Studies on the Potential Role of Secretin in the Islet Cell Tumor Diarrheogenic Syndrome

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THE SYNDROMES associated with functioning tumors of the pancreatic islet cells enjoyed years of clinical anonymity. Gradually their hormonal conspiracy has been unmasked with the recognition of a wide variety of syndromes associated with the overproduction of one or more hormones. Bioassay and radioimmunoassay technics have documented the excessive production of insulin,[30] gastrin,[12] glucagon,[22] serotonin,[24] ACTH, MSH[19] and parathormone.[24] Data were presented in 1968 implicating a secretin-like hormone as responsible for the watery diarrhea, hypokalemia and achlorhydria of the islet cell tumor diarrheogenic syndrome.[38] Since that time very few new cases have been reported and little additional support for secretin as the etiologic polypeptide has been forthcoming. Indeed, based on studies in canine Thiry-Vella loop preparations, a combination of gastrin and glucagon has been suggested as the responsible mechanism in the diarrheogenic syndrome.[1,2,3] In spite of the controversy surrounding the responsible hormone, most authors have agreed that excessive production of succus entericus, inhibition of intestinal absorption or a combination of both offer the most attractive explanation for the massive water diarrhea associated with these particular nonbeta islet cell tumors.[4]

In order to more clearly define both the mechanism and the responsible polypeptide in this syndrome, jejunal water and electrolyte transport was studied in 12 human volunteers during the intravenous administration of either secretin or a combination of gastrin and glucagon. In addition, alcohol-phosphoric acid extracts of pancreatic tumors from two patients with documented islet cell tumor diarrheogenic syndrome were characterized by bio and immunoassay. This experimental data will be presented to provide further evidence that a tumor elaborated secretin-like polypeptide is the principle etiologic hormone of the islet cell tumor diarrheogenic syndrome.

Methods

Intestinal perfusion of a 30 cm. segment of jejunum was performed in 12 healthy volunteers using the triple-lumen tube technic.[7] After an overnight fast a composite triple-lumen polyvinyl tube was passed by mouth and positioned fluoroscopically with the infusion port just beyond the ligament of Trietz (Fig. 1). The proximal and distal sampling sites were 15 and 45 cm. distal to the infusion port respectively. Thus, there was a 15 cm. mixing segment and a 30 cm. jejunal test segment. Each liter of isotonic perfusion solution contained 145 mEq-Na, 5 mEq-K, 140 mEq-Cl, 10 mEq-MCO_3 and polyethylene glycol (PEG) (Mol. Wt. 4000; Carbowax, Union Carbide, New York, N.Y.) 5 Gm., as a nonabsorbable marker. Twenty microcuries of ^{24}Na were added to each liter of perfusate to facilitate the determination of unidirectional (lumen-to-blood vs blood-to-lumen) movements of sodium. Solutions were maintained at 37°C and infused by peristaltic pump (Harvard Apparatus Co., Model 600–900, Millis, Mass.) calibrated to deliver 10 ml./min. at the infusion port. Fluid was collected at the proximal sampling site by siphonage at a constant rate of

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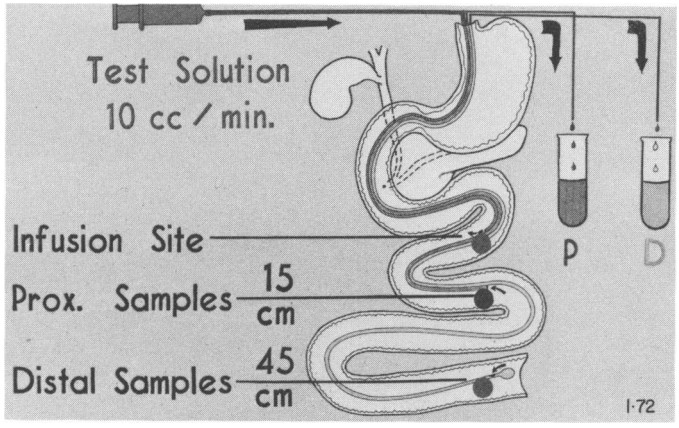

FIG. 1. Triple lumen perfusion technic. Each liter of isotonic perfusate contained: Na, 145 mEq; K, 5 mEq: Cl. 140 mEq, and HCO_3, 10 mEq. Fluid from the proximal (P) and distal (D) sampling sites was collected by siphonage.

