Title
THE UTILIZATION OF THE BRANCHED CHAIN OF ISOBUTYRIC ACID STUDIED WITH C14

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Irving Gray, Patricia Adams and Heinrich Hauptmann

June 9, 1950

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Abstract

THE UTILIZATION OF THE BRANCHED CHAIN OF ISOBUTYRIC ACID STUDIED WITH C^{14}

Irving Gray*, Patricia Adams and Heinrich Hauptmann**

Radiation Laboratory and Department of Chemistry,
University of California, Berkeley+

ABSTRACT

June 9, 1950

A mechanism for the breakdown of isobutyric acid in rats is proposed based on the fate of the methyl groups of the branched chain traced by means of the compound labeled in the carboxyl group and methyl groups with C^{14}.

---

* Major, Medical Service Corps, United States Army.

** Rockefeller Fellow. While on leave from the Faculdade de Filosofía, Ciéncias e Letras, Universidade de São Paulo, São Paulo, Brasil.

+ The work described in this paper was sponsored by the Atomic Energy Commission.

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Recent work has shown that isocaproic and isobutyric acids are degraded *in vitro* by kidney or liver enzyme preparations following the classic scheme of β-oxidation. This has been accomplished by manometric measurements of oxygen uptake (1) and by counter-current distribution separation of the reaction products (2). A mechanism for the formation of propionic acid from isobutyric acid has been proposed (2). On the other hand, Coon and Gurin (3) have reported that leucine is first degraded to isovaleric acid and then β-oxidized to acetic acid and a 3-carbon fragment. It seemed of interest to investigate the behavior of these compounds *in vivo* by the application of radioactive tracer techniques. This paper is a report on experiments carried out with carboxyl- and methyl-labeled isobutyric acid.

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EXPERIMENTAL

Sodium isobutyrate-1-C^{14} was prepared by the reaction of isopropylmagnesium bromide with $C^{14}O_2$ following the directions of Calvin and coworkers (4) for the preparation of acetic acid-1-C^{14}. The sodium isobutyrate-3-C^{14} was prepared in a similar manner using methyl-labeled isopropyl bromide and inactive CO_2. The specific activity of the sodium isobutyrate-1-C^{14} was 1.72 μc/mg., and that of the sodium isobutyrate-3-C^{14} was 1.50 μc/mg. The yield for the carboxyl-labeled material was 97.5% based on the radioactive barium carbonate employed++. The yield for the methyl-labeled material was 48% based on the isopropyl bromide. The preparations were carried out on a 20 mmole scale.

The sodium salt of the acid, ca. 0.075 mg./g. body weight, was injected intraperitoneally into a 200 g. (Curtis-Dunning strain) rat that had been fasted for twenty-four hours previously. The animal was immediately placed in a metabolism cage, and the expired carbon dioxide,

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++ One μc = 2.20×10^6 dis./min. The counters used were calibrated using accurately standardized barium carbonate prepared by the Oak Ridge National Laboratories. The efficiency of the counters used was about 5%.

(1) A.L. Graffin and D.E. Green, J. Biol. Chem. 176, 95 (1948).

(2) W.A. Atchley, J. Biol. Chem., 176, 123 (1948).

(3) M.J. Coon and S. Gurin, J. Biol. Chem., 180, 1159 (1949).

(4) M. Calvin, C. Heidelberger, J.C. Reid, B.M. Tolbert and P.E. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, 1949.

feces and urine collected. This carbon dioxide was collected at specified time intervals in 1 N sodium hydroxide and converted to barium carbonate. The specific activity of the barium carbonate was determined according to the method of Yankwich, et.al. (5,6). Geiger-Müeller or "Nucleometer" (a windowless proportional counter) counters were used, depending on the specific activities being measured.

After five hours the animal was sacrificed. The liver was removed and ground in a glass mortar with sand until very finely divided. The ground mass was fractionated to obtain the total lipid, amino acid and protein, and glycogen.

