RENIN PROTEINURIA IN THE RAT

II. Evidence that Renin Does Not Interfere with the Tubular Resorption of Purified Human Hemoglobin or Bovine Albumin

By L. J. RATHER, M.D., and T. ADDIS,* M.D.

(From the Department of Pathology, Stanford University School of Medicine, San Francisco, and the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles)

Plates 40 and 41

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In a previous paper (1) it was suggested that the proteinuria which occurs in the rat following the intraperitoneal injection of renin was due to constriction of the efferent glomerular arteriole, a consequent increase in the intraglomerular filtration pressure, and the passage of more protein through the glomerular membrane. It was shown that inactivation of renin with respect to its pressor activity led, under the conditions of the experiments, to a loss of its capacity to induce proteinuria. Brandt and Gruhn (2), using rabbits instead of rats, have advanced an alternative hypothesis. According to its terms the proteinuria is not due to an increase in the amount of protein passing through the glomerular membrane, but to a decreased resorption of a normal amount by the cells of the proximal convoluted tubule. The action of renin, according to their view, is to inhibit the normally occurring tubular resorption of protein. Although their data indicated that a rise of intraglomerular pressure did occur after the administration of renin they did not think that this could account for the observed proteinuria. They based this conclusion on estimates of the hemoglobin permeability of the glomerulus in two rabbits of the series. Since the ratio of hemoglobin clearance to creatinine clearance was not altered by the intraperitoneal administration of renin, they concluded that there was no increase in the amount of hemoglobin passing through the glomerulus, and, therefore, no increase in the passage of other proteins.

There is both direct and indirect evidence for the renal tubular resorption of protein (3–6) although it has not been demonstrated that there is a continual passage of protein, or split-protein, from the renal tubule cells back into the blood stream. The direct evidence referred to is the appearance of particulate protein within the renal tubule cells, shortly after the parenteral injection of the same protein. The subsequent fate of these particles has not been thoroughly investigated. Smetana's findings (4, 5) indicated the indefinite per-

* Deceased June 4, 1949.

sistence of the protein-dye complex, which he used in his studies, in the cytoplasm. On the other hand we (6) have studied the particles which appear in the renal tubule cells of the rat after the intraperitoneal injection of purified human hemoglobin and have found that within a few hours there is a release of histochemically demonstrable iron. This suggests that other proteins may similarly be rapidly digested. Since the presence of these "athrocytosed" particles is the only direct evidence for the tubular resorption of protein we thought it would be of interest to determine whether or not their formation would be inhibited by the simultaneous administration of renin along with intraperitoneal injection of hemoglobin or albumin. The findings would have an obvious bearing on the controversy sketched above.

Methods

A description of the methods used in the collection and analysis of the urine is given in a previous paper (1). Young female rats of about 150 gm. body weight were given intraperitoneal injections of purified human hemoglobin (furnished by Sharp and Dohme, Inc.) or bovine serum albumin, with or without added renin (hog renin furnished by Eli Lilly and Co.) as shown in Table I. Urine volumes and total hemoglobin or albumin excretions were measured and the findings are recorded in Table I. The rats were killed by exsanguination under ether anesthesia and the kidneys weighed to the nearest milligram on a torsion balance. Sections of the kidneys were cut at 8 microns and stained with hematoxylin and eosin. No attempt was made to identify the intracellular particles by histochemical techniques, since this was not necessary in the case of hemoglobin, owing to its peculiar staining qualities, and not possible in the case of bovine albumin. It was assumed that the particles appearing in the renal epithelium after the parenteral administration of bovine albumin were composed largely of that protein. This assumption is strengthened by the recent work of Oliver (8).

