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REVIEW

A Review of Some Recent Developments in the Chemistry of the Gastrins[1]

RODERIC A. GREGORY

Physiological Laboratory, University of Liverpool, Liverpool L69 3BX, England

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An account is presented of the history, distribution, and chemistry of the several forms and fragments of the gastric hormone gastrin and gastrin-like peptides which have been identified in gastric, intestinal, nervous, and tumor (gastrinoma) tissues, and also in the circulation.

Endocrinology as we know it today began in 1902 in University College, London, with the discovery by Bayliss and Starling (*1*) of the duodenal hormone secretin and their appreciation that it exemplified a second means in addition to the nervous system of communication between different organs of the body, namely, by blood-borne "chemical messengers." Three years later, in St. Bartholomew's Hospital Medical School, London, Edkins (*2*) discovered a second hormone in the stomach, where it was involved in the stimulation of gastric secretion by food; he called it "gastrin." Edkins' results could not be confirmed by his contemporaries; there ensued nearly half a century of controversy and doubt concerning the "gastrin hypothesis," as it was called before the existence of the hormone was confirmed by physiological experiments. By 1959, when Tracy and I began our work on gastrin (*3*), it had long been evident that the digestive system had become something of a Cinderella in the world of endocrinology. Although secretin, gastrin, and several other hormones had been thought to exist in the gastrointestinal tract, none had been isolated; their chemical natures could only be guessed at, and the physiological properties of each were the subject of uncertainty and speculation, since knowledge of them was based upon the study of relatively crude extracts.

The isolation of gastrin in the form of what was later shown to be a heptadecapeptide amide was achieved early in 1962 (*4*). It was a remarkably fortunate circumstance that only a few months previously, when success was in sight, we had met George Kenner for the first time, having learned from others of his great expertise in the

[1] This account is intended to make clear something of the great part which Kenner played in the unfolding of more than 15 years of the gastrin story. I hope it also conveys to the reader my great admiration for his brilliance as a scientist and his sterling qualities of modesty, kindness, and sensitivity as a person. I owe him an immense debt of collaborative support and encouragement which alas cannot be repaid but only recorded in these words.

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field of peptide chemistry. There began between us a very close and happy collaboration which continued unbroken for 16 years. Thanks very largely to Kenner's participation in our work, gastrin became the first of the gastrointestinal hormones to be sequenced and synthesized, not only from pig but also from man, dog, cat, sheep, and cow. The provision from Liverpool to workers throughout the world of supplies of natural gastrins and of synthetic human gastrin, which was first prepared by Kenner and his group, has made possible a vast number of studies on the role of the hormone in normal and pathological conditions of digestion. The physiology and pathology of gastrin will not be described in detail here as they have been intensively reviewed elsewhere (*5*).

DISTRIBUTION

The prime physiological source of the hormone is the pyloric antral mucosa of the mammalian stomach, where it occurs in amounts of about 2–20 μg/g fresh tissue. Relatively small amounts of gastrin (10% or less of antral content) are found in the upper part of the small intestine, chiefly the duodenum. It has been reported that in man the total amount of gastrin in the duodenum is about as much as that in the pyloric antrum, but this needs confirmation. A malignant tumor which produces gastrin (gastrinoma) arises from islet cells in the human pancreas and rather rarely from traces of similar tissue in the nearby wall of the duodenum; it metastasizes chiefly to the liver (and rarely to other organs), and the metastases also produce gastrin. Gastrinoma is the basis of the clinical syndrome first described in 1955 by Zollinger and Ellison (*6*) in which severe and intractable peptic ulcer, due to hypersecretion of gastric juice, was observed to be associated with the presence of a non-β-cell islet tumor of the pancreas. It was established in 1960 by Gregory *et al.* (*7*) that the condition was due to the continuous and inappropriate production by such tumors of a gastrin-like stimulant of gastric secretion; the secretagogue was later isolated and chemically identified as human antral gastrin by Gregory *et al.* (*8*). Such tumors or their metastases (which are sometimes very large) have proved to be a very valuable source of the various forms and fragments of the human variety of the hormone. Although the human heptadecapeptide amide form of gastrin was originally isolated from a small number of human stomach specimens removed at operations for duodenal ulcer, the procedure is no longer popular with surgeons; and this source of the human hormone has unfortunately virtually ceased.

Several forms and fragments of the hormone have been identified immunologically by radioimmunoassay of plasma or serum (the latter is usually preferred for technical reasons and gives values identical with those of plasma), and an important diagnostic feature of the Zollinger–Ellison syndrome is that the level of circulating gastrin is abnormally high. It is also greatly elevated in some cases of pernicious anemia. Small amounts of gastrin have recently been detected by radioimmunoassay and also by immunocytochemistry in the pituitary gland and in certain nerves, notably the vagus. A gastrin-like peptide, the COOH-terminal octapeptide amide of the duodenal hormone cholecystokinin, is widely distributed in the cerebral cortex in relatively large amounts and has been isolated from this source (*9*); the functions of the peptides in these situations are at present unknown.

