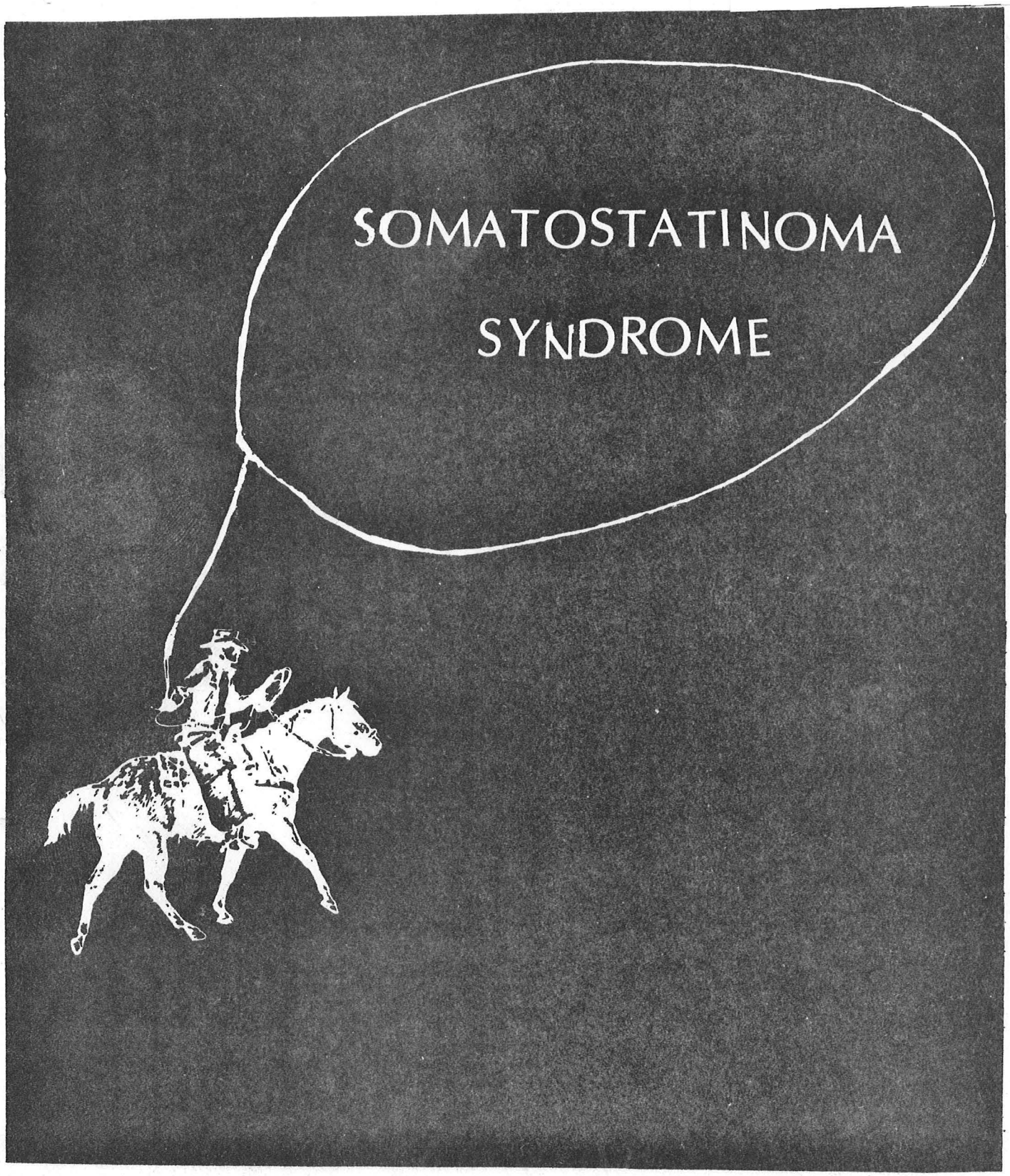

INTERNAL MEDICINE GRAND ROUNDS - PARKLAND MEMORIAL HOSPITAL

APRIL 26, 1979

GUENTER J. KREJS, M.D.

PROLOGUE

THE FOUR MODES OF FUNCTION SUGGESTED FOR SOMATOSTATIN:

1. NEUROHORMONE REGULATING PITUITARY ENDOCRINE SECRETION
2. NEUROTRANSMITTER IN CENTRAL AND PERIPHERAL NERVOUS SYST.
3. PARAHORMONE ACTING LOCALLY IN THE GI-TRACT AND PANC. ISLETS
4. CONVENTIONAL HORMONE REACHING TARGET ORGAN VIA BLOOD CIRCULATION

APRIL 26, 1979

INTRODUCTION

By some the 1970's will probably be remembered for the discovery of somatostatin, its isolation, sequencing and synthesis. Prompt availability of synthetic somatostatin for research purposes rapidly increased the knowledge of the pharmacological and possibly physiological actions of the peptide. The question soon arose whether there would be a disease related to the production of this peptide. While production by tumors has been described for many hormones, this is certainly not the rule (remember for instance the first hormone ever discovered:secretin; a secretinoma has yet not been discovered). As for somatostatin, two patients were described (2,3) in whom a pancreatic tumor was incidentally found during cholecystectomy and in whom somatostatin was identified by immunocytochemical studies of resected tissue. Three other patients with malignant islet cell tumors that contained somatostatin have been reported in abstracts or other brief communications (4-6).

Today I would like to present to you the first case (1) in whom the diagnosis of somatostatinoma was made preoperatively on the basis of the clinical picture and then verified by radioimmunoassay showing marked hypersomatostatinemia.

The workup and management of the patient to be presented was only possible due to the cooperation and help of a great number of my colleagues. Their names and contributions are acknowledged in Table 1.

TABLE 1. LIST OF PHYSICIANS AND SCIENTISTS COLLABORATING IN THE DIAGNOSTIC AND THERAPEUTIC MANAGEMENT OF PATIENT E.L.

THOMAS ALDOR, M.B., B.S. F.R.C.P. (C)	WINNIPEG	REFERRING GASTROENTEROLOGIST
ROGER UNGER, M.D. MICHAEL CONLON, PH.D. VIRGINIA HARRIS, B.A.	DALLAS	SLI-RIA AND SLI CHARACTERIZATION STUDIES
LELIO ORCI, M.D. MARIELLA RAVAZZOLA, PH.D. DANY BAETENS, M.D.	GENEVA, SWITZERLAND	IMMUNOCYTOCHEMICAL STUDIES
JOHN BROWN, M.D.	VANCOUVER	GIP, MOTILIN
PHILIP RASKIN, M.D.	DALLAS	GLUCAGON, DIABETES MANAGEMENT
ARTHUR RUBENSTEIN, M.D.	CHICAGO	C-PEPTIDE
SAMI SAID, M.D.	DALLAS	VIP
JOHN WALSH, M.D.	LOS ANGELES	VIP
SAMUEL WELLS, M.D.	DURHAM	CALCITONIN
EDWIN EIGENBRODT, M.D.	DALLAS	HISTOLOGY, EM
MURRAY GORDON, M.D.	DALLAS	ANGIOGRAPHY
DEAN KUMPURIS, M.D.	DALLAS	SMALL BOWEL MOTILITY
DANIEL NORMAN, M.D.	DALLAS	CALCIUM METABOLISM
CHARLES PAK, M.D.	DALLAS	CALCIUM METABOLISM
GLENN DAVIS, M.D.	DALLAS	GCRC PHYSICIAN
PHILLIP GORDON, M.D. DENIS McCARTHY, M.D. STEPHEN COLLINS, M.D.	NIH BETHESDA, MARYLAND	TUMOR TREATMENT PROTOCOL
JOHN CHIPMAN, M.D.	DALLAS	GROWTH HORMONE

CASE REPORT

E.L. is a 52 year old Canadian railroad inspector, who in 1966 and 1967 experienced episodes of melena which were attributed to peptic ulcer disease, although no ulcer was found by x-ray and endoscopic examination. In 1970 he had hematemesis and melena requiring blood transfusions, and again no ulcer was found. In 1973 he was troubled by postprandial eructations and abdominal pain, as well as periumbilical cramping after heavy meals. In 1974 a large single gallstone was shown on oral cholecystogram and, on barium meal examination, a filling defect was detected in the second portion of the duodenum (Fig 1).

