1. Dolman, C. E., Can. J. Microbiol., 1956, v2, 189.
2. McCoy, K. L., Kennedy, E. R., J.A.M.A., 1960, v174, 35.
3. Rogers, D. E., Ann. Int. Med., 1956, v45, 748.
4. Fisher, M. W., Manning, M. C., J. Immunol., 1958, v81, 29.
5. Fisher, M. W., Nature, 1959, v183, 1692.
6. Kekwick, R. A., Biochem. J., 1940, v34, 1248.
7. Mainland, D., Murray, I. M., Science, 1952, v116, 591.
8. Quie, Paul G., Wannamaker, Lewis W., Pediatrics, 1964, v33, 63.
9. Campbell, B., Petersen, W. E., Die Umschau, 1958, v14, 435.

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In vitro Inhibition of Intestinal Fluid and Electrolyte Transfer by a Non-Beta Islet Cell Tumor. (31488)

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Zollinger and Ellison(1) described a syndrome of intractable peptic ulceration and gastric hypersecretion associated with non-Beta islet cell pancreatic neoplasms. Subsequently, Verner and Morrison(2) pointed out that non-Beta islet cell tumors may also be associated with profuse watery diarrhea and hypokalemia without gastric hypersecretion or peptic ulceration. Although the ulcerogenic mechanism has been partially clarified by isolation of a gastrin-like substance from these neoplasms(3), the diarrheogenic mechanism remains obscure. This report presents evidence which suggests that the diarrhea in a patient with a non-Beta islet cell tumor of the pancreas resulted from the tumor or its products inhibiting the net intestinal absorption of fluid and electrolytes.

The patient who stimulated this study was a 31-year-old male with a non-Beta islet cell pancreatic adenocarcinoma, intractable diarrhea (15-40 bowel movements per day with stool volumes as high as 8 liters per 24 hours) and hypokalemia without peptic ulceration or gastric hypersecretion. Balance studies performed on this patient demonstrated excessive fecal losses of fluid and electrolytes. Mouth-to-anus transit time measured with an indigo carmine marker on 2 occasions was normal. Continuous aspiration from the patient's distal ileum demonstrated a pronounced increase in the volume of fluid passing from the small intestine into the colon. These excessive volumes were obtained during continuous simultaneous gastric aspiration, thus excluding a significant gastric contribution to the excessive ileal fluid. The clinical studies will be reported in detail later.

Materials and methods. Liver metastases, histologically proven to be a non-Beta islet cell adenocarcinoma of the pancreas, were obtained at laparotomy and immediately frozen. Assay of the tumor for gastrin-like activity was negative.* A sample of the patient's serum, an autopsy specimen of histologically normal liver tissue and pancreatic tissue from a patient with an accidentally ligated pancreatic duct were also obtained and frozen. Prior to each experiment, the tumor, liver and pancreatic tissue were thawed, homogenized and extracted with distilled water such that the extracts contained 100 mg of tissue per ml of distilled water.

Fluid and electrolyte transfer: Experiments were carried out on adult, male Golden Syrian hamsters (210-240 g) maintained on a commercial diet, (Wayne Lab-blox) with unrestricted access to food and water. The animals were sacrificed and the small intestine was immediately removed and everted according to the method of Wilson and Wiseman (4). The combined jejunum and ileum was

* Kindly performed by Dr. M. I. Grossman, Veterans Admin. Center, Los Angeles, Calif.

divided into 3 segments of approximately equal length. Each sac was filled with 1.0 ml of Krebs-Ringers-bicarbonate-glucose solution and was suspended vertically in 25 ml of the same solution. The mucosal solution was continuously gassed with 95% O_2-5% CO_2 and the sacs incubated for one hour at 37°C. One of the extracts to be tested or the patient's serum was added to the solution bathing the mucosal surface. The composition (mM/l) of the solution on both sides of the gut sac at the beginning of the incubation period was Na-145, K-5.9, Ca-1.3, Mg-1.0, Cl-125, HCO_3-26, SO_4-1.0, P-3.0, glucose-15.1. The pH was 7.4. Concentrations of the tumor, liver, and pancreas extracts used were 180 mg/l of bathing solution; concentration of the patient's serum was 21 ml/l. In no instance did the addition of any substance to the mucosal solution alter the concentration of sodium, chloride, potassium or glucose or the pH of the bath. Sodium and potassium concentrations were measured simultaneously with an Instrumentation Laboratories flame photometer. Chloride concentration was measured with an Aminco-Cotlove Chloride Titrator. Fluid volume change was determined gravimetrically by taking the difference between the volume initially inside the sac and the volume in the sac at the end of the incubation period. Total concentration of electrolytes in the bath did not change during incubation. No gross leaks from the sacs could be detected. Net mucosal-to-serosal transfer was taken as the amount of a substance on the serosal side of the sac at the end of the experiment in excess of that initially present/mg dry wt sac/hour.