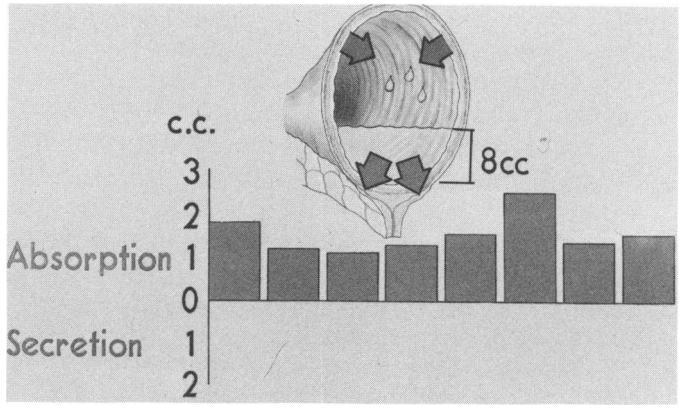

FIG. 2. Control human jejunal water absorption.

1 ml./min. As much fluid as could be obtained was siphoned at the distal sampling site and the collections were delayed 15 minutes to allow for the transit time across the study segment. A steady state was achieved over a 60-minute period and then five sequential 15-minute samples were obtained to serve as control periods. After appropriate controls had been collected, a constant intravenous infusion of either secretin (Jorpes, 4 U/Kg./hr.) or combination of pentagastrin (Ayerst, 4 U/Kg./hr.) and glucagon (Lilly, 5 ug/Kg./hr.) was begun and a second series of five sequential, 15-minute samples were obtained.

Sodium and potassium were analyzed by flame photometry (IL Model 143, Instrumentation Laboratory, Lexington, Mass.), chloride electrotitrimetrically (Radiometer Corp.), and bicarbonate manometrically (Natelson Microgasometer 650, Scientific Industries Inc., Queens Village, N.Y.). Polyethylene glycol was measured turbidometrically at 650 mu.[13] ^{24}Na was counted in a sodium iodide well (Nuclear-Chicago Corporation, Cincinnati, Ohio). Absorption or secretion of water and electrolytes was calculated with reference to PEG, using standard formulae.[7] The results are expressed as microequivalents of electrolyte or milliliters of water absorbed or secreted per minute per 30-cm. segment of jejunum.

The data were analyzed for statistical significance using the paired T test.

Tumor Extraction

Diarrheogenic islet cell tumor extracts were prepared by Dr. Ronald Chance (Eli Lilly Co., Indianapolis, Ind.) using an alcohol phosphoric acid extraction procedure.[5,14] According to this method, the tissue was homogenized in 82% ethanol which had been adjusted to pH 2.85 with 85% phosphoric acid. After stirring for 2 hours at 4°C the homogenate was strained through cheese cloth. The filtrate was then adjusted to pH 8.5 with ammonium hydroxide and centrifuged in the cold. After adjusting the supernate to pH 6.5 with 5 N H_2SO_4, two volumes of cold ethanol and five volumes of cold ether were added. The solution was allowed to stand overnight at 4°C without stirring and centrifuged the following morning. The yields of powdered extract from the two primary tumor portions were 2.1% and 2.7%.

Radioimmunoassay for gastrin in the tumor extracts were performed using the technic of McGuigan.[23] Insulin and glucagon determinations were kindly performed by the research laboratories of the Eli Lilly Company.

Results

During the control periods, the jejunum continuously absorbed water at a rate of 1.68 ml./min./seg, sodium at 232 uEq/min./seg and potassium at 12.5 uEq/min./seg (Table I). These levels of absorption remained constant during control perfusions up to 3 hours in duration. Dur-

TABLE 1. *Jejunal Water and Electrolyte Transport during the Control Period Compared to That during the Intravenous Administration of Either a Gastrin-glucagon Combination or Secretin*

	Control (N = 20)	Gastrin Glucagon (N = 5)	Secretin (N = 5)
	Absorb		Secrete
Net:			
Water cc/min/30 cm	1.68	0.18	1.70
K^+ ueq/min/30 cm	12.5	2.6	9.6
NA^+ ueq/min/30 cm	232	18.9	290
Unidirectional ^{24}NA:			
Blood to lumen ueq/min/30 cm	585	527	992
Lumen to blood ueq/min/30 cm	768	542	774

ing the intravenous infusions of a combination of pentagastrin (Ayerst, 4 ug/Kg./hr.) and glucagon (Lilly, 5 ug/Kg./hr.) control absorption was inhibited. Water absorption was reduced to 0.18 ml./min./seg. (Fig. 3), sodium to 18.9 uEq/min./seg and potassium to 2.6 uEq/min/seg. The mechanism responsible for the decreased absorption was determined from the changes in ^{24}Na counts relative to the net sodium change. The gastrin-glucagon combination significantly inhibited movement of water and electrolytes from bowel lumen-to-blood ($p < .05$). Transport from blood-to-lumen did not differ from that during the control period.

