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Authors
Kritchevsky, David
Abraham, S.

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The labeled glycerol obtained from the eggs of a hen continuously fed sodium acetate-1-C^{14} for 7 days has been degraded by two methods. The general pattern of activity distribution is 86-88% in the terminal carbon atoms and 12-14% in the middle carbon atom. A mechanism that might explain these results has been suggested.

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(*) This work was sponsored by the U.S. Atomic Energy Commission.

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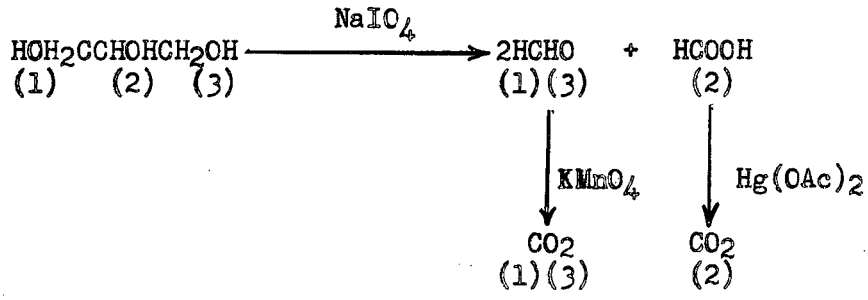
The distribution of radioactivity in the eggs and in the yolk products following the continuous feeding of sodium acetate-1-C^{14} to a laying hen has been described (1,2). This report describes the distribution of radioactivity in the glycerol obtained from eight of the ten yolks. These yolks represent seven eggs layed by the hen and two large ova and the combined small ova obtained after sacrifice of the animal.

The glycerol samples were all obtained by saponification of the tribenzoate derivatives of the original glycerol samples. Two methods of degradation have been employed and in several cases both methods were used on the same sample, with duplication of results. The methods used were either lead tetraacetate oxidation (3) which leads to the following products:

$$\underset{(1)\ (2)\ (3)}{HOH_2CCHOHCH_2OH} \xrightarrow{Pb(OAc)_4} \underset{(1)(3)}{2HCHO} + \underset{(2)}{CO_2}$$

$$2HCHO \xrightarrow{KMnO_4} \underset{(1)(3)}{CO_2}$$

or sodium periodate oxidation (4) which proceeds in the following manner:



By these methods it is impossible to distinguish between carbon atoms 1 and 3 of glycerol, but the symmetry of the molecule obviates the necessity for this distinction.

TABLE I

Activity of Glycerol Carbon Obtained in Degradation

Yolk No.	C1,C3 *	%(C1 + C3)	C2	%(C2)
1	4	100	0	0
2	23	88	6	12
3	lost			
4	lost			
5	31	86	10	14
6	34	86	11	14
7	68	86	22	14
8	23	88	6	12
9	30	88	8	12
10	4.6	86	1.5	14

* All activities given as counts/min./mg.$BaCO_3$

Experimental: - All radioactivity determinations were performed using $BaCO_3$ plates prepared after the procedure of Entenman et al. (5). Counting data were obtained by using an end window counter and are expressed as counts/min./mg.$BaCO_3$ using 40.0 mg. as a standard weight. The total glycerol activity was determined using the wet combustion method described by Van Slyke and Folch (6). Each radioactive glycerol sample was diluted to 250 mg. with inactive glycerol and dissolved in absolute ethanol to insure complete mixing. The ethanol was removed under reduced pressure at room temperature. Representative experiments using both methods of oxidation are described below.

Lead Tetraacetate Oxidation: - A portion of the diluted glycerol (62 mg.) was dissolved in 2 ml. of distilled water and 1 ml. of the resulting solution was oxidized with 1.42 g. of $Pb(OAc)_4$ for 4 hours at 40-42° using the method already described (3). The evolved carbon dioxide was collected in sodium hydroxide and precipitated as barium carbonate. This carbon dioxide represents carbon atom 2 of glycerol. The solution was then made basic and the formaldehyde representing carbon atoms 1 and 2 was distilled and oxidized to carbon dioxide in a manner described below.

Two experiments using inactive glycerol revealed that 99.0% and 99.7% of the theoretical amount of carbon dioxide is liberated under these conditions.

