

17202. Renal Lesions in Chronic Hypertension Induced by Unilateral Nephrectomy in the Rat.*

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Chronic experimental hypertension induced in the rat resembles that observed in other species with the exception that the incidence of hypertension following operative procedures on one kidney or unilateral nephrectomy is greater than in other species. In the dog, for example, chronic hypertension ensues rarely following application of a clamp to the renal artery, removal of one kidney, or other manipulations, if the opposite kidney be left intact.¹⁻³ In the rat, on the other hand, approximately 20% of animals develop hypertension following application of a figure-of-eight ligature to one kidney or unilateral nephrectomy.⁴ The present study was undertaken to discover the factors which determine whether or not a given animal will develop hypertension following unilateral operation in order to elucidate further the role of the kidney in the pathogenesis of this disorder. The effect of unilateral nephrectomy on the weight of the remaining kidney and the changes produced in this kidney by hypertension; the changes produced in the heart by removal of the remaining kidney; and the influence of various types of diet on the survival period of the bilaterally nephrectomized rat were also studied.

Methods. Healthy piebald rats (Evans-McCollum strain), one to 2 years old, were selected from our breeding colony. The blood pressures were determined daily for a period of 10 days by the plethysmographic method.⁵

Under ether anesthesia, the right kidney was removed through a lumbar incision from 110 animals, the blood pressures of which ranged between 110 and 120 mm of mercury. Subsequently, blood pressure determinations were recorded 3 times weekly. The left kidney was then removed and the nephrectomized animals were subjected to various dietary regimes to determine the effects of these diets on the survival period. When the rat died the heart was removed. The weights of each kidney and of the heart were recorded after fixation in 10% formalin. A cross section of the central portion and a longitudinal section of either the distal or the proximal portion of each kidney and a longitudinal or transverse section including both ventricles of the heart were imbedded in paraffin, cut, and stained with hematoxylin and eosin.

Results. *Effect on the blood pressure of unilateral nephrectomy.* The mean blood pressure levels of 100 rats, 3 months following removal of the right kidney, are recorded in Table I. The figures indicate that 23% of the animals remained normotensive or manifested no greater increase in blood pressure over the preoperative level than the limits of experimental error (± 10 mm of mercury). The largest group, 57%, assumed an intermediate position with blood pressures up to 150 mm of mercury, which are definitely above normal. The remaining 20% of the rats developed hypertension of notable degree with blood pressures above 150 mm of mercury. These are essentially the same effects previously found to follow the application of a figure-of-eight ligature to one kidney, the other kidney being left intact. Following the removal of the second kidney, the blood pressure in those animals which survived to the fifth and sixth days

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¹ Grollman, A., Factors Regulating Blood Pressure, Trans. Second Conference, Macy Foundation, New York, 1948.

² Goldblatt, H., *Physiol. Rev.*, 1947, **27**, 120.

³ Ogden, E., Tripp, E., and Constant, G., *Fed. Proc.*, 1949, **8**, 120.

⁴ Grollman, A., Harrison, T. R., and Williams, J. R., Jr., *Am. J. Physiol.*, 1943, **139**, 293.

⁵ Williams, J. R., Jr., Harrison, T. R., and Grollman, A., *J. Clin. Invest.*, 1939, **18**, 373.

TABLE I.
Incidence of Lesions in the Kidney and Blood Pressure of 100 Rats Following Unilateral Nephrectomy.

Mean blood pressure in mm Hg	Up to 120	120 to 130	130 to 140	140 to 150	Over 150
No. of rats	8	15	29	28	20
Incidence of lesions in ablated kidney	0	+	++	+++	++++

TABLE II.
Effect of Various Diets on the Survival of Rats Following Bilateral Nephrectomy.

No. of rats	Diet	Survival in days	
		Range	Avg
20	Regular	2.0 to 4.5	3.0 $\pm$ 0.4*
20	Dialyzed (sodium and potassium "free")	3.2 to 6.0	4.5 $\pm$ 0.4
20	"Protein-free" diet (glucose, starch, lard)	2.9 to 5.5	4.6 $\pm$ 0.5
20	Dialyzed food plus 2% NaCl	2.8 to 5.0	4.3 $\pm$ 0.5
20	" " " " KCl	2.5 to 4.5	3.7 $\pm$ 0.3

* Standard deviation.

was in each case found to be elevated 30 to 50 mm of mercury over the highest level attained prior to the removal of the second kidney. Bilateral nephrectomy in the rat, as in the dog,⁶ thus results in elevation of the blood pressure to hypertensive levels if the animals survive for 5 days or more in good condition.