DISCUSSION

The results of these experiments are summarized in the accompanying figures and tables. The curves for the rate of elimination of C^{14} as $C^{14}O_2$ are shown in Fig. 1. The amount eliminated after five hours approaches a value of 80-85% for the carboxyl-labeled compound and 45-50% for the methyl-labeled compound. In the case of the carboxyl-labeled isobutyrate, the final amount and the general shape of curve "A" is similar to the curves observed by Jones and co-workers (7) for the straight chain fatty acids. Based upon the amount of activity of the isobutyrate injected, the ratio of the specific activities of the excreted $C^{14}O_2$ should

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(5) P.E. Yankwich, Science, 107, 681 (1948).

(6) P.E. Yankwich and J.W. Weigl, Science, 107, 651 (1948).

(7) H.B. Jones, personal communication.

be 1.82. We see from Table I that the ratio is 1.76.

There is, however, a definite difference in the initial rates of excretion of labeled carbon dioxide in the breath of the animals injected with sodium isobutyrate-1-C^{14} and sodium isobutyrate-3-C^{14}, that of the animal injected with the isobutyrate-1-C^{14} being the higher.

The difference in these rates together with the amount of radioactivity incorporated into the fraction of the liver leads us to believe that the isobutyrate is degraded to CO_2 and a 3-carbon fragment. This degradation may proceed in either of two ways. The first involves a direct decarboxylation to CO_2 and a 3-carbon fragment, in this case acetone. The second involves β-oxidation to a malonic acid derivative followed by decarboxylation to give CO_2 and a 3-carbon fragment, propionic acid.

If the latter were the major reaction, the propionic acid formed from the carboxyl-labeled isobutyrate would have one-half the activity of that formed from the methyl-labeled material, since it has been shown (8) that propionate is a direct precursor of liver glycogen. The glycogen formed in the liver of the animal given the carboxyl-labeled isobutyrate should have one-half the activity of that from the animal given the methyl-labeled isobutyrate. From the data in Table I we see that the reverse is true.

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(8) J.M. Buchanan, A.B. Hastings and F.B. Nesbett, J. Biol. Chem., 150, 413 (1943).

Table I

C^{14} Content of Tissues and Liver Fractions of Rats Injected with Sodium Isobutyrate-1-C^{14} and Sodium Isobutyrate-3-C^{14}

Fraction	Compound Injected**			
	Sodium Isobutyrate-1-C^{14}		Sodium Isobutyrate-3-C^{14}	
	Specific Activity dis/min/mg. dry tissue	% of Injected Dose	Specific Activity dis/min/mg. dry tissue	% of Injected Dose
Breath	3700.	87.	2100.	47.
Urine	10170.	1.48	3350.	0.25
Total lipid of liver	186.	0.11	960.	0.4
Total fatty acids of liver	--*	--*	650.	0.3
Non-saponifiable	--*	--*	650.	0.03
Liver protein	110.	0.32	562.	1.41
Liver glycogen	926.	0.65	300.	0.07

* The specific activity was so low as to make these values insignificant.

** These results are the average of three rats for each compound. Individuals did not vary more than 5% from the mean.

Assuming direct decarboxylation, all $C^{14}O_2$ excreted in the breath of the animal injected with methyl-labeled isobutyrate would result from further oxidation of the 3-carbon fragment. Since acetone is a symmetrical molecule, half of the CO_2 would be expected to show radioactivity. Actually, 87% of the radioactivity of the carboxyl-labeled isobutyrate and 47% of the methyl-labeled isobutyrate was excreted in the breath. We assume, then, that the remainder, 13% and 3%, respectively, goes to the metabolic pool. It has been shown that CO_2 is a precursor of both liver glycogen (8,9,10) and urea (11,12). Since the ratio of the $C^{14}O_2$ available in these two cases is 13:3 or 4.3, we should expect that the ratio of the specific activities of the liver glycogen and the urea would be about the same. We see from the data in Table I that the ratios are 3.09 and 3.03, respectively. The deviation of the value of 3.09 from the theoretical value may be attributed to the incorporation of radioactive pyruvate (see below) into the liver glycogen. We cannot, at present, explain the slight deviation of the value for urea from the theoretical.

