Ultraviolet-Absorbing Compounds in Urine of Normal Newborns and Young Children

Joel M. Vavich and R. Rodney Howell

Ultraviolet-absorbing compounds excreted in the urine of normal newborns, infants, and children have been measured by high-pressure anion-exchange chromatography. The chromatograms increased in complexity with increasing age of the subject. Preliminary data indicate that the excretion of pseudouridine and uridine was significantly higher in newborns and infants than in older children and adults. Excretion of hippuric acid was low in the newborn. Excretion of ergothioneine was lower in older children than in newborns, infants, or adults. However, hypoxanthine excretion was constant in all groups.

Additional Keyphrases pseudouridine • uridine • hippuric acid • hypoxanthine • ergothioneine • column chromatography • effects of diet • creatinine, reliability of, as excretion index

A high-pressure anion-exchange chromatographic system (Mark II), developed at Oak Ridge National Laboratory and capable of resolving over 100 peaks from urine (1, 2), is being evaluated as a tool for screening children for metabolic disease. In an initial investigation, we chromatographed samples from normal children one day to nine years old.

The importance of using age-specific control patients for such studies was suggested by the report of large variation with age in the excretion of the uv-absorbing compound uric acid (3). The use of adults as controls for a pediatric population is justified only when it has been demonstrated that age does not affect the factor under measurement.

Procedures

Urine Collection and Patient Groups

Urine was obtained from nine normal newborn males by placing a pediatric urine collector (Sterilon Corp., Braintree, Mass.) over the PHisoHexand Zephiran chloride-washed genitalia. Stroking the child paravertebrally usually resulted in reflex voiding (Perez reflex) within a few minutes (4). Males were used because the normal vaginal discharge of newborn females makes clean collections difficult. Collections were made when the infants were 12 h old (to minimize the effects of birth trauma and maternal medication) but before circumcision, which was usually done at 24 to 36 h. Two uncircumcised males had collections at 55 and 72 h.

All nine subjects were physically normal at birth, had a normal course in the nursery, and are clinically normal at this time (2 to 10 weeks of age). Variations in the pregnancy, labor, and delivery included 38 to 42 weeks of gestation, 4 to 11 h of labor, and Apgar scores of 8 to 10. Age and weight data are shown in Table 1. One child was delivered by Caesarian section; the others were all vaginal deliveries with vertex presentations. Two mothers were mildly pre-eclamptic and were treated with hydrochlorothiazide and phenobarbital near delivery. One mother had a urinary tract infection during pregnancy and was treated with sulfamethoxazole. Two mothers received meperidine-HCl just before delivery. One mother was taking chlordiazepoxide-HCl and chlorpromazine during pregnancy. There were no obvious differences in the urinary chro-
matograms of the nine newborns despite these differences in the mothers' histories, hospital course, and medications.

All the newborns began feeding 24 h after delivery, taking about 120 ml of evaporated milk, diluted 2:3, every 4 h. They also were treated prophylactically with ophthalmic penicillin ointment and 1 mg of vitamin K intramuscularly during the first 24 h.

The older children and infants included two females and nine males who had been admitted to the Pediatric Surgery service with the diagnoses of hernias (seven subjects), hand infections requiring incision and drainage (three subjects), or a small benign muscle tumor requiring excision (one subject). They were otherwise normal. All urines were obtained at admission to the hospital, before any drug therapy or surgery. In addition, two older children on a low-fat diet, one with progeria and one with essential familial hyperlipemia, Type I, were included in this study since their chromatograms were indistinguishable from those of the other children. The diet was not controlled for the other 11 patients. They had been on normal random diets at home just before urine was collected (Table 1).

Random urine specimens were obtained from all pediatric age groups because it was impractical to collect 24-h uncontaminated urines during the newborn period. In practice such urines can be collected only by restraining normal infants and collecting the urines on ice; such restraint of normal infants is ethically unwarranted. Since accurate, timed collections during the newborn period would involve continuous prolonged observation, they were not attempted. In general, only one urine specimen was obtained from each newborn. Our experience with collecting urines on large numbers of children suggests that single, random urines are the only type usually available. For these reasons, we have dealt with random fresh urines.

