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Metabolic fates of isovaleric acid and isovalthine in rats

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Abstract

1. Isovaleric acid-1-C14, -4-C14, or C14-CaCO₃ with or without non-isotopic isovaleric acid was orally administered to rats and the incorporation of these isotopes into liver cholesterol, fatty acid, or urinary isovalthine was examined. 2. Isopropyl group of isovaleric acid was more efficiently utilized for cholesterol synthesis than carboxyl group, and also for cholesterol synthesis than for fatty acid. These results indicate that isovaleric acid is cleaved into two fragments before it is utilized for cholesterol synthesis. 3. Carbon dioxide was used for the synthesis of liver cholesterol and of liver fatty acid. Isovaleric acid seems to enhance the incorporation of carbon dioxide into cholesterol. 4. All the experimental rats received isotopic or non-isotopic isovaleric acid excreted isovalthine, but no radioactivity was found in it. Thus, isovaleric acid residue of urinary isovalthine molecule is not derived from isovaleric acid administered, and carbon dioxide is not the carbon source of urinary isovalthine. 5. Suspicious metabolism of isovaleric acid or of carbon dioxide was discussed. 6. Isotopic isovalthine which was synthesized from ($\pm$) α -bromoisovaleric acid-4-C14 is administered to rat and it was found that the isotope did not incorporate into cholesterol or fatty acid of liver and of brain. About 15% of isotopic isovalthine was recovered in urine up to the next day after injection. The large part of isovalthine was missing.

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METABOLIC FATES OF ISOVALERIC ACID AND ISOVALTHINE IN RATS

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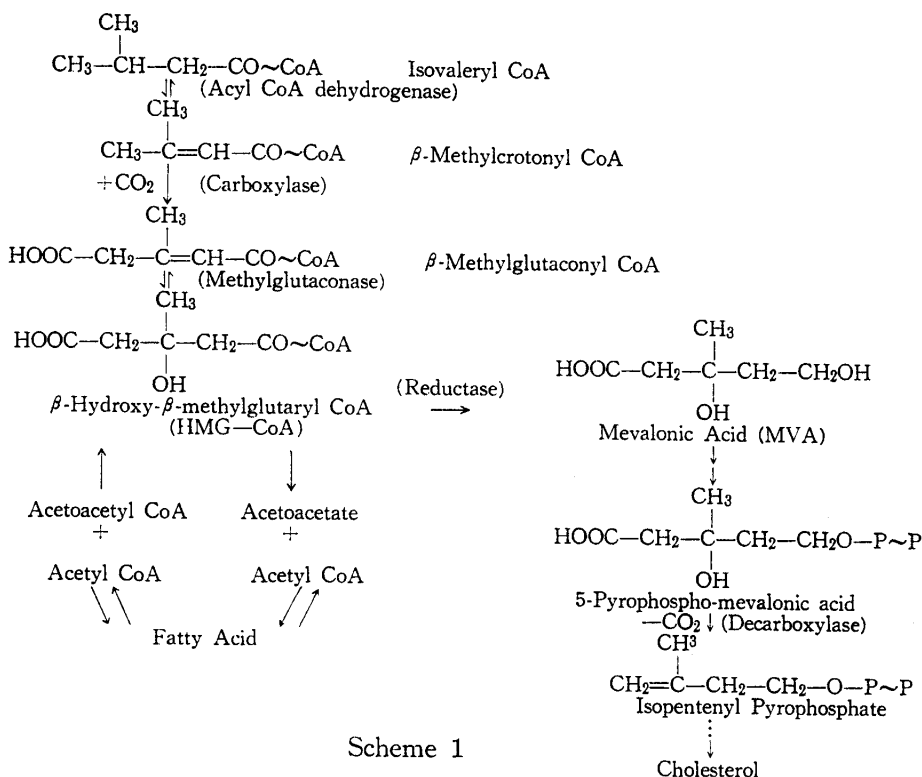
Isovaleric acid is known to be a precursor of cholesterol biosynthesis¹⁻³. ZABIN and BLOCH^{2, 4} have reported that isopropyl group of isovaleric acid incorporates more efficiently into cholesterol than its carboxyl group, and that the incorporation of carbon dioxide into cholesterol is more markedly increased in the presence of isovaleric acid than in its absence. Isovaleric acid is also known to be cleaved into acetoacetate and acetyl CoA⁵, and these metabolic pathways have now been summarized generally as shown in the scheme 1.

If HMG-CoA derived from isovaleryl CoA is considered to incorporate directly into cholesterol according to the scheme 1, the incorporation efficiency of isopropyl group and of carboxyl group in the isovaleric acid molecule should be almost equal. Furthermore, the carbon dioxide which is fixed by the β -methylcrotonyl CoA carboxylase reaction (EC 6. 4. 1. 4) should be eliminated by the 5-pyrophosphomevalonate decarboxylase reaction (EC 4. 1. 1. 33) and should never incorporate into cholesterol. These considerations appear to be not in accord with the experimental results of ZABIN and BLOCH^{2, 4}.

In addition to the above obscurities on the isovalerate metabolism, the following curious phenomena have been found in this laboratory:

1. Isovaleric acid is a strong inducer of isovalthinuria, but isovaleric acid-1-C¹⁴ or 4-C¹⁴ administered does not incorporate into urinary isovalthine in guinea pigs^{6, 7}. 2. Although isovalthine is not cleaved by amino acid oxidase⁸ or by cystathionase⁹, isovalthine administered to some animals is recovered only 40% in urine and biliary excretion of isovalthine is quite small.

Under these circumstances, the experiments of ZABIN and BLOCH^{2, 4} are retested by using isovaleric acid-1-C¹⁴, -4-C¹⁴, or C¹⁴-CO₂ in rats to see if these isotopic carbons incorporate into liver cholesterol, liver fatty acids, and urinary isovalthine. And the fate of C¹⁴-isovalthine administered to rats is also examined.



MATERIALS AND METHODS

Isovaleric acid-1- C^{14} and -4- C^{14} were synthesized as described in a previous paper⁷, and diluted with non-isotopic isovaleric acid. The final specific activity of sodium isovalerate-1- C^{14} used was 1.9×10^5 cpm/mg, and that of -4- C^{14} was 2.6×10^4 cpm/mg. Calcium carbonate- C^{14} was prepared from commercial sodium carbonate- C^{14} by mixing calcium chloride, and its final specific activity was 1.23×10^4 cpm/mg. Isovalthine- C^{14} was synthesized from ($\pm$)- α -bromoisovaleric acid-4- C^{14} and L-cysteine according to the method reported elsewhere¹⁰. The final specific activity of C^{14} -isovalthine used was 8.1×10^4 cpm/mg.

Each experimental group consisting of two male rats in a metabolic cage was fed on MF solid food (Oriental Yeast Inc., Tokyo). The urine was collected in a bottle containing toluene and hydrochloric acid. Temperature of the animal room was kept at around 23°C during these experiments.

Sodium isovalerate-1- C^{14} was administered per os 95 mg/rat/day for three days, and -4- C^{14} 90 mg/rat/day for three days. Calcium carbonate- C^{14} was administered per os 100 mg/rat/day for two days in one group, and another group received 125 mg non-isotopic sodium isovalerate besides calcium carbonate-

C¹⁴. Isovalthine-C¹⁴ was injected intraperitoneally 50 mg/rat/day for two days.

Isovalthine-containing fraction of urine was prepared as reported previously¹¹. The fraction was developed two dimensionally on filter paper according to the method of UBUKA¹², and the radioactivity of isovalthine was examined by autoradiography as described previously¹³.

Rats were killed by decapitation on the next day after the last isotope administration, and the liver cholesterol and fatty acids were prepared by the usual method as follows. The alcoholic-KOH digest of liver was extracted with petroleum ether by means of Soxhlet apparatus, and digitonin precipitate of the extract was dissolved in pyridine and then extracted again with petroleum ether. Free cholesterol thus obtained was counted by a gas-flow counter. The residual alcoholic KOH digest of the liver was acidified with sulfuric acid, and fatty acid was extracted with petroleum ether by means of Soxhlet. The extract was dried and counted.

RESULTS AND DISCUSSION

I. Incorporation of Isotopic Isovaleric Acid and Carbon Dioxide into Liver Cholesterol and Fatty Acids

In accord with the report of ZABIN and BLOCH², the present results shown in Table 1 (a) and (b) indicate that isopropyl group of isovaleric acid is more efficiently utilized for cholesterol synthesis than its carboxyl group suggesting the preliminary cleavage of isovaleric acid into two fragments on the occasion of its conversion to cholesterol, and that isopropyl group of isovaleric acid is more efficiently utilized for the synthesis of cholesterol than for that of fatty acid. In another words, these data are suggesting of the different metabolic fate of isopropyl group and of carboxyl group.

Table 1 (c) shows that carbon dioxide incorporates into liver cholesterol and fatty acids. It seems, though it cannot be said with confidence by these data, that incorporation of carbon dioxide into liver cholesterol is enhanced by isovaleric acid administered simultaneously.

In the present knowledge of biochemistry, only possible pathway in which CO₂ is fixed into acetyl CoA may be the reaction of acetoacetate formation from HMG-CoA which is derived from isovaleryl CoA by the reaction sequences of acyl CoA dehydrogenase (EC 1. 3. 2. 2), β -methylcrotonyl CoA carboxylase (EC 6. 4. 1. 4), and β -methylglutaconase (EC 4. 2. 1. 18). The formation of acetoacetate-1-C¹⁴ from isovaleric acid and CO₂-C¹⁴ is indeed demonstrated by rat liver slices¹⁴, and acetoacetate is reported to be utilized, though less efficiently than acetate or isovalerate, for cholesterol synthesis by rat liver slices without preliminary formation of two carbon fragments³. Liver is well known to be unable

Table 1
(a) Sodium isovalerate-1-C¹⁴ (1.9×10^5 cpm/mg) 95mg/rat/day for 3 days

Rats	Body Weight	Liver Weight	Liver Cholesterol		Liver Fatty Acids	
	gm	gm	mg	cpm/mg	mg	cpm/mg
1a	165	} 9.1	5.2	277	212	225
1b	130					
2a	145	} 8.5	6.4	787	346	625
2b	140					

(b) Sodium isovalerate-4-C¹⁴ (2.6×10^4 cpm/mg) 90mg/rat/day for 3 days

Rats	Body Weight	Liver Weight	Liver Cholesterol		Liver Fatty Acids	
	gm	gm	mg	cpm/mg	mg	cpm/mg
1a	170	} 9.8	3.22	905	53	395
1b	170					
2a	180	} 10.5	3.36	1510	38	525
2b	140					
3a	180	} 13.4	6.30	580	183	218
3b	192					

(c) Calcium carbonate-C¹⁴ (1.23×10^4 cpm/mg) 100mg/rat/day for 2 days

Rats	Body Weight	Liver Weight	Liver Cholesterol		Liver Fatty Acids	
	gm	gm	mg	cpm/mg	mg	cpm/mg
1a } without	190	} 8.5	6.7	158	81	104
1b } isovalerate	140					
2a } with	175	} 8.2	2.5	216	49	108
2b } isovalerate 125mg/rat/day	180					

to cleave acetoacetate. Then it should be considered that an active form of acetoacetate formed from HMG-CoA is directly utilized for the formation of cholesterol or fatty acid. But there still remains a question why HMG-CoA formed from isovalerate is not utilized directly for the synthesis of MVA. As described in the preface, if HMG-CoA derived from isovalerate is considered to be utilized directly for cholesterol synthesis, CO₂ fixed into β -methylcrotonyl CoA should be eliminated by 5-pyrophosphomevalonate decarboxylase reaction. Then it follows that liver is making vain efforts for HMG-CoA synthesis in such a manner that HMG-CoA formed from isovalerate is once cleaved and again resynthesized. If this vain effort is considered to be actually occurring, isovalerate may be expected to be utilized for cholesterol synthesis less efficiently than acetate as shown in vitro system by BRADY and GURIN³. But the in vivo experiment of ZABIN and BLOCH² is opposed to the above expectation.

If an active acetoacetate formed from isovalerate via HMG-CoA is also utilized directly for fatty acid synthesis, then it should be considered that CO_2 -incorporation into fatty acid is also enhanced by isovalerate administered simultaneously. But the data of Table 1 (c) do not seem to support the above consideration. Furthermore, the data of ZABIN and BLOCH⁴ appear to indicate that $\text{CO}_2\text{-C}^{14}$ administered with isovalerate is less utilized for the synthesis of fatty acid than for that of cholesterol. But this might be due to the different pool size between fatty acid and cholesterol.

