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Alkaline and acid phosphatase determinations in the eccrine sweat glands of patients with cystic fibrosis of the pancreas

Robert Lewis Kollmorgen
University of Nebraska Medical Center

ALKALINE AND ACID PHOSPHATASE DETERMINATIONS IN THE ECCRINE SWEAT GLANDS OF PATIENTS WITH CYSTIC FIBROSIS OF THE PANCREAS

ROBERT L. KOLLMORGEN

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INTRODUCTION

Cystic fibrosis of the pancreas is a generalized, hereditary disease of children and adolescents due to dysfunction of the exocrine glands (1). Pathologic changes in the pancreas attracted early attention and gave the disease its name. Later it was learned that there was a widespread defect of mucus secretions throughout the body and the name "mucoviscidosis" was suggested (2).

In the fully manifested cases there is chronic pulmonary disease, pancreatic deficiency and abnormally high sweat electrolyte concentrations. Gradation in or absence of involvement of the various organs or glandular systems is characteristic of this disorder and leads to many variations in the clinical picture.

In the mucus-producing glands the basic change consists of an accumulation of presumably abnormal secretions leading to dilatation of the secretory gland. This accumulation may lead to obstruction of the small intestine by inspissated meconium resulting in intestinal obstruction in the newborn, so-called meconium ileus (3). Obstruction of the pancreatic ducts leads to secondary degeneration of the exocrine parenchyma of the pancreas and pancreatic achylia (3). Obstruction of the bronchi by accumulated secretions leads to secondary bronchopneumonia and chronic pulmonary involvement which is present at some time in the course of the disease in virtually all patients with cystic fibrosis.

The incidence of cystic fibrosis is estimated as being about 1 in 1000 live births (4). Since this condition is familial and thought to be transmitted as a recessive trait, as many as 2 to 18% of the population may harbor the specific gene (5).

Abnormally high chlorides and sodium in sweat are present in almost all patients with cystic fibrosis (6). Patients with this disease have a concentration of these ions in the sweat 2 to 5 times greater than controls (4). To date no other disease has been found in which sweat electrolytes are increased, with the exception of untreated adrenal insufficiency; therefore, the "sweat test" because of its simplicity and reliability has come into general use as the main diagnostic procedure in this disease.

The basic defect in cystic fibrosis is unknown as yet. Deficiencies in autonomic nervous system function (4) or absence of an enzyme (7) or some other metabolic error (4) have been proposed in general terms to explain the generalized exocrine gland dysfunction.

PURPOSE

Dent (8) has defined an inborn metabolic error as "a permanent inherited condition due to a primary enzyme abnormality. As a result one or more chemical compounds may follow an altered metabolic pathway and may be found in some body fluids in greatly increased or decreased quantities." Because of the abnormal amounts of sodium and chloride ions in the sweat of patients with cystic fibrosis and the accessibility of sweat glands, our purpose was to perform enzyme studies on sweat glands in an attempt to determine whether we could uncover a metabolic error.

METHODS AND TECHNIQUES

Skin plugs 3 millimeters in diameter were obtained from the back just below the iliac crest by the punch drill biopsy technique (9). One percent procaine was used to infiltrate the area and 3 to 6 skin plugs were taken at once.

Immediately on removal from the body these small pieces of skin were dipped in tragacanth jelly and then placed on a frozen layer of tragacanth covering the surface of a microtome sample holder at -18 C°. Freezing is almost instant. Serial sections, 32 microns thick, were cut by a microtome in a cryostat at -18 C° (10-19). As cut the sections were transferred serially with sharp pointed forceps to a suitable holder (10). When filled, the section holders were placed in a glass evacuation tube inside the cryostat (10).which can also serve as a storage container. The evacuation device was connected by tubing to a vacuum pump located outside the cryostat. The frozen serial sections can be dried in 1 to 4 hours by applying a vacuum of 0.1 mm. Hg (10,12, 13, 14, 15, 19). The evacuation tube contains a stopcock which can be closed after the frozen sections are dried and then detached from the vacuum line. Tissues can be stored at -18 C° in this manner or the evacuation tube can be removed from the cryostat and connected to the vacuum line outside the cryostat and brought to room temperature. Tissues must be at room temperature before the vacuum is broken and dissection is started, or moisture will

condense on the sections and ruin them (10).

