

# *Acta Medica Okayama*

---

*Volume 23, Issue 6*

1969

*Article 4*

DECEMBER 1969

---

## Experimental isovalthinuria. VII. Experiments with radioactive acetate, valine, and leucine

Yoshio Fjii\*

\*Okayama University,

Copyright ©1999 OKAYAMA UNIVERSITY MEDICAL SCHOOL. All rights reserved.

# Experimental isovalthinuria. VII. Experiments with radioactive acetate, valine, and leucine\*

Yoshio Fjii

## Abstract

1. For the settlement of carbon origin of urinary isovalthine, acetic acid-2-C14, valine-U-C14 or leucine-U-C14 was administered to rats together with isovaleric acid as an isovalthinuria inducer, and urinary isovalthine excreted was tested by autoradiography. As the results of which, it was found that these isotopic compounds were not the precursor of urinary isovalthine. Although the isovalthinuria inducing effect of isovaleric acid was fairly diminished by these isotopic compounds, urinary isovalthine was detected by paper electrophoresis. 2. Some metabolic products of these isotopic compounds were also inquired in urine and some tissues. The results were as follows: a) Acetic acid incorporated into urea, aspartate, serine, glutamate, proline, glycine, alanine, ornithine, ethanolamine, r-amino-butyric acid (brain only), cholesterol and fatty acids. b) Valine incorporated into urinary glutamate and glycine, and tissue cholesterol and fatty acids. Valine was rapidly excreted in urine and found in a very small amount in liver digest. c) Leucine incorporated into urinary aspartate, serine, glutamate and glycine, and tissue cholesterol and fatty acids. 3. Several important problems of isovalthine studies to be elucidated were discussed.

---

\*PMID: 4246440 [PubMed - indexed for MEDLINE] Copyright ©OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama **23**, 497—503 (1969)

**EXPERIMENTAL ISOVALTHINURIA**  
**VII. EXPERIMENTS WITH RADIOACTIVE ACETATE,**  
**VALINE, AND LEUCINE**

Yoshio FUJII

*Department of Biochemistry, Okayama University Medical School, Okayama,  
Japan (Director: Prof. S. Mizuhara)*

*Received for publication, July 18, 1969*

Isovalthine is a new sulfur-containing amino acid found in the urine of some hypercholesterolemic patients and normal cat (1, 2). The method of induction of isovalthinuria has been studied extensively in some normal animals (3, 4, 5, 6). The sulfur atom of isovalthine has been found to originate from cysteine or methionine (7). However, the carbon origin of isovaleric acid residue in the isovalthine molecule has not been elucidated. Isovaleric acid is a strong inducer of isovalthinuria in some normal animals (3, 6), but isovaleric acid -1-C<sup>14</sup> or -4-C<sup>14</sup> administered has never incorporated into urinary isovalthine (8, 9, 10).

For the elucidation of the carbon origin of isovalthine, further experiments were carried out by using sodium acetate -2-C<sup>14</sup>, valine-U-C<sup>14</sup>, and leucine-U-C<sup>14</sup> to see if these radioactive compounds incorporate into urinary isovalthine. As reported in a previous paper (6), acetic acid, valine, or leucine was not an inducer of isovalthinuria by itself. So each labeled compound was administered with non-labeled isovaleric acid as an inducer. As the results of the present experiments, all isotopic compounds tested have incorporated into tissue protein, cholesterol, fatty acids and several urinary amino acids, but not into urinary isovalthine.

**MATERIALS AND METHODS**

Sodium acetate-2 C<sup>14</sup>, valine-U-C<sup>14</sup>, and leucine-U-C<sup>14</sup> used were the commercial products obtained from The Radioactive Center, Amersham, England.