In contrast a constant intravenous infusion of secretin (Jorpes, 4 U/Kg./hr.) produced an abrupt reversal of jejunal fluid and electrolyte transport. Water (Fig. 4), sodium and potassium were actually secreted by the jejunal mucosa at rates of 1.7 ml./min./seg, 290 uEq/min./seg and 9.6 uEq/min./seg, respectively (Table 1). Movement of water and electrolytes from lumen-to-blood did not change significantly during the 75-minute hormone infusion. The enhanced secretion of succus entericus was caused solely by a marked increase ($p < .05$) in the transport of water and electrolytes from blood-to-lumen.

Thus a combination of gastrin and glucagon inhibited the transport of nearly 90% of the water that is normally absorbed in the basal state. The intravenous administration of secretin on the other hand actually produced secretion of an additional 1.7 ml. of water per minute by the jejunal segment. In all studies, the jejunal absorption of fluid and electrolytes promptly returned to normal at the termination of the hormone infusions.

Tumor Extract Studies

For the first time in our experience a positive secretin-like response was demonstrated from the primary pancreatic tumors of two patients with the diarrheogenic

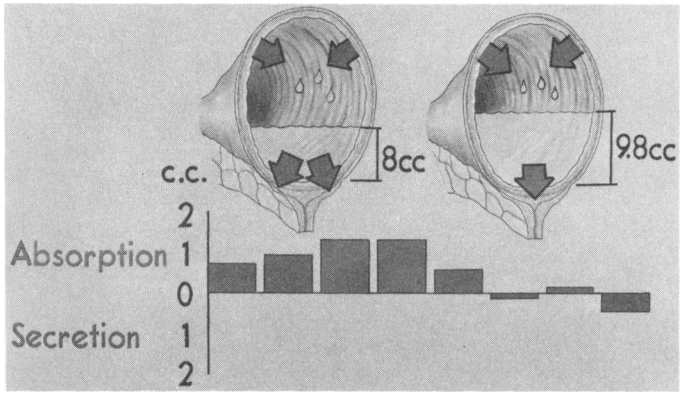

FIG. 3. The effect of intravenous gastrin and glucagon on human jejunal water absorption (mean, 5 studies). The first four periods represent control absorption. The remaining four periods demonstrate the hormone effect.

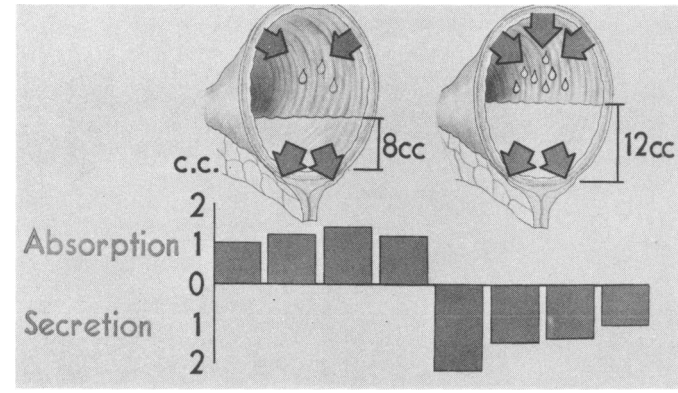

FIG. 4. The effect of intravenous secretin on human jejunal water absorption (mean, 5 studies). The first four periods represent control absorption. The remaining four periods demonstrate the hormone effect.

syndrome. Both patients had the characteristic clinical and laboratory manifestations of severe watery diarrhea, hypokalemia and achlorhydria. A total of 15 Gm. of non-beta islet cell tumor from the first patient, who was treated in 1969 at Ohio State University, was available for extraction. We are greatly indebted to Dr. Martin J. Salwen, Director of Pathology, Monmouth Medical Center in Long Branch, N.J. for generously supplying us with 30 Gm. of pancreatic tumor from a second patient with proved diarrheogenic syndrome, treated by Drs. Alberto Dodde and Elias Abou-Lehof of that same institution.[28]

Extracts of these tumors were studied in anesthetized dogs using a modification of the bioassay model of Dr. T. M. Lin.[38] These experiments were carried out while the animals were receiving a constant intravenous infusion of pure natural secretin (Jorpes) at a rate of 0.25 U/Kg./hr. Bile and pancreatic juice were collected separately under oil, at sequential 10-minute intervals. After a constant rate of pancreatic secretion had been established in each experiment, 25 units of Jorpes secretin were injected intravenously. After demonstrating a satisfactory pancreatic response to secretin (Fig. 5), flow rates were allowed to return to baseline and the tumor extracts were administered as a bolus injection. A final dose of 25 units of Jorpes secretin was injected at the conclusion of testing to assure adequate terminal pancreatic responsiveness. Volume, bicarbonate and total protein concentrations were determined in all samples.

