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Estimation of the Urinary Galactitol Level in Children by Capillary Gas Chromatography

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ABSTRACT

A rapid and efficient capillary gas chromatography was utilized to measure urinary galactitol in 133 non-galactosemic children on a free diet. The children aged from 12 days to 14 years were divided by age into six groups. The urinary galactitol concentration was found to be the highest (64.04 mmol/mol creatinine) in the neonatal group and the lowest (7.12 mmol/mol creatinine) in the group over 2 years old. It is concluded that the urinary concentration of galactitol is strongly age-dependent.

Key words: *Urinary galactitol, Galactosemia, Capillary gas chromatography*

The main pathway of galactose metabolism in humans is the conversion of galactose to glucose in several enzymatic steps. Galactose is reduced to galactitol through alternate pathways catalyzed by aldose reductase and L-hexonate dehydrogenase. Galactitol is not further metabolized but excreted by the kidney[10,13]. Measurement of urinary galactitol is important for understanding the metabolism of galactose and in the diagnosis and monitoring of diet therapy in galactosemia[2,7]. The purpose of our investigation was to define the urinary galactitol in normal children through the use of procedures that could be used in study of galactosemia, especially in the neonatal screening of galactosemia. The normal urinary galactitol level in nongalactosemic Japanese children must be determined, especially neonate, because we are specially concerned with galactosemic patients aged under 1 month or 1 month. There has not yet been a report on normal values of urinary galactitol in Japanese children. Therefore, we measured urinary galactitol in 133 non-galactosemic children and observed age-dependent phenomenon of urinary galactitol concentration.

MATERIALS AND METHODS

Urine samples were collected from 133 non-galactosemic children on a free diet (aged 12 days to 14 years old, male to female ratio = 71:62) at the outpatient department of our hospital. They were divided by age into six groups: group 1 was under 1 month of age; group 2 was 1—3 months of age; group 3 was 4—6 months of age; group 4 was 7—11 months of age; group 5 was 1—2 years of age; and group 6 was over 2 years of age. Appropriate studies have ruled out galactose metabolic disorder, liver and kidney diseases. Urine samples were measured immediately after urine collection or stored at -20°C until assay.

Galactitol, mannitol, ethylacetate, pyridine and acetic anhydride were obtained from Nacalai Tesque Inc., Kyoto, Japan. Ion exchange columns, SAX (OH^-) and SCX (H^+) (bed volume = 2.8 ml) were purchased from Analytic International Inc., Harbor city, USA. These were washed with 3 ml methanol twice and 3 ml distilled water twice before use. Gas chromatography: A Shimadzu GC-14 A gas chromatography equipped with a hydrogen flame ionization detector and interfaced with a Shimadzu C-R4A chromatopac data system was employed. The column was a J & W Science CAT capillary column (DB1 phase, 60 m×2.25 mm ID, film thickness 25 μm). The primary pressure of Helium gas was 5 kg/cm^2 and the carrier pressure was 2 kg/cm^2. The temperature of column, injector and detector were set at 230°C, 300°C and 250°C, respectively.

We modified the method described by Allen to measure urinary galactitol[1]. Two 1.0 ml aliquots of urine were taken into screw-capped test tubes. To one tube, 100 μl of internal standard solution (1 mg/ml mannitol) was added, the other being analyzed directly. The samples were then transferred to ion exchange columns (SAX and SCX). The galactitol was eluted with 1 ml distilled water twice and the eluate was evaporated to dryness at 40°C by vacuum evaporator. 100 μl of pyridine and 100 μl of acetic anhydride was added to the residue in order to be acetylated. After heating in a stoppered tube at 100°C for 1 hour, the mixture was dried in a vacuum evaporator. The dry residue was reconstituted in 50 μl of ethylacetate, of which 1 μl was

