed that they were heptadecapeptide amides of identical amino acid sequence; they differed only in that in one (Type II) the single tyrosine residue present was sulphated while in the other (Type I) it was not. Subsequently, Tracy and I isolated similar pairs of peptides from the antral mucosa of several other mammalian species, including man; their structures were determined, and total synthesis accomplished, by Kenner and his co-workers (Gregory, 1970). The structures of these peptides (Table 3) showed that there were trivial substitutions in the body of the molecule, referable to single base changes in the codon triplets, but the C-terminal sequence remained unchanged.

Our original survey (Gregory & Tracy, 1963, 1964) of the properties of the natural porcine peptides revealed that they possessed a remarkable range of actions on gastrointestinal structures (as was to become evident subsequently for secretin and the other gastrointestinal hormones). Most

of the effects we studied had already been noted by others using crude antral extracts but had been attributed to the presence of other biologically active substances besides gastrin, such as secretin, pancreozymin and substance P. The list of actions of gastrin has been greatly extended by the studies of many others (Thompson, 1969; Grossman, 1970*a*) using at first our natural material and later synthetic preparations provided by Kenner or made available commercially by Imperial Chemical Industries Ltd (Table 4). In man, the dose of the heptadecapeptide amide required for a half-maximal gastric acid secretory response is about 1 ng kg^{-1} min^{-1}

TABLE 4. Actions of gastrin

I. *The following are produced by low doses*

1. Stimulates secretion of water and electrolyte by stomach, pancreas, liver and Brunner's glands
2. Inhibits absorption of water and electrolytes from ileum
3. Stimulates secretion of enzymes from stomach and pancreas
4. Stimulates the lower oesophageal (cardiac) sphincter and stomach muscle
5. Inhibits tone of the sphincter of Oddi
6. Increases gastric mucosal blood-flow
7. Releases histamine and induces histidine decarboxylase activity in gastric mucosa of rats
8. Stimulates incorporation of amino acids into protein in gastric mucosa

II. *The following are produced by high doses*

1. Stimulates the growth of gastric mucosa
2. Stimulates insulin release
3. Inhibits gastric secretion however excited (dogs only)
4. Stimulates smooth muscle of gut, gall-bladder, uterus

intravenously, which makes it about 1500 times more potent than histamine on a molar basis; it is also a stimulant of pancreatic enzyme secretion nearly as powerful as the duodenal hormone CCK-PZ (see later). Among the many other effects which have been described, perhaps the most interesting are those of 'trophic' character; growth of gastric mucosa and pancreas is stimulated by daily doses and cell division and protein turnover increased. These actions may be implicated in conditions such as the Zollinger–Ellison syndrome (see later) and duodenal ulcer, where an increase in the mucosal mass and parietal cell population is known to occur.

During the synthesis of porcine gastrin Tracy and I were afforded the opportunity of studying the physiological properties of several synthetic fragments of the molecule (Tracy & Gregory, 1964). We discovered that the C-terminal tetrapeptide amide Trp-Met-Asp-Phe-NH_2, common to all gastrins, possessed the full range of physiological actions of the total

molecule, although its potency was somewhat lower; the remainder of the molecule had no intrinsic activity, and the C-terminal tripeptide amide was apparently inactive (Table 5). Lin & Southard (1973) have recently shown that the latter has slight activity (about 1/30000 of the total molecule) and that even the dipeptide amide Asp-Phe-NH_2 slightly stimulates gastroduodenal motility. Tracy and I also observed at this time that removal of the C-terminal amide group of the tetrapeptide amide, or oxidation of the methionine residue, virtually inactivated the molecule; and it was suggested (Gregory & Tracy, 1966) that one or other of these changes would prove to be the mode of inactivation of the circulating hormone. Subsequent studies indicate that circulating gastrin is chiefly disposed of in kidney (Clendinnen, Davidson, Reeder, Jackson & Thompson, 1971) and intestinal mucosa (Temperley, Stagg & Wyllie, 1971) by deamidation (Walsh & Laster, 1973).

TABLE 5. Structure-function relations of human little gastrin (heptadecapaptide)

Type I

Sequence	Activity
Pyr-Gly-Pro-Trp-Leu-$(Glu)_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2	1
Pyr-Gly-Pro-Trp-Leu-$(Glu)_5$-Ala-Tyr-Gly	Nil
Trp-Met-Asp-Phe-NH_2	1/10
Met-Asp-Phe-NH_2	1/30,000
Asp-Phe-NH_2	Nil
Trp-Met-Asp-Phe-NH_2	Nil
↓ o	
Trp-Met-Asp-Phe-OH	Nil

A study of the physiological activities of thirty-three analogues, chiefly of the tetrapeptide amide (Morley, Tracy & Gregory, 1965) defined structure-function requirements in this simple molecule. Among these compounds was *t*-BOC-β-Ala-Trp-Met-Asp-Phe-NH_2 which is now marketed by ICI as 'Pentagastrin' and has replaced histamine in clinical tests of gastric secretory function. Morley (1968) later examined more than 500 analogues of the tetrapeptide and further defined the structural conditions for gastric secretory activity.

Cholecystokinin–pancreozymin

It is appropriate at this point to introduce another duodenal hormone, which belongs to the 'gastrin' rather than the 'secretin' family. Ivy & Oldberg (1928) discovered by physiological experiment the presence in the duodenal mucosa of a hormone liberated by food (especially fat) which caused contraction and emptying of the gallbladder; they named it

'cholecystokinin' (CCK) and distinguished it from secretin. As the result of the work of Harper & Vass (1941), who had shown that in anaesthetized cats peptone in the duodenum still stimulated pancreatic enzyme secretion after all nervous connexions had been divided, Harper & Raper (1943) discovered a second duodenal hormone besides secretin for the control of pancreatic secretion. It stimulated the output of pancreatic enzyme in a manner closely similar to that of vagal excitation, which until then had been thought to be the only mechanism for the release of pancreatic enzyme (Mellanby, 1925). They named it 'pancreozymin' (PZ); it was separated from secretin and partially purified (Crick, Harper & Raper, 1949). The presence in the preparation of what was described as a 'cholecystokinetic agent' enabled it to be used clinically to produce evacuation of the gallbladder (Duncan, Harper, Howat, Oleesky, Varley & Scott, 1953).

