Augmented renin substrate, while not excluded, seems an unlikely explanation, since increases in normal substrate concentrations do not yield an increase in pressor activity comparable to that seen following nephrectomy.

A possible explanation is the appearance from postnephrectomy plasma of a renin incubation product with twice the chromatographic mobility of the prenephrectomy peptide.

The alterations in substrate protein which follow nephrectomy would appear to involve portions of the peptide chain distant from the point of renin action, since the rