The Isolation and Identification of Galactitol from the Urine of Patients with Galactosemia*

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A sensitive and convenient method for the quantitative determination of serum and urinary sugars by gas-liquid chromatography has been recently developed (1). The method is based upon the immediate and stoichiometric formation of the sugar O-trimethylsilyl ethers. These volatile derivatives are easily separated on polar or nonpolar gas chromatographic columns (2, 3).

During the routine investigation of the sera and urines from two newly admitted galactosemia patients, it was observed that the urine, in both cases, contained unusually high amounts of two nonreducing sugars. The first was found to give a single gas chromatographic peak in the monosaccharide region. It has now been identified as galactitol. The second sugar, a disaccharide, proved to be sucrose, and it has been found in the urine of a number of infants we have examined. It is suggested that an alternate route of galactose metabolism, reduction to galactitol, is present in the body, and that this pathway may be of special importance in galactosemia patients.

EXPERIMENTAL PROCEDURE AND RESULTS

Materials—Galactitol, m.p. 187.5–188.5°, was obtained from Nutritional Biochemicals Corporation, Cleveland. Galactitol hexaacetate, m.p. 168–169°, was prepared in this laboratory from commercial galactitol by the method of Abdel-Akher, Hamilton, and Smith (4).

Gas-Liquid Chromatography—The materials, columns, and conditions cited in this study have been reported earlier (1–3).

Galactosemia Patients—A 2-month-old white male infant, K.D., was admitted to the hospital with a distended abdomen, enlarged liver and spleen, and well developed cataracts. Urinalysis revealed significant proteinuria and reducing substance (negative to glucose oxidase). The patient, who had previously been fed a condensed milk formula, was placed on Nutramigen for 2 days after admission. Subsequently, he was given a meat base formula.

Serum Analysis—A serum sample was obtained from the first patient (K.D.) before dietary lactose was withdrawn. A significant amount of galactose (Table I, 0.05%) was detected in the serum by gas chromatography (Fig. 1). Galactose is a mixture of a γ isomer (possibly a furanose structure) and the α and β anomeric pyranose forms. Glucose is seen only as the two pyranose anomers, α and β (3). The retention time of β galactose (third peak) on the 15% ethylene glycol succinate polyester column is identical with that of galactitol (3); consequently, it was necessary to examine the sample on a nonpolar column, e.g. SE-52 (3). As a result, a small but significant concentration of galactitol (0.008%) was also detected in the serum (Fig. 2, Peak 5). The value of a “two-phase” (polar and nonpolar) study of identity in gas chromatography is clearly illustrated by this example. Serum from the same patient, K.D., was acquired 52 days after his initial admission. It was free of galactose and contained a normal concentration of glucose (Table I, 0.078%). At this time the lenses of the child were markedly improved in contrast to their original appearance.

The serum of the second galactosemia patient (S.H.) was obtained 48 hours after the time of lactose withdrawal. Examination of this serum revealed a normal glucose content (Table I, 0.098%), and no sign of galactose or galactitol.

Urinalysis—The urine of both patients was collected 48 hours after the removal of dietary lactose. It is interesting to note that galactose was not observed in the urine, suggesting that it is rapidly cleared by the kidney. Instead, a conspicuous single peak was found in the monosaccharide area in the urine obtained from both patients. Gas chromatographic analysis of the disaccharide area on an SE-30 column at 230° disclosed the presence of prominent amounts of a single peak accompanied by trace amounts of additional unknown peaks. A qualitative test of the urine for reducing sugars was negative. The single peak located in the monosaccharide region essentially ruled out the presence of an aldohexose because, in all cases of this class studied previously, days after birth with a history of vomiting, a convulsion (presumably from hypoglycosemia), and poor weight gain. He displayed hepatomegaly, dehydration, and cataracts. Urinalysis disclosed a highly positive proteinuria and reducing substance (negative to glucose oxidase). The patient, who had previously been fed a condensed milk formula, was placed on Nutramigen for 2 days after admission. Subsequently, he was given a meat base formula.

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at least two or more separable anomers or isomers were observed (3). The lack of retention on a mixed bed ion exchange resin eliminated the possibility of a sugar with strongly ionizable groups. Except for the hexitols, very few classes of sugars exhibit these combined properties. The retention times of the O-trimethylsilyl ether of the unknown compound on both a polar (15% ethylene glycol succinate polyester) and a nonpolar (3% SE-52) column (3) were in precise agreement with those of authentic galactitol (Fig. 3). The urine of both galactosemia patients contained similarly high concentrations of galactitol (Table I, 0.225 and 0.133%). The amount of galactitol in the urine of Patient K.D. at the time of his second admission was considerably diminished (Table I, 115 days old, 0.02%). After the initial discovery of galactitol in the urine of the second patient, daily samples of urine were analyzed. A gradual decrease in galactitol content from an initial value of 0.133% to 0.013% after 9 days was found (Table I).

