Ulcerogenic Tumor Syndrome of the Pancreas Associated with a Nongastrin Acid Secretagogue

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Among 30 patients with islet cell neoplasms or hyperplasia who exhibited marked gastric acid hypersecretion and peptic ulceration and/or diarrhea, fasting plasma gastrin concentrations were less than 150 pg/ml in 11 patients, whereas the remaining 19 patients had hypergastrinemia. Plasma extracts from seven of these 11 patients were assayed for acid secretagogue activity in rats. All seven plasma extracts had secretagogue activity that was not found in the plasma extracts of ten patients with ordinary duodenal ulcer disease. Each of the tumor or pancreatic tissue extracts obtained from nine patients exhibited secretagogue activity in rats even though tissue gastrin content was 101.9 pmol (213.8 ng) · g^{-1} or less. The secretagogue activity of the tumor extracts was confirmed in conscious gastric fistula dogs. The tumors' secretagogue activity, in contrast to gastrin, was destroyed by trypsin. It was eluted between porcine motilin and human gastrin I from a Sephadex G-50 (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ) superfine column and was not retained by CM-cellulose, at pH 8.5. Its retention time during reverse phase HPLC on a C_{18} column also differed from those of G17 and G34. Thus, this secretagogue activity appeared mediated by a small, acidic peptide with a molecular size of about 2000 to 3000 daltons. The present study indicates that plasma and tumor extracts of these 11 patients contain a gastric acid secretagogue activity mediated by a nongastrin peptide. We suggest that what may be a distinct clinical entity associated with endocrine neoplasms of the pancreas should be considered in the face of excessive acid hypersecretion without fasting hypergastrinemia.

ALTHOUGH AN ASSOCIATION BETWEEN peptic ulceration and islet cell tumors of the pancreas without hypoglycemia was recognized several decades ago,[1,2] Zollinger and Ellison were the first to indicate that gastric hypersecretion of acid in interdigestive state was a significant pathophysiological factor responsible for the syndrome associated with peptic ulceration and/or diarrhea and nonbeta islet cell tumor of the pancreas.[3] They suggested that a humoral factor released from the tumor could be responsible for gastric acid hypersecretion in these patients.[3] Subsequently the tumors associated with this syndrome were found to contain gastrin.[4–6] With successful development of radioimmunoassay for gastrin it became known that these patients had high circulating gastrin levels[7,8] and the term "gastrinoma syndrome"[9] came into use. However, we recently found that some patients with marked gastric acid hypersecretion associated with islet cell tumors did not have hypergastrinemia but their tumor extracts contained a nongastrin acid secretagogue.[10]

This communication contains our latest observations on the clinical and laboratory features of these patients, as well as the biologic and chemical characteristics of the tumor extracts.

Materials and Methods

Thirty consecutive patients with hypersecretion of acid, peptic ulceration, and/or watery diarrhea were evaluated. Among these 30 patients, the basal acid output of 24 patients with an intact stomach was greater than 15 mEq/hour. In 19 patients, fasting plasma or serum gastrin concentration was 159 pM (334 pg/ml equivalent to human gastrin I) or higher with a mean ± standard error of 370.5 ± 53.2 pM (777 ± 112 pg/ml), and in the remaining 11 patients the gastrin level was lower than 68.2 pM (143 pg/ml). Three diagnostic investigations were routinely performed in these 11 patients including upper gastrointestinal endoscopy, gastric secretory test to determine basal and maximum acid output in response to intramuscular

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injection of pentagastrin, 6 $\mu g \cdot kg^{-1}$ as described previously,[11] and determination of plasma gastrin concentrations in response to intravenous injection of pure porcine secretin (2 clinical units $\cdot kg^{-1}$).[12] In addition, plasma gastrin concentration was determined in response to ingestion of a meal in some of these patients and patients with duodenal ulcer disease. Serial peripheral venous blood samples were obtained in heparinized tubes before and after ingestion of a standard meal consisting of cooked ground beef (60 g), two slices of bread, 150 ml of brewed coffee, and 120 ml of orange juice. Plasma was separated from the whole blood by refrigerated centrifuge at 3000 rpm and was stored at −20 C for later radioimmunoassay of gastrin and secretin.

Radioimmunoassay Procedures

Radioimmunoassay of plasma was carried out as described previously[13] with minor modification. In each incubation mixture, an aliquot of 0.2 ml of plasma sample was incubated in a final volume of 1.5 ml in 50 mM Tris HCl, pH 7.8 containing 0.1% BSA and 0.02% NaN_3 with antigastrin serum (R-5-9a) at a final dilution of 1:250,000. Human gastrin-I was used as standards. The standards (3 to 500 pg/tube) and ^{125}I-gastrin (5000 cpm/tube) was dissolved in 10% charcoal treated hormone-free plasma as described.[13] The reaction mixture was incubated at 4 C for 72 hours, then the bound and free counts were separated by adding plasma- and dextran-coated charcoal.[14] Both bound and free counts were then counted in an LKB model 1274 RIA Gamma Counter (LKB Instruments, Inc., Gaithersberg, MD). The antiserum was highly specific for gastrins and reacted equally well with both sulfated and nonsulfated forms of human G-34 and G-17. Cross reactions with CCKs were low with CCK-33 and CCK-39 cross reacted at 10%, while CCK-8 reacted at 5% of gastrin. Pentagastrin cross reacted by only 2%. Thus, the antiserum was specific for C-terminal region of gastrin. Other unrelated gastrointestinal and pancreatic hormones and peptides, including secretin, VIP, somatostatin, pancreatic polypeptide, neurotensin, peptide YY, motilin, bombesin- or gastrin-releasing peptide, insulin, and glucagon, that were tested did not cross react with the antigastrin serum at all. The 95% confidence assay limit calculated by dividing 2 standard deviation of binding ratio determined at zero unlabeled gastrin (bound to total ratio, B/T; n = 4) by the initial slope of the standard curve of B/T *versus* unlabeled gastrin dose was 1.2 pg/assay tube or 2.8 pM in the plasma.

Radioimmunoassay of secretin was carried out by the ethanol extraction method as described.[15]

Preparation of Plasma Extracts and Tumor Extracts

The plasma samples were extracted on a column of XAD-2 resin (3 ml bed in a 5 ml disposable pipet) in the same manner as described for other peptides,[13–15] and each column was percolated with 2.5 to 5.0 ml of plasma. The combined eluates then were dried and reconstituted in Dulbecco's phosphate buffered saline (DPBS) containing 1% bovine serum albumin for bioassay. Plasma samples were also obtained from 10 patients with ordinary duodenal ulcer disease with normal plasma gastrin and basal acid output of less than 10 mEq $\cdot hr^{-1}$. They were extracted by the same method.

