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THE METABOLISM OF SODIUM ISOBUTYRATE-1-C^{14} AND SODIUM ISOCAPTOATE-1-C^{14} IN THE RAT

Heinrich Hauptmann and Irving Gray

December 1, 1949

Berkeley, California

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ABSTRACT

The rates of expiration of $C^{14}O_2$ from rats injected with sodium isobutyrate-1-C^{14} and sodium isocaproate-1-C^{14} has been measured. The radioactivity of the fat, protein and glycogen isolated from the liver and of the excreta has been determined. The results are consistent with the idea that isobutyric acid is decarboxylated while isocaproic acid is degraded by β-oxidation with formation of acetic acid.

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** Major, Medical Service Corps, United States Army.

+ 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 classical scheme of β-oxidation. This has been accomplished by manometric measurements of the oxygen uptake (1) and by counter-current distribution separation of the reaction products (2). 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-labeled isobutyric and isocaproic acids.

EXPERIMENTAL

Sodium isobutyrate-1-C^{14} and sodium isocaproate-1-C^{14} were prepared by the reaction of isopropylmagnesium bromide and isoamylmagnesium bromide, respectively, with $C^{14}O_2$ following the directions of Calvin and co-workers (3) for the preparation of

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(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) Calvin, Heidelberger, Reid, Tolbert and Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, 1949.

acetic acid-1-C^{14}. The specific activity of the sodium isobutyrate was 1.72 μc/mg, and that of the sodium isocaproate was 1.79 μc/mg. The yields were 97.5% and 95.5%, respectively, based on the barium carbonate employed. The preparations were carried out on a 20 mmole scale.

The sodium salt of the acid 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, feces and urine were collected. This carbon dioxide was collected at specified time intervals in 1N sodium hydroxide and converted to barium carbonate. The specific activity of the barium carbonate was determined according to the method of Yankwich, et. al. (4, 4a). Geiger-Müeller tubes or "Nucleometer" (a windowless proportional counter) were used, depending on the specific activities being measured.

After five hours the animal was sacrificed and the liver and one kidney were removed. The kidney and feces were dried, burned, and the carbon dioxide collected as barium carbonate and the specific activity determined. The urine was dried and weighed, and then redissolved in distilled water, spread uniformly over a glass plate and counted. The liver was ground in a glass mortar with sand until very finely divided. The ground mass was fractionated according to the procedure in Figure 1.

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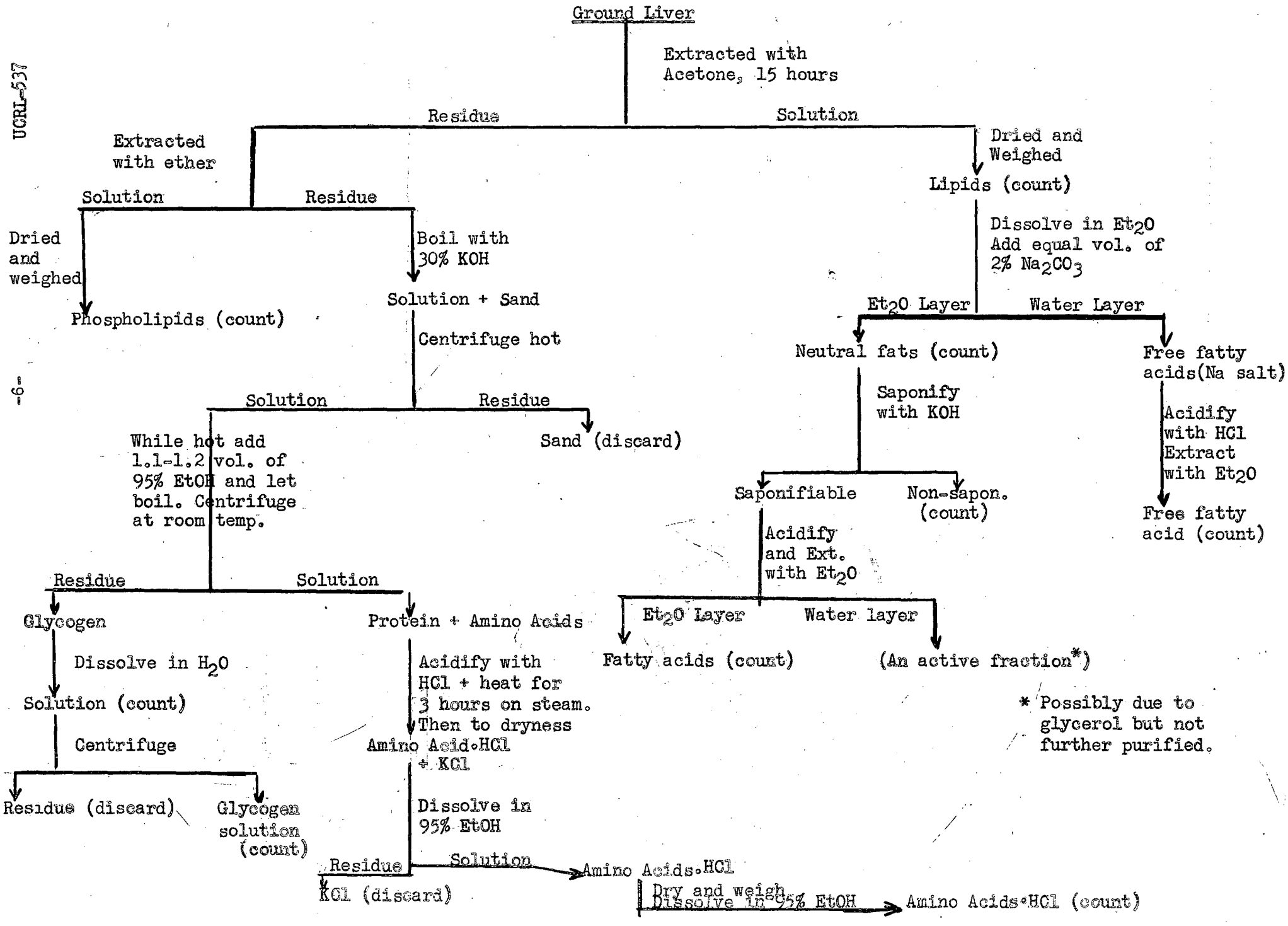
(4) P. E. Yankwich, Science, 107, 681 (1948).

