Appearance of C-14 in Fumarate and Lactate Following the Injection of DL-Methionine-2-C-14.*† (19205)

LAWRENCE M. MARSHALL AND FELIX FRIEDBERG.
(Introduced by Arnold H. Maloney.)
From the College of Medicine, Howard University, Washington, D. C.

Although the glucogenic activity of methionine in phlorizinized dogs was proposed by Vars(1) the relationship between the butyryl part of the molecule and the common intermediates frequently elaborated in the course of the conversion of amino acids to glycogen has not been investigated. It therefore seemed of interest to test the possibility of C-14 appearing in the intermediates of the Krebs cycle when methionine labeled on the alpha-carbon was administered to the intact animal. The present report presents qualitative evidence that C-14 occurs in fumarate and lactate following the intraperitoneal injection of DL-methionine-2-C-14.

Procedure and results. Mice weighing 20 g were injected intraperitoneally with 20 mg (equivalent to 0.04 mc) DL-methionine-2-C-14 suspended in isotonic saline. After 4, 7, and 10 hours, the animals were sacrificed and the livers removed. The procedure for the precipitation of protein and the extraction of organic acids followed that previously described(2). Inert fumaric, succinic, and aconitic acids were added as carriers to the acetone extract and the extracts were chromatographed. The silica gel columns of Isherwood(3) and the procedure of Marvel and Rands for improving resolution on these columns(4) were used. The developing liquid was delivered from a reservoir so arranged that the n-butyl alcohol-chloroform mixture would gradually and automatically increase with respect to the concentration of alcohol (5). Effluent acids were titrated in alternate fractions throughout the chromatogram and fractions not titrated were measured for radioactivity. For chemical identification, fractions near the chromatographic peaks that had not been titrated were measured spectrophotometrically at 230 millimicrons. Since the extinction coefficient of succinic acid is insufficiently high in the ultraviolet to distinguish it from aliphatic acids in general, this acid was examined in the infrared region between 1650 and 1790 cm-1. It showed an absorption pattern identical to that of chemically pure succinate. Lactic acid was identified by the use of p-hydroxydiphenyl(6). The effluent was collected throughout the study by means of the Technicon fraction collector and absorbance was measured on Beckman spectrophotometers Models DU and IR-2. As an index of radio-chemical purity, the degree to which activity followed titrable acidity was determined (Fig. 1). In 2 experiments, each acid was passed through a second chromatographic column. The radioactivity for each acid occurred at the same position and none of the activity was transferred to other por-

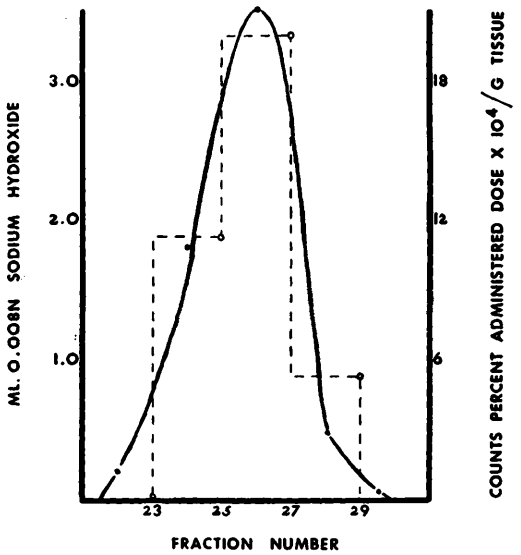

FIG. 1. A comparison of radioactivity and titratable acidity of alternate fractions within the effluent chromatographic zone for the fumaric acid chemically derived from aspartate.

* DL-methionine-2-C-14 was supplied by Tracerlab, Inc.

† Supported in part by a Grant from the Damon Runyon Memorial Fund.

TABLE I. Radioactivity of Certain Organic Acids of Liver Following the Intraperitoneal Injection of DL-Methionine-2-C-14.

Acid	Time after administration 10 hr	7 hr	4 hr
	Counts*		
Fumaric	12.8	24.6	26.4
Succinic-lactic	4	23.8	48.8
Cis-aconitic	5.2	12	7.4

* Expressed as % administered counts $\times 10^4$/g liver.

tions of the effluent. The tissue proteins were examined for labeled aspartic acid. Protein residues from liver and kidney were washed 3 times with 5% trichloroacetic acid containing 1% inert DL-methionine. They were hydrolyzed with 10 times their weight of 6N hydrochloric acid after the addition of 5 mg inert DL-aspartic acid. The freed aspartic acid and its carrier were converted to fumaric acid by means of dimethyl sulfate in alkaline medium(7). The fumaric acid was separated chromatographically. Lactate was degraded by ceric sulfate(8) and the fumarate by acid and alkaline permanganate(9).

The results for liver samples 4, 7, and 10 hours after the administration of the amino acid are indicated in Table I. The succinate and lactate were released together on the silica gel columns, and radioactivity followed titrable acidity with this chromatographic zone. The values shown were corrected for background. The smallest observed count was more than twice the background and the counting error was not greater than 5%. Quantities of wet liver used ranged from 0.8 to 1 g.

Effluent succinate-lactate, as well as fumarate from carcass, showed a similar relationship with respect to acidity and radioactivity. When effluent samples of the succinate-lactate zone from carcass were twice chromatographed with N-amyl alcohol, 30% of the activity was associated with the lactate. No attempt was made to study the aconitate chromatographic zone in this way(1).