Jorpes & Mutt, having completed the isolation of secretin, proceeded to purify the CCK and PZ activities also present in their crude porcine duodenal extract. They had previously made considerable progress along this line, and had commented (Jorpes & Mutt, 1961): 'It is quite remarkable how closely the two hormone activities follow each other through all these steps of purification...the strength of the pancreozymin in the meantime having increased approximately 600-fold over that of the original standard preparation of Crick, Harper & Raper.' This proved to be still true even when a 10–20,000-fold enrichment was achieved and an essentially pure product obtained (Jorpes & Mutt, 1966).

Mutt & Jorpes (1967), examining the structure of what they henceforth termed cholecystokinin-pancreozymin (CCK-PZ), reported that the probable C-terminal sequence was Gly-Trp-Met-Asp-Phe-NH_2, i.e. identical with gastrin; and Jorpes (1968) reviewing their isolation of secretin and CCK-PZ provided incontrovertible chemical evidence that both cholecystokinin and pancreozymin activities were exerted by a single basic peptide having thirty-three amino acid residues, the C-terminal heptapeptide sequence of which was almost identical with that of gastrin II. In both, the single tyrosine residue was sulphated, but in CCK-PZ a methionine residue was interposed between tyrosine and glycine (Tables 6 and 7). These two structural features are responsible for the characteristically powerful effect of CCK-PZ on the gallbladder; removal of the sulphate group or alteration of the spacing between tyrosine and glycine (Ondetti, Rubin, Engel, Plůsčec & Sheehan, 1970) reduces CCK activity to a level comparable with that of the gastrins, either of which (sulphated or unsulphated) has only a very weak effect on the gallbladder (Gregory & Tracy, 1964). This structure-function relation does not seem to hold for PZ activity, for both gastrins have equal activity, which is almost as great as that of CCK-PZ itself.

Our further acquaintance with structure-function relations in the C-terminal sequence of CCK-PZ has been broadened in a novel manner by the studies of Erspamer and his colleagues (Anastasi, Bernardi, Bosisio, De Castiglioni, Geoffredo, Bertaccini & Erspamer, 1971) on the activities of synthetic analogues of caerulein, a decapeptide amide first isolated from the skin of an Australian frog *Hyla caerulea* (Anastasi, Erspamer & Endean, 1967). The C-terminal octapeptide sequence of caerulein differs from that of CCK-PZ only in that threonine is substituted for methionine (Table 7). It is not surprising to find that the range of biological activities of gastrin,

TABLE 6. The amino acid sequence of porcine cholecystokinin (Mutt & Jorpes, 1971)

Lys-Ala-Pro-Ser-Gly-Arg-Val-Ser-Met-
Ile-Lys-Asn-Leu-Gln-Ser-Leu-Asp-Pro-
Ser-His-Arg-Ile-Ser-Asp-Arg-Asp-
Tyr-Met-**Gly-Trp-Met-Asp-Phe-NH_2**
|
(SO_3H)

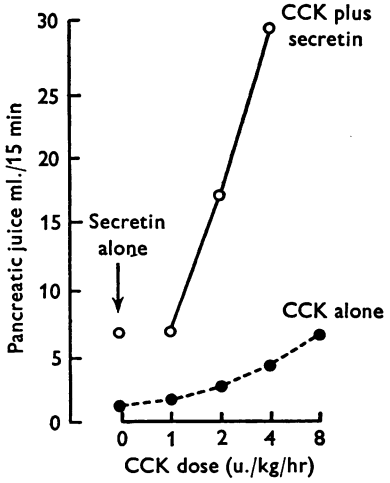
TABLE 7. The complete amino acid sequence of caerulein (top) and the C-terminal sequences of CCK (middle) and little gastrin II (bottom)

Pyr-Gln-Asp-Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH_2
|
SO_3H
-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2
|
SO_3H
-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2
|
SO_3H

CCK-PZ and caerulein is qualitatively almost identical; the cholecystokinetic activity of caerulein is identical with that of the C-terminal octapeptide of CCK-PZ, which is, surprisingly, about 10 times greater than that of the total molecule (Jorpes, 1968). The complete sequence of CCK-PZ has been revealed (Mutt & Jorpes, 1971) but total synthesis has not yet been reported. A second CCK-PZ ('CCK-variant'), which is six residues longer at the N-terminus, has also been found by Mutt & Jorpes (1968).

The similarity of CCK-PZ to gastrin extends to the stimulation of gastric acid secretion. In cat and rat, CCK-PZ is a full agonist and can stimulate gastric acid secretion as strongly as gastrin. In dog and man it is a partial agonist; given alone it evokes only a small response and given in conjunction with gastrin it inhibits the response to the latter (Grossman, 1973*b*). For these reasons, the role of CCK-PZ in the 'intestinal phase' of

gastric secretory stimulation by meals is not yet clear. This component of the response certainly involves a hormonal mechanism (Gregory & Ivy, 1942; Sircus, 1953); gastrin-like activity is extractable from the upper small intestine (Emas & Fyrö, 1968) and is released by feeding gastrectomized subjects (Stern & Walsh, 1973), but whether the release of CCK-PZ which no doubt occurs at the same time contributes to stimulation of gastric acid secretion or inhibits it is not understood. In this respect species differences would seem likely to be found.



Text-fig. 1. Dose–response lines for pancreatic secretion from pancreatic fistulas of dogs in response to CCK alone or CCK plus a contant background of secretin (redrawn from Grossman, 1971).

A remarkable feature of the action of CCK-PZ on pancreatic secretion is its interaction with secretin. Each augments the primary action of the other (Text-fig. 1) and maximal bicarbonate and enzyme responses to combinations of the two hormones are larger than to either alone (Henriksen, 1968). Physiological studies (Meyer, Way & Grossman, 1970; Meyer, Spingola & Grossman, 1971) suggest that the amounts of secretin and CCK-PZ released by a meal are small and that it is by this process of amplification through interaction that the large bicarbonate and enzyme responses to feeding are attained (Grossman, 1971).

