Ccoproporphyrin in Urine of Newborns with Meconium Aspiration Syndrome

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We evaluated coproporphyrin in the first urine passed by newborn infants with and without meconium aspiration, by spectrophotometric analysis and thin-layer and "high-performance" liquid chromatography. Urines of newborn infants without meconium aspiration contained only very small quantities of coproporphyrin, detected, after partial purification, by "high-performance" liquid chromatography. Urines of newborn infants with meconium aspiration contained large quantities of coproporphyrin, identified by all three techniques. Urinary coproporphyrin as measured spectrophotometrically correlates well with the "urinary meconium index," and the method is simple, rapid, and reliable, even for samples containing hemoglobin.

The first urine passed by newborns with meconium aspiration shows an absorption peak at 405 nm. Using this observation as a starting point, we developed a rapid biochemical test for meconium aspiration, based on the determination of the urinary meconium index (UMI). When the value of the UMI exceeds 1, there probably has been meconium aspiration (I, 2).

This test is unreliable when the urine contains hemoglobin, which gives a shift peak at 415 nm and makes impossible the direct spectrophotometry of about 10% of the urine specimens. Therefore we attempted to identify and to quantify the substance responsible for the peak at 405 nm, in an attempt to improve the specificity of the biochemical diagnosis of meconium aspiration.

Coproporphyrin was investigated because it is present in meconium (3–5) and has properties similar to those of substances contained in the first urine of newborns who have inhaled meconium-stained amniotic fluid: absorption in the Soret band between 400 and 410 nm and destruction by light (6, 7).

Materials and Methods

Specimens

Urine specimens were collected from 74 male newborns admitted to the intensive-care unit. We obtained the first urine, uncontaminated with meconium, by attaching an adhesive bag to the infant at birth. These specimens were immediately frozen at −20 °C and protected from light.

After thawing, the UMI of each sample was determined as previously described (I, 2). A test-strip method (Ames Division, Miles Laboratory, Inc., Elkhart, IN 46515) was used to identify urines containing hemoglobin, and such specimens were discarded.

Identification of Coproporphyrin

In the first stage of investigation, 10 urines of the 74 collected were selected for coproporphyrin identification. They were separated into two groups, each containing five specimens. For the first group (group I) the UMI values were <0.50; i.e., they were from newborns without meconium aspiration. The second group (group II) consisted of specimens with a UMI between 2 and 5, i.e., from newborns who had inhaled meconium.

We used three different analytical techniques for the identification of coproporphyrin: spectrophotometry, thin-layer chromatography, and "high-performance" liquid chromatography. The samples were hydrochloric acid extracts obtained after selective extraction with diethyl ether of urine made acid with acetic acid according to the method of Bellet (8) and Dobriner et al. (9).

Both the samples and their hydrochloric extracts were examined spectrophotometrically between 380 and 430 nm, with a DB-GT spectrophotometer (Beckman Instruments Inc., Fullerton, CA 92634).

For the chromatographic analysis we used the lutidine method (10), Merck silica gel plates (Merck GmbH, Darmstadt D-6100, F.R.G.), a mobile phase consisting of 2.5-lutidine/water (6/4, by vol), in a chamber saturated with ammonia vapor. The following samples were applied to the plates: 20 μL of a 5 g/L standard solution of coproporphyrin (Sigma Chemical Co., St. Louis, MO 63178); 20 μL of the hydrochloric acid extracts corresponding to urine of groups I and II; and 20 μL of these same extracts with added coproporphyrin standard. After 6 h of migration, the resolved coproporphyrin could be seen under ultraviolet (375 nm) light.

Further to identify the porphyrin, we added 1 mL of the hydrochloric acid extracts, corresponding to urine from groups I and II, to 10 mL of a 95/5 (by vol) methanol/sulfuric acid mixture. After 12 h at ambient temperature, the mixture was filtered and the porphyrin methyl esters were extracted into 10 mL of chloroform, which was washed with four 100-mL portions of water, filtered, and evaporated. The residue, containing the porphyrin methyl esters, was redissolved in 50 μL of chloroform, and 10 μL of the solution was analyzed by "high-performance" liquid chromatography (11, 12) on a 300 × 4 mm column of silica of 10 μm average particle size (Waters Associates, Inc., Milford, MA 01757). The detector was an LC 65 spectrophotometer equipped with a microcell (Perkin-Elmer Corp.). After elution for 15 min with a mixture of ethyl acetate and cyclohexane (55/45 by vol), the porphyrins were identified by comparing retention times with those of a standard mixture of porphyrin methyl esters (uroporphyrin, heptacarboxylate porphyrin, hexacarboxylate porphyrin, pentacarboxylate porphyrin, coproporphyrin, protoporphyrin, all purchased from Porphyrin Products, Logan, UT 84321).

