SERUM AND URINE L-XYLULOSE IN PENTOSURIC AND NORMAL SUBJECTS AND IN INDIVIDUALS WITH PENTOSURIA TRAIT*

Irwin M. Freedberg, David S. Feingold, and Howard H. Hiatt

Department of Medicine, Harvard Medical School, and
Department of Medical Research, Beth Israel
Hospital, Boston, Mass.

Received December 4, 1959

Pentosuria is a recessively inherited (Lasker, Enklewitz, and Lasker, 1936) metabolic error characterized by the urinary excretion of relatively constant amounts of L-xylulose. Following the elucidation of the glucuronic acid oxidation pathway of carbohydrate metabolism (see Horecker and Hiatt, 1958, for a review of the reactions involved) much evidence has appeared to support the hypothesis that pentosuria is the result of a block in this pathway beyond the L-xylulose step (Touster, Hutcheson, and Rice, 1955; Touster, Mayberry, and McCormick, 1957; Hiatt, 1958; Hiatt, in press). The enzymatic defect has yet to be demonstrated; however, since pentosuria is a benign as well as uncommon disturbance, and since the enzymes involved in L-xylulose metabolism have thus far been found only in mammalian liver, opportunities to define the abnormality with precision will be exceedingly sparse. It is the purpose of this communication to present additional strong evidence for a disturbance in glucuronic acid metabolism in pentosuria. The data to be presented exclude the suggestion (Knox, 1958) that pentosuria may result from a disturbance in the capacity of the renal tubules to reabsorb L-xylulose. A second objective of this note is to point out that under appropriate conditions a chemical expression of pentosuria trait can be demonstrated in heterozygous individuals.

* This work was supported by grants from the National Cancer Institute, U. S. Public Health Service, and the American Cancer Society.

This study was carried out by measuring serum and urine levels of L-xylulose with an enzyme first described by Touster, Reynolds, and Hutcheson (1956) and purified by Hickman and Ashwell (1959). Serum and urine L-xylulose was measured enzymatically before and after glucuronolactone administration in three pentosuric individuals, four subjects considered heterozygous for pentosuria on the basis of their family histories, and five normal subjects. Because pentosuria has been described almost exclusively in Jews, the control group was limited to non-Jews. Details concerning the protocols of the studies the pentosuric heterozygotes, and the enzymatic assay are described in Table I. All pentosuric individuals had more than 1 mg. of L-xylulose per 100 ml. of serum in the fasting state. In sharp contrast, in only one of the other subjects was L-xylulose detectable in fasting serum. Following the administration of glucuronolactone, which has long been known to increase urinary L-xylulose in pentosuric subjects (Enklewitz and Lasker, 1935), increases in serum levels were found in pentosuric subjects comparable to those observed by Bozian and Touster (1959) with a chromatographic assay. In normal individuals little or no L-xylulose was found in the serum following glucuronolactone, while the levels in heterozygous subjects were intermediate between those of the pentosuric and the normal groups. Glucuronolactone also led to the expected rise in urinary L-xylulose in pentosuric subjects and to the appearance of small but significant levels of the pentose in the urine of normal individuals. Again, the pentosuric heterozygotes had urinary levels intermediate between those of the other two groups.

If one assumes that these individuals had average glomerular filtration rates, one can calculate that virtually all L-xylulose entering the glomerular filtrate was excreted in the urine of the normal and pentosuric subjects. Thus, a "defect" in renal tubular reabsorption of L-xylulose appears to exist in all subjects, but it is clearly not the metabolic error which distinguishes the pentosuric from the normal

Table I

Serum and Urine L-Xylulose Levels Before and After D-Glucuronolactone

Subject	Serum L-Xylulose*		Urine L-Xylulose*	
	Fasting	Maximal	Fasting	Maximal
	mg. per 100 ml.		mg. per hour	
Pentosurics				
A	1.2	7.2		320
B	1.3	9.9	106	549
C	1.7	14.7	88	450
Pentosuric Heterozygotes#				
D	**	1.26	0	
E	**	3.90	0	
F	0.18	0.71	0	81
G			0.1	63
Normal Subjects				
H	**	**	0	9
I	**	**	0	22
J	**	0.22	0	16
K	**	0.29	0.6	13
L	**	0.15	0.3	18

After fasting blood and urine samples were obtained, 5 (subject A), 10 (B and C), or 25 (all other subjects) grams of D-glucuronolactone were given by mouth. Blood and urine were collected at intervals for at least 3 hours thereafter. Maximal serum and urine pentose levels were reached within 30-90 minutes and the first or second hours, respectively, following glucuronolactone administration.

D and E are mothers of pentosurics, F is one of five siblings, three of whom have pentosuria, and G is the son of a pentosuric.

Less than 0.1 mg. per 100 ml.

Blood samples were obtained from an antecubital vein. Serum and urine specimens were stored at -20° until analyzed. Two ml. of serum was deproteinized with 0.2 ml. of 70 per cent perchloric acid, and 1 ml. of the supernatant solution was brought to pH 7.0 with 2 N KOH. The insoluble potassium perchlorate was removed by centrifugation at 2° C., and an aliquot of the clear supernatant solution was used in the assay. Urine samples were brought to pH 7.0 prior to analysis. The assay mixture included 40 μmoles of Tris-maleate buffer, pH 7.5, 5 μmoles of $MgCl_2$, 1 μmole of cysteine, 0.1 μmole of TPNH, urine or deproteinized serum, and an excess of the ammonium sulfate fraction of guinea pig liver L-xylulose dehydrogenase described by Hickman and Ashwell (1959) in a final volume of 1.0 ml. The reaction was begun by the addition of enzyme and was followed spectrophometrically at 340 mμ. With the enzyme preparation used no significant reaction occurred with D-glucuronolactone, D-glucuronic acid, L-gulonolactone, or D-xylulose.

individual. On the other hand, the high serum L-xylulose levels in pentosuria are consistent with an impairment in the metabolism of the sugar.

The data on the heterozygous subjects demonstrate that pentosuria is another in the group of inborn errors of metabolism in man in which the carrier state can be demonstrated chemically (Allison and Blumberg, 1958). If one accepts the concept that pentosuria is the result of an abnormality in the enzyme which mediates the conversion of L-xylulose to xylitol, our data indicate that the single normal gene in heterozygous individuals produces sufficient enzyme for the metabolism of the L-xylulose produced under ordinary conditions. However, the loading of this pathway of metabolism, as is accomplished when a large quantity of D-glucuronolactone is administered, results in an inability of the complement of enzyme present to metabolize all of the L-xylulose produced. Indeed, even in the normal individual under these conditions some L-xylulose escapes metabolism, but less than is the case in the heterozygote.

We should like to express our gratitude to Drs. Gilbert Ashwell and Margaret Lasker for helpful advice.

References

Allison, A. C., and Blumberg, B. S., Amer. J. Med., 25, 933, 1958.

Bozian, R. C., and Touster, O., Nature, 184, 463, 1959.

Enklewitz, M., and Lasker, M., J. Biol. Chem., 110, 443, 1935.

Hiatt, H. H., Biochim. Biophys. Acta, 28, 645, 1958.

Hiatt, H. H., in The Metabolic Basis of Inherited Disease, McGraw-Hill, New York, in press.

Hickman, J., and Ashwell, G., J. Biol. Chem., 234, 758, 1959.

Horecker, B. L., and Hiatt, H. H., New England J. Med., 258, 177, 225, 1958.

Knox, W. E., Amer. J. Human Genetics, 10, 385, 1958.

Lasker, M., Enklewitz, M., and Lasker, G. W., Human Biol., 8, 243, 1936.

Touster, O., Reynolds, V. H., and Hutcheson, R. M., J. Biol. Chem., 221, 697, 1956.

Touster, O., Hutcheson, R. M., and Rice, L., J. Biol. Chem., 215, 677, 1955.

Touster, O., Mayberry, R. H., and McCormick, D. B., Biochim. Biophys. Acta, 25, 196, 1957.