Immunological studies

Radioimmunoassay, introduced by the classical work of Solomon Berson* and Rosalyn Yalow (1966) is now recognized as an outstanding

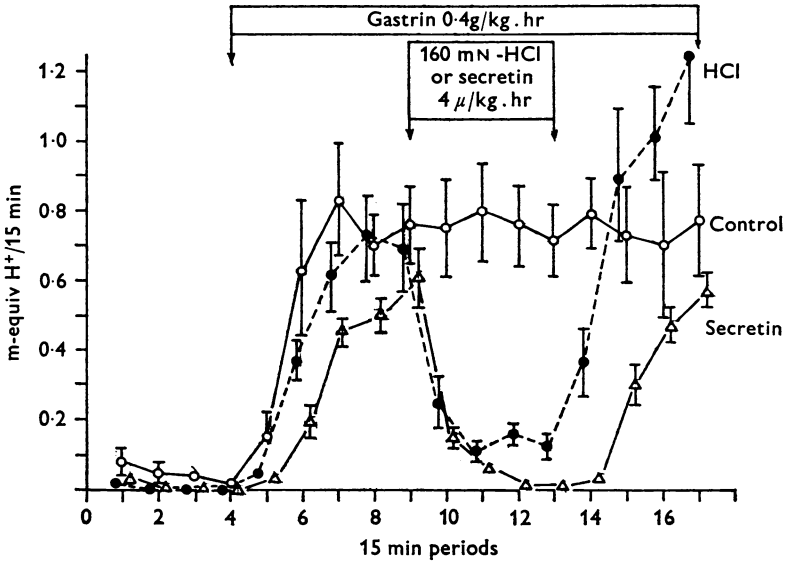
* Died 11 April 1972.

methodological advance in medical science. It provides a means of assay, often of unparalleled sensitivity and specificity, for any substance to which an antibody can be raised; and foremost among its applications so far has been to the study of the protein and peptide hormones in body fluids and tissues. Certainly one of the most important consequences of the isolation of the gastrointestinal hormones has been to open the way for the application of immunological techniques to the study of their relationships in health and disease.

McGuigan (1968*a*) was the first to succeed in establishing a sensitive and specific radioimmunoassay for gastrin; he coupled the 2–17 portion of synthetic human heptadecapeptide amide to bovine serum albumin and raised an antibody in rabbits with the conjugate. McGuigan (1968*b*) also used the antibody to identify by immunofluorescent staining the gastrin or 'G cell' in the mid-region of the pyloric antral glands (Pl. 2). The subsequent study of this cell by conventional techniques, including electron microscopy, has among other things thrown light on a long-standing problem. It had been recognized since Pavlov's time that accumulation of acid in the stomach inhibited further acid secretion; and although Oberhelman *et al.* (1952) identified the antrum as the source of the inhibition, the mechanism was at first supposed to be due either to a reflex or to the release of an antral chalone. The work of Schofield (1966), then in Newcastle, showed that neither of these postulates was tenable, leaving only a direct action of acid on the hormone-secreting cell as a reasonable explanation. However, whether the cell was so situated in the antral glands as to make such a mechanism feasible was not known. It is now revealed that the cell has access to the lumen and possesses microvilli by which it presumably senses a change in acidity there and adjusts its output of hormone accordingly (Pl. 3).

From the large and rapidly growing body of new information brought to the gastrin story by radioimmunoassay (Grossman, 1974) a further example may be cited which is particularly apposite to the present review because it reveals an aspect of the interaction between secretin and gastrin which could not have been detected in any other way. In the conscious dog provided with a denervated pouch of the gastric fundus, a steady acid secretory response evoked by infusion of gastrin is strongly inhibited by secretin; acidification of the duodenum, which releases the animals own secretin, has the same effect (Text-fig. 2, Johnson & Grossman, 1968). The inhibition is non-competitive and is clearly due to actions of the hormones at their target sites on the parietal cell. However, what can only be shown by radioimmunoassay is that when the gastrin originates endogenously, e.g. by feeding, secretin lowers the level of circulating gastrin (Hansky, Soveny & Korman, 1971; Thompson, Reeder, Bunchman,

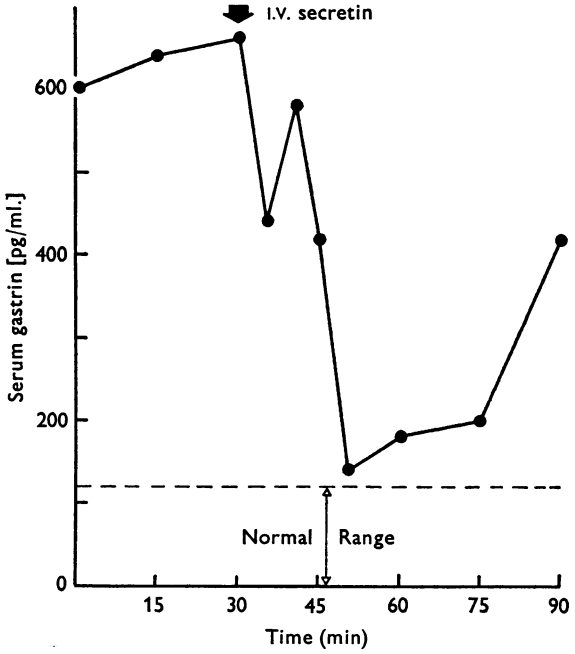
Becker & Brandt, 1972). Thus secretin, in addition to providing the alkaline pancreatic juice which neutralizes the acid gastric contents as they pass into the duodenum, also (1) inhibits the action of gastrin on the parietal cell and (2) inhibits the release of gastrin from the antral G cells. The effect of secretin on the G cell can be further demonstrated in a novel manner. In many cases of Addisonian pernicious anaemia the antral glands are spared from atrophy and the level of circulating gastrin is extremely high (McGuigan & Trudeau, 1970). This is probably not solely due to the absence of acid from the gastric juice, for there is hyperplasia of the G cells (Creutzfeldt, Arnold, Creutzfeldt, Feurle & Ketterer, 1971). In such patients secretin causes a prompt and sustained fall in the level of circulating gastrin (Text-fig. 3, Hansky, Korman, Soveny & St John, 1971).



Text-fig. 2. Effects of HCl infused intraduodenally or secretin injected I.V. on Heidenhain pouch secretion stimulated by intravenous gastrin (Johnson & Grossman, 1968).

Radioimmunoassays for secretin, CCK-PZ and the other duodenal peptides are still in the stage of development in several laboratories, but have not yet come into general use like that for gastrin, owing to such methodological difficulties as restricted supplies of the pure hormones and problems associated with radiolabelling (Berson & Yalow, 1972). However, Polak, Bloom, Coulling & Pearse (1971) and Bussolatti, Capella, Solcia, Vassallo & Vezzadini (1971) have successfully identified the secretin or

'S cell' in the duodenal mucosa by immunofluorescent staining; it is a small pyramidal cell situated in the junctional zone between villus and crypt with apical projections into the lumen. The number of S cells has been reported to be greatly increased in coeliac disease (Polak, Pearse, van Noorden, Bloom & Rossiter, 1973). The GIP cell has been similarly localized by Polak, Bloom, Kuzio, Brown & Pearse (1973) in the midzone of the duodenal and jejunal glands. VIP, which stimulates intestinal secretion and inhibits gastric acid secretion, has recently been implicated in a rare but now well recognized syndrome first described by Verner &



Text-fig. 3. Serum gastrin response to the rapid I.V. injection of secretin (2 u./kg body wt.) in a patient with pernicious anaemia (Hansky *et al.* 1971).

