Identification of the Diarrheogenic Hormone Associated with Non-Beta Islet Cell Tumors of the Pancreas

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ISLET cell tumors of the pancreas have proven to be a multipotential source of hormones capable of producing a variety of clinical syndromes. The identification of insulin,[26] gastrin,[11] serotonin[28] and glucagon activity, singly or in combination with other hormones,[15] in these tumors has made them prime suspects in the production of new gastrointestinal clinical syndromes. A different hormone arising from certain of these non-beta islet cell tumors has been postulated as the cause of a rather uncommon syndrome of watery diarrhea, hypokalemia and achlorhydria.[19, 27] The laboratory evidence gained from intensive studies of two patients with these clinical findings suggests a secretin-like hormone of islet cell origin as the etiological factor in the production of this clinical triad.

Diarrhea with Islet Cell Tumors

Diarrhea, with or without ulceration, has long been associated with non-beta islet cell tumors of the pancreas.[33] Gregory's proof[11] that these tumors elaborated the potent gastric secretagogue, gastrin, provided a sound explanation for the production of diarrhea as a result both of marked gastric hypersecretion with neutralization of the pancreatic enzymes and irritation of the mucosa, and a direct stimulatory action of the hormone on the intestinal tract. As more cases were reported, certain irregularities became apparent, such as the absence of ulcers in the presence of marked gastric hypersecretion and diarrhea. In some patients the diarrhea was actually a watery diarrhea instead of steatorrhea.[20] Gradually it was appreciated that some patients with non-beta islet cell tumors did not have gastric hypersecretion and ulcer but were serious clinical problems because of severe watery diarrhea leading to a marked potassium loss and death from hypokalemic nephropathy.[25, 29]

Murray *et al.*,[23] in 1961, found achlorhydria in a patient with watery diarrhea and a non-beta islet cell tumor, thereby substantiating previous impressions that hypersecretion and hyperacidity were absent in these unusual conditions. With the documentation of achlorhydria, excess acid was invalidated as the mechanism of diarrhea for this group of patients. Matsumoto and associates,[19] after studying such a patient operated upon by Longmire, reported that Grossman was unable to find gastrin activity in the pancreatic tumor. They introduced the term "pancreatic cholera" in order to differentiate the characteristics of this small group of patients from those who

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have gastrin-producing tumors. Marks and associates [18] in a recent thorough review of these cases called attention to the rarity of this condition and emphasized the significance of the achlorhydria. They referred to this entity as the WDHA Syndrome (watery diarrhea, hypokalemia and achlorhydria), and stated that these criteria are necessary for including a case in this group of patients.

The following two cases are presented to support the concept that certain islet cell tumors produce a secretin-like hormone whose dual stimulating and inhibiting effects may explain the triad of watery diarrhea, hypokalemia and achlorhydria.

Case Reports

Case 1. B. O., a 24-year-old woman was referred to The Ohio State University Hospital in May 1967 by Dr. Ronald L. Scott and Dr. Davis A. Baltz of Vallejo, California, because of a possible islet cell tumor of the pancreas. She had a 4-year history of unexplained, episodic, watery diarrhea. These episodes, occurring at 2 to 6-month intervals, often lasted longer than 2 weeks with up to 15 stools per day. Hospitalization was often required for correction of the resultant prostration and severe electrolyte imbalance, particularly hypokalemia. It is interesting that the patient had no episodes of diarrhea during two pregnancies in this 4-year period.

Early in 1965, the patient was discovered to have left renal lithiasis and serum calcium levels which were persistently elevated above 11 mg/100 ml., leading to the diagnosis of hyperparathyroidism. Because of an intrauterine pregnancy diagnosed at the time of hospitalization, neck exploration had to be deferred until November 1965. At operation, four enlarged parathyroid glands were found, three and one-half of which were removed. The microscopic examination was reported as being compatible with primary hyperplasia of the parathyroids. Following this procedure, the serum calcium levels returned to normal and remained so for 14 months.

In January 1967, the patient was hospitalized after 17 days of severe watery diarrhea and found to have persistently elevated calcium levels between 12.0 and 12.6 mg./100 ml. (normal 8.4 to 10.8 mg./100 ml.) with low phosphorus levels in the range of 2.5 mg./100 ml. (normal 2.4 to 4.5 mg./100 ml.). Serum potassium ranged from 3.0 to 1.6 during this episode despite replacement of up to 120 mEq. per day. Following correction of fluid and electrolyte imbalance, the patient underwent re-exploration of the neck and the anterior mediastinum, but no parathyroid tissue could be found. However, coincidentally with remission of the diarrhea after operation, the serum calcium levels returned to low normal values. On April 3, 1967, the serum calcium was 10.2 mg./100 ml. but one week later during a recurrent attack of diarrhea the calcium level was again elevated to 15 mg./100 ml.

The patient's family history was significant only on her father's side. The paternal grandmother had hyperparathyroidism. The paternal grandfather, three uncles and three of five aunts had all been treated for peptic ulcer disease. Most significantly, the father and one of his brothers had ulcerogenic tumors of the pancreas.

On admission to The Ohio State University Clinical Research Unit, the patient was asymptomatic and remained so for 10 days. She appeared pale, but cheerful, and no abnormalities could be detected on physical examination. Initial hemoglobin, hematocrit, white blood cell count and urinalysis were normal. Serum sodium was 141, potassium 3.8, chloride 110 and CO_2 combining power 26 mEq./l. Fasting blood sugar, uric acid, blood urea nitrogen (BUN), creatinine and amylase were all normal. Serum calcium levels of 9.0 to 9.8 mg./100 ml. and phosphorus levels of 3.7 and 3.9 mg./100 ml. were normal, as was the magnesium value of 1.97 mEq./l. Thymol turbidity, cephalin flocculation, alkaline and acid phosphatases and icteric index were normal. The total proteins of 6.4 Gm./100 ml. were slightly low with a normal albumin of 5.4 and a decreased globulin of 1.0 Gm./100 ml. Serum protein electrophoresis showed an increase in the beta globulins and a decrease in the gamma fraction. Although the protein-bound iodine of 8.7 μg./100 ml. was slightly elevated (normal 4 to 8 μg./100 ml.), the uptake of radioactive iodine was normal. The plasma cortisol level of 29.0 mg./100 ml. was slightly elevated (normal 8 to 24 mg./100 ml.) and rose to 60.5 mg./100 ml. one hour after the intramuscular administration of 25 units of adrenocorticotropic hormone (ACTH). Three separate determinations of urinary 17-ketosteroids ranged between 2.3 and 3.6 mg./24 hours (normal 5 to 15 mg./24 hrs.) while the 17-hydroxysteroids were between 3.3 and 3.6 mg./24 hours (normal 2 to 9 mg./24 hrs.). Three determinations of urinary 5-hydroxyindole acetic acid ranged from 1.4 to 4.3 mg./24 hours (normal 1 to 8 mg./24 hrs.). Urinary potassium levels were always in the low range from 7 to 24 mEq./24 hours. An intestinal absorption test with D (+) xylose was within normal limits and several stool

TABLE 1. *Preoperative Gastric Analyses—Case 1*

Date	Study	Vol cc.	pH	Conc. mEq./l.	Output mEq.
5/9/67	2 hr. basal	170	8.00	0	—
5/10/67	Basal value	47	5.54	4	0.19
	Histamine stimulation*				
	15 min.	45	8.17	0	
	30 min.	9	5.86	50.6	
	45 min.	35	1.47	75.0	
	60 min.	20	1.20	97.5	Total 5.00
5/17/67**	Basal value	98	7.40	0	0.00
	Histamine stimulation*				
	15 min.	10	7.30	0	
	30 min.	4	7.00	0	
	45 min.	8	2.50	18.1	
	60 min.	42	2.00	30.0	Total 1.40

* Histamine acid phosphate 0.04 mg./Kg. subcutaneously.
** Simultaneous duodenal and gastric aspiration.

examinations disclosed no bacterial or parasitic pathogens or neutral fats. During the patient's first 10 days of hospitalization, when diarrhea was not a problem, there was an average loss of 42.5 mEq./day of potassium in the stool.

