THE ENZYMATIC DEFECT IN HISTIDINEMIA

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Two siblings with positive urinary ferric chloride tests not due to phenylketonuria were recently referred to our laboratory for study.* Elevated urinary and blood levels of histidine were found. The children's intelligence and rate of development are normal except that both have a speech defect. A detailed biochemical and clinical investigation of the members of this family will shortly be published elsewhere.

In 1961 Ghadimi et al. first described elevated blood and urinary levels of histidine in two sisters, the elder with a speech defect, and proposed that they had a new inborn error of histidine metabolism. The children also had positive urinary ferric chloride tests which were thought to be due to other abnormal metabolites of histidine in the urine. Auerbach et al.(1961), in a recent investigation of another case of histidinemia, identified imidazolepyruvic, imidazolelactic and imidazoleacetic acids in the urine and attribute the positive ferric chloride test to the presence of imidazolepyruvic acid. They observed that urocanic acid was metabolized normally via formiminoglutamic acid following its intravenous administration and concluded that urocanase and the subsequent enzymes in this pathway were not impaired in histidinemia. On the basis of this evidence and the pattern of urinary metabolites, they suggested that the metabolic

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defect in this disease is the lack of histidase, the enzyme which catalyzes the conversion of histidine to urocanic acid. However, direct demonstration of the enzymatic defect was not made.

The present paper reports the results of our studies on the enzymatic nature of the biochemical defect in histidinemia.

Histidase activity has been demonstrated in mammalian liver (Takeuchi, 1941; Sera, 1951; Tabor and Mehler, 1955) and in guinea pig skin (Schwarz, 1961). We have also studied the distribution of this enzyme in mammalian tissues, including man, and have found it to be present in highest concentrations in liver and the epidermal layer of the skin. In fact, histidase activity in human skin per gram fresh weight is at least half as high as that found in liver. It was, therefore, evident that the suspected enzymatic defect in histidinemia might be demonstrated in skin biopsies of our patients. Homogenates were prepared from finger tip skin samples (stratum corneum) and incubated with histidine to determine the rate of urocanic acid formation by a modification of the spectrophotometric method of Tabor and Mehler (1955). In addition to its accessibility as a source of the enzyme, the epidermis has the advantage that it lacks urocanase activity and thus urocanic acid is not further metabolized during the histidase assay procedure.

The two siblings with histidinemia had no demonstrable histidase activity in their epithelial skin (Table I). In contrast, normal children and adults had histidase activity which was easily measured. Furthermore, there was no evidence of an inhibitor to account for the differences observed, since the combination of homogenates of epidermis from the affected child (W. B.) with that from a normal adult did not reduce the latter enzyme activity. It is of interest that the sister of the affected children had a level of enzyme activity which was considerably below the range of values in the control group of children. The level of histidase activity in the father's skin was also below the range of the control group.

However, the mother's level, although lower than the average female, was within the range observed in the normal adult control group.

TABLE I

HISTIDASE ACTIVITY IN THE EPIDERMIS OF A FAMILY WITH HISTIDINEMIA AND IN NORMAL INDIVIDUALS

Subjects	Epidermal Histidase Activity [a]	
	μg of histidine metabolized /hr/g fresh weight [b]	
Case 1 (W. B.); 6 years, female [c]	0	
Case 2 (E. B. Jr.); 5 years, male	0	
Sister (L. B.); 4 years	276	
Mother (R. B.); 25 years	318	
Father (E. B.); 31 years	193	
	Average values	Range
Control - Children (8) [d]	740	(579-910)
Control - Adults		
Female (12)	413	(249-731)
Male (12)	502	(263-732)

[a] Incubation conditions - 0.4 ml. aliquots of dialyzed 2% homogenates of the stratum corneum of finger tip skin were added to 0.5 ml. of 0.1 M pyrophosphate buffer, pH 9.2, 0.05 ml. of 0.1 M glutathione and 0.05 ml. of 0.1 M L-histidine (or water in the control tubes), with additional water added to give a final volume of 1.2 ml. After incubation at 37°C for 2 hours, perchloric acid was added and the amount of urocanic acid formed was determined spectrophotometrically at 270 mμ.

Urocanic acid formation was found to be linear with time and proportional to the concentration of homogenate employed. Urocanic acid incubated for 2 hours in place of histidine was completely recovered. Boiled homogenate and the incubation mixture without homogenate produced no urocanic acid. A detailed description of this method to measure skin histidase activity will be published.

[b] The values are expressed on a fresh weight basis. Protein determinations by the method of Lowry et al. (1951) showed little variation in the protein concentration of the homogenates and averaged 9 mg./ml.

[c] Histidase activity on three occasions was found to be zero. Under these assay conditions, values below 20 are not significant.

[d] The numbers in parentheses represent the number of individuals.

The blood concentration of histidine and the presence of urinary histidine and imidazolepyruvic acid in the members of this family are shown in Table II. Only the two children with the enzymatic defect had an elevated level of histidine in the blood and excreted histidine and imidazolepyruvic acid in the urine. The sweat of one of the affected children (W. B.) was analyzed for urocanic acid and none was found. However, similar analyses on the sweat from the parents and normal individuals showed urocanic acid to be present, although the concentration was considerably reduced in the father. The lack of urocanic acid in the sweat of the affected child is not unexpected in view of the lack of histidase activity in the skin.

TABLE II

PLASMA HISTIDINE, URINARY HISTIDINE AND IMIDAZOLEPYRUVIC ACID IN A FAMILY WITH HISTIDINEMIA

Subjects	Plasma Histidine	Urine Histidine	Urine Imidazole-pyruvic Acid
	mg.%		
Case 1 (W. B.)	17.3	+++	+++
Case 2 (E. B. Jr.)	13.4	+++	+++
Sister (L. B.)	0.9	–	–
Mother (R. B.)	1.4	–	–
Father (E. B.)	3.3	+	–
Normal values	<2.0	–	–

Histidine was determined by a modification of the method by La Du and Michael (1960) at pH 7.8 as suggested by R. C. Baldridge. The imidazolepyruvic acid was measured as the enol-borate complex.

The above demonstration that histidase is missing in the skin of patients with histidinemia establishes that this metabolic disease is due to the absence of this enzyme and that it properly belongs to the group of inborn errors of metabolism. The biochemical disturbance in histidine

metabolism is so pronounced that there is little doubt that the enzyme deficiency also exists in other tissues, including liver, where the enzyme normally occurs.

The metabolic disturbance in these children is therefore similar to that in the families reported by Ghadimi et al.(1961) and by Auerbach et al. (1961). It should be pointed out that a different type of defect in histidine metabolism, presumably due to a faulty renal reabsorption of histidine with histidinuria but without an increase in the level of plasma histidine concentrations, has been recently reported by Bessman and Baldwin (1962).

The similarities in the biochemical findings in histidinemia and phenylketonuria are apparent. A striking difference in the consequences of having these diseases is that mental retardation is apparently not associated with the defect in histidine metabolism as is generally found with the defect in phenylalanine metabolism. It would be of considerable importance to learn the biochemical basis for this difference.

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