Preparative Biosynthesis of ^{14}C-Orotic Acid

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When a metabolic pathway is blocked by a mutation impairing the enzyme catalysing one of the steps, the corresponding substrates accumulate. This phenomenon can be used for the preparation of metabolites. We tried to use this method to obtain ^{14}C labelled orotic acid, using a uracil requiring mutant of E. Coli defective at the level of orotidilate decarboxylase. When incubated with a crude extract of a fully derepressed sample of this mutant ^{14}C aspartate has been completely transformed in ^{14}C orotic acid.

EXPERIMENTAL.

(a) *Bacterial strain:* the uracil requiring mutant of E. Coli has been generously provided to us by Dr. John C. Gerhart (Berkeley, California).

(b) *Preparation of the crude extract:* the mutant has been grown as described by J. C. Gerhart and H. Holoubek [1]. After centrifugation, the bacteria have been resuspended in Tris-HCl 10^{-2} M pH 7.4, until an optical density of 0.5 at 546 mμ is attained, and then are disrupted for three times 30 secondes in a Biosonik III oscillator. In order to verify the derepression of the bacteria the specific activity of the extract in aspartate transcarbamylase has been determined, using the method of Hunninghake and Grisolia [2] for aspartate transcarbamylase determination, and the method of Lowry *et al.* [3] for protein estimation.

(c) *Incubation of ^{14}C-aspartate with the extract:* 10 μCi of ^{14}C aspartate uniformly labelled (80 mc/mmole) have been incubated at 37° C in the presence of 7.5 μmoles of Tris-HCl buffer pH 7.4, 7.5 μmoles of carbamylphosphate, 7.5 m μmoles of NAD and 125 μg of bacterial extract (expressed in proteins) is a total volume of 0.75 ml. Every ten minutes 0.1 ml samples have been taken off and analysed for orotic acid and precursors in the following way : proteins have been precipitated by addition of 0.4 ml of ethanol and incubation 2 mn at 100° C. After centrifugation, the supernatant has been evaporated; the residue has been redissolved in 1 ml of water, and 0.1 ml of samples have been analysed by paper and thin layer chromatography according to Gordon *et al.* [4], simultaneously with samples of ^{14}C aspartate

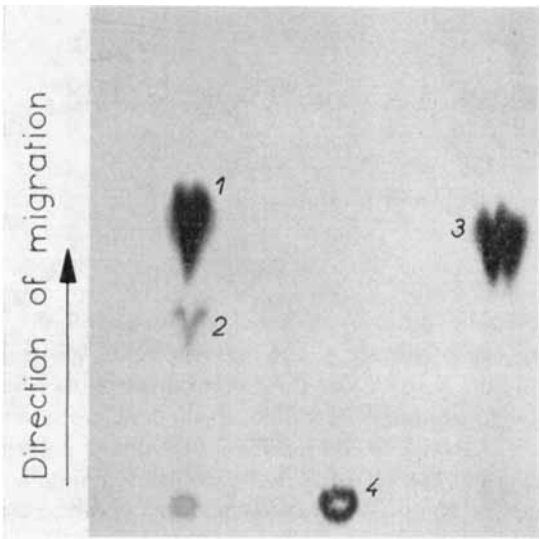

FIG. 1. 1. — ^{14}C orotic acid and 2. — ^{14}C dihydroorotic acid obtained in biosynthesis; 3. — ^{14}C orotic acid and 4. — ^{14}C aspartic acid as controls.
Incubation : 2 hours; Solvent, Isopropanol, pyridine, water, acetic acid (8/8/4/1)

and ^{14}C orotate as controls. The chromatograms have been exposed overnight to Kodirex films.

RESULTS.

The following autoradio-gram, corresponding to a two hours incubation, shows the separation of aspartate, dihydro-orotic acid and orotic acid.

The disappearance of aspartate, and appearance of orotic acid, during the incubation can be observed. It has been verified that the intermediary spot corresponds to dihydro-orotic acid, using unlabelled compound detected by bromophenol blue.

In these conditions a nearly complete transformation of ^{14}C aspartate in ^{14}C orotate is obtained after two hours of incubation. 100 μCi samples of ^{14}C aspartate have been treated in this way with a yield of 96 μCi of ^{14}C orotate. The final product has been also controled by spectrophotometry.

Since one atom of carbone of the orotic ring is coming from the carbamylphosphate, the specific radio-activity of the final product would be increased by using ^{14}C labelled carbamylphosphate.

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