The tumors or pancreatic tissues were obtained from 11 patients, including nine patients with plasma gastrin concentrations less than 68.2 pM (143 pg/ml), one patient with proved gastrinoma whose plasma gastrin concentration exceeded 455 pM (956 pg/ml), and one patient with adenocarcinoma of the pancreas without hypersecretion or hypergastrinemia. In four patients, including three patients with islet cell cancer of the pancreas and the patient with pancreatic adenocarcinoma, tumor tissues were obtained within four hours after they were pronounced dead. In six of the seven remaining patients who had hypersecretion of acid but not hypergastrinemia, tumors or pancreatic tissues were obtained during surgical exploration of the abdomen. At surgical exploration, when examination of the pancreas failed to reveal a tumor, the distal one third of the pancreas was surgically resected for histological examination. Both tissues were also processed for immunohistochemistry of neuron-specific enolase[16,17] and gut peptides as described previously.[10,18] The tissues were processed for extraction of gastric acid secretagogues. The tissues were cut into 1-cm^3 cubes immediately after they were obtained and were boiled in water for ten minutes. The tissue and water extracts were cooled at 4 C, separated, and stored at −60 C until further processing. The tissues were thawed before they were homogenized in five volumes of H_2O with pH adjusted to 9.2 with NH_4OH. Mercaptoethanol was added to 0.1% and the homogenates were stirred for one hour at 4 C and were centrifuged at 29,100 $\times g_{av}$ in a Beckman J2-21 centrifuge (Beckman Instruments Incorporated, Fullerton, California) for 20 minutes at 4 C. The supernates were collected and the pellets were extracted again with five volumes of the alkaline H_2O with mercaptoethanol and centrifuged. The supernates were pooled and freeze dried. The volume of the extracts and the weight of the freeze-dried materials were both recorded. Before bioassay and radioimmunoassay for gastrin, the freeze-dried material was weighed and redissolved in DPBS containing 0.1% BSA.

Bioassays of Tumor and Plasma Extracts

Bioassays in anesthetized rats. After fasting for 24 hours, except for access to drinking tap water, Sprague-Dawley rats weighing 280 to 300 g were anesthetized by intraperitoneal injection of 25% urethane, 0.7 ml/100 g

of weight. As described previously,[10] after one-hour collection of gastric effluent, pentagastrin 100 ng was administered intravenously. If gastric secretion of acid was not increased by pentagastrin, the experiment was discarded. This occurred very rarely. When the gastric acid secretion returned to the level before pentagastrin injection for 30 minutes, a tumor extract equivalent to 0.4 to 0.5 g wet weight of tumor tissue or plasma extract equivalent to 5 to 8 ml of plasma was administered intravenously to study acid secretory response. For determination of acidity, 2 ml of each ten-minute gastric effluent was added to 20 ml of deionized water in a plastic cup and was stirred. Using an automatic titrator (Fisher Titrimeter II Titration System; Fisher Scientific Company, Pittsburgh, Pennsylvania), the acidity of the diluted perfusate was titrated with a standard solution of 0.01 N NaOH using an endpoint pH of 7.4. The rate of acid secretion was expressed in microequivalents per ten minutes. Acid secretagogue activity was defined as follows; 1 unit is equal to 50%, 2 units 100%, 3 units 150%, 4 units 200%, 5 units 250%, 6 units 300%, and so on, of the acid output per hour produced by intravenous injection of 100 ng of pentagastrin.

Bioassays in conscious dogs. In three dogs prepared with gastric cannulas,[19] tumor extracts obtained from islet cell carcinomas of two patients (Patients 8 and 9) were tested to determine whether the extracts stimulate gastric secretion of acid in this species. The tumors were obtained during abdominal exploratory laparotomy. After 18 hours fasting, the dogs were placed on Pavlov stands. The gastric cannulas were opened and were gently irrigated with lukewarm tap water. A small plastic catheter was placed in a peripheral vein of a foreleg and was kept open by slow infusion of 0.15 M NaCl at a rate of 0.6 ml per minute. After gastric juice was collected continuously in a plastic tube in 15-minute samples, either pentagastrin or tumor extracts were administered intravenously and the collection of gastric juice was continued. The acidity of gastric juice was determined by titration of an aliquot of 0.5 ml with a standard solution of 0.05 N NaOH using end point pH of 7.4. The acid output was expressed in milliequivalent per 15 minutes.

Partial Purification of Acid Secretagogue Activity from the Tumor Extract

The tumor extract with acid secretagogue activity was partially purified successively by gel filtration, ion exchange chromatography, extraction with C_{18} SEP-PAK cartridge, and reverse-phase high performance liquid chromatography (HPLC). The gel filtration step was carried out by percolating 2.8 ml of sample containing 84 mg of the freeze-dried tumor extract (equivalent to 2.5 g tumor) through a column (3 × 34 cm) of Sephadex G-50 (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ), superfine packed and run in 2% ammonium bicarbonate solution. Fractions of 2.8 ml were collected and the presence of peptide or protein in each was monitored by absorbance at 220 nm and 280 nm. An aliquot of 0.5 ml was taken from each fraction and aliquots from every three fractions were pooled and freeze-dried before being redissolved in 1 ml of DPBS/BSA for bioassay. Fractions containing acid secretagogue activity were then pooled and freeze-dried. The sample (5 mg) was redissolved in 20 mM NH_4HCO_3 and then applied onto a column of carboxymethyl cellulose (CM-cellulose; CM-32, Whatman, 0.9 × 18 cm) equilibrated in 20 mM NH_4HCO_3, pH 8. The column was washed with 30 ml of the same buffer before being eluted with a linear gradient of ammonium bicarbonate from 20 to 500 mM, 100 ml in each reservoir. Fractions of 3.3 ml were collected and monitored for protein at A_{220} nm. The bioactivity was not retained by the column and was eluted by 20 mM NH_4HCO_3. These fractions were pooled and freeze dried. The freeze-dried material was then dissolved in 2 ml H_2O and subjected to extraction on a C_{18} SEP-PAK cartridge (Waters Assoc., Milford, MA). Before application of the sample, the SEP-PAK cartridge was washed with 10 ml isopropanol followed by 5 ml of 2-propanol/acetonitrile/0.1 M triethyl-ammonium formate pH 3 (5/2/3,v/v/v), which is the solvent B of HPLC, and then with 10 ml of H_2O. The sample was allowed to percolate through the cartridge, washed twice with 3 ml of H_2O, and eluted with 5 ml of 60% solvent B in H_2O (v/v) followed with 5 ml of solvent B. The bioactivity was found to be located only in the 60% solvent B eluate, which was then lyophilized. The material was redissolved in 35% solvent B in H_2O and was subjected to reverse-phase HPLC on a Varian MCH-10 C_{18} (Varian Assoc., Walnut Creek, CA) column (4 × 300 mm). The column, run at 0.5 ml/minute, was eluted for 10 minutes isocratically with 35% solvent B, increased to 40% B over the next 50 minutes, then raised to 47% B in 5 minutes and held at that concentration for 30 minutes before elevating to 100% B in 10 minutes. This system resolved most of the gut peptides and separated peptides capable of stimulating gastric acid secretion in anesthetized rats including CCK8, CCK33, G17, and G34 into well-resolved peaks. Fractions were collected every four minutes (2 ml) and each was freeze-dried before bioassay.