FORMS AND FRAGMENTS OF GASTRIN

The various forms and fragments of gastrin which have so far been recognized in the circulation or tissues of origin may be enumerated as follows:

1. "Little gastrin" (LG), 17 amino acid residues
2. "Big gastrin" (BG), 34 amino acid residues
3. "Minigastrin" (MG), 14 amino acid residues
4. "Big, big gastrin" (BBG), MW about 20 000
5. "Component I" (CI), size intermediate between BG and BGG
6. NH_2-terminal fragment of LG (G17NT), 13 amino acid residues
7. "Tryptic peptide" fragment of BG (G34NT), 17 (?) residues
8. NH_2-terminal fragment of porcine BG (? 1–30)
9. COOH-terminal tetrapeptide amide

Of these 1, 2, 3, and 6 have been isolated from antral mucosa or gastrinoma tissue and fully characterized chemically. They all occur as pairs of otherwise identical peptides in which the single tyrosine residue is sulfated or unsulfated. Substance 4 was originally identified immunologically as a component of serum, and corresponding material was believed to have been found by the same method in partially purified gastrinoma and antral mucosa extracts; present evidence strongly suggests that serum BBG is an artifact due to interference by protein in the radioimmunoassay. Peptide 5 has been well documented immunologically and chemically in the circulation and in antral and gastrinoma extracts, but has not yet been isolated. Gastrins 7 and 8 have been identified immunologically and chemically in antral mucosa extracts but not yet isolated. The presence of 9 in the circulation and in antral and gastrinoma extracts is suspected but not yet established.

LITTLE GASTRIN

The pair of peptides which Tracy and I isolated in 1962 from porcine antral mucosa had identical amino acid compositions, but one (I) was less acidic than the other (II), thus enabling them to be separated chromatographically and electrophoretically. Kenner chose to sequence gastrin II, which was available in greater amount than gastrin I. The difficulties presented by the fact that both ends of the molecule were blocked were surmounted (*10*), and the sequence was elucidated late in 1963 (Table 1). Synthesis was commenced forthwith; by the time (May 1964) our full account of the method of isolation and physiological properties of pure gastrin was published (*11*), synthesis was well advanced and was completed in September 1964. Kenner and his colleagues had guessed that the synthesis of one gastrin might reveal the difference between it and the other, and they were right. When the final synthetic product was purified by the same chromatographic and electrophoretic methods as had been used for isolation of the natural material (*12*), it turned out to be not gastrin II but the less acidic gastrin I. Kenner immediately recalled that (1) tyrosine *sulfate* had been described as a constituent of urine and of a fibrinopeptide released during blood clotting, and (2) during the sequencing work a dipeptide alanyl-tyrosine found among

TABLE 1

THE AMINO ACID COMPOSITION OF THE LITTLE GASTRINS (HEPTADECAPEPTIDES) ISOLATED FROM GASTRIC ANTRAL MUCOSA

Man	Pca–	Gly–	Pro–	Trp–	Leu–	Glu–	Glu–	Glu–	Glu–	Ala–	Tyr(R)–	Gly–	Trp–	Met–	Asp–	Phe-NH_2
Hog					–Met–											
Cat									–Ala–							
Dog					–Met–			–Ala–								
Cow					–Val–				–Ala–							
Sheep					–Val–				–Ala–							

R = H or SO_3H

the products of enzymatic degradation of gastrin had shown surprising mobility on high-voltage paper electrophoresis. Within a day or two it had been established that the sole difference between G I and G II was sulfation of the tyrosine residue; the point was elegantly confirmed by conversion of the one into the other by mild hydrolysis and sulfation (*11*). All the forms or fragments of gastrin which contain the tyrosine residue are found in pairs, the proportions of the two forms varying in different species. Sulfation makes little or no difference to the physiological activity of the gastrins, but it is essential for the characteristic activity of the gastrin-like peptide cholecystokinin (see later).

Over the next few years, similar pairs of heptadecapeptide amides were isolated from the antral mucosa of man, dog, cat, cow, and sheep (*13*). The sequences of these were established (Table 1), and total syntheses of the unsulfated forms accomplished, by Kenner and his group (*14–16*). In the case of dog and cat gastrins, there were particular problems because of the very small amounts of pure material available. The sequence of dog gastrin was established by total synthesis of all five of the possible variants indicated by comparison of the quantitative amino acid analysis with other known sequences. These were then compared with the natural peptide by enzymatic degradation and high-voltage electrophoresis of the fragments; the result indicated unambiguously the correct sequence (*15*). In the case of cat gastrin where only about 250 μg was available, a similar problem was solved by mass spectrometry (*16*). The synthesis of cat gastrin by Kenner, Mason, and Ramage (1977) has not been published.

BIG GASTRIN

In 1968 Tracy and I (*17*) detected by bioassay of fractions from partially purified antral extracts subjected to gel filtration on Sepadex G50, gastrin of considerably larger size than the heptadecapeptide. It was obviously likely to be a precursor form of the hormone. Isolation of this "bigger" gastrin in amounts sufficient for chemical characterization by the methods available at the time proved extremely difficult, partly because of the very small amount of material present; it eventually proved necessary to extract at least 20 kg of antral mucosa (600 stomachs) to obtain 1 mg. While this work was in progress, Yalow and Berson (*18*) reported that in patients with the Zollinger–Ellison syndrome or pernicious anemia, in which circulating levels of gastrin are very

high, the predominant form of immunoreactive gastrin was considerably larger ("big gastrin," BG) than the heptadecapeptide ("little gastrin," LG), as indicated by gel filtration of serum samples. They further showed that when such sera were briefly digested with trypsin, BG was converted into LG, from which they surmised that LG might be the COOH-terminal portion of BG, with a cleavage point at an arginine or lysine residue.

It was clear that Yalow and Berson's BG must be the circulating counterpart of the "bigger" gastrin which Tracy and I had previously identified in antral extracts. Early in 1972 we isolated (*19*) from a small accumulation of gastrinoma tissue, and also from porcine antral mucosa, pairs of gastrin peptides of the same size and chromatographic and electrophoretic behavior as the BG immunoreactivity in serum; they were potent stimulants of gastric secretion.

The preliminary studies of Kenner and his colleagues on the human peptides (*19*) established that the amino acid constitutions of the two members of the pair were identical and included two lysines but no arginine. Tryptic digestion released two basic peptides containing one or two lysines together with free lysine and a strongly acidic peptide which had the amino acid composition of LG and was fully active physiologically. It was initially ninhydrin-positive but gradually became ninhydrin-negative. These observations were interpreted to mean that trypsin cleaved the BG molecule either between (20%) or on the carboxyl side (80%) of a pair of lysine residues, releasing free lysine and LG having initially NH_2-terminal glutaminyl or glutamyl, which slowly cyclized to the pyrollidone form. It was also noted at this time that BG would not react with phenylisothiocyanate, indicating the probable presence of NH_2-terminal pyroglutamyl and preventing the use of the Edman method of sequencing. This and the very small amounts of pure material which were available greatly hindered progress in sequencing BG; but in 1973 we were greatly fortunate in being able to engage the interest of Dr. Ieuan Harris of the MRC Molecular Biology Laboratory, Cambridge, in the problem, and at the same time to offer him a supply of the pyrollidone carboxylyl peptidase prepared (*20*) and kindly donated by Dr. R. F. Doolittle of San Diego.

Using the Dansyl–Edman method on the deblocked peptide, Harris provided a complete sequence for human BG I. Porcine BG presented much greater difficulty because the Doolittle enzyme, in addition to removing the NH_2-terminal pyroglutamyl residue, also cleaved the peptide at residues 14–15. However, Harris succeeded in obtaining a complete sequence for porcine BG I. Harris's sequences were reported, with his permission, by Gregory and Tracy in 1975 (*21*).

A peptide corresponding to the sequence assigned by Harris to human BG I was synthesized jointly by Wünsch in Munich and by Kenner in Liverpool (*22*). The products from each laboratory were found to have physiological activity equivalent to that of the natural material, and they also showed immunoreactivity equivalent to that of the natural material with an antibody directed toward the COOH-terminal sequence (the active region) of the molecule. However, in 1977 Dockray in this laboratory succeeded in preparing an antibody which was characterized using the "tryptic peptide" fragment of natural BG (human and porcine) and synthetic NH_2-terminal fragments provided by Kenner. The antibody was thus proved to be specific for the NH_2-terminal region of the molecule. This antibody showed excellent binding of natural human and

porcine BG, sulfated or unsulfated, and as expected it showed no binding whatever of LG. However, it unexpectedly showed no significant binding of the synthetic human BG made by either Wünsch or Kenner, indicating unequivocally that there was some difference between the natural and synthetic products in the NH_2-terminal region of the molecule (*23*).

By this time, the total amount of human BG in existence had dwindled to 250 μg, and the prospect of obtaining starting material to prepare more was only a very distant one. In efforts to elucidate the nature and precise location of the discrepancy, Kenner and his colleagues synthesized a considerable number of NH_2-terminal fragments representing possible variants of the proposed sequence, and these were examined for immunoreactivity with the NH_2-terminal antibody. Kenner also synthesized porcine BG I according to the proposed sequence; the product showed binding to the NH_2-terminal antibody which was significantly better than that of the synthetic human BG, but still far lower than that shown by natural BG.

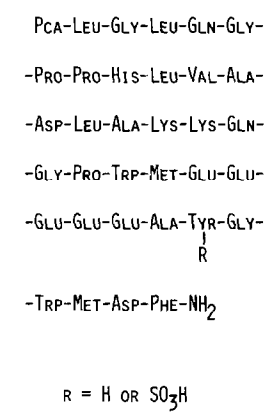

FIG. 1. Sequence of porcine big gastrin (revised 1978).

At the time of Harris's sudden and tragic death in March 1978 the problem remained unsolved, though further studies of the natural and synthetic products were being considered. As now recounted in an accompanying paper in this volume, the resequencing of porcine BG by Hood in July 1978 and very recent independent confirmation by Kenner's former pupil Agarwal and his group (*24*) using nucleotide sequencing has provided unequivocal evidence that Harris's sequence was correct except for a simple inversion of residues 7–9, the correct sequence being –Pro–Pro–His– (Fig. 1). The NH_2-terminal 1–12 fragment (and more recently the entire molecule) synthesized in Liverpool according to this revised sequence shows binding to Dockray's antibody equivalent to that of the natural molecule. The human BG sequence has not yet been reviewed by the methods of Hood and Agarwal, but work along this line is in progress.

The final outcome of this story would have given Kenner the greatest satisfaction; for its shows, as he strongly believed, that synthesis is an essential test of postulated structure. It shows also, as he greatly appreciated, that the production of region-specific antibodies to different parts of a molecule can provide a most powerful means of testing

by radioimmunoassay the correspondence of natural and synthetic products, particularly where the available amount of the natural material is very small.

MINIGASTRIN

Tracy and I (*25*) discovered in gastrinoma tissue very small amounts of a pair of gastrins (sulfated and unsulfated) which were potent stimulants of gastric secretion and on Sephadex filtration were clearly smaller than LG. The quantitative amino acid analyses made for us, together with other evidence, led us to conclude that they were the COOH-terminal tridecapeptide amides of LG, viz., Leu–Glu_5–Ala–Tyr–Gly–Trp–Met–Asp–Phe-NH_2; they were given the trivial name "minigastrin" (MG).