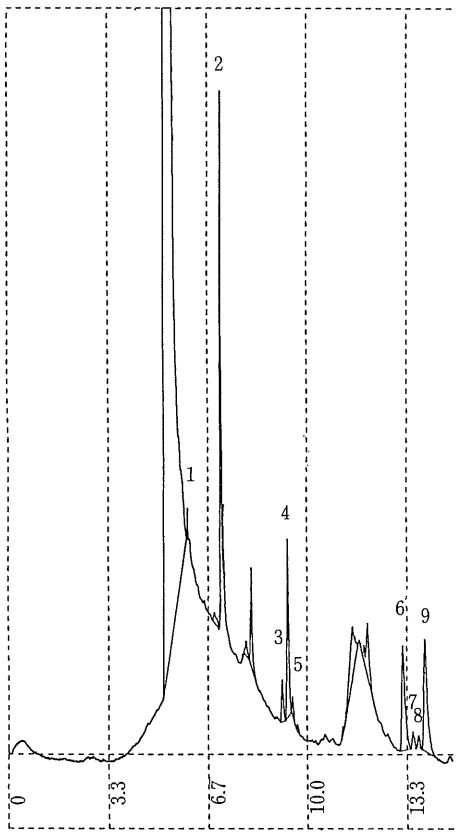

Fig.1. Chromatogram of a urine sample of non-galactosemic child. Peaks: 1: glycerol; 2: erythritol; 3: adonitol; 4: arabinitol; 5: xylitol; 6: inositol; 7: mannitol; 8: sorbitol; and 9: galactitol. Retention time of mannitol and galactitol was 13.6 and 14.0 minutes, respectively.

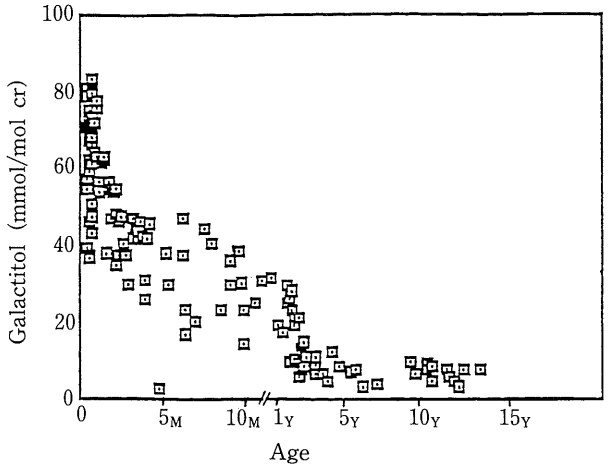

Fig.2. Age dependent reference values for galactitol in the urine of 133 non-galactosemic children on a free diet. Data are expressed in terms of creatinine.

injected into gas chromatograpy. Mannitol served as an internal standard to quantify the galactitol in urine. The ratio Gs to Ms-(Mc × (Gs/Gc)) is used, with a ratio of galactitol to mannitol peak areas in the calibration standard, in order to calculate the galactitol concentration. Where Gc and Mc are the peak areas for galactitol and endogenous mannitol respectively. Gs and Ms stand for the peak areas for the substances in the run with the addition of a known concentration of mannitol. The creatinine was measured according to Heinegard's method[6]. T-test and analysis of variance were used. Statistical significance was assigned when $p<0.05$.

RESULTS

A typical gas chromatogram of urinary polyols of a non-galactosemic individual is shown in Fig.1. GC analysis for each specimen was completed within 15 minutes and galactitol was separated satisfactorily. The peaks of galactitol and mannitol were identified by mass-spectrometry. The urinary galactitol concentration was expressed in term of creatinine. The mean value of urinary galactitol was 64.04 ± 13.95 mmol/mol creatinine for group 1 (n=34), 50.28 ± 13.61 mmol/mol creatinine for group 2 (n=22), 38.15 ± 8.78 mmol/mol creatinine for group 3 (n=17), 28.08 ± 9.44 mmol/mol creatinine for group 4 (n=17) 15.76 ± 7.76 mmol/mol creatinine for group 5 (n=20), and 7.12 ± 2.15 mmol/mol creatinine for group 6 (n=23). The mean urinary concentration of galactitol shows a decreasing tendency from group 1 toward group 6. Urinary concentration of galactitol decreased significantly ($p<0.01$ between group 1 and group 2, $p<0.01$ between group 2 and group 3, and $p<0.01$ between group 3 and group 4) below 1 year old (Fig.2). No significant difference was observed between male and female ($p>0.05$).

