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The metabolism of leucine in tissue culture of skin fibroblasts of maple-syrup-urine disease

KROOTH AND WEINBERG[1] have studied the metabolic defect of galactosemia in skin fibroblasts grown in tissue culture. To apply this approach to other metabolic diseases, it is necessary that the enzyme under study be normally present in skin and that it retain its activity under conditions of tissue culture. In order to be certain that the enzyme in the normal skin fibroblast is under the same genetic control as that responsible for the metabolic defect, it is necessary to demonstrate that the skin fibroblasts grown from patients with the disease either have no activity or markedly reduced activity.

In maple-syrup-urine disease, the enzymic defect is that of oxidative decarboxylation of the ketoacids of leucine, isoleucine and valine[2–4]. Enzymes capable of performing this function are widely distributed in the tissues of the experimental animal[5], suggesting that such activity may also be present in the skin.

In the present study, skin specimens were obtained from four normal subjects and from two patients with maple-syrup-urine disease. The specimens were grown

TABLE I

Results reported as observed counts/min. No correction for self absorption. Ketoacids measure transamination activity; $^{14}CO_2$ measures decarboxylation.

Source of skin		*No. of "generations"**	*Ketoacid*	$^{14}CO_2$
Normal	I	approx. 15	—	885
	II	7	5475	1183
	III	6	3909	458
	IV	approx. 150	2057	126
Maple-syrup-urine disease				
DoRa		13	3537	0
WaBi		9	4909	0

* "generations" signifies subcultures.

in tissue culture as a monolayer[6] in modified Eagle's medium (Microbiological Associates) with 30% fetal calf serum.

To measure the enzyme activity, the cells from three or four bottles (about $3 \cdot 10^5$–$6 \cdot 10^5$ cells per bottle) were harvested by incubating in 0.1% trypsin for 20 min at 37°. The cells were washed in buffer and then incubated for 75 min with 150 000 counts/min of DL-[1-^{14}C]leucine (30 μg) as previously described[7]. The ketoacid was isolated as the 2,4-dinitrophenylhydrazone and the liberated CO_2 was trapped in alkali and precipitated as $BaCO_3$.

The results are presented in Table I. The radioactivity in the ketoacid fraction reflects the transamination activity and is the same in the normal skin fibroblast and in that grown from patients with maple-syrup-urine disease. In contrast, decarboxylase activity is absent in the maple-syrup-urine disease cultures whereas it is readily demonstrable in the normal. These results parallel those obtained with the peripheral leucocyte[7]. It is of some interest that decarboxylase activity at a reduced level can still be demonstrated in normal skin maintained in culture for 150 generations.

One of the difficulties in investigating maple-syrup-urine disease has been the limitation of available tissue for study. The enzyme has proved unstable in autopsy material[8]. The amount of "tissue" obtainable as peripheral leucocytes is very small. This problem should be resolved by the use of tissue culture.

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