Preliminary Study of Carbohydrates in the Urine of Manganese-deficient Guinea Pigs at Birth

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ABSTRACT Carbohydrate components of urine of manganese-deficient and control newborn guinea pigs have been determined before the animals had access to extra-uterine nourishment. Small amounts of the sugars arabinose, fructose, fucose, galactose, glucose, lactose, mannose, ribose and xylose were found in the urine of newborn guinea pigs of both manganese-deficient and control dams. Equating urine specimens on the basis of creatinine concentration revealed that slightly higher amounts of ribose occurred in the manganese-deficient progeny. The most striking difference in the carbohydrate components of urine was a threefold higher concentration of free myo-inositol in the urine of control animals at birth. Compounds were determined by paper chromatography techniques. Some speculation is made about the reduced myo-inositol content of urine of manganese-deficient young, the synthesis of glucuronic acid in fetal development, and the relation of these to connective tissue defects known to occur in manganese-deficient guinea pigs.

Earlier studies of the influence of a maternal deficiency of manganese in guinea pigs have revealed that the progeny have skeletal abnormalities at birth which are believed due to defective cartilage matrix (1). The acid mucopolysaccharides present in rib and epiphysal cartilage were significantly reduced in the manganese-deficient animals. More recent studies have shown that the young of guinea pigs receiving low intakes of manganese also have abnormalities of the pancreas. Animals which survived only a few hours had aplasia or marked hypoplasia of pancreatic tissue (2). Some congenitally deficient animals continued on the low manganese diet survived to adult age, and when these animals were given glucose intravenously, glucose tolerance was found to be abnormal (3).

In the present study, free sugars and sugar alcohols have been investigated in bladder urine of manganese-deficient and control guinea pigs at birth, as one means of exploring possible metabolic variations in the manganese-deficient animals.

The urinary excretion of sugars has been determined by several groups of investigators, and information available at this time deals mainly with the excretion of sugars by human subjects. Tower and his associates (4, 5) have investigated the excretion of pentoses in neuromuscular diseases; Woolf and Norman (6) have reported lactose, galactose, glucose, fructose, arabinose and xylose present in urine in early infancy; Howarth and Macdonald (7) have investigated reducing sugars in the urine of premature babies; and Bickel (8) has studied melitiruria in both infants and children. The excretion of several sugars occurred more often in infants under 10 days of age, and interpretations of their presence in urine included lack of kidney function in the young infant and immature liver function. The origin of some of these sugars was uncertain.

Great progress has been made in the past 15 years on knowledge of the occurrence of carbohydrates in tissues and their participation in carbohydrate-protein linkages. Glegg et al. (9) reported the presence of galactose, glucose, mannose and fucose in reticulin fibers, suggesting that these fibers contain a carbohydrate-protein complex. Neutral sugars present in fetal urine of fetal calf serum include galactose and mannose in the ratio of 3:2 (10). Recent work has revealed that galactose and xylose are linked with serine forming a carbohydrate-protein linkage of heparin.

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and protein (11). Chondroitin 4-sulfate from bovine nasal septa has been investigated and was found to be linked to protein through a glycosidic linkage between xylose and serine, with galactose also present as a constituent of the carbohydrate-protein complex (12). Arabinose- and xylose-containing complexes in human brain were first reported by Stary et al. in 1962. Two distinct polysaccharide peptides, a hyaluronic peptide containing arabinose and a chondroitin sulfate peptide containing xylose, were identified from protease-digested brain preparation in 1966 (13).

Carbohydrates appear to play an important role in the development of many tissues, and it was, therefore, believed worthwhile to determine their occurrence in the urine and tissues of newborn guinea pigs born to control and manganese-deficient dams. Preliminary data on the sugar and sugar alcohol components of urine are reported at this time.

MATERIALS AND METHODS

Information dealing with rations and general procedures for studying manganese deficiency in the guinea pig have been described in former papers (1, 14). Low manganese rations provided less than 3 ppm of the trace element, and control animals received 125 ppm manganese as the sulfate.