Following the bolus injection of 40 mg. of the extract from one primary tumor and 80 mg. from the other a prompt and substantial rise in pancreatic output was produced. This response closely paralleled that observed after the injection of Jorpes secretin. There was an increase in volume and bicarbonate concentration, with a reciprocal fall in total protein concentration (Fig. 6). Previous studies, using this same bioassay model, have

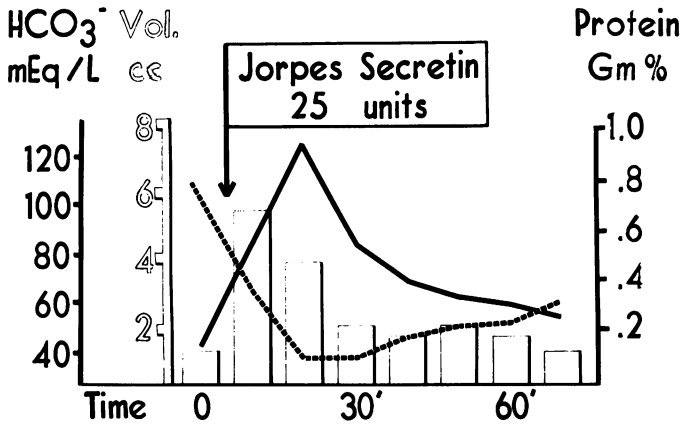

FIG. 5. Response of pancreatic juice bicarbonate (solid line), protein concentration (dashed line) and volume in the anesthetized canine bioassay model after the intravenous administration of Jorpes secretin.

demonstrated that serotonin, gastrin, prostaglandin E-1, glucagon, cholecystokinin-pancreozymin and histamine do not give this "classic" pancreatic secretin response.[38]

The extracts were also tested in two conscious dogs by Dr. T. M. Lin (Eli Lilly Co., Indianapolis, Ind.).[20] These chronic preparations made it possible to determine the effect of the tumor extracts on gastric acid secretion as well as biliary and pancreatic output. In contrast to the bolus technic used in the anesthetized preparation, a continuous intravenous infusion of 1 or 2 mg./Kg./hr of both tumor extracts was given. These infusions produced an increase in pancreatic juice volume and bicarbonate concentration. There was a reciprocal decrease in protein concentration during the administration of the extract from Case 2. The protein concentration rose when the Case 1 extract was given, however. At the same dosage, gastric acid secretion was inhibited by both tumor extracts. The extract from Case 1 also demonstrated a mild hyperglycemic effect; but none was seen with the administration of the Case 2 extract.

Increasing concentrations of both tumor extracts were assessed by radioimmunoassay. These studies documented that the concentrations of gastrin, glucagon, and insulin present were much less than that found in normal pancreas. The amounts were so small that they could not have produced a hormonal effect when given in the bioassay models.

Discussion

A specific diarrheogenic hormone of islet cell origin, first postulated in 1961 and strongly implicated by the studies of Gardner, Peskin, Cerda and Brooks, was identified 7 years later as secretin or a secretin-like substance.[9, 10, 38] This observation was based on extensive clinical and laboratory studies of two patients with the clinical triad of fulminating watery diarrhea, life-threatening hypokalemia and basal achlorhydria which could be overridden by the administration of histamine. The first indication of secretory over-activity by the pancreas and biliary tract came during the operation on the first patient when it was noted that the duodenum continually refilled with secretions and, more importantly, that the gallbladder was tensely distended. Analysis of the otherwise very dilute bile aspirated from the enlarged gallbladder revealed elevated levels of chloride and bicarbonate, a finding that had been described only after secretin infusion in animals.[35] Strikingly similar results were obtained when bile removed from the enlarged gallbladder of the second patient was analyzed. The prompt return of normal acid values in the patient who did not have metastatic tumor provided further support that an inhibitory substance, presumably with secretin-like characteristics, had been elaborated by the non-beta islet cell tumor. The solid pancreatic tumor from the first case and the hepatic metastases removed from the second were prepared for biological assay according to a method of extraction provided by Dr. T. M. Lin of Eli Lilly Laboratories. While there was no response from the solid pancreatic tumor, the hepatic metastasis produced a prompt rise in the volume of pancreatic juice and bile which closely paralleled that seen after the injection of pure natural (Jorpes) secretin. There was also a rise in the output and concentration of pancreatic juice bicarbonate together with a fall in the concentration and output of chloride. Decreased pancreatic juice enzyme concentrations were reflected in the lowered concentrations of pancreatic juice amylase and total proteins. Of the control substances tested, including cholecystokinin-pancreozymin, glucagon, gastrin pentapeptide, histamine, serotonin and prostaglandin E-1, only Jorpes and synthetic secretin evoked similar responses. Unfortunately, diarrhea could not be produced in conscious dogs despite the prolonged