RESULTS

The results are summarized in Table I and representative histological findings are shown in Figs. 1 to 5. In Experiment I there was a remarkable increase in the kidney weight of the single rat given hemoglobin plus renin, as compared with the four controls given hemoglobin alone, and the one control given renin alone. Fig. 1 shows the renal epithelium of the proximal convoluted tubule to be loaded with athrocytosed particles identifiable as hemoglobin. Figs. 2 and 3 show representative portions of the control kidneys. In Experiments II and III the mean kidney weight of the rats given renin and hemoglobin was greater than that of the animals given hemoglobin alone. In Experiment II this increase was statistically significant ($P < 0.01$) and in Experiment III the increase was of doubtful statistical significance ($P < 0.2 > 0.1$). A quantitative study of the number of athrocytosed particles in the renal epithelial cells was not carried out, but, judging from inspection of several sections, the number was significantly greater in the kidneys of the rats given renin in addition to hemoglobin. In Experiment IV there was no significant difference ($P < 0.8 > 0.7$) between the mean kidney weights of the control and experimental groups and

TABLE I

Exp.	Intraperitoneal injection	No. of rats	Weight of kidneys expressed as per cent of normal	Mean hemoglobin or albumin excretion after initial injection	Mean urine volume after initial injection
				mg.	*cc. per hr.*
Exp. I	10 mg. renin in 10 cc. saline at 7.00 a.m. 5 mg. renin in 16 cc. of 6.2 per cent hemoglobin at 10.00 a.m. Kill at 9.00 p.m.	1	146	49	1.1
Renin control I	As above with 16 cc. of saline instead of hemoglobin at 10.00 a.m.	1	86	0	3.8
Hemoglobin control I	No renin given. 16 cc. 6.2 per cent hemoglobin at 10.00 a.m. Kill at 9.00 p.m.	4	92	168	2.7
Exp. II	6.5 cc. 6.2 per cent hemoglobin at 9.30 a.m. 5 mg. renin in 5 cc. saline at 11.30 a.m. Kill at 4.30 p.m.	6	111	42	1.3
Control II	As above with 5 cc. of saline without renin at 11.30 a.m.	6	100	55	1.5
Exp. III	5 mg. renin in 16 cc. 6.2 per cent hemoglobin at 9.30 a.m. Kill at 4.30 p.m.	6	120	53	—
Control III	As above without renin.	6	105	67	—
Exp. IV	6.5 cc. 6.2 per cent hemoglobin at 9.00 a.m. 5 mg. renin in 5 c.c. saline at 11.00 a.m. Kill at 2.00 p.m.	6	103	57	1.4
Control IV	As above with 5 cc. saline without renin at 11.00 a.m.	5	102	52	1.3
Exp. V	16 cc. 6.2 per cent bovine albumin at 9.30 a.m. on day before kill. 16 cc. 6.2 per cent bovine albumin at 9.30 a.m. on the following day plus 5 mg. renin in 5 cc. saline at 12.30 p.m. Kill at 3.30 p.m.	6	129	405	2.1
Control V	As above with saline without renin at 12.30 p.m.	6	110	139	1.7

no difference, ascertainable by simple inspection, in number of intracellular particles. In Experiment V, in which bovine albumin was injected both with and without renin, there was again a significant ($P < 0.01$) increase in the mean kidney weight of the rats given renin. The histological preparations showed a greater number of particles in the rats given renin. Figs. 4 and 5 are

not representative of the state of the whole kidney (as are Figs. 1 to 3) since the particles were distributed in a patchy manner throughout the descending limb of the proximal convolution. Zones where the particles were concentrated were chosen for photography in order to show something of their morphology, but this gives an erroneous notion as to their frequency of occurrence. Table I shows that the administration greatly enhanced the proteinuria following the injection of bovine albumin, but failed to augment the excretion of hemoglobin.

DISCUSSION

It is clear from our results that the amount of intracellular particulate material in the renal epithelium is increased when renin is injected intraperitoneally along with hemoglobin or bovine albumin. The data show also that there is a significant increase in the mean kidney weight of the rats given renin as compared with those given protein alone. Such an increase in weight may be due to the presence of a larger amount of athrocytosed protein or to distension of the tubular lumina by a viscous fluid with a high protein content. It seems probable that both factors are operative, but probably the latter was more important in producing the extreme increase in weight which occurred in Experiment I. The intraluminal accumulation of a concentrated solution of hemoglobin may effectively block the tubules and lead to oliguria, as we have previously demonstrated (6). This has a bearing on the conclusions drawn by Brandt and Gruhn (2), since, for a determination of hemoglobin clearance to be valid, all hemoglobin not resorbed by the tubule cells must pass down the tubule lumen and appear quantitatively in the urine during the period of collection. Brandt and Gruhn give no data on the weight or histology of the kidneys from the two rabbits whose hemoglobin permeability they estimated from hemoglobin clearances determined before and after the administration of renin. If, in their experiments, hemoglobin accumulated in distended or plugged tubules their estimated clearances would fall below the true values, and the conclusion which they drew (that there was no increased passage of protein through the glomerular membrane) would be based on error.