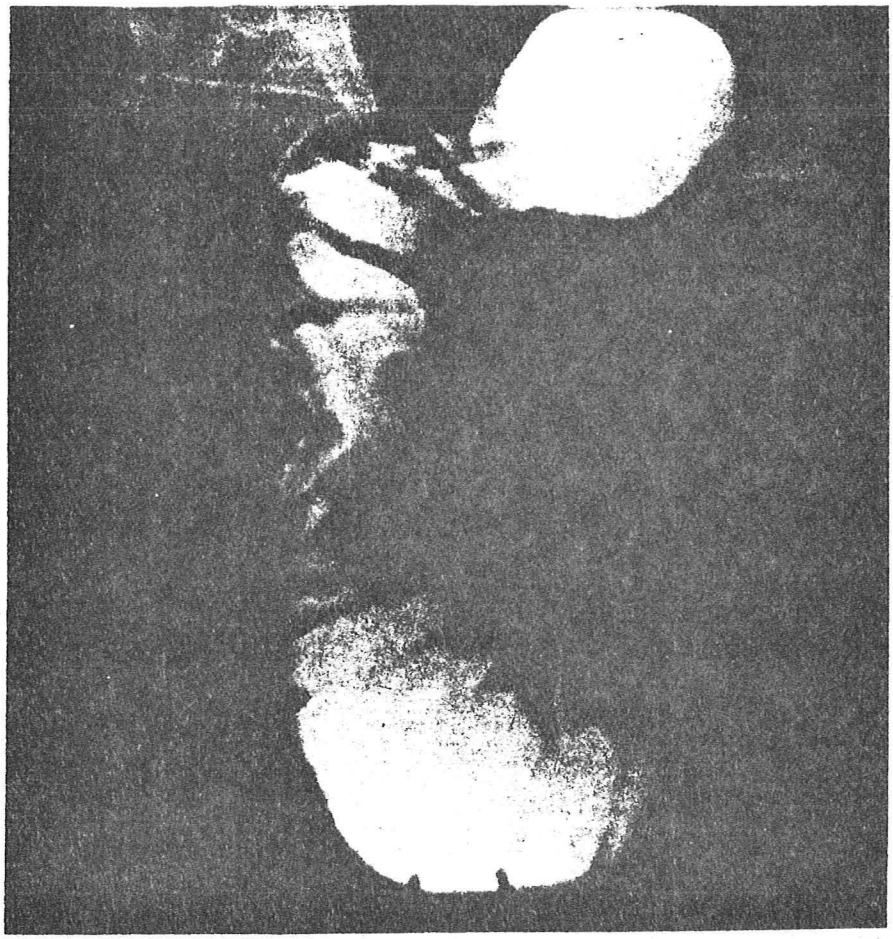

Figure 1: Duodenal C-loop with filling defect (3 x 4cm) in the second portion (film dated January 24, 1974)

At endoscopy this filling defect was described as broad-based sessile polyp located just proximal to a normal appearing papilla of Vater. Subsequently in 1974, a cholecystectomy (large single gallstone), truncal vagotomy and pyloroplasty were performed, but no ulcer was found. During the pyloroplasty, a firm lobulated tumor mass, measuring about 3 x 4 cm, was palpated digitally in the area of the ampulla.

It was not fixed and appeared to be in the wall of the medial portion of the second part of the duodenum. It was not possible to biopsy it or see it through the pyloromyotomy; the pyloroplasty was therefore completed and the duodenum and head of the pancreas mobilized by Kocher maneuver and the tumor inspected through a duodenotomy in the second part of the duodenum. No mucosal lesion was noted. A Vim-Silverman needle biopsy was performed through the duodenal wall, but the biopsy did not survive processing. A subsequent barium meal confirmed the filling defect on the concavity of the duodenal loop above the level of the ampulla. This was unchanged on another barium meal in 1976.

The patient was relatively well until 1977, when he returned complaining of a dry mouth and belching. Another barium meal study showed no change of the duodenal filling defect. Late in 1977, he developed weight loss (84 to 75 kg, height 178 cm) associated with occasional vomiting two hours after meals. He also complained of abdominal discomfort relieved by eructation and loose stools. One of three stools was positive for occult blood. Sudan stain was positive for fat globules on a stool smear. Blood sugar was 246 mg/dl (3 hours after a meal), and a subsequent glucose tolerance test showed a fasting blood sugar of 180 and one and two hour values of 324 and 303 mg/dl, respectively, with 4% glucosuria. Except for an alkaline phosphatase at the upper limit of normal, blood chemistry tests were normal.

The patient's weight fell further to 73 kg. Repeat endoscopy showed duodenal mucosa stretched over what appeared as an extrinsic lesion, distorting the medial wall above the ampulla. Biopsies at that time revealed normal duodenal mucosa and cytology brushings were negative for tumor cells. The patient was begun on 14 units of Lente insulin per day and a 2,000 calorie diabetic diet, but he continued to note fatigue, together with periodic vomiting and diarrhea, consisting of 3-4 loose, foul smelling stools daily. Endoscopic retrograde cholangiopancreatography was performed and showed a normal pancreatic duct. Biopsies of the tumor at ERCP showed tissue consistent with an endocrine lesion such as gastrinoma or a carcinoid. The duodenal aspirate following secretin injection (1 clinical unit/kg) revealed a normal volume (4.1 ml/kg/80 min, normal >2) and bicarbonate output (0.27 meq/kg/80 min, normal >0.2). Serum gastrin was 99 pg/ml (normal <200), and 5-HIAA was not elevated in a 24-hour urine collection. A 72-hour stool collection yielded 20 g of fat per day on a 100 g fat diet. Pancreatic enzyme replacement was started, and stool frequency declined to one per day, but weight loss continued to 68 kg.

The clinical picture of combined exocrine and endocrine pancreatic insufficiency (steatorrhea and diabetes mellitus), and cholelithiasis in the presence of a duodenal tumor of endocrine type, was felt to be suggestive of a somatostatinoma. This was confirmed by somatostatin radioimmunoassay, which showed a plasma level in excess of 2,000 pg/ml (fasting level 88 ± 8 pg/ml in 10 normal controls) (see below). Subsequently the patient was admitted to the General Clinical Research Center of The University of Texas Health Science Center at Dallas for further workup (August 1978).

Physical exam was essentially negative on admission. The patient's weight had dropped to 64 kg. Abnormal laboratory results included hemoglobin 12.9 g/dl, hematocrit 36%, total bilirubin 1.3 mg/dl, and alkaline phosphatase 136 U per liter (30-115).

Endoscopy revealed a patent pyloric channel (pyloroplasty) and a 3 x 4 cm intramural mass located on the medial side of the second portion of the duodenum. The overlying mucosa was of normal appearance, and multiple biopsies showed normal duodenal mucosa. Vigorous duodenal motility was also noted during endoscopy.

Ultrasonography revealed a 4-5 cm mass in the region of the head of the pancreas. Liver spleen scan showed multiple filling defects in the liver consistent with metastases. Angiography revealed a hypervascular mass in the region of the head of the pancreas (2 x 3 cm) and multiple areas of hypervascular stain in the late arterial phase of hepatic artery injections, compatible with liver metastases. At peritoneoscopy, multiple white implants with hyperemic margins slightly raised above the liver surface were seen. Light microscopy of biopsies taken from the hepatic implants at peritoneoscopy showed tumor formed by small masses and cords of cells separated by a fibrovascular stroma. The tumor cells were cuboidal in shape, though some assumed columnar appearance. The tumor nuclei appeared to occupy more than half of the cell volume. No mitotic figures were identified. Secretory granules were seen in the tumor cells on electronmicroscopy performed at Parkland Hospital.

After completion of most of the special studies that I will subsequently discuss the patient was referred to the National Institutes of Health in Bethesda, Maryland (September 1978) for treatment. After several weeks of intravenous hyperalimentation and further investigation the patient was taken to surgery. A preoperative repeat angiogram suggested a rapid increase in size of the liver metastases and this was also reflected by the plasma somatostatin level which had doubled, (from around 10,000 to 20,000 pg/ml) during this period. A 5 x 5 cm tumor in the head of the pancreas was resected by a Whipple's procedure (follow up see page 22).

SOMATOSTATIN

Somatostatin is a cyclic polypeptide consisting of 14 amino acids (tetradecapeptide, molecular weight 1818).

The original observation that growth hormone secretion is under dual control of both stimulating and inhibiting factors was made in this institution by Krulich and associates in 1968 (7-9). At that time it had been proven that the hypothalamus controls and regulates the secretion of two pituitary hormones namely thyrotropin (TSH) and the gonadotropin, luteinizing hormone (LH). A hypothalamic releasing factor for growth hormone (somatotropin) was postulated on the basis of a series of experimental and clinical observations. The nature of this postulated hypothalamic releasing factor for growth hormone, however, had remained elusive. Searching to demonstrate the presence of this still hypothetical somatotropin releasing factor Krulich et al (7-9) incubated anterior pituitaries in vitro with crude sheep hypothalamic extract and measured growth hormone release into the medium. When crude extract was fractionated on Sephadex G-25, a consistent picture was obtained with a zone containing GH-releasing activity and a zone that produced a strong inhibition in GH release.

In 1972, Guillemin*and coworkers (10,11) succeeded to isolate the active principle using hypothalami from 500,000 sheep. The hypothalamic tetradecapeptide was called somatostatin in the belief that it was a specific hypothalamic factor which modulates the release of growth hormone (somatotropin) analogous to the factor which inhibits release of prolactin. The purity and yield of their isolation procedure permitted Guillemin and his colleagues to determine the amino acid sequence of somatostatin (12). This breakthrough then led to the production of a biologically active synthetic replica which in turn allowed the development of a radioimmunoassay (13,14) and a host of pharmacologic and physiologic studies.

Routine protein synthesis techniques are used to produce somatostatin which is now available to a great number of investigators. It is of interest that another method

* Nobel Prize 1977

for the synthesis of somatostatin has been brought about by the advances in molecular genetics. Synthesis of somatostatin can be performed by *E. coli* bacteria whose genetic code was changed (15).