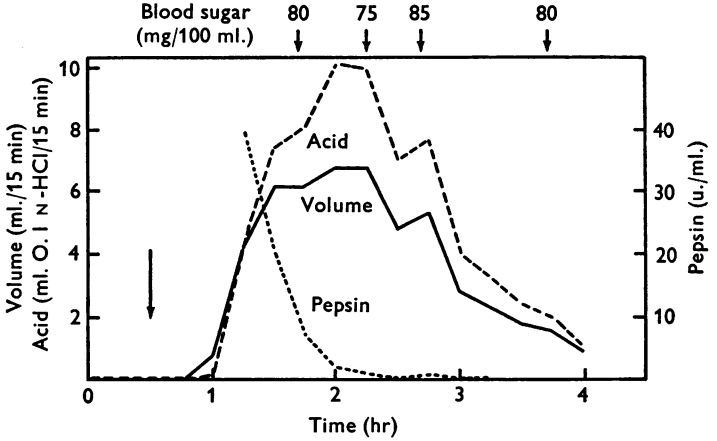
Morrison (1958), in which a pancreatic tumour is associated with severe watery diarrhoea, hypokalaemia and inhibition of gastric secretion. Bloom, Polak & Pearse (1973) reported that in six such patients there were raised plasma levels and/or a high tumour content of VIP as measured by a specific radioimmunoassay. In two cases tumour material gave a positive immunofluorescent reaction to VIP antibody.

The Zollinger–Ellison syndrome

In the last phase of this account I should like to discuss recent studies related to this remarkable and now well known example of inappropriate

hormonal secretion which have led to important advances in our knowledge of the nature of circulating gastrin.

The American surgeons Robert Zollinger of Columbus, Ohio and the late Edwin Ellison of Milwaukee were not the first to note the occasional clinical association of severe and intractable peptic ulceration, due to continuous hypersecretion of gastric acid, and the presence of a pancreatic tumour of islet-cell character distinct from the well known insulinoma; but they were the first (Zollinger & Ellison, 1955) to perceive the element of causality in the relationship and to suggest that the tumour, by continuously elaborating a powerful gastric secretagogue, was the basis of the condition. Perhaps because the tumour was found in the pancreas



Text-fig. 4. Response of a completely denervated pouch of the gastric fundus in a conscious dog to the subcutaneous injection of ZE tumour extract. From Gregory *et al.* (1960).

and not in the stomach, and glucagon had at the time come into prominence as a second pancreatic islet hormone, it was proposed as the possible causative agent; but this was soon disproved. Attempts to demonstrate the presence of a secretagogue in such pancreatic tumours were unsuccessful and by 1960 it was being questioned whether in fact the pancreatic tumour had anything to do with the gastric hypersecretion. Early in that year, Tracy and I received a small fragment of a solitary tumour removed at operation from the pancreas of a patient showing the typical clinical picture of the syndrome. Extracting it by the method we were at that time using for the preparation of gastrin from antral mucosa, we had no difficulty in demonstrating that the histamine- and insulin-free extract contained a powerful stimulant of gastric acid secretion (Gregory, Tracy, French & Sircus, 1960, Text-fig. 4). Soon afterwards it was shown

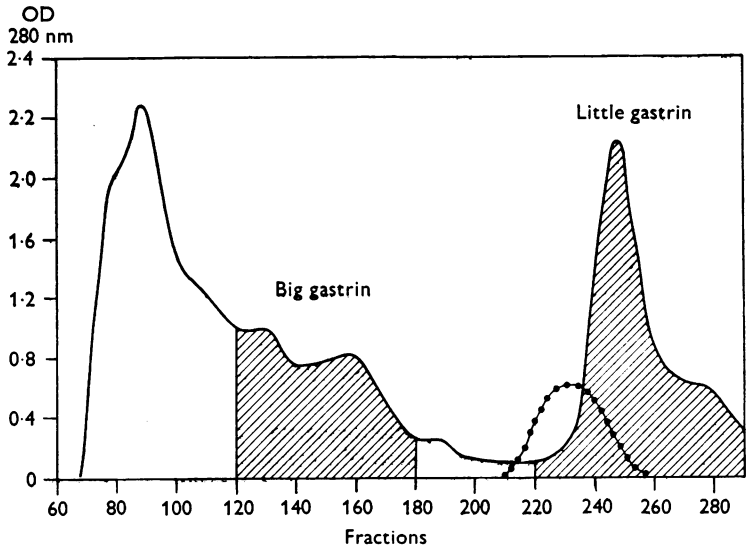
(Grossman, Tracy & Gregory, 1961) that liver and lymph gland metastases of such tumours also produced the secretagogue; similar observations have since been made by ourselves and others in a very large number of cases. Such crude tumour extracts showed the full range of physiological actions characteristic of pure gastrin (Jackson, Blair, Dawson, Reed & Watts, 1963; Gregory & Tracy, 1964) and it became obvious that the active principle must be closely similar to, if not identical with, the antral hormone.

Until 1968, when radioimmunoassay for gastrin became available, a clinical diagnosis of the syndrome could only be conclusively established by extraction and bio-assay of tumour tissue found at operation or post-mortem examination, and accumulation of material from the many specimens sent to us for such a service enabled us eventually to establish that such tumours contained a pair of heptadecapeptide amides identical in proportions, amino acid composition and sequence with the pair of peptides we had previously isolated from human antral mucosa (Gregory, Grossman, Tracy & Bentley, 1967; Gregory, Tracy, Agarwal & Grossman, 1969). These findings were complemented by the report of McGuigan & Trudeau (1968) and later others, e.g. Yalow & Berson (1970*a*) that in cases of the ZE syndrome the level of circulating immunoreactive gastrin was raised, often to extremely high values.

