AN ELECTROPHORETIC STUDY OF URINARY PROTEIN IN THE RAT*

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The administration of renin to the rabbit or the rat causes marked changes in the composition of the urine (1–3). The adult female rat normally excretes 0.1 to 0.2 mg. of protein in its urine per hour, while the adult male rat excretes from three to five times this amount (4). However, in the hour following the intraperitoneal injection of 4 Goldblatt dog units of hog renin the excretion of protein increased to an average value of 31.7 mg. (5). Gilson (6) fractionated the urinary protein of normal rats with ammonium sulfate, and found approximately equal quantities of albumin and globulin. Wicks (7) found traces of albumin and globulin in normal mouse urine, but concluded that the greater portion of the urinary protein consisted of nucleoprotein. Parfentjiv and Perlzweig (8) reported that the protein present in the urine of normal male mice was a chondromucoid substance. The nature of the protein excreted in the urine following renin administration is not known. The present report describes an electrophoretic study of normal urinary proteins in the rat, and of the proteins appearing in the urine following the administration of renin.

Methods

Adult male and female albino rats of the Slonaker-Addis strain weighing from 150 to 200 gm. were used in these experiments. These animals were maintained on a diet containing 17 per cent protein and adequate quantities of all of the known essential food factors.

Preparation of Normal Rat Urinary Proteins.—Groups of fifteen animals of the same sex were taken from the colony at 4:00 p.m. and placed in individual metabolism cages. The voided urine was collected during the ensuing 17 hours. The technique used for the collection of uncontaminated urine has been described elsewhere (2). During the overnight urine col-

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selection period, the animals subsisted on a solution containing 15 per cent glucose, 0.4 per cent sodium chloride, and 0.5 per cent of a solution of B vitamins (betaplexin (R)). The pooled overnight urine was clarified by filtration at 2°C. This crystal clear filtrate was placed in cellophane bags and dialysed for 24 hours against running distilled water in the cold. The dialyzed urine was then lyophilized, weighed, and stored in a desiccator over P₂O₅ for later use in the experiments described below.

Preparation of Urine after Renin Administration.—Animals that were to receive renin were treated precisely as indicated above. At 9:00 a.m., after termination of the overnight urine collection period, the bladders of these animals were emptied by allowing them to inhale ether while pressure was exerted over the bladder area. They were then injected intraperitoneally with 4 Goldblatt dog units (9) of hog renin¹ in 4 ml. of 0.85 per cent sodium chloride solution. The urine excreted during the following 60 minutes was collected. An aliquot of this urine was removed for the estimation of total protein by a modification of Kingsley’s biuret method (10). The individual urine samples were then frozen, and in some instances lyophilized, after dialysis. Urine excreted following renin administration will be referred to as “renin urine.”

Preparation of Serum.—Blood was obtained by simultaneously severing the abdominal aorta and the inferior vena cava while the animal was under light ether anesthesia. The blood was allowed to clot at room temperature for 60 minutes, and after centrifugation the serum was removed with a pipette. After aliquots were taken for total protein determinations (10) the individual sera were stored in the frozen state.

Electrophoretic Analysis.—All electrophoretic analysis were carried out at 0°C. in the Perkin-Elmer Tiselius apparatus, employing a 2 ml. cell. Photographs of the ascending and descending boundaries were obtained by the scanning method. All samples were dialyzed against buffer for 2 to 4 days prior to analysis. Two buffers were employed: the usual 0.1 M veronal buffer, pH 8.6 (11) and the 0.025 M veronal buffer, pH 8.6, described by Miller and Golder (12). The more dilute buffer, which was used for most of the studies, permits better resolution of albumin and α₁-globulin in rat serum.

Serum samples were diluted 3.5 times with buffer before dialysis, “renin urine” was dialyzed without dilution, and normal or renin urinary proteins were dissolved in buffer in concentrations of 30 to 50 mg. per ml. prior to dialysis. In some instances, normal urinary proteins were added to normal serum and to “renin urine” to obtain information on their mobility characteristics in the presence of other proteins. The proportions employed are described below. It should be noted that prior freezing or lyophilization was not found to have any significant effect on the electrophoretic patterns of rat serum or “renin urine.”

Calculations of the percentage distribution of proteins in the samples analyzed were made from enlargements of the ascending boundaries. Estimations of the electric mobilities of the proteins present were obtained from the descending patterns.