The feeding of rats and the collection of urine were carried out by the same procedure as described in a previous paper (10). Body weight of each rat and volume of urine excreted were described in Table II. Each labeled compound was diluted with respective non-labeled material and orally administered together with sodium isovalerate (30 mg/rat/day) for 5 days. Each experimental group was consisted of two rats and the amount of labeled compound administered was as follows: sodium acetate-2-C<sup>14</sup>, 30 mg/rat/day (total 1 mc for two rats); valine-U-C<sup>14</sup>, 20 mg/rat/day (total 0.2 mc for two rats); leucine-U-C<sup>14</sup>, 20 mg/rat/day

(total 0.1 mc for two rats). After collecting the urine for 7 days, rats were killed by decapitation, and the liver, kidney and brain were removed. The extraction and the measurement of radioactivity of total tissue cholesterol and fatty acids were carried out by the same procedure as described in a previous paper (10). After extraction of lipids, each tissue digest was made weakly acidic with conc. HCl and transferred on a column containing Diaion SK-1 (H-form of sulfonated cation exchanger, mesh 100). The column was washed with deionized water and then eluted with 2N-NH<sub>3</sub>. The ammonia eluate was evaporated to dryness under reduced pressure, and the residue was stored for amino acid analysis.

The collected urine of each group was adjusted to around pH 5 with acetic acid and transferred to a column (A) containing Diaion SK-1(H-form, mesh 100) and the column was washed with deionized water. The effluent and washings were combined and dried under reduced pressure. The residue was hydrolyzed in 6N HCl for 20 hrs on a sand bath, and the hydrolysate was evaporated to dryness under reduced pressure. The amino acids in the residue (Fraction I) was collected by using another Diaion SK-1 column (B) according to the same procedure as described above.

Urinary NH<sub>2</sub>-free amino acids and peptides attached to the column (A) was eluted with 2N NH<sub>3</sub>. The ammonia eluate was dried and dissolved in 0.2M AcOH and filtered. The filtrate was transferred on a column containing Amberlite IR-4B (acetate form, mesh 100—200) and washed with 0.2M AcOH. The effluent and washing were combined and dried. The residue (Fraction II) contains mainly basic and neutral amino acids and peptides. The Amberlite column was then washed with 2M AcOH and 2N HCl in that order, and each effluent was dried. The 2M AcOH effluent (Fraction III) contains mainly acidic amino acids and peptides, and the 2N HCl effluent (Fraction IV) strong acidic amino acids and peptides.

The measurement of radioactivity and the quantitative determination of the tissue and urinary amino acids were carried out simultaneously by an automatic amino acid analyzer (Beckman Model 120 B) connected with Tri-Carb Flow Monitor System (Packard Instrument Co. Inc., Model 3141) according to their Manual.

## RESULTS

### 1. *Urinary Isovalthine*

Isovalthine was usually found in the Fraction III, and the same was true in this experiment. But the Fraction III of all three experimental groups have contained too small amount of isovalthine to be detected by amino acid analyzer (sensitivity 0.1  $\mu$  mole). On paper electrophoresis (sensitivity 0.01  $\mu$  mole), however, isovalthine was detected. So two dimensional paper electrophoretogram, which was prepared by the method of UBUKA (11), was exposed to autoradiography by the same procedure as described in a previous paper (7), and it was found that isovalthine was

not labeled in all the three groups. Thus the carbon of urinary isovalthinnne was not derived from acetic acid, valine, or leucine. As will be discussed later, acetic acid, valine, and leucine have diminished the isovalthinnria-inducing-effect of isovaleric acid.

## 2. *Amino Acids in Urine and Tissue Digest*

### (a) $\text{AcOH}\cdot 2\text{-C}^{14}$ + Isovaleric Acid Group

The labeling pattern of amino acids in urine and tissue digest is summarized in Table 1. Specific activities of alanine and glycine in the tissue digest could not be calculated accurately. Some ninhydrin negative compounds were detected to be labeled, but their nature was not characterized.

Table 1 Specific Activity (cpm/ $\mu$  mole) of Amino Acid, etc. in Urine and Tissue Digest. ( $\text{AcOH}\cdot 2\text{-C}^{14}$ +IVA Group)

	Urine	Liver	Kidney	Brain
Urea	5817			
Serine	+	152	262	73
Aspartic Acid	527			
Glutamic Acid	267	604	448	275
Proline	775			
Glycine	126	+	+	+
Alanine	262	+	+	+
Ornithine		185	185	101
Ethanolamine		98	98	56
$\gamma$ -Aminobutyric Acid				292

### (b) Valine- $\text{U-C}^{14}$ + Isovaleric Acid Group

Valine was the only labeled amino acid in tissue digest. In the Fraction I of urine, however, glutamic acid and glycine were found to be slightly labeled, and two unknown highly labeled ninhydrin negative compounds were seen near the peaks of aspartate and cystine. Some unknown labeled ninhydrin negative compounds were also found in urine and tissue digest.