It is desirable to dissect the sweat gland fragments in a room free of harmful fumes and dust and kept at constant temperature and humidity. The operator wears a mask to prevent blowing away the sections.

Unstained sections were then examined under a dissecting microscope at 40 magnifications. The microscope lamp was positioned 8 to 10 inches to the side and the reflecting mirror was covered with photographic dark paper to give a black background. The various parts of the sectioned skin plug can be identified by direct application of multiple stain for frozen sections to alternate sections of the dried tissue and viewed by substage lighting. Coils of the sweat glands in the adjacent unstained section can be recognized by their snow-white appearance against a grey-white background of the surrounding tissue. Dissection was done with a hypodermic needle and scalpel (Bard Parker blade No. 11) (10). Sweat gland fragments weighing 1 to 5 micrograms were dissected from the dried slices.

The size of the sweat gland coils appears to be related to the age of the child; younger children having smaller coils of sweat gland.

Fragments of dissected sweat gland were then transferred to a quartz fiber balance and weighed (10,20). These fragments became the basis for individual enzyme determinations.

ENZYME METHODS

Alkaline phosphatase was determined by the method used by Bonting et al (21), which measures the amount of p-nitrophenol released by enzymatic activity from substrate at pH 10.0.

Acid phosphatase was determined by the method outlined by Hershey et al (22), using the same substrate at pH 5.4 and measuring the amounts of p-nitrophenol resulting from enzyme activity.

The principle of these tests is that the compound, p-nitrophenyl phosphate, is colorless, but upon hydrolysis of the phosphate group in the presence of an alkali, the yellow salt of p-nitrophenyl is liberated. Thus, the end product is itself an indicator of the amount of splitting and hence is a measure of phosphatase activity, as indicated by the following reaction:

$$\underset{\text{(colorless in acid and alkali)}}{\text{p-nitrophenyl phosphate}} + H_2O \xrightarrow{\text{phosphatase}} \underset{\substack{\text{(colorless in acid}\\ \text{yellow in alkali)}}}{\text{p-nitrophenol}} + H_3PO_4$$

Due to the limited number of patients available, a relatively small number were used for study of each enzyme.

RESULTS AND CONCLUSIONS

As indicated in table I, the content of alkaline and acid phosphatase in sweat glands in patients with cystic fibrosis is not different from the content in glands of normal individuals. No data on alkaline phosphatase in isolated normal sweat glands has been found in the literature. Alkaline phosphatase activity in dermis has been reported by Hershey and Mendle (23) to the amount of 190 mM/Kg/hour.

Determinations by the same method by Bonting (21) on various structures of the kidney shows values of 0.56 M/Kg/hour in glomeruli; 2.76M/Kg/hour in distal and 5.48 M/Kg/hour in proximal tubules.

Acid phosphatase in normal sweat glands as determined by Hershey (22) showed mean values of 0.39 M/Kg/hour.

These enzymes were selected for study on the basis of their possible involvement in the disordered metabolism. This experiment also establishes a technique to be followed for other future enzyme studies on sweat glands.

	Alkaline Phosphatase		Acid Phosphatase	
	cystic fibrosis	normal	cystic fibrosis	normal
	0.37	0.19	0.33	0.98
	0.26	0.35	0.35	0.96
	0.23	0.42	0.90	0.74
	0.22	0.29	0.61	0.87
	0.31	0.25	0.71	0.43
	0.47	0.41	0.44	0.66
	0.27	0.48	0.77	0.73
Mean	0.34	0.29	0.62	0.76
Standard Deviation	0.15	0.16	0.29	0.43
Range*	0.12-0.63	0.10-0.82	0.12-1.30	0.24-1.82

Table I

Enzyme activity of alkaline and acid phosphatase in sweat glands. Moles of substrate split per kilogram of dry sweat gland.

*Each value listed in the table is the average of three individual determinations.

SUMMARY

1. The basic defect in cystic fibrosis of the pancreas is unknown as yet. It is an hypothesis that this is an inborn metabolic error due to an enzyme abnormality.

2. A technique was devised to obtain sweat gland specimens for enzyme determinations from living patients with this disease.

3. Alkaline and acid phosphatase activity of sweat glands was determined on normals and patients with cystic fibrosis of the pancreas. No significant differences were found.

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