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Prenatal Diagnosis and a Case Report of Isovaleric Acidaemia

M. E. Blaskovics, W. G. Ng and G. N. Donnell
Children's Hospital of Los Angeles Regional Center and the Division of Medical Genetics, Los Angeles, California 90027, USA

Once considered a 'frontier' procedure, prenatal diagnosis via amniocentesis, fibroblast cultures and enzyme activity determinations are now regularly done in some centres. Where potentially lethal inherited disorders have previously been recognized in a family the availability of prenatal diagnosis now allows options where before there were none.

This report presents a previously undescribed patient with isovaleric acidaemia and a procedure developed for prenatal diagnosis of his sibling. Isovaleric acid accumulates as a consequence of decreased activity of the dehydrogenase enzyme involved in the metabolism of L-leucine (Figure 1). Defects in the metabolism of L-leucine have been recognized at almost every step in its degradation.

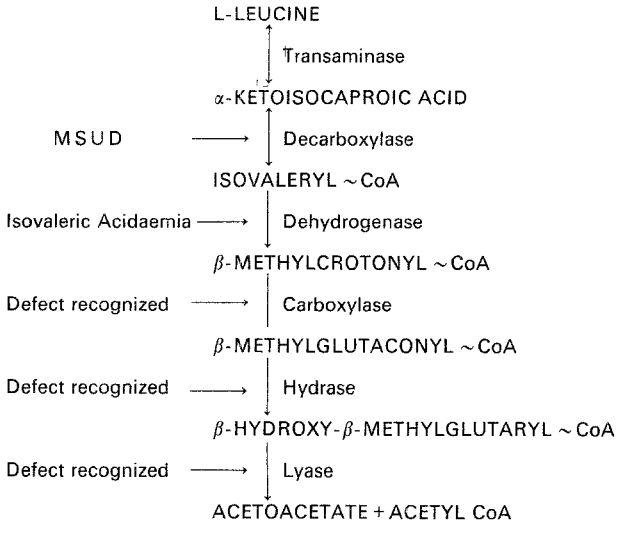

Figure 1 Enzymatic defects in the leucine pathway

The mother's first pregnancy was uncomplicated and she was delivered at term of a female infant. Although the infant was normal at birth and when discharged at three days of age, she was readmitted for severe respiratory distress and emesis and died at eight days of age. The infant was noted to have an unusual odour prior to death. Death was ascribed to bronchopneumonia, but pathological studies demonstrated no significant pulmonary findings.

R.M.A. (Figure 2), the propositus, now 13 years old, is the product of the mother's second pregnancy, which also was uncomplicated. The parents could not recall details of this patient's neonatal period; but he had been in hospital three times before the age of one-and-a-half years because of severe emesis, acidosis and coma. He usually responded to hydration with glucose water and alkalization, becoming alert after 48–72 h of treatment. The diagnosis of isovaleric acidaemia (IVA) was suspected by one of the authors (M.E.B.) because of the history and confirmed when the private physician admitted the patient with an episode of emesis and lethargy, secondary to an upper respiratory infection.

The patient was six years of age at the time (1968) and had been in hospital 14 times previously with a comparable clinical course of cyclic vomiting and lethargy progressing to coma. Despite the numerous illnesses he remains intellectually normal. Earlier extensive evaluations, including amino acid chromatography of blood and urine, were unrevealing. The diagnosis was confirmed by gas-liquid chromatography according to the method described by Perry *et al.* (1970) (Figure 3). Plasma and urine were steam-distilled, neutralized, lyophilized and then reacidified with acetone–formic acid (9:1). The column composition was 12% diethylene glycol succinate and 2% phosphoric acid on chromosorb W (AC). The plasma IVA concentration was 84 mg/100 ml as compared to a normal value of zero. During the past eight years plasma IVA has ranged from less than 0.1 mg/100 ml in good health to between 10 and 61 mg/100 ml with illnesses. Diet has always been unrestricted and the family reports no problem with a breakfast consisting of a four-egg omelette, toast and a glass of milk when he is well.

A request for prenatal diagnosis was made shortly after the mother became pregnant for the fourth time.

