

Peptide-secreting adenomata with special reference to the Zollinger-Ellison syndrome

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FOR 25 YEARS an association between the pancreas and peptic ulceration has been known, has fascinated but remains ill-understood (Dragstedt, 1942). The experimental formation of an external pancreatic fistula in dogs always results in the development of a duodenal ulcer. When instead, the main and accessory pancreatic ducts are ligated, a duodenal ulcer forms in only one-third of the animals. On the other hand, total extirpation of the pancreas is rarely followed by the development of duodenal ulcer. This association between the pancreas and peptic ulcer was highlighted when, 12 years ago, the syndrome of the occurrence of a non-beta-cell tumour of the pancreas with intractable peptic ulceration and massive gastric hypersecretion was detailed by Zollinger & Ellison (1955).

With the passing of time, however, it has become apparent that this syndrome is only one of a number resulting from the growth of adenomata in tissues which have developed out of the undifferentiated mesodermal cells of the primitive coelom of the embryo. Thus these adenomata, benign or malignant, are found in the pancreas, in the upper alimentary tract, in bronchi, thyroid and parathyroid glands. Each adenoma is characterized by the capacity for the production of one or more substances recognizable as amino acid groups or polypeptides which are physiologically active. They may be elaborated both sequentially and simultaneously. Thus far among the agents recognized have been gastrin, adrenocorticotrophic-like substance, parathyroid-stimulating substance, tryptamine, 5-hydroxytryptophan, bradykinin, blood glucose reducing agents, antidiuretic substance, thyroid gland stimulating substance and a glucagon-like agent. From this list it is clear that a number of substances, which have a demonstrable action in the body, have not yet been chemically identified. Indeed it was only very recently that, by

study of the amino-acid constitution of material extracted from a tumour in a case of Zollinger-Ellison syndrome that the gastric secretagogue substance elaborated by these tumours has been identified as having the structure of the naturally occurring antral gastrin (Gregory *et al.*, 1967). It is probable that many if not all, of the other agents will be shown to be of the same chemical constitution as hormones elaborated in the normal body and the action of which as they present they appear to mimic.

Clinical syndromes

The clinical syndromes in the spectrum of the group of diseases which I have suggested should be known as peptide-secreting adenomata (Sircus, 1967) represent the uncontrolled activity of these various substances. In view of the multipotentiality of production of these peptides by any one adenoma, the clinical syndromes in a particular patient often undergo variation with the passing of time. Thus one subject with a metastasized pancreatic adenoma in my care, presented in the first instance with a profound diarrhoea, largely the product of a continuous flow of gastric juice from the unstimulated stomach containing up to 60 mEq of hydrogen ions every hour. The pancreatic islets were destroyed by the progression of the disease and she became diabetic. Subsequently an insulin-like activity appeared and she suffered in place of the diabetes, from profound spontaneous hypoglycaemia. Later she experienced intense flushing and borborygmi found to be associated with excessive production of bradykinin and hydroxytryptamine. Finally and just before her death, when this patient was weighing only 35 kg she was consuming 4000-5000 calories every 24 hr as a result of hyperphagia which was not altered by intravenous infusion of glucose and may possibly have been due to the production of a

substance augmenting metabolism and perhaps adrenocorticotrophic.

One of the most challenging aspects of these tumours is the consideration of the significance of the multipotentiality for production of polypeptides. Every cell in the body has the same information coded on the same complement of DNA. Why each cell does not continuously turn out biologically active amino acid groups is unknown. One possible explanation is that there is built into cells a repressor system which given a neoplastic process, becomes distorted and permits the unmasking of new coding instructions (Lipsett, 1965). On the other hand, the production of amino acid sequences in the peptide chains which are biologically active may be merely an expression of chance. The rate of production of such molecules is extremely high. For example, the liver cell produces 6000 molecules of albumin per second. Accident alone may possibly, therefore, account for the production of chain lengths with sequences of amino acids that have the action of known hormones. Thus in the naturally occurring hormone gastrin, the end terminal tetrapeptide sequence is adequate to reproduce all the known physiological effects of the total molecule.