Sucrose was found to be the major companion sugar of galactitol in the urine of both galactosemia patients on the basis of comparison of retention times of authentic and urinary O-trimethylsilyl derivatives on several columns (3). Both urine samples from Patient K.D. contained high concentrations of sucrose (Table I, 0.2 and 0.138% at 63 and 115 days of age, respectively). This persistent sucroruria may have been the result of the large amount of sucrose found in Nutramigen. The results observed for Patient S.H., who was given Nutramigen for 48 hours and subsequently transferred to a meat base formula, exhibited a sudden drop of sucrose content from the 49th to the 50th day (Table I, 0.14 and 0.003%, respectively).

**Separation of Authentic Galactitol and Sucrose by Paper Electrophoresis**—The effectiveness of the paper electrophoresis technique of Frahn and Mills (5), modified for use with the Wieland-Pfleiderer instrument (Brinkmann Instrument Company, New York), was investigated for the separation of galactitol from sucrose. Whatman No. 3MM paper (35 × 40 cm) soaked in 0.1 M Na2B4O7 buffer, pH 9.2, was streaked with 0.5 ml of an aqueous solution of 25 mg each of galactitol and sucrose, and a potential of 25 volts per cm was applied. The galactitolborate anion complex separated from that of sucrose by more than 5 cm in 3 hours. Galactitol borate traveled just behind the anion front, identified by spots of phenol red indicator, while the corresponding sucrose-anion complex did not move more than 5 cm from the origin. To locate the sugars, the dried paper was cut into strips and the rolled strips were each extracted twice with distilled water at 80°. The combined extracts were treated with sufficient Dowex 50 H+ to acidify the solution. The sugar along with boric acid was filtered, and water was evaporated under reduced pressure. Anhydrous methanol (50 ml) was added to the residue, and the volatile methyl borate was evaporated from the flask. The addition of methanol and evaporation were repeated twice more. The residues were dissolved in water, and aliquots of each were prepared for gas-liquid chromatographic analysis (1). The crude galactitol found near the anion front was recrystallized from aqueous ethanol, m.p. 187–188° (uncorrected).

**Isolation and Identification of Urinary Galactitol**—A 125-ml sample of urine from Patient S.H., containing an estimated 0.133% of galactitol and 0.11% of sucrose, was placed in a 500-ml Erlenmeyer flask and brought to a boil on a hot plate. The urine was cooled, and 3.0 g of urease powder, type II (800 Sumner units per g), were added. The solution was incubated at 50° for 30 minutes, and the contents of the flask were again boiled for 5 minutes, this treatment precipitated most of the protein. Further soluble protein from the urease preparation was removed by the addition of 20 ml of 2% ZnSO4 and 20 ml of

<table>
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<th>Urine</th>
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**TABLE 1**

**Analysis of serum and urine sugars from male galactosemia patients**

![Fig. 1. A comparison of the retention times of characteristic peaks obtained from gas chromatography of the sugar O-trimethylsilyl ethers from the serum of a galactosemia patient (K.D.) before institution of diet therapy, from a standard solution of galactose, and from a standard solution of glucose. A 15% ethylene glycol succinate polyester phase supported by Chromosorb W, 80 to 100 mesh, in a glass column 8 feet × 1 1/4 inch, was employed. A Barber-Colman model 10 instrument, equipped with an argon ionization detector (A-4183), was maintained at a temperature of 155° and an argon inlet pressure of 18 p.s.i. See Sweeney et al. (3) for further details.**
Fig. 2. Gas chromatography of the sugar O-trimethylsilyl ethers from the serum of a galactosemia patient (K.D.) on a nonpolar 3% SE-52 phase at 160°. The dimensions of the column and conditions are identical with those described in Fig. 1 and Reference 3.

Fig. 3. The identification of galactitol in the urine of a galactosemic patient, K.D., by the comparison of peak retention times for urinary sugar preparations (upper records) and authentic galactitol (lower records) on two columns, 15% ethylene glycol succinate polyester and 3% SE-52 silicone gum (1, 3).

1.8% Ba(OH)_2. The precipitate was removed by filtration and washed with water. The ions in the filtrate were removed by rapid treatment of the solution with 100 g of mixed bed resin. The mixture was filtered through glass wool, and water was evaporated under reduced pressure.