The adult subjects were eight normal males from Oak Ridge National Laboratory whose urine was collected for 24 h. The values in Table 1 are from a composite urine containing equal volumes of each of the eight separate urines. It therefore represents an "average normal urine," but has more peaks than routinely seen in a random adult urine.

Table 1. Data on Samples and Results of Chromatography of Normal Urines

<table>
<thead>
<tr>
<th>Substance, age, wt., mg</th>
<th>No. samples</th>
<th>Subjects' age</th>
<th>No. samples</th>
<th>Endurance</th>
<th>Serum creatinine, mg</th>
<th>Serum uric acid, mg</th>
<th>Epiphenalin</th>
<th>Pseudoephedrin</th>
<th>Uric acid (mg)</th>
<th>Hypertonic</th>
<th>Hypotonic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine specimens</td>
<td>75 ± 8 yr</td>
<td>16-72 h</td>
<td>9 ± 9 yrs.</td>
<td>2.7-4.54</td>
<td>3.46 ± 0.87</td>
<td>42.41</td>
<td>0.82 ± 0.22</td>
<td>0.92-1.05</td>
<td>&lt; 1.5</td>
<td>&lt; 1.5</td>
<td>&lt; 1.5</td>
</tr>
<tr>
<td>Fresh specimens</td>
<td>75 ± 8 yr</td>
<td>16-72 h</td>
<td>9 ± 9 yrs.</td>
<td>2.7-4.54</td>
<td>3.46 ± 0.87</td>
<td>42.41</td>
<td>0.82 ± 0.22</td>
<td>0.92-1.05</td>
<td>&lt; 1.5</td>
<td>&lt; 1.5</td>
<td>&lt; 1.5</td>
</tr>
<tr>
<td>Composite specimens</td>
<td>75 ± 8 yr</td>
<td>16-72 h</td>
<td>9 ± 9 yrs.</td>
<td>2.7-4.54</td>
<td>3.46 ± 0.87</td>
<td>42.41</td>
<td>0.82 ± 0.22</td>
<td>0.92-1.05</td>
<td>&lt; 1.5</td>
<td>&lt; 1.5</td>
<td>&lt; 1.5</td>
</tr>
</tbody>
</table>

Urine Preparation

Fresh urine specimens (normal to routine urinalysis) were filtered to remove debris, and were adjusted to pH 4.4 and to a concentration of 40 g of solids per liter. They were frozen at −47°C until chromatography. Uric acid precipitated from newborn urines under these conditions; this problem was eliminated by freezing newborn urine at a
Table 2. Column Operating Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column dimensions, cm</td>
<td>0.62 × 150</td>
</tr>
<tr>
<td>Resin</td>
<td>Bio-Rad A27</td>
</tr>
<tr>
<td>Resin size, μ</td>
<td>12-13</td>
</tr>
<tr>
<td>Sample size, ml</td>
<td>2.0-4.0</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>25°, increasing to 60° after 11 1/2 h</td>
</tr>
<tr>
<td>Operating pressure, lb/in.</td>
<td>1500-3000</td>
</tr>
<tr>
<td>Flow rate, ml/h</td>
<td>38</td>
</tr>
<tr>
<td>Eluent</td>
<td>0.015 to 6.0M ammonium acetate-acetic acid buffer, pH 4.4</td>
</tr>
</tbody>
</table>

concentration corresponding to 10.4 g of solids per liter. An amount of urine equivalent to 1 ml at a concentration of 40 g/liter was always chromatographed (e.g., 2.0 ml of 20 g/liter or 4.0 ml of 10 g/liter). A 5-ml sample-application loop was required for chromatography of urine from newborns.

Analyzer

The chromatographic system was as previously described (1) with the specific operating parameters noted in Table 2.

Calculations

Data are expressed either in terms of the heights of a peak in absorbance units or as a ratio of the peak height to the height of the creatinine peak. Peak shape does not vary significantly between chromatograms, and peak height is a good index of concentration. Calculation of concentrations in terms of milligrams, however, is not appropriate now since certain peaks are known not to be completely pure. Methods used at the Oak Ridge National Laboratory to identify urinary constituents and to establish peak purity have been previously described (5).