Besides the hypothalamus, somatostatin has been found in numerous other areas of the central nervous system as well as in peripheral autonomic ganglia and plexus. The numerous examples of neurones containing somatostatin thus satisfy one of the criteria for a neurotransmitter or neuromodulator (6).

During such localization studies of somatostatin in central nervous tissue Dubois and others (17,18) used liver, spleen, pancreas and intestine as presumably negative control tissues. However, somatostatin was either found in high concentration in tissue extracts or identified by immunocytochemical studies in the digestive tract, particularly in the pancreas.

Figure 2 shows the somatostatin content of the digestive tract.

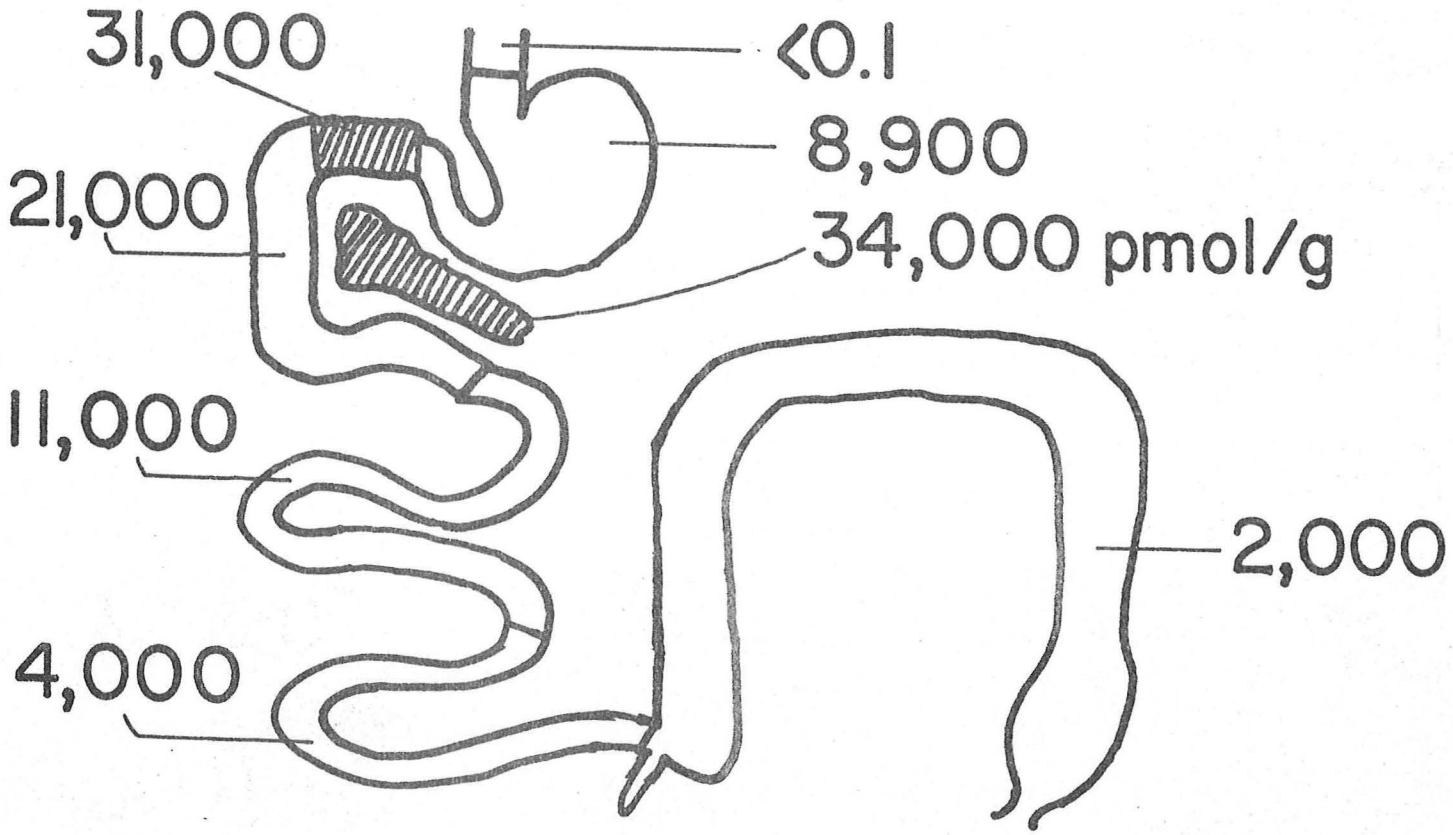

Figure 2: Somatostatin content (pmol/g tissue) of the digestive tract (modified from Bloom, S.R., 1978, GASTROINTESTINAL HORMONES)

In the pancreas somatostatin was localized to the D cells (17-23). In the normal islets of Langerhans of mammals, B cells are mainly located in the center of the islet, and a so-called "mantle" of A and D cells is seen on the islet periphery (23-25). This type of islet of Langerhans is observed in the body and tail of the pancreas. In the head and uncinate process, the islets also contain many pancreatic polypeptide-producing cells.

Ultrastructurally the D cells have been characterized by their large (200-400 nm) weakly osmiophilic secretion granules. The core of the secretion granule exhibits a fine homogeneous granulation and its membrane is closely applied.

PROOF OF DIAGNOSIS IN E.L.

The diagnosis of somatostatinoma in patient E.L. was proven by 1) somatostatin radioimmunoassay, and 2) immunocytochemical studies in tumor tissue obtained at peritoneoscopy (liver metastases) and surgery (primary tumor).

1. Radioimmunoassay (1,14)

The patient's fasting plasma level of SLI ranged from 9,000 to 13,000 and averaged 9,800 ± 600 pg/ml (mean ±SEM)(July, August 1978).

For comparison the fasting SLI plasma level of healthy subjects (n=10) was found to be 88 ± 8 pg/ml (mean ±SEM) and after a 1,000 calorie hamburger meal, SLI plasma levels rise to 135 ± 26 pg/ml (P. Raskin, unpublished observation).

When we infused somatostatin in (probably pharmacological) doses (8 μg/kg/h) in healthy subjects (26) plasma SLI levels rose to 2,062 ± 192 pg/ml (n=20), which is about one-fifth of the patient's fasting levels.

Thus, SLI in the patient was over 100 times the normal average. Extreme and constant hormonal excess is consistent with a malignant functioning islet cell tumor with metastatic spread.

To characterize plasma SLI,gel filtration chromatography of plasma samples was carried out on Biogel P-10 and Sephadex G-200 columns.

The SLI of canine plasma is undistinguishable from synthetic somatostatin, but it circulates bound to high molecular weight components (150,000) that are saturable by endogenous somatostatin (27). Although SLI in human plasma has been less thoroughly characterized, a typical elution profile on Biogel P-10 columns (pH 7.4) of plasma from a normal subject (Figure 3, upper panel) indicates that bound SLI also represents the predominant form in human plasma. If free somatostatin is present, it is below the detection limit of the radioimmunoassay (3 pg/tube). When the plasma samples were chromatographed under a dissociating condition, e.g. in 2 M acetic acid, however, over 90% of the total SLI was eluted from the column in the same position as synthetic somatostatin, thus confirming the earlier report by Kronheim et al (28). The lower panel on Figure 3 shows the elution profile of a basal plasma sample from the patient. In addition to a peak of somatostatin at the void volume of the column, multiple peaks of SLI were observed in the molecular weight regions 10,000-15,000, 2,000-3,500 and in the molecular weight region of free somatostatin. Chromatography of a plasma sample on Sephadex G-200 indicated that the amount of SLI eluted in the >150,000 molecular weight zone was not greater than in plasma of normal subjects.

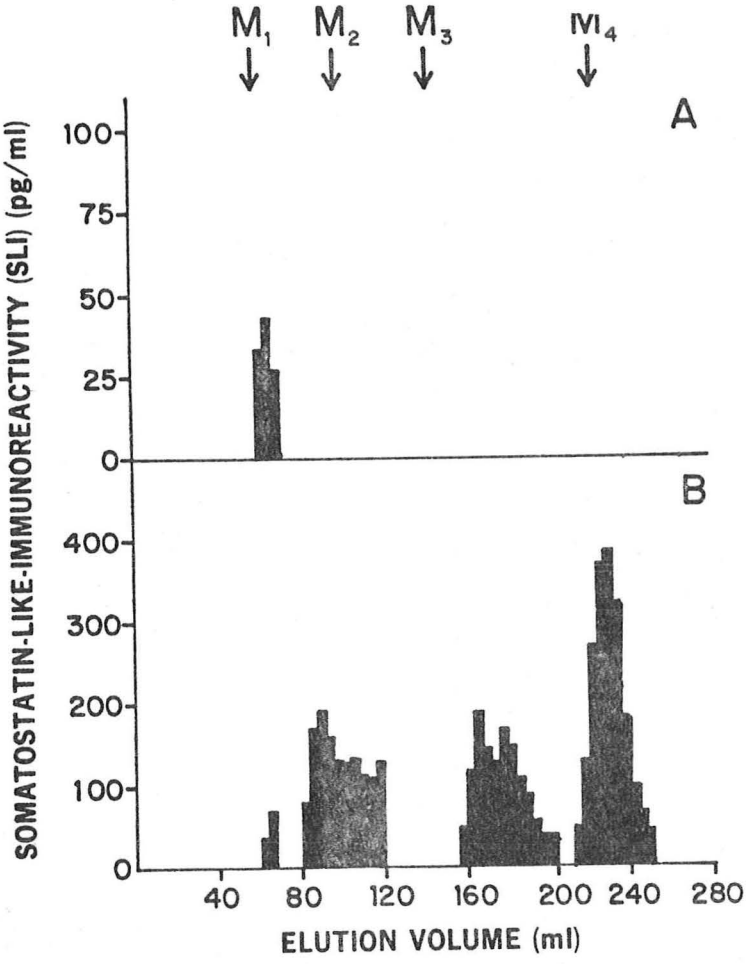

Figure 3: Elution profiles of SLI from the plasma of a healthy control subject (A), 4 ml plasma, 100 pg/ml SLI, and the patient with somatostatinoma (B), 3.0 ml plasma, >2,000 pg/ml SLI, on Biogel P-10 column at pH 7.4. The column was calibrated with markers of known molecular weight: M_1 (blue dextran; void volume), M_2 (cytochrome-c), M_3 (glucagon), and M_4 (somatostatin).