Gastrin radioimmunoassay has proved to be of great importance in the diagnosis and effective treatment of the syndrome as originally defined, i.e. where the gastrin originates from a pancreatic tumour. The tumours are usually small, they may be multiple, and they frequently metastasize; where they are highly active, medical treatment is of no avail and only total gastrectomy can be relied upon to preserve the patient from recurrence of peptic ulceration and catastrophes such as perforation and haemorrhage with eventual death. The increasing clinical use of radioimmunoassay has not only revealed a greater incidence of gastrinoma – as the condition is perhaps now appropriately termed – in a comparatively mildly active form than had originally been suspected, but in conjunction with immunofluorescent staining has led to the recent discovery by Polak, Stagg & Pearse (1972) of a clinical condition in which hypergastrinaemia, with consequent gastric hypersecretion and peptic ulceration, was due not to a gastrinoma but to hyperplasia of the antral G cells (Pl. 4). This important observation not only clarifies the diagnostic implications of hypergastrinaemia in a case of suspected gastrinoma, but may also have significance for the problem of duodenal ulceration in general if a more moderate degree of G cell hyperplasia should prove to be at all common.

The nature of circulating gastrin

Discussing the gastrin peptides in 1964, I remarked (Gregory, 1966), 'We have termed the peptides we isolated "gastrins" I and II, but we do not mean to imply by this that either is considered to be the same form as the hormone is when released from antral mucosa. Clearly, there may be present in antral mucosa other "gastrins" composed of part of the peptides we have isolated, or indeed incorporating them, or the active parts, within a larger molecule. This consideration must apply also to the substance produced by Zollinger–Ellison tumours.' These words were unconsciously prophetic; by 1968 Tracy and I had become aware that

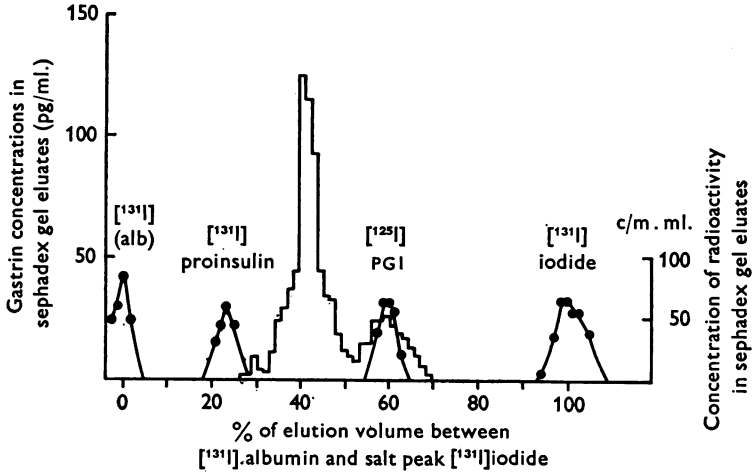


Text-fig. 5. Fractionation of a partially purified extract of hog antral mucosa on Sephadex G50. –●–●–●– Salt zone.

there could be detected in our partially purified antral extract gastrin-like activity which was of considerably larger size than the heptadeca-peptides. On Sephadex G50 filtration of a partially purified antral extract almost all of the gastrin activity present (heptadecapeptide) emerged in the vicinity of the 'salt zone'; but there was a very small amount of activity also detectable midway between the salt zone and the protein 'void volume' (Text-fig. 5). We undertook to isolate this 'bigger' gastrin surmising that if it was not merely an artifact due to binding of a little heptadecapeptide to some larger material, it might be a precursor form of the hormone. We were prepared to travel hopefully, realizing that we

might eventually arrive at nowhere in particular! For technical reasons the task proved so difficult as to make our isolation of the heptadecapeptides seem like child's play, but we slowly made progress.

Early in 1970, we learned from Berson & Yalow that having set up gastrin radioimmunoassay they were applying to serum gastrin the methods of immunological analysis already used by them and others to demonstrate heterogeneity of circulating hormones, e.g. parathyroid hormone and insulin (Berson & Yalow, 1971). Serum samples, mainly from cases of the



Text-fig. 6. Distribution of immunoreactive gastrin components in Sephadex G50 fractions of Be plasma (ZE syndrome) eluted with 8M urea in veronal buffer. [^{125}I]PG 1, radio-iodinated porcine little gastrin I. From Yalow & Berson (1971).

ZE syndrome or pernicious anaemia with high gastrin levels were subjected to filtration on Sephadex G50 or to starch gel electrophoresis and the distribution of gastrin immunoreactivity compared with that of the heptadecapeptide in column effluent fractions or gel segments (Yalow & Berson, 1970*b*, 1971). The results (Text-fig. 6) showed clearly that no more than 50% of the total immunoreactivity present corresponded to the heptadecapeptide or as they termed it 'little gastrin' (LG): the remainder, usually predominating in amount, corresponded to a larger (and less acidic) molecule which they termed 'big gastrin' (BG). If serum containing BG was digested with trypsin, then the BG disappeared and LG took its place; from this they inferred that BG might consist of LG covalently linked through a lysine or arginine residue to a basic peptide. In antral mucosa, the amount of BG present was low compared with that of LG;

in the few ZE tumours they had the opportunity of examining, the proportion of BG was considerably higher.

It was obvious that Yalow and Berson's BG was probably the circulating counterpart of the 'bigger' gastrin we had identified in our hog antral extract, and early in 1972 we succeeded in isolating from this source a pair of gastrin peptides which corresponded in their behaviour on Sephadex and starch gel to serum BG. At the same time, having accumulated over the previous four years a further quantity of ZE tumour larger than before, we extracted this by a method closely similar to that which we had evolved for the isolation of the porcine BG peptides. There was now isolated, in addition to the heptadecapeptides previously described, a pair of BG peptides closely similar in amino acid composition to, and identical in chromatographic and electrophoretic behaviour with, the porcine BG peptides and with Yalow & Berson's serum BG (Gregory & Tracy, 1972, 1973). The complete amino acid sequences of both have been determined by our chemist colleagues and synthesis of human BG I is now progressing. The peptides contain no arginine, but two residues of lysine; tryptic

TABLE 8. The partial amino acid sequence of the human and porcine BG peptides

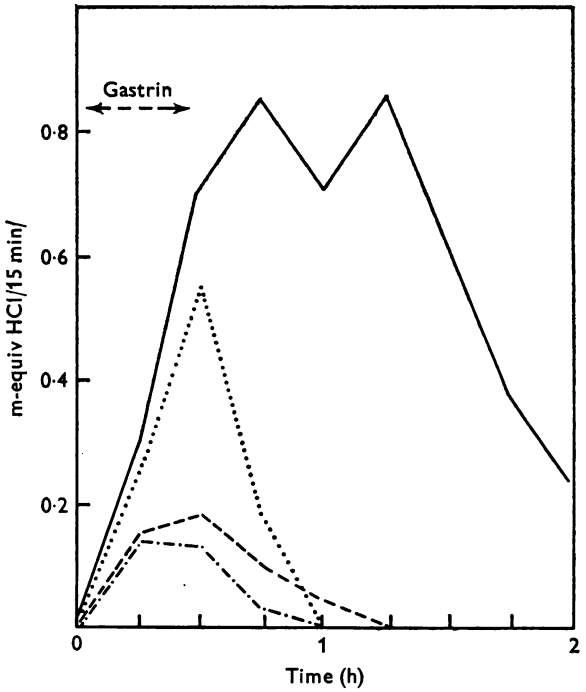
Human Pyro-(Leu$_2$, Gly$_2$, Pro$_3$, Gln$_1$, His$_1$, Ser$_2$, Val$_1$, Ala$_1$, Asp$_1$)-Lys-Lys
-Gln-Gly-Pro-Trp-Leu-Glu$_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH$_2$
|
R