Another important aspect is the possibility that the combination of amino acids which are elaborated by the tumours may differ from normally produced and physiologically active peptides in only slight degree, but sufficiently so as to impede the activity of the normal substances. This is suggested as an explanation of the occurrence of a histamine-fast achlorhydria in the presence of morphologically normal parietal cells which is found in some examples of those pancreatic adenomata associated with a potassium-losing watery diarrhoea. That is, the achlorhydria may be the result of the production of an analogue of gastrin which by blocking the receptor sites on the parietal cells effectively impedes the stimulation of hydrogen ion productions.

To return to the Zollinger–Ellison syndrome, the particular one in the spectrum of peptide-secreting adenomata with which we are specially concerned, the pathological morphology in this condition has been found to range from hyperplasia of certain islet-cells in the pancreas with histochemical and microscopic properties which distinguish them from beta and alpha cells, through multiple micro-adenomas, single adenoma, to frankly malignant adenocarcinoma. Often the grouping of the cells in ribbons and rosettes and the considerable scattered fibrous tissue make the appearances difficult to distinguish from those found in carcinoid disease but

for the absence of the characteristic staining. *The nature of the cells* remains undetermined. The most recent studies favour describing them as delta cells and as originating from the clear cells of the epithelium of the main pancreatic ducts (Feyrter, 1953; Becker, 1966).

On the other hand, mixed forms have been reported in which are also β cells producing insulin, and α cells producing glucagon-like substance. The primary tumour may appear as a nodule or mass, superficial to the head of the pancreas or in an ectopic site in the wall of the upper alimentary tract.

Determination of the significance of the tumours in terms of the relationship to the physiological production of gastrin by the human antrum constitutes another challenge. No one has yet succeeded in identifying the cell of origin of gastrin in the antrum.

Up to now, by the time of diagnosis of the syndrome, most subjects have already developed metastases in the liver. However, the tumour and its metastases are fortunately very slow in growth. The first case treated in 1955 by Zollinger & Ellison by a total gastrectomy, is still alive and very well, despite that during a cholecystectomy for cholelithiasis in 1965, another tumour in the tail of the pancreas was revealed. It is for this reason that the condition is always worth a vigorous surgical approach despite the presence of liver metastases.

The adenoma of the pancreas found in this syndrome, often co-exists with endocrine adenomata in other organs when the syndrome alters to the more complex one of multiple endocrine adenomatosis. However, whenever the Zollinger–Ellison syndrome is recognizable within the multiple presentation, then either an adenoma or islet hyperplasia and micro-adenomata are found in the pancreas or at ectopic sites.

Disordered physiology

The disordered physiology has been unfolded step by step over the past five years. In their original communication, Zollinger and Ellison suggested that the tumours might be elaborating some substance which acts upon the stomach to create the gastric hypersecretion. This hypothesis was supported firstly, by the extraction of a gastric secretagogue from a pancreatic adenoma (Gregory *et al.*, 1960) and secondly by the demonstration of a secretagogue in the circulation of a subject with the syndrome by injection of serum into a dog equipped with a fundic gastric pouch (Sircus, 1964a, b). Subsequently this provided

the basis for a diagnostic bio-assay using a modification of the Lai procedure based on the Schild rat preparation (Lai, 1964). In this procedure a semiquantitative assessment is obtained of the acid secretory response to the intravenous injection of plasma from a suspect subject. The standard is provided either by a known amount of gastrin or of peptavlon.

Thus far we have assayed eighteen subjects suspected of having the Zollinger–Ellison syndrome and, where the subsequent clinical enquiry has reached a level of definitive diagnosis, the test has been accurate (Thomson, Sircus & Cleator, 1968). A number of the suspect cases have yet to be subjected to final proof by laparotomy or autopsy. Recently an immunofluorescence assay utilizing antibody developed in rabbits and guinea-pigs by repeated injection of protein-linked tetrapeptide of gastrin has been reported and may well replace the rat bio-assay method as a basis for diagnosis of the disorder (McGuigan, 1967).

Clinical manifestations

The clinical manifestations of the syndrome are largely the result of the pouring into the circulation of the hormone gastrin though, as stated above, additional polypeptide production may well give rise to other features which are not essential to the diagnosis of the syndrome. The most important aspect of the drive upon the stomach resulting from the constant outpouring of gastrin is in its effect upon the parietal cells of the stomach and, in certain cases, on other cells as well. So massive is the hypersecretion in a typical case, that the usual homeostatic forces controlling gastric secretion are overwhelmed. Such patients may produce between 20 and 100 mEq of hydrogen ions in the resting state for each hour of the day and night. Modification of this pattern is frequently a function of the resulting dehydration and hypochloroemia slowing down the activity of the parietal cells until this electrolyte and fluid imbalance is corrected. The acute effects upon parietal cell function are in due course followed by chronic effects, the main one being a trophic effect resulting ultimately in hyperplasia of the parietal cell mass.