Sodium Periodate Oxidation: - The diluted glycerol (92 mg.) was dissolved in 8 ml. of distilled water and after 8 ml. of 0.37 M sodium periodate was added, the resulting oxidation mixture was allowed to stand at room temperature for one hour. After one hour 16 ml. of 1 N hydrochloric acid and 30 ml. of 1.2 N Na_2HAsO_3 were added, the solution made basic to phenol red, and the formaldehyde (representing carbon atoms 1 and 3 of glycerol) was distilled. One g. of $KMnO_4$ was added to the distillate and the solution heated under reflux in a current of carbondioxide-free

air. The carbon dioxide resulting from this oxidation was collected in sodium hydroxide and precipitated as barium carbonate.

Experiments using inactive glycerol showed that 94-96% of the theoretical amount of formic acid is formed in this reaction. Van Slyke-Folch oxidation of a 62 mg. sample of glycerol yielded 99-100% theoretical amounts of carbon dioxide.

Comparison of Methods: - The tetraacetate oxidation of glycerol No. 10 showed a specific activity of 1.5 for carbon atom 2 of the glycerol. In the periodate oxidation, after the formaldehyde had been distilled, the residue was acidified and the formic acid distilled. Oxidation of this formic acid with mercuric acetate yielded barium carbonate of specific activity of 1.6. The specific activities of the glycerol from yolks 1, 2 and 6 were 2.7, 17.4 and 26.2, respectively. To compare this with degradation data we find:

Yolk 1: $C1 + C2 + C3 = 4 + 0 + 4 = 8 \quad 8/3 = 2.6$

Yolk 2: $C1 + C2 + C3 = 23 + 6 + 23 = 52 \quad 52/3 = 17.6$

Yolk 6: $C1 + C2 + C3 = 34 + 11 + 34 = 74 \quad 74/3 = 26.3$

Discussion: - The salient feature of the combustion data is that in all but one case 86-88% of the total glycerol activity is in the terminal carbons and 12-14% in the middle carbon atom. The lone discrepancy is in the first yolk glycerol and it must be pointed out that were there even one count in carbon 2 the percentages would be 89% for C1 + C3 and 11% for C2. The low level of activity in this sample could cause such an error.

The fact that the hen was being fed labeled acetate throughout the initial labeling experiment (1) can account for the constant labeling observed. These data suggest an equilibrium between the formation and recycling of the glycerol. Such a scheme would involve the conversion of the carboxyl-labeled acetate to a symmetrical, carboxyl-labeled succinate via the Krebs cycle. The conversion of

carboxyl-labeled acetate to carboxyl-labeled succinic acid in rats has recently been demonstrated (7). This, in turn, could go to a carboxyl-labeled pyruvate by way of oxalacetate. Such a labeled pyruvate would give a one-labeled dihydroxyacetone monophosphate which, in turn, would give a glycerol labeled in the terminal carbon atoms. The resulting glycerol-1,3-C^{14} could recycle to give pyruvate labeled in both the methyl- and carboxyl-carbon atoms. The irreversible decarboxylation of this pyruvate would yield a methyl-labeled acetate fragment which would enter into the Krebs cycle to give glycerol-2-C^{14} by the steps outlined above. The $C^{14}O_2$ resulting from decarboxylations of intermediates in the metabolic scheme would again be fixed in such a manner as to give rise to terminal labeled glycerol. According to current concepts, these decarboxylations could in no case account for the labeling of the middle carbon atom.

Such a scheme could account for the labeling pattern as observed and the earlier inclusion of the carboxyl-labeled acetate moiety can explain the higher activity of the terminal carbon atoms. Had the original feeding experiment not been stopped after exhaustion of the labeled feed, the glycerol from subsequent yolks might eventually have approached uniform labeling. The suggested scheme is presented graphically in Figure 1.

Other investigators have suggested a symmetrical three-carbon intermediate in the glycolytic scheme. This has been postulated in the case of conversion of glucose-1-C^{14} to lactic acid by Lactobacillus casei (8,9). A symmetrical three-carbon intermediate has also been postulated in the alcoholic fermentation of glucose by yeast (10).

Inasmuch as such a symmetrical three-carbon atom intermediate has not been demonstrated in animal experiments, the above scheme has not taken such a step into account. It should be borne in mind, however, that a mechanism involving a symmetrical three-carbon intermediate (dihydroxyacetone or dihydroacetone diphosphate)

could also be operative to give labeling as observed.