Specific activity of valine was as follows: urine, 17774 cpm/ $\mu$  mole; kidney, 3601 cpm/ $\mu$  mole; brain, 83 cpm/ $\mu$  mole. Surprisingly, very small amount of valine was found in liver digest and the specific activity was not calculated. These results will suggest the rapid excretion of valine in this instance. This might be due to the effect of isovaleric acid administered simultaneously.

(c) Leucine-U- $C^{14}$ +Isovaleric Acid Group

The specific activity of leucine in urine and tissue digest was as follows: urine, 6410 cpm/ $\mu$  mole; liver, 963 cpm/ $\mu$  mole; kidney, 781 cpm/ $\mu$  mole; brain, 621 cpm/ $\mu$  mole.

Aspartic acid, serine, glutamic acid and glycine in the Fraction I of urine were found to be slightly labeled, but leucine was the only labeled amino acid in tissue digest. Some unknown labeled ninhydrin negative compounds were also found in urine and tissue digest.

3. *Tissue Cholesterol and Fatty Acids*

As shown in Table 2, the isotopic carbons of valine and leucine are used for the synthesis of cholesterol and fatty acids, though it seems less than that of acetic acid.

Judging from the amount of radioactivity used, leucine (0.1 mc) seems

Table 2 Incorporation of Radioactive Compounds into Tissue Lipids  
(a) AcOH-2- $C^{14}$ +IVA Group (Urine volume: 170 ml/two rats/7 days;  
Body weight of each rat: 185 & 180 gm)

Tissues	Tissue Weight (two rats)	Cholesterol		Fatty Acids	
		mg	cpm/mg	mg	cpm/mg
Liver	7.40	4.9	1263	75.1	1347
Kidney	2.25	6.7	2161	112.7	1091
Brain	3.35	7.0	715	171.2	563

(b) Val-U- $C^{14}$ +IVA Group (Urine volume: 210 ml/two rats/7 days;  
Body weight of each rat: 210 & 220 gm)

Tissues	Tissue Weight (two rats)	Cholesterol		Fatty Acids	
		mg	cpm/mg	mg	cpm/mg
Liver	10.85	14.7	248	286	64
Kidney	2.80	7.0	69	73.8	86
Brain	2.95	11.5	33	171.5	55

(c) Leu-U- $C^{14}$ +IVA Group (Urine volume: 165 ml/two rats/7 days;  
Body weight of each rat: 180 & 180 gm)

Tissues	Tissue Weight (two rats)	Cholesterol		Fatty Acids	
		mg	cpm/mg	mg	cpm/mg
Liver	9.6	5.3	125	123.4	137
Kidney	2.7	6.4	91	104.2	147
Brain	3.2	8.6	153	109.7	89

to be a better precursor for the synthesis of lipids than valine (0.2 mc). This may be due to the ketogenic character of leucine, though the rapid excretion of valine should also be taken into account.

#### DISCUSSION

One of the most important problems remaining in the isovalthine studies is the settlement of carbon origin of isovaleric acid residue in the isovalthine molecule. The following compounds, most of which are known to have intimate relation with cholesterol metabolism, have been found to induce isovalthineria in some normal animals (3, 4, 5, 6): isovaleric acid,  $\beta$ -methylcrotonic acid,  $\beta$ -methylglutaconic acid, palmitic acid, methionine, cysteine, bile acids,  $\Delta^4$ -cholestenone, some hypocholesterolemic agents, glucocorticoids, ACTH, and epinephrine. But these inducers are not always sure. Even when most strong inducer such as isovaleric acid, bile acid or glucocorticoid is administered, occasionally no isovalthineria is observed. Furthermore, some antagonistic phenomena are seen between some strong inducers. For example, the simultaneous administrations of the following combination never induce isovalthineria (6): isovaleric acid + dehydrocholic acid, isovaleric acid + dexamethasone, dehydrocholic acid + dexamethasone. It is also known that some non-inducers diminish or inhibit the isovalthineria-inducing effect of strong inducers (6). Thus the exact condition necessary for isovalthineria induction and the mechanism of induction are still ambiguous at present. Therefore, it is quite hazardous to select an isotopic compound tested for the settlement of carbon origin of isovalthine.