Porcine Pryo-(Leu$_4$, Gly$_2$, Pro$_3$, Gln$_1$, His$_1$, Val$_1$, Ala$_2$, Asp$_1$)-Lys-Lys
-Gln-Gly-Pro-Trp-Met-Glu$_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH$_2$
|
R

Pyro = pyroglutamyl
R = H or SO_3H

digestion liberates heptadecapeptide in which the N-terminus is not pyroglutamyl, but glutaminyl, together with two other peptides of almost identical composition (Dancsi, 1972; B. Mason, personal communication). The partial sequence of the human and porcine BG peptides is shown in Table 8. Yalow & Berson's serum BG emerged on Sephadex G50 filtration between pro-insulin (mol. wt. approximately 9000) and insulin (mol. wt. approximately 6000), from which they concluded (Yalow & Berson, 1970*b*) that the mol. wt. of BG was probably approximately 7000. Our pure human BG peptides emerge in an identical position, but the minimal mol. wt. calculated from the amino acid composition is approximately 3870. The most likely explanation would appear to be that either the position on Sephadex is a further example of the anomalous behaviour shown in such circumstances by many other substances, or the BG peptides exist in solution as dimers.

Studies on the half-life in circulation and acid-stimulating properties of the pure human BG peptides have been made by Walsh, Debas & Grossman (1973, 1974) using preparations supplied by us. As in the case of the heptadecapeptides, the two BG forms (sulphated and unsulphated) do not differ in properties, but they differ greatly from the LGs in circulating half-life. That for BG is 15·8 min; that for LG is 3·2 min. The



Text-fig. 7. Response of a Heidenhain pouch to approximately equimolar amounts of BG and LG given by I.V. infusion for the first 30 min of each experiment. The dog was receiving 1 μg per hour carbachol throughout each experiment. Symbols: –·–·–, 0·5 μg LG 1; ––––, 1·0 μg BH 1; ....., 16 μg LG 1; ———, 30 μg BG 1 (Gregory & Tracy, 1973).

consequence is that when large equimolar doses are used in non-steady-state conditions a much greater and more prolonged acid secretory response is obtained to BG than to LG (Text-fig. 7, Gregory & Tracy, 1973). However, when steady-state conditions are studied and secretory responses are related to circulating gastrin levels determined by radioimmunoassay, it is found that a nearly 5 times greater molar increment of circulating BG is required, compared with LG, to evoke the same response (Walsh *et al.* 1974). Thus, although BG is usually the predominant circulating form, it may in fact be the LG also present which is making the greater

contribution to the stimulation of acid secretion; even if the BG:LG ratio were as high as 5:1, which would be unusual, the two would contribute equally. An interesting consequence of the difference in half-life times is that since both forms have the same volume of distribution (about 25% of the body weight) the antral G cell would have to secrete LG 5 times faster than BG to maintain equal levels of the two in circulation. No comparisons of LG and BG in respect of other physiological actions have as yet been made.

TABLE 9. The amino acid sequence of the human minigastrins (tridecapeptides)

$$\text{Leu-(Glu)}_5\text{-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH}_2$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \text{R}$$

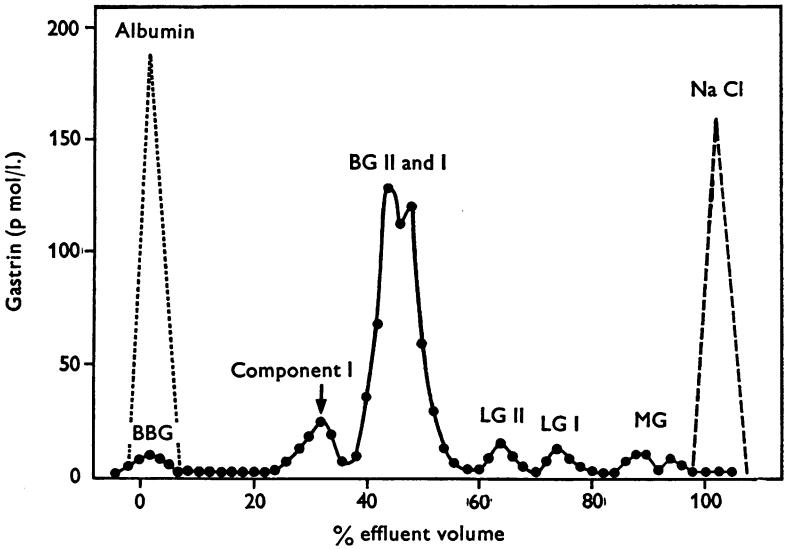
$$\text{R} = \text{H or } SO_3H$$

Minigastrins. During a study of the gastrins present in a very large ZE tumour recently made available to us by the kind efforts of Drs Morton Grossman and Edward Passaro of Los Angeles, we discovered in the heptadecapeptide fraction a pair of tridecapeptides (sulphated and unsulphated), present in the same proportions (1:2) as are the heptadecapeptides in this and previous ZE tumours and in human antral mucosa (Table 9). Both these peptides are potent stimulants of gastric acid secretion; J. H. Walsh (personal communication) reports that they have about half the potency of the heptadecapeptides and a slightly shorter half-life in circulation. We were initially inclined to suppose that they might be artifacts, perhaps formed from larger gastrins by post-mortem autolysis, but there is evidence that this is not so.

