Ultrafiltration of Urine Specimens for Electrophoresis of Mucopolysaccharides, Monica C. Hsieh and Helen K. Berry (Metabolic Disease Center, Children's Hosp. Med. Center, Eliaud and Bethesda Aves., Cincinnati, OH 45229)

Mucopolysacchariduria is a feature of mucopolysaccharide (MPS) storage diseases: Hurler's (MPSI), Hunter's (MPSII), and Sanfilippo's (MPSIII) syndromes. The first step in differential diagnosis is identification of MPS by electrophoresis on cellulose acetate membranes (1).

Before electrophoresis, MPS is concentrated by precipitation with quaternary salts such as cetylpyridinium chloride or a dye such as Alcian blue (1). These tedious and time-consuming methods require periods of standing, extensive washing, and centrifugation. Low-molecular-mass species may not be isolated by these procedures.

We describe a procedure for simple, rapid ultrafiltration in a 10-mL stirred "Omegacell" (Pharmacia LKB Biotechnology Inc., Piscataway, Nj) to concentrate urine for MPS electrophoresis. After preliminary screening (2), 3 mL of each MPS-positive specimen is placed in an Omegacell and ultrafiltered through a membrane with a 10,000-Da cutoff. The cell is connected to an air line to exert a pressure of approximately 2.7 kg/cm². With magnetic stirring, 3 mL of urine can be concentrated to 0.3 mL in 10 to 15 min. Filtrate and retentate can be retested for presence of MPS (2). For most specimens, MPS was retained by the membrane; in a few, MPS was found in both filtrate and retentate. When the process was repeated with use of a 3000-Da cutoff membrane, about 45 min was required to reduce the volume of the specimen to 0.1 of the original.

Electrophoresis was carried out in a 0.1 mol/L barium acetate buffer system (pH 6.0) with constant current, 5 mA per 15 × 2.5 cm strip. After staining the strips in Alcian blue (1) and destaining in water, we transferred them to glycerol/water (1/9 by vol) and soaked them for ≥1 h. This removes residual dye and prevents breaking of the strips.

The electrophoretic pattern was identical regardless of molecular size. In a patient with MPS I, dermatan sulfate retained by the 10-kDa-cutoff membrane migrated with the dermatan sulfate that was filtered through the 10-kDa-cutoff membrane but retained by the 3-kDa-cutoff membrane. The procedure may be useful in monitoring patients for MPS of decreased molecular size after bone-marrow transplant.

The stirred cell can be re-used if it is cleaned and stored in glycerol/water (1/19 by vol) at 4 °C. Distilled water (5 mL) should be filtered through the membrane before re-use.

References

Direct Determination of Arginine-Vasopressin in Urine, A. Panzali, C. Signorini, R. Ferrari, and A. Albertini (1 Cattedra di Chimica e 2 di Cardiologia, University of Brescia, Italy)

Arginine-vasopressin (AVP) is measured, either in plasma or in urine, for differential diagnosis of chronic hypotremia, diabetes insipidus, ectopic AVP production, and psychogenic water intoxication.

Different RIA techniques have been applied to assay of AVP in extracts of plasma and urine (1, 2). However, the extraction procedures are complex and recoveries are poor and variable. Furthermore, the extraction procedure—reportedly essential for determination of low AVP concentrations in plasma—is not necessarily a prerequisite for assay of urine.

Our aim here was to develop a simple, rapid assay for urinary AVP without extraction and to compare it with methods in current use.

Twenty-four-hour specimens of human urine were collected in the presence of 10 mL of 6 mol/L HCl as preservative; aliquots were diluted two- and fourfold with the RIA buffer from a commercially available RIA kit (Bühlmann Laboratories AG, Basel, Switzerland), the pH of which was adjusted to 7.5 with drops of 2 mol/L NaOH and directly assayed with the kit. We extracted AVP from another urine aliquot by the modified method of Mehra (3), applying 500 μL of acidified, twofold-diluted urine to a Sep-Pak C₁₈ cartridge that had been washed with 3 mL of methanol, then with 3 mL of water. Weakly bound urine components were eluted with 3 mL of water and AVP with 1 mL of acidic (pH 3) ethanol. The ethanolic eluate was evaporated and reconstituted with 100 μL of 0.05 mol/L NaOH plus 900 μL of AVP RIA buffer. Analytical recovery of AVP added to urine and eluted from the column was 89–95% for various AVP concentrations.

To measure the immunoreactive AVP with or without extraction, we modified the RIA kit by using a longer incubation after the second antibody addition (60 min vs 30 min) and washing the precipitate with 1 mL of de-ionized water to decrease nonspecific binding.

Intra-assay CVs for direct analysis of control urines (n = 27, mean = 51.2 pg/mL) were 7.7% and 6.7% for samples diluted two- and fourfold, respectively.

Serial twofold dilutions of 10 urine samples showed a good linear response to dilution. Moreover, the curves for the dilutions of the urine specimens paralleled the standard curve, confirming the immunological identity of measured product and synthetic AVP (Figure 1).