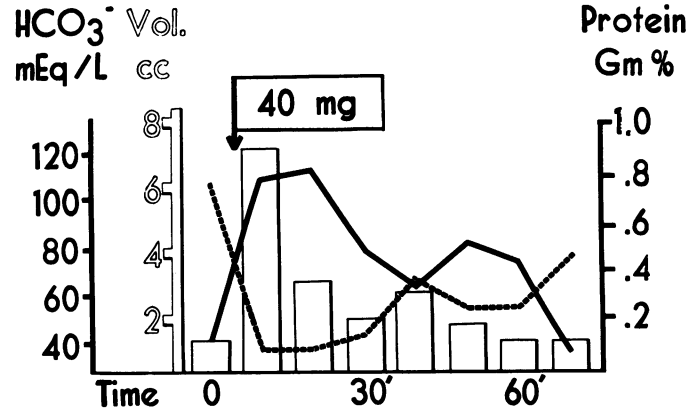

FIG. 6. Response of pancreatic juice bicarbonate (solid line), protein concentration (dashed line) and volume in anesthetized canine bioassay model after the intravenous administration of tumor extract.

infusion of large doses of Jorpes secretin. However, explosive diarrhea did occur in two conscious animals receiving continuous intravenous secretin, after the rapid intravenous administration of the active tumor extract.

In further studies, Tompkins, *et al.*, demonstrated that the effects of these same active extracts upon the flow as well as the bicarbonate and chloride secretion of hepatic bile in test animals were qualitatively similar to those of Jorpes and synthetic secretin.[33] Thin layer chromatography of the bile samples from both patients was felt to mitigate against the production of free or unusual bile acids by the liver as a possible cause of the diarrhea in this syndrome. Furthermore, they were able to produce explosive watery diarrhea by administering 1 unit Kg. of Jorpes secretin to a patient who had had an indwelling T-tube placed during choledochotomy several days previously. Total bile collections during the baseline and secretin-stimulated periods (4 hours before the onset of diarrhea) revealed the characteristic increases in bile volume, bicarbonate and chloride secretion. Diarrhea had been observed by Wormsley in two patients during clinical studies on the effects of secretin in man.[36]

In the 4 years which have elapsed since the original suggestions that secretin is the polypeptide elaborated in the islet cell tumor diarrheogenic syndrome, there have been surprisingly few papers written to confirm or deny the concept. Professor J. Erik Jorpes in a written communication dated March 28, 1969 reported that his colleague, Viktor Mutt, had found "substantial secretin-like activity" in a diarrheogenic tumor sent to them by Dr. Bengt Ihre, though little else is known about the specific details of the case.[15] Tompkins and Kraft (Unpublished Data, 1971) bioassayed two extracts of hepatic metastases removed from a 55-year-old man under the care of Drs. H. William Scott and David H. Law at Vanderbilt University. On two occasions, they were able to demonstrate an increase in the volume of pancreatic juice together with a rise in bicarbonate concentration and a fall in the protein concentration as well as a diminished specific gravity and total solids in the bile, all of which were physiologically similar to the responses of pure natural secretin in this preparation. The results of further investigations aimed at defining the etiologic polypeptide in this syndrome have been less consistent.

