PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 192–194 (1978)

Pancreatic Secretory Isoenzyme of Alkaline Phosphatase (40312)

WALTER P. DYCK,[1] A. M. SPIEKERMAN, AND CHARLES R. RATLIFF

Section of Gastroenterology, Department of Internal Medicine and Section of Biochemistry, Department of Clinical Pathology, Scott and White Clinic, Temple, Texas 76501

Alkaline phosphatase exists in a wide variety of tissues in different molecular forms. Characterization of these isoenzymes is possible on the basis of their resistance to various physical and chemical manipulations. As early as 1944, Nothmann (1) reported that ligation of the pancreatic duct in dogs produced an increase in serum alkaline phosphatase, but there have been few attempts to measure this enzyme in pancreatic juice. Warnes and Bulmer (2) demonstrated the presence of alkaline phosphatase in the duct system, islet cells, and acini of the human pancreas. Warnes *et al.* (3) extracted alkaline phosphatase from normal human pancreas and pancreatic tumors and showed that these enzymes have distinctive isoenzyme characteristics when compared with the enzymes of the small intestine and of normal serum.

The present study was designed to examine the isoenzyme characteristics of alkaline phosphatase in canine pancreatic secretory fluid. The availability of pure human pancreatic juice from a patient with a traumatic fistula allowed us to conduct similar observations in this fluid.

Methods. Six adult mongrel dogs, weighing 14–18 kg, were previously prepared with gastric and pancreatic fistulas fitted with Thomas cannulas in the stomach and duodenum (4). Animals were not studied until 3–4 weeks after this operation and were deprived of food but not water for approximately 18 hr prior to each study. A continuous iv infusion of 0.15 *M* sodium chloride was given at a rate of 50 ml/hr. Observations were carried out in conscious animals during continuous intravenous infusion of secretin, 0.5 U/kg per hr. The secretin used in these studies was from a single batch purchased from the Gastrointestinal Hormone Research Unit, Karolinska Institute Chemistry Department, Stockholm, Sweden. The gastric cannula was kept open during all observations to prevent the entry of acid into the duodenum. The duodenal cannula was opened and a glass cannula was inserted into the pancreatic duct under direct vision. Pancreatic secretion was collected continuously as 10-min specimens.

Pancreatic juice also was collected from a patient who had an established posttraumatic pancreatic fistula that was draining clear, alkaline juice, 400–600 ml/day, with a bicarbonate concentration of 68 meq/liter and an amylase concentration of 120,000 Somogyi U/100 ml. Fluid was collected by direct cannulation of the fistula with a sterile catheter after appropriate skin cleansing to minimize the liklihood of bacterial contamination.

Alkaline phosphatase, expressed in international units, was determined by the method of Roy (5) with thymolphthalein monophosphate as the substrate.

Isoenzyme characterization, based on different susceptibilities of alkaline phosphatase isoenzymes to inhibition by urea and L-phenylalanine (6–8) and heat inactivation (9), was performed in all specimens. The method of Kind and King (10) was used for alkaline phosphatase measurements in these isoenzyme studies.

Isoenzymes present in the human pancreatic fistula fluid were examined by acrylamide gel electrophoresis and compared to the electrophoretic behavior of alkaline phosphatase of known human origin from liver, bone, and intestine. Liver alkaline phosphatase was obtained from the serum of patients with known liver disease and intestinal alkaline phosphatase was purchased from Dade Corporation. Bone alkaline phosphatase was obtained from shavings of bone extracted with butanol to remove insoluble material and break the protein–lipid bond. The alkaline phosphatase obtained from the pancreatic fistula fluid was concentrated ten

[1] Reprint requests to: Dr. W. P. Dyck, 2401 South 31st Street, Scott and White Clinic, Temple, Texas 76501.

0037-9727/78/1592-0192$01.00/0

fold before electrophoresis. All samples to be electrophoresed were dialyzed for twelve hours against two changes of electrophoresis buffer. Alkaline phosphatase isoenzymes were separated by Raymond's method of continuous polyacrylamide gel electrophoresis in a vertical cell (11).

Results. At the low dose of secretin infusion utilized in the canine studies, pancreatic secretory volumes varied from 5 to 10 ml/10 min. The mean (± SEM) alkaline phosphatase concentration in specimens from all six animals (87 collections) was 15.4 ± 1.1 mU/ml. Alkaline phosphatase concentration in pancreatic fistula fluid collected from the patient was 17.8 mU/ml.