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(9) A.K. Solomon, B. Vennesland, F.W.Klemperer, J.M. Buchanan and A.B. Hastings, J. Biol. Chem., 140, 171 (1941).

(10) B. Vennesland, A.K. Solomon, J.M. Buchanan and A.B. Hastings, J. Biol. Chem., 142, 379 (1942).

(11) D. Rittenberg and H. Waelsch, J. Biol. Chem. 136, 799 (1940).

(12) E.A. Evans, Jr. and L. Slotin, J. Biol. Chem. 136, 805 (1940).

To carry the idea of direct decarboxylation further, the acetone formed from carboxyl-labeled isobutyrate would have no activity, whereas that from the methyl-labeled isobutyrate would be labeled in the methyl group. This compound is further oxidized through pyruvate (carboxyl and β-labeled) to acetic acid and CO_2. One-half the activity is now in the acetic acid and one-half is in the CO_2 (see above). As this $C^{14}O_2$ is produced, we get the delayed rise in the curve for the excretion of $C^{14}O_2$ in the breath of the animal injected with the methyl-labeled isobutyrate. One-half of the acetic acid formed in the animal given the methyl-labeled isobutyrate was itself labeled in the methyl group. This accounts for the activity of the fatty acids (13,14) and sterols (14,17) from the liver of this animal, whereas these components from the liver of the animal given the carboxyl-labeled isobutyrate contained an insignificant amount. The same reasoning leads to an explanation of the radioactivity of the liver protein. Greenberg and Winnick (15) found that the protein of animals fed methyl- or carboxyl-labeled acetic acid contained more activity than those fed radioactive bicarbonate. The difference in radioactivity which we report is greater than reported by these investigators, but this can again be attributed to the incorporation of pyruvate (16).

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(13) D. Rittenberg and K. Bloch, J. Biol. Chem., 160, 417 (1945).

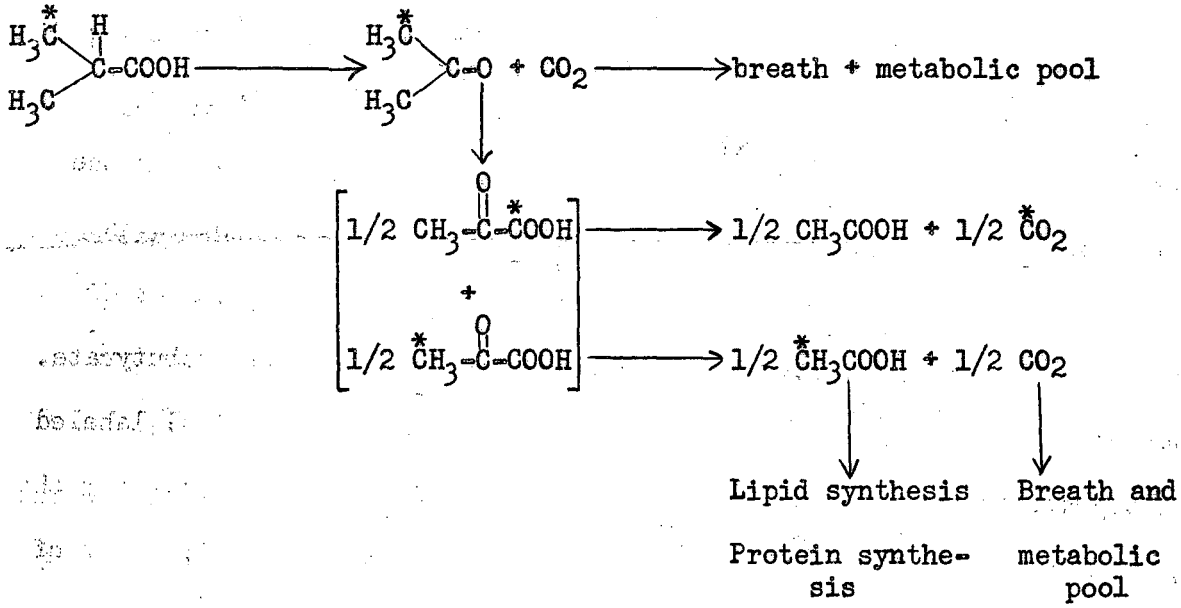
(14) Konrad Bloch, Physiol. Rev., 27, 574 (1947).

