A Simple and Rapid Method for the Paper Electrophoretic Determination of Urinary Proteins

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A method is described for the simple, rapid, and accurate determination of paper electrophoretic patterns of urinary proteins using polyethylene glycol (Carbowax 20 M) for the concentration of the urine. The electrophoretic patterns of sera with normal and abnormal protein constituents did not change when added to a protein-free urine and subjected to the method described. The electrophoretic determination of urinary proteins as a routine procedure may give an insight into the physical type of permeability disturbance in the kidneys of a patient.

A number of attempts have been made to determine urinary proteins by electrophoresis, in diseases with renal leakage of normal or abnormal serum proteins (1-5). It appears, however, that none of the methods used in these determinations were simple enough to permit paper electrophoresis of urinary proteins as a routine procedure. The advantages of performing such determinations on a large scale in order to estimate the permeability defect of the glomerular membrane are obvious. It remains to be seen whether these defects differ in different renal diseases or stages of diseases and whether or not a typical pattern can be correlated with such conditions. Hardwicke and Soot-hill (4) believe that certain leakage patterns can be related to certain specific morphologic alterations (e.g., membranous glomerulonephritis).

It must be remembered in this connection that a proteinuria of identical magnitude can be caused by a large number of mildly protein-

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leaking nephrons on one hand or by a small number of nephrons with severe protein leakage on the other. In such circumstances, however, the ratio between proteins of a relatively small size (e.g., albumin) and those of a large size (e.g., gamma globulin) would differ considerably. In addition, the question of loss of antibody gamma globulin into the urine in certain renal diseases can be approximated if electrophoretic determinations of urinary proteins are done routinely. It appeared therefore important to develop a method for the simple, rapid, and inexpensive electrophoretic determination of urinary protein.

**Procedure**

The urine specimens are centrifuged for 30 min. at 2000 r.p.m., decanted, and filtered through Whatman No. 40 paper. The protein concentration of each specimen is determined by the Biuret method (6). Calculations are then made to determine the concentration necessary to bring the protein in the urine to approximately 5 gm./100 ml., which is needed to obtain a distinct paper electrophoretic pattern. The initial volume of urine is selected so as to give a final volume of approximately 1 ml. after concentration. This amount is then encased in a bag of Visking* cellulose dialysis tubing which is then suspended in a bath of 40% polyethylene glycol (Carbowax† 20 M, mol. wt. 20,000) dissolved in Veronal buffer, pH 8.6 and ionic strength 0.075. The bath is agitated continuously by a magnetic stirrer at 4°. In approximately 5 hr., the necessary concentration of the urine is reached and the specimen can be applied to the paper strip. Occasionally a cloudy precipitate appears in the urine after concentration. This precipitate can be removed by centrifugation for 5 min. at 3000 r.p.m. It does not contain protein and its removal does not affect the electrophoretic pattern.

The Beckman-Spinco Durrum§ cell was used for the present electrophoresis studies and the Beckman RB Analytrol§ for the quantitative analysis of the paper strip after staining with alcoholic bromphenol blue.

In order to see whether the procedure itself alters the protein pattern in the urine, 0.5 ml. of a serum with 8.5 gm./100 ml. of proteins which contained an abnormal myeloma protein was added to 9.5 ml. of a urine which by itself showed no electrophoretic mobility when con-

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*Visking Corp., Chicago, Ill., seamless dialyzer tubing 1½ in. wide.
†Union Carbide Chemical Co., Charleston, W. Va.
‡15.4 g. sodium barbital, 2.76 g. barbituric acid to 1000 ml. H₂O.
§Beckman Instruments, Inc., Palo Alto, Calif.
centrated 10 times. This prepared sample was handled as described above and subjected to paper electrophoresis at the same time as the original serum. The results are shown in Table 1. The table indicates that the procedure itself does not introduce an error beyond the inherent error of the paper electrophoretic method. Other experiments using sera containing normal and abnormal proteins, and normal and abnormal concentrations of proteins again did not show any significant deviation from the type of results shown in Table 1.

The dialysis of the urine against polyethylene glycol (Carbowax 20 M) dissolved in distilled water did not yield different results from the dialysis against polyethylene glycol dissolved in the Veronal buffer. It was felt, however, that the latter method was preferable to maintain a uniform buffer system throughout the procedure. Carbowax 6000 (mol. wt. 6000) diffuses through the membrane of Visking tubing, as can be shown by precipitation with 20% trichloroacetic acid. We were able to show, as previously reported by Tombs et al. (7) that the diffusing polyethylene glycol did not alter the electrophoretic pattern of the urinary protein. It was felt, nevertheless, that the use of a non-diffusing Carbowax would be preferable. Therefore, we used Carbowax 20 M (mol. wt. 20,000) in our studies, which does not diffuse through the membrane of the Visking tubing used in our laboratory. It thus appears that this method does not lead in itself to any changes in the protein pattern.

Most recently, Hardwicke and Soothill (4) and Slater and McKay (8) have shown with immunochemical methods that normal urine may contain a gamma globulin of a very small molecular size, possibly derived directly from tubular protein breakdown. While the presence of such a protein will require further confirmation, it is obvious that such a moiety of small molecular size would not appear in a paper electrophoretic pattern since it might be lost into the bath fluid during the concentration process. Since urinary protein determinations will serve

Table 1. Results of Electrophoresis by Using Polyethylene Glycol for Concentration of Urine

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Original serum (%) of total protein</th>
<th>Urine plus serum (%) of total protein</th>
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<tbody>
<tr>
<td>Albumin</td>
<td>26.3</td>
<td>25.2</td>
</tr>
<tr>
<td>Alpha-1 globulin</td>
<td>3.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Alpha-2 globulin</td>
<td>4.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Beta globulin</td>
<td>6.8</td>
<td>7.0</td>
</tr>
<tr>
<td>Gamma globulin</td>
<td>59.4</td>
<td>59.4</td>
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essentially to determine plasma protein clearances and the permeability of the glomerular membrane, the loss into the urine of proteins of such small molecular weight, which do not appear in the plasma protein pattern, may be considered an advantage rather than a disadvantage.

References