However, when the tridecapeptide amide which had kindly been synthesized for us by Dr. J. S. Morley of Imperial Chemical Industries Ltd., was compared with the natural product by our colleague Dr. M. I. Grossman of Los Angeles, there was revealed a considerable difference in physiological activity, the synthetic material being apparently about twice as potent as the natural product. It was suggested that there was an error in the postulated structure, which (as in the later case of BG) had only been revealed by synthesis and comparison with the natural product, and was to be explained as follows. It is customary to dispense pure gastrin and gastrin-like peptides on the basis of their absorbance at 280 nm in aqueous solution, which can be calculated from the tyrosine and tryptophan content and is chiefly due to the latter. It was argued that if minigastrin were in reality the COOH-terminal *tetra*decapeptide of LG, it would contain 2 Trp and the actual amount of the natural material so dispensed would be only about half that of the synthetic tridecapeptide, containing 1 Trp, hence the apparent difference in potency. Harris kindly determined for us the NH_2-terminal residue of the very small amount of natural minigastrin left by that time, and established that it was tryptophan (and the next residue leucine). The correct structure of human minigastrin was thus established as:

$$\text{Trp–Leu–Glu}_5\text{–Ala–Tyr}(\text{R})\text{–Gly–Trp–Met–Asp–Phe-NH}_2 \qquad (\text{R} = \text{H or } SO_3\text{H}).$$

"True" minigastrin was synthesized by Kenner and co-workers and proved to have the expected potency (*26*).

Minigastrin is beyond reasonable doubt a minor circulating form of the hormone in man; Rehfeld *et al.* (*27*) found in the sera of patients with the Zollinger–Ellison syndrome or pernicious anemia and also in normal subjects after meals a pair of immunoreactive components which corresponded in position on Sephadex filtration to samples of natural material provided by us. Small amounts have also been identified immunologically in antral mucosa and serum of pig, dog, and man. However, in the cat the situation is different. The major circulating immunoreactive forms of gastrin correspond in size to LG and MG (*28*, *29*) and in contrast to other species there is only a very small amount of BG in circulation; the antral mucosa contains BG, LG, and MG immunoreactivity in about the same proportions as in man, dog, and pig. Circulating MG appears to arise in the circulation from LG by enzyme activity (*29*); Tracy and I in work so far unpublished have incubated pure porcine LG (5–10 mg) in cat plasma or

serum and have isolated from the digest minigastrin (the NH_2 terminus of which was checked by Harris) and unchanged LG.

BIG, BIG, GASTRIN

Yalow and Berson (*30*) reported the presence in human serum and in simple aqueous extracts of jejunal mucosa of an immunoreactive component which emerged in the void volume on Sephadex G50; they named it "big, big, gastrin" (BBG). On tryptic digestion rather miscellaneous immunoreactive material of smaller size appeared to be formed. The amount of BBG found in serum from fasting individuals was not increased by feeding; the MW was estimated by ultracentrifugation to be about 20 000 (*31*). It was also reported that similar material could be identified immunologically in partially purified extracts of antral mucosa and gastrinoma tissue. However, the subsequent studies of Rehfeld (*32*) and others have brought strong evidence to show that serum and mucosal BBG is an artifact, but that immunoreactive material of the size of BBG may be present in gastrinoma tissue.

COMPONENT I

Rehfeld and Stadil (*33*) identified in serum samples which were filtered on very long columns of Sephadex G50 so as to obtain great resolution, an immunoreactive component emerging slightly in advance of BG, but substantially later than the void volume. On tryptic digestion it was converted into material of the size of LG; they used a COOH-terminal antibody, so it would appear likely that CI is a peptide longer than BG by extension at the NH_2 terminus, and probably has some physiological activity. It has been partially purified by Tracy (unpublished studies) and by Rehfeld *et al.* (*27*) but has not yet been isolated.

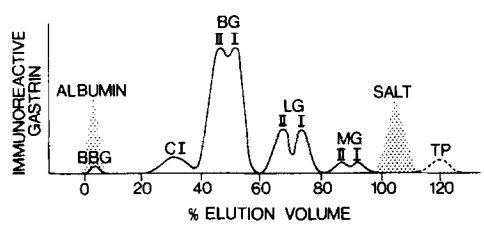

FIG. 2. Schematic elution profile showing position and approximate relative amounts of gastrin components as they might be seen after gel filtration of human postcibal serum on Sephadex G50. The tetrapeptide (TP) is included to show its position in the elution profile (not to scale).

Figure 2 shows the various immunoreactive gastrins (BBG, CI, BG, LG, MG and tetrapeptide) as they would appear in an elution profile after gel filtration on Sephadex G50 superfine.

THE NH_2-TERMINAL HEPTADECAPEPTIDE FRAGMENT

In 1967, Tracy and I (*17*), while preparing large batches of porcine LGs, discovered the presence in them of small amounts of a pair of physiologically inactive peptides

which proved to be the NH_2-terminal tridecapeptide fragments of the porcine LGs (sulfated and unsulfated), viz.

$$\text{Pca–Gly–Pro–Trp–Met–Glu}_5\text{–Ala–Tyr–Gly} \qquad (R = H \text{ or } SO_3H).$$

The peptide was ninhydrin-negative, and COOH-terminal glycine was confirmed by hydrazinolysis. What had been removed, presumably by enzymatic cleavage, was the active COOH-terminal tetrapeptide amide; our efforts at the time to find this important fragment in the extracts did not succeed. More recently, Dockray and Walsh (*34*) obtained an antibody reacting specifically with the NH_2-terminal region of LG. Using this antibody together with another reacting to the COOH-terminal region of gastrin in a double radioimmunoassay, they were able to identify, in the circulation of normal subjects and also in that of patients with the Zollinger–Ellison syndrome, an immunoreactive component corresponding exactly to the natural fragment we supplied to them.