Sircus, Cleator and colleagues presented the clinical data of two patients with the islet cell tumor diarrheogenic syndrome.[31] One had an islet cell adenoma, while the other had only islet cell hyperplasia. Bioassays using cat, dog and rat preparations were carried out on tumor extracts as well as on plasma from these patients.[6] The tumor extract from the first case caused an increase in the output of pancreatic juice, compatible with a secretin-like effect, in both the dog and the cat preparations. An unexplained finding in the second case was a positive rat bioassay for serum gastrin. Similarly, in the case of Gjone, *et al.*, there were positive Lai Rat and PSU (pourvoir secretagogue urinaire) tests preoperatively, which they postulated may have been on the basis of competition by the diarrheogenic hormone for the gastric acid secretory receptor site.[11] In neither case, however, were the tumor extracts found to contain gastrin activity. The primary pancreatic tumor removed from Gjone's patient was extracted by Professor W. S. Rosenthal (New York Medical College), according to a method developed there for separating *gastrone*, and assayed for "inhibitory activity" in Heidenhain pouch dogs.[29] It was concluded that these extracts contained a "gastrone-like substance." Secretin activity could not be demonstrated when the extract was injected in a dosage that showed gastric secretory inhibition. The latter experiment was carried out in an anesthetized dog in which the major pancreatic duct had been cannulated.

More recently, Barbezat and Grossman[3] have postulated a different concept, based on their observations from intestinal absorption studies in dogs prepared with Thiry-Vella loops. In this preparation, a combination of gastrin and glucagon decreased the net absorption of sodium and water, and increased the secretion of bicarbonate in the ileum. Intravenous infusions of synthetic secretin had no effect on either jejunal or ileal transport of fluids and electrolytes. Further studies in intact dogs demonstrated that an intravenous infusion of a combination of pentagastrin and glucagon produced explosive diarrhea within one to five hours. Secretin was not tested in the intact animals.

In view of the known species differences to the various gastrointestinal hormones,[8] the effect of either a combination of gastrin and glucagon or secretin on intestinal water and electrolyte transport was tested in 12 normal human volunteers in an attempt to define both the mechanism and etiologic hormone capable of producing the watery diarrhea associated with the syndrome. The human intestinal perfusion technic [7] has been used in recent years to elucidate the mechanism of such diarrheal diseases as cholera, sprue, cholerrheic enteropathy and the ulcerogenic tumor syndrome.[37] Since diarrhea can be defined as a malabsorption of water and electrolytes, it has generally been accepted that the demonstration of either diminished absorption or increased secretion by these technics can be equated with the clinical manifestation of diarrhea. The experiments using normal controls demonstrated that the concurrent infusion of gastrin and glucagon actually decreased the absorption of fluid and electrolytes in the jejunum. The mechanism for these changes appears to be a direct inhibition of the transport from lumen-to-blood.

Contrary to the findings of Barbezat and Grossman in their animal studies, intravenous secretin in man

prompted an excessive secretion of water, sodium and potassium into the jejunal lumen. The mechanism by which secretin effected these changes would appear to be entirely different than that seen with the gastrin and glucagon combination since the absorptive capacity of the jejunum remained normal during the secretin infusion. If the secretin data are extrapolated for the entire length of the jejunum, the water losses in 24 hours would be greater than 12 liters. Even if the ileum and colon were able to absorb 5-6 liters of this excessive succus entericus, the residual 6-7 liters would approximate the diarrhea observed clinically. By similar reasoning, the potassium losses for the entire jejunum would exceed 75 mEq./day. Currently available evidence suggests that potassium is absorbed from the intestine by a passive process along electrochemical gradients. In the ileum and colon the electrical gradient is increasingly more negative thus favoring potassium loss into the lumen. In the large intestine potassium is normally secreted. This suggests that the actual potassium losses would be even greater than that calculated on the basis of the jejunal fluid secretion.

In view of the results reported by Grossman and Barbezat, it would be anticipated that extracts of diarrheogenic islet cell tumors would contain gastrin and/or glucagon. The availability of sensitive radioimmunoassays made it possible to rule out all but trace amounts of either gastrin, glucagon or insulin in the two tumors that were bioassayed. Furthermore, the few patients reported to have alpha cell glucagonomas have not been troubled with diarrhea, nor has it been a problem when glucagon has been used therapeutically in cardiovascular surgery or to control the symptoms of the beta cell adenoma.