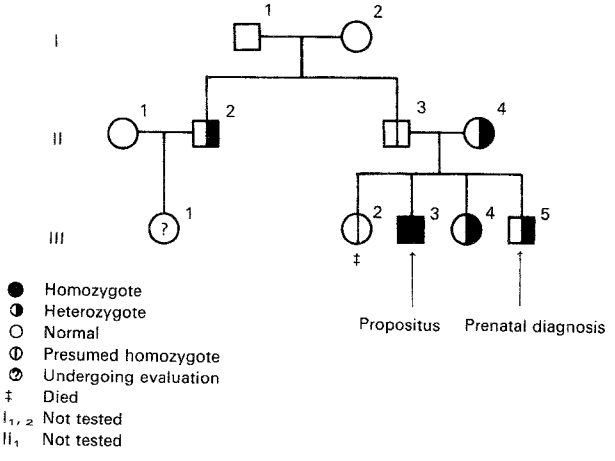

Figure 2 Isovaleric acidaemia family pedigree

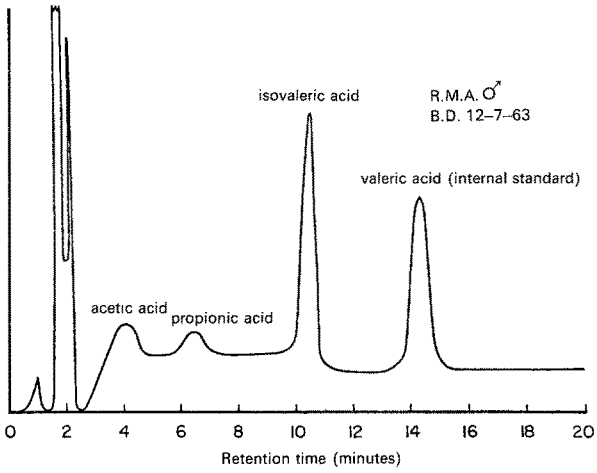

Figure 3 Gas liquid chromatograph isovaleric acidaemia

The need for total family involvement was discussed and arrangements were quickly made for the entire family to have skin biopsies for fibroblast cultures. The family served as controls for the fetus. The procedure used was minimally modified from Shih *et al.* (1973). The diagnostic method relied upon the fact that the number two carbon of L-leucine is obligatorily metabolized to CO_2 (Figure 4). The conversion of radioactively labelled L-leucine 2-^{14}C to radioactive CO_2 reflects the 'openness' of the metabolic pathway. L-Leucine-2-^{14}C (specific activity of 54 mCi/mmol) was purchased from Schwarz/Mann. The cultured cells, 0.5 to 1.5 million, were incubated with either 0.5 or 1.0 μCi of L-leucine-2-^{14}C in Krebs–Ringer bicarbonate buffer at pH 7.4, at a final glucose concentration of 100 mg/100 ml and a total volume of 0.22 ml. The incubation was done within a liquid scintillation vial utilizing a micro-CO_2 collecting system designed in our laboratory (Ng, 1976). After a 2–3 h incubation period 0.1 ml of H_2SO_4, 0.5 mol/l, was added to the incubation mixture and 1.0 ml hyamine injected into the enclosed vial. After an additional hour of $^{14}CO_2$ collection, 10 ml of toluene scintillator (Spectrafluor PPO-POPOP, Amersham/Searle) was added to the hyamine and radioactivity determined by liquid scintillation counting. The results are summarized in Table 1. The patient's fibroblasts liberated negligible quantities of $^{14}CO_2$. Both parents and the sibling S.A. produced half normal amounts of $^{14}CO_2$. These data support a recessive mode of inheritance and suggest the sibling is also a heterozygote.

