STUDIES ON THE MECHANISM OF EXPERIMENTAL PROTEINURIA*

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PLATES 30 AND 31

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Proteinuria may be produced in the experimental animal in a variety of ways. One of the simplest and most convenient methods is injection of the kidney enzyme, renin.

Pickering and Prinzmetal (1) were the first to notice that proteinuria was increased following renin administration to the rabbit. Brandt and Gruhn (2) confirmed this finding, and by studying the simultaneous excretion of injected hemoglobin, concluded that renin does not significantly alter the glomerular permeability, but rather increases proteinuria by diminishing tubular reabsorption of protein from the glomerular filtrate. Shortly after this, Addis, Barrett, Boyd, and Ureen (3) discovered that renin produced an intense, though transient, proteinuria in the rat. Since inactivation of renin, with respect to its pressor effect, led to a loss of its capacity to induce proteinuria, and since repeated injections of renin, at suitable intervals, led to a diminution in the proteinuric as well as the pressor property of renin, these workers concluded that this form of proteinuria is dependent on the pressor action of renin. Rather and Addis (4) reported that the athrocytosis of simultaneously administered hemoglobin or bovine albumin by the cells lining the proximal convoluted tubule is not inhibited by renin, and stated that Brandt and Gruhn’s hypothesis, that renin inhibits the tubular reabsorption of protein, is not supported by their experiments. More recently, Lippman, Ureen, and Oliver (5) have restudied the effect of renin on the excretion of injected hemoglobin. From a combination of morphological and functional data these workers conclude that renin causes an increase in glomerular permeability to hemoglobin and decreases the tubular reabsorption of this substance. Hence, conflicting views are expressed concerning the mechanism whereby renin produces an increased excretion of protein in the urine.

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The present study was instituted to clarify certain views expressed above, and presents convincing evidence that the proteinuric property of renin is in no way dependent upon its pressor action.

Methods

Male and female albino rats of the Slonaker-Addis strain were used.

Blood Pressure Determinations.—Blood pressure was determined by direct cannulation of the carotid artery. Continuous kymographic tracings were obtained with an inked pen arm connected to a float which rested on the mercury column in one arm of a U tube manometer. The other arm of the manometer was connected to the animal through a No. 20 gauge tygon tube. The system was adequately heparinized prior to use and a pressure bottle, which could be opened into the apparatus, permitted the introduction of additional heparin into the animal and allowed for calibration of the system. Though this apparatus was rather insensitive to pulse pressure variations, an accurate recording of the mean arterial blood pressure could be obtained. Light ether anesthesia was maintained during the blood pressure determinations.

Operations.—Adrenalectomy was performed by the paravertebral approach under light ether anesthesia. All adrenalectomized animals were maintained on 1 per cent sodium chloride solution drinking fluid and fed the usual stock diet for a period of 5 days before being used in the experiments.

Protein Determinations.—Urines were analyzed for protein by a modification of Kingsley's biuret method (6).

Urine Collection.—Animals were taken from the colony at 4 p.m. and placed in individual urine collection cages. During the ensuing 17 hours, the rats subsisted on a 0.4 per cent sodium chloride solution containing 15 per cent glucose and 0.5 per cent of a solution of B vitamins (betaplexin (R)). Beginning at 9 a.m. the following morning, the animals received hog renin diluted in 0.89 per cent sodium chloride solution in the amount and by the route desired. Uncontaminated urine was collected in the manner described by Addis et al. (3). Control animals received equivalent volumes of 0.89 per cent sodium chloride solution.

RESULTS

Large doses of renin can be administered intramuscularly to the rabbit (7) and the dog (8) without significantly altering the mean arterial blood pressure. It was anticipated that this route of administration might provide a means of separating the pressor effect of renin from its proteinuric effect in the rat.

11 rats were used in this experiment. Each animal was anesthetized with ether and its carotid artery cannulated as described above. After a suitable base line period, 4 Goldblatt dog units of hog renin in 0.25 ml. of 0.89 per cent sodium chloride solution was injected into the muscles of the thigh. The mean arterial blood pressure was recorded for a period of 60 minutes. At the conclusion of each experiment a pressor response was elicited by an intravenous injection of 1 unit of renin in order to be certain that the renin was active and the animal normally responsive. Blood pressure changes of less than 10 mm. mercury were not considered significant since control animals, whose blood pressures were measured in a similar manner, showed frequent spontaneous alterations of this degree. Of the 11 animals studied, we are indebted to Drs. Harry Goldblatt, Erwin Haas, and Hildegard Lamfrom for the renin used in this study.
one had a blood pressure elevation of 18 mm. mercury and one of 13 mm. mercury. Blood pressures of the remaining 9 rats showed no significant change in the hour following the intramuscular injection of 4 units of renin. 4 additional animals had their blood pressure recorded for 120 minutes following the intramuscular administration of renin. In none of these animals was a significant blood pressure elevation recorded during the 2 hour period.

From these data we conclude that the intramuscular administration of renin results in little or no blood pressure elevation in the rat during a period of 120 minutes.

We then studied the proteinuria of rats receiving renin intramuscularly.

The rat normally excretes from 0.2 to 0.8 mg. of protein per hour in its urine (Table I). In the hour following the intraperitoneal injection of 4 units of renin, an average of 31.7 mg. of protein is excreted (9). It has been shown that the peak of proteinuria following the intraperitoneal injection of renin occurs during the 1st hour, and that animals excrete very little additional protein during the 2nd hour after injection (3). Following the intramuscular injection of 4 units of renin, the rat excretes an average of 10 mg. of protein in the 1st hour; another group of animals excreted 46 mg. in a 2 hour period; while a third group excreted 43 mg. in a 3 hour period.

Thus, a massive proteinuria occurs in the rat following the intramuscular injection of renin, and the peak of this proteinuria is reached during the 2nd hour following its injection. Little or no protein is excreted during the 3rd hour after injection. We conclude that when renin is administered intramuscularly, massive proteinuria occurs in the absence of significant blood pressure elevation.

A second line of evidence pointing to a dissociation between the pressor and proteinuric properties of renin has been obtained from studies on the adrenalectomized rat.

After observing the base line blood pressure, 5 normal control animals were given 1.0 Goldblatt dog unit of renin in 0.5 ml. of 0.89 per cent sodium chloride solution intravenously.

<table>
<thead>
<tr>
<th>Units of renin</th>
<th>Route of administration</th>
<th>No. of rats</th>
<th>Duration of collection after renin administration</th>
<th>Mean total protein excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Intraperitoneal</td>
<td>131</td>
<td>1 hrs.</td>
<td>0.60</td>
</tr>
<tr>
<td>4</td>
<td>Intraperitoneal</td>
<td>96</td>
<td>1 hrs.</td>
<td>31.7</td>
</tr>
<tr>
<td>*5</td>
<td>Intraperitoneal</td>
<td>44</td>
<td>2 hrs.</td>
<td>35.1</td>
</tr>
<tr>
<td>4</td>
<td>Intramuscular</td>
<td>25</td>
<td>1 hrs.</td>
<td>10.0</td>
</tr>
<tr>
<td>*4</td>
<td>Intramuscular</td>
<td>9</td>
<td>2 hrs.</td>
<td>46.0</td>
</tr>
<tr>
<td>4</td>
<td>Intramuscular</td>
<td>9</td>
<td>3 hrs.</td>
<td>43.0</td>
</tr>
</tbody>
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* Data taken from Addis et al. (3).
9 bilaterally adrenalectomized rats were similarly treated. The recorded pressure curves were then tabulated; the data are presented in Table II. The blood pressure response of the normal animals varied from 30 to 80 mm. of mercury with an average rise of 61 mm. of mercury. Blood pressure elevations of adrenalectomized rats were from 25 to 63 mm. of mercury and the average rise was 46 mm. of mercury.

Though there may be a decrease in the magnitude of the pressor response to intravenous renin in the adrenalectomized animals, when the curves of these

<table>
<thead>
<tr>
<th>Base blood pressure</th>
<th>Maximum response</th>
<th>Net rise</th>
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<tbody>
<tr>
<td>mm. Hg</td>
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<td>mm. Hg</td>
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<tr>
<td>Normal</td>
<td></td>
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<tr>
<td>120</td>
<td>175</td>
<td>55</td>
</tr>
<tr>
<td>115</td>
<td>145</td>
<td>30</td>
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<tr>
<td>100</td>
<td>180</td>
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<td>110</td>
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<td>70</td>
</tr>
<tr>
<td>100</td>
<td>170</td>
<td>70</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>113</td>
<td>63</td>
</tr>
<tr>
<td>75</td>
<td>112</td>
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<td>87</td>
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<td>58</td>
</tr>
<tr>
<td>75</td>
<td>125</td>
<td>50</td>
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</table>

are compared to curves from normal animals little qualitative difference from the control tracings is seen (Text-fig. 1).

It has been demonstrated previously, that the intraperitoneal injection of renin fails to increase proteinuria in the salt-maintained adrenalectomized rat (9, 10). Since intravenous renin elicits a normal pressor response in the adrenalectomized rat, a study was made of the proteinuric effects of intravenous renin in normal and adrenalectomized animals.

It can be seen from Table III that adrenalectomy abolishes the proteinuric effect of intravenous renin just as it does that of intraperitoneal renin. Hence, intravenous renin elicits a pressor response in the adrenalectomized rat, but the elevation in blood pressure is unaccompanied by a significant rise in protein excretion.
TEXT-Fig. 1. Kymographic tracings of the blood pressure response to 1.0 unit of renin administered intravenously. Tracing 1 is the response of a normal female rat to renin. Tracings 2, 3, and 4, are responses of adrenalectomized female rats, 5 days postoperative to renin. The space between the heavy lines represents 2.5 minutes in each tracing.

TABLE III
Proteinuria Following the Intravenous Administration of One Dog Unit of Renin to Normal and Adrenalectomized Rats

<table>
<thead>
<tr>
<th></th>
<th>No. of Rats</th>
<th>Mean proteinuria (mg./hr. ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15</td>
<td>9.98 ± 5.68</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>22</td>
<td>1.00 ± 1.06</td>
</tr>
</tbody>
</table>

* Using Fischer's method for small number observations the P value for the difference between these means is < 0.001.

Renal Excretion of Dye-Labelled Protein

In the second phase of this study information was obtained on the effect of renin on glomerular permeability by studying the renal excretion of dye-labelled protein.

The dye T-1824 (Evans's blue) forms a stable, blue, complex with the plasma proteins. When injected into the blood stream in low concentrations, the dye attaches preferentially to albumin. As the concentration of the dye is increased, the plasma albumin becomes saturated and the dye then attaches to the plasma globulin, selecting first alpha globulin. Rawson (11) has shown that the concentration of free dye in the equilibrium system between dye-protein complex, plasma protein, and free dye is infinitesimally small. Allen and Orahovats (12) have described the forces binding T-1824 to plasma protein and consider the renal clearance of T-1824 as a measure of the clearance of plasma protein. We have taken advantage of this property of T-1824 as a means of studying the excretion and intrarenal distribution of native plasma protein under controlled experimental conditions.

At 5 p.m. animals were placed in metabolism cages as described above. The following morning all rats received 25 mg. of T-1824 in 0.5 ml. of 0.89 per cent sodium chloride solution intravenously. 5 minutes after the administration of T-1824, experimental animals re-
received 4 Goldblatt dog units of hog renin in 4 ml. of 0.89 per cent sodium chloride solution intraperitoneally. Control animals received sodium chloride solution only, intraperitoneally. Kidneys were removed under ether anesthesia from groups of animals at suitable intervals following the renin injection. The kidneys were placed in 10 per cent formalin solution, and 24 hours later, unstained frozen sections were cut, studied, and photographed. Other animals were treated in precisely the same way only their voided urine was collected for a 60 minute period. It was uniformly found that renin-treated animals excrete an intensely blue urine, while the urine excreted by control animals had the usual pale yellow color with an occasional faint blue tinge. (Fig. 4).

Before going further, it was necessary to establish that T-1824 in the serum and in the urine of renin-treated rats was, in fact, completely attached to protein. If this could be proved it would be reasonable to assume that the T-1824, in the lumen of the renal tubules, exists similarly as a dye-protein complex. Animals were injected intravenously with 25 mg. of T-1824 as described above. 5 minutes later they were bled by severing the abdominal aorta while the animals were under light ether anesthesia. The blood was allowed to clot at room temperature and was then centrifuged. The blue serum was pipetted off. Serum proteins were precipitated both by the cadmium sulfate method (13) and by addition of sodium tungstate and sulfuric acid (14). In each instance, after centrifugation of the precipitated protein, the supernatant fluid was clear and colorless while the precipitated proteins were intensely blue and remained so after repeated washings with the protein precipitant. T-1824 alone, in aqueous solution at pH 7.4, is not precipitated by the addition of sodium tungstate and sulfuric acid.

It has been pointed out that the rat normally excretes about 0.5 mg. of protein in its urine per hour, but an average of 31.7 mg. is excreted in the hour following renin administration. The proteins in the blue urine of the renin treated rats were precipitated in the same manner as described above for the serum proteins. Again, the supernatant fluid was colorless, or, at the most, tinged yellow from the urinary pigments, while the precipitated proteins were colored intensely blue. Thus, the T-1824 present in both the serum and urine of the experimental rat is attached to protein. In the discussion to follow, it will be assumed that the T-1824 seen in the lumen of the renal tubule on histological examination is similarly protein-bound.

Description of Histological Sections

(a) Saline Treated Controls.—Frozen sections of the kidneys of rats taken 5 and 15 minutes after the administration of 25 mg. of T-1824 reveal blue dye in the peritubular capillaries and, to some extent, in the capillary forming the glomerular tuft. No dye can be seen in the tubular lumina or in the cells lining the tubules. At 30 minutes the dye can no longer be seen in the renal capillaries. Sections of the kidney at 30 and 60 minutes are completely colorless, except for a faint suggestion of color in an occasional collecting tubule (Fig. 6). At the end of 90 minutes, a few pale blue granules make their appearance in an occasional cell lining the proximal convoluted tubules. At the end of 120 minutes, a few more such granules are seen. By 24 hours, however, very definite, small, intensely blue granules are seen in the majority of the cells lining the proximal convoluted tubules (Fig. 9). Gilson (15) and Dock (16) have reported similar 24 hour observations.

(b) Renin-Treated Animals.—An entirely different picture is seen in the kidneys of animals that have received T-1824 and renin. At 5 and 10 minutes after renin injection, blue dye is seen in the peritubular capillaries and occasionally in the glomerular capillaries (Fig. 1). Occasional collecting tubules contain traces of dye. At the end of 15 minutes the collecting tubules are loaded with the blue dye-protein complex, and, here and there, cross-sections of proximal convoluted tubules are seen, the cells of which are being invaded from the luminal
border by a homogeneous rather intensely blue material. This material usually occupies the luminal half of the cell and the opposite half contains no dye whatsoever. A faint blue dye can be seen in the lumina of approximately one third of the proximal convoluted tubules. At 30 minutes the most striking change is in the formation of small, blue granules in the mid-zone of cells of the proximal convoluted tubules (Fig. 2). The luminal half of the cells still contain the homogeneous blue material described above. More of the proximal tubule cells contain dye at 30 minutes than at 15 minutes, and this progresses until at 90 minutes nearly all the cells lining the proximal convoluted tubules contain large dye granules (Fig. 3). At 30 minutes, the collecting tubules are still packed with dye-protein and, progressively, as time goes on, more of the dye-protein appears in the lumina of the proximal convoluted tubules. The granules in the cells of the proximal convoluted tubules grow larger at 60 (Fig. 5) and 90 minutes, and larger still at 120 minutes (Fig. 7). The homogeneous dyed material in the luminal half of the cell tends to disappear after 60 minutes and is usually gone at 90 minutes. At both 60 and 90 minutes many of the proximal convoluted tubule lumina are filled with dye-protein complex.

**DISCUSSION**

It has been postulated that renin causes massive proteinuria in the rat by increasing the blood pressure in the glomerular capillaries (3). We have shown that when renin is administered intramuscularly, proteinuria occurs, although the mean arterial pressure does not significantly depart from the normal. Further, when renin is given intravenously to the normal rat, massive proteinuria occurs, while a similar blood pressure elevation, produced in the adrenalectomized animal by intravenous renin, fails to result in increased urinary protein excretion. Clearly, if increased proteinuria can be induced by renin without blood pressure elevation, and if the blood pressure can be elevated without producing increased proteinuria, the massive proteinuria observed to follow intraperitoneal renin administration in the normal rat must occur as a result of some mechanism other than a rise in intraarterial pressure. It is conceivable that glomerular capillary pressure may be elevated without elevation of the mean systemic arterial pressure, but there is no evidence to support such a view.

When T-1824 is injected into the normal animal, little or no dye appears in the urine, and sections of the kidney taken as late as 60 to 90 minutes after the dye is administered fail to reveal dye in the collecting tubules or in the cells lining the proximal convoluted tubules. When renin has been administered after the dye injection, an entirely different picture is obtained. The urine is colored intensely blue. Dye-protein complex appears in the collecting tubules, and the cells of the proximal convoluted tubules are invaded by dye as early as 15 minutes after injection. By the end of 90 minutes the cells of the convoluted tubule are packed with blue globules and blue dye-protein complex can be seen in the lumen of the proximal convoluted tubules. It has been demonstrated that the T-1824 in the serum and the urine is firmly and completely attached to protein. It is safe to assume that the T-1824 appearing in
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the tubular lumen is similarly attached to protein. It is currently held that protein enters the urine by way of the glomerulus only (17). If this is so, the permeability of the glomerulus to dye-protein complex in renin-treated animals must be strikingly increased. One might argue that glomerular permeability to dye-protein is not necessarily enhanced, but that the overwhelming amount of dye-protein in the tubular lumina and in the voided urine following renin injection is the result only of impaired tubular reabsorption of this material. Two lines of argument may be developed against this position. Firstly, it can be seen in the sections that dye actually exists in the cells lining the proximal convoluted tubules, and that the dye appears to travel from the luminal to the capillary border of these cells. Secondly, if the glomerulus normally allows the passage of dye-protein in the amounts indicated by the renin-treated animals, and if the collecting tubules and the urine are kept relatively free of this material by complete tubular reabsorption of the complex, some evidence of the dye-protein should be seen in its passage through the tubule cells from lumen to capillary and some evidence of dye-protein complex awaiting reabsorption in the lumen of the proximal convoluted tubule might be expected. As can be seen in Figs. 6 and 8, there is no indication of dye in the tubular lumen or in the cells lining the proximal convoluted tubules of non-renin-treated animals. It seems unreasonable that reabsorption of filtered dye-protein proceeds so rapidly that no trace of dye-protein exists in frozen sections of the kidney taken as late as 60 minutes after injection of the dye.