In three cases studied in our unit my colleague, Dr G. P. Crean has counted the parietal cell population of the stomachs removed by surgery. In each of the three he found a different situation. In one case there appeared to be selective hyperplasia of the parietal cells, the pepsin cells either being normal or somewhat depressed. The

ratio of parietal to peptic cells was 6 : 1 as opposed to a probable 1 : 1.3 in normal subjects. It is particularly interesting that this patient despite a massive production of hydrogen ions at no time developed peptic ulceration. It may be that there has to be a critical quantitative relationship between the production of hydrogen ions and proteolytic enzymes before proteolytic digestion can occur, which is the case in most of the reported subjects with the syndrome, the oesophagus, duodenum and jejunum as well as the stomach being the site of massive and recurrent and intractable ulceration. The production of hydrogen ions outstrips the output of bicarbonate ion by the pancreas, and gross morphological damage occurs in the duodenum and upper jejunum. In another of our subjects, Dr Crean found that there were three times as many pepsin cells as parietal cells. This patient had had a previous vagotomy and this might have influenced the hyperplasia effect which appears in this subject to have been selective for pepsin cells. Despite the vagotomy she proceeded to recurrent multiple ulceration of the stomach and duodenum. In the third subject who had had both repeated partial gastrectomies and a vagotomy, the ratio of parietal to pepsin cells was equal.

The trophic action on the parietal and peptic cell mass is such that there can occur a thickening of the mucosa in the glandular area resulting in the appearance radiologically mimicking Menetrier's disease.

The basal or resting production of acid is often so high that adding the stimulation of a 'maximal' dose of histamine may not increase the basal production at all or by not more than 60%. This, however, is by no means a pathognomonic feature as sometimes the ratio of maximal acid output to basal may be that found in normal or in duodenal ulcer subjects. Secondary to the outpouring of unbuffered hydrogen ions into the duodenum and upper jejunum the pH of the intraluminal contents is often such as to cause inactivation of lipase and steatorrhoea. The sheer volume of water alone would account for diarrhoea but the addition of a chemical jejunitis, and the probable action of the gastrin molecule upon the motility of the small bowel, explain why it is a frequent and disabling complaint. The extent of water and electrolyte losses in some cases has led to death from inadequately corrected dehydration, hypochloroemia and hypokalaemia. In particular cases additional syndromes such as pseudo-carcinoid, pseudo-Cushing's and pseudo-hyperparathyroidism bring their own particular metabolic problems.

Diagnosis of a case

The diagnosis of a case firstly depends on the recognition of certain criteria which should arouse clinical suspicion (Table 1). Likewise criteria can now be adopted on which certain diagnosis may be founded (Table 2).

TABLE 1
Clinical basis for diagnostic suspicion

'Primary' ulcers of jejunum
Severe peptic ulceration plus gastric rugae hypertrophy
Severe diarrhoea plus gastric rugae hypertrophy
Recurrent peptic ulceration despite apparently adequate surgery
Severe peptic ulceration with recurrent bleeding and perforations
Intractable oesophagitis with peptic ulceration without hiatus hernia
Severe peptic ulceration accompanied by intractable diarrhoea or steatorrhoea
Any of above together with a family history of endocrine disease
Any of above together with clinical evidence of hyperparathyroidism or of pituitary disease
Ulcer or diarrhoea syndromes accompanied by any combination of:
Hypokalaemic myasthenia
Alimentary hypermotility
Skin flushing
Hyperphagia
Spontaneous hypoglycaemia
Hyperthyroidism
Diabetes mellitus
Excess uptake of ⁷⁵ Selenium methionine in pancreatic scan