X-rays showed indentation of the lesser curvature side of the gastric antrum suggesting a mass either in the body of the pancreas or in the left hepatic lobe. There was no evidence of abnormal gastric secretion or ulcer. A small bowel series demonstrated a slightly dilated jejunum with dilution of barium indicative of excessive small bowel fluid. The transit time was normal. An intravenous pyelogram showed two small calculi in the left renal pelvis, each approximately 4 mm. in diameter. Only after a double dose (6 Gm.) of Telepaque® did the gallbladder visualize faintly on oral cholecystogram; no calculi were seen. Scanning of the parathyroid glands and the pancreas after intravenous administration of 250 μc. of Selenomethionine® (^{75}Se) failed to identify parathyroid tissue or to outline any tumor masses in the pancreas. A liver scan with 110 μc. of ^{198}Gold disclosed no metastatic foci. Several bioassays of fasting morning serum specimens by the Lai rat method were negative for gastrin activity.

Using fluoroscopic tube placement, repeated basal and augmented histamine gastric analyses were performed preoperatively by Dr. Richard D. Ruppert of the Division of Gastroenterology (Table 1). There was no evidence of gastric hypersecretion in these studies. Indeed, there was virtual resting achlorhydria that could be effectively overridden by histamine. The decreased gastric response to histamine on May 17, 1967, is interesting in that the patient's symptom-free period ended that afternoon with the onset of a typical episode of watery diarrhea. Resistance of gastric secretion to stimulation with histamine had been noted in September 1964 and again in July 1966 during attacks of diarrhea.

During the current bout of diarrhea, she averaged four to six stools per day over a 7-day period, losing 150 mEq. of potassium or more in an average of 2,000 Gm. of stool each day. Repeated stool examinations were negative for neutral fats. Her serum potassium fell to 2.8 mEq./l. by the third day of the diarrhea and remained low despite as much as 200 mEq. per day of oral and intravenous potassium supplementation. As with previous exacerbations, the serum calcium levels became elevated to 11.2 mg./100 ml. for the first time since admission. The patient's difficult-to-control and dangerous situation emphasized the necessity of exploring the pancreas.

At operation on May 24, 1967, a nodular, baseball-sized tumor in the superior margin of the neck of the pancreas and an olive-sized, encapsulated tumor within the substance of the tail of the pancreas were removed by resection of the body and tail of the pancreas. Prior to the resection, blood was aspirated from a prominent vein which drained the large tumor. The Lai bioassay of this venous blood was negative for evidence of a gastric secretagogue. Similarly, extracts of the tumor failed to show gastrin activity by the same test. No metastatic foci of tumor were seen at operation or found in biopsy specimens taken from the liver and

regional lymph nodes. The jejunum was fairly large, measuring about 4 to 5 cm. in width. The remainder of the small bowel was slightly edematous and contained a watery fluid.

In an effort to determine if there were any microscopic changes which might help explain the watery diarrhea and excessive loss of potassium, multiple biopsies were taken from many sites, including the fundus and antrum of the stomach, the mid-jejunum and the lower ileum. These specimens were histologically normal.

Microscopic examination * (Fig. 1) of the surgically removed pancreas showed the large tumor to be composed of papillary and solid groups of tall columnar cells which in some areas appeared shrunken while the cells in other areas appeared to be filled with light-staining transparent material. The tall columnar cells contained oval nuclei and an eosinophilic, poorly defined cytoplasm. The tumor was poorly encapsulated and the cells showed trabecular or rosette-like arrangements.

Sections through the smaller tumor showed cells which were of low columnar or cuboidal type and appeared in small masses separated by a scant stroma. In a few areas the tumor was composed of a single row of larger cells having an appearance similar to those described in the large tumor mass.

Random sections through the remainder of the pancreas showed many large but morphologically normal islets. In addition there were many islet cell adenomas of the small or nonfunctioning cell type. The cells were cuboidal in shape with clear cytoplasm and prominent, vesicular nuclei. Other adenomas showed cells of the ductal type. Sections taken taken from a nodule near the tail of the pancreas showed small, darkly staining islet cells separated by a deeply eosinophilic hyalinized connective tissue. Similar hyalinized adenomas were also found in other sections of the pancreas.

In conclusion, review of all pancreatic specimens showed multiple adenomas together with islet cell hyperplasia. The large tumor differed from the small tumors in the region of the tail of the pancreas in that the former was composed mostly of tall columnar cells with rosette or trabecular pattern.

During the operative procedure, it was noted that the duodenum repeatedly became distended with fluid and that the gallbladder was considerably enlarged, although there was no obstruction to the common bile duct. As part of a study to analyze bile taken from normal patients as well as those with gallstones, bile was aspirated from the gallbladder for complete chemical analysis. The visual impressions of a very dilute, non-viscous bile that was light green in color were borne out by subsequent analysis (Table 3). Despite dilution of the sodium and potassium concentrations in the gallbladder bile, there was a paradoxical elevation of 2½ times the chloride and 4 times the bicarbonate concentrations which had been found in bile aspirated from the gallbladders of 10 surgical patients with normal biliary tracts.

The diarrhea ceased within 24 hours after the tumors were removed. Serum electrolyte determinations on the second postoperative day were: sodium 142, potassium 3.5, chloride 106, CO_2 combining power 29 mEq./l. Serum calcium levels initially dropped to 7.6 mg./100 ml. but returned to a normal 9.4 mg./100 ml. prior to discharge. Phosphorus levels were always in the normal range. Glucose tolerance tests were normal during the postoperative period.

Five days after operation, two separate basal gastric analyses showed low pH and high normal acid concentrations. In a repeat augmented histamine test one month after operation, there was a good response to histamine (Table 2). Following drainage of an abscess in the lesser omental sac on the 18th postoperative day, the patient's recovery was uneventful. At discharge she was having one to two well-formed stools daily.

On February 4, 1968, the patient was admitted for follow-up evaluation to the Surgery Service of The University of California Medical Center, San Francisco. At this time, 8½ months after operation, she had gained 35 pounds and was having only one normal stool per day. The surgical scars were well-healed and there were no other significant physical findings. Hemoglobin, hematocrit, white blood cell count and differential and urinalysis were normal. Serum sodium was 143, potassium 4.5, chloride 108, CO_2 combining power 20 mEq./l. Fasting blood sugar, BUN, creatinine, alkaline phosphatase, total protein and prothrombin time were normal. The serum calcium of 10.4 mg./100 ml. and phosphorus of 3.3 mg./100 ml. were normal.

Roentgenograms of the chest and skull were normal. In abdominal films taken in the supine and upright positions, there was a slight lumbar scoliosis with convexity to the left, a normal intestinal gas pattern and calcification overlying the left kidney. On oral cholecystogram the gallbladder was well visualized, of normal size and without calculi. Upper G. I. series showed some deformity of the retrogastric area and duodenal sweep probably related to the previous operative procedure.

Gastric analyses disclosed normal resting acid

* The authors are grateful to Dr. Emmerich von Haam, Professor, Department of Pathology, The Ohio State University College of Medicine for the histologic descriptions.

levels and a good response to stimulation with Histalog® (Table 2).