THE COOH-TERMINAL TETRAPEPTIDE AMIDE

The physiological properties and structure–function relationships of the amide, the minimal fragment of the gastrin molecule possessing significant activity, were established by Tracy and Gregory (*35*) using synthetic fragments which became available during the synthesis of LG by Kenner or were made by request. It was first discovered that the synthetic fragment Z–Gly–Trp–Met–Asp–Phe-NH_2, prepared for coupling to an NH_2-terminal sequence, had all the physiological properties of the natural material though in lower potency. Our chemist colleagues then provided, at our request, the tetrapeptide amide, the tripeptide amide, and the tetrapeptide. The first of these was as active as the pentapeptide, the second had virtually no activity, and the third was completely inactive. We then showed that oxidation of methionine in the tetrapeptide amide also inactivated it, although we did not succeed in reducing the methionine and so restoring the activity. Later, Agarwal kindly synthesized desamido-LG for us, which as expected proved to be completely inactive, and incidentally proved of great value for characterizing the reactivity of COOH-terminal antibodies. Later in Kenner's program of synthesis it was possible to establish that the 1–13 sequence of the LG molecule had no intrinsic physiological activity. Structure–function studies of 33 analogs of the tetrapeptide amide by Morley *et al.* (*36*) led to the choice of BOC-β-Ala–Trp–Met–Asp–Phe-NH_2 as a stimulant of gastric acid secretion for clinical use in place of histamine, later marked as "Pentagastrin." A later study by Morley (*37*) of more than 500 analogs of the tetrapeptide further defined the structure–function relations in this simple molecule.

Present interest in the tetrapeptide amide derives chiefly from the possibility that it may exist as an independent entity in the antral mucosa and in the circulation; it has also been suggested recently that it may function as a neurotransmitter in the nervous system (*38*). The only evidence at present for such speculations is (1) the fact that the NH_2-terminal fragment of LG, which would result from the removal of the tetrapeptide amide, has been isolated from antral mucosa and also identified immunologically in the circulation, and (2) the fact that when simple aqueous extracts of antral mucosa or

brain are filtered on columns of Sephadex G50 and the effluent fractions monitored by a COOH-terminal radioimmunoassay, there can be detected a very small peak of gastrin-like immunoreactivity in a position corresponding to that which would be occupied by a small molecule of the size and composition of the tetrapeptide (Fig. 2). Whether this material is in fact the tetrapeptide or whether it is an artifact has not yet been satisfactorily established; whether there is any tetrapeptide to be found in the circulation must await the development of a sensitive radioimmunoassay, for all the gastrin antibodies so far obtained show relatively poor reactivity to the small tetrapeptide molecule.

THE NH_2-TERMINAL FRAGMENT OF BG ("TRYPTIC PEPTIDE")

At present very little that is based on experimental evidence can be said about the biosynthesis of gastrin. The reason for this lies in the difficulty experienced in attempting to apply to this problem the methods which have proved conspicuously successful in other cases. For instance, partly because of the heterogeneity of the cell population in antral mucosa and the physical character of the tissue, and partly for reasons not yet clear, attempts to obtain preparations of surviving "gastrin cells" which will manufacture new gastrin and release it in response to appropriate stimulation have met with little or no success. Again, the classical study of Steiner *et al.* (*39*) using insulinoma slices, which led to the discovery of proinsulin, might well be repeated with gastrinoma tissue, but for clinical reasons such tumors are now seldom removed. The extraction and translation of mRNA from antral or gastrinoma tissue has not been reported; but there is now good reason to expect that important progress along this line may soon result from the nucleotide sequencing studies of Noyes *et al.* (*24*).

In the meantime, by analogy with what is known of the biosynthesis of other peptide hormones (*40*), it seems highly probable that Component I may be regarded as the "pre-pro" form of the hormone, giving rise to BG, the "pro" form, which in turn releases LG by a tryptic cleavage at the two lysine residues in the middle of the molecule. The NH_2-terminal "tryptic peptide" (or peptides) thus released can be detected by Dockray's NH_2-terminal-specific BG antibody; we have found that antral extracts contain amounts of tryptic peptide which are, as would be expected, comparable with those of the LG present. Isolation of the material is well advanced. Finally, the fact that the NH_2 terminus of BG is pyrollidone carboxylic acid, like that of LG, obviously suggests that BG has been cleaved from a larger molecule (probably Component I) in a manner similar to the derivation of LG from BG, i.e., by a tryptic-like cleavage on the COOH-terminal side of a pair of basic amino acids.

PORCINE BG III (?NH_2-TERMINAL 1–30 FRAGMENT)

When BG was being isolated from porcine antral mucosa (*24*), the first stage at which the two major forms of the hormone were resolved was when the partially purified extract was fractionated on aminoethylcellulose (AE). There were in fact three components having uv absorption at 280 nm; the first two were associated with gastrin activity, and from them were isolated BG I and II. The third component was inactive; and although it was suspected that it might represent some form or fragment of the hormone, attempts to isolate gastrin-like material from it were a total failure.

However, when the production of porcine BG was recently resumed, it was possible to guide the purification by region-specific radioimmunoassays of both the NH_2 and COOH termini of the gastrin peptides present. When this was done at the stage of AE fractionation, the result was unequivocal; the first two peaks reacted with both antibodies, but the third peak reacted only with the NH_2-terminal antibody and not at all with the COOH-terminal antibody. This can only mean that BG III, as it is provisionally called for the present, has an NH_2-terminal region which is indistinguishable from that of BG I or II, but a COOH-terminal region which is altered or defective in some way. The isolation of BG III is now almost complete; it is physiologically inactive, has a stronger charge than BG II (from its position on AE), and is slightly smaller as indicated by its position on Sephadex G50. These observations, taken together, suggest that BG III may be a big gastrin lacking a short COOH-terminal segment; the obvious possibility is that it lacks the COOH-terminal tetrapeptide amide and is thus the "BG" counterpart of the NH_2-terminal fragment of LG.