The crude urinary residue, containing the suspected galactitol and sucrose, was dissolved in a minimum volume of water (2.5 ml) and the total quantity was subjected to five separations by paper electrophoresis as described for the model system. The zone corresponding to galactitol was cut from the sheet for each individual separation. The paper strips were extracted with water, and the combined extracts were acidified, filtered, and freed from boric acid with methanol. The residue was dissolved in a minimum amount of hot water and 5 volumes of absolute ethanol. Crystals appeared on cooling, usually within 24 hours; the melting point was 185-186°. Recrystallization from aqueous ethanol increased the melting point to 186-187° (uncorrected). The infrared spectrum of this material was identical with that of authentic galactitol (Fig. 1).

Galactitol Hexaacetate—The urinary galactitol was acetylated with 1.5 ml of acetic anhydride and 0.1 ml of concentrated sulfuric acid. After solution of the residue, the mixture was heated at 70° for 10 minutes. The product was recovered by slowly pouring the reaction mixture into a beaker of ice water. Galactitol hexaacetate was isolated from the aqueous mixture with three extractions of diethyl ether; the combined ether extract was thoroughly washed with water and dried, and the solvent was removed by evaporation. The derivative was recrystallized from ethyl acetate, m.p. 167.5-168.5°. The infrared spectrum of the urinary galactitol hexaacetate was identical with that of authentic galactitol hexaacetate prepared in this laboratory (Fig. 5).

**DISCUSSION**

The discovery of galactitol in the urine of persons unable to metabolize galactose properly is believed to be the first report of the occurrence of appreciable amounts of a hexitol in human urine. One may tentatively assume that the galactitol arose from galactose by reduction of the anomeric carbon atom. Galactitol has not been detected in the urine of any other patients (children and adults) examined in our laboratory. An interesting feature of the urine of the two galactosemia patients analyzed in this report is the complete absence of galactose. The urine was collected in each case 2 days after removal of the subjects from dietary lactose. In contrast to the absence of galactose, an unusually high concentration of galactitol was found in the urine of both cases initially. In one patient, S.H., the data suggest that galactitol is removed from the depots at a slow rate. In this connection, galactitol is reported to diffuse across cell membranes more slowly than galactose (6).

The possible discovery of an alternate route for the disposition of unmetabolizable galactose in the galactose 1-phosphate uridylyltransferase-deficient individual raises the interesting question whether the ability to reduce galactose is due to the action of a constitutive or an inductive enzyme system. It should be interesting to determine whether the ability to reduce galactose changes with age. An explanation for the apparent amelioration...
of clinical symptoms in older galactosemia patients was advanced by Isselbacher (7), who reported the presence in mammalian tissue of an enzyme, uridine diphosphate galactose pyrophosphorylase, which could overcome the block in galactose utilization. Nevertheless, measurements of galactose oxidation in galactosemic subjects, in which galactose-1-14C was used, revealed (8) that only in a small subgroup of galactosemia patients did the ability to metabolize galactose rise. In the majority of the post-pubertal cases observed by Segal, Blair, and Topper (8), the inability to metabolize galactose-1-14C was retained although the clinical condition was dramatically improved. Since galactose labeled in carbon atom 1 with 14C does not permit evaluation of the galactitol route by the examination of respiratory CO2, it will be of great interest to examine mature galactosemia patients further in this regard. Segal and Blair (9) investigated the metabolism of galactose-1-14C in normal humans and noted a discrepancy in the level of blood 14C and that of combined glucose and galactose. These workers suggested that unknown metabolites of galactose were present in blood.

Studies of rat lenses have shown that “sugar cataract” formation is accompanied by an accumulation of hexitols in the lens (10, 11). Significant quantities of sorbitol, xylitol, and galactitol have been isolated from the lenses of diabetic rats and from those fed xylose or galactose, respectively. Van Heyningen (10) has further observed that the reduction of galactose is catalyzed by a dehydrogenase that requires reduced triphosphopyridine nucleotide as a coenzyme. In addition to lens, the only other animal tissues known to contain a similar enzyme system are placenta and seminal vesicle (12). Nevertheless, van Heyningen has reported the presence of galactitol in heart and leg muscle of the galactose-fed rat, although at lower concentration than found in the lens (10).

SUMMARY

Galactitol has been identified in the urine of two infants with galactosemia. This is believed to be the first report of the occurrence of a hexitol in human urine. Excretion of galactitol continues for a prolonged period of time after dietary galactose has been removed. The sucrosuria of the two galactosemia patients is attributed to dietary sucrose.

REFERENCES

The Isolation and Identification of Galactitol from the Urine of Patients with Galactosemia
W. W. Wells, T. A. Pittman and T. J. Egan