Case 2. L. F., a 47-year-old mother of three was admitted to the Emory University Hospital on July 4, 1967, under the care of Dr. William Waters, III, after 2 days of protracted vomiting and anuria.

The patient's present illness began in October 1966 with the onset of diarrhea and gradual weight loss. The diarrhea consisted of four to five watery and mucoid stools per day containing no blood or pus and was refractory to treatment with Kaopectate® and paregoric. Outpatient evaluation in November 1966 disclosed a hemoglobin of 9.1 Gm./100 ml. and a normal urinalysis. A 2-hour postprandial blood sugar was 300 mg./100 ml. Serum protein electrophoresis was normal. Stool cultures showed no unusual organisms. A urine culture was positive for Monilia. Upper gastrointestinal series including a small bowel study showed no lesions but the gallbladder did not fill on oral cholecystogram. Sigmoidoscopy showed no abnormalities.

The physician's impression was that the patient had malabsorption and she was admitted to a hospital on November 5, 1966. Vital signs were normal and physical examination was unremarkable. Hematocrit was 32% with a normal white blood cell count and differential. An oral glucose tolerance test showed a typical diabetic curve with the fasting value of 174 mg./100 ml. rising to 324 mg./100 ml. two hours after glucose administration. The urine remained consistently negative for sugar, but occasionally contained small amounts of acetone. The serum iron content was 16 μc./100 ml. (normal 50 to 180 μc./100 ml.). Protein-bound iodine in the serum was 20 μc./100 ml. The D (+) xylose absorption test was normal with 7.9% of the ingested dose excreted in 5 hours. Serum lipase determination was 0.4 units/100 ml. (normal 0 to 0.3). Absorption studies using radioactive triolein and oleic acid revealed that 92% of the triolein was absorbed (normal 95%). No report was given for the oleic acid. Serum protein electrophoresis was normal with a nonspecific beta-globulin elevation. Serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were 28 and 17 units, respectively; lactic dehydrogenase (LDH) was 330 units (normal 200 to 500). The patient was discharged, and a nonroughage diet and several medications were prescribed for the suspected diagnosis of malabsorption syndrome.

Despite medications her complaints of diarrhea and weakness persisted over the next 6 to 7 months. In June 1967, the patient was given four tablets of cortisone per day, tapered to two a week and discontinued in the last 2 weeks of June. It is reported that she did not gain much relief from diarrhea while on this medication.

The hyperglycemia became more pronounced and the patient was treated with an oral hypoglycemic agent, presumably tolbutamide. On July 2, 1967, a few hours after taking the first dose of this medication, she became nauseated and dizzy, and was hospitalized at another hospital because of leg cramps and dark urine.

On admission the patient had an hematocrit of 50% and a white blood cell count of 17,300. Urinalysis showed 4+ albuminuria with hematuria and pyuria, but no glycosuria. Serum sodium was 126, potassium 2.7, chloride 100.8 and CO_2 combining power 13.5 mEq./l. BUN level was 40 mg./100 ml. Blood glucose levels ranged from 131 to 264 mg./100 ml. The urinary output was only 40 cc. in 2 days despite treatment with mannitol. Because of lack of response to treatment, the patient was transferred to the Emory University Hospital on July 4, 1967, for further evaluation.

Additional history obtained at Emory University showed a weight loss from 118 to 93 pounds in the preceding 6 months. The patient was allergic to chocolate, morphine and sulfa drugs. At one week of age she had undergone operation, presumably for correction of pyloric stenosis. She had had Cesarean section for placenta praevia in the past and stated that she had passed the menopause earlier in 1967. On admission the patient appeared cachectic with a blood pressure of 84/60 mm. Hg, pulse rate 92, respiratory rate 20 and oral temperature 98° F. Physical examination was generally unremarkable except for hepatomegaly to two fingerbreadths below the costal margin. The hematocrit was 42% and the white blood cell count 14,800 with 93% neutrophils. The urinalysis showed no glucose or acetone, but had 4+ albumin, sheets of renal epithelium and red blood cell casts. Serum chemistry determinations included a sodium of 120, potassium 2.7, chloride 90 and CO_2 combining power 9.5 mEq./l., BUN 71 mg./100 ml. and blood sugar 318 mg./100 ml. The serum was negative for acetone. Later on the day of admission, her serum potassium fell to 1.9 mEq./l. but there was relatively little change in the serum sodium and chloride levels. Blood pH at this time was 7.31 with pCO_2 21.9. Serum amylase was slightly elevated at 136 units (normal 40 to 110). Serum alkaline phosphatase was greater than 50 units, although the total bilirubin was only 0.58 mg./100 ml. SGOT was 83 units, LDH 265 units, cholesterol 160 mg./100 ml. and uric acid 11.6 mg./100 ml. The serum calcium was 9.1 mg./100 ml. and the phosphorus 4.8 mg./100 ml. Serum magnesium values were 1.2 and 1.8 mEq./l. Serum protein electrophoresis was normal with

TABLE 2. Postoperative Gastric Analyses—Case 1

Date	Study	Vol cc.	pH	Conc mEq./l.		Output mEq.
5/29/67	2 hr. basal—AM	316	2.11	17.5		5.53
	2 hr. basal—PM	35	1.45	69.0		2.42
6/6/67	12 hr. basal	200	1.50	47.5		9.50
6/7/67	12 hr. basal	470	1.60	55.5		26.10
6/29/67	Basal value	30	2.14	20.0		0.60
	Histamine stimulation*					
	15 min.	10	1.56	32.7		
	30 min.	25	1.12	83.0		
	45 min.	25	0.98	111.5		
	60 min.	22	0.94	112.0	Total	7.66
2/5/68***	Basal value	160	2.7	10.1		1.62
	Histalog®**					
	15 min.	40	1.4	59		
	30 min	45	1.1	93		
	45 min.	50	0.99	103		
	60 min.	45	1.1	96	Total	16.01

* Histamine acid phosphate 0.04 mg./Kg. subcutaneously.
** 50 mg. intramuscularly.
*** University of California, San Francisco Medical Center.

total proteins of 6.6 Gm./100 ml. The D (+) xylose absorption test showed excretion of greater than 6.8% of the administered dose. Stool samples were negative for ova and parasites. A quantitative fecal fat analysis while the patient was receiving 100 to 125 Gm. of fat daily averaged 1.2 Gm. per day over a 3-day period.

The catecholamine level in a 24-hour collection of urine was 45 μc. per 24 hours (normal range 30 to 200 μc.), while the vanillylmandelic acid level was 9.3 mg. (normal 0.7 to 6.8 mg.). A 24-hour collection of urine for 5-hydroxyindole acetic acid was reported as negative.

Roentgenologic evaluation of the patient's chest and abdomen was normal. An upper gastrointestinal series and a small bowel study suggested malabsorption on the basis of slow transit time and fluid-filled loops of bowel. Barium enema was normal and sigmoidoscopic examinations to 17 cm. disclosed no abnormalities.

Radioisotope scanning of the liver showed slight enlargement and two areas of decreased activity, one in the right upper lateral margin and the second in the anterior-superior margin. There was increased activity in the region of the spleen. The scan was interpreted as being compatible with abscess, hepatoma or metastatic neoplasm. A tube biopsy specimen from the jejunum showed normal villi with no features of a "sprue-like" syndrome.

Gastric analysis produced a basal volume of 110 cc. with pH 7.82 and no hydrogen ion present. In the hour after stimulation with Histalog®, the volume of gastric juice was 84 cc. with pH 1.51 and total hydrogen ion concentration 60 mEq./l. The pH values in subsequent analyses of eight basal aspirations ranged between 7.65 and 8.0.