(4a) P. E. Yankwich and J. W. Weigl, Science, 107, 651 (1948).

Figure 1

Liver Fractionation Procedure



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 Figure 2. The amount eliminated after five hours approaches a common value of 80-85%. Both this final amount and the general shape of the curve are similar to those observed by Jones and co-workers (5) for the straight chain fatty acids. This is in good agreement with the observations (1,2) that the fatty acids of the iso-series are oxidized essentially in the same manner as the straight chain acids.

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 and sodium isocaproate, that of the animal injected with the isobutyrate being the higher. Further experiments are felt to be necessary in order to decide the significance of these observations. However, it is not impossible that the difference may be caused by an additional mechanism of decarboxylation in the case of isocaproic acid.

Considerable differences (see Table I) are indicated in the C^{14} content of the various organs, liver fractions and urine, while the excretion in the feces is low in both cases. When sodium isobutyrate is given, the greatest amount of activity in the liver is found in the glycogen, while the lipid and protein activity is low. Exactly the opposite is true for the animal injected with sodium isocaproate. In this case, there is an accumulation of activity in the fats and proteins as well as in the urine.

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(5) H. B. Jones, unpublished; personal communication.

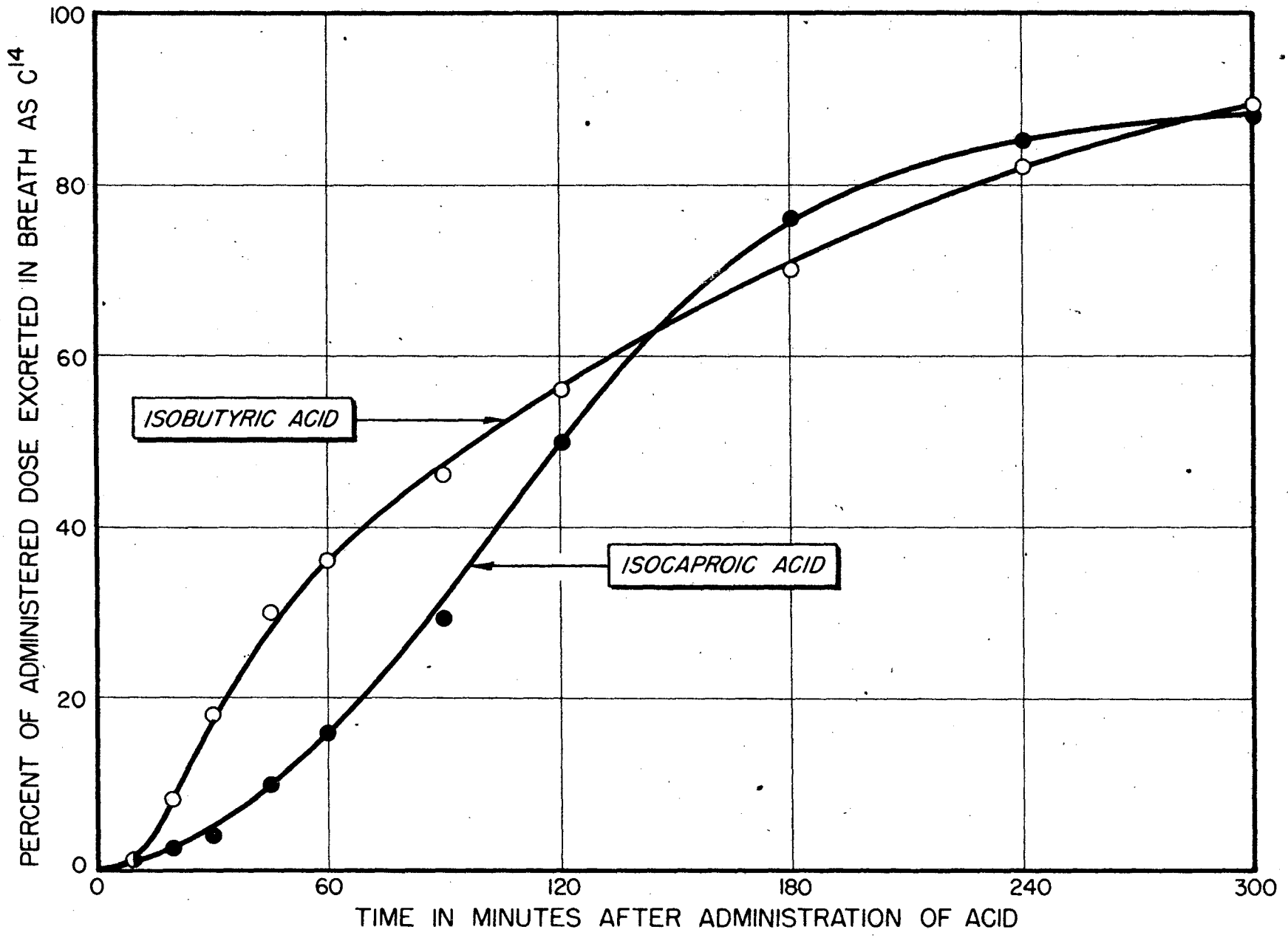

Figure 2 - Rate of Elimination of C^{14} in the Breath

Table I

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

	Compound Injected			
	Sodium isocaproate-1-C^{14} *		Sodium isobutyrate-1-C^{14} **	
	Specific Activity dis/min/mg of Tissue	% of Injected Dose in Tissue	Specific Activity dis/min/mg of Tissue	% of Injected Dose in Tissue
CO_2	--	85	--	87
Feces	400	0.03	40	0.07
Kidney	3000	1.1	50	0.03
Urine	6500	2.2	8500	1.48
Acetone extract of liver	946	0.5	186	0.11
Liver glycogen	300	0.14	926	0.65
Liver protein	500	1.0	110	0.32
Fatty acids from neutral fats of liver	315	0.1	+	
Nonsaponifiable fraction of neutral fats of liver	900	0.1	+	

* Average of two animals
** Average of three animals
} In all cases deviation of individuals from the mean was less than 5%.

\+ Since the acetone fraction was so low in activity, no further fractionation of lipids was carried out.

