The Isolation of Crystalline Erythritol from Normal Human Urine*

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With the finding that xylitol is an intermediate in the glucoronic acid pathway (2, 3), experiments were undertaken to determine whether this pentitol occurs in human urine. Instead of xylitol, the analyses led to the isolation of 2-arabitol from pentosuric urine as well as 2-arabitol from normal urine (4). During the fractionation of urinary polyols on Dowex 1-borate columns, eluates were also found to contain a substance with the paper chromatographic behavior of a tetritol. Purification of these fractions yielded crystalline erythritol.

This paper describes the procedures used in the isolation and identification of erythritol from the urine of two normal fasting men.

EXPERIMENTAL

Materials—Erythritol was obtained from the Mann Research Laboratories, Inc. L-Threitol was generously contributed by Dr. N. K. Richtmyer, National Institute of Arthritis and Metabolic Diseases.

Methods—All urine samples of the two adult male subjects were preserved with toluene and were refrigerated until used. They were analyzed within a few days of collection. The separation of polyols was made by the method of Khym and Zill (5), with 0.08 M H₂BO₃-0.004 M Na₂B₄O₇ serving to elute the erythritol from the Dowex 1-borate columns. Eluates from the columns were analyzed as follows: for ketopentoses, the Dische-Borenfreund cysteine-carbazole reaction (6); for substances containing α-glycol groups, the titrimetric periodate procedure of Voris et al. (7) and the colorimetric periodate procedure of Bailey (8). For analysis of eluates by paper chromatography, sodium ions were removed by treatment with Amberlite IR-120 (H+), and, after addition of methanol, the boric acid was distilled off as methyl borate (5). Separations on paper chromatograms were effected in benzene-n-butyl alcohol-pyridine-water (1:5:3:3, upper layer), ethyl acetate-pyridine-water (2:1:2, upper layer), and 80% n-propyl alcohol. Polyols were detected with periodate-benzidine dip reagents (9). Most chromatograms were run in descending solvent, and when ascending development was used, the RF values of polyols agreed with those reported previously (4).

RESULTS

Summary of Isolation Experiments—Evidence for the presence of erythritol in urine has been obtained in 4 experiments. One preliminary run on each of the two subjects yielded fractions containing a tetritol, as indicated by paper chromatographic analysis of eluates from the Dowex columns. Although the tetritol and erythritol have similar RF values, the urinary substance usually was closer to the latter. An additional experiment on the urine of each subject during fasting was carried through to the isolation of crystalline erythritol.

Isolation of Crystalline Erythritol from Urine of J. N. C. during Fasting—Urine, 450 ml, was deionized by passage through a 2.5-cm diameter column containing both Amberlite IR-120 (H+), 25-cm height, and Duolite A-4 (CO₃⁻), 57-cm height, the column then being washed with 500 ml of water. The combined eluates were evaporated in a vacuum (<50°) to a 5-ml volume. After addition of an equal volume of 0.02 M Na₂B₄O₇, the sample was chromatographed on Dowex 1-borate as indicated in Fig. 1. The eluates between 220 ml and 320 ml were pooled, freed of eluting agent, concentrated to dryness, and analyzed by paper chromatography. The polyol present in largest amount had the same mobility as erythritol in the solvents given under "Methods." Since other periodate-positive substances, including ketoses, were present, further purification was effected by preparative chromatography on Whatman 3MM paper, with ethyl acetate-pyridine-water (2:1:2, upper layer) as developer. After elution of the tetritol with water, the extract was passed through 1-ml columns of Amberlite IR-120 (H⁺) and Duolite A-4 (CO₃⁻) and evaporated to dryness. The dry weight was 19 mg, approximately the amount of tetritol indicated by paper chromatographic assay. After several months in a freezer, crystallization occurred. Trituration with 0.5 ml of cold absolute ethanol gave 5.4 mg of white crystals, m.p. 110-111°. Threitol melts at 88° (10). Mixture with authentic erythritol gave a melting point intermediate between those of the urinary and authentic preparations. The isolated material was recrystallized from absolute ethanol-ether and then from absolute ethanol. Melting points (uncorr.) taken simultaneously on a Kofler hot stage were: urinary tetritol, 116-117°; erythritol, 116.5-117.5°; mixture, 116.5-117°. Dr. H. M. Powell found that the x-ray diffraction pattern of the isolated

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FIG. 1. Fractionation of normal urine (450 ml) on a Dowex 1-borate column (1.1 X 17 cm). The eluent was 600 ml of 0.004 M Na₂B₄O₇, 0.08 M H₃BO₃, 100 ml of 0.005 M Na₂B₄O₇, 100 ml of 0.01 M Na₂B₄O₇, 300 ml of 0.02 M Na₂B₄O₇, and then 300 ml of 0.04 M Na₂B₄O₇. Every third 10-ml fraction was analyzed initially, additional fractions then being analyzed as necessary. △, periodate reactivity, calculated as pentitol; ●, cysteine-carbazole reactivity, xylitol as standard.

The biosynthesis of erythritol is not difficult to envisage in view of the reported biochemical roles of tetrooses, the most likely precursors of the polyol. Perhaps the most significant fact is that 3-deoxy-1-phosphate is an intermediate in the pentose phosphate pathway (22). In addition, Uehara et al. (23, 24) have presented evidence that minced muscle can convert dihydroxyfumaric acid into 3-deoxy-1-phosphate. Charalampous (25) has described a liver enzyme that catalyzes the aldol condensation between formaldehyde and dihydroxyacetone phosphate to yield erythrose-1-phosphate. Also of possible relevance is the conversion of hydroxypyruvic acid to erythrose-1-phosphate by yeast extracts (26).

Erythritol might arise from the reduction of erythrulose or erythrose, or from the hydrolysis of an erythritol phosphate. Many microbial enzymes catalyze the interconversion of l-erythrose and erythritol (27), but the tetrose acted upon by known polyol dehydrogenases of mammalian liver is l-threitol (28, 29). We have examined liver extracts for the presence of an erythritol dehydrogenase. Some time ago we obtained good evidence for such an enzyme, linked specifically to diphosphopyridine nucleotide, in rat liver extracts (1). A purification procedure involving acidification, neutralization, and then treatment with alcohol-ether, a purification procedure that is effective with the Blakley polyol dehydrogenase (30), led to almost complete loss of erythritol reactivity. Unfortunately, we have been unable to obtain very active extracts in recent months.

The isolation of erythritol from urine brings to mind studies of other polyols in mammalian metabolism. The important roles of glycerol and inositol are obvious, and recent work on xylitol and arabitol have already been mentioned. Sorbitol is considered to be the intermediate in the conversion of fructose to fructose in man and the formation of the fructose found in the fetal blood of ungulates (32). Further study of the metabolism of erythritol in animals would therefore appear warranted.

SUMMARY

Erythritol has been isolated in crystalline form from the urine of two fasting men and its identity established by chromatography, melting point, and x-ray diffraction pattern.

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