At autopsy immediately after birth, it was frequently observed that the bladders of guinea pigs were greatly distended and that 0.5 to 5.0 ml of urine could be collected by syringe. Urine specimens were collected in this manner before the young received extraneous nourishment. Six pooled samples of urine were prepared for the present study representing urine from a total of 9 manganese-deficient animals and 12 controls. All deficient animals tested had severe signs of manganese deficiency based on their ataxic condition. The average birth weight of deficient animals was 111 g and control young averaged 113 g. Each of the pooled urine preparations was diluted to 25 ml, and duplicate aliquots were removed for creatinine determinations (15). Pooled urine preparations were treated with urease for 24 hours at 5°. Each was then concentrated in a rotary evaporator at 35° and reduced to a volume of 3–5 ml. The total concentrated preparation was desalted by passing one-half portions, adjusted to pH 5, over resin columns containing Dowex 50-X8 (200–400 mesh), hydrogen form. Carbohydrates were removed in 100 ml of boiled deionized water using a flow rate of 1 ml/3 minutes. The combined eluate for each pooled urine preparation was then concentrated to a volume of 2 ml by means of a rotary evaporator and was next deproteinized using zinc sulfate and barium hydroxide. The protein-free filtrate was concentrated to dryness in a vacuum oven, and the carbohydrates were taken up in pyridine by heating at 100° for 10 minutes. After the pyridine was removed by drying in a vacuum oven, the final product was dissolved in 2 ml of 10% isopropyl alcohol. Preliminary tests, carried out on comparable urine preparations, revealed that aliquots equal to 160 μg of creatinine were satisfactory for most chromatograms.

Descending paper chromatography, using the following solvent systems was employed to separate urine components: Ethyl acetate–pyridine–water (40:11:6); phenol saturated with water (80:20); n-butanol–pyridine–water (6:4:3); and isopropyl alcohol–ammonia–water (70:10:20). For separation of mannose, fructose and arabinose, flavogrost was added to the ethyl acetate–pyridine–water solvent mixture at the rate of 140 mg/100 ml (16). Detecting procedures included silver nitrate, triphenyltetrazolium chloride, aniline hydrogen phthalate, p-anisidine·HCl and phloroglucinol solution (16). Because of the number of spots encountered in the urine specimen chromatograms and the wide difference in Rf values of the carbohydrate components, running times using the ethyl acetate–pyridine–water (40:11:6) solvent mixture varied from 22 hours to 400 hours. Standards of the various compounds present in urine were mixed with the prepared samples and also spotted separately and simultaneously for each paper chromatogram. Procedures for

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4 Whatman no. 1 filter paper no. 58451-1, Van Waters and Rogers, Incorporated, San Francisco, California.
expressing carbohydrate components of urine on a semiquantitative basis consisted of comparing five concentrations of the standard with the spotted urine sample and judging concentrations on the basis of visual comparisons and densitometer recordings.

RESULTS

As a result of preparing numerous paper chromatograms using the ethyl acetate–pyridine–water (40:11:6) solvent mixture and applying urine preparations equivalent to as much as 640 μg creatinine, it was found that ribose was the fastest migrating carbohydrate present. As seen in table 1 and figure 1, the urine of manganese-deficient guinea pigs contained a somewhat larger amount of ribose. This was observed for each of the three pooled urine preparations tested. A total of 9 sugars: arabinose, fructose, fucose, galactose, glucose, lactose, mannose, ribose and xylose have been tentatively identified by their mobility rates and reactions to the various detecting agents used. Quantities of sugars present in the urine of deficient and control animals at birth were much alike except in the case of ribose. Satisfactory separation of mannose and fructose was accomplished by the use of flavognost, and arabinose also separated from the two sugars under these conditions. By lengthening the running time and using the same solvent mixture (ethyl acetate–pyridine–water, 40:11:6), good separation was obtained for xylose and fucose and between glucose and galactose.

Slowly migrating carbohydrate components were first studied using ethyl acetate–pyridine–water and by increasing the running time to 200 hours and up to 400 hours. Figure 2 illustrates the migration of standard myoinositol under these conditions and the comparative amounts of myoinositol in the urine of manganese-deficient and control guinea pigs at birth. Of the three sets of urine specimens tested, the manganese-deficient sample in each case contained less myoinositol than the control specimen, varying from one-half to one-fourth the amount of the cyclitol found in matching control animals.

Spots tentatively identified as ribose and myoinositol by one-dimensional chromatography were removed and rechromatographed to investigate the possible presence of more than one compound. Findings were negative. Earlier work which included tests of inositol in urine, nerve and testis had revealed that the compound migrating similarly to standard myoinositol stimulated growth of Saccharomyces cerevisiae when added to an inositol-free medium. To date, quantitative differences in the myoinositol content of urines of manganese-deficient and control guinea pigs at birth based on one-dimensional chromatography tests