DISCUSSION

Because galactitol is related to galactosemia and cataracts, increasing attention has been drawn to its concentrations in urine and tissues in recent years[5,8,11,14]. However, few investigations on normal urinary galactitol value have been conducted. Studies on urinary galactitol value in non-galactosemic children have been conducted, to our knowledge, only by Pfaffenberger, et al and Jansen, et al[9,12]. Pfaffenberger, et al divided subjects into neonate, juvenile (4—14 years) and adult. Urinary galactitol in neonate was not measured satisfactorily due to interference of large amounts of myo-inositol. In Jansen's research, galactitol in urine was analyzed in 48 healthy children (aged from 0 to 15 years old). Galactitol was urinated from normal children and urinary concentration of galactitol decreased as children grew up and was apparently age-dependent. However, Jansen's research was insufficient to show galactitol levels in children under 1 year of age. We measured urinary galactitol in 133 non-galactosemic Japanese children by capillary gas chromatography and extended Jansen's previous research by including more non-galactosemic children and dividing them into six groups. In our study, the highest urinary galactitol concentration and the lowest was 64.04

mmol/mol creatinine and 7.12 mmol/mol creatinine for group 1 (0—1 month) and group 6 (over 2 years old), respectively. The galactitol value was close to that reported by Jansen, et al in group 5 (1—2 years old) and group 6 (over 2 years old). Galactitol values in other groups could not be compared with that in their study because of different group classification. Jansen and his associates divided children under 1 year of age only into two groups (0—3 months and 3—12 months groups) probably due to a limit in the number of children under 1 year of age. We divided them into four groups (0—1 month group, 1—3 months group, 3—6 months group and 6—12 months group). Urinary galactitol concentration decreased subatantially with increasing age.

Like Jansen, et al, in our study urinary galactitol was expressed in terms of creatinine. Because urine volume varies considerably according to water intake, urinary galactitol value and its range also differs markedly. It is essential to correct urinary galactitol level with creatinine to prevent erroneous estimation. Creatinine has also been used by many other researchers to correct urinary galactitol level[3,7,9,12,16]. If we had expressed our data as concentration without correction, any difference between patients or heterozygotes and controls would have disappeared. It is well-known that urinary excretion of creatinine increases only slightly with age (0—6 months: 15.3 ± 4.0 mg/kg/day; and 6—7 years: 20.2 ± 0.9 mg/kg/day), and this may be one of the factors that make the creatinine corrected value of urinary galactiotol decrease with age. However, this influence of creatinine on urinary galactitol value can be estimated by comparing the age-dependent curve of creatinine with that of urinary galactitol. Jansen, and his associates[9] showed that the slope of the age-dependency curve of urinary galactitol was much steeper than may be expected from the increase of creatinine excretion during the first years of life. In our study, the slope of urinary galactitol was much steeper than that of Jansen, et al. It is concluded, therefore, that urinary galactitol excretion is strongly age-dependent. We also collected urine samples during 9:00 am to 11:00 am for all the subjects to prevent the influence of circadian change of creatinine excretion.

The strong age-dependency for urinary galactitol may be related to the relative high intake of galactose from milky foods in the infant. It suggests also a relative low capacity to convert galactose into glucose resulting from a not yet maturated liver function and an increased conversion of galactose to galactitol simultaneously in this period[9]. Clinically, the phenomenon of transient galactosemia which usually recovers within 10 months after birth may also indicate that the ability of galactose metabolism matures with age[4,15].

