Urinary Albumin and Retinol-Binding Protein in Diabetes, Robert Beetham,1 Anne Silver,2 and Anne Dowmany (1 Dept. of Chem. Immunology, Westminster Hospital, Page St., London SW1P 2AR; and Depts. of 2 Nephrology and 3 Chem. Pathol., St. Bartholomew’s Hospital, London EC1A 7BE, U.K.)

To provide information on proximal renal tubular involvement in diabetic nephropathy, we have measured both retinol-binding protein (RBP) and albumin in urine from diabetics. A mid-stream specimen of urine was provided by virtually all 84 patients attending three successive diabetic clinics, regardless of age, degree of diabetic control, or type of diabetes. Albumin and RBP in the urine were measured by radioimmunoassays (1, 2), and output was related to creatinine concentration. Reference intervals for the albumin/creatinine ratio and RBP/creatinine ratio were those described previously for ages 17–65 (1, 2).

Values for albumin excretion vs RBP excretion are plotted for the 84 patients in Figure 1. A significant number of patients had both above-normal albumin and RBP excretion (including seven with albuminuria >30 mg/mmol of creatinine, suggesting a 24-h excretion of >200 mg); 17 patients had increased values for RBP with normal values for albumin, as opposed to only seven with the reverse.

![Graph](image)

**Fig. 1.** Urinary albumin and RBP excretion by 84 diabetic patients. *Broken lines denote the upper limit of the two reference intervals.*

This was a preliminary retrospective study in a group of unselected diabetic patients, with no data available as to age or drug ingestion. Nevertheless, our data make necessary a closer look at the premise that tubular function is normal in diabetic nephropathy (3). In view of the increased RBP excretion in pregnancy (4), we find it interesting that the specimen with the very increased RBP excretion but normal albumin excretion was from a pregnant patient.

**References**

**Source of Error in the Assay of Urinary Orotic Acid, P. Kamoun, M. Coudé, C. Deprun, and D. Rabier (Dept. d’Investigation Métabolique des Enfants Malades, Hôpital des Enfants Malades, 149 rue de Sèvres, 75743 Paris Cedex 15, France)**

Colorimetry of orotic acid in urine involves a series of chemical reactions after protein has been removed from the sample. Orotic acid is purified by rapid liquid–liquid column chromatography (1) and the dried residue of the chloroform–amyl alcohol eluate is used in the following procedures. Orotic acid is brominated to dibromobarbituric acid, which is then condensed to dimethylaminobenzaldehyde to form 5-(p-dimethylbenzylidene) barbituric acid (2). This colored product is extracted into an immiscible solvent and its absorbance determined. In the method of Kesner et al. (1) no blank was used.

We have performed 100 such assays of urinary orotic acid, but with use of a blank in which the bromide water is replaced by distilled water. In doing so, we encountered some compounds that also form colored derivatives with p-dimethylaminobenzaldehyde; the absorbance of the blanks ranged from 0.012 to 0.220 A (mean 0.063, SD 0.041). Adding 40 nmol of orotic acid to each urine sample before liquid–liquid chromatography gave a constant absorbance difference of 0.480 A. For 90 urine assayed by the method with the blank, the mean (±SD) urinary excretion of orotic acid was 1.48 ±1.30 (range 0.1 to 5.0) mmol/mol of creatinine; by the method of Kesner et al. it was 4.81 ± 2.84 (range 1.2 to 15.0) mmol/mol of creatinine. We believe the values obtained by the latter method are overestimated, and we recommend use of the blank in this assay.

**References**
To minimize the effects of these inhibitors by simple dilution, I increased the sample/reagent ratio to 1/5 (20 μL in 1.0 mL) at the same time prolonging the incubation at 37 °C to 6 h to obtain adequate sensitivity. Because of the large dilution, sample blanks were negligible (<0.010 A; 0.3 U/L) and were not corrected for. Non-enzymic substrate hydrolysis as a result of the prolonged incubation was, however, substantial (0.070 A; 2.0 U/L) and appropriate reagent blanks were required. Incubation was terminated by adding 1.0 mL of 0.4 mol/L glycine–NaOH, pH 10.5.

Results by my method and method I were compared with those obtained after gel filtration (II). Linear regression analysis of results from 64 randomly collected urines from diabetic patients yielded the following equations: y (my method) = 0.982x + 0.2 (r = 0.995, range 0.9–28.9 U/L) and y (method I) = 0.834x + 0.4 (r = 0.985, range 1.1–28.2 U/L) when compared with method II. Mean results by methods I, II, and mine were 9.77, 11.23, and 11.26 U/L, respectively. Thus results by method I were significantly lower (up to 27%; paired t-test, p < 0.001), while those by my method were not significantly different from those by method II (p > 0.5).

Evidently simple dilution is as effective as gel filtration in decreasing the effects of inhibitors in urines. My method is further simplified because sample blanks are not necessary as a result of the large dilution except when specimens are very pigmented.

References

Revised Method of 1H-NMR Urinalysis for Detecting Inborn Errors of Metabolism, Hideaki Yamamoto,1 Naoya Koda,2 Shuichi Yamaguchi,3 Yoshikatsu Eto,3 and Kikumaro Aoki2 (1 Dept. of Radiology, 2 Div. of Endocrinol. & Metab., Saitama Children’s Medical Center, Iwatsuki, Saitama, Japan; 3 Dept. of Pediatrics, Jikei Univ. Sch. Med., Minatoku, Tokyo, Japan; and 4 Research & Development Dept., Aiiku Maternal and Child Health Ctr., Minatoku, Tokyo, Japan. Address correspondence to S. Y.)

Urinalysis by nuclear magnetic resonance (NMR) is currently successfully applied to screening or diagnosis of inborn errors of metabolism (2–4). In the method previously described by ourselves and others, we could not detect signals located in the field of suppressed water signals (chemical shift: 3.5 to 5.5 ppm). We have devised a new method of sample preparation to reveal these missing signals.

Previously (2), we mixed 0.45 mL of urine with 0.05 mL of trimethylsilylpropionate-2,2,3,3-d4 (TSP) 10 g/L in 2 H2O as internal standard, and performed NMR by a homogated decoupling method (method A). We now take a portion of lyophilized urine sample and dissolve it in 0.5 mL of dimethyl sulfoxide (DMSO) containing trimethylsilylane (TMS) 10 mL/L; the decoupling method was not used (method B). 1H-NMR spectra were recorded at 9.45 MHz with a JEOL FX-90Q NMR spectrometer (2). Chemical shifts (ppm) were referenced to TSP or TMS. For a patient with glycolate kinase deficiency, who excreted glycolate in urine (5), 1H-NMR spectra by method A (Figure 1A) showed only signals for water in the shift region of 3.5 to 5.5 ppm, but the spectra by method B showed signals of glycerol (3.30, 4.38 ppm) and water (3.50 ppm) (Figure 1B). Glycerolactone was not detected by method A, but was detected by method B. The water signal in method B was from a contaminant in the DMSO solution and was shifted with respect to that in method A, owing to the chemical interaction of water and DMSO in method B. Except for glycerol, we could detect signals such as those for glycolic acid (3.90 ppm) that were not apparent in the previous procedure and chemical compounds that have multiple signals (e.g., gly- cine: 2.17, 2.46, 3.80 ppm). We therefore recommend that NMR urinalysis for detecting inborn errors of metabolism should be performed by both methods A and B.

Fig. 1. 1H-NMR spectra of glyceroluria
A method A; B method B. Signals of glycolic are indicated by X

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References