Microscopic studies of the right kidneys. The structural deviations from normal observed microscopically in the right kidneys of the rats whose blood pressure levels are recorded in Table I could be grouped as absent, minimal, and slight. There was a conspicuous absence of any acute or chronic inflammatory change involving the glomeruli, tubules, renal pelvis, stroma or blood vessels. The minimal and slight changes observed consisted of an increase in the connective tissue stroma of occasional glomeruli and hyaline change of some glomeruli, of varying degrees or complete. Glomeruli with such changes were few and only in occasional kidneys were they associated with changes in the tubules. Comparison of the incidence of these structural changes in the kidneys with the blood pressures of the animals from which they were removed indicated with some degree of certainty that glomeruli with hyaline change were more numerous and that the associated tubular changes were conspicuous in those rats whose blood pressures were in

the hypertensive range. The structural changes were absent or minimal in the kidneys of animals, the blood pressures of which were not affected by nephrectomy.

Survival following removal of the second kidney. The survival times of the rats following removal of their second kidney are recorded in Table II. Removal by dialysis of the salts present in the regular diet or the use of a salt-free, protein-free diet increased the average survival times from 3 to 4.5 days. That this prolongation in the survival time is due chiefly to the removal of potassium salts from the diet is shown by the fact that the addition of sodium chloride to the dialyzed diet did not affect appreciably the period of survival, whereas the addition of potassium chloride reduced it to a period comparable to that observed with the regular diet. Complete abstinence from food or the administration of 0.9% saline in lieu of drinking water also reduced the survival period markedly. The longest survival observed under optimal conditions was 6 days. Comparable results have been reported for the dog.⁶ The urea content of the blood of 18 of the bilaterally nephrectomized rats just prior to or at death varied from 500 to 1090 mg per 100 cc and averaged 748 mg %. These values are appreciably higher than those reported by

⁶ Grollman, A., Vanatta, J., and Muirhead, E. E., *Am. J. Physiol.*, 1949, **157**, 21.

TABLE III.
Relation of Weight of Kidneys and Heart to Ultimate Blood Pressure.

Ultimate mean blood pressure in mm Hg	Up to 120	120 to 130	130 to 140	140 to 150	Over 150
	Avg wt in g per 100 g of body wt				
Right kidney	0.46	0.46	0.48	0.50	0.49
Left "	0.46	0.48	0.50	0.53	0.55
Heart	0.31	0.33	0.34	0.36	0.48

others,⁷⁻⁸ and reflect the much longer survival period of our animals which are comparable to those reported by Rosenkrantz,⁹ who administered aluminum hydroxide to prolong the life of his rats. The longer survival of our animals on a regular diet, we attribute to the fact that removal of the second kidney was accomplished with a minimum of trauma.

Studies on the remaining kidney and heart. The average weight in grams of each kidney and heart calculated per 100 g of body weight are recorded in Table III. The weights of the right kidneys were essentially the same in all animals regardless of the ultimate blood pressure attained following unilateral nephrectomy. The weights of the left kidneys, on the other hand, were increased in proportion with the elevation of blood pressure. It will be noted that removal of kidneys which on microscopic examination were normal resulted in no increase in weight of the remaining kidney whereas the kidneys of animals manifesting microscopic changes underwent maximum enlargement. There was thus no correlation between the weight of the first kidney removed and the ultimate blood pressure but a definite correlation between that of the remaining kidney and the blood pressure. The weights of the hearts increased proportionately to the elevation of blood pressure. This hypertrophy is not reflected exactly by the data of Table III since some loss of substance was likely to occur during the survival following removal of the second kidney. Microscopic studies of the left kidneys disclosed no striking differences from the right kidneys of the same rats. Occa-

sional glomeruli seemed larger and the tubules perhaps more spacious. This might account for the slight increase in weight of the kidneys. Changes also occurred in the left kidneys of those rats in which lesion of the right kidneys were minimal or slight. In these, glomeruli with hyaline change were more numerous and the associated tubules were dilated by a homogeneous, bright pink material and were lined by flat cells. These changes were marked only in those rats which developed hypertension. Typical appearances of the kidneys are shown in Fig. 1. Microscopic studies of the hearts disclosed minimal or slight hypertrophy of the left ventricle. The increase and variation in size of the myocardial fibers were most marked in the hypertensive rats. Acute changes were superimposed in all of the hearts which presumably occurred during the period following removal of the second kidney. They consisted of varying degrees of dilatation of the capillaries with focal areas of extravasation of blood and obliteration of the striations of the myocardial fibers to a degree of waxy change. These changes are comparable to those observed in the nephrectomized dog.¹⁰ No acute or chronic inflammatory lesions were noted in the stroma nor were there any obvious changes in the blood vessels.