VARIANTS OF LG AND BG IN ANTRAL AND GASTRINOMA TISSUES

In extracts of antral and gastrinoma tissue we have found small amounts of little and big gastrins in addition to the two major forms (sulfated and unsulfated) previously described in this account. Thus, during the first isolation of human BG from gastrinoma tissue there was clearly present at the stage of chromatography on AE a third LG and a third BG (*19*); these were physiologically active and showed amino acid analyses typical of LG and BG, respectively. Again, during a later isolation of LG and BG from a massive gastrinoma, two minor LG components were isolated in small amount in addition to the usual LG I and II. They had the typical amino acid composition of LG and the tyrosine was sulfated in each case; for this reason we named them LG IIA and IIB (*24*). How these minor components differ from the major forms of LG and BG is not known; they might well be dismissed as probably of artifactual nature but for the remarkable study of Rehfeld *et al.* (*41*), which indicates that the circulating forms of CI, BG, LG, and MG are highly heterogeneous. They obtained serum containing a large amount of immunoreactive gastrin from a patient with the Zollinger–Ellison syndrome and filtered it on a large column of Sephadex G50, so as to obtain fractions of effluent corresponding to CI, BG, LG, and MG. These fractions were concentrated and rerun on large high-resolution columns; the procedure was repeated three times for each gastrin peak and the columns monitored throughout by radioimmunoassay. It was possible to resolve the different forms of gastrin as follows: There were six component I's, six big gastrins, four little gastrins, and four minigastrins. These would include of course the sulfated and unsulfated forms in each case. Very probably, the other differences in the subforms or variants are trivial and of little importance; but their existence cannot be ignored in any account of the heterogeneity of the hormone.

GASTRIN-LIKE PEPTIDES IN GUT AND NERVOUS SYSTEM

The duodenal hormone cholecystokinin (CCK), which causes contraction of the gallbladder, was isolated in 1966 from porcine material by Mutt and Jorpes (*43*). It was

later shown to be a basic peptide of 33 residues having the same active COOH-terminal pentapeptide amide sequence as gastrin (Fig. 3). As would be expected, the two hormones have an identical range of physiological actions, although their relative potency with respect to these varies widely. The CCK molecule contains two lysines and three arginines, which are potential sites of trypsin-like cleavage; of the numerous fragments which might thus be produced, the best known is the COOH-terminal octapeptide amide (CCK8). Since the sulfated tyrosine residue is one of the structural features essential for the characteristically powerful effect of CCK on the gallbladder, CCK8 is almost the minimal active fragment. It is available commercially in synthetic form, has all the actions of the total molecule, and as a matter of fact is considerably more potent than the latter. Because pure natural CCK is in extremely short supply and

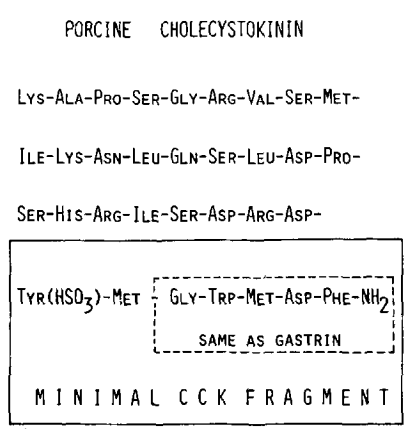

FIG. 3. Amino acid structure of cholecystokinin showing the sequence common to gastrin and the minimal sequence having characteristic CCK activity.

the entire molecule has not yet been synthesized, the octapeptide amide has been widely used for physiological studies and as a stimulant of gallbladder contraction in clinical practice. Until very recently there was a lack of good evidence for the independent existence of any fragment of CCK in tissues or circulation; but it now seems clear that CCK8 and very probably other forms of the hormone must be added to the group of peptides of established or putative hormonal status which are found both in nervous tissue (central and peripheral) and in gastrointestinal endocrine cells; these are substance P, stomatostatin, neurotensin enkephalin, vasoactive intestinal peptide, bombesin, and gastrin.

In 1975, Vanderhaegen *et al.* (*44*) reported the presence in brain tissue, particularly the cerebral cortex, of gastrin-like immunoreactivity; but Dockray (*45*) showed that the pattern of cross-reactivity was that of a COOH-terminal fragment of CCK rather than gastrin, and gel filtration studies indicated that the main component corresponded to CCK8. He also identified immunoreactivity corresponding to CCK8 in intestinal extracts (*46*). In both brain and gut extracts there were identified smaller amounts of immunoreactive material corresponding to: (1) intact CCK, (2) the COOH-terminal dodecapeptide, and (3) the COOH-terminal tetrapeptide. Similar studies, leading to similar conclusions, were made independently by Rehfeld (*47*). The latter investigator

also showed that there is a small amount of gastrin in the pituitary gland and that CCK8 is most abundant in the postcentral gyrus and temporal lobe of the cerebral cortex.

We decided to attempt to isolate the CCK8-like material from both brain and gut, and have completed the first part of the work (*9*). Sheep brains were extracted as for gastrin, monitoring the procedures by a radioimmunoassay for CCK8. Purification by chromatography, column electrophoresis, and gel filtration led to the resolution on columns of DEAE-cellulose of one minor and two major components, SB I, II, and III Fig. 4). SB II and III were each obtained in yields of 150–300 μg from 100 kilograms