Thus, the SLI in our patient's plasma was distributed in four main peaks on gel filtration chromatography: these were a 1600-dalton peak, corresponding to synthetic somatostatin, a 3,000-dalton and 12,000-dalton peak corresponding to compounds that may represent precursors of somatostatin and correspond to peaks present in extracts of normal somatostatin containing tissues (29-42) but not previously found in plasma, and fourthly, a >150,000-dalton peak, corresponding to the fraction in which all measurable SLI appears when plasma of normal subjects is chromatographed by these techniques. This component is believed to represent somatostatin bound to large molecular weight plasma components (27). The latter was not increased above normal in this somatostatinoma patient.

Extracts of the primary tumor contained ≈ 5 μg/mg net tissue weight. The somatostatin content of normal pancreas has been reported to be 253 ± 43 (range 31-640)

pg/mg tissue (43). SLI was distributed into the same 1,600, 3,000 and 12,000 molecular weight fractions present in the plasma of this patient and in extracts of normal dog pancreas (41). Multiple molecular forms of SLI were also reported in extracts of the tumor of the patient described by Larsson et al (2). Analogous to insulinomas, glucagonomas and other polypeptide releasing pancreatic tumors (44-46), the recognition of such large molecular weight forms of SLI may aid in the diagnosis of pancreatic endocrine neoplasms.

2. Morphology

Liver metastasis: On electron microscopy, the cytoplasm of the tumor cells, obtained by biopsy of a liver metastasis at peritoneoscopy, was filled with small electron dense secretory granules of various shapes. Immunofluorescent staining of the biopsy material with several different antisera (see Table 2) revealed a positive reaction only with antisomatostatin antiserum. All control tests were negative, confirming the specificity of the immunofluorescent reaction.

Primary pancreatic tumor:

Light microscopy. The primary pancreatic tumor was composed of solid nests and cords of cells forming anastomosed ribbons or follicular structures. The latter were surrounded by cuboidal or columnar cells and filled with PAS positive material. Broad and thin bands of connective tissue penetrated the tumor tissue. No amyloid was detected.

Immunofluorescence. The results of immunofluorescence study are summarized in Table 2. Virtually all tumor cells displayed a positive reaction with the antisomatostatin antiserum. The immunofluorescence appeared consistently brighter at the periphery of the blocks of tumor tissue, whereas the central part displayed a weaker staining (Fig 4a,b). Exposure to antigen-absorbed antiserum completely abolished the immunoreaction (Fig 4). Calcitonin positive cells showing bright to weak fluorescence were also found, being much more numerous in one of the two blocks examined. Control tests showed a total inhibition of the specific antisomatostatin and anticalcitonin staining.

Electron microscopy. At the ultrastructural level the tumor was characterized by two populations of well granulated cells (Fig 5). The granules of one type were large and contained material that varied from dense and homogeneous to pale and flocculent. The other type of tumor cells showed polymorphic dense granules of small size, like the tumor cells of the liver metastasis. Junctional complexes were usually seen joining adjacent cells of the follicle-like structures. The apical parts of these cells were characterized by a well developed cell web and numerous microvilli.

Immunocytochemistry. With the antisomatostatin antiserum, using the two steps method, gold particles indicating antigen-antibody reaction sites, were consistently found on the secretory granules of all cells, irrespective of the granule size, shape and electron density (Fig 5). By contrast, with the anticalcitonin antiserum, gold particles were localized only within the population of small granule cells. Each antiserum staining was abolished by the homologous but not by the heterologous antigen.

Somatostatin and calcitonin immunoreactive material thus coexisted in the secretory granules of a minor population of tumor cells. However, since not all granules were labeled and consecutive sections were not examined, it cannot be decided whether somatostatin and calcitonin immunoreactivity are stored in the same secretory granules.

TABLE 2. Immunofluorescence Microscopy of Tumor Tissue

ANTISERUM	DILUTION	PRIMARY TUMOR	METASTASIS
Anti-somatostatin[a]	1:200	+	+
Anti-calcitonin[b]	1:300	+	-
Anti-insulin[c]	1:200	-	-
Anti-glucagon[d]	1:100	-	-
Anti-pancreatic polypeptide[e]	1:100	-	-
Anti-glicentin[f]	1:50	-	-
Anti-secretin[g]	1:10	-	-
Anti-gastrin[h]	1:50	-	-
Anti-ACTH[i]	1:10	-	-
Anti-TRH[j]	1:50	-	-

[a]provided by Dr. S. Ito, Niigata, Japan

[b]provided by Dr. J. McIntyre, London, England and directed against residues 16-19, 29-32 of calcitonin M

[c]provided by Dr. P.H.Wright, Indianapolis, Indiana, USA

[d]provided by Dr. R. Donald, Christchurch, New Zealand

[e]provided by Dr. R. Chance, Indianapolis, Indiana, USA

[f]provided by Dr. A.J. Moody, Bagsvaerd, Denmark

[g]provided by Dr. N. Yanaihara, Shizuoka-Shi, Japan

[h]provided by Dr. W. Gepts, Brussels, Belgium

[i]provided by Dr. J.A. Edwardson, London, England and directed against residues 17-39 of human ACTH

[j]provided by Dr. J.P. Leppäluoto, Oulu, Finland

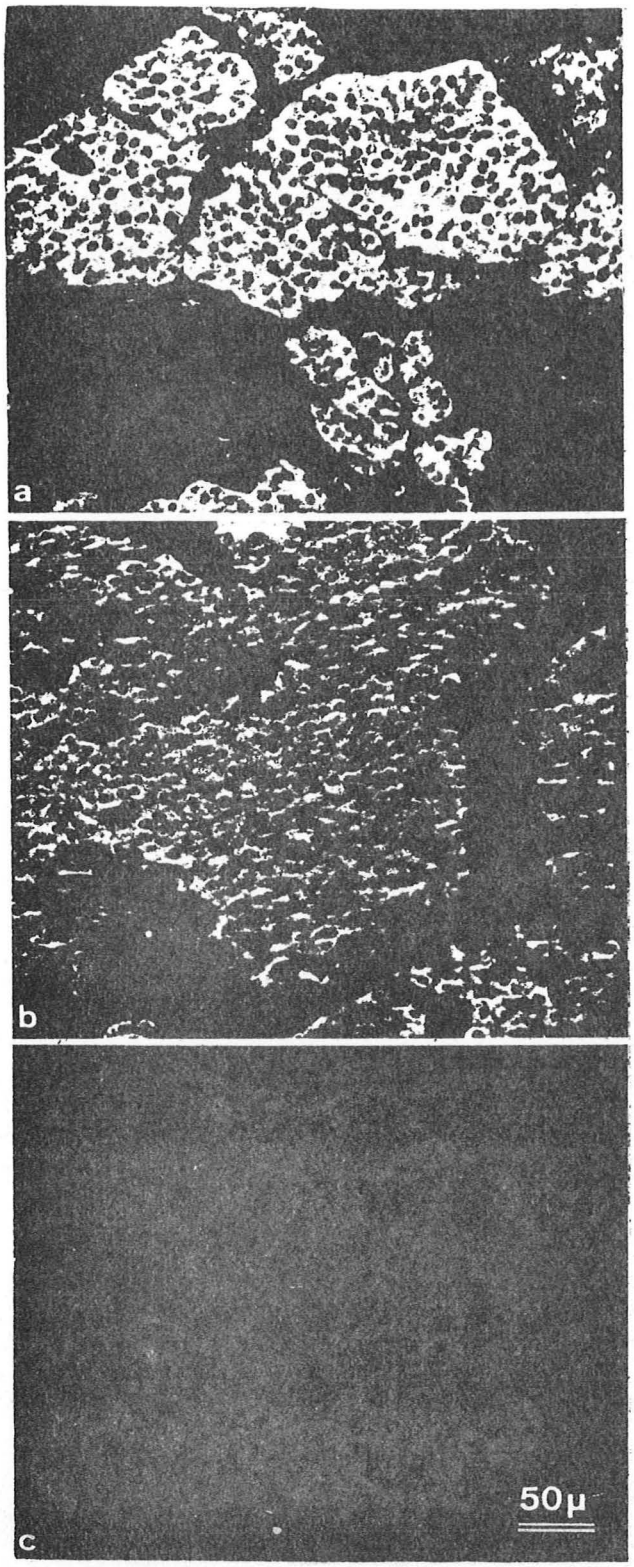

Figure 4: Paraffin sections of the primary pancreatic tumor stained with the indirect immunofluorescence method with anti-somatostatin serum. a. Clusters of brightly positive cells surrounded by abundant connective tissue. b. Masses of tumor cells showing a weak immunofluorescent reaction. c. Somatostatin immunostaining appears completely abolished on the section consecutive to b. which has been stained with an anti-somatostatin serum absorbed with synthetic somatostatin (X 200).