Although dehydration and electrolyte imbalance were corrected by the third hospital day and BUN returned to normal by the seventh day, severe diarrhea, averaging 3,260 cc./day with a urinary output of 3,920 cc./day, posed a constant problem. These losses required replacement of an average of 4,230 cc./day by intravenous routes in addition to 3,060 cc. per day orally. Analysis of one liquid stool late in the course of the illness showed a total potassium loss of 109 mEq./day.

Because of the unrelenting clinical course and laboratory evidence, including the positive liver scan, an exploratory laparotomy was performed to determine the cause of the fulminating diarrhea. It was believed that the patient had a non-insulin secreting islet cell tumor of the pancreas, although the possibility of lymphoma was also considered.

At operation on August 8, 1967, by Dr. J. Richard Amerson, multiple tumor implants were noticed in the liver, especially in the left lobe, and in the mesenteric lymph nodes. The pancreas appeared edematous, but no definite tumor could be

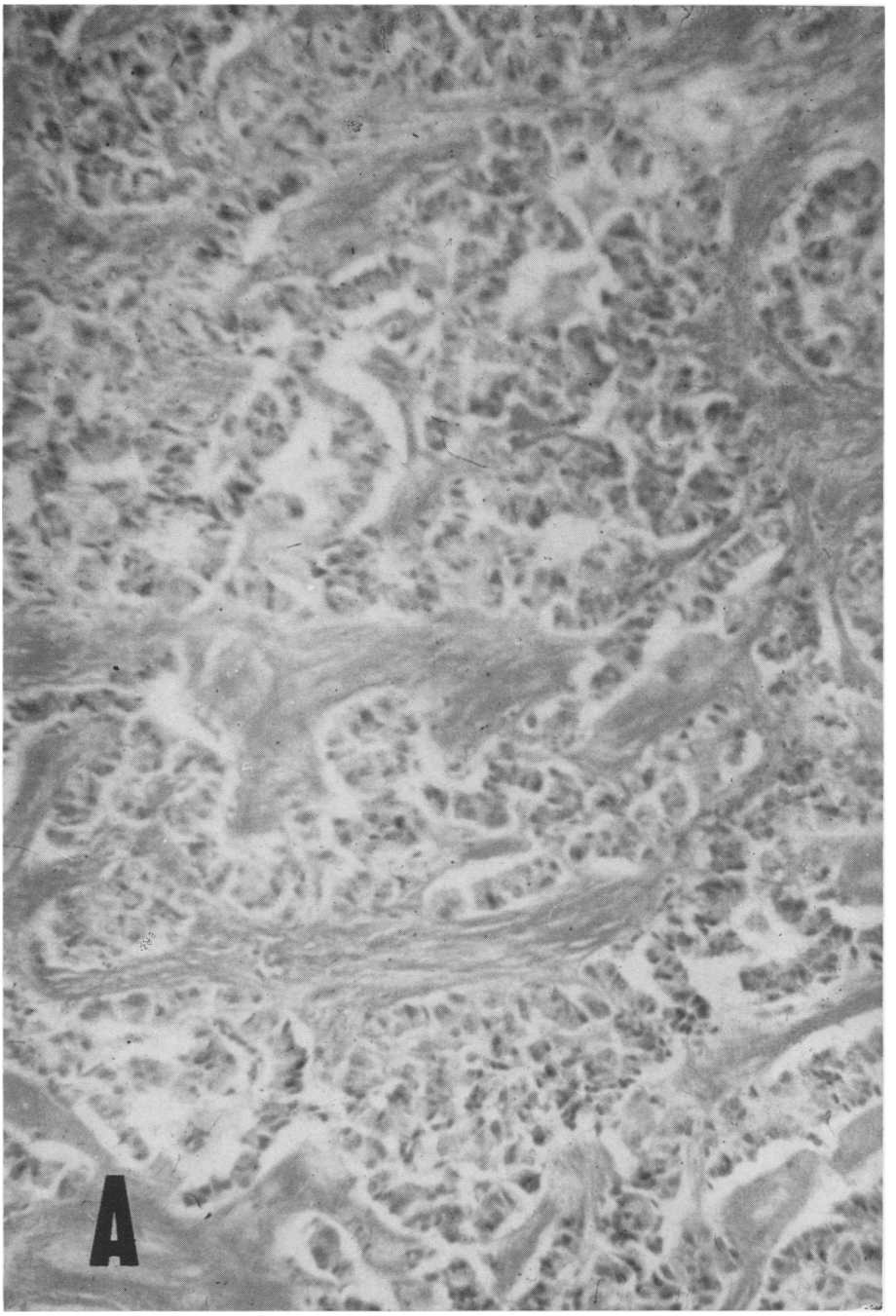

FIG. 1A. Section of the large non-beta islet cell tumor removed from the pancreas in Case 1, showing columnar cells in trabecular and rosette-like formation.

palpated. The gallbladder was enlarged, soft and filled with fluid. Biopsy specimens from a small nodule at the edge of the left lobe of the liver, as well as from one of the lymph nodes were submitted for frozen section. A diagnosis of metastatic adenocarcinoma, suggestive of islet cell tumor of the pancreas or carcinoid tumor, was given by the pathologist. The metastatic tumors were composed of tall columnar cells arranged in trabecular patterns as well as gland-like formations (Fig. 1).