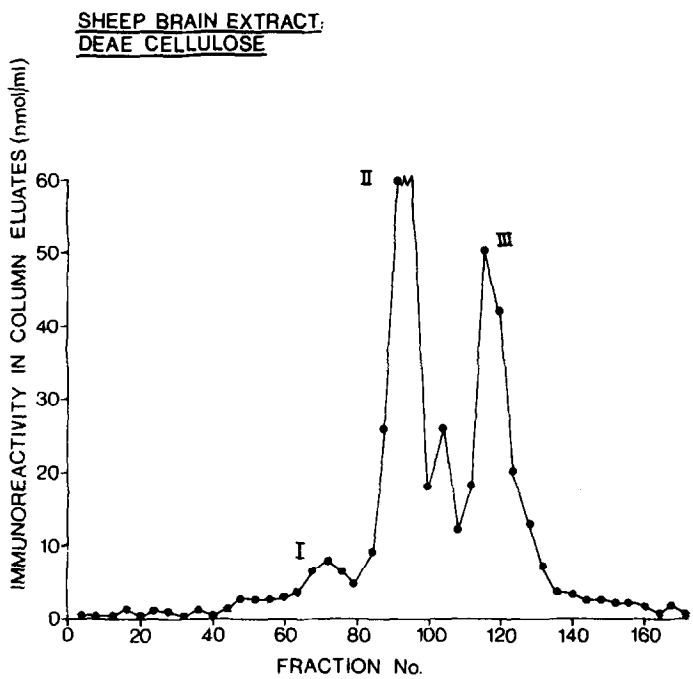

FIG. 4. Separation of SB I, II, and III from a partially purified extract of sheep brain by gradient elution on a column of DEAE-cellulose.

fresh brains; the yield of I, which was not homogeneous, was only about 50 μg. SB II and III were shown to possess the characteristic physiological activities on gallbladder and pancreas of CCK8. Sequence studies by Harris and Runswick of the MRC Laboratory of Molecular Biology, Cambridge, established that SB III was authentic CCK octapeptide amide and was indistinguishable from synthetic material kindly provided by Dr. M. Ondetti of the Squibb Institute. SB II had an identical amino acid sequence, including COOH-terminal phenylalanine-amide (identified as DNS-Phe-NH_2 after seven cycles of Edman degradation) and sulfated tyrosine (identified by amino acid analysis following enzymatic hydrolysis), but was less acidic than SB III and was easily separated from it by DEAE chromatography, electrophoresis at pH 10, and tlc. The nature of the difference between SB II and SB III remains unknown; the corresponding peptides are being prepared in larger amounts from ox brain, and it is hoped that this

will lead to elucidation of the difference. The remaining part of the study, namely, the isolation of the corresponding peptides from the intestine, is well advanced. The amount of the peptides in hog intestine is much less than that in brain and as many as four immunoreactive CCK8-like components have been separated upon chromatography on DEAE cellulose.

The functional role of CCK8 in gut and brain is at present unknown; but in the latter case it is tempting to speculate that it may have some role in the nature of a neurotransmitter. However, Rehfeld (*47*), recognizing the presence in brain extracts of what may be the COOH-terminal tetrapeptide amide, has suggested that this may in fact be "the principal chemical transmitter in the family of CCK- and gastrin-like peptides, and the larger forms may merely be biosynthetic precursors." It is likely to be very difficult to identify chemically the tetrapeptide amide in brain tissue; but it may be easier to find and locate an enzyme capable of effecting the specific cleavage of the tetrapeptide from a larger molecule, and perhaps also to find and locate the deamidase which would be the obvious mechanism for rapid inactivation of the tetrapeptide amide following its release and action.

Finally in the light of these speculations on the role of gastrin-like peptides in the nervous system, it is of particular interest to note that immunoreactive gastrin has been identified in the vagus nerves of cats, dogs and humans by Uvnäs-Wallensten *et al.* (1977, *48*); no CCK-like material was found. The abdominal portion of the nerve contained the gastrin, which was identified as chiefly the heptadecapeptide, with a very small proportion of big gastrin and a still smaller amount of Component I; the thoracic and cervical portions of the nerve contained only minute amounts of gastrin. The total amount present was 16–73 *p*mol per gram fresh nerve. In addition, the nerve contained detectable amounts of the NH_2-terminal tridecapeptide fragment of LG.

REFERENCES

1. W. M. Bayliss and E. H. Starling, *J. Physiol.* (*London*) **28**, 325 (1902).
2. J. S. Edkins, *Proc. Roy. Soc. Lond. Ser. B* **76**, 376 (1905).
3. R. A. Gregory and H. J. Tracy, *J. Physiol.* (*London*) **149**, 70P (1959).
4. R. A. Gregory, "Surgical Physiology of the Gastro-Intestinal Tract" (Symposium), pp. 57–70 (A. N. Smith, Ed.). Royal College of Surgeons, Edinburgh.
5. R. A. Gregory, *Harvey Lect. Ser.* **64**, 121 (1970); R. A. Gregory, *J. Physiol.* (*London*) **241**, 1 (1974); J. H. Walsh and M. I. Grossman, *N. Engl. J. Med.* **292**, 1324, 1337 (1975).
6. R. M. Zollinger and E. H. Ellison, *Ann. Surg.* **142**, 709 (1955).
7. R. A. Gregory, H. J. Tracy, J. M. French, and W. Sircus, *Lancet* **1**, 1045 (1960).
8. R. A. Gregory, H. J. Tracy, K. L. Agarwal, and M. I. Grossman, *Gut* **10**, 603 (1969).
9. G. J. Dockray, R. A. Gregory, J. I. Harris, J. B. Hutchinson, and M. J. Runswick, *Nature* (*London*) **274**, 711 (1978).
10. H. Gregory, P. M. Hardy, D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Nature* (*London*) **204**, 931 (1964).
11. R. A. Gregory and H. J. Tracy, *Gut* **5**, 103 (1964).
12. J. C. Anderson, M. A. Barton, R. A. Gregory, P. M. Hardy, G. W. Kenner, J. K. MacLeod, J. S. Morley, J. Preston, and R. C. Sheppard, *Nature* (*London*) **204**, 933 (1964).
13. R. A. Gregory, H. J. Tracy, and M. I. Grossman, *Nature* (*London*) **209**, 583 (1966); K. L. Agarwal, J. Beacham, P. H. Bentley, R. A. Gregory, G. W. Kenner, R. C. Sheppard, and H. J. Tracy, *Nature* (*London*) **219**, 614 (1968); R. A. Gregory, H. J. Tracy, M. I. Grossman, D. de