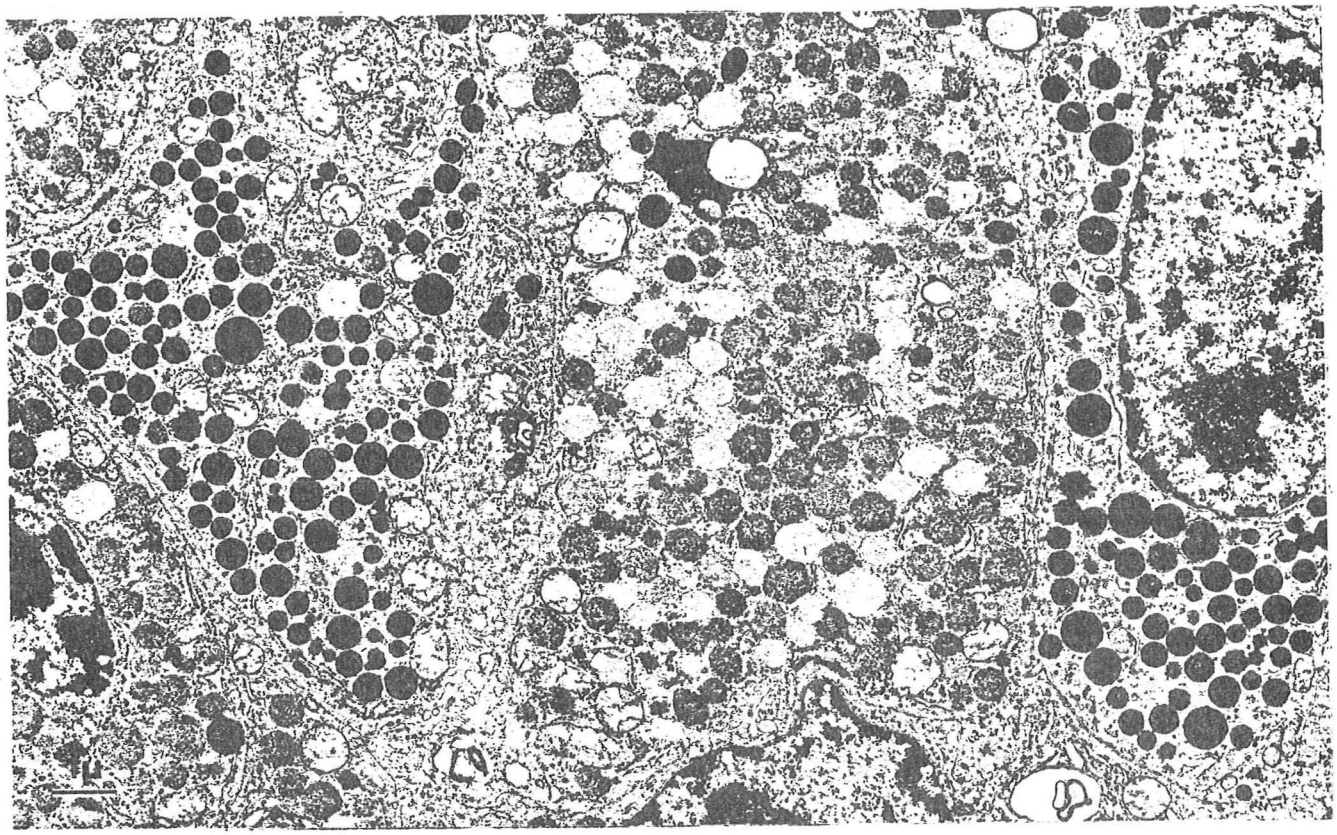

Figure 5: Low power electron micrograph showing several tumor cells. Note the large size and the variable degree of electron density of the secretory granules (X 8,000).

PLASMA CALCITONIN IN PATIENT E.L.

As may be expected from the morphological studies, plasma calcitonin was elevated in the patient's fasting plasma: 4,650 pg/ml (normal <120). High calcitonin levels were also observed in the patient reported by Galmiche et al (6) and we have recently seen a patient with a VIP-producing malignant islet cell tumor who also had a high plasma calcitonin concentration (47). Elevated serum calcitonin may be a marker of a variety of endocrine tumors.

ACTIONS OF SOMATOSTATIN

A. ENDOCRINE FUNCTIONS

1. Hypothalamic and Pituitary Function

In pharmacological doses somatostatin is a powerful inhibitor of human growth hormone secretion, suppressing the response to insulin-induced hypoglycemia (48),

L-Dopa (49), arginine infusion (50), exercise (51) and sleep (52).

In our patient, both insulin-induced hypoglycemia (blood sugar 30 mg/dl) and arginine infusion failed to stimulate growth hormone secretion with the plasma concentration remaining below 0.8 ng/ml.

Although somatostatin is known to suppress TRH-stimulated TSH secretion in euthyroid subjects (48,50,53) we were unable to demonstrate suppression of the TSH response to TRH in our patient. The reason for this is not clear but it is possible that the suppression of TSH secretion by somatostatin in acute experiments is not seen in the presence of persistently elevated levels of somatostatin.

Plasma levels of prolactin, FSH, LH and cortisol were normal in our patient which is in agreement with reports that somatostatin has no effect on these hormones (48, 49,53).

2. Pancreatic Islet Cell Function

One of the three cardinal symptoms in our patient was diabetes mellitus.

Somatostatin lowers basal and stimulated serum concentrations of insulin (54), glucagon (54) and pancreatic polypeptide (55). The combined suppression of insulin and glucagon release should give rise to a syndrome of mild diabetes with impaired glucose tolerance, but, because of concomitant suppression of glucagon, neither severe endogenous hyperglycemia nor hyperketonemia are to be expected (56).

In our patient islet cell function was investigated by oral glucose tolerance test, arginine infusion and the response to a mixed meal (1,000 calorie hamburger meal). Basal levels of glucagon (IRG) and C-peptide* in all three tests were normal.

During the glucose tolerance test (Figure 6) there was no suppression of IRG and the rise in C-peptide appeared to be normal. Basal SLI levels rose fourfold by 120 minutes after glucose ingestion.

During the arginine infusion test IRG rose more than fourfold and there was a 50 mg/dl rise in blood glucose (Fig 7). C-peptide levels also rose: however, the normal C-peptide response to arginine infusion has not been reported. SLI levels rose more than fourfold in response to arginine infusion.

The excessive rise in glucagon levels in response to arginine stimulation simulated the A-cell response of ordinary diabetes. Thus, despite the enormously increased levels of somatostatin, the A-cells were resistant to it.

After the mixed meal (Fig 8) there was a normal rise in IRG and although somewhat delayed, C-peptide rose in a normal fashion. SLI showed a dramatic (eightfold) increase in response to the meal.

The C-peptide response in these tests suggests that B-cell function was only moderately impaired. In fact, while in our General Clinical Research Center the patient's diabetes could be satisfactorily controlled with carbohydrate restriction and without insulin injection.

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*Direct measurement of immunoreactive insulin was impossible in this patient due to insulin antibodies presumably resulting from insulin therapy. For the same reason it was impossible to perform the pancreatic polypeptide radioimmunoassay.

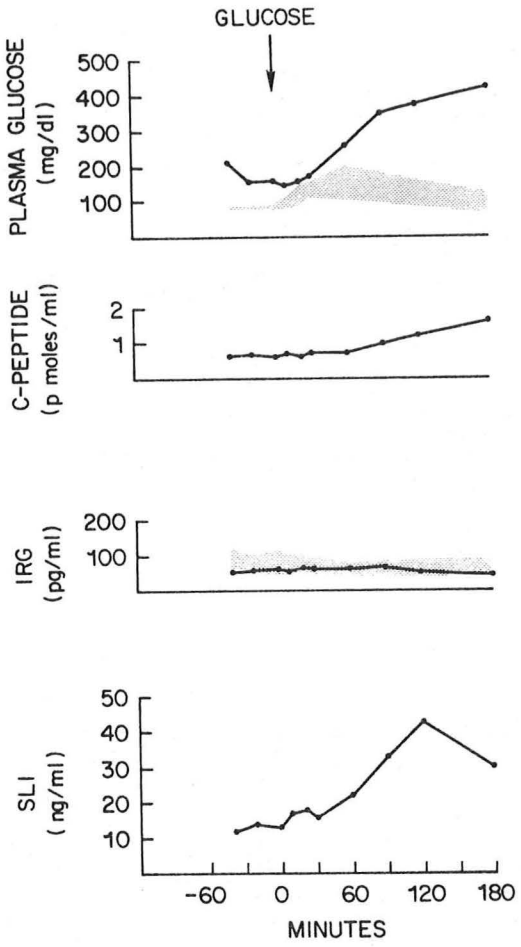

Figure 6: Plasma glucose, C-peptide, immunoreactive glucagon (IRG) and somatostatin-like immunoreactivity (SLI) response to an oral glucose load in the patient with somatostatinoma. The shaded areas represent the response of nine normal subjects (mean ±SD) to the same glucose load (57).