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td><strong>Carbohydrate components of urine of guinea pigs at birth</strong></td>
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<tr>
<th>Standard compound</th>
<th>Comparative information of the amounts of carbohydrate components of urine in manganese-deficient and control guinea pigs at birth based on one-dimensional chromatography tests</th>
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<tbody>
<tr>
<td>d (−) Ribose</td>
<td>2x quantity in manganese-deficient animals</td>
</tr>
<tr>
<td>d (+) Xylose</td>
<td>No distinguishable difference</td>
</tr>
<tr>
<td>L Fucose</td>
<td>No distinguishable difference</td>
</tr>
<tr>
<td>d (−) Arabinose</td>
<td>No distinguishable difference</td>
</tr>
<tr>
<td>d (−) Fructose</td>
<td>No distinguishable difference</td>
</tr>
<tr>
<td>d (+) Mannose</td>
<td>No distinguishable difference</td>
</tr>
<tr>
<td>d (+) Galactose</td>
<td>No distinguishable difference</td>
</tr>
<tr>
<td>Unidentified</td>
<td>No distinguishable difference</td>
</tr>
<tr>
<td>Unidentified</td>
<td>No distinguishable difference</td>
</tr>
<tr>
<td>Myoinositol</td>
<td>3x quantity in control animals</td>
</tr>
<tr>
<td>Unidentified</td>
<td>Higher amount in manganese-deficient animals</td>
</tr>
<tr>
<td>Unidentified</td>
<td>Uncertain</td>
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<tr>
<td>Unidentified</td>
<td>Higher amount in manganese-deficient animals</td>
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![Image](https://academic.oup.com/jn/article-abstract/96/3/283/4778833) by guest on 13 March 2019
The question of the suitability of using creatinine excretion as a basis for comparing animals of the two ration groups. Much information dealing with creatinine excretion by subjects of various ages has been reviewed by Peters and Van Slyke (17). There appears to be general agreement that creatinine excretion in infants bears the same relation to muscle mass that it does in adults. Pigs at birth have been shown by paper chromatography alone.

The identity of additional slowly migrating components, particularly the prominent spot shown in figure 2 for urine of manganese-deficient animals, has not been tested adequately to report at this time and must await additional test material.

**DISCUSSION**

The decision to explore metabolic differences in the newborn guinea pig by analyses of urinary components posed the problem of the suitability of using creatinine excretion as a basis for comparing animals of the two ration groups. Much information dealing with creatinine excretion by subjects of various ages has been reviewed by Peters and Van Slyke (17). There appears to be general agreement that creatinine excretion in infants bears the same relation to muscle mass that it does in adults. Pigs at birth have been shown by paper chromatography alone.

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does in adults. Therefore, creatinine elimination was accepted as the best available method of comparing carbohydrate components of the urine at birth. Manganese deficiency per se has not been found to influence creatinine excretion.

The finding that manganese-deficient guinea pigs at birth excreted approximately one-third the myoinositol found in the urine of control animals is believed to be of importance and may identify a handicap of metabolism during fetal growth.

One of the functions of myoinositol is that of a precursor of free glucuronic acid (18) which is needed for synthesis of supporting structures. The second precursor, uridine diphosphate glucuronic acid, is dependent on glucuronyl transferase to yield the free glucuronic acid and also requires UDPG dehydrogenase to form from UDP-glucose (18). Brown et al. (19) have studied the activity of both enzymes in fetal, newborn and adult guinea pig liver. They state, "Defects have been demonstrated in two enzymatic steps in the glucuronide-synthesizing system in the fetal and newborn guinea pig. The glucuronyl transferase activity as well as the UDPG dehydrogenase activity are markedly deficient in the fetus and gradually increase during the first few days of life." Dutton (20, 21) has also reported the early fetal guinea pig liver to have negligible glucuronide-synthesizing capacity.

The extent to which each of the two pathways for formation of glucuronic acid functions during the progressive development of the fetal guinea pig is not known; however, it seems reasonable that myoinositol may play a more important part in the synthesis of glucuronic acid and in the ultimate synthesis of supporting structures than is generally recognized. If the reduced concentration of myoinositol in the urine of manganese-deficient guinea pigs at birth is due to an inhibition in the synthesis of the cyclitol, it will be of great interest to know where this block occurs. The studies of Chen and Charalampous (22) and Eisenberg (23, 24) will be especially helpful in determining whether there is inhibition in cyclization of glucose 6-phosphate to d-myoinositol 1-phosphate or in the conversion of the latter to free myoinositol by a highly specific phosphatase. These speculations offer many challenges for the future.

The appearance of 9 sugars in the glomerular filtrate of both manganese-deficient and control guinea pigs at birth is believed to reflect both the participation of these carbohydrates in tissue synthesis of this species at birth and some defect in tubular reabsorption. The significance of the small differences in ribose noted earlier is unknown and should have additional study.

LITERATURE CITED