TABLE 2
Biochemical basis for diagnostic suspicion

Continually aspirated basal acid gastric secretion over 15 mEq/hr
Increment over basal output of acid of maximal stimulation by histamine less than 100%
Failure of partial gastrectomy or/and vagotomy to reduce basal and stimulated secretion by more than 60%
Achlorhydria despite parietal cells in gastric biopsy
Hypoalbuminaemia with faecal loss of excess protein in peptic ulcer subject
Abnormal serum and urine calcium values in peptic ulcer subject
Diarrhoea and steatorrhoea diminish or disappear with gastric aspiration
Positive 'gastrin' test by biological and immunological assay of serum
Abnormal circulating bradykinin and tryptamine values in peptic ulcer subject
Evidence of excessive stimulation of parathyroids, of glucagon-like activity, of thyroid stimulating substance, of glucose reducing substance, of adrenal stimulating substance in special assays of patients' serum

Management

In management for most cases the ultimate treatment will be by total gastrectomy. It may be difficult to know, when a solitary lesion is found in the pancreas, whether or not it has already metastasized to the liver. In removing the stomach, the target organ, the stimulation of which is the mechanism responsible for the death of most patients, is removed.

In the Milauwaukee registers of 450 cases maintained by Ellison (Wilson & Ellison, 1966) 248 operated cases are reported. Where the stomach has been left behind altogether, in an up-to-ten years follow-up, only 27% of 106 cases were alive. If a sub-total gastrectomy had been carried out, 53% of 164 cases were alive. If a sub-total gastrectomy was done in the first place and then a total, 73% of sixty-three cases were alive and, finally, where total gastrectomy was carried out as a first choice, which was in fifteen cases, 87% are still living. Sometimes there are circumstances in which total gastrectomy cannot be entertained. When this is so two possibilities offer some assistance. One is the use of prolonged high dosage anti-cholinergic drugs. There is yet no knowledge as to whether this can bring about effective control for more than a limited period. In one case in the author's care, a vagotomy had been effectively carried out which resulted in a fall of acid gastric secretion to far below normal levels but the effect lasted for only 12 months after which time the further production of gastrin recreated the gastric hypersecretion. Irradiation of the stomach can destroy the parietal and pepsin cells sufficiently to bring about achlorhydria for at least a limited period but there is no knowledge yet as to how long this persists. Usually irradiation effects on the stomach do not totally destroy the mass of parietal and pepsin cells which soon regenerate.

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Appendix

Investigations for suspect peptide-secreting adenomata

The following list of investigations are pertinent to diagnosis and management of suspect cases of peptide secretory adenomata including those presenting as the Zollinger-Elison syndrome. Items marked with a single asterisk are those desirable for contributing to knowledge of the subject but not essential for diagnosis.

Gastric secretion

1. *Unstimulated basal secretion* (Screened intubation with Levine or Portex Ryle's tube)

(a) Continual suction aspiration over 3 hr after discarding resting secretion. Measure volume per hour and titrate acidity in mEq in an aliquot of each hour's collection.

(b) Nocturnal secretion, 20.00 hours to 08.00 hours continuous aspiration. Volume and concentration of total acid measured of each hour's collection. (N.B. Nocturnal secretion testing is conventional but probably adds nothing to 1(a).)

*Part of each aliquot from 1(a) and 1(b) should be buffered as soon as possible to pH 3-4 and stored at -10°C until ready for pepsin assay.

*A further part of each aliquot should be stored in refrigerator for possible determination of albumin content, of intrinsic factor content, and of mucus characteristics.

2. *Stimulated gastric secretion*

The output (Vol. \times Conc.) in response to a 'maximal' dose of:

(a) Histamine acid phosphate
0.04 mg/kg B.W. subcutaneously,

or/and

(b) Peptavlon (pentagastrin, I.C.I.)
6.0 μg /kg B.W. subcutaneously,

collected in each case for 1 hr after previously withdrawing and discarding resting secretion.

*Store an aliquot for determination of Pepsin, albumin and intrinsic factor as described above for unstimulated secretion.

3. *If achlorhydria demonstrated*

(a) Repeat 1 and 2 above after correcting any fluid and electrolyte disturbance by infusions.

(b) Obtain biopsy of gastric mucosa with Crosby capsule or Rubin tube and stain for parietal cells (Marks & Drysdale, 1957)

*If opportunity presents determine subsequently the effect of 20 mg prednisolone daily for 10 days on acid output.

Biochemistry screening

In consideration of association with multiple endocrine adenomatosis.

(i) Parathyroids:

Serum calcium.

Inorganic phosphorus.

Protein electrophoresis.

Urinary 24-hr output of calcium.

Tubular reabsorption of phosphate.

'Parathormone' activity in serum (Treacher, 1966)

Determination in interested laboratory of effect

on blood calcium of experimental animal of intravenous injection of sample of patient's serum.