It is also noteworthy that a needle biopsy of the patient's quadriceps muscle revealed thickening of the capillary basement membrane (2219 ± 349 Å) (diabetic range above 1600 Å (59). This finding also makes the patient's diabetes indistinguishable from the common forms of diabetes mellitus.

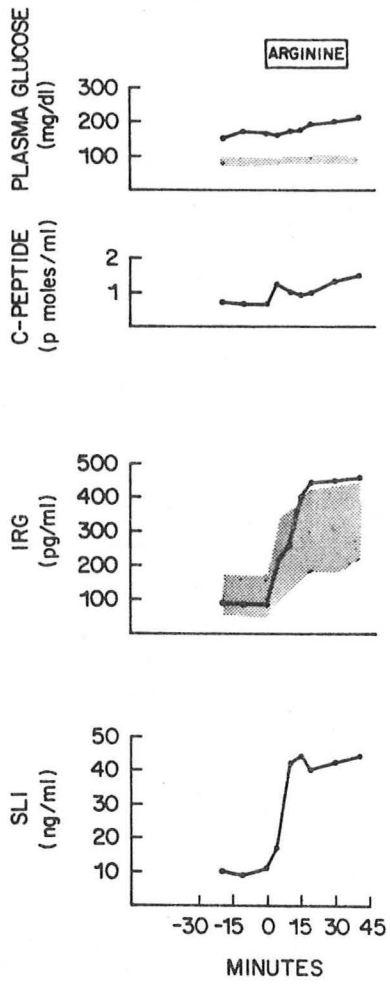

Figure 7: Plasma glucose, C-peptide, IRG and SLI response to intravenous arginine in the patient with somatostatinoma. The shaded areas represent the response of 28 normal subjects (mean ±SD) to a similar intravenous infusion of arginine (58).

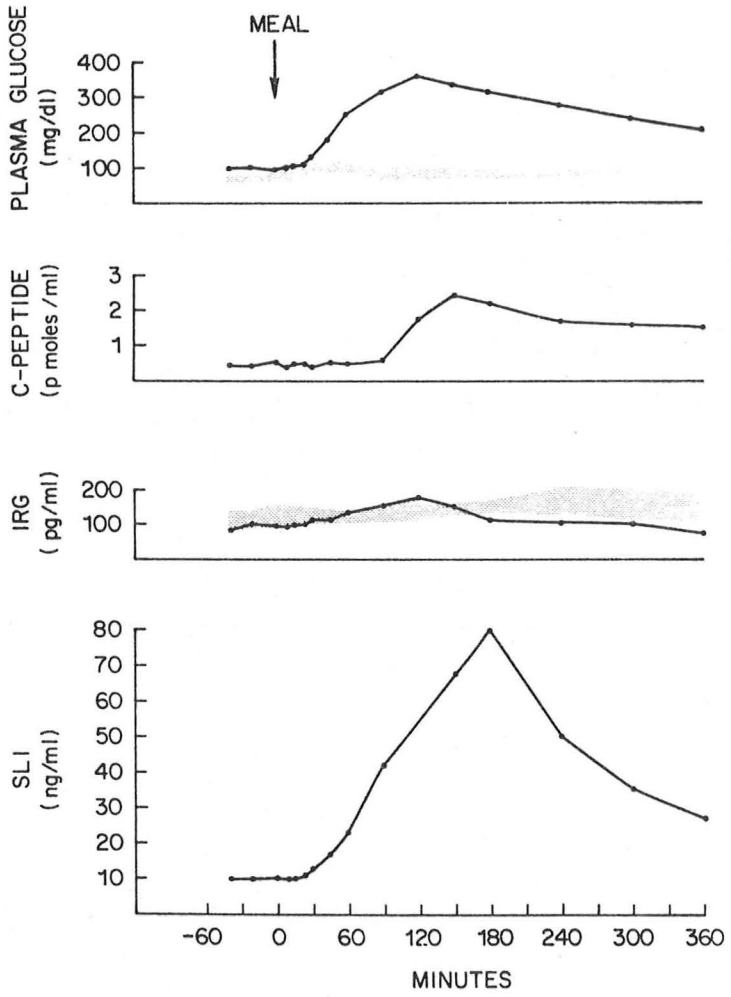

Figure 8: Plasma glucose, C-peptide, IRG and SLI response to a mixed meal in the somatostatinoma patient. The shaded areas represent the response of ten normal subjects (mean ±SD) to the same meal.

3. Gastrointestinal Hormones

Somatostatin suppresses the release of gastrin (60), CCK (61), secretin (62), motilin (63), GIP (64) and GLI (65). This may lead to a variety of gastrointestinal manifestations, including hypochlorhydria, indigestion, postprandial fullness, eructations, vomiting, abdominal pain, maldigestion, steatorrhea, diarrhea, and susceptibility to cholelithiasis secondary to reduced postprandial gallbladder contraction.

Table 3 shows gastrin and GIP responses to the mixed meal. Gastrin levels were normal. Gastrin rose to 210 pg/ml at a time when plasma somatostatin increased several fold (Fig 8). GIP levels were suppressed and did not rise in response to the meal in patient E.L.

TABLE 3. Postprandial serum concentration of gastrin and GIP. Blood samples were drawn during the mixed meal study depicted in Figure 8. GIP radioimmunoassay was performed by Drs. A. Shulkes and J. Walsh, Los Angeles, California (normal fasting level: <60 pmoles/l).

		Time (min) after meal						
	Basal	10	20	45	90	120	240	360
GASTRIN pg/ml	79	113	147	189	210	180	151	138
GIP fmoles/l	<10	<10	<10	<10	<10	<10	<10	<10

B. GASTROINTESTINAL FUNCTION

1. Gastric Acid Secretion

Somatostatin has been shown to be a potent inhibitor of gastric acid secretion (60,66).

Gastric acid analysis in patient EL revealed a BAO of 0.04 meq/h and a MAO (following pentagastrin) of 1.92 meq/h. Despite the patient's truncal vagotomy and pyloroplasty these numbers appear low and may be considered hypochlorhydric (maximal acid output following truncal vagotomy and pyloroplasty has been reported to average 6.7 ± 4.3 (range 2.6 to 16.0 meq/h) (67).

The patient's relative hypochlorhydria may be explained by a direct inhibitory influence of somatostatin on the parietal cells. However, with hypochlorhydria and a history of vagotomy one might expect very high postprandial gastrin levels and thus, this patient's normal gastrin values (Table 3) may indeed reflect an inhibitory effect of somatostatin on gastrin release.

Suction biopsy from the body of the stomach showed normal mucosal architecture with parietal cells appearing normal in number and morphology. There were no signs of superficial or atrophic gastritis. Histological examination of the resected antrum (Whipple's procedure) revealed no abnormalities.

2. Gastric Emptying

Several observations suggest that somatostatin may play a role in controlling motor activity of the digestive tract. Inhibition of gastric emptying has been described by several investigators (63,68,70).

In patient E.L. gastric emptying was studied by scintigraphy using a ^{99m}Tc-labeled meal (71). Gamma counts from the stomach were recorded with a computer via the gamma camera every five minutes for one hour. In the patient 100% of the activity was still in the stomach at 60 minutes. In normal controls 52 ± 2% (mean ±SEM) of the

radioactivity is found to remain in the stomach at 60 minutes following the meal. The vagotomy and pyloroplasty in the patient would even be expected to enhance gastric emptying (72). Thus, delayed gastric emptying in this patient is consistent with the known action of high circulating levels of somatostatin.

3. Small Bowel Motility

The effect of somatostatin on small bowel motility is very complex and has not been investigated to a great extent. Somatostatin may inhibit motility by suppressing the release of such gastrointestinal hormones as motilin, gastrin, and cholecystokinin that are involved in the control of intestinal motor activity.

For the purpose of this Grand Rounds I would like to pick two motility parameters to discuss. One is intestinal transit time and the other is the interdigestive myoelectric complex.

When we studied normal controls by small bowel perfusion during somatostatin administration (26) we found that somatostatin almost doubled transit time in the test segment (Fig 9). Intestinal transit time was not studied in patient E.L.

Prolongation of transit time by somatostatin may be of benefit in diarrheal states that are at least in part caused by an intestinal motor disorder. One possible application is the malignant carcinoid syndrome and we have, in fact, markedly reduced daily stool weight in such a patient during somatostatin infusion (74).