Rehfeld and his colleagues in Copenhagen (Rehfeld & Stadil, 1973; Dencker, Håkanson, Liedberg, Norryd, Oscarson, Rehfeld & Stadil, 1973; Rehfeld, Stadil & Vikelsøe, 1974) have made similar gel filtration studies to those of Berson & Yalow, using serum not only from ZE or pernicious anaemia patients but also from normal subjects after meals. They use very long columns, which give increased resolution, and they have analysed the nature of the separated components by chromatography on aminoethylcellulose, using as 'markers' samples of purified peptides supplied by us. Their results can be summarized as follows (Text-fig. 8). The 'big gastrin' component predominates; it is composed of the BG peptides I and II. A smaller paired component corresponds to little gastrins I and II, and a still smaller and later paired component corresponds to minigastrins I and II. Emerging just before 'big gastrin' is a smaller peak which they term 'component I'; it is unpaired. Material closely

corresponding to component I has been purified from our ZE tumour extract by Tracy; it has very low biological and immunological reactivity.

To complete this survey of the circulating gastrins thus far identified, there must be mentioned 'big, big' gastrin (BBG) a minor immunoreactive component discovered by Yalow & Berson (1972) in serum and extracts of jejunal mucosa. It emerges in the void volume on Sephadex G50 columns (Fig. 19) and has a mol. wt. about 20,000; it is the predominant form of gastrin found in fasting serum of man, hog and dog but does not increase on feeding like the other components (Yalow & Wu, 1973). There is present in our ZE tumour and hog antral extracts a void volume fraction which contains immunoreactivity apparently corresponding to BBG (Yalow & Wu, 1973; Rehfeld *et al.* 1974). So the present 'score' of circulating forms of gastrin amounts to eight: pairs of 'big', 'little' and 'mini' gastrins, a single 'component I' and BBG.



Text-fig. 8. Immunoreactive serum gastrins. From Yalow & Berson (1972) and Rehfeld *et al.* (1974).

There is undoubtedly more to be unearthed in this corner of the gastrin field. For instance, evidence has recently been obtained which indicates that in duodenal ulcer patients the value for circulating gastrin measured by direct bioassay of serum substantially exceeds that obtained by radioimmunoassay using an antibody raised to the heptadecapeptide, but not when the antibody has been raised to the much smaller pentagastrim molecule (Herring & Blair, 1969; Colin-Jones & Lennard-Jones, 1972;

Byrnes, Coleman & Lazarus, 1971); this suggests that there may be in circulation active C-terminal gastrin fragments too small to react significantly with the heptadecapeptide antibody. As a matter of fact, in 1967 Tracy and I (unpublished studies) isolated from hog antral mucosa small amounts of the pair of 1–13 fragments (sulphated and unsulphated) of the gastrin heptadecapeptides; we were unable to find the remaining piece, the active C-terminal tetrapeptide amide. However, Dockray & Walsh (1974) have recently identified in the serum of ZE patients a component which corresponds immunologically and chromatographically to the 1–13 fragment, so it is possible that the tetrapeptide may also have been in circulation.

It will clearly be a complex and difficult, but rewarding, task to unravel the functional and biosynthetic relationships of all these different forms of circulating gastrin; and when similar techniques of immunological analysis can be applied to the other gastrointestinal hormones, we may expect that equally fascinating, though no doubt substantially different, pictures will likewise emerge.

It may well be thought that this account of some of the recent advances which have taken place in our knowledge of the gastrointestinal hormones has been more concerned with chemistry (including immunology) and physics than with physiology; but I should like to hope that it might have pleased Bayliss and Starling to see how these older disciplines have been brought to serve the physiologist in capturing and studying those elusive substances, the first of which their joint genius brought to us in this College seventy-one years ago.

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EXPLANATION OF PLATES

PLATE 1

The discovery of gastrin (Edkins, 1905)

PLATE 2

Porcine antral mucosal cells stained by peroxidase-labelled gastrin antibody (× 500) (McGuigan & Greider, 1971).

PLATE 3

Three G cells (human) located in the upper portion of a pyloric gland. Microvilli project into the lumen (approx. × 15,000) (Rubin, 1972).

PLATE 4

Extensive G cell hyperplasia, each pyloric gland (cross-section) being composed almost entirely of G cells. A considerable proportion of the latter are submaximally fluorescent, suggesting high gastrin output and turnover. Indirect immunofluorescent staining with antihuman gastrin antibody (× 140) (Cowley *et al.* 1973).

On the Chemical Mechanism of Gastric Secretion.

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(Communicated by Professor C. S. Sherrington, F.R.S. Received May 13,— Read May 18, 1905.)

It has long been known that the introduction of certain substances into the stomach provoke a secretion of gastric juice. This is regarded as in no sense depending upon mere mechanical stimulation of the mucous membrane, and it has been thought that the nervous mechanism of the gastric glands may be susceptible to certain local chemical stimuli.

On the analogy of what has been held 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 digested food in the stomach, a substance may be separated from the cells of the mucous membrane which, passing into the blood or lymph, later stimulates the secretory cells of the stomach to functional activity. The following observations support this view :—