Table 1 Diagnostic studies—isovaleric acidaemia. Production of $^{14}CO_2$ from leucine-2-^{14}C in skin cultured fibroblasts*

Name	*Relationship*	*$^{14}CO_2$ cpm/10^6 cells*		*$^{14}CO_2$ cpm/mg cell protein*	
		1 μCi Leu-2-^{14}C	*0.5 μCi Leu-2-^{14}C*	*1 μCi Leu-2-^{14}C*	*0.5 μCi Leu-2-^{14}C*
R.M.A.	Propositus†	65	negligible	348	negligible
S.A.	Sister	10778	5226	53658	25891
H.H.A.	Mother	11724	5649	60229	29307
G.A.	Father	17000	6617	77299	30078
Mrs R.	Normal control	26937	13594	96309	48602

* Incubation at 37 °C for two hours
Leucine-2-^{14}C, sp. activity of 54 mCi/mM
† Isovaleric acidaemia

Amniotic cells for culture were obtained during the 14th week of pregnancy according to the date of the mother's last menstrual period. The results of studies with these cells (Table 2) clearly indicated a non-affected fetus; however, although we suspected heterozygosity we could not be certain because of the wide variation in activity determined from our control cells. After birth, skin fibroblast studies (Table 2) confirmed that the infant is a carrier for the condition based upon less than half the normal production of $^{14}CO_2$. He is clinically normal as would be expected for known hetero-

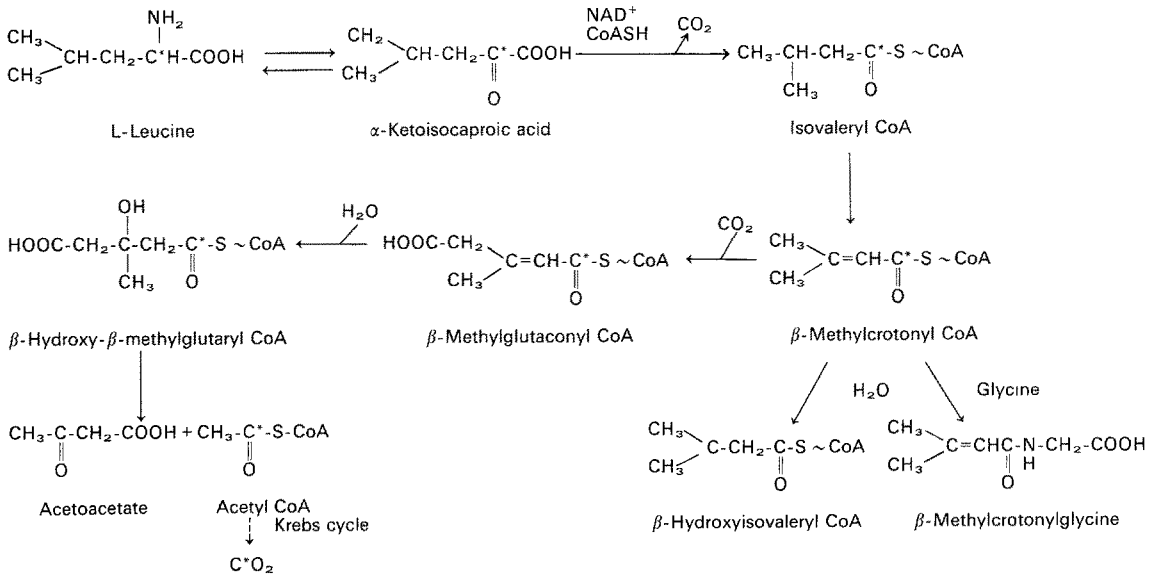

Figure 4 Leucine degradation pathway (Note: Asterisk indicates the labelled carbon atom in position 2)

Table 2 Isovaleric acidaemia—antenatal diagnosis. Leucine-2-^{14}C oxidation in cells derived from amniotic fluid and skin obtained postnatally

	Prenatal analysis	*Postnatal analysis*
	$^{14}CO_2$ *cpm/10^6 cells/3 h (1 μCi Leu-2-^{14}C)*	$^{14}CO_2$ *cpm/10^6 cells/2 h (0.5 μCi Leu-2-^{14}C)*
Fetus	50972	6561
Normal cultured amniotic cells—A	18291	—
Normal cultured amniotic cells—B	89820	—
Normal skin cultured fibroblasts	—	22315

zygotes in this family. The findings in our studies were confirmed independently in Dr Vivian Shih's laboratory in Boston, Massachusetts.

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