Alkaline Phosphatase—Correlation of Histochemical Demonstrability in Pancreatic Tissue with Presence in Pancreatic Juice. (19057)

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Gomori(1) and subsequently others(2-4), have reported that the histochemical method for demonstration of alkaline phosphatase produces a strongly positive reaction in the centro-acinar and intralobular duct cells of the dog. It has also been shown that pure pancreatic juice from the dog contains alkaline phosphatase(4,11).

In the present studies observations on the histochemical reaction for alkaline phosphatase have been extended to the pancreas of other species and samples of the pancreatic juice of these same species have been examined for their content of alkaline phosphatase.

Pancreatic tissue and pancreatic juice from man, dog, cat, guinea pig and rabbit were studied. The histochemical demonstration of alkaline phosphatase was accomplished by Gomori's method(5) after fixation of the tissue in 80% ethyl alcohol. Alkaline phosphatase in the pancreatic juice was determined by the Shinowara modification(6) of the Bodansky method with color development according to the procedure of Holman(4). In the animals, pancreatic juice was obtained by direct cannulation of the major duct and stimulation with purified secretin.* Pancreatic juice was obtained from 2 human patients with pancreatic fistulas. In each case it was demonstrated during subsequent surgery that the pancreatic fistula did not communicate with the duodenum. Thus, in neither the animal nor the human experiments was there contamination with duodenal juice, which is known to contain alkaline phosphatase(7). The animal tissues were removed before death and immediately placed in the fixative. The human pancreatic tissue was obtained by surgical biopsies.

The findings were as follows (Table I). The intralobular ducts and centroacinar cells of the dog, rabbit and human showed a positive staining reaction for alkaline phosphatase and the pancreatic juice of these species contained the enzyme. In the guinea pig and cat the enzyme was not demonstrable in either the tissue or the juice. The acinar cells of all the species examined were negative for alkaline phosphatase.

Comment. In an earlier study we have shown that elevation of the level of alkaline phosphatase in the blood does not cause an increase in the amount in the pancreatic juice (8). Taken together with the present evidence, this indicates that the alkaline phosphatase of the pancreatic juice is supplied by the pancreas itself and that, at least for this component, the intralobular ducts and centro-acinar cells have a secretory function. The evidence supporting the possibility that the intralobular ducts and centro-acinar cells have other secretory functions has been reviewed elsewhere(9). In the cat alkaline phosphatase is demonstrable in the walls of the blood vessels after freezing-drying fixation of the pancreas, but the intralobular ducts and centro-acinar cells are negative just as they are with alcohol fixation(10).

Summary. In man, dog and rabbit alkaline

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TABLE I. Alkaline Phosphatase in Pancreatic Tissue and Pancreatic Juice of Various Species.

Species	Reaction of intralobular duct to stain for alkaline phosphatase	Pancreatic juice	
		No. of specimens	Alkaline phosphatase (units/100 cc)
Dog	+	124	.4 to 41
Human	+	2	2.1 to 3.8
Rabbit	+	8	2.6 to 4.4
Guinea pig	0	3	0
Cat	0	10	0

phosphatase is demonstrable histochemically in the intralobular ducts and centro-acinar cells of the pancreatic tissue and is present in the pancreatic juice. In the cat and guinea pig this enzyme is present neither in the intralobular ducts and centro-acinar cells nor in the pancreatic juice.

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Development of Resistance to 4-Amino-N^{10}-Methyl Pteroylglutamic Acid (Amethopterin) by *Streptococcus faecalis*.* (19058)

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In children with acute leukemia, whose disease initially responds well to treatment with 4-amino-N^{10}-methyl pteroylglutamic acid (amethopterin), there develops in the leukemic process, after 6 to 18 months, refractoriness to therapy(1-4). Prevention of this development of resistance would be of the greatest clinical importance, since in the absence of resistance these children could be treated again and again and the disease suppressed indefinitely. The development of resistance to amethopterin in a previously sensitive strain of mouse leukemia has been reported by us(5), and was later repeated in another strain of leukemia by Law and coworkers(6). With these two strains of amethopterin-fast leukemias, studies are in progress on the mechanisms of resistance in the hopes of finding technics for preventing its development. In order to provide another system for the study of resistance to amethopterin, attempts were made to develop an amethopterin-fast strain of *Streptococcus faecalis*, an organism ordinarily requiring folic acid, and very sensitive to the inhibitory action of amethopterin.

Method. Streptococcus faecalis (ATCC No. 8043) was grown on Difco folic acid assay broth in the presence of 5 mγ/ml of pteroyl-

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