GLUTARIC ACID: A PRODUCT OF TRYPTOPHAN METABOLISM[1]

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Previous work in this laboratory (Gholson and Henderson 1958) has shown that a major portion of a test dose of DL-tryptophan-7a-C^{14} is metabolized by the rat to $C^{14}O_2$ via acetate-1-C^{14}, 3-hydroxyanthranilic acid-2-C^{14} (3-OHAA) being a probable intermediate (Gholson and Henderson 1959). Consideration of possible metabolic sequences between 3-OHAA-2-C^{14} and acetate-1-C^{14} suggested some five-carbon dicarboxylic acids as plausible intermediates. A number of these compounds have been tested by the "metabolite overloading" technique (Rothstein and Miller 1954a) in the rat with tryptophan-7a-C^{14} and 3-OHAA-H^3. Isotopes from both of these compounds were incorporated into excreted glutaric acid, but not into any other compound so far tested by this technique.

EXPERIMENTAL

DL-tryptophan-7a-C^{14} was prepared from aniline-1-C^{14} (Henderson, Nystrom and Rao 1958). Tritium-labeled 3-OHAA was prepared by the Wilzbach (1957) procedure. Trans-glutaconic acid (Kohler and Reid 1925) and β-ketoglutaric acid (Adams, Chiles and Rassweiler 1925) were prepared by established methods.

Fifty mg. of tryptophan-7a-C^{14}, or five mg. 3-OHAA-H^3, together with 400-500 mg. of the test acid were injected intraperitoneally into rats weighing 200-300 gm., in two or three equal doses, three hours apart, and the urine was collected for 24 hours. The acidified urine was passed through a column of Dowex 50-H^+ to remove tryptophan and its cationic metabolites. The effluent was chromatographed on a silicic acid column by a modification of the procedure of Marvel and Rands (1950).

RESULTS AND DISCUSSION

When DL-tryptophan-7a-C^{14} and unlabeled glutaric acid were administered to rats, carbon-14 labeled glutaric acid was excreted in the urine.

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Figure 1 shows the correlation between the radioactivity and acid titration peaks when urinary glutaric acid was co-chromatographed with carrier glutarate.

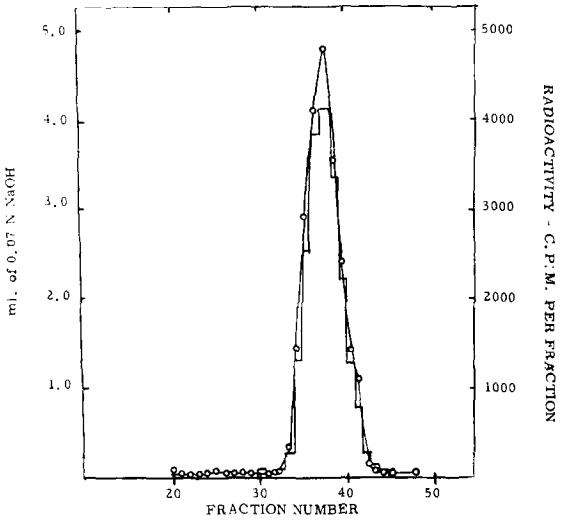
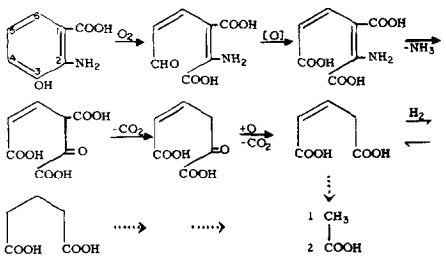


Figure 1
CORRELATION BETWEEN GLUTARIC ACID TITRATION VALUES (CURVE) AND C^{14} CONTENT (BARS) OF 10 ML URINE FRACTIONS FROM A SILICA GEL COLUMN

The material in the peak tubes was isolated and crystallized from boiling benzene. The specific activity did not change during repeated recrystallization. Acetate-1-C^{14} having three times the total activity of the tryptophan administered failed to produce detectable labeling in excreted glutarate. This failure of acetate to label glutarate was previously observed (Rothstein and Miller 1954b) and supports the view that glutarate is formed more directly from tryptophan than via acetate.

Better evidence in support of this hypothesis was provided by the finding that an established tryptophan metabolite, 3-OHAA, labeled with tritium, produced glutarate-H^3. Retention of the tritium in glutaric acid suggests a direct conversion to a five-carbon unit rather than an initial degradation followed by resynthesis. The conversion of both tryptophan and 3-OHAA to glutarate, which is known to be an acetate precursor (Hobbs and Koeppe 1958), also strongly suggests that 3-OHAA is an intermediate in the oxidative degradation of tryptophan.

A hypothetical pathway for the formation of glutaric acid from 3-OHAA via cis-glutaconic acid is shown in the following scheme.



In a metabolite overloading experiment with ring labeled tryptophan, the glutaconate recovered from the urine contained no radioactivity. The failure of tryptophan-7a-C^{14} and glutaric acid-1,5-C^{14} (Rothstein and Miller 1954b) to label trans-glutaconic acid by no means disproves the proposed pathway, since exogenous glutaconate may not equilibrate with that endogenously formed. Also, the relatively unstable cis isomer is the expected product from 3-OHAA, while trans-glutaconic is produced by the usual synthetic methods (Malachowski 1929) and was used in both these studies and those of Rothstein and Miller (1954b).

Malonic, β-ketoglutaric and β-hydroxybutyric acids were tested by metabolite overloading as possible glutarate and, therefore, tryptophan metabolites. All of these acids were recovered in the urine, but none was labeled by tryptophan-7a-C^{14}. However, these negative results cannot be interpreted as excluding any of these compounds from the pathway of glutarate catabolism (Rothstein and Miller 1954a).

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