Intestinal motility: Two techniques were used to evaluate the effects of the various substances on intestinal motility. To monitor intrasac pressure, the sacs were prepared as they were when transfer was being studied except a polyethylene cannula was inserted into the upper end of the sac. The cannula was connected to a strain gauge transducer, and intrasac pressure was recorded under the same conditions as when fluid and electrolyte transfer was measured. An attempt was made to demonstrate an effect on intestinal motility using the guinea pig ileum. Circular muscle strips of isolated ileum were suspended in a 10-ml organ-bath according to the method of Harry(5). The muscle bath containing Tyrode's solution was stirred by continuous gassing with 95% O_2-5% CO_2. The bath and all solutions were kept at 37°C. Contractions were recorded using a strain gauge transducer connected to an ink-writing polygraph giving an amplification of 1:20. The effects of the extracts and serum on smooth muscle contraction were recorded using the following concentrations of extracts of tumor, liver and pancreas: 90, 180, 360, 900 and 1800 mg/l. The patient's serum was tested in concentrations of 10, 21, 42, 105 and 210 ml/l. After adding the test substance to the bath, contractions were recorded for 3 minutes or until the tracing returned to baseline value. The bath was then changed and after one minute, changed again. The procedure was then repeated.

Results. Table I presents the data on the net mucosal-to-serosal transfer of fluid, sodium, potassium and chloride by everted gut sacs from various portions of the hamster small intestine. In sacs from the distal third, addition of the tumor extract was associated with a significant decrease ($p<0.01$) in the net transfer of fluid, sodium and chloride but not potassium. No such decrease was observed when liver or pancreas extracts were added to sacs from the distal third. Neither the addition of the tumor nor liver or pancreas extracts altered the net transfer of fluid or electrolytes in sacs from the proximal or middle portions of the hamster small intestine. Addition of the patient's serum produced no observable effect on the net transfer of fluid or electrolytes in any portion of the intestine studied.

There was no change in intrasac pressure when the tumor extract was added to the mucosal solution. Addition of liver, pancreas or patient's serum also produced no observable effect. There was no stimulation of the ileal smooth muscle by the tumor extract when it was present in the bath at concentrations from 90-1800 mgm/l. Likewise, no response was observed with liver, pancreas and patient's serum. Histamine (5μg/ml) produced the anticipated contraction.

TABLE I. Effect of Extracts and Patient's Serum on Net Fluid and Electrolyte Transfer in Various Portions of Everted Hamster Intestine.

Substance transferred	Portion of hamster small intestine		
	Proximal third	Middle third	Distal third
Fluid (μl/mg dry wt sac/hr)			
Control	5.2 ±2.4 (7)*	8.6 ±2.5 (10)	10.3 ±1.4 (10)
Tumor extract	5.4 ±2.0 (3)	8.8 ±1.4 (6)	7.2 ± .2 (6)†
Liver "	5.8 ±1.5 (7)	9.6 ±1.0 (8)	9.5 ±1.7 (8)
Pancreas "	4.4 ±1.3 (6)	9.9 ±2.1 (6)	12.0 ±1.8 (6)
Patient's serum	4.7 ±1.2 (8)	8.3 ±1.5 (10)	9.1 ±1.6 (10)
Sodium (μM/mg dry wt sac/hr)			
Control	.49 ± .27 (7)	.98 ± .38 (10)	1.20 ± .17 (10)
Tumor extract	.64 ± .24 (3)	1.00 ± .18 (6)	.87 ± .10 (6)†
Liver "	.64 ± .14 (7)	1.20 ± .21 (8)	1.15 ± .20 (8)
Pancreas "	.38 ± .16 (6)	1.13 ± .30 (6)	1.40 ± .18 (6)
Patient's serum	.49 ± .29 (8)	.93 ± .25 (10)	1.15 ± .17 (10)
Potassium (μM/mg dry wt sac/hr)			
Control	.04 ± .03 (7)	.04 ± .02 (10)	.04 ± .01 (10)
Tumor extract	.04 ± .01 (3)	.04 ± .01 (6)	.05 ± .03 (6)
Liver "	.04 ± .01 (7)	.05 ± .02 (8)	.04 ± .01 (8)
Pancreas "	.02 ± .01 (6)	.03 ± .01 (6)	.04 ± .01 (6)
Patient's serum	.02 ± .01 (8)	.04 ± .01 (10)	.04 ± .01 (10)
Chloride (μM/mg dry wt sac/hr)			
Control	.39 ± .24 (7)	.84 ± .33 (10)	1.13 ± .16 (10)
Tumor extract	.51 ± .20 (3)	.95 ± .24 (6)	.79 ± .09 (6)†
Liver "	.42 ± .10 (7)	.97 ± .14 (8)	1.14 ± .16 (8)
Pancreas "	.19 ± .10 (6)	.97 ± .24 (6)	1.34 ± .19 (6)
Patient's serum	.30 ± .18 (8)	.81 ± .21 (10)	1.04 ± .19 (10)

* Values expressed as mean ± standard deviation. No. of experiments in parentheses.
† Significantly less ($p < 0.01$) than control, liver extract and pancreas extract.