Discussion. The results of the present study are compatible with the view that the maintenance of a normal blood pressure is dependent on the presence in the organism of an adequate renal function.¹¹ It is impossible, on the other hand, to harmonize the present observations with the view that hy-

⁷ Durlacher, S. H., and Darrow, D. C., *Am. J. Physiol.*, 1942, **136**, 577.

⁸ Bondy, P. K., and Engel, F. L., *Proc. Soc. Exp. Biol. and Med.*, 1947, **66**, 104.

⁹ Rosenkrantz, J. A., *Proc. Soc. Exp. Biol. and Med.*, 1946, **63**, 155.

¹⁰ Muirhead, E. E., Vanatta, J., and Grollman, A., *Arch. Path.*, in press.

¹¹ Grollman, A., *Special Publications N. Y. Acad. Sci.*, 1946, **3**, 99; Factors Regulating Blood Pressure, First Conference, Macy Foundation, New York, 1947.

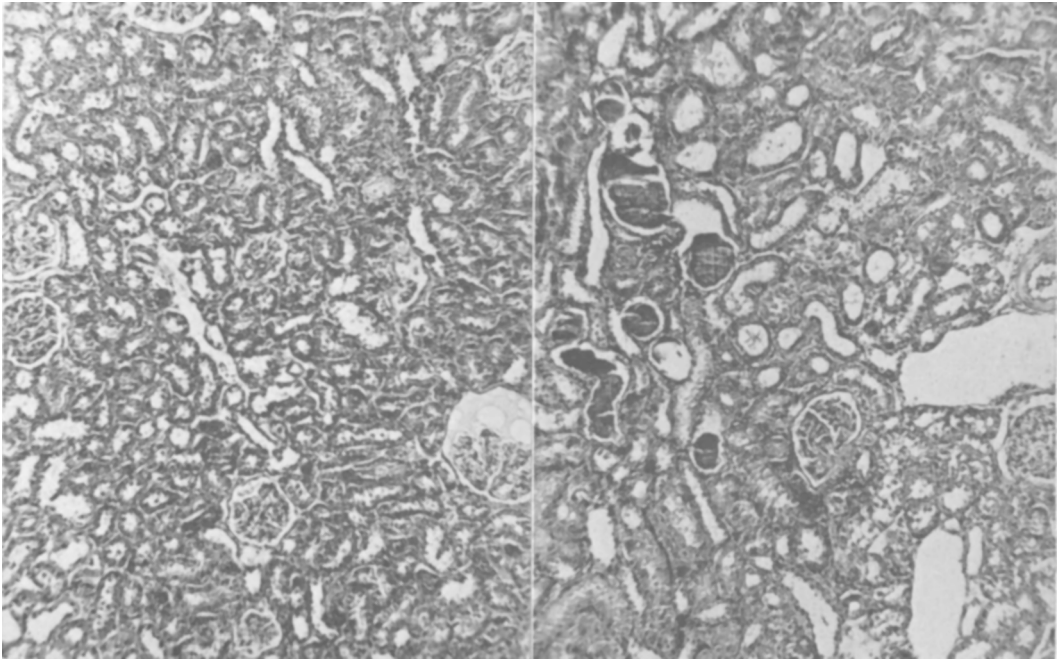


FIG. 1.

Microscopic changes in glomeruli and tubules of the right kidney (left) removal of which was followed by permanent elevation of blood pressure to 160 mm Hg. More extensive changes are seen in the left kidney (right) of the same rat (R-64-48) removed 10 weeks later. $\times 60$.

pertension results from the elaboration of some pressor agent unless one makes the unlikely assumption that removal of one kidney puts such a strain on the other that it excretes a pressor agent.¹² When both kidneys are unimpaired and one kidney is removed, the other may compensate for the removed kidney and no hypertension results. On the other hand, when both kidneys are slightly impaired and one kidney is removed, an elevation in blood pressure ensues. The fact that unilateral nephrectomy in the rat more frequently results in hypertension than the same procedure does in the rabbit¹³ or in the dog¹⁴ suggests that the rat has less reserve of kidney function than do these other experimental animals. Microscopic examination of presumably normal kidneys supports this hypothesis. However, the lesions present in the rat are not, as commonly stated, a result of preexistent pyelonephritis. They resemble

(Fig. 1) those described by the authors¹⁵ in rats with chronic hypertension, but are much less extensive. As regards the nature of the lesions, they apparently are not vascular in origin¹⁶ but are probably secondary to nutritional deficiencies in early life. Such deficiencies induce renal damage¹⁷ which, if extensive, results in hypertension.¹⁸ This would account for the observation that the incidence of spontaneously occurring hypertension in rats varies and depends on the dietary conditions under which they have been reared. A group of rats, for example, which as weanlings had been used in dietary deprivation studies, were found to have an

¹⁵ Halpert, B., and Grollman, A., *Arch. Path.*, 1947, **43**, 559.