Lactate from a single entire carcass was degraded to acetaldehyde(7) and oxidized to acetic acid .75% of the activity of the lactate occurred in the acetate indicating that a large part of the activity was in the non-carboxyl carbons. The results from the degradation(9) of fumaric acid indicated that 52% of the radioactivity was located on the carbon dioxide representing one methine carbon. 48% appeared on the formate derived from one methine along with both carboxyl carbons.

When the fumaric acid derived from aspartate by treatment with dimethyl sulfate was chromatographed(2,3) on silica gel columns, the effluent zone for fumaric acid assayed 74 counts per million counts of administered dose. Progressive changes in titratable acidity accompanied changes in radioactivity within the chromatographic zone for this acid (Fig. 1).

Discussion. The C-14 appears largely in the methine carbons of fumarate and the non-carboxyl portions of lactate. This would seem to indicate that carbon dioxide fixation alone does not explain(10) the path of conversion of DL-methionine to the acids of the Krebs cycle. Two possible pathways of methionine dissimilation have been suggested in the literature. The first describes alpha-amino butyric acid(11) as the intermediate which results on loss of the methyl group of methionine. Deamination of the intermediate occurs *in vivo* (12) but not in enzyme preparations(13). The second pathway postulates that deamination occurs before demethylation. The intermediate gamma-methiol butyric acid, is formed in kidney slices(14) and methyl mercaptan is produced simultaneously.

In the absence of carbon dioxide fixation the metabolic significance of the small amounts of radioactivity found in the organic acids can be evaluated only when the detailed fate of the butyryl part of methionine becomes known. Therefore, we do not attempt in this paper to suggest a pathway by which the C-14 of methionine could appear in lactate or fumarate.

Summary. Qualitative data indicate that C-14 appears in fumarate and lactate following the intraperitoneal injection of DL-methionine-2-C-14.

1. Vars, H. M., PROC. SOC. EXP. BIOL. AND MED., 1933, v31, 129.

2. Marshall, L. M., Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1949, v179, 1127.

3. Isherwood, F. A., *Biochem. J.*, 1946, v40, 688.
4. Marvel, C. S., and Rands, R. D., *J. Am. Chem. Soc.*, 1950, v72, 2642.
5. Donaldson, K., Tulane, V., and Marshall, L. M., *Anal. Chem.*, in press.
6. Markus, R. L., *Arch. Biochem.*, 1950, v29, 159.
7. Friedberg, F., and Marshall, L. M., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 446.
8. Winnick, T., *J. Biol. Chem.*, 1942, v142, 451.
9. Allen, M. B., and Ruben, S., *J. Am. Chem. Soc.*, 1942, v64, 948.
10. Wood, H. G., *Cold Spring Harbor Symposia on Quantitative Biology*, 1948, v13, 201.
11. Dent, E., *Science*, 1947, v105, 335.
12. Waelsch, H., and Miller, H. K., *J. Biol. Chem.*, 1942, v145, 1.
13. Carroll, W. R., Stacy, G. W., and Du Vigneaud, V., *J. Biol. Chem.*, 1949, v180, 375.
14. Waelsch, H. and Borek, E., *J. Am. Chem. Soc.*, 1939, v61, 2252.

Received July 30, 1951. P.S.E.B.M., 1951, v78.

Effect of Cortisone and ACTH on Adrenals in Experimental Diphtheria, Shiga, and Meningococcus Intoxication. (19206)

RODERICK MURRAY AND SARA E. BRANHAM.

From the Laboratory of Biologics Control, National Microbiological Institute, National Institutes of Health, Public Health Service, Bethesda, Md.

Involvement of the adrenal glands is a pronounced feature of the intoxication produced in animals by a number of bacterial toxins and in the course of many bacterial infections.

The possible sparing of the adrenals by cortisone was studied in the case of the toxin of *Shigella dysenteriae* (Shiga) and virulent meningococcus infection in mice, and in guinea pigs given a fatal dose of diphtheria toxin. Because of the interrelationship of ascorbic acid and adrenocortical function, experiments were conducted in which cortisone and ascorbic acid were both administered to the same animal even though mice are known to have the ability, not possessed by guinea pigs, to produce their own ascorbic acid. In addition the effect of ACTH was studied in guinea pigs receiving 1 MLD of diphtheria toxin.

Methods. The cortisone used was the dry acetate or free alcohol which was prepared for use by dissolving a weighed amount in the smallest quantity of acetone required to effect complete solution and then quickly bringing up to full volume with physiological saline solution so that the required dose was contained in 0.5 ml. A drop of "Tween 80" per 100 ml helped stabilize the suspension. The ACTH solution was freshly prepared prior to injection by dissolving a weighed amount of the dried powder in sufficient physiological saline solution so that the required dose was contained in 0.5 ml. Weights of ACTH are expressed in terms of Armour Standard No. LA-1A. The animals used in these experiments were guinea pigs weighing 240-280 g and pure line CFW strain white mice weighing 16-20 g. All guinea pigs were weighed daily. All animals were autopsied at death or after sacrifice.

Effect of cortisone and ACTH on guinea pigs given 1 MLD of diphtheria toxin. The hemorrhagic appearance of the adrenals has long been cited as the most conspicuous finding in guinea pigs dying from diphtheria toxin. The course of intoxication following the administration of 1 MLD of diphtheria toxin has been closely observed in this laboratory for a great many years, an experience which provided a background for the detection of any deviation from the expected course which could be attributed to cortisone, cortisone plus ascorbic acid, or ACTH. In 3 experiments all guinea pigs were given 1 MLD of diphtheria toxin subcutaneously. In Exp. 1 and 2 ACTH was given twice daily (0.17 mg) or cortisone daily (0.34 mg) for 15 days, or until death. In Exp. 1 the initial dose was given subcutaneously at the same time as the toxin.