SUMMARY

The labeled glycerol obtained from the eggs of a hen continuously fed sodium acetate-1-C^{14} for seven days has been degraded by two methods. The general pattern of activity distribution is 86-88% in the terminal carbon atoms and 12-14% in the middle carbon atom. A mechanism that might explain these results has been suggested.

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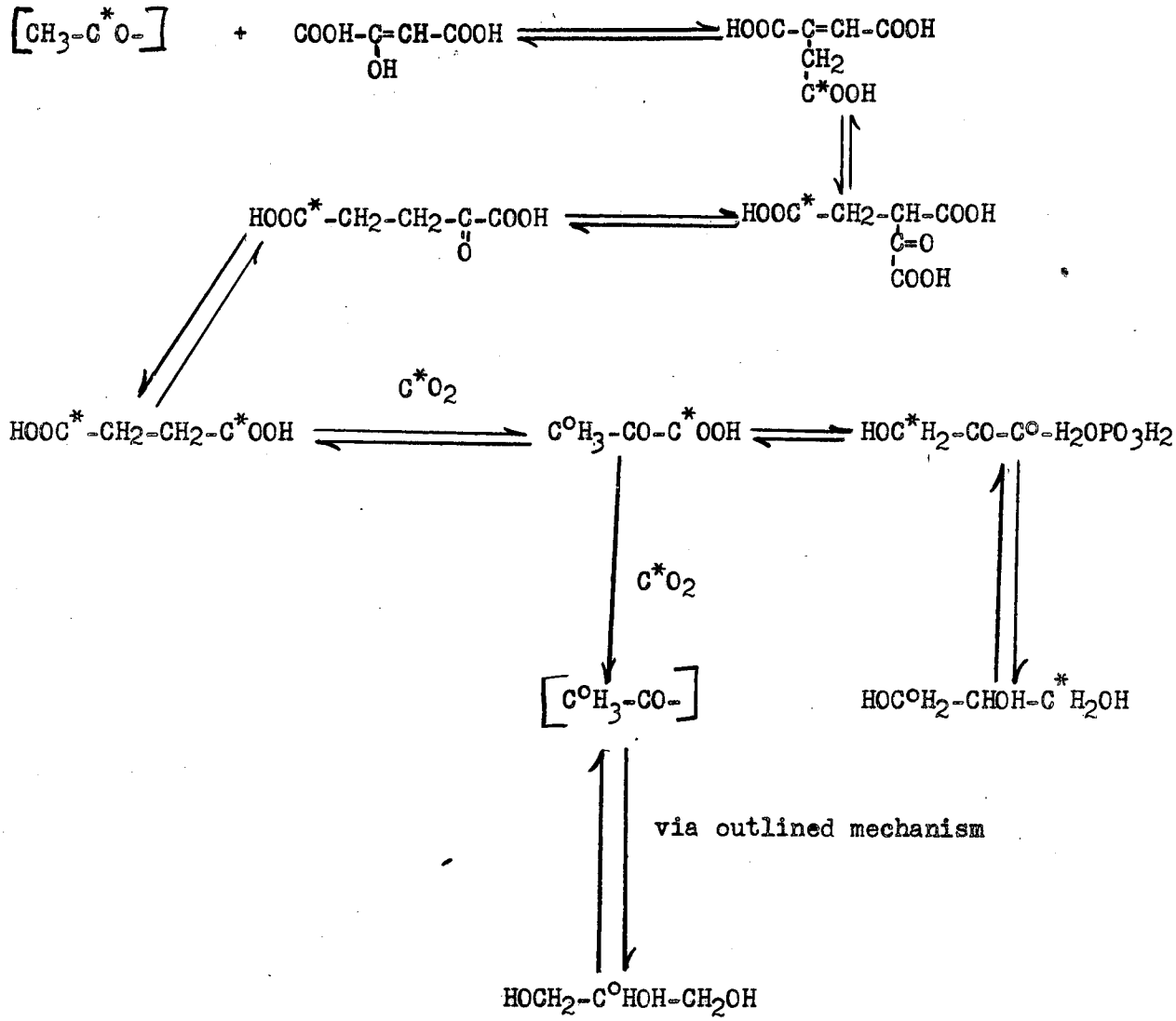
FIGURE 1 - SUGGESTED SCHEME TO ACCOUNT FOR OBSERVED LABELING



* denotes atom labeled in original feeding

o denotes atom labeled in recycling