We have no real knowledge that dye-protein complex is handled by the cells lining the renal tubules in a manner analogous to undyed native protein. It is possible that the site of reabsorption or even the rate of reabsorption of protein from the tubule lumen may be grossly altered as a result of its attachment to several molecules of T-1824. This does not alter the fact that renin administration results in an outpouring of dye-protein into the renal tubule and entrance into the cells lining the proximal convoluted tubules, while little or no evidence of dye-protein exists in the tubular cells or lumina of non-renin-treated controls. We conclude that the preponderant basis for renin proteinuria is an increase in glomerular permeability. Our data does not permit us to comment on the role of tubular reabsorption of protein, other than to say that if reabsorption is reduced its effect on the over-all increase in proteinuria must be relatively small.

SUMMARY

When renin is administered intramuscularly to the rat, massive proteinuria occurs without a significant elevation of mean arterial blood pressure. The intravenous administration of renin to normal rats results in a great increase in urinary protein excretion. This response to renin is abolished by bilateral adrenalectomy. While the adrenalectomized rat fails to respond to intravenous
renin with increased proteinuria, it does exhibit a normal elevation in mean arterial blood pressure. It is concluded that in the rat, the proteinuric property of renin is not related to the ability of this compound to elevate arterial blood pressure.

The passage of plasma proteins has been followed through the kidney and into the urine by attaching them to the dye T-1824 (Evan's blue). The intraperitoneal injection of renin causes a massive, transient proteinuria in the rat. From a study of frozen sections of the kidney of rats whose plasma proteins are labelled with T-1824, it is concluded that the preponderant basis for renin proteinuria is an increase in glomerular permeability.

We wish to thank the Glesby Brothers Grain and Milling Company, Monrovia, California, for supplying us with grain products; The William R. Warner Division of Warner-Hudnut, Inc. for a grant-in-aid; and Mrs. Ruth Lackey for preparing the frozen sections.

BIBLIOGRAPHY
EXPLANATION OF PLATES

The photomicrographs were made by Mr. Dale Gillette.

PLATE 30

Unstained, frozen sections of rat kidney. All animals have received 25 mg. of T-1824 intravenously.

Fig. 1. 10 minutes after the intraperitoneal injection of hog renin. Blue dye-protein complex in the peritubular capillaries. × 570.

Fig. 2. 30 minutes after the intraperitoneal injection of hog renin. Proximal convoluted tubule with dye-protein complex in the lumen. Note the homogeneous blue material occupying the luminal half of the tubule cells, and the early formation of small blue granules in the middle third of the cells lining the tubule lumen. × 570.

Fig. 3. 90 minutes after the intraperitoneal injection of hog renin. Large discrete blue globules occupying the middle third of the cells of the proximal convoluted tubules. × 570.

Fig. 4. The blue urine was excreted by rats that had received renin in addition to T-1824. The colorless urine was excreted by control animals that had received T-1824 only.
(Sellers et al.: Experimental proteinuria)
PLATE 31

Unstained, frozen sections of rat kidney. All animals have received 25 mg. of T-1824 intravenously.

Fig. 5. 60 minutes after the intraperitoneal injection of hog renin. Intensely colored granules occupying the middle third of the cells of the proximal convoluted tubules. × 535.

Fig. 6. Control for the animal whose kidney is shown in Fig. 5. This rat received 25 mg. of T-1824 intravenously and 4 ml. of 0.89 per cent sodium chloride solution intraperitoneally. 60 minutes later the kidney was removed. Note complete absence of dye in this section. × 535.

Fig. 7. 120 minutes after the intraperitoneal injection of hog renin. Protein complex fills the lumina of the proximal convoluted tubules. Discrete, large globules occupy the full width of the cells of the proximal convoluted tubules. × 535.

Fig. 8. Control for animal in Fig. 7. Note almost complete absence of dye in this section. × 535.

Fig. 9. Frozen section of kidney removed 24 hours after animal had received 25 mg. of T-1824 intravenously. The dye particles appear to occupy the capillary third of the cells of the proximal convoluted tubules. × 535.
(Sellers et al.: Experimental proteinuria)