All these differences are consistent with the observations made *in vitro* (1,2) that isocaproic acid is metabolized with the formation of acetic acid. The latter cannot be formed from isobutyric acid which is degraded to carbon dioxide and propionic acid (2). Besides being excreted in the breath, the carbon dioxide enters the metabolic pool and is incorporated into the glycogen (6,7,8). The resulting propionic acid should not contain the radioactive carbon if the sequence of reactions *in vivo* is the same as that postulated for the *in vitro* degradation. But even if propionic acid-1-C^{14} had been formed, it would also be incorporated into the liver glycogen (8).

The β-oxidation of isocaproic acid leads to inactive isobutyric acid and acetic acid-1-C^{14}. The metabolic pathways which the latter follows are well known. It may be completely transformed into acetoacetate (9) which is decarboxylated and excreted in the urine.

Furthermore, it has been postulated (10) that acetic acid is incorporated into the fats to a considerable extent. A particular confirmation of our whole scheme can be found in the fact that not only the neutral fats contain a considerable amount of radioactivity, but that the highest concentration of C^{14} is found in the unsaponifiable matter. Acetic acid is well known as a precursor for sterol synthesis, while carbon dioxide does not contribute appreciably to either sterol or fat synthesis.

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

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

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

(9) S. Weinhouse, G. Medes and N. F. Floyd, J. Biol. Chem., 158, 411 (1945).

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

The same reasoning leads to an explanation of the difference in the radioactivity of the liver protein of the animals given isobutyrate and isocaproate. Greenberg and Winnick (11) found that the proteins of animals fed methyl- or carboxyl-labeled acetic acid contain more radioactivity than those fed radioactive bicarbonate. The difference in radioactivity which they report is of the same order of magnitude as that found in these experiments. Their results are therefore consistent with this interpretation of the observed differences in the metabolism of isobutyric and isocaproic acids.

Further information is expected from work in progress with the branched chain fatty acids labeled with C^{14} in other positions of the chain.

Acknowledgment: We wish to express our thanks to Prof. Melvin Calvin for his kind interest in this work. This work was done under the auspices of the Atomic Energy Commission.

SUMMARY

The rates of expiration of $C^{14}O_2$ from rats injected with sodium isobutyrate-1-C^{14} and sodium isocaproate-1-C^{14} has been measured. The radioactivity of the fat, protein and glycogen isolated from the liver and the excreta has been determined. The results are consistent with the idea that isobutyric acid is decarboxylated while isocaproic acid is degraded by β-oxidation with formation of acetic acid.

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