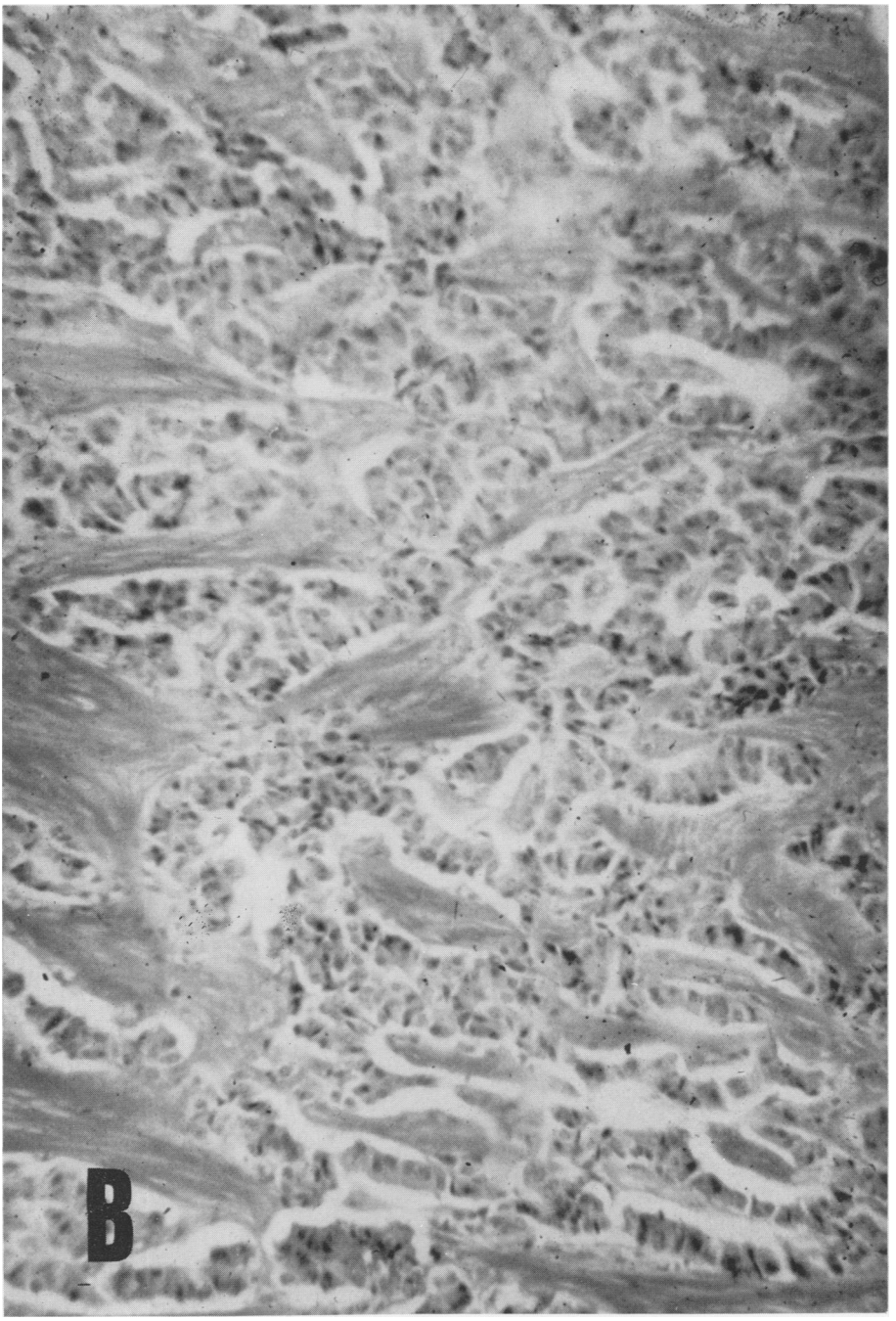

FIG. 1B. Section of the hepatic metastasis from the non-beta islet cell tumor in Case 2, showing large columnar cells arranged in ribbons as well as a glandular pattern. (Hematoxylin and eosin stain: original magnification 250×)

Postoperatively the patient's diarrhea continued unabated. On August 15, 1967, the patient underwent a second operation in the hope that a sufficient amount of tumor tissue could be removed by left hepatic lobectomy to bring the diarrhea under control. At that time, the right lobe was also found to be extensively involved with metastatic tumor. A large nodule in the left lobe and several

TABLE 3. *Gallbladder Bile Analyses*

Determination	Case 1	Case 2	Control*
Sodium (mEq./l.)	156	145	187.4 ± 38.7
Potassium (mEq./l.)	2.4	2.6	11.5 ± 4.5
Chloride (mEq./l.)	111	90	41.6 ± 21.9
Bicarbonate (mEq./l.)	26.2	48.1	8.8 ± 7.9
Bilirubin, total/direct (mg./100 ml.)	52.5/50	9.2/7.4	300/ –
Bile acids (mEq./l.)	21.2	9.5	143/4 ± 55.9
pH	7.65	7.73	6.5–9.0
Osmolality (milliosmols/l.)	281	276	300
Specific viscosity (Centipoise)	1.029 (29° C)	1.096 (25°)	H_2O = 1.000
Specific gravity	1.016	1.010	1.033
Water conc. (Gm./100 ml.)	97.5	97.5	82
Total solids (Gm./100 ml.)	3.8	3.8	16.6

* Mean of 10 normal patients.

lymph nodes were excised for further study. Since all the tumor could not be removed, the operative procedure was not extended.

At the time of operation, particular attention was paid to the gallbladder which was previously noted to be enlarged. In view of the unusual chemical findings in the bile analysis of Case 1, it had been preoperatively planned to aspirate bile from this patient's gallbladder for a comparable analysis, and this was done. The results of the chemical analyses were similar to those in Case 1 (Table 3). Culture of the bile showed a light growth of diphtheroids.

In view of the patient's extensive liver metastases, and the previous experience of a favorable response to irradiation, in amounts of 4,800 Roentgen units, in patients with diarrhea secondary to gastric hypersecretion (2, 22), it was elected to attempt palliation using this mode of therapy. Postoperatively the patient received 2,000 r to the upper abdomen, but symptoms did not abate and she died suddenly one month after the second operation.

At autopsy the head of the pancreas was enlarged and contained a 2 × 2 × 3 cm. hard, white infiltrating mass. The liver was enlarged, weighing 2,620 Gm. Multiple large metastatic tumor nodules, varying in size from 2 cm. to 6 cm. in diameter, were scattered throughout the right and middle lobes of the liver. The moderately enlarged lymph nodes of the para-pancreatic and mesenteric chains contained white tumor infiltrates. The left adrenal gland was enlarged and weighed 40 Gm. It contained a 2 cm. metastatic tumor nodule invading both cortex and medulla. All parathyroid glands were found to be two or three times larger than normal.

The final diagnosis from autopsy was non-beta islet cell carcinoma of the pancreas with metastases to the liver, regional lymph nodes and left adrenal gland.

All available tumor tissue from the hepatic metastases was frozen for subsequent analysis. An extract made from the tumor tissue was negative for gastrin activity when assayed by the Lai rat method.

Sections * taken through the head and midportion of the pancreas showed large collections of flat to columnar-like, pink-to-gray staining cells with abundant cytoplasm and pleomorphic, hyperchromatic nuclei. Multiple mitotic figures were present. The cells were arranged in large, poorly encapsulated, round nodules with unremarkable acinar tissue intervening between them. Multiple small foci of similar cells were interspersed through the fibrous tissue of the pancreatic parenchyma. Gomori chromium hematoxylin staining of the tumor tissue confirmed its islet cell nature and showed no evidence of beta cell granularity.

Discussion

Both patients presented with watery diarrhea, hypokalemia and achlorhydria. Gastrin activity was not present in either non-beta islet cell tumor. The first significant clue to secretory overactivity of the pancreas and biliary tract in these patients was the observation in Case 1 that the duodenum refilled with secretions during the operative procedure and that the gallbladder

* We are indebted to Drs. Wallace G. Campbell and Michel N. Haddad of the Emory University Department of Pathology for providing us with these microscopic descriptions. Emory Nos. A-67-304-34 & A-67-304-38.

was obviously enlarged. Bile was aspirated from the gallbladder for complete chemical analysis in the Biliary Research Laboratory of the Department of Surgery. The unexpected finding of high chloride and bicarbonate levels (Table 3) in the otherwise very dilute bile led to the suspicion that an unusual choleretic agent was being liberated by the islet cell tumor.

A review of choleretic hormones investigated in the past disclosed that significantly elevated levels of both chloride and bicarbonate in bile had been described only after secretin infusions in animals.[30] Other choleretic hormones such as pancreozymin-cholecystokinin have been shown not to significantly elevate bicarbonate or chloride levels in bile, while it has recently been demonstrated that gastrin significantly raises the bicarbonate concentration but has no effect on the chloride level. Histamine, which has a significant choleretic action, decreases bicarbonate levels while raising the chloride concentrations in bile.[32]

The dilated gallbladder found in Case 1 was quite consistent with observations previously described after secretin administration. This phenomenon is the basis for the secretin test of biliary function in humans.[1] The suggestive chemical findings in the bile of Case 1 prompted the subsequent aspiration of bile for complete analysis from the obviously distended gallbladder in Case 2. The strikingly similar chemical results in the bile of the latter case were indeed encouraging (Table 3).

The third exciting similarity in both patients was basal achlorhydria, not refractory to histamine. The ability to override the achlorhydria in both patients by histamine stimulation suggested that an inhibitory substance might be elaborated by the tumors in these patients. Although postoperative gastric analyses were not performed in Case 2 because of the presence of large amounts of metastatic tumor and because of her early demise, it was possible to carry out several postoperative gastric acid determinations in Case 1. The surprising and prompt return of normal acid values in this patient within 5 days after operation strongly implied that an inhibitory factor had been removed with the excision of the pancreatic tumors. Gastric analysis performed 8½ months after operation continued to show normal amounts of free acid (Table 2).

