Preliminary Notes

Metabolism of the white blood cells in maple-syrup-urine disease

Maple-syrup-urine disease is an inherited metabolic anomaly characterized clinically by early onset of feeding problems and neurological symptoms with eventual mental retardation and death, and a maple-syrup odor to the urine. It has been suggested that the biochemical defect may be at the step involving oxidative decarboxylation of the branched-chain keto acids of the respective amino acids: leucine, isoleucine and valine. This was based on finding an elevation in the branched-chain amino acids in the plasma, normal transaminase activity in tissues obtained at post-mortem, the accumulation of branched-chain keto acids in the urine and the absence of an excess of metabolites located further down the degradative pathway[1-3]. The enzymes necessary for the oxidative decarboxylations of the branched-chain keto acids are widely distributed in animal tissues including the white blood cell[3]. This suggested that the enzymic defect might be directly demonstrable in the white blood cell of the peripheral blood of a patient.

The opportunity to test this hypothesis presented itself with the birth of a sibling to the previously reported case. The patient revealed the typical biochemical abnormalities by the second week of life. At the time of these studies she was 1 year old and had been on a synthetic amino acid diet since the diagnosis had been made. The plasma levels of the branched-chain amino acids were within normal limits; methionine, however, was elevated. There was no increased excretion of keto acids[4].

Blood was drawn into a heparinized syringe and the red blood cells sedimented with fibrinogen. The supernatant was centrifuged and the white blood cells resuspended in sodium phosphate buffer. Aliquots were transferred to 10-ml Erlenmeyer flasks with a center well. The number of white blood cells per flask approximated that which had been present in 1 ml of plasma. The incubation medium consisted of 0.8 ml 0.067 *M* sodium phosphate buffer, pH 7.4, 0.03 ml 0.15 *M* $MgSO_4$, and 2 μg of crystalline catalase (100 units/mg). To this was added one of three substrates: 150,000 counts/min DL-[1-^{14}C]leucine (30 μg), 250,000 counts/min L-[^{14}C]isoleucine (322 μg) or 150,000 counts/min DL-[1-^{14}C]valine (14 μg). The center well contained 0.1 ml 1% Na_2CO_3 in 1 *N* KOH. Control flasks contained all the above except for the white blood cells. The flasks were incubated with shaking for 75 min at 37°.

Following incubation the contents of the center well were precipitated as $BaCO_3$, washed and transferred to weighed planchettes for detection of radioactivity. The total amount of $^{14}CO_2$ was calculated by relating the weight of $BaCO_3$ on the planchette to the amount of carrier carbonate added to the center well. Results were corrected for self-absorption.

The incubation medium was treated with 2,4-dinitrophenylhydrazine, extracted into *m*-xylene, re-extracted into Na_2CO_3 which was acidified and finally extracted into ethyl acetate for transfer to a planchette. Radioactivity was determined in a gas-flow counter. This is reported as the "keto acid" fraction.

The results are presented in Table I. The "keto acid" fraction is probably represented primarily by branched-chain keto acids. This was verified for leucine by paper chromatography in the patient and in one control case. The presence of small amounts of other organic acids cannot be excluded. The formation of "keto acids"

TABLE I

TRANSAMINATION ("KETO ACIDS") AND DECARBOXYLATION (CO_2) IN MμMOLES AS DETERMINED IN THE WHITE BLOOD CELLS OF NORMAL 1-YEAR-OLD INFANTS AND IN A PATIENT WITH MAPLE-SYRUP-URINE DISEASE

		Leucine		*Isoleucine*		*Valine*	
		"Keto acids"	*CO_2*	*"Keto acids"*	*CO_2*	*"Keto acids"*	*CO_2*
Control infants	1	1.86	1.80	2.0	7.4	0.31	0.77
	2	2.47	1.47	3.0	3.7	0.03	0.42
	3	1.72	0.62	2.9	1.7	0.12	0.83
	4					0.20	0.30
Maple-syrup-urine disease	5	1.50	0.01	3.0	0	0.46	0.03

is about the same in the normal infant and in the patient indicating active transamination. However, the accumulation of $^{14}CO_2$ by the patient's cells was drastically reduced for all three amino acids indicating grossly deficient or absent decarboxylation. This supports the hypothesis that the three amino acids share one enzyme in the degradative pathway which is deficient in maple-syrup-urine disease. It excludes the possibility that only one amino acid was primarily involved and that the accumulation of this keto acid interfered secondarily with the decarboxylation of the other two.

The diagnosis in the present case of maple-syrup-urine disease was delayed for almost two weeks after birth because the urine failed to show an appreciable accumulation of keto acids. It was therefore desirable to learn whether this enzymic function could be demonstrated in the white blood cell of the young infant. Four infants were tested with [1-^{14}C]leucine (3 at 2½ days and 1 at 6 days of life). The production of $^{14}CO_2$ was 0.87, 1.71, 0.43 and 0.38 mμmoles, respectively. It therefore seems possible to make an early and specific diagnosis of maple-syrup-urine disease by studying the metabolism of the peripheral white blood cell.

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[1] R. G. WESTALL, J. DANCIS AND S. MILLER, *A.M.A. J. Diseases Children*, 94 (1957) 571.
[2] J. H. MENKES, *Pediatrics*, 23 (1959) 348.
[3] J. DANCIS, M. LEVITZ AND R. G. WESTALL, *Pediatrics*, 25 (1960) 72.
[4] S. SNYDERMAN, J. DANCIS, P. NORTON AND L. E. HOLT, JR., *A.M.A. J. Diseases Children*, in the press.

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