The bioassay evidence presented in 1968, which favored a secretin-like substance as the etiologic polypeptide, was based on the results of pancreatic and biliary responses to extracts of metastatic tumors from a patient with the diarrheogenic syndrome.[38] Although impressive secretin-like responses were obtained from these extracts, similar results were not obtained when extracts from primary tumors were assayed. The lack of response from the primary tumor extracts may well have been due to the method of extraction and/or the documented lability of the secretin molecule. As outlined previously, the extraction method has subsequently been altered. By using the phosphoric acid-alcohol extraction procedure,[14] good yields of powdered extracts were obtained from both tumors. Bioassay studies of these extracts in both chronic canine preparations and also acute anesthetized dogs have documented a secretin-like effect on the pancreatic secretions. This effect is demonstrated by not only the increased volume but also the increased bicarbonate concentrations. A reciprocal decrease in protein concentrations was seen in all the bioassay studies using the extract from the Case 2 tumor. Case 1 tumor extract produced a similar effect in the anesthetized dogs, but caused an increase in the conscious chronic preparations. We are unable to explain the differences in protein concentration response between the acute anesthetized preparation and the conscious chronic model. Furthermore, we were able to characterize the effects of these extracts on gastric acid secretion as secretin-like in character.

Kraft, Tompkins and Zollinger [18] in 1969 characterized the outstanding clinical and laboratory features of the diarrheogenic islet cell tumor syndrome on the basis of 27 case reports collected from the available literature. Fifteen patients met each of the established criteria of the syndrome as developed by Marks and by Matsumoto (profuse watery diarrhea, hypokalemia, absence of gastric hypersecretion and a non-gastrin producing islet cell tumor). These were designated *proved* cases. In the remaining 12 patients, the hypokalemia and/or absence of gastric hypersecretion was implied rather than measured. These patients were designated as *probable* cases.

The watery diarrhea approximated 6 liters/day, and was present on the average, more than 3 years before a pancreatic tumor was diagnosed. The serum potassium levels averaged 2.2 mq/L during episodes of diarrhea and could be stabilized only after spontaneous remission of the diarrhea, total excision of the tumor or, in some cases, after treatment with steroids. Absence of gastric hypersecretion is essential to the diagnosis of diarrheogenic tumor; and of the 15 *proved* patients, 11 had basal achlorhydria, two had lower than normal acid values and two were in the range of normal. Five of the achlorhydric patients were found to be refactory to histamine stimulation; four of whom were later found to have unresectable metastatic tumors. Certainly the augmented histamine gastric analysis must be considered a vital key in the diagnosis of a diarrheogenic non-beta islet cell tumor of the pancreas.

Approximately 60% of these patients had elevated serum levels of calcium. In two patients there was recurrent hypercalcemia even after the removal of parathyroid adenomas or hyperplastic parathyroid glands. This was not corrected until there was either spontaneous cessation of the diarrhea or removal of the islet cell tumor. Four other patients did not undergo neck exploration, but became normocalcemic following excision of the pancreatic tumor and control of the diarrhea. Similar findings have since been reported by Patterson,[26] Lopes [21] and Sircus.[31] The pathogenesis of this phenomenon remains to be elucidated. Similarly, it has not been determined why approximately one-third of the patients in Kraft's series had hyperglycemia; although he theorized that this phenomenon might be related to the uncontrollable hypokalemia.

The tensely dilated gallbladder, a hallmark of this syn-

drome, has been observed in five cases. Chemical analysis of the dilute bile removed from three of these, by the method of Potter; revealed high concentrations of bicarbonate and chloride such as that which occurs following the injection of secretin into animals. In Kraft's series, 15 of the patients had malignant tumors, 25% of which had metastasized at the time of operation. Whether benign or malignant, the adenomas were usually discrete, ranging in size from 1.5 to 7 cm. in diameter. Operations ranging in magnitude from tumor excision to total pancreatectomy were carried out in 14 patients. Total removal of the primary tumor, whether it was benign or malignant, led to control of the diarrhea and improvement in the general condition of 11 patients. Of the patients undergoing surgical treatment for benign tumors, all were alive and well and had not experienced recurrent symptoms when their cases were reported. The average length of survival in patients with malignant disease was only one year.

Verner, in 1969, published findings which closely paralleled these in the proceedings of an international symposium on islet cell tumors held in Erlangen.[34] Since that time, however, fewer than 10 *proved* cases have been collected from the literature, written communications, and personal experience.[11,16,17,21,25,26,27,28,31,32] It is only by recognition of the clinical syndrome associated with fulminating watery diarrhea and hypokalemia that the diagnosis can be more frequently made. As the syndrome is more frequently recognized, it should be possible to obtain sufficient tumor tissue for investigations to prove both physiologically and biochemically the identity of the diarrhogenic hormone, or hormones, be it secretin, a combination of known hormones or a "chemical messenger" not as yet identified.

Conclusions

1. The intravenous administration of a combination of gastrin and glucagon inhibits basal water and electrolyte absorption in the human jejunum.