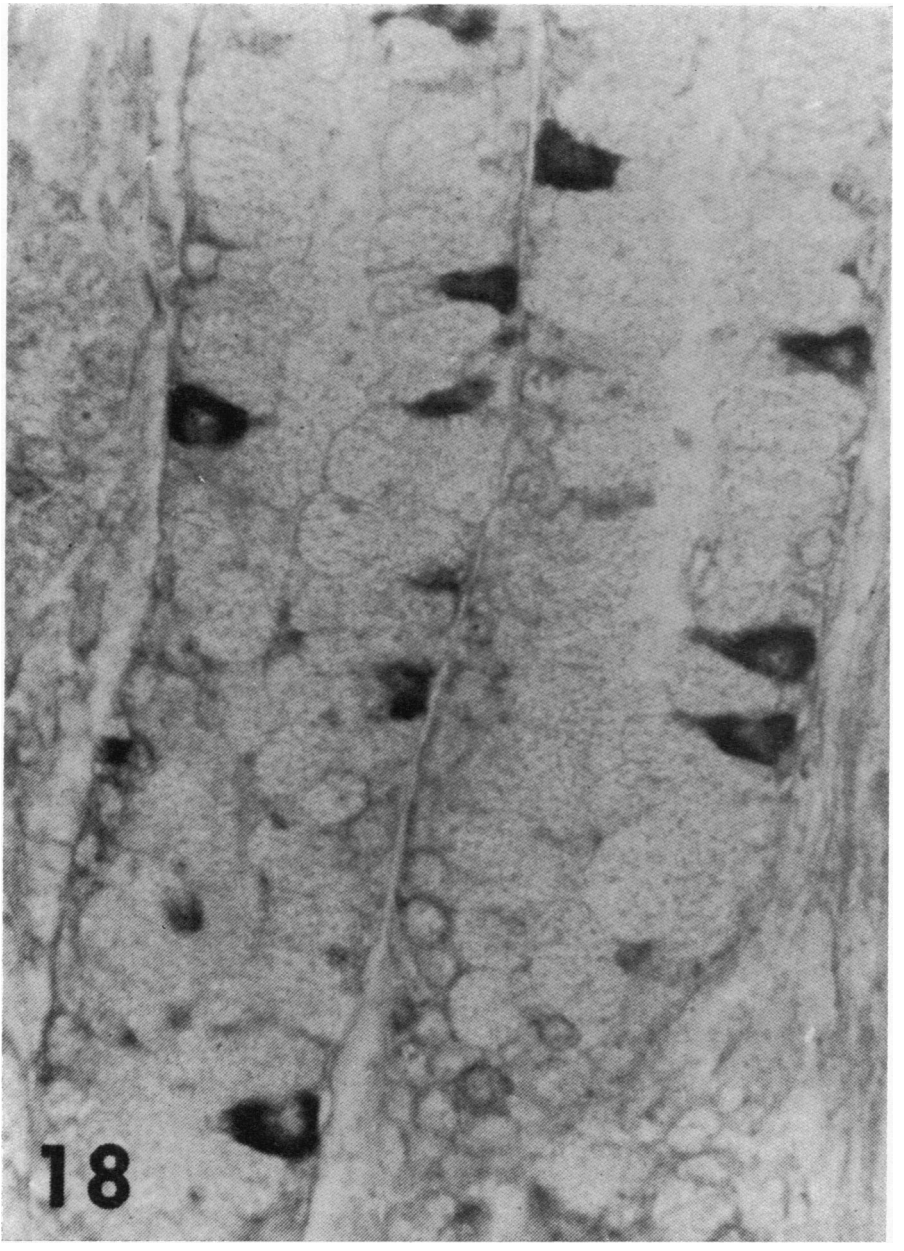
If an extract in 5 per cent. dextrin of the fundus mucous membrane be injected into the jugular vein, there is no evidence of secretion of gastric juice. If the extract be made with the pyloric mucous membrane, there is evidence of a small quantity of secretion. With dextrin by itself there is no secretion.

Extracts of fundus mucous membrane in dextrose or maltose give no secretion ; extracts of pyloric mucous membrane give marked secretion ; dextrose or maltose alone bring about no secretion.

If extracts be made with commercial peptone, it is found that no secretion occurs with the fundus mucous membrane, a marked secretion with the pyloric mucous membrane ; the peptone alone gives a slight secretion.

If the extracts be made by boiling the mucous membrane in the different media, the effect is just the same, that is to say, the active principle, which may be called "gastrin," is not destroyed by boiling.

Finally, it may be pointed out that such absorption as occurs in the stomach apparently takes place in the pyloric end. With the pig's stomach, in which the true cardiac region differs from the typical fundus region in having only simple glands as in the pyloric, extracts of the cardiac region in general have the same efficacy in promoting secretion, as do pyloric.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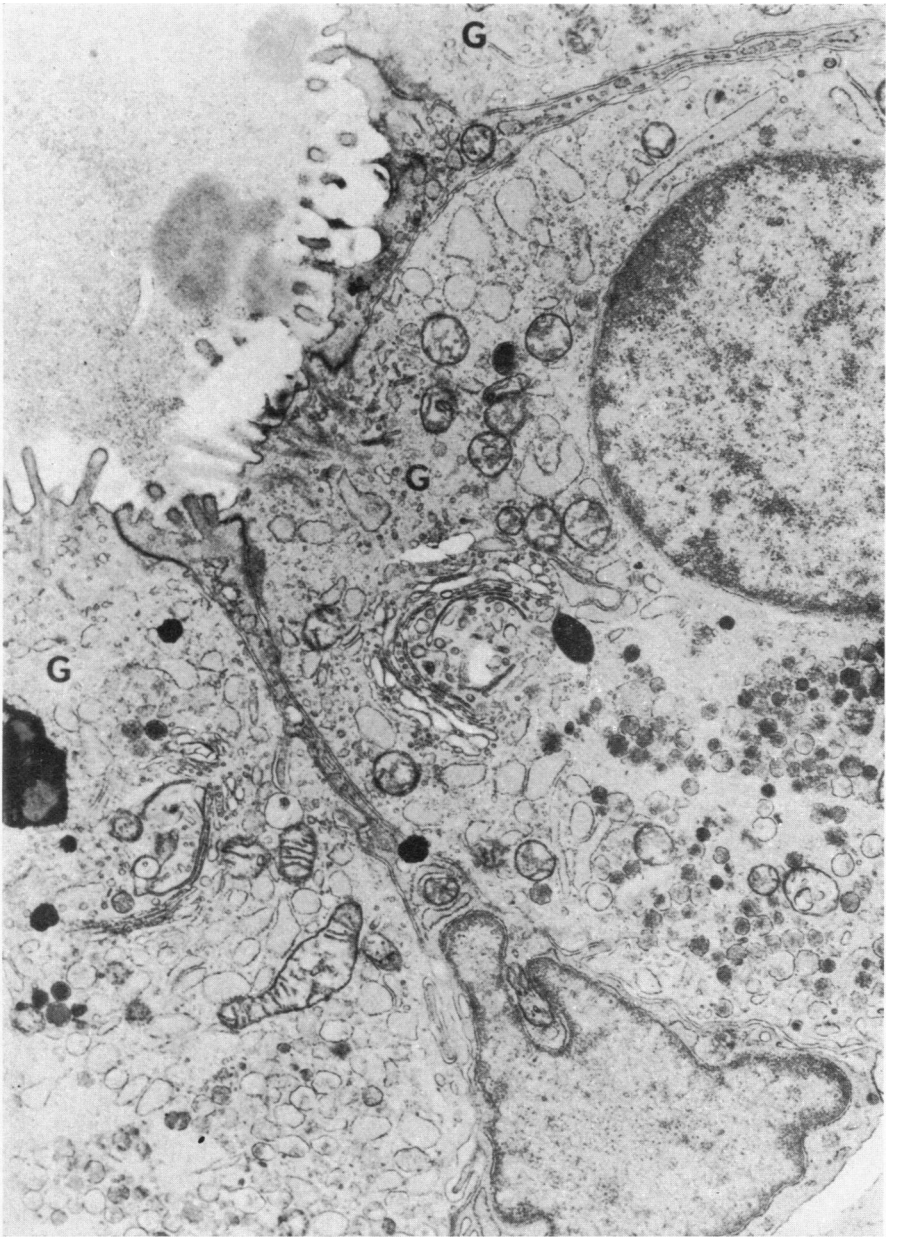
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