RESULTS

The Influence of Renin on Urinary Excretion of Proteins.—The difference in protein excretion between male and female normal rats of the Slonaker-Addis strain has been described previously (4). Fig. 1 shows that this protein, in both male and female rats, consists of two major components. Experiments elucidating the nature of these proteins will be described below.

The administration of renin resulted in a tremendous increase in protein

¹ We are indebted to Drs. Harry Goldblatt, Erwin Haas, and Hildegard Lamfrom for the renin used in this study.
excretion in the rat (2). All of the serum proteins appear to be excreted, with the possible exception of $\alpha_2$-globulin. Thus, electrophoretic patterns of urinary proteins following renin administration differed strikingly from those of normal urinary proteins, but bore a close resemblance to normal serum protein patterns (Fig. 2). The percentage distribution and electric mobilities of the various protein components in normal serum and "renin urine" are given in Table I. The mobilities of the various urinary proteins appeared to be slightly greater than their analogs in the serum. However, the only significant difference in mobility occurred in the comparison of serum and urinary "albumin" ($P < 0.01$). The apparent differences in mobility of the "globulin" fractions were not significant ($P > 0.6$).

The Nature of Normal Urinary Proteins.—As pointed out above, normal rat urine usually exhibited two major protein fractions when submitted to electrophoresis patterns of normal rat urine. (A), (B), male (1, major fraction; 2, minor fraction) (C), (D), female. Arrows pointing to the right indicate ascending patterns; arrows pointing to the left indicate descending patterns.

In unpublished experiments performed in 1949, Dr. Eloise Jameson and Mr. Harry Barnett in collaboration with some of the authors (A. L. Sellers, S. Smith, J. Marmorston, and H. C. Goodman) obtained substantially the same result.
phoretic analysis (Fig. 1). The larger and less rapidly moving fraction constituted from 65 to 100 per cent of the total protein present. The electric mo-

Fig. 2. Electrophoresis patterns of serum and urinary proteins. (A), normal rat serum; B), (C), (D), rat urine following renin injection in each of three separate animals.

bilities calculated from four separate samples were $3.69 \pm 0.51$ s.d. cm$^2$/volt/sec. $\times 10^4$ for the major fraction and $5.87 \pm 0.36$ cm$^2$/volt/sec. $\times 10^4$ for the lesser fraction. No significant differences were noted between the urinary protein of male and female rats. The mobility of the major fraction most closely
resembles that of serum $\alpha_2$-globulin, while the lesser fraction has a mobility that lies approximately midway between serum $\alpha_1$-globulin and serum albumin.

It seemed likely that the electric mobilities of these normal urinary proteins might be somewhat different in the presence of other rat proteins, and that further information regarding their identity might be obtained by conducting electrophoretic analysis on renin urine and normal serum samples to which lyophilized normal urinary protein had been added.

Individual samples of normal rat serum, diluted with 2.5 parts of buffer, were divided into 3.5 ml. aliquots. After dialysis, an electrophoretic pattern was obtained on one aliquot consisting of normal serum only. To the other aliquot of serum, 75 mg. of the normal urinary protein was added, the solution dialyzed, and an electrophoretic diagram of the mixture was obtained (Fig. 3, (A), (C)). It may be seen that normal urinary protein added to serum produced its peak primarily at the $\alpha_1$-globulin position with some overlap at the $\beta$-globulin border. The albumin peak of normal rat serum did not appear to be increased by the addition of normal rat urinary protein.

In a similar fashion, individual samples of renin urine were divided into 3.5 ml. aliquots. After dialysis, an electrophoretic pattern was obtained on one aliquot consisting of renin urine only. To the other aliquot, 75 mg. of normal urinary protein was added and an electrophoretic diagram was similarly obtained. Again, the normal urinary proteins added primarily at the "$\alpha_1$-globulin" position with some overlap at the "$\beta$-globulin" border (Fig. 3, (B), (D)).

It can be concluded from these experiments that the proteins contained in normal urine have electrophoretic mobilities similar to serum $\alpha_1$- and $\beta$-globulins.