Our data do not show any augmentation of hemoglobin excretion by renin. This might be due to hemoglobin remaining behind in the tubule cells or to plugging of the tubular lumina. When bovine albumin was used, however, there was a threefold increase in the amount of protein excreted in the 3 hour period following the injection, as compared with the controls. This is clearly significant. Possibly the difference in behavior between hemoglobin and bovine albumin is due to the larger portion of the renal tubule which is capable of taking up hemoglobin. The intracellular particles appearing after the injection of bovine albumin are found largely within the medullary portion of the cortex within the straight descending portion of the proximal tubule. In contrast, intracellular particulate hemoglobin is found distributed throughout practically

the entire proximal convulution as well. The total number of intracellular particles visible after the injection of hemoglobin is vastly in excess of the number visible after the injection of bovine albumin, whether or not renin is given.

The most reasonable explanation for the increased athrocytosis of the two proteins studied is that, due to the mediated pressor effect of renin on the efferent arteriole of the glomerulus, there is an increase in the amount of protein passing through the glomerular membrane and more protein thus becomes available for athrocytosis. An alteration of glomerular permeability due to a direct effect of renin on the glomerular membrane is a possibility not explored in these experiments. The fact that we have found no inhibition of athrocytosis by renin does not necessarily prove that tubular resorption of protein is not inhibited. The relation between the tubular resorption of protein and athrocytosis is not a simple one (7) and it is possible that all phases of the process are not microscopically visible. Athrocytosis is, nevertheless, the only direct evidence of renal tubular resorption of protein. Therefore the hypothesis of Brandt and Gruhn, that renin inhibits this resorption, is not supported by our experiments.

SUMMARY

The intraperitoneal injection of renin together with purified human hemoglobin or bovine albumin increases the weight of the kidneys and increases the number of athrocytosed particles of protein in the cells of the proximal convoluted tubules. In the case of bovine albumin administration there is a threefold increase in protein excretion in those rats given renin as compared with those given bovine albumin alone. A possible explanation of these effects is given.

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EXPLANATION OF PLATES

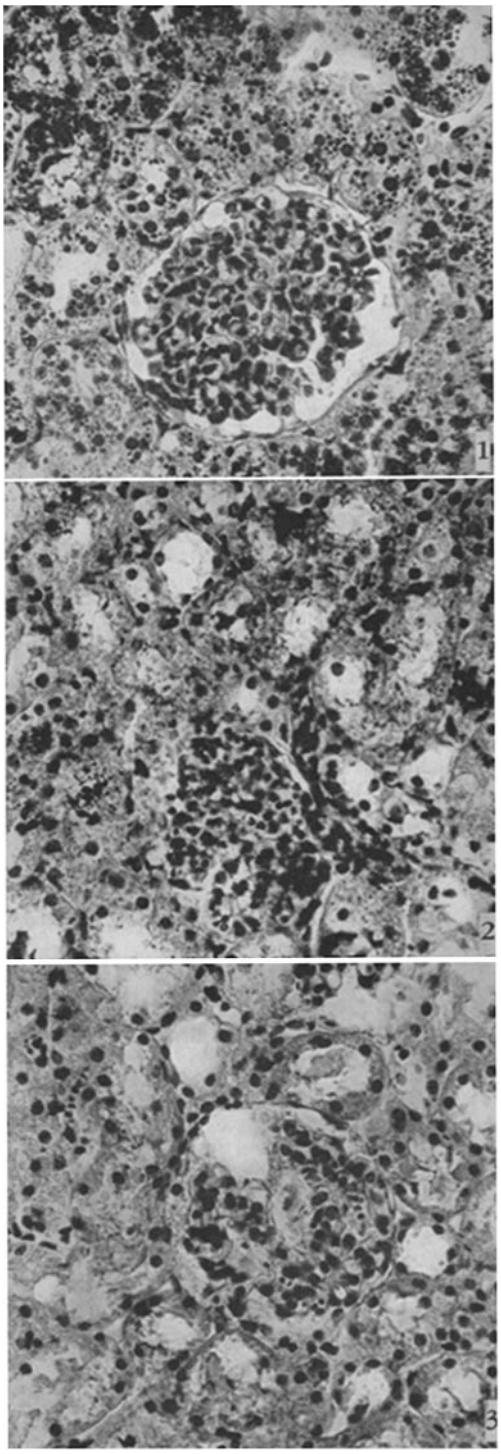
The sections were all stained with hematoxylin and eosin.