Figure 1 shows the percentage of alkaline phosphatase remaining in pancreatic juice from each of the six dogs after incubation of specimens with urea or phenylalanine or after heat inactivation. There was relative uniformity among the animals in that the isoenzyme exhibited relative resistance to phenylalanine inhibition, intermediate inhibition by urea, and marked thermal lability.

Isoenzyme characteristics of alkaline phosphatase in canine and in human pancreatic secretory fluid are compared in Fig. 2. The mean alkaline phosphatase activity remaining after phenylalanine inhibition was 67% in canine pancreatic juice compared to 85% in human juice. The mean values after urea

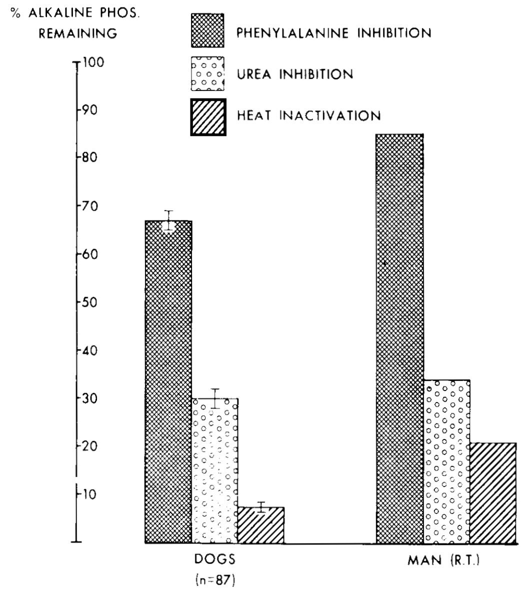

FIG. 2. Mean percent of alkaline phosphatase remaining in canine pancreatic juice and the percentage of enzyme remaining in human pancreatic fistula fluid after incubation with urea or L-phenylalanine or after heat inactivation. Bars at left represent mean ± SEM of all collections from six dogs.

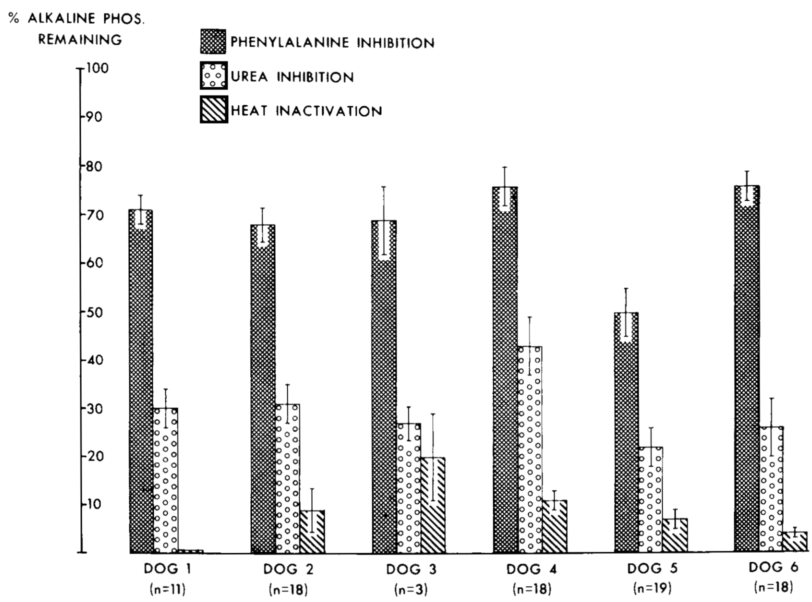

FIG. 1. Percentage alkaline phosphatase remaining in pancreatic juice after incubation with urea or L-phenylalanine or after heat inactivation. Each bar represents the mean ± SEM of all 10-min collections in a single animal during continuous intravenous infusion of secretin, 0.5 U/kg per hr. n = number of observations in each mean.

inhibition were 30% and 34%, respectively, and after heat inactivation were 7.5% and 21% respectively.

Figure 3 shows the electrophoretic mobility of the alkaline phosphatase isoenzyme in the human pancreatic fistula fluid compared to mobilities of isoenzymes derived from other human tissue sources. The pancreatic enzyme exhibited a pattern of mobility clearly different from that of any of the isoenzymes of other tissues sources.

