Acta Medica Okayama

Volume 24, *Issue* 1 1970 *Article* 9

FEBRUARY 1970

No evidence of isovalthinuria in isovaleric acidemia– brief note

H. Kodama* Y. Fujii†

S. Mizuhara‡ K. Tanaka**

*Okayama University,

†Okayama University,

‡Okayama University,

**Okayama University,

Acta Med. Okayama **24**, 107—109 (1969)

NO EVIDENCE OF ISOVALTHINURIA IN ISOVALERIC ACIDEMIA-BRIEF NOTE

H. KODAMA, Y. FUJII, S. MIZUHARA and K. TANAKA*

Department of Biochemistry, Okayama University Medical School, Okayama
** The Third Department of Internal Medicine, University of Tokyo Faculty of Medicine, Tokyo*

Received for publication, August 18, 1969

Isovaleric acidemia, an inborn error of leucine metabolism, has been characterized by a marked accumulation of isovaleric acid in serum and an increased urinary excretion of N-isovalerylglycine, and the genetic defect has been suggested to be at the level of isovaleryl CoA dehydrogenase (1, 2, 3).

On the one hand, the following facts have been found in Okayama. When isovaleric acid is administered to some normal animals, isovalthine (4) is excreted in their urine (5, 6). However, when isovaleric acid-1-C^{14} or -4-C^{14} is administered to some animals, isovalthine excreted has never been labeled (7, 8, 9).

In these circumstances, it is quite interesting to know whether the patient with isovaleric acidemia excretes isovalthine or not.

The urine samples of the patients tested are shown in Table I. The urine samples of each patient were combined separately and each sample (B. A.: 379 ml; S. A.: 275 ml) was made weakly acidic (around pH 5) with dilute hydrochloric acid and filtered. The filtrate was transferred on a column containing 100 ml of Diaion SK-1 (H-form of sulfonated cation

Table 1. Urine Samples of the Patients Tested.

Patients	Clinical State	24 Hour Urine Volume (ml)	Volume of Urine Used for Analysis (ml)	Dried Residue (gm)
B. A.	Coma	940	80	0.6
3 1/2 years old	Lethargy	760	260	2.0
	Remission	116	39	1.5
		1816	379	4.1
S. A.	Remission	625	100	2.0
5 1/4 years old	Remission	615	85	0.7
	Remission	160	90	0.4
		1400	275	3.1

exchanger, mesh 100, Mitsubishi Kasei Co., Tokyo) and the column was washed with 1 l of water. The effluent and washing were combined and evaporated to dryness under reduced pressure (Fraction IV). The column was then eluted with 800 ml of 2N-NH_3 and the ammonia eluate was dried under reduced pressure. The dried residue was dissolved in 100 ml of 0.2M acetic acid and filtered. The filtrate was transferred on a column containing 50 ml of Amberlite CG-4B (acetate form, mesh 100—200) and the column was washed with 500 ml of 0.2M acetic acid. The effluent and washing were combined and dried (Fraction I). The Amberlite column was then washed with each 500 ml of 2M acetic acid and 2N-HCl successively. The 2M acetic acid eluate (Fraction II) and 2N-HCl (Fraction III) were dried separately.

Fraction IV was hydrolyzed in 6N-HCl for 12 hrs, and the hydrolysate was dried, dissolved in 100 ml of water, and filtered. The free amino acids in the filtrate was collected by using Diaion SK-1 column as described above.

Fraction I contains mainly NH_2-free basic and neutral, Fraction II acidic, Fraction III strong acidic amino acids and peptides. Fraction IV is the hydrolysate of NH_2-covered amino acids and peptides. Isovalthine is usually found in Fraction II. In this experiment, however, all fractions of both samples were analyzed before and after hydrolysis on an automatic amino acid analyzer (Beckman Model 120-B, 150 cm column, 0.2M Na citrate buffer of pH 3.24 at 30°)[10] and by two dimensional paper electrophoresis.[11] The minimum quantity of isovalthine determined accurately by our amino acid analyzer is 0.1 μ mole and detected by paper electrophoresis 0.01 μ mole. As the results of these analyses, isovalthine was not found in any of the fractions tested.

Judging from our studies on experimental isovalthinuria, (5, 6) the serum isovaleric acid level of the patients, especially in the state of acidosis, (1) seems to be sufficient for the induction of isovalthinuria. Nevertheless, isovalthine was not detected even in the urine of an acidotic patient (B. A.)

The following considerations should be taken into account for the present result.

(1) The volume of urine tested might be too little to detect isovalthine. But 200 ml of urine was enough to estimate the isovalthine quantity in the studies on experimental isovalthinuria (5, 6, 12, 13).

(2) Isobutyric acid and some other lower fatty acids were known to disturb the isovalthinuria inducing effect of isovaleric acid (5). So the high content of isobutyric acid etc. in the serum of the patients 1 might have

disturbed the isovalthine formation.

(3) Isovaleric acid is further metabolized in the normal animals, but not in the isovaleric acidemics. So some metabolic changes of isovaleric acid in the animal body might be necessary for the induction of isovalthinuria. The fact that isovaleric acid-1-C^{14} or-4-C^{14} administered to some normal animals never incorporated into urinary isovalthine (7, 8, 9) might allow this assumption.

The mechanism of induction of isovalthinuria and the direct precursor of isovaleric acid residue in the isovalthine molecule are still unknown.

REFERENCES

1. Tanaka, K., Budd, M. A., Efron, M. L. and Isselbacher, K. J.: *Proc. Nat. Acad. Sci.* **56**, 236, 1966
2. Tanaka, K. and Isselbacher, K. J.: *J. Biol. Chem.* **242**, 2966, 1967
3. Budd, M. A., Tanaka, K., Holmes, L. B., Efron, M. L., Crawford, J. D. and Isselbacher, K. J.: *New Engl. J. Med.* **277**, 321, 1967
4. Ohmori, S. and Mizuhara, S.: *Arch. Biochem. Biophys.* **96**, 170, 1962
5. Fukutome, K.: *Acta Med. Okayama* **16**, 241, 1962
6. Shimomura, T.: *Acta Med. Okayama* **19**, 227, 1965
7. Fukutome, K.: *Acta Med. Okayama* **16**. 247, 1962
8. Ohmori, S., Ubuka, T., Shimomura, T., Azumi, T. and Horiuchi, K.: *Acta Med. Okayama* **18**, 351, 1964
9. Kodama, H., Azumi, T., Shimomura, T. and Fujii, Y.: *Acta Med, Okayama* **20**, 107, 1966
10. Ohmori, S.: *Arch. Biochem. Biophys.* **104**, 509, 1964
11. Ubuka, T.: *J. Biochem.* **52**, 440, 1962
12. Ubuka, T.: *Acta Med. Okayama* **17**, 273, 1963
13. Horiuchi, K.: *Clinica Chim. Acta* **12**, 325, 1965