PLATE 40

FIG. 1. Experiment I, renin plus hemoglobin. Many large droplets of hemoglobin in cortical cells. × 287.

FIG. 2. Experiment I, typical control, given hemoglobin alone. × 287.

FIG. 3. Experiment I, control given renin without hemoglobin. × 287.

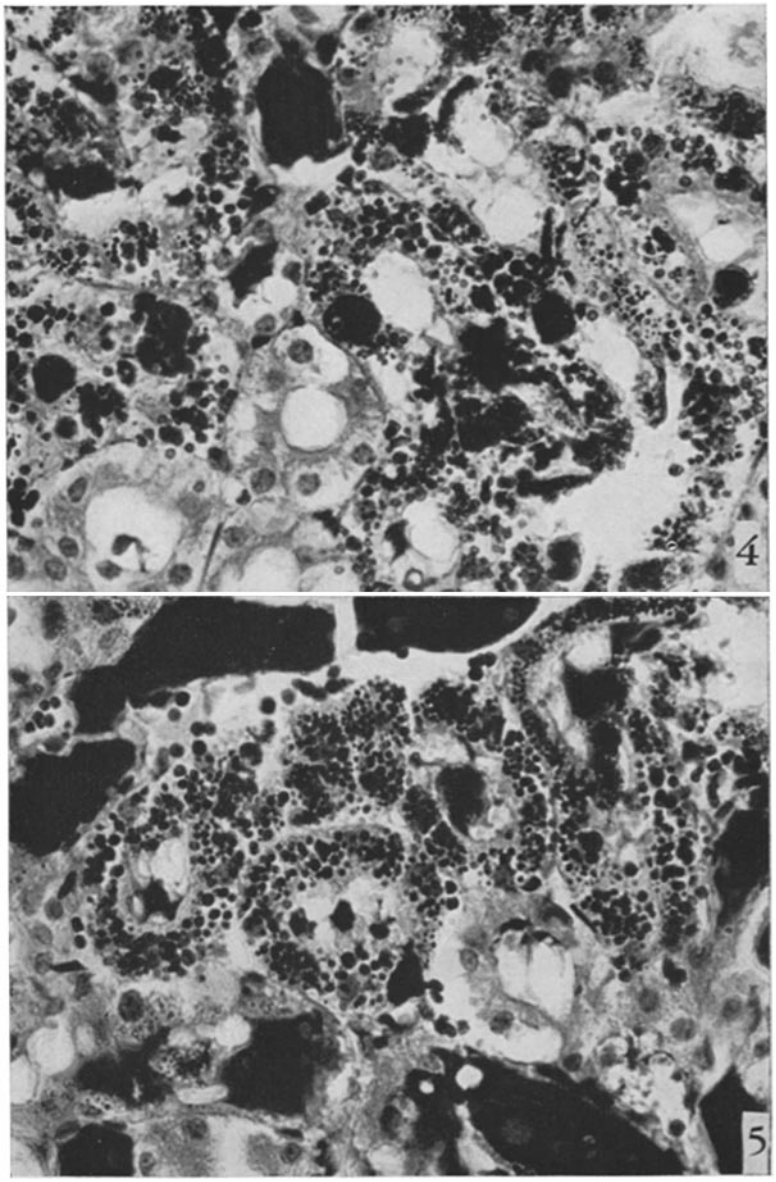


(Rather and Addis: Renin proteinuria in rat. II)

PLATE 41

FIG. 4. Experiment V, bovine albumin alone. The intracellular droplets are chiefly in the descending limb of the proximal convolution. × 440.

FIG. 5. Experiment V, bovine albumin plus renin. No increase in number of droplets in this particular zone (descending limb of the proximal convolution). × 440



(Rather and Addis: Renin proteinuria in rat. II)