VALOIS, AND R. LICHTER, *Experientia* **25**, 345 (1969); K. L. AGARWAL, G. W. KENNER, AND R. C. SHEPPARD, *J. Amer. Chem. Soc.* **91**, 3096 (1969).
14. G. W. KENNER AND R. C. SHEPPARD, *Proc. Roy. Soc. London Ser. B* **170**, 89 (1968).
15. K. L. AGARWAL, G. W. KENNER, AND R. C. SHEPPARD, *Experientia* **25**, 345 (1969).
16. K. L. AGARWAL, G. W. KENNER, AND R. C. SHEPPARD, *J. Amer. Chem. Soc.* **91**, 3096 (1969).
17. R. A. GREGORY, *J. Physiol. (London)* **241**, 1 (1974).
18. R. S. YALOW AND S. A. BERSON, *Gastroenterology* **60**, 203 (1971).
19. R. A. GREGORY AND H. J. TRACY, *Lancet* **2**, 797 (1972).
20. R. F. DOOLITTLE, "Methods in Enzymology," Vol. 25, pp. 231–244 (S. P. Colowick and N. O. Kaplan, Eds.). Academic Press, New York, 1972.
21. R. A. GREGORY AND H. J. TRACY, "International Symposium on Gastrointestinal Hormones 1974," pp. 13–24 (J. C. Thompson, Ed.). Univ. of Texas Press, Austin, 1975.
22. A. M. CHOUDHURY, G. W. KENNER, S. MOORE, R. RAMAGE, P. M. RICHARDS, W. D. THORPE, L. MORODER, G. WENDLBERGER, AND E. WÜNSCH, "Proceedings, 14th European Peptide Symposium, Belgium, 1976," pp. 257–261. Editions de L'Université de Bruxelles, 1977.
23. G. J. DOCKRAY, R. A. GREGORY, AND G. W. KENNER, *Gastroenterology* **75**, 556 (1978).
24. B. N. NOYES, M. MEVARECH, R. N. STEIN, AND K. L. AGARWAL, *Proc. Nat. Acad. Sci. U.S.* **76**, 1770 (1979).
25. R. A. GREGORY AND H. J. TRACY, *Gut* **15**, 683 (1974).
26. R. A. GREGORY, H. J. TRACY, J. I. HARRIS, M. J. RUNSWICK, S. MOORE, G. W. KENNER, AND R. RAMAGE, *Hoppe-Seyler's Z. Physiol. Chem.*, **360**, 73 (1979).
27. J. F. REHFELD, F. STADIL, AND J. VIKELSØE, *Gut* **15**, 102 (1974).
28. K. UVNÄS-WALLENSTEN AND J. F. REHFELD, *Acta Physiol. Scand.* **98**, 217 (1976); J. F. REHFELD AND K. UVNÄS-WALLENSTEN, *J. Physiol. (London)* **283**, 379 (1978).
29. E. L. BLAIR, E. R. GRUND, P. K. LUND, AND D. J. SANDERS, *J. Physiol. (London)* **273**, 561 (1977).
30. R. S. YALOW AND S. A. BERSON, *Biochem. Biophys. Res. Commun.* **48**, 391 (1972).
31. R. S. YALOW AND N. WU, *Gastroenterology* **65**, 19 (1973).
32. J. F. REHFELD, *Gastroenterology* **73**, 469 (1977).
33. J. F. REHFELD AND F. STADIL, *Gut* **14**, 369 (1973).
34. G. J. DOCKRAY AND J. H. WALSH, *Gastroenterology* **68**, 222 (1975).
35. H. J. TRACY AND R. A. GREGORY, *Nature (London)* **204**, 935 (1964).
36. J. S. MORLEY, H. J. TRACY, AND R. A. GREGORY, *Nature (London)* **207**, 1356 (1965).
37. J. S. MORLEY, *Proc. Roy. Soc. London Ser. B* **170**, 97 (1968).
38. J. F. REHFELD, *J. Biol. Chem.* **253**, 4022 (1978).
39. D. F. STEINER, D. CUNNINGHAM, L. SPIGELMAN, AND B. ATEN, *Science* **157**, 697 (1967).
40. M. A. PERMUTT AND A. ROUTMAN, *Biochem. Biophys. Res. Commun.* **78**, 855 (1977).
41. J. F. REHFELD, F. STADIL, J. MALSTRØM, AND M. MIYATA, "International Symposium on Gastrointestinal Hormones 1974," pp. 43–58 (J. C. Thompson, Ed.). Univ. of Texas Press, Austin, 1975.
42. J. E. Jorpes, *Gastroenterology* **55**, 157 (1968).
43. V. MUTT AND J. E. JORPES, *Eur. J. Biochem.* **6**, 156 (1968).
44. J. J. VANDERHAEGHEN, J. C. SIGNEAU, AND W. GEPTS, *Nature (London)*, **257**, 604 (1975).
45. G. J. DOCKRAY, *Nature (London)* **264**, 568 (1976).
46. G. J. DOCKRAY, *Nature (London)* **270**, 359 (1977).
47. J. F. REHFELD, *J. Biol. Chem.* **253**, 4016, 4022 (1978).
48. K. UVNÄS-WALLENSTEN, J. F. REHFELD, L.-I. LARSSON, AND B. UVNÄS, *Proc. Nat. Acad. Sci. USA* **74**, 5707 (1977).