The second motility parameter is the interdigestive migrating complex (75). The cyclic recurrence of the phenomenon and the migration of the activity front down the intestine is thought to clean up the remnants of the preceding meal and function as the "housekeeper" of the small intestine (76).

This migrating myoelectric complex is abolished by feeding or infusion of gastrin, cholecystokinin and insulin.

An interesting observation was recently made in dogs where somatostatin infusion was shown to double the frequency of the interdigestive myoelectric complex. Also, after feeding, somatostatin converted the fed-type pattern to the fasting-type pattern with the onset of migrating complexes (77).

Small bowel motility was evaluated in patient E.L. by using a four-lumen polyvinyl tube with side openings 6 cm apart in the proximal small bowel. The tubes were constantly perfused with water at a slow rate and connected to external Statham transducers and a multichannel recorder.

In patient E.L. six migrating myoelectric complexes were observed in the fasting state at a mean interval of 38 min (range 33 to 48), complexes lasted for 274 sec (range 220 to 284) and propagation velocity was 2.1 cm/min (range 1.2 to 3.2). There was myoelectrical quiescence between the migrating myoelectrical complexes. However, the lack of a crescendo activity before and a decrescendo activity following the complexes may have been due to the patient's vagotomy. In 11 normal subjects migrating myoelectric complexes were observed at intervals of 120.3 $\pm$ 14.0 min, the duration of complexes was 420 $\pm$ 36 sec and the propagation velocity was 3.9 $\pm$ 0.5 cm/min. The patient did not tolerate eating with the tube in place and the motility pattern of the fed state could not be studied.

The results of this patient's motility study are thus consistent with experimental results observed in the canine intestine (77).

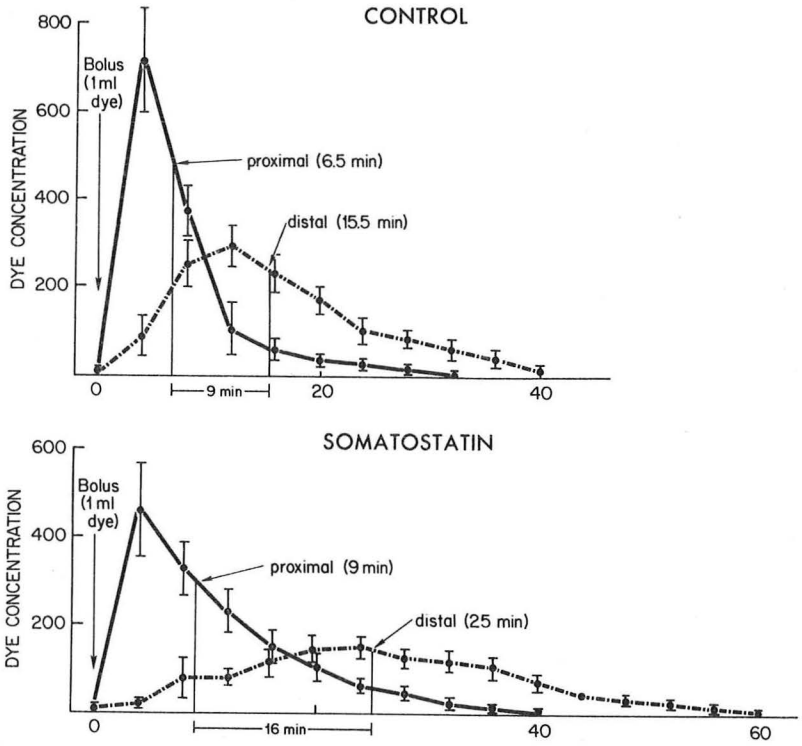

Figure 9: Dye dilution curves for estimating mean transit time in a 30 cm jejunal test segment. Ordinate gives normalized dye concentration (^{3}H-mannitol, ^{14}C urea and BSP were used). Abscissa gives time in minutes. Dye concentration was measured every 4 minutes at the proximal and distal sampling site. Mean transit time is estimated between the vertical lines that separate half the area under each curve (73). Somatostatin prolonged mean transit time from 9 to 17 minutes. Atropine (bottom) prolonged transit time from 9 to 19 minutes.

It is possible that this motility disturbance played a role in the patient's abdominal discomfort and dyspeptic symptoms.

4. Small Intestinal Absorption

Somatostatin reduces blood levels of sugars, Ca isotope and triglycerides after oral test meals (78-81). This suggests but does not prove reduction of absorption. One physiological role proposed for somatostatin by Dr. Unger and his associates (82) is the regulation of nutrient influx from the gastrointestinal tract into the circulation in coordination with the insulin-mediated regulation of nutrient disposal to the tissues. Thus the islets of Langerhans with its A, B and D cells would have control over all routes of nutrient flux: influx from storage sites (glucagon), disposal to tissues (insulin) and nutrient uptake by the intestine (somatostatin).

To test whether somatostatin directly affects small bowel absorption we have used a steady-state perfusion technique in the jejunum of helathy volunteers. The test solutions contained various nutrients and perfusion was performed during either saline (control) or somatostatin infusion (26).

Figure 10 shows the effect of somatostatin on glucose absorption from the human jejunum. Glucose absorption is decreased by somatostatin with a reduction of V_{max} by about 30 percent.

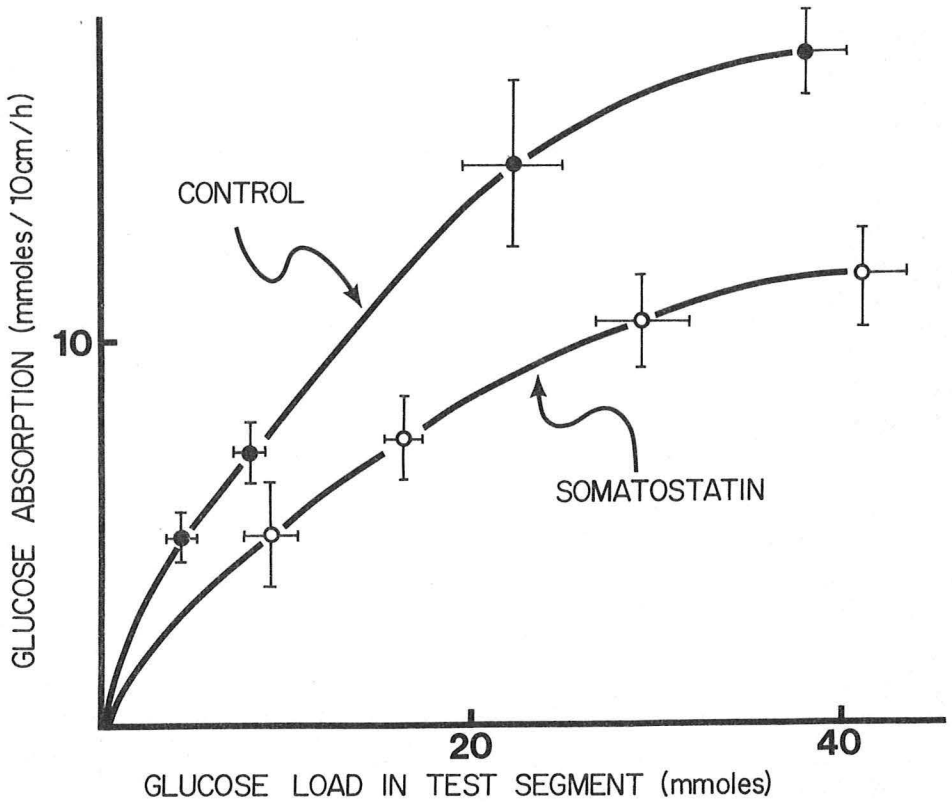

Figure 10: Kinetic curves for glucose absorption from 10 cm test segments of human jejunum. Each point represents mean ±SEM of 5 observations. Lineweaver-Burk analysis showed reduction of the V_{max} for glucose absorption (17.9 and 11.4 mmoles/10 cm/h for control and somatostatin periods, respectively) while the km values were similar (8.2 and 9.3 mmoles, respectively).

Table 4 compares jejunal absorption of sugars, amino acids and oleic acid in healthy subjects and the patient. The values observed in the patient are similar to the absorption rates seen in healthy subjects when somatostatin is given by continuous intravenous infusion (8 µg/kg/h).

TABLE 4. SMALL BOWEL PERFUSION STUDY

NET ABSORPTION FROM TEST SEGMENT
(PERCENT OF TOTAL AMOUNT OF SOLUTE ENTERING TEST SEGMENT)

	HEALTHY SUBJECTS		PATIENT
ABSORBED SOLUTE	CONTROL	SOMATOSTATIN INFUSION	"NO INFUSION"
GLUCOSE	65	36	49
FRUCTOSE	36	24	29
XYLOSE	18	16	14
GLYCINE	35	21	20
LYSINE	22	18	14
OLEIC ACID	57	47	49

In our patient pancreatic exocrine function was assessed by a secretin test, a Lundh meal and stool fat analysis.

In response to exogenous secretin the patient's duodenal aspirate was normal with respect to volume (3.8 ml/kg/80 min, normal >2.0) but bicarbonate concentration and output were borderline low (conc: 60 meq/liter, normal >70; output: 0.2 meq/kg/h, normal >0.2).