(ii) Pancreas cell adenoma:

Pre-breakfast blood sugar.

Prolonged (18 hr) fasting blood sugar.

Plasma insulin assay in suitable laboratory (Morgan, 1966).

Tolbutamide test if initial investigation suggestive.

(iii) Carcinoid disease:

5-HIAA urinary excretion (Zeitlin & Smith, 1966).

Tryptamine (Zeitlin & Smith, 1966).

Hydroxytryptophan (Zeitlin & Smith, 1966).

If initial clinical and biochemical evidence is suggestive:

Plasma kinin (10 ml of venous blood, special containers supplied with protocol from Mr I. J. Zeitlin, G.I. Unit Pharmacology Research Laboratory, Western General Hospital, Edinburgh).

(iv) Pituitary disease:

ACTH (Danellis, Lipscomb & Nelson, 1960).
Growth hormone (Morgan, 1966).

(v) Glucagon-producing tumours:

Glucagon (Samols *et al.*, 1963, 1967).

In cases presenting with diarrhoea

For evidence of electrolyte disturbance

Serum potassium.

Total body potassium ^{42}K study where total body counter available.

Serum magnesium.

Serum chloride.

Serum sodium.

Serum albumin.

24 hr urine potassium excretion.

24 hr faecal potassium and magnesium losses.

For evidence of malabsorption

Fat excretion in 3-day stool collection or/and

If steatorrhoea

Isotope labelled-fats absorption study.

Xylose test.

Pancreatic function test.

(a) Secretin-pancreozymin stimulation for vol., HCO_3 , lipase.

(b) Selenium-technetium isotope scanning for uptake by pancreas and liver.

Biopsy of small intestine with capsule or Rubin tube for structural integrity of S.I. mucosa.

If hypoalbuminaemia

B.S.P. test of liver function.

P.V.P. and/or labelled-albumin study for presence of protein-losing gastroenteropathy.

Plasma screening for circulating gastric secretagogue

Remove 40 ml of fasting venous blood, add heparin and centrifuge. Divide the resultant plasma into aliquots of 3 ml each, place in separate containers, pack round with carbon dioxide snow in metal box and despatch by express post or other air or land transport (if under 48 hr travelling time) to centre with biological assay method available. Include statement of clinical basis for diagnostic suspicion.

(Dr W. Sircus, Gastro-Intestinal Unit, Clinical Research Office, Western General Hospital, Crewe Road, Edinburgh 4, Scotland.)

*(The same material could be studied in appropriate laboratories for effect on small bowel motility and of transport of water, electrolytes and other substances through the small bowel mucosa.)

Radiological investigations

Plain film abdomen for evidence of Hepatomegaly.

Calcification in pancreas and suprarenal areas.

Barium meal and follow through; possible findings:

Oesophagitis.

Local or generalized giant hypertrophy of gastric rugae.

Gastric, duodenal, jejunal ulcers (especially second part duodenum).

Distortion of duodenal loop by space-occupying lesion of pancreas.

Ectopic pancreas in duodenum.

Disorganized S.I. pattern.

Ileal ulceration or tumour.

If suspected multiple endocrine adenomatosis

Radiological screening of:

Sella turcica.

Parathyroids/skeletal changes.

Suprarenal delineation studies.

Where available, technetium and selenium scanning of liver and pancreas for identification of tumours.

Alternatively

Selective arteriography of mesenteric vessels.

If hepatomegaly

Liver biopsy and divide into two:

(a) Light microscopy of tissue of one part.

(b) Electron microscopy of other where technique available and if (a) is positive for tumour.

Proven case

(a) If explorative laparotomy reveals tumour—apart from specimen for histological diagnosis—

mass of removed tumour should be stored immediately at -10°C and a laboratory carrying out gastrin extraction procedures contacted regarding possible interest (Professor R. A. Gregory, Department of Physiology, University of Liverpool).

(b) In the event of death of known case presenting as Zollinger–Ellison syndrome—as rapidly as possible (by prior agreement with general practitioner and next-of-kin) remove all primary and secondary tumour masses and store in deep freeze -10°C without delay. Contact laboratory carrying out gastrin extraction procedures and arrange for transport of material.

This final advice relates to the necessity to identify the chemical structure of the 'gastrin-like' material elaborated by these tumours.

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