2. In contrast intravenous secretin produces secretion of water and electrolytes by the human jejunal mucosa.

3. Alcohol-phosphoric acid extracts of two diarrheogenic islet cell tumors contained only trace amounts of gastrin, glucagon and insulin by radioimmunoassay.

4. The tumor extracts produced an increase in pancreatic juice volume and bicarbonate concentration with a reciprocal decrease in total protein concentration. These effects are uniquely secretin-like in the canine bioassay model.

5. All patients with chronic or intermittent "watery" diarrheal diseases should be studied for evidence of hypokalemia and basal achlorhydria. Hypercalcemia and impaired glucose tolerance commonly accompany the diarrheogenic islet cell tumor syndrome.

6. Proof of the humoral etiology of this syndrome awaits the availability of sufficient tumor tissue to allow extraction, purification, and amino acid analysis for secretin as well as other polypeptides.

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Discussion

Dr. Josef E. Fischer (Boston): I would like to confirm the findings of Dr. Sanzenbacher, Dr. Zollinger, and their group. We recently had the opportunity last year to take care of a patient who, I think, fits the criteria of this category—syndrome—exactly.

[Slide] We were fortunate enough to be able to get samples pre- and postoperatively, and from the tumor. Gastrin at that time—(the lowest level of sensitivity) was 50 picograms per ml., which did not change pre- and postoperatively. We did notice in the assay that there was a high nonspecific binding, indicating a circulating peptide which was present preoperatively. Prostaglandins were not in evidence, both pre- and postoperatively, and Dr. Scratcherd, of Dr. Wilfred Sircus' unit, was kind enough to test—this actually should be secretin-like activity—in a bioassay, which was present preoperatively and not present postoperatively.

[Slide] The second slide shows a tracing of the bioassay carried out by Dr. Scratcherd which indicates that at this point, which is C, this is an extract of the fibrosed pancreas which we sent to him, indicating that there is some secretin-like activity.

Dr. R. Tompkins (Los Angeles): I liked Dr. Sanzenbacher's paper very much, because it confirmed a very strong prejudice that I have had for several years, and I would like to take the opportunity to make a few comments about his observations.

First of all, I think he should be congratulated on bringing these studies into the human. As he has pointed out in his presentation, there are species differences in the action of these hormones. The opponents of the secretin theory have cited the failure to produce diarrhea in dogs with constant infusions of secretin. However, they have ignored, to a certain extent, the production of diarrhea which does occur in humans who are undergoing secretin infusions for various reasons.

Wormsley (Gastroenterology, 54:117, 1968) has observed this in secretin testing of pancreatic function. I had the misfortune to produce this in an unfortunate patient while testing her bile chemistries postoperatively. She had her attack of explosive, watery diarrhea while visiting with friends in the lobby of the hospital about 3 hours after the secretin injection (Surgery, 66:131, 1969).

Furthermore, there has been some emphasis recently on the effects of many of the hormones and the prostaglandins upon cyclic AMP, the suggestion being made that cyclic AMP increase in the intestinal mucosa leads to diarrhea. These studies have involved glucagon, and glucagon has certainly been shown to increase cyclic AMP in the tissues.

However, I want to point out that secretin has been observed by workers at NIH to be twice as active in the production of adenyl cyclase, and hence cyclic AMP levels, as glucagon in many of their test systems.

I would like to ask Dr. Sanzenbacher if he would comment if there is evidence for other peptides in the extract of these tumors, and if he is accumulating enough of this tumor tissue to use for amino acid sequencing, which will be the final proof needed for the etiological agent.

Dr. Lawrence W. Way (San Francisco): This interesting paper has firmly established at least one conclusion: Extracts from this tumor (and the other mentioned by Dr. Fischer) stimulated pancreatic secretion similar to that evoked by secretin.

I would like to raise a few questions regarding methodology, because I still wonder whether it has been shown that secretin can stimulate the small bowel to secrete. First, use of the triple lumen tube at this level of the bowel is sometimes difficult because of reflux of the marker into the stomach. It may be impossible to obtain a steady state.

Secondly, I would be interested in the anion concentrations of the jejunal secretion that was stimulated by secretin infusion in the human volunteers. It is interesting that fluid aspirated from the proximal small bowel in several patients afflicted with the diarrheogenic syndrome has had high chloride concentrations. The injection of secretin into the experimental subjects in this study would maximally stimulate the pancreas to secrete a large volume of bicarbonate. The physiological studies on jejunal secre-