Under these circumstances, another possibility could not be ruled out, as proposed by COON¹⁴, that an active form of acetone derived directly from isovaleryl CoA or β -methylcrotonyl CoA might be the actual substrate for the carboxylation. In this connection, it is worthy of notice that β -methylcrotonate and isovalerate show their similar conversion efficiencies in steroid synthesis and are the most efficient sterol precursors among the branched chain acids tested such as HMG, or β -methylglutaconate, or β -hydroxyisovalerate¹⁵.

Isovalthine was found to be excreted in all the experimental groups of (a) and (b) in Table 1, but no radioactivity was observed on autoradiograms. Thus it is again confirmed that isovaleric acid residue of isovalthine molecule is not derived from isovaleric acid administered. One group of rats which received $\text{CO}_2\text{-C}^{14}$ and non-isotopic isovaleric acid (Table 1 (c), 2a and 2b) also excreted isovalthine, but radioactivity was not found in it. Thus carbon dioxide is not a carbon source of urinary isovalthine. It should be added here that isovalthineuria is induced in guinea pigs by the administration of isovalerate, β -methylcrotonate, or β -methylglutaconate, but not by that of HMG, acetoacetate, or acetate¹⁶. Thus the metabolic pathway of isovalerate via HMG seems to be somewhat doubtful.

Further investigation will be needed for the elucidation of exact metabolism of isovaleric acid.

II. Fate of Isotopic Isovalthine

One rat is used for this experiments and brain lipids are also examined in this case.

As shown in Table 2, isovaleric acid residue of isovalthine molecule is not utilized for the synthesis of cholesterol and fatty acids in rat liver and brain.

Table 2
 C^{14} -isovalthine (8.4×10^4 cpm/mg) 50mg/rat/day for two days (intraperitoneal)

	Fresh Weight	Cholesterol		Fatty Acids	
	gm	mg	cpm/mg	mg	cpm/mg
Liver	5.6	6.1	0~2	124	6~10
Brain	1.6	3.3	4~7	47	2~3

Urinary isovalthine was determined on an automatic amino acid analyzer according to the procedure reported previously¹¹, and it was found that only 15% of isovalthine administered was recovered in urine. Urinary isovalthine was purified and counted by the same procedure described before³ and its specific activity was found to be almost equal to that of isovalthine-C¹⁴ administered.

By using guinea-pigs or rabbits, unpublished data of this laboratory showed that usually around 35% of isovalthine administered intraperitoneally was recovered in urine on the next day after injection and a few per cent on the second day. When rabbits having bile fistula received isovalthine via ear vein, around 35% of isovalthine was recovered in urine on the next day after injection but trace amount in bile. Thus the large part of isovalthine administered is missing. It has been reported that isovalthine is neither affected by amino acid oxidase⁸ nor by cystathionase⁹. Therefore the exact metabolic fate of isovalthine is still unknown.

SUMMARY

1. Isovaleric acid-1-C¹⁴, -4-C¹⁴, or C¹⁴-CaCO₃ with or without non-isotopic isovaleric acid was orally administered to rats and the incorporation of these isotopes into liver cholesterol, fatty acid, or urinary isovalthine was examined.

2. Isopropyl group of isovaleric acid was more efficiently utilized for cholesterol synthesis than carboxyl group, and also for cholesterol synthesis than for fatty acid. These results indicate that isovaleric acid is cleaved into two fragments before it is utilized for cholesterol synthesis.

3. Carbon dioxide was used for the synthesis of liver cholesterol and of liver fatty acid. Isovaleric acid seems to enhance the incorporation of carbon dioxide into cholesterol.

4. All the experimental rats received isotopic or non-isotopic isovaleric acid excreted isovalthine, but no radioactivity was found in it. Thus, isovaleric acid residue of urinary isovalthine molecule is not derived from isovaleric acid administered, and carbon dioxide is not the carbon source of urinary isovalthine.

5. Suspicious metabolism of isovaleric acid or of carbon dioxide was discussed.

6. Isotopic isovalthine which was synthesized from ($\pm$)- α -bromoisovaleric acid-4-C¹⁴ is administered to rat and it was found that the isotope did not incorporate into cholesterol or fatty acid of liver and of brain. About 15% of isotopic isovalthine was recovered in urine up to the next day after injection. The large part of isovalthine was missing.

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