Treatment of Tumor Extract with Trypsin

Fourteen mg (equivalent to 0.42 g tumor) of tumor extract of Patient 8 (Table 1) was dissolved in 2 ml of 40 mM sodium phosphate, pH 8.2, containing 0.1% bovine serum albumin and 150 mM NaCl, and was treated with 250μg of L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (Sigma) for 30 minutes at 37 C. The reaction mixture was then heated at 100 C for ten minutes. After being cooled, 500μg of soybean trypsin

TABLE 1. *Clinical Features, Fasting Plasma Gastrin Concentrations, and Pathologic Condition in Patients with Gastric Acid Hypersecretion but Without Hypergastrinoma*

Patient No.	Age/ Sex	Acid Output (mEq/hr) BAO	Acid Output (mEq/hr) MAO	Clinical Features	Plasma Gastrin (pg/ml)	Pathologic Condition
1*‖	62/M	5.3	12.3	Stomal ulcer, Esophageal ulcer, GI bleeding	109	¶Malignant APUDoma of pancreas with distant metastases
2‖	39/M	40.0	65.0	Duodenal ulcer	100	APUDoma cell hyperplasia of pancreas
3	50/F	54.8	59.0	Stomach ulcer	82	Malignant APUDoma of the pancreas with distant metastasis
4	59/F	30.6	81.9	Duodenal ulcer	60	APUDoma cell hyperplasia of pancreas
5†‖	51/F	1.6	17.5	Stomal, ulcer, GI bleeding	76	†Malignant APUDoma of pancreas with regional and distant metastases
6‡‖	55/F	13.9	15.0	Stomal ulcer, GI bleeding	135	Malignant APUDoma of pancreas with regional and distant metastases
7	63/M	64.9	78.0	Watery diarrhea, Duodenal ulcer, Positive secretin test	68	
8	51/M	53.9	64.7	Watery diarrhea, Duodenal ulcer	142	Malignant APUDoma of pancreas with distant metastasis
9	64/M	21.6	55.3	Watery diarrhea, Duodenitis	28	Malignant APUDoma of pancreas with distant metastasis
10*	44/M	9.1	20.8	Stomal ulcer, GI bleeding	42	Benign APUDoma of pancreas
11	78/M	27.0	43.0	Obstructive jaundice	44	Malignant APUDoma in head of pancreas

* Hemigastrectomy with truncal vagotomy.
† Subtotal gastrectomy with truncal vagotomy.
‡ Whipple procedure without vagotomy.
‖ These four patients were reported in part in a previous communication.[10]
¶ Postmortem examination.

inhibitor (Sigma) was added. A control tumor extract was similarly treated without addition of trypsin in the incubation at 37 C. In addition, the extract of a typical gastrinoma and 200 ng of human gastrin-I was similarly treated and bioassayed in anesthetized rats for comparison.

Results

Clinical and Pathologic Features, Plasma Gastrin Concentration, and Gastric Acid Secretion

Of the 11 patients with marked gastric acid hypersecretion but without hypergastrinemia as summarized in Table 1, 10 had peptic ulcer disease with epigastric pain and 4 of these 10 patients also had watery diarrhea. The initial problem in one patient (patient 11) was obstructive jaundice resulting from a malignant APUDoma in the head of the pancreas. The basal acid outputs of seven patients (patients 2 to 4, 7 to 9, and 11) with intact stomachs were greater than 21 mEq/hour with a mean ± standard error (SE) of 39.7 ± 6.9; maximal acid outputs ranged from 43 to 81.9 mEq/hour with a mean ± SE of 64.7 ± 5.9. In four patients who had undergone either hemigastrectomy (patients 1, 6, and 10 with truncal vagotomy) or subtotal gastrectomy (patient 5 with truncal vagotomy), three patients with hemigastrectomy showed basal acid outputs exceeding 5 mEq/hour. Plasma gastrin concentrations of these 11 patients ranged from 12.7 pM (26.6 pg/ml) to 67.6 pM (141.8 pg/ml) with a mean ± SE of 36.6 ± 5.1 pM (76.8 ± 10.7 pg/ml). Pathology of the pancreas was confirmed by surgical laparotomy in nine patients and by postmortem examination in one. Seven of these ten patients had malignant APUDoma of the pancreas with distant metastases, mostly to the liver. One of these seven patients had extensive metastases to distant organs including the liver, lungs, heart, bones, and right kidney (patient 1). One patient (patient 10) had a benign APUDoma (adenoma) and two others (patients 2 and 4) had pancreatic APUD cell hyperplasia. To date one remaining patient (Patient 7) has not been surgically explored to study pancreatic histology (Table 1). Criteria for diagnosis of islet cell hyperplasia include presence of large islets greater than 300 μ in diameter, irregular, circumferential outlines, considerable variation in size of islet cells, and many cells with large hyperchromic nuclei.[20] APUD cells were defined by immunohistochemistry of

TABLE 2. *Results of Immunohistochemistry of Pancreatic Tumors or Tissues of Patients Containing a Nongastrin Acid Secretagogue as Described in Table 1*

Patient	NSE	GAS	CCK	SEC	MOT	VIP	INS	GLU	SOM	HPP
1*	+	+	+	−	+	+	+	+	+	+
2†	+	+	−	−	−	−	+	+	+	+
4†	+	+	+	+	+	+	+	+	+	+
5*	+	+	−	+	+	+	−	+	−	−
6*	+	+	−	+	+	+	+	+	+	+
8*	+	+	+	+	−	+	−	−	−	+
9*	+	+	−	−	+	+	+	+	+	+
11*	+	+	+	−	+	+	+	+	+	+

* Islet cell cancer.
† Islet cell hyperplasia.
\+ Positive stain for specific antiserum.
− Negative stain for specific antiserum.

