The Isolation of Crystalline Erythritol from Normal Human Urine*

OSCAR TOUTZER,† SHIRLEY O. HECHT, AND W. M. TODD‡

From the Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee, and the Department of Biochemistry, Oxford University, Oxford, England

(Received for publication, December 3, 1959)

With the finding that xylitol is an intermediate in the glucuronate xylulose 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 L-arabitol from pentosuric urine as well as D-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 H₃BO₃-0.004 M Na₂B₄O₇ serving to elute the erythritol from the Dowex 1-borate columns. Eluates were analyzed as follows: for ketones, the Dieche-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).

* Supported in part by a research grant from the National Science Foundation. A preliminary account was presented at the Federation of American Societies for Experimental Biology, Atlantic City, April, 1959 (1).
† Fellow of the John Simon Guggenheim Foundation (1957–8), and Investigator of the Howard Hughes Medical Institute.
‡ Postdoctorate Fellow of the United States Public Health Service (1958–9).

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 threitol 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 filtrates 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°. Erythritol 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 Koller 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
Phosphate to yield erythrulose-1-phosphate. Also of possible release is the conversion of hydroxypropylidone to L-erythrulose 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-erythrulose and erythritol (27), but the tetritol 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 dihydrophosphopyridine 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 glucose to fructose in male accessory organs (31) and in 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.

Acknowledgments—The senior author is indebted to Sir Hans Krebs and Dr. Paul Kent for their hospitality during his stay at Oxford University in 1957–8. Dr. H. M. Powell of Oxford very kindly carried out the crystallographic examination of the urinary erythritol. Miss Diana Shanklin and Mr. Allen Rosen- thal assisted in the analyses of the urine of W. M. T.

REFERENCES

16. RABBERGER, M., AND LANDSEIDEL, Monatsh., 21, 571 (1900).
The Isolation of Crystalline Erythritol from Normal Human Urine
Oscar Touster, Shirley O. Hecht and W. M. Todd


Access the most updated version of this article at http://www.jbc.org/content/235/4/951.citation

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/235/4/951.citation.full.html#ref-list-1