Results and Discussion

Chromatogram complexity. Newborn chromatograms are considerably less complex than adult chromatograms. The difference between urine from a newborn and that from an adult is shown in Figure 1. As can be seen in Table 1, the number of peaks generally increases with increasing age of the patient, which may simply reflect the increasing complexity of the diet as the child grows older. The study of the effects of various diets will clarify this point.

Creatinine. After variation in urine concentration was corrected by adjustment to 40 g of solids per liter, creatinine peak height varied as shown in Figure 2. Creatinine is eluted in a portion of the chromatogram where there is a dramatic change in pH of the effluent. Since the molar absorptivity (e) of creatinine is markedly affected by pH, calculation of creatinine concentration is similarly affected. The “creatinine peak” also contains other compounds. In spite of these variables, we have found that the creatinine concentrations as calculated from the uv chromatogram and from the Jaffe reaction bear a constant relationship. In 14 urines studied, creatinine concentrations were calculated from both the uv chromatogram and the Jaffe reaction. The values produced by the conventional Jaffe reaction were 21.8 ± 5.3% of those calculated from the uv chromatogram. We have used the values calculated from the chromatogram in our chart and tables. The developmental trends would be the same regardless of which values are used for creatinine.

The decrease of creatinine excretion as the newborn enters infancy has been previously reported (6). The increase of creatinine excretion as the infant grows is presumably due to increasing muscle mass (7).

Excretion data for the other compounds discussed below are presented as a ratio of peak height to the creatinine peak height since such creatinine...
ratios are very widely used (8) and because this normalization decreases the standard error and range for these data. No notable difference in the general shape of the curves in Figures 3 and 4 is produced by plotting absolute absorbance values, however.

We recognize that 24-h urine collection is a more accurate technique than creatinine ratios. The daily creatinine excretion can vary as much as 25%, although it is usually less (7, 9, 10). As previously mentioned, however, timed collections are very difficult in the newborn and infant. Random collections, in which creatinine is used as a reference compound ["the daily excretion of which is independent of urine volume, little influenced by diet, and only slightly increased by physical exertion" (9)] would seem to be the best alternative.

**Pseudouridine.** The height of the pseudouridine peak in the newborn and infant is about twice that for older children and adults (Figure 3). This peak appears to represent a single compound.

**Uridine.** Similarly, the absorbance of the uridine peak in the samples from newborns and infants is about twice that of older persons (Figure 3). The identity of this peak, however, is not confirmed.

**Uric acid.** Uric acid excretion was not quantitated because, in this system, measurement of its large absorbance in the young child necessitates dilution of the urine to the point at which other peaks are difficult to measure. Although this peak does not absolutely represent a single substance, it predominately reflects uric acid.

**Hippuric acid.** The peak corresponding to hippuric acid is not consistently present in the newborn and is small when detectable. It increases in size in urine from the infant and older child and is highest in urine from the adult. This probably reflects increasing amounts of benzoate in the diet (Figure 4). This peak essentially reflects only hippuric acid.

**Hypoxanthine.** The hypoxanthine peak was constant in height for urine from all age groups studied (Figure 4). The "purity" of this peak is also quite good.

**Ergothioneine.** Ergothioneine peak height was maximal in urine from the adult. Newborn and infants excreted an intermediate amount, older children the least (Figure 4). The significance of this observation is unclear. The peak is known to contain several components, and further purification may change the apparent excretion pattern.
There is great variation in the excretion of some compounds such as uric acid, carbohydrates, and amino acids in infants and children (3, 6, 8). These differences persist whether the excretion rate is expressed per 24 h or on the basis of body weight or surface area. As we plan to investigate genetic-metabolic variation in the urinary excretion of uv-absorbing compounds, it is essential that the normal patterns be established in newborn infants as well as young children. These preliminary data suggest that there are marked differences in the excretion of uv-absorbing urinary components between the child and the adult.

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References