Dragstedt *et al.*[9] have demonstrated that secretin inhibits basal gastric acid output as well as acid stimulation by feeding. Similar findings have led Grossman[12] to speculate that secretin might be of eventual value in the treatment of duodenal ulcer. Secretin is not usually able to completely inhibit insulin or histamine-stimulated gastric secretion. However, in 12 proven cases of non-beta islet cell tumors with watery diarrhea, hypokalemia and achlorhydria collected to date, five of the six patients with malignant islet cell tumors had histamine-fast achlorhydria. This suggests that such malignant tumors may be capable of more marked production of the responsible hormone.

Recent studies by Gardner and Cerda[7] have demonstrated that an extract of an hepatic metastasis from a non-gastrin producing, non-beta islet cell tumor is capable of inhibiting the intestinal transport of fluid and electrolytes as measured in everted sacs of hamster distal ileum. Using the same experimental model, Gardner, Peskin, Cerda and Brooks[8] have shown that commercial preparations of gastrin, secretin and pancreozymin possess the same inhibitory properties. From these experiments, they concluded that one of these hormones might be the active agent in the production of the watery diarrhea by preventing fluid and electrolyte absorption in the distal gastrointestinal tract. Recently Johansen *et al.*[13] have reported that biopsies of the rectal mucosa in children, taken 15 minutes after the administration of two units of secretin/Kg. of body weight, demonstrated displacement of the nuclei toward the luminal sur-

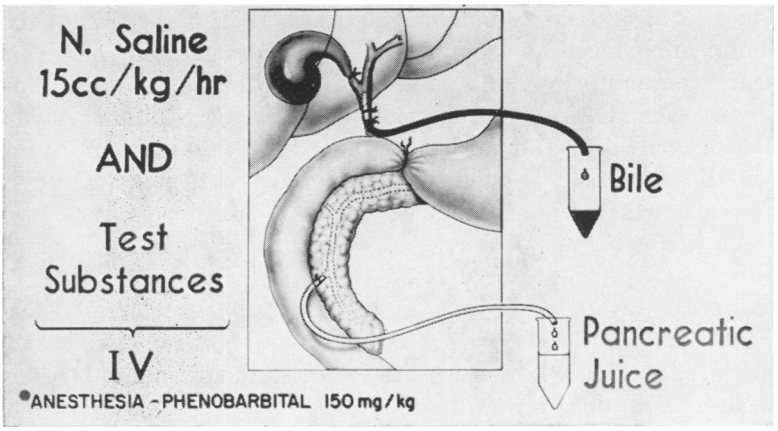

FIG. 2. The experimental model, modified from T. M. Lin, for the bioassay of secretin • and other test substances in anesthetized dogs.

face with a "fuzzy" change in the area between nuclei and subepithelial connective tissue. They postulated that such structural alterations could result in a modification of cell "porosity" to fluid and electrolytes leading to the accumulation of fluid and electrolytes in the lower intestinal tract.

In this connection, it is of interest that Greenough,[10] in studies of a different disease entity in 1965, proposed that biliary and pancreatic juice hypersecretion could be responsible for the diarrhea of cholera. He postulated that an excess of secretin, released from the duodenum by large numbers of cholera vibrios, might cause biliary and pancreatic hypersecretion in amounts as high as 8 liters per day.

Wormsley,[31] in a recent article dealing with the effects of single injections and constant infusions of secretin in man, noted that two subjects suffered attacks of explosive, painless diarrhea one or more hours after the completion of a 1-hour intravenous infusion of 10 to 25 units of Boots secretin/Kg. of body weight. The absence of cramping pain suggests that secretin does not act by increasing gut motility. This suggestion is supported by the finding of normal or delayed transit times on barium studies in our two patients. We have been unable to produce similar diarrhea in dogs by the constant intravenous infusion of as much as 25 units of Jorpes secretin/Kg./hour, carried out for as long as 53 hours. However, on the basis of the other experimental studies cited above, carried out in humans as well as in the laboratory, it appears that secretin is capable of the production of watery diarrhea with attendant electrolyte disturbances.

The intriguing observation, in Case 1, of hypercalcemia occurring simultaneously with diarrhea and unrelieved by nearly total parathyroidectomy and the finding at autopsy in Case 2 of uniform parathyroid hyperplasia, raises the possibility of still another hormone being produced by these tumors. In a review of the proven cases of non-beta islet cell tumors with diarrhea, achlorhydria and hypokalemia collected to date, over half have shown hypercalcemia which was not necessarily related to the onset of diarrhea. In a recent report, Kofstad *et al.*[14] stated that the hypercalcemia associated with a malignant tumor of the pancreas in their patient, was probably due to a parathormone-like hormone which, however, had no influence on the tubular reabsorption of phosphorus. Since no parathormone assays were done in this case or are known to have been performed on any of these islet cell tumors, demonstration of the presence of this hormone in these patients must await further studies.

In order to prove or disprove the presence of a hormone with secretin-like activ-

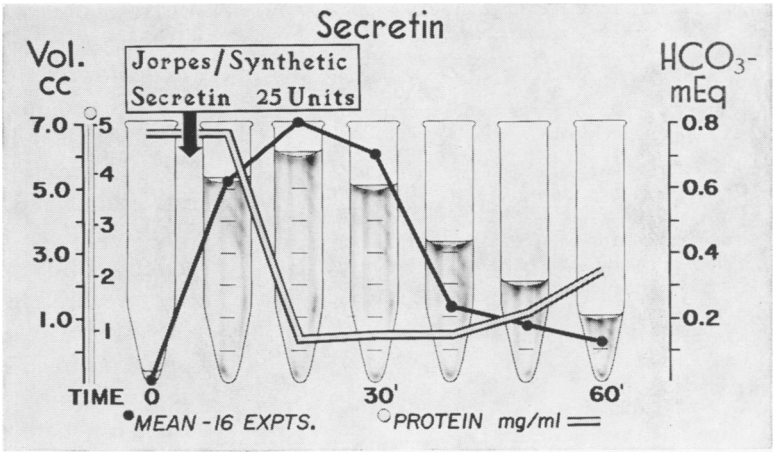

FIG. 3. Mean response • of pancreatic juice bicarbonate (solid line), protein concentration (open line), and volume in anesthetized dogs following intravenous administration of 25 units of Jorpes or synthetic (Squibb) secretin.

ity, the pancreatic tumor tissue from Case 1 and the liver metastases from Case 2 were prepared for bioassay in dogs.

Islet Cell Tumor Bioassay for Secretin

The 54 Gm. of pancreatic tumor removed from Case 1 were divided into 32 and 22 Gm. portions and extracted separately. The total of 218 Gm. of liver metastases from Case 2 were divided into 178 and 40 Gm. portions and extracted in two separate procedures. The method of extraction kindly provided by Dr. T. M. Lin of Eli Lilly Laboratories, Indianapolis, Indiana, was modified for small quantities. According to this method, the frozen tissue was homogenized in 0.4% hydrochloric acid and filtered. The filtrate was then saturated with sodium chloride and centrifuged in the cold. The precipitate was defatted and dehydrated with acetone. The yield of powdered extract from the four tumor portions ranged between 2.5 and 5.0 per cent of the weight. This powder was refrigerated until placed in water to make a 5% solution by weight and filtered just prior to injection into the bioassay animals.