Discussion. Our results point to an impaired net absorption of fluid and electrolytes by the small intestine as the source of diarrhea in the patient studied. Data using the everted gut sacs clearly demonstrate the capacity of the tumor extract, but not the control substances or patient's serum, to inhibit the net mucosal-to-serosal transfer of fluid, sodium and chloride across sacs from the distal portion of the hamster intestine. The results obtained by monitoring intrasac pressure fail to show a rise in the intrasac pressure as the mechanism by which the tumor extract diminished fluid and electrolyte transfer. Matsumoto *et al*(6) reported that an aqueous extract of lyophilized islet cell tumor tissue from a patient similar to ours had no effect on water or glucose transport in Hakim's(7) canine small bowel mucosal preparation. These authors did mention that "certain aspects of net Na^+ movement were different" in the presence of the tumor extract, but that these studies were of a preliminary nature. Furthermore, they reported that the tumor extract had no effect on the short-circuit in a "frog skin preparation." They implied that their failure to demonstrate an effect of the tumor extract on fluid and glucose transport may have resulted from factors such as inactivation of the diarrheogenic substance by their extraction procedure, failure to explore a concentration range, or failure to test the effect of the extract at various levels of the intestine. This latter procedure is important since, as demonstrated in our studies, the tumor extract altered fluid and electrolyte transfer only in sacs taken from the distal portion of the hamster small intestine.

The patient's mouth-to-anus transit time was normal, suggesting that hypermotility was not the cause of the diarrhea. In addition, neither the tumor, liver or pancreas extracts nor the patient's serum stimulated strips of isolated guinea pig ileum. It is unlikely that these negative findings resulted from our using an inadequate dose since concentrations 10 times greater than those at which the tumor inhibited intestinal transfer failed to affect motility. Espiner and Beaven (8) reporting a patient similar to ours,

demonstrated increased contractions in strips of guinea pig ileum exposed to their patient's serum, but not to a saline extract of the patient's tumor, to normal human serum or to an endometrial extract. No concentration range was reported as having been explored for the substances which had no effect on their preparation.

Summary. Clinical observations in a patient with a non-Beta islet cell pancreatic adenocarcinoma demonstrated excessive fecal losses of fluid and electrolytes and excessive volumes of fluid from the distal ileum during continuous gastric aspiration. Intestinal transit time was normal. *In vitro* studies demonstrated the capacity of a distilled-water extract of an hepatic metastasis to inhibit the net mucosal-to-serosal transfer of fluid, sodium and chloride across everted gut sacs from the distal portion of the hamster small intestine. No effect of the tumor extract on *in vitro* intestinal motility was observed. These results suggest that the patient's diarrhea resulted from the tumor or its products inhibiting the net absorption of fluid and electrolytes in the patient's small intestine.

1. Zollinger, R. M., Ellison, E. H., Ann. Surg., 1955, v142, 709.
2. Verner, J. B., Morrison, A. B., Am. J. Med., 1958, v25, 374.
3. Gregory, R. A., Tracy, M. J., Gut, 1964, v5, 103.
4. Wilson, T. H., Wiseman, G., J. Physiol., London, 1954, v123, 116.
5. Harry, J., Brit. J. Pharmacol., 1963, v20, 399.
6. Matsumoto, K. K., Peter, J. B., Schultze, R. G., Hakim, A. A., Franck, P. T., Gastroenterology, 1966, v50, 231.
7. Hakim, A., Lester, R. G., Lifson, N., J. Appl. Physiol., 1963, v18, 409.
8. Espiner, E. A., Beaven, D. W., Quart. J. Med., 1962, v31, 447.

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Antiprolactin and Experimentally-Induced Hepatic Metastases.* (31489)

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Hypophysectomy has been previously observed in our laboratory to inhibit the development of artificially-induced hepatic metastases following intraportal injection of known numbers of Walker tumor cells in female Sprague-Dawley rats(1). This effect was markedly minimized in hypophysectomized rats by intraperitoneal injection of ovine prolactin but not somatotropin, thyrotropin, adrenocorticotropin or luteinizing and follicle stimulating hormones administered either singly or in combination(2). This information, as well as other experimental (3,4) and clinical(5,6) evidence suggesting that prolactin may play a role in the induction and dependence of some mammary tumors prompted us to investigate the effect of a physiologically active antiprolactin preparation on the growth of hepatic metastases in our model system.

Materials and methods. Twelve adult white rabbits received subcutaneous and intraperitoneal injections of 8 mg of ovine prolactin† suspended in Freund's complete adjuvant weekly for 3 weeks. An intravenous booster of 0.25 mg prolactin in saline was administered 3 weeks later. Hemagglutination titers determined according to the method of Levy and Sampliner(7) and agar-gel diffusion were performed on blood samples obtained prior to immunization and 10 days following the booster dose. Only sera from animals exhibiting a titer greater than 1:600 and distinct precipitin bands (Fig. 1) were utilized in the experiments. Antiprolactin (APS) and normal rabbit sera (NRS) were dispensed in small sterile containers and kept frozen until used.

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