The first trial by using variously labeled isovaleric acid has failed as described in the preface. Isovaleric acid is the most sure and strong inducer, and isovalthine molecule is consisted of isovaleric acid and cysteine. Nevertheless, labeled isovaleric acid administered never incorporates into urinary isovalthine excreted. On the other hand, *in vitro* experiments, guinea-pig liver homogenate can synthesize labeled S-(isopropyl carboxymethyl) glutathione (GSIV) from labeled isovaleric acid and glutathione, and the cleavage of the labeled GSIV by kidney glutathionase gives labeled isovalthine (12, 13, 14). Thus, *in vitro* experiments, labeled isovaleric acid can be changed to labeled isovalthine. The discrepancy found between *in vivo* and *in vitro* experiments will serve a warning on the studies of metabolic pathway.

In the second trial described in this paper, labeled acetic acid, valine, and leucine are used, because they are known to give isovaleric skeleton

during their metabolism. Although these labeled compounds are non-inducers by themselves and diminish the inducing effect of isovaleric acid, a small amount of isovalthine is excreted and found to be not labeled in all cases. The isotopic carbon of these compounds, however, are found to incorporate into some other amino acids and lipids. Therefore, it may be concluded that acetic acid, valine, or leucine is not a precursor of isovalthine.

#### SUMMARY

1. For the settlement of carbon origin of urinary isovalthine, acetic acid- $2\text{-C}^{14}$ , valine- $\text{U-C}^{14}$  or leucine- $\text{U-C}^{14}$  was administered to rats together with isovaleric acid as an isovalthine inducer, and urinary isovalthine excreted was tested by autoradiography. As the results of which, it was found that these isotopic compounds were not the precursor of urinary isovalthine.

Although the isovalthine inducing effect of isovaleric acid was fairly diminished by these isotopic compounds, urinary isovalthine was detected by paper electrophoresis.

2. Some metabolic products of these isotopic compounds were also inquired in urine and some tissues. The results were as follows:

a) Acetic acid incorporated into urea, aspartate, serine, glutamate, proline, glycine, alanine, ornithine, ethanolamine,  $\gamma$ -amino-butyric acid (brain only), cholesterol and fatty acids.

b) Valine incorporated into urinary glutamate and glycine, and tissue cholesterol and fatty acids. Valine was rapidly excreted in urine and found in a very small amount in liver digest.

c) Leucine incorporated into urinary aspartate, serine, glutamate and glycine, and tissue cholesterol and fatty acids.

3. Several important problems of isovalthine studies to be elucidated were discussed.

#### REFERENCES

1. MIZUHARA, S. and OHMORI, S., *Arch. Biochem. Biophys.* **92**, 53, 1961
2. OHMORI, S. and MIZUHARA, S.: *Arch. Biochem. Biophys.* **96**, 179, 1962
3. FUKUTOME, K.: *Acta Med. Okayama* **16**, 241, 1962
4. UBUKA, T.: *Acta Med. Okayama* **17**, 273, 1963
5. HORIUCHI, K.: *Clin. Chim. Acta* **12**, 325, 1965
6. SHIMOMURA, T.: *Acta Med. Okayama* **19**, 227, 1965
7. UBUKA, T., HORIUCHI, K., SHIMOMURA, T. and MIZUHARA, S.: *Acta Med. Okayama* **18**, 65, 1964



8. FUKUTOME, K.: *Acta Med. Okayama* **16**, 247, 1962
9. OHMORI, S., UBUKA, T., SHIMOMURA, T., AZUMI, T. and HORIUCHI, K.: *Acta Med. Okayama* **18**, 351, 1964
10. KODAMA, K., AZUMI, T., SHIMOMURA, T. and FUJII, Y.: *Acta Med. Okayama* **20**, 107, 1966
11. UBUKA, T.: *J. Biochem.* **52**, 440, 1962
12. KUWAKI, T., OHMORI, S. and MIZUHARA, S.: *Biochim. Biophys. Acta* **78**, 553, 1963
13. KUWAKI, T.: *Acta Med. Okayama* **18**, 333, 1964
14. KUWAKI, T.: *J. Biochem.* **57**, 126, 1965