Discussion. When Nothmann (1) found that ligation of the pancreatic duct in dogs resulted in a progressive increase in serum alkaline phosphatase activity, he assumed that this increased activity was of pancreatic origin. Subsequent studies (12, 13) have shown a significant increase in alkaline phosphatase concentration in duodenal juice after CCK-pancreozymin stimulation. The demonstration, by histochemical techniques, of this enzyme in various cellular components of the human pancreas (2) and the subsequent identification of distinctive isoenzyme characteristics of pancreatic alkaline phosphatase (3) are consistent with the presence of this enzyme in pancreatic secretory fluid.

Our data are in agreement with the findings of Warnes *et al.* (3) who showed that pancreatic alkaline phosphatase was much more sensitive to heat inactivation and urea inhibition than was the enzyme from the small intestine, but, in contrast, was largely unaffected by L-phenylalanine.

The question of whether increased serum total alkaline phosphatase values may at times reflect a predominant increase in the pancreatic isoenzyme remains unanswered and must await isoenzyme characterization studies in subjects with acute inflammatory as well as neoplastic disease of the pancreas. Additional techniques, such as acrylamide gel electrophoresis, will doubtless aid in further refining our means of identifying the source of different serum isoenzymes (14).

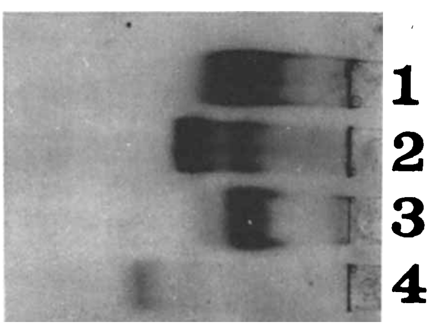

FIG. 3. Electrophoretic patterns (acrylamide gel) of alkaline phosphatase isoenzymes from human sources: 1, bone; 2, mixed liver and intestine; 3, intestine; and 4, pancreatic fistula fluid. Vertical electrophoresis in pH 9.0 Trismalein acid buffer (0.283 *M* and 0.019 *M*, respectively) at 4°; 300 V for 3 hr; stained with sodium α-naphthyl acid phosphate (1 hr).

Summary. Alkaline phosphatase activity was measured in hormonally stimulated pancreatic juice from six dogs and in pancreatic fistula fluid from a human subject. Isoenzyme characterization studies, based on different susceptibilities to urea and L-phenylalanine inhibition and to heat inactivation indicated similarities between canine and human pancreatic secretory alkaline phosphatase. Compared to intestinal alkaline phosphatase, the pancreatic isoenzyme was much more sensitive to heat inactivation and urea inhibition but much more resistant to L-phenylalanine inhibition. The electrophoretic mobility of the enzyme present in human pancreatic juice was different from that of human hepatic, bone, or intestinal alkaline phosphatase.

1. Nothmann, M. M., Proc. Soc. Exp. Biol. Med. **57**, 15 (1944).
2. Warnes, T. W., and Bulmer, D. J., Anat. **106**, 410 (1970).
3. Warnes, T. W., Timperley, W. R., Hine, P., and Kay, G., Gut **13**, 513 (1972).
4. Thomas, J. E., Proc. Soc. Exp. Biol. Med. **46**, 260 (1941).
5. Roy, A. V., Clin. Chem. **16**, 431 (1970).
6. Bahr, M., and Wilkinson, J. H., Clin. Chem. Acta **17**, 376 (1967).
7. Horne, M., Cornish, C. J., and Posen, S., J. Lab. Clin. Med. **72**, 905 (1968).
8. Kreisher, J. H., Close, V. A., and Fishman, W. H., Clin. Chim. Acta **11**, 122 (1965).
9. Ratliff, C. R., Hall, F. F., Culp, T. W., Gevedon, R. E., and Westfall, C. L., Amer. J. Gastroenterol. **58**, 22 (1972).
10. Kind, P. R. N., and King, E. J., J. Clin. Pathol. **7**, 322 (1954).
11. Raymond, S., Ann. NY Acad. Sci. **121**, 350 (1964).
12. Warnes, T. W., Hine, P., and Kay, G., Gut **10**, 1049 (1969).
13. Dyck, W. P., Martin, G. A., and Ratliff, C. R., Gastroenterology **64**, 599 (1973).
14. Smith, I., Lightstone, P. J., and Perry, J. D., Clin. Chim. Acta **19**, 499 (1968).

Received January 16, 1978. P.S.E.B.M. 1978, Vol. 159.