(15) D.M. Greenberg and T. Winnick, Arch. Biochem., 21, 166 (1949).

(16) E. Baldwin, "Dynamic Aspects of Biochemistry", Macmillan Co., New York, 1947.

(17) E. Borek and D. Rittenberg, J. Biol. Chem., 179, 843 (1949).

Based upon the above discussion, we postulate the following reaction scheme:



Acknowledgement: We wish to express our thanks to Prof. Melvin Calvin for his kind interest in this work.

SUMMARY

A mechanism for the breakdown of isobutyric acid in rats is proposed based on the fate of the methyl groups of the branched chain traced by means of the compound labeled in the carboxyl group and methyl groups with C^{14}.

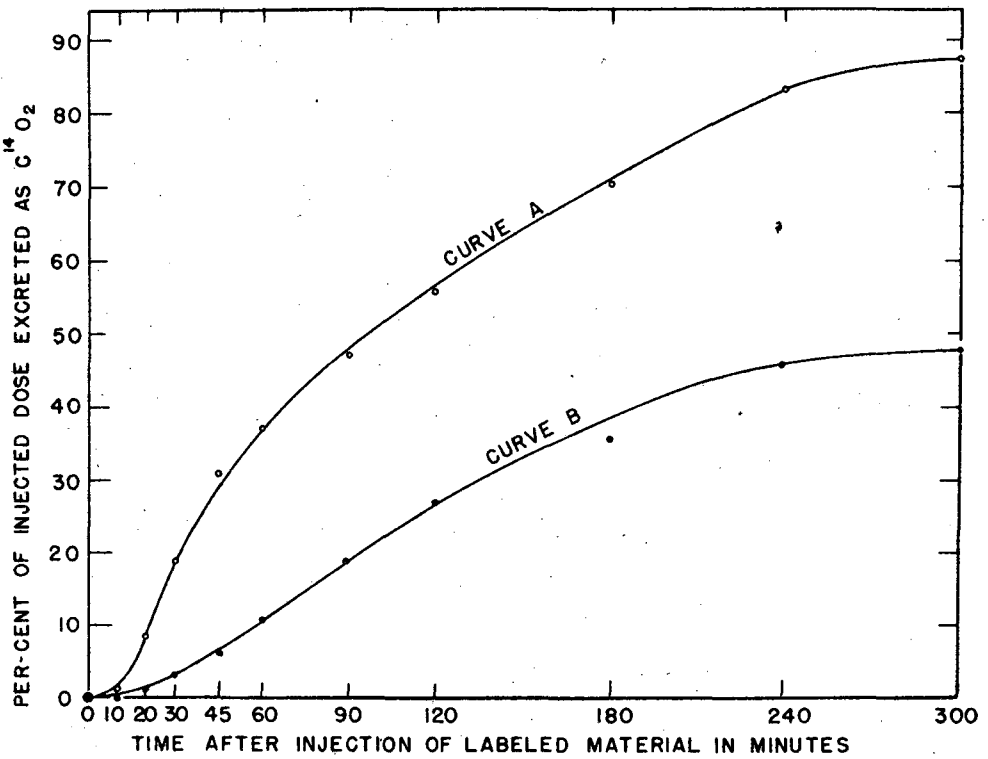

PER-CENT OF INJECTED DOSE EXCRETED AS $C^{14}O_2$
0
10
20
30
40
50
60
70
80
90
CURVE A
CURVE B
0 10 20 30 45 60 90 120 180 240 300
TIME AFTER INJECTION OF LABELED MATERIAL IN MINUTES
MU 392

FIG. 1