Using the method described in this paper, we measured urinary galactitol levels in several galactosemic patients. They excreted about 2—5 times more galactitol depending upon different subtypes from urine than non-galactosemic children of the same age. Among them, a patient with galactokinase deficiency excreted 10 times more galactitol than control children (unpublished data).

It is interesting to note such age dependent phenomenon in galactose metabolism and this phenomenon should be taken into consideration in the diagnosis of galactosemia in children. We conclude that urinary galactitol is measurable in normal children and that a significant difference exists between age groups. Urinary galactitol concentration is strongly age dependent and decreases with increasing age.

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REFERENCES

1. **Allen, J.T., Holton, J.B. and Gillett, M.G.** 1981. Gas-liquid chromatographic determination of galactitol in amniotic fluid for possible use in prenatal diagnosis of galactosemia. Clin. Chim. Acta **110:** 59–63.
2. **Allen, J.T.** 1988. Early moring galactitol level in relation to galactose intake: a possible method of monitoring the diet in galactokinase deficiency. J. Inher. Metab. Dis. **11 Suppl. 2:** 243–245.
3. **Blau, K.** 1972. Increased mannitol excretion in controlled hereditary galactosemia. Clin. Chim. Acta **38:** 441–445.
4. **Chetham, C.H.** 1989. Transient galactosemia. Arch. Dis. Child. **64:** 637–638.
5. **Haga, H. and Nakajima, T.** 1989. Determination of polyol profiles in human urine by capillary gas chromatography. Biomedical Chromatography **3:** 68–71.
6. **Heinogard, D. and Tiderstrom, G.** 1973. Determination of serum creatinine by a direct colorimetric method. Chin. Chim. Acta **43:** 305–309.
7. **Itakura, K., Suyama, I. and Isshiki, G.** 1988. Estimation of urinary galactitol and its clinical application. Proceedings of Metabolic Disorder Screening Conference **11:** 99–100 (in Japanese).
8. **Jokobs, C.** 1988. Elevated plasma galactitol levels in patient with congenital cataracts without apparent enzyme defect. Eur. J. Pediatr. **147:** 446–449.
9. **Jansen, G., Maskiet, F.A.J., Schierbeek, H., Berger, R. and Slik, W.V.D.** 1986. Capillary gas chomatographic profiling of urinary, plasma, and erythrocyte sugars and polyols as their trimethysilyl derivatives, preceded by a simple and rapid prepurfication method. Chin. Chim. Acta **157:** 227–294.
10. **Kang, U.G., Nolan, D. and Frey, P.A.** 1975. Uridine diphosphate galactose 4-epimerase. J. Biol. Chem. **250:** 7099–7105.
11. **Mount, J.N. and Laker, M.F.** 1981. Estimation of-sugar alcohol by gasliquid chromatography using a modified acetylation procedure. J. Chromatography **226:** 191–197.
12. **Pfaffenberger, C.D., Szafranek, J., Horning, M.G.**

and Horning, E.C. 1975. Gas chromatographic determination of polyols and aldoses in human urine as polyacetates and aldonitrile polyacetates. Anal. Biochem. **63:** 501–512.

13. **Stanton, S.** 1989. Galactosemia, p.453–480. *In* C.R. Scriver, A.L. Beaudet and W.S. Sly(eds.), The Metabolic Basis of Inherited Disease. 16th ed. Mc Graw-Hill Inc Company, New York.
14. **Starnbolian, D.** 1986. Cataracts in patient with heterozygous for galactokinase defiency. Invest. Ophthalmol. Vis. Sci. **27:** 429–433.
15. **Taylor, C.J.** 1988. Transient neonatal galctosemia. Arch. Dis. Child. **64:** 637–638.
16. **Wong, N.G., Donnell, G.N. and Bergren, W.R.** 1975. Mannitol excretion in galactosemia patients. Clin. Chim. Acta **64:** 39–44.