Two preliminary experiments were carried out using a conscious dog with previously implanted pancreatic and biliary cannulae. The animal received a constant intravenous infusion of pure natural secretin * at a rate of 0.8 units/Kg. body weight /hour. After a constant rate of pancreatic secretion had been established in each experiment, 1 Gm. of the extract from the hepatic metastases of Case 2 was injected intravenously. Following injection of the extract there was a prompt and definite additional rise in the pancreatic juice volume and bicarbonate output. More significantly, the dog passed a formed stool within 10 minutes of the injection, followed by retching within 16 minutes and explosive passage of liquid stool 30 to 35 minutes after injection of the extract. This gastrointestinal response was not noted after extract administration to any of the subsequent anesthetized animals. Because of technical objections arising from the use of a chronically prepared, conscious dog, it was decided to employ a modification of the bioassay model suggested by Lin.[16]

Fourteen mongrel dogs, averaging 17.5 (±3.5) Kg. body weight, were anesthetized with intravenous sodium phenobarbital (150 mg./Kg. after fasting for 24 hours. A PE-240 catheter was placed in a jugular vein and hydration was maintained with normal saline at a rate of 15 cc./Kg./hr. The same catheter was used for subsequent

* Obtained from Professor J. Erik Jorpes, Department of Chemistry, Karolinska Institutet, Stockholm, Sweden.

TABLE 4. *Substances Bioassayed for Pancreatic Response in Anesthetized Dogs**

	Amount
A. No volume increase	
Serotonin	15.2 mg.
Gastrin pentapeptide	0.015 & 0.25 mg.
Prostaglandin E_1	0.25 & 0.50 mg.
Bronchial carcinoma	1,400 mg.
Villous adenoma of rectum	300 mg.
Glucagon-producing hepatic metastasis	1,600 mg.
Case 1—pancreatic tumor	800 mg.
Case 2—hepatic metastasis (2nd oper.)	670 mg.
Case 2—normal liver (postmortem)	1,000 mg.
Normal liver (postmortem)	1,000 mg.
B. Intermediate volume increase—increased protein, decreased bicarbonate	
Stage I gastrin	20 & 33 mg.
Glucagon	1 & 1.5 mg.
Pancreozymin-CCK	75 Ivy Units
Histamine phosphate	1 & 2 mg.
C. Classic volume increase—increased bicarbonate, decreased protein	
Pure natural secretin (Jorpes)	25 units
Synthetic secretin (Squibb)	25 units
Case 2—hepatic metastasis (postmortem)	500 mg.

* Modified from method of T.M. Lin
Anesthesia 150 mg/Kg., Phenobarbital I.V.

injection of test samples. Laparotomy was performed through a midline abdominal incision and the pylorus was ligated with umbilical tape (Fig. 2). The cystic duct was then ligated and the common bile duct was cannulated with a polyethylene catheter (PE-240). The major pancreatic duct was cannulated extraduodenally with a polyethylene catheter (PE-200); the minor duct was not disturbed. The abdomen was then closed in routine fashion, with the catheters led out through the incision. Bile and pancreatic juice were collected in graduated centrifuge tubes over 10-minute intervals and the volumes measured. After baseline flow rates had been established, 25 units of pure natural secretin were injected rapidly into the jugular catheter. After demonstrating a satisfactory pancreatic response to secretin, the various test substances were studied. The flow rates were allowed to return to baseline before each succeeding test substance was injected. At the conclusion of testing in each dog, a final dose of 25 units of Jorpes secretin was injected. In all animals, the pancreas was found to respond to this terminal secretin stimulation, even as long as 7 hours after preparation of the model.

Bicarbonate determinations were performed on fresh 10-minute bile and pancreatic juice samples using the Natelson microgasometer method.[24] Pancreatic juice electrolytes were done by flame photometry and amylase was determined by the Caraway method.[5] In addition, the total enzymatic content of the pancreatic juice was estimated by the measurement of total proteins according to the method of Lowry.[17]

The two extracts prepared from the firm pancreatic tumor removed from Case 1 were injected three times into two dogs. In the first dog, extract weighing 1.6 Gm. (obtained from 32 Gm. of the pancreatic tumor tissue) was injected in two equal doses of 800 mg. each, with no response. In the second dog, 1 Gm. of extract from 22 Gm. of the same tumor was injected as a single dose and again there was no pancreatic response.

Extracts prepared from the cellular liver metastases in Case 2 were tested in three animals. The largest amount of tissue, 178 Gm., yielded 7.1 Gm. of extract. Of this extract, 500 mg. and 450 mg. were injected into two dogs and produced a prompt rise in the output of pancreatic juice and bile which was strikingly similar to that produced by Jorpes secretin (Figs. 3, 4). The 800 mg. of powder obtained from the separate extraction of 40 Gm. of liver metastases were injected into a third dog and again produced a secretin-like volume response.

In addition to the volume response produced by the extracts, there was a rise in pancreatic juice bicarbonate concentration and output, with a concomitant fall in the

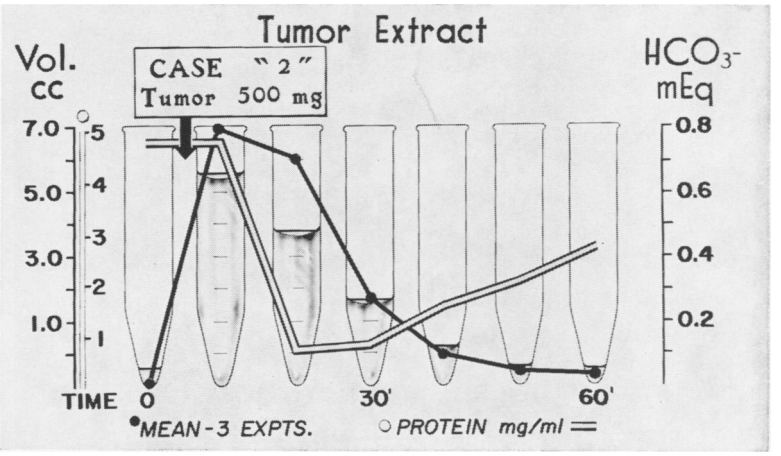

FIG. 4. Mean response • of pancreatic juice bicarbonate (solid line), protein concentration (open line) and volume in anesthetized dog bioassay model following intravenous administration of tumor extract from Case 2.

concentration and output of chloride. A fall in enzyme concentration of the pancreatic juice was reflected by the decreased pancreatic juice amylase and total protein concentration noted after the administration of these tumor extracts.

Pure secretin,* as synthesized by Drs. Miklos Bodansky, M. A. Ondetti and others,[4] was also tested in our bioassay preparation in order to more clearly define the secretin response. Following intravenous administration of 25 units of this pure synthetic hormone, the increase in volume, bicarbonate concentration in the pancreatic juice, with a fall in total protein concentration, paralleled the response to infusion of Jorpes secretin as well as the tumor extract from Case 2.

To rule out nonspecific tissue factors as well as chemical contaminants of the extraction process which might stimulate a pancreatic response, extracts of several tissues were tested in the bioassay model (Table 4). Most significant was the finding that neither extracts from 194 Gm. of liver obtained at autopsy from a patient who died of ruptured abdominal aortic aneurysm, nor extracts of 205 Gm. of the grossly and microscopically uninvolved liver surrounding the metastases in Case 2 produced any response in the test animals. It is also necessary to point out that no activity was found in the extracts of 22 Gm. of the hepatic metastasis taken at the second operation in Case 2. The inactivity of this tissue as well as that of the pancreatic tumor of Case 1 might be explained on the basis of a limited amount of starting material for extraction. Another likely reason for differences in extract activity is a variation in the activity of the tumors themselves. This is best evidenced by comparing the cyclical self-limiting diarrhea in Case 1 to the fulminating, unremitting diarrhea associated with the malignant tumor and cellular hepatic metastases of Case 2.