Additional evidence for the globulin character of the major portion of normal urinary proteins was obtained by studying the fractional precipitation of this material by increasing concentrations of ammonium sulfate. The results obtained are shown in Table II. It is apparent that the major proportion of the

## Table I

<table>
<thead>
<tr>
<th>Component</th>
<th>Electric mobility</th>
<th>Percentage composition</th>
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<tbody>
<tr>
<td></td>
<td>Normal serum</td>
<td>Renin urine</td>
</tr>
<tr>
<td>Albumin</td>
<td>6.01 ± 0.19</td>
<td>6.89 ± 0.54</td>
</tr>
<tr>
<td>$\alpha_1$-Globulin</td>
<td>5.38 ± 0.12</td>
<td>5.48 ± 0.35</td>
</tr>
<tr>
<td>$\alpha_2$-Globulin</td>
<td>4.30 ± 0.27</td>
<td>12.4 ± 1.8</td>
</tr>
<tr>
<td>$\beta$-Globulin</td>
<td>2.81 ± 0.20</td>
<td>3.09 ± 0.44</td>
</tr>
<tr>
<td>$\gamma$-Globulin</td>
<td>1.97 ± 0.11</td>
<td>2.14 ± 0.47</td>
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* The results are expressed as the mean ± the standard deviation.
‡ $1 \times 10^3$ cm.$^2$/volt/sec.
§ Average of 15 rats.
|| Average of 6 rats.

The albumin peak of normal rat serum did not appear to be increased by the addition of normal rat urinary protein.

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Fig. 3. Electrophoresis patterns of (A) normal rat serum; (C) normal rat serum plus 75 mg. of normal urinary protein; (B) renin urine; and (D) renin urine plus 75 mg. of normal urinary protein. Arrows indicate position of added protein.

<table>
<thead>
<tr>
<th>Ammonium sulfate (per cent saturation)</th>
<th>Protein precipitated (per cent of total)</th>
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<tbody>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>25</td>
<td>5.0</td>
</tr>
<tr>
<td>50</td>
<td>66.7</td>
</tr>
<tr>
<td>100</td>
<td>28.3</td>
</tr>
<tr>
<td>Supernatant</td>
<td>0</td>
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</tbody>
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* Urinary protein pooled from 15 male rats and 15 female rats was used for these studies. The ammonium sulfate was added to a 2 per cent solution of the dialyzed, lyophilized urine.

proteins present in the urine of both male and female rats have solubility characteristics similar to serum α- and β-globulins; i.e., precipitation at 50 per cent
saturation with ammonium sulfate. A small additional portion of 5 to 11 per cent had the solubility characteristic of serum $\gamma$-globulin, i.e., precipitation at 25 per cent saturation with ammonium sulfate. In addition, some protein having the solubility characteristics of serum albumin appeared to be present in the urine of the male rat.

**DISCUSSION**

Results of the present investigation suggest that the major portion of the urinary proteins normally excreted by rats of the Slonaker-Addis strain are similar in their electrophoretic characteristics to serum $\alpha$- and $\beta$-globulins. Occasionally, albumin or $\gamma$-globulin may be present in small amounts. These conclusions are based on determinations of electrophoretic mobility as well as on studies of the fractional precipitation of urinary proteins by ammonium sulfate.

The urinary proteins normally excreted by the rat have been shown to come from the kidney and do not represent contamination from the lower urinary tract (4). Furthermore, there is no evidence to indicate that proteins are added to the urine by tubular secretion. It appears most probable, therefore, that proteins normally enter the urine by passage through the glomerular membrane. If, as indicated in these studies, the glomerular membrane of the rat normally allows passage of large serum globulin molecules, it would seem likely that the much smaller serum albumin particles would enter the glomerular filtrate even more readily. The relative paucity of albumin in normal urine may be interpreted to mean that this protein is more completely reabsorbed by the cells lining the convoluted tubules than is serum globulin.

Following renin administration, a massive proteinuria occurs (2). The proteins excreted are similar in electric mobility and relative distribution to those of normal rat serum, although "albumin" is present in a higher relative concentration in urine than in serum and has a somewhat greater mobility. The action of renin can be explained as being due either to a marked increase in glomerular permeability, so that the tubules are suddenly presented with more plasma protein than they are able to reabsorb, or to inhibition of the tubular reabsorption of protein. The data that have been presented would appear to be consistent with either possibility.

**SUMMARY**

The nature of the proteins present in the urine of the normal rat has been investigated by electrophoretic analysis and by fractional precipitation of these proteins by ammonium sulfate. Components similar to serum $\alpha$- and $\beta$-globulin constitute the major portion of the urinary protein in both male and female rats.

Following the intraperitoneal injection of renin, a massive proteinuria oc-
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curs. The proteins excreted are similar in proportion and electric mobility to those of normal rat serum.

BIBLIOGRAPHY