Quantification of Coproporphyrin

In the second stage of the study, the amount of urinary coproporphyrin was determined on the 64 remaining samples by spectrophotometry in hydrochloric acid solution after extraction as previously described (8, 9). This, briefly, is as follows. Two milliliters of urine, acidified with 0.4 mL
of acetic acid, was mechanically agitated with 10 mL of diethyl ether for 10 min. Eight milliliters of the organic layer was removed and mixed with 3 mL of 1.2 mol/L hydrochloric acid. After 10 min of stirring, the hydrochloric acid layer, which contained the coproporphyrin, was separated and its absorbance measured at 402, 430, and 380 nm. The amount of coproporphyrin was then calculated from the corrected absorbance at 402 nm:

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\text{Corrected } A_{402 \text{ nm}} = \frac{2A_{402 \text{ nm}} - (A_{430 \text{ nm}} + A_{380 \text{ nm}})}{1.835}
\]

The factor 1.835 is the correction factor described by Rimington (7). The correlation between UMI values and urinary coproporphyrin concentrations was measured.

Results

Figure 1 shows the absorption spectra of one sample from group I (left) and one from group II (right) after selective extraction of coproporphyrin. The samples had UMI values of 0.25 and 2.50, respectively.

The absorption spectra of all hydrochloric extracts in group II exhibited a peak at 402 nm. By contrast, no samples in group I had an absorption peak between 380 and 430 nm.

After thin-layer chromatography, the coproporphyrin standard was visible under ultraviolet light as a rose-colored fluorescent spot (RF 0.30). An identical spot was observed in the hydrochloric extracts of urine in group I and not in the urine extracts in group II. Adding coproporphyrin standard to the urine extracts in group II did not result in any additional spots on the chromatogram.

Chromatograms obtained after "high-performance" liquid chromatography of the chloroform extracts showed that urine of both groups contained mainly coproporphyrin, but greater amounts of this porphyrin were present in the urines of group II (Figure 2).

Figure 3 shows the good correlation between the values for UMI and urinary coproporphyrin, as obtained by the spectrophotometric method. We determined that a concentration of urinary coproporphyrin of 130 nmol/L corresponds to a UMI value of 1.

To evaluate whether hemoglobin interferes with the spectrophotometric method, we measured the concentration of the coproporphyrin in urine from 10 newborn infants before and after adding hemoglobin (to give final concentrations of 0.15, 0.30, 0.60, and 1.20 g/L). This addition had no discernible effect. Urine containing 0.15 g of hemoglobin per liter did, however, give a highly positive reaction with the Ames test strip.

Discussion

Both meconium and old blood will stain amniotic fluid dark brown, so that clinical diagnosis based solely on the appearance of amniotic fluid is highly subjective. A reliable biochemical test for meconium staining would thus be welcomed by obstetricians.

In a previous study (1), we showed that the first urine passed by newborn infants who have inhaled meconium-stained amniotic fluid shows a characteristic peak at 405 nm. We suggested that this peak could have been produced by coproporphyrin, which is known to be present in meconium (4), and which might have crossed the alveolar barrier. We took particular care to avoid external contamination of our urine samples by meconium. Thus the peak at 405 nm that was demonstrated by our three different analytical methods must have resulted from the presence of urinary coproporphyrin.

Quantification of coproporphyrin in urine by "high-performance" liquid chromatography is time-consuming and requires special equipment. By contrast, spectrophotometry is simple, rapid, and reliable. It gives a good correlation with UMI values and should lead to an increase in the specificity of the biochemical diagnosis of meconial aspiration, even when the urine contains hemoglobin.
It would be interesting to identify coproporphyrin isomers so as to ascertain their origin. The principal component in meconium is coproporphyrin I; coproporphyrin III is the major form in urine from adults.