¹⁶ Halpert, B., and Grollman, A., *Proc. Soc. Exp. Biol. and Med.*, 1946, **62**, 273.

¹⁷ Heller, H., and Dicker, S. E., *Proc. Roy. Soc. Med.*, 1947, **40**, 351; Dessau, F. I., and Oleson, J. J., *Proc. Roy. Soc. Exp. Biol. and Med.*, 1947, **64**, 278.

¹⁸ Harteroft, W. S., and Best, C. H., *Brit. Med. J.*, 1949, **1**, 423.

¹² Goldblatt, H., *The Renal Origin of Hypertension*, Chas. C. Thomas, Springfield, 1948.

¹³ Grollman, A., *Am. J. Physiol.*, 1942, **142**, 666.

¹⁴ Grollman, A., *Am. J. Physiol.*, 1946, **147**, 647.

incidence of about 20% of hypertension, whereas those maintained on adequate diets rarely developed the disorder spontaneously. The existence of lesions comparable to those observed would account for the rises in blood pressure observed in the rabbit and dog following unilateral nephrectomy.^{6,13,14}

Summary. The incidence of hypertension following unilateral nephrectomy is recorded in 100 rats. The development of hypertension was shown to be dependent on the status of the kidneys at the time of removal of the first kidney. If lesions are present, hypertension develops; if absent or minimal, the animals remain normotensive. The bearing of these observations on current theories as

to the pathogenesis of experimental hypertension are discussed.

The effects of unilateral nephrectomy on the weight of the heart and remaining kidney and the influence of various types of diet on the survival period following removal of the remaining kidney were also determined. Enlargement of the heart as a result of hypertrophy, as well as of the remaining kidney, may be correlated with the degree of hypertension induced by unilateral nephrectomy. Removal of potassium from the diet markedly prolongs the survival period following bilateral nephrectomy.

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17203. Limiting Essential Amino Acids of Some Legume Seeds.

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Pulses are one of the most important sources of vegetable proteins in human diets and knowledge of their amino acid composition is of interest especially in countries where there is insufficient consumption of animal products. The present experiments are part of a study on the nutritional problem in Venezuela, where legumes play a very important role in human nutrition.

The experiments were performed with young rats of the Sprague-Dawley strain. Each group consisted of 2 male and 2 female animals, housed individually in screen-bottomed cages. In most cases the animals were put on the experimental diet at the age of 3 weeks and weighed about 40 g each; in some cases animals weighing about 100 g were used. The diets used were similar to those described by Russell *et al.*¹ and contained 10% of protein and 4-5% of fat, with the exception of the soybean diets which contained 7% of fat. Food and water were given *ad libitum*. All animals received 3 drops of liver extract 3 times weekly by dropper;

this amount was sufficient to promote maximum growth on a soybean-corn diet when tested by a method previously described.² Each group received the unsupplemented diet for 3 weeks, immediately followed by the supplemented diet for the same time. In some cases the diets were given for 10 periods only. Animals were weighed every 5 days and food consumption was recorded. Digestibility was determined for all the legumes studied. Since good correlation between growth and protein efficiency was observed in accordance with the results of other authors,³ only weight changes are reported.

The legume seeds were obtained from the Genetical Division of the Ministry of Agriculture in Maracay or were purchased from the local market. All seeds were ground and autoclaved at 10 lb. pressure for 20 minutes with the exception of the kidney and hyacinth beans, which were soaked in water over night, autoclaved for 30 minutes at 10 lb. pressure,

¹ Russell, W. C., Taylor, M. W., Mehrhof, T. G., and Hirsch, R. R., *J. Nutr.*, 1946, **34**, 491.

² Jaffé, W. G., and Elvehjem, C. A., *J. Biol. Chem.*, 1947, **169**, 287.

³ Hegsted, D. M., and Worcester, J., *J. Nutr.*, 1947, **33**, 685.