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Abstract

FUKUTOME has once reported that isovaleric acid is an isovalthinuria inducer but isovaleric acid-1-C14 administered to a dog does not incorporate into urinary isovalthine and glutamic acid is most strongly labeled among acidic amino acids excreted. Recently, however, KUWAKI has found that liver homogenates of some animals can synthesize C14-labeled S-(isopropylcarboxymethyl) glutathione (GSIV) from isovaleric acid-1-C14 and glutathione, and that GSIV can be converted into isovalthine by kidney homogenate or glutathionase preparation4. For the elucidation of the above discrepancy, FUKUTOME's experiments were repeated by using isovaleric acid-methyl-C14 or-1-C14, and it was again found that these isotopic compounds did not significantly incorporate into urinary isovalthine.

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BRIEF NOTE

ON THE CARBON ORIGIN OF ISOVALERIC ACID RESIDUE OF URINARY ISOVALTHINE*

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FUKUTOME[1, 2] has once reported that isovaleric acid is an isovalthinuria inducer but isovaleric acid-1-C^{14} administered to a dog does not incorporate into urinary isovalthine and glutamic acid is most strongly labeled among acidic amino acids excreted. Recently, however, KUWAKI[3] has found that liver homogenates of some animals can synthesize C^{14}-labeled S-(isopropylcarboxymethyl) glutathione (GSIV) from isovaleric acid-1-C^{14} and glutathione, and that GSIV can be converted into isovalthine by kidney homogenate or glutathionase preparation[4]. For the elucidation of the above discrepancy, FUKUTOME's experiments were repeated by using isovaleric acid-methyl-C^{14} or-1-C^{14}, and it was again found that these isotopic compounds did not significantly incorporate into urinary isovalthine.

Isovaleric acid-methyl-C^{14} was synthesized according to the method of ZABIN and BLOCH[5] by using 11.3 g of ethyl cyanoacetate and 1 mc of acetone-1, 3-C^{14} diluted with 5 ml of cold acetone. The yield of methyl-labeled isovaleric acid was 5.7 g and it showed 9.9×10^4 cpm/mg.

Isovaleric acid-1-C^{14} was synthesized according to the method of LESLIE[6] by using 822 mg of isobutylbromide and 2 mc of sodium carbonate-C^{14} (80 mg) diluted with 35 mg of cold sodium carbonate. Two ml of cold isovaleric acid were mixed into the above reaction products and distilled. The yield of carboxyl-labeled isovaleric acid was 1.7 g and it showed 7.9×10^5 cpm/mg.

Two groups each consisting of two male guinea pigs weighing about 400 g received per os 50 mg of each isotopic isovaleric acid per animal per day for 6 days. Basal diet, conditioning of room temperature, urine collection, and preparation of urinary acidic amino acid fractions were essentially the same as

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described in a previous paper[7].

The urine of each group was collected for seven days after the start of administration, and urinary isovalthine in the acidic amino acid fraction was determined and collected at the same time by using preparative column (1.8× 150 cm) of an automatic amino acid analyzer (Beckman Model 120 B) provided with stream divider accessory. The isovalthine containing fractions collected in a fraction collecter were mixed, deionized by passing a sulfonated resin column, dried in stainless planchets, and counted in a gas-flow counter. Results obtained are summarized in Table 1.

Table 1

C^{14}-Isovaleric Acid administered			Urinary Isovalthine	
	cpm/mg	total dose (mg)	total amounts excreted (mg)	cpm/mg
methyl-C^{14}	9.9×10^4	600	1.99	11.7
carboxyl-C^{14}	7.9×10^5	600	11.40	19.4

The specific activities of urinary isovalthine obtained from two groups were about one thousandth and one ten-thousandth of that of the original isotopic isovaleric acid administered. Therefore, it should be considered that the isovaleric acid administered was only an isovalthinuria-inducer and did not directly incorporate into urinary isovalthine. Studies on the carbon origin of isovaleric acid residue in the urinary isovalthine molecule will be continued further.

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