In response to the Lundh meal, however, there was only a minimal rise in amylase and lipase concentration suggesting either failure to release the hormones in the duodenal mucosa that induce pancreatic secretion or a direct inhibitory effect of somatostatin on the pancreas (increased postprandial SLI levels: see Fig 8). This abnormal test result explains the patient's third cardinal symptom, namely steatorrhea. That the steatorrhea was of pancreatic etiology is further supported by the decrease of fecal fat on pancreatic enzyme replacement (Table 5).

TABLE 5. FECAL FAT ON 100 g FAT DIET IN PATIENT E.L.

	Stool Weight (g/24h)	Stool Fat (g/24h)
OFF MEDICATION	520	31
PANCREATIC ENZYME REPLACEMENT THERAPY	357	4

Table 6 summarizes the comparison between the known pharmacological actions of somatostatin and the findings in the patient with malignant somatostatinoma syndrome.

TABLE 6.

PHARMACOLOGICAL ACTIONS OF SOMATOSTATIN		FINDINGS IN PATIENT E.L.
	REDUCTION OR INHIBITION OF	
CARDINAL SYMPTOMS	Gallbladder motility (CCK-PZ release)	Gallstones
	Insulin release	Mild diabetes
	Pancreatic bicarbonate and enzyme secretion	Steatorrhea
	OTHER ENDOCRINE FUNCTIONS	
	GH-release	Absent growth hormone response to arginine and insulin
	TSH release	Normal TSH response to TRH
	Glucagon	Normal glucagon response to arginine
	Gastrin	Normal gastrin (relative hypo-gastrinemia?)
	GIP	No postprandial rise of GIP
	Renin activity	Not studied
	Erythropoietin	Not studied (normocytic, normochromic anemia)
	OTHER GASTROINTESTINAL FUNCTIONS	
	HCl and pepsin secretion	"Relative hypochlorhydria" (vagotomy)
	Gastric emptying	Delayed despite vagotomy and pyloroplasty
	Small bowel absorption of sugars, amino acids and fatty acids	Reduced in comparison to normal controls
	Intestinal motility	Abnormal
	Salivary secretion	Not studied (dry mouth)

The clinical features observed in the six patients with somatostatinoma syndrome that have thus far been reported are listed in Table 7.

TABLE 7. Review of clinical manifestations in 6 patients with somatostatinoma. (+) present, (-) absent, NS: not studied, NR: not reported, W: Whipple's procedure, CH: Chemotherapy with streptozotocin, EL: Exploratory laparotomy

Author	Patient's Age and Sex	Patient's Home Country	Cholelithiasis	Diabetes Mellitus	Steatorrhea	Diarrhea	Hypochlorhydria	Anemia	Weight loss	Liver Metastases	Localisation of Primary Pancreatic Tumor	Additonal Features	Treatment	Outcome
Larson (2)	55 f	Sweden	+	+	+	+	+	NR	+	+	Head	none	W	Died postop.
Ganda (3,84)	46 f	USA	+	+	NS	-	-	+	+	-	Head	none	W	Asymptomatic 24 months later
Kovacs (4)	54 m	Canada	-	+	NS	-	NS	NR	+	+	Tail	ACTH prodcution	EL	Died postop.
De Nutte (5)	NR	Belgium	+	+	+	-	NS	NR	+	+	NR	none	CH	Not known
Galmiche (6)	70 f	France	+	+	+	+	+	NR	+	+	NR	Calcitonin production	W	Died postop.
Present Case (1)	52 m	Canada	+	+	+	+	+	+	+	+	Head	Calcitonin production	W,CH	Clinical and biochemical improvement

FOLLOWUP OBSERVATIONS IN E.L.

Following intravenous hyperalimentation the patient's plasma somatostatin level had risen from about 10,000 to 20,000 pg/ml at the time of surgery. This was accompanied by

a marked increase in the size of the liver metastases as followed by angiography.

After resection of the primary tumor plasma SLI dropped to 17,000 pg/ml and decreased further after subsequent chemotherapy (Table 8).

An interesting observation was made when the patient's plasma after tumor resection was again subjected to gel filtration chromatography. While pure somatostatin was the major fraction of total plasma SLI before surgery (1600 dalton), postoperatively the precursor form (3,000 dalton) is predominating. This suggests that the liver metastases mainly release the high molecular precursor form of somatostatin while the primary pancreatic tumor released the pure decatetrapeptide.

Complications during his workup and treatment included a postoperative wound infection which resolved, an a-v fistula in a liver metastasis and a hepatic artery aneurysm, both of which were successfully occluded by balloon and embolization.

TABLE 8. COURSE OF PLASMA SLI (PG/ML) IN PATIENT E.L.

Aug./Sept. 1978	Diagnostic workup	9,000 - 13,000
October 1978	Preoperatively, (after six weeks of intravenous hyperalimentation)	20,000 - 25,000
November 1978	Postoperatively	17,000
December 1978	Chemotherapy	14,000
January 1979	(streptozotocin by	11,000
February 1979	hepatic artery infusion)	7,000

The patient is clinically improving, has gained 4 kg since surgery, and his insulin requirements which had risen to 40 U Lente per day preoperatively are now down to 7 U in April, 1979. Followup angiography showed 30% shrinkage of the liver metastases in April, 1979.

EARLY DIAGNOSIS

The early diagnosis of the somatostatinoma syndrome is clearly difficult. A benign somatostatinoma, such as that reported by Ganda et al. (3), in which plasma SLI was not measured, might well produce only intermittent hypersomatostatinemia. In this event a provocative test with a substance such as tolbutamide, a powerful stimulus of pancreatic somatostatin release (85), may be required to expose the abnormality, as was first employed by Nutte et al. (5). The early features are nonspecific and common, a

triad of dyspepsia, mild diabetes and cholelithiasis. Both diabetes, which was present in all cases, are common in the older age group in which all of the somatostatinomas have thus far been found.

Steatorrhea in the absence of pancreatitis may be a clue to the diagnosis, but the only patient without evidence of metastases did not have steatorrhea (3).

Perhaps the early diagnosis of benign somatostatinoma may require localization of the primary tumor. In the present case, the tumor was present at least 4 years prior to the definite diagnosis. During that period, symptoms were limited to postprandial eructation and periumbilical cramping after heavy meals and were attributed to the patient's gallstones. Late in 1977, however, the relatively abrupt onset of severe weight loss, steatorrhea, diabetes and the finding of a serum alkaline phosphatase at the upper limit of normal may herald the development of hepatic metastases and of more severe hypersomatostatinemia. When a presumably endocrine pancreatic tumor distorts the duodenum (Fig 1), its removal is urgently indicated and studies of survival in patients with the Zollinger-Ellison syndrome indicate that this location is associated with the most favorable prognosis. With the availability of ultrasonography and arteriography, pancreatic tumors are more easily detected. Since somatostatinoma may not be rare, one wonders if blood somatostatin should be measured in the workup of a diabetic patient with dyspepsia, or gallstones.

A plasma SLI radioimmunoassay is available at several centers. A survey of patients with such diseases as gallstones, diabetes mellitus, and exocrine pancreatic insufficiency may reveal if such a screening is clinically relevant. No disease other than the somatostatinoma syndrome is known to cause severe hypersomatostatinemia, although juvenile-type diabetes may be associated with modest elevations (86,87). If basal levels of SLI are normal in a patient with a pancreatic tumor, a provocation test with tolbutamide or another stimulus may be of value.

SUMMARY

Knowledge of the pharmacological actions of somatostatin allowed recognition of the cause of this patient's syndrome and availability of a radioimmunoassay confirmed the diagnosis of somatostatinoma. Cardinal symptoms were cholelithiasis, diabetes and steatorrhea associated with a pancreatic tumor. Further pathophysiological studies showed - with a few exceptions - that the patient's endocrine and gastrointestinal function was consistent with the effects of somatostatin excess predicted from experimental observations.

Six patients with somatostatinoma - including the present case - have been reported. The true incidence of the disease, however, is not known at this time, since general awareness of this syndrome among physicians has probably not been reached.

Future experience will have to show whether a provocation test with tolbutamide or screening of plasma somatostatin levels by radioimmunoassay may be beneficial in the early recognition of this disease.

APPENDIX

Potential Clinical Use of Somatostatin

The original publication by Guillemin and co-workers about the discovery of somatostatin ends with the following statement: "Should SRIF be active in humans, its possible clinical significance, particularly in the treatment of acromegaly and the management of juvenile diabetes, has not escaped our attention"(10).

Limitations for clinical use are caused by two characteristics of somatostatin: its short-lived action and its multiple effects. Attempts are being made to produce somatostatin analogues that are long acting and only enhance certain actions of the peptide (88).

1. Diseases for which clinical studies or single case reports have suggested a potential benefit:

Acromegaly (48,89, 90)

Diabetes mellitus (91-93)

Insulinoma, glucagonoma, VIPoma, gastrinoma: preoperative or palliative use suggested (88 ,94)

Malignant carcinoid syndrome (74,95)

Upper gastrointestinal bleeding due to peptic ulcer disease or esophageal varices (96-98)

2. No benefit:

Extrapyramidal disorders and EEG abnormalities (99)

Experimental pancreatitis in rats (100)

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