Several other hormones and chemical substances known to be capable of producing one or more of the observed responses (achlorhydria, choleresis, pancreatic juice hypersecretion or diarrhea) were also tested in physiologic, pharmacologic and occasionally pathologic amounts (Table 4). Only pancreozymin, glucagon, stage 1 gastrin and histamine gave a pancreatic volume response, but the secretin-like elevation of bicarbonate concentration coupled with the decrease in total proteins was not present (Fig. 5). Using the method of Code,[5] an assay of the tumor extract (Case 2) for histamine activity was negative.

Only the extract of the hepatic metas-

* Kindly furnished by the Squibb Institute for Medical Research, New Brunswick, New Jersey.

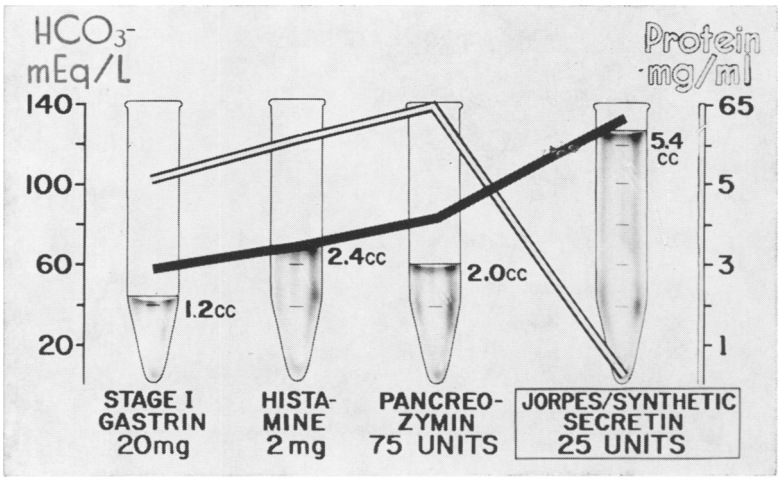

FIG. 5. Typical response of pancreatic juice bicarbonate concentration (solid line), protein concentration (open line) and volume at 10 minutes following intravenous administration of 4 test substances.

tases obtained at postmortem from Case 2 showed the classic secretin-like pancreatic response of high volume of juice with an increased bicarbonate concentration and a decreased enzyme content as evidenced by total protein.

Conclusion

Evidence for the production of a secretin-like hormone by these non-beta islet cell tumors is provided by the abnormal bile analyses, by the disappearance of achlorhydria in Case 1 after the tumor had been removed, and by pancreatic stimulation, with an extract of the tumor in Case 2, which produced a bicarbonate-rich and enzyme-poor fluid. It is known that the pancreatic islets arise from the dorsal bud of the pancreas which has a common cell of origin with the antrum and the duodenum. This provides an anatomical rationale for the production of the antral hormone gastrin, as well as the duodenal hormone secretin, by tumors of these islet cells.

It is disappointing that only one of the two patients' tumors showed activity, but variable hormone concentration and release have long been characteristic of endocrine tumors. The analysis of sufficient amounts of islet cell tumor of the pancreas, especially hepatic metastases, obtained from future patients with watery diarrhea, hypo-

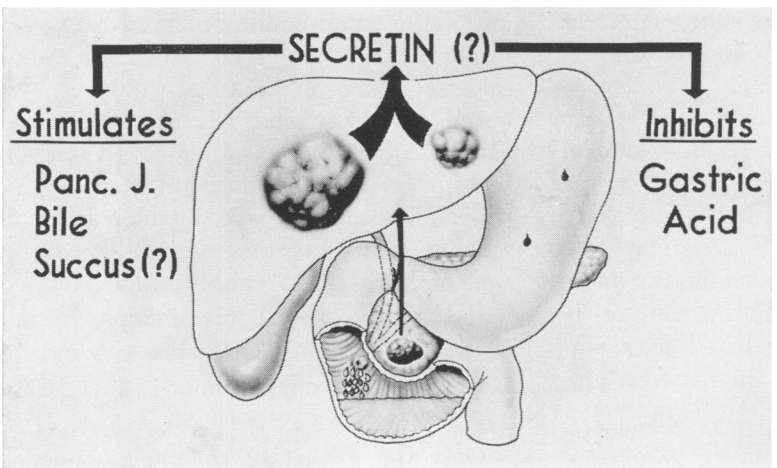

FIG. 6. The clinical features of watery diarrhea, achlorhydria and hypokalemia associated with non-beta islet cell tumors may be explained on the basis of a secretin-like hormone.

kalemia and achlorhydria is needed to confirm the presence of secretin in these tumors. In this period of burgeoning biochemical progress, it appears that secretin, the first substance to which the same hormone was applied in 1902, will be definitely linked with its own tumor and clinical syndrome.

Summary

1. Two patients with watery diarrhea, hypokalemia and gastric achlorhydria were found at operation to have non-beta islet cell tumors of the pancreas and gallbladders distended with very dilute bile. Chemical analysis of the bile showed paradoxically elevated bicarbonate and chloride concentrations consistent with a secretin-like effect.

2. Preoperative gastric anacidity in both patients was overcome by histamine stimulation. In the surviving patient (Case 1), removal of all grossly identifiable tumor tissue resulted in the prompt rise of gastric acid to normal levels, where it has remained for nearly 9 months. Inhibition of gastric secretion is consistent with a secretin effect.

3. Metastatic liver tumor from Case 2 was extracted and administered to dogs as a bioassay. A classic secretin-like pancreatic response was obtained with a sevenfold increase in the volume of juice, an increase in the bicarbonate concentration and a decrease in the protein concentration. Of the control substances tested, including pancreozymin, glucagon, gastrin pentapeptide, histamine, serotonin and prostaglandins, only Jorpes and synthetic secretin produced a similar response.

4. Diarrhea could not be produced in conscious dogs despite continuous large dosages of Jorpes secretin injected intravenously for as long as 53 hours. However, explosive diarrhea did occur in two conscious animals receiving continuous intravenous secretin, following the rapid intravenous administration of the active tumor extract.

5. These studies support the concept that the islet cells of the pancreas, having an embryonic origin from a foregut cell common to the duodenum and antrum, possess the potential for elaboration of several hormones. Secretin, to which the name hormone was first applied in 1902, appears to be one of these hormones. Further studies of extracts of tumors from patients with watery diarrhea, hypokalemia and achlorhydria may establish these clinical findings to be attributable to secretin-producing islet cell tumors of the pancreas.

Acknowledgments

The prostaglandin E_1 used in the studies described was generously supplied by Dr. John E. Pike of The Upjohn Company, Kalamazoo, Michigan; the glucagon-producing alpha cell tumor tissue by Dr. Malcolm H. McGavran, Professor of Surgical Pathology at Washington University in St. Louis, Missouri; and the bronchial carcinoma by Dr. Karl P. Klassen, Director of The Ohio State University Division of Thoracic Surgery.

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Discussion

Dr. Lester R. Dragstedt (Gainesville): Dr. Zollinger and his associates have now given us a look at the cells that produce gastrin and secretin. To be sure, these cells have been changed by malignant transformation, but they may well retain enough characteristics so that they can be identified by histochemists and cytologists. The gastrin cells occur chiefly in the antrum of the stomach, while the secretin cells occur in the first part of the duodenum. There is no evidence that the normal pancreatic islets produce secretin and the evidence that they produce gastrin is scant and contradictory.

It is, therefore, probable that the pancreatic tumors that produce gastrin and secretin arise from heterotopic or embryologically misplaced cells that occur normally in the stomach