NSE, Neurone-specific enolase; GAS, Gastrin; CCK, Cholecystokinin; SEC, Secretin; MOT, Motilin; VIP, Vasoactive Intestinal Polypeptide; INS, Insulin; GLU, Pancreatic glucagon; SOM, Somatostatin; HPP, Human pancreatic polypeptide.

the tumor or pancreatic tissues using neuron-specific enolase (NSE) immunostaining method[16,17] and specific antisera, as described previously,[10,18] to various gut and pancreatic peptides including gastrin, secretin, cholecystokinin, human pancreatic polypeptide, motilin, insulin, and glucagon. The study was carried out in the tissues obtained from eight of these 11 patients (Table 2). The tissues of these eight patients contained positive cells for NSE, and several of the peptides so tested.

Plasma Gastrin and Secretin Responses to Exogenous Secretin and a Standard Meal

In only one of these 11 patients (Patient 7; Table 1) was there an increase in plasma gastrin concentration in response to intravenous injection of secretin 2 CU · kg^{-1}: from 52.4 pM (109.9 pg/ml) to 277.2 pM (581.6 pg/ml) (Fig. 1). The increase paralleled the marked increase in gastric acid output. Periodical determinations of plasma gastrin concentration in this patient (patient 7) on five separate occasions in a 1-year period were always below 45.5 pM. On each occasion hypersecretion of acid was confirmed. After intravenous injection of secretin, plasma secretin levels exceeded 1000 pM (3056 pg/ml), whereas his postprandial level was about 10 pM (30.6 pg/ml) (Fig. 2). However, a computerized tomography of the abdomen failed to show any abnormality in the pancreas or other organs in the abdomen. The plasma secretin levels in response to intravenous secretin or a standard meal in five other patients so studied were similar to those observed in patient 7.

After a meal, plasma gastrin concentration increased significantly in all five patients with ordinary duodenal ulcer disease. However, in four of the six patients with gastric acid hypersecretion whose fasting plasma gastrin levels were within normal limits, there was no measurable increase in postprandial plasma gastrin levels. Three (patients 3, 8, and 9) of the four patients had a malignant APUDoma of the pancreas with metastases to the liver (Table 1), and the remaining patient (patient 7) had a marked rise in plasma gastrin concentration after intravenous injection of secretin (Figs. 1 and 2).

Immunoassay for Gastrin in Tissue Extracts and Bioassays of Tumor Extracts and Plasma Extracts

Immunoreactive gastrin contents of tumor or pancreatic tissue extracts were determined in ten patients, including one patient with a gastrinoma syndrome and nine patients with marked hypersecretion without hypergastrinemia (Fig. 3). In the patient with a proved gastrinoma, the gastrin content was greater than 5000 pmoles

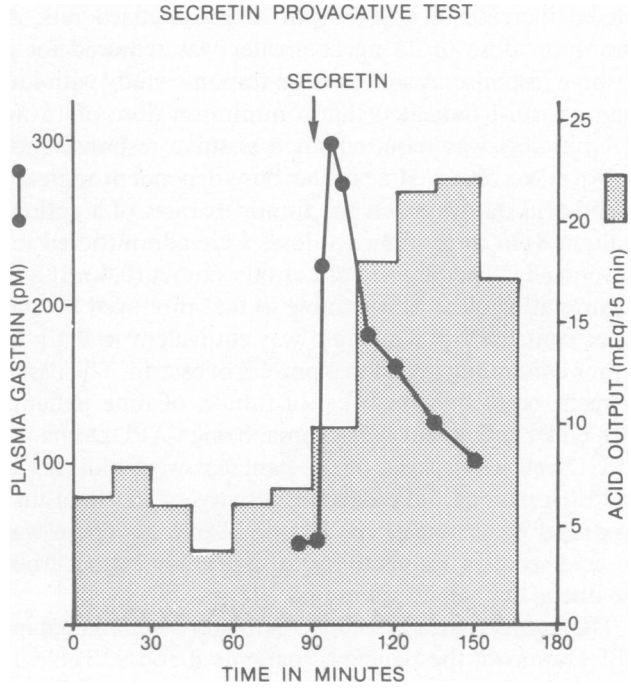

FIG. 1. Effect of intravenous secretin. Two clinical units · kg^{-1} on plasma gastrin concentration and gastric acid output in a patient (patient 7, Table 1) with marked gastric acid hypersecretion, watery diarrhea, and duodenal ulcer.

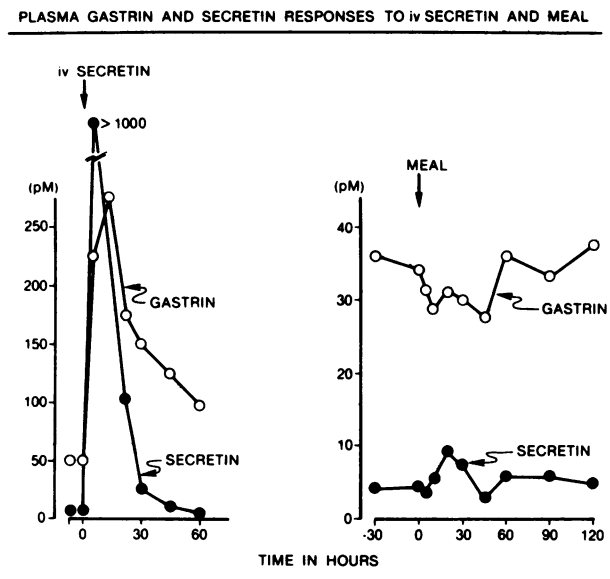

FIG. 2. Plasma secretion and gastrin responses to intravenous injection of porcine secretin, two clinical units · kg^{-1}, and ingestion of a meal in patient 7 with marked gastric acid hypersecretion but without fasting hypergastrinemia.

(10.49 μg) per gram of tumor, whereas the gastrin content in all nine patients' tissue extracts contained less than 101.9 pmoles (213.8 ng) per gram of tissue weight. In a patient with pancreatic ductal cell adenocarcinoma, gastrin was absent in the tumor extract (not shown).

As shown in Figure 4, pentagastrin produced a dose-related increase in acid output in anesthetized rats. A minimum dose of 25 ng or greater was required for a positive response. A similar dose-response study with human gastrin-I indicated that a minimum dose of 15 ng (7.1 pmoles) was required for a positive response (not shown). We observed a similar dose-dependent increase in the acid output when the tumor extracts of a patient (patient 8) in three different doses were administered intravenously. The largest dose of this extract (0.4 ml) had a stimulating effect comparable to that produced by 100 ng of pentagastrin. This dose was equivalent to 0.1 g of tumor containing less than 4 pmoles of gastrin. The tissue extracts, equivalent to 0.4 g of tumor, of nine patients with either malignant APUDoma, benign APUDoma, or APUD cell hyperplasia of the pancreas were bioassayed and each patient had biological activity greater than that produced by pentagastrin, 100 ng (Table 3). There was no acid secretagogue activity in the tumor extract from the ductal cell adenocarcinoma.

The tumor extracts of two patients with malignant APUDomas of the pancreas (patients 8 and 9; Table 1) were tested in three conscious dogs with gastric cannulas because an adequate amount was available from each patient for the study. The intravenous injection of an extract equivalent to 1.3 g of tumor from patient 8 evoked gastric acid response in all three dogs (Fig. 5). A marked hypersecretion of acid in response to the tumor extract was observed in dog 1. The extract from patient 9 evoked an acid response only with a dose equivalent to 5 g of tumor (data now shown).

Fresh plasma extracts equivalent to 5 to 8 ml of plasma from 7 patients were tested in anesthetized rats. All seven plasma extracts stimulated acid secretion (Table 3). One of these seven plasma extracts was obtained from patient 7 (Table 1) who was not surgically explored. However, the plasma extracts from 10 patients with ordinary duodenal ulcer failed to stimulate acid secretion.

Partial Purification and Characterization of the Acid Secretagogue in Tumor Tissue

The acid secretagogue activity in the tumor of patient 8 was completely extracted by an alkaline water solution.

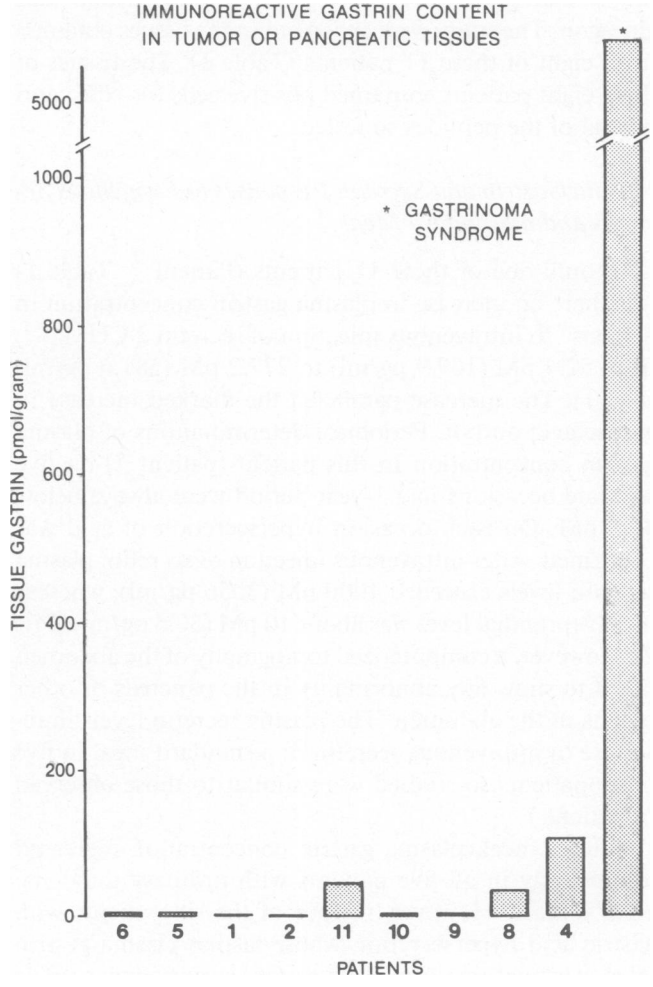

FIG. 3. Immunoreactive gastrin contents in islet celleoplasms and pancreas with islet cell hyperplasia. Patient 12 had a proved gastrinoma syndrome.

Subsequent extraction of the residue with 0.5 N HAc failed to yield any acid secretagogue activity. With gel filtration, its elution volume situated between porcine motilin and human gastrin I suggests a molecular size of 2000 to 3000 daltons (Fig. 6). Although G-34 would have been eluted in this region, there was no gastrinlike immunoreactivity eluted in the fractions containing the major bioactivity. It is probably an acidic compound because it was not retained by CM-cellulose at pH 8.5. After treatment with TPCK-trypsin, it lost its bioactivity, while under the same conditions trypsin could not abolish the bioactivity of human gastrin I (Fig. 7) or a gastrinoma extract (Fig. 8). Thus the acid secretagogue appeared to be a peptide containing trypsin-sensitive peptide bonds involving basic amino acids and is distinguishable from gastrin. A similar trypsin-sensitivity was observed with the extract of tumor from patient 1, which was reported previously.[10] In two other patients (patients 6 and 9), similar effects of trypsin on the bioactivity of their tumor extracts were observed. Further purification of the material on reverse-phase HPLC yielded four bioactive peaks with retention times corresponding to 56 to 60 minutes, 69 to 72 minutes, 89 to 92 minutes, and 101 to 104 minutes, respectively. Except for the peak eluted at 69 to 72 minutes, which overlapped with standard G17-I, the other peaks were well resolved from various forms of CCK and G34 (Fig. 9). The content of gastrin (2 pmoles) in the fractions eluted between 69 to 72 minutes, however, was unable to account for the bioactivity observed. These results further suggest that the tumor contains multiple forms of a nongastrin acid secretagogue.

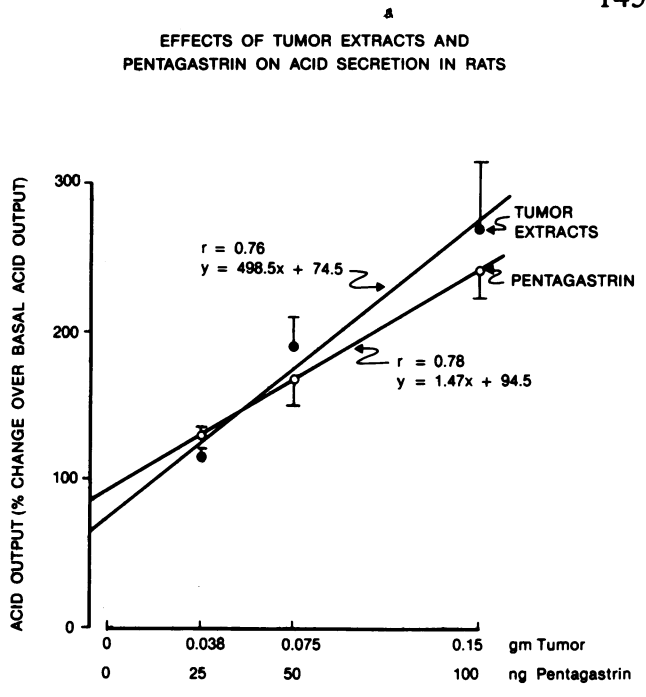

FIG. 4. Responses of acid secretion in four anesthetized rats to intravenous injections of pentagastrin and a tumor extract.

Discussion

The present study indicates clearly that there are patients with pancreatic endocrine neoplasms who have marked gastric acid hypersecretion without hypergastrinemia. We have thus confirmed our initial observations on 4 patients whose acid hypersecretion was not explained by high levels of circulating gastrin or a gastrinlike secretagogue.[10] It is significant that in this report some of the patients had an intact stomach, whereas in our previous report the patients had undergone partial gastrectomy.[10] The collection of gastric juice in the patients with an intact stomach was probably more complete than in patients with partial gastrectomy. The presence of acid secretagogue activity both in tumor or pancreatic tissue extracts and plasma extracts suggests strongly that a nongastrin acid secretagogue released from these neoplastic tissues may evoke hypersecretion of acid. Their clinical and pathologic features otherwise were indistinguishable from those of Zollinger–Ellison.[3,9]

A nongastrin acid secretagogue activity in the tumor extracts was demonstrated in rats and in dogs. In rats the stimulatory effect on acid secretion by the tumor extract was dose dependent. The factor mediating this acid secretagogue activity is not gastrin nor gastrinlike but rather a peptide which, unlike gastrin, contains trypsin-sensitive peptide bonds involving basic amino acids. Incubation with trypsin destroys its secretagogue activity. Human synthetic gastrin (G-17) or the extract of a gastrinoma,

TABLE 3. *Results of Acid Secretagogue Activity in Anesthetized Rats in Response to Tissue Extracts and/or Plasma Extracts*

Patient	1	2	4	5	6	7	8	9	10	11
Tissue (unit*)	14	6	2.9	4.5	13.2	NT	6.5	3.8	8.8	12.1
Plasma (unit)	3	2	2.3	NT†	13.1	2	4.3	9	NT	NT

* 1 unit, 50% of acid output produced by pentagastrin 100 ng. 2 units, 100%; 3 units, 150%; 4 units, 200%; 5 units, 250%; 6 units, 300%; Increment in units hereafter represent 50% further increase over the prior unit.

† NT, not tested.

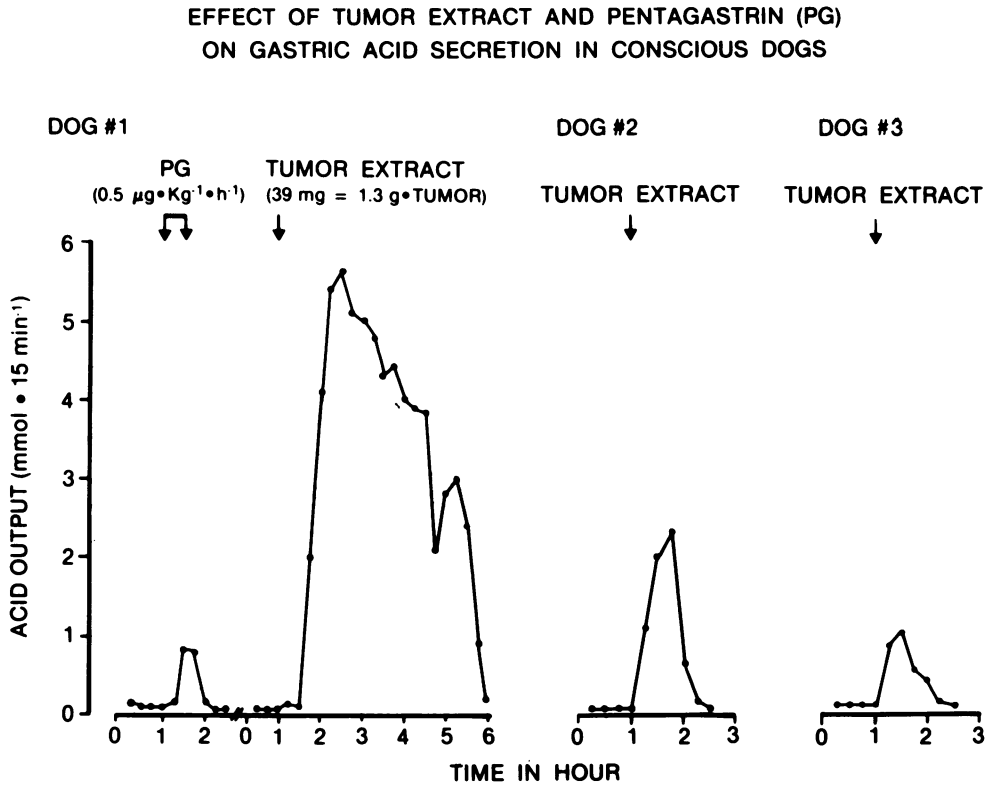

FIG. 5. Effect of a tumor extract and pentagastrin (PG) on gastric acid secretion in conscious dogs with gastric cannulas.

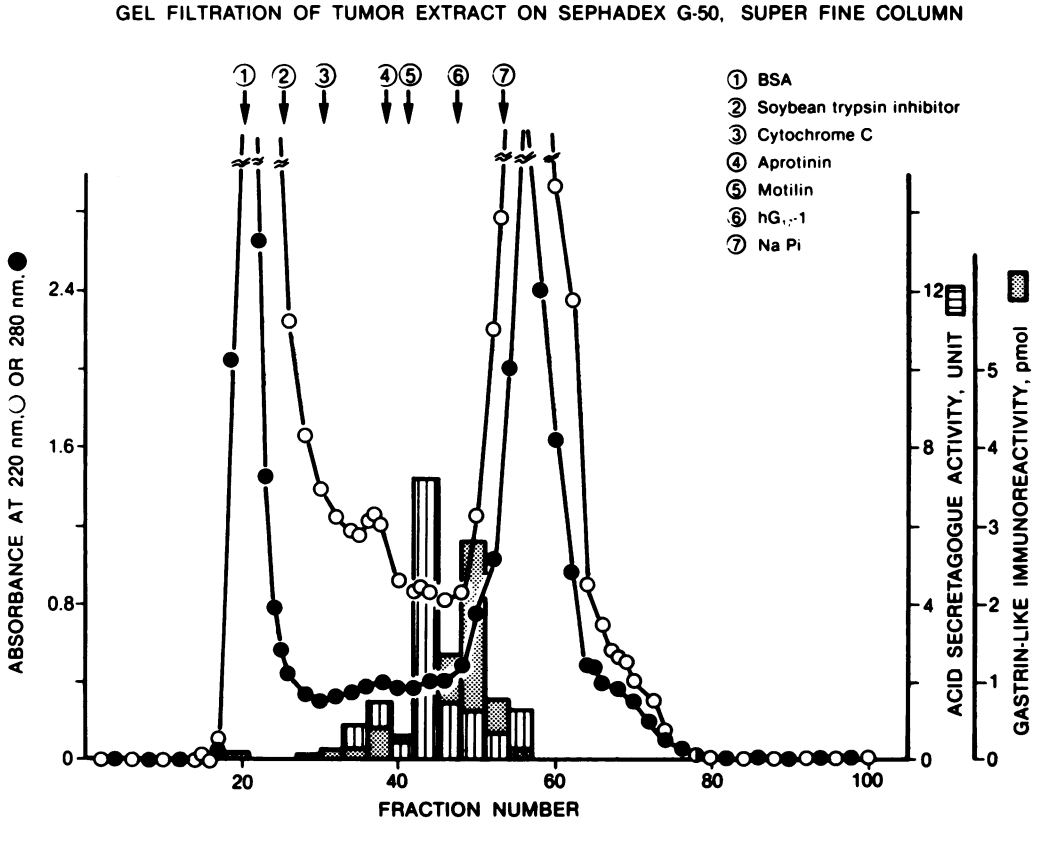

FIG. 6. Gel filtration of a tumor extract (patient 8) on a column of Sephadex G-50 (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ), superfine (3 × 32 cm) packed and run in 2% ammonium bicarbonate solution. The numbers in the circles with arrows denote the elution position of the standard proteins or peptides shown on the upper right corner. Other symbols are open circles, absorbance at 220 mm; filled circles, absorbance at 280 mm; hatched bars, bioactivity in unit as defined in the text and Table 3; and filled bars, gastrinlike immunoreactivity.

on the other hand, was not inactivated by trypsin. In our earlier study,[10] we showed that the secretagogue activity in tumor tissue was not destroyed by incubation with rabbit antigastrin serum. The same amount of antiserum abolished the activity of human synthetic gastrin. Furthermore, in one of the four patients in that study,[10] the secretagogue activity in the plasma disappeared after surgical removal of the tumor, the extract of which contained secretagogue activity. This particular patient has remained free of hypersecretion of acid and peptic ulceration since removal of the tumor in February 1983. Her plasma has also remained free of the secretagogue activity. Partial purification of the secretagogue suggests that it is an acidic peptide with a molecular size of 2000 to 3000 daltons. The peptide with acid secretagogue activity may be a peptide or a family of peptides originating from gastrointestinal mucosae. The presence of enterooxyntin in hog intestinal mucosal extracts has been well recognized. However, it has not been proved to be a hormone. The secretagogue found in our patients is probably not the enterooxyntin isolated by Wider et al.[21] because its molecular weight of 13,000 daltons is much higher than the bioactivity found in the extract of our tumors.

There was a rise in plasma concentration of gastrin in response to intravenous injection of secretin in only one of our patients (patient 7). Although hypergastrinemia in response to secretin has been considered one of the diagnostic criteria for the Zollinger–Ellison or gastrinoma syndrome,[12,22] plasma gastrin increases markedly only with plasma secretin levels far in excess of those reached after a meal. Gastrin levels did not increase in this patient (patient 7) or in two other patients whose plasma secretin levels increased postprandially. Furthermore in patient 7, whose plasma gastrin increased after the injection of secretin, gastrin did not increase in plasma when porcine secretin was administered intravenously in doses of 0.03 or 0.06 CU $\cdot$ kg^{-1} $\cdot$ h^{-1}, which usually mimics the postprandial plasma secretin level.[23] During a 1-year period of observation, blood samples were obtained from the same patient at the time when a gastric secretory study was done to observe hypersecretion on five different occasions. Plasma gastrin levels were always less than 110 pg/ml. In addition, plasma samples obtained on ten separate dates showed gastrin concentration less than 110 pg/ml during the period of 1 year. This level of plasma gastrin could not account for the acid secretagogue activity found in his plasma (Table 3). It is quite possible, therefore, that hypersecretion of acid in some of the patients with normal or marginally elevated plasma levels of gastrin who exhibited secretin-induced hypergastrinemia may result from a nongastrin secretagogue in circulation. In three other patients with islet cell cancer of the pancreas, no discernible increase in plasma gastrin occurred after ingestion of a meat meal. These observations strongly suggest that the mechanism of gastric acid hypersecretion in these patients did not involve gastrin because their fasting gastrin levels were within normal limits and post-

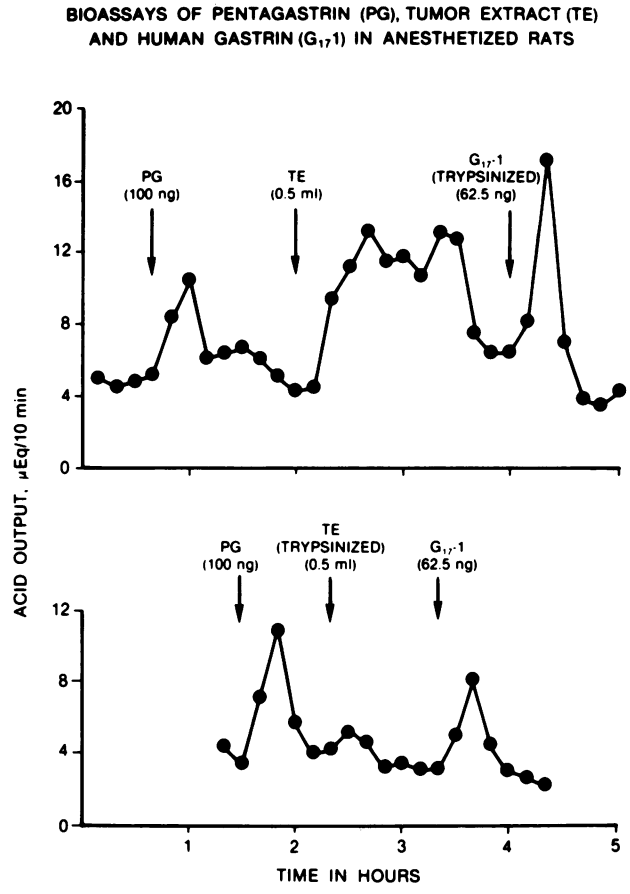

FIG. 7. Effect of L-1-tosylamide-2-phenylethyl chloromethyl ketone treated trypsin on secretagogue activity of a tumor extract (patient 8) and synthetic human gastrin-17 in anesthetized rats. PG represents pentagastrin and TE represents tumor extract.

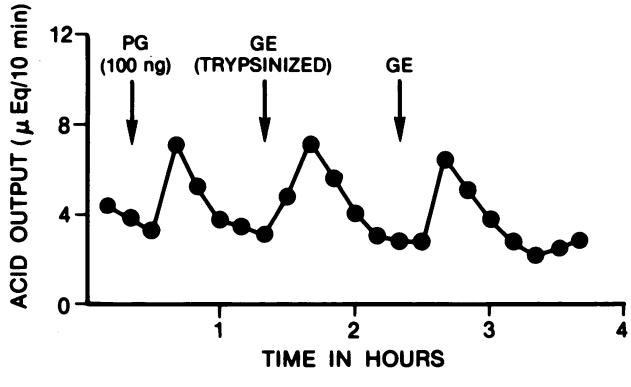

FIG. 8. Effect of TPCK-treated trypsin on secretagogue activity of a gastrinoma extract (GE) in an anesthetized rat.

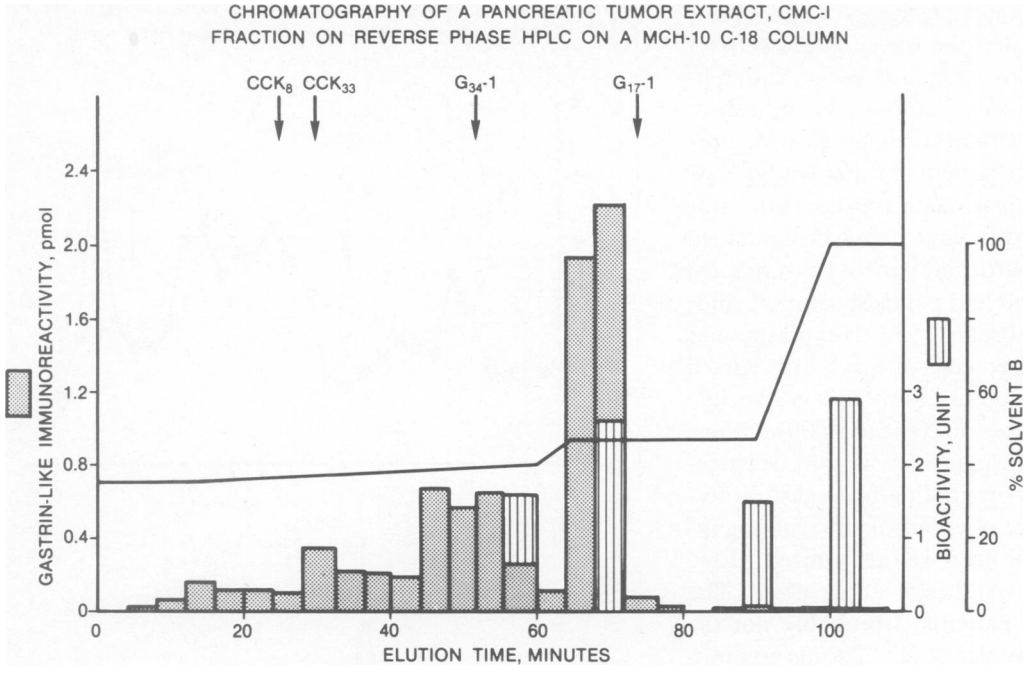

FIG. 9. Chromatography of patient 8's pancreatic tumor extract, CMC-I fraction on reverse-phase HPLC on a MCH-10 C-18 column. Fractions #40 to #52 of G-50 fractions (Fig. 6) were pooled and lyophilized, and chromatographed on a CM-cellulose column. The fractions not retained by CM-cellulose were lyophilized, extracted with C-18 SEP-PAK cartridge, and then subjected to HPLC as described in Methods.

prandial increases in plasma secretin levels or exogenous secretin in physiological dose did not increase circulating gastrin levels.

In recent years, unexplained marked acid hypersecretion in patients with pancreatic neoplasms but without hypergastrinemia has been recognized by others[24,25] in some patients with peptic ulcer disease. One recent report[25] described the finding of a nongastrin acid secretagogue in a pancreatic tumor extract. Apparently there are patients with clinical features similar to Zollinger–Ellison or gastrinoma syndrome but without associated fasting hypergastrinemia or with only a transient hypergastrinemia in response to a pharmacological dose of secretin. Such patients require further investigation to determine whether or not they have an endocrine neoplasm of the pancreas. In one patient (patient 9; Table 1), because of the clinical features without an associated hypergastrinemia, a computed tomographic scan of the abdomen revealed a neoplasm in the tail of the pancreas with metastatic lesions in the liver. Until the circulating secretagogue can be measured by radioimmunoassay or a sensitive bioassay, measurement of gastric acid secretion is the only laboratory marker that leads us to suspect this clinical entity. Thus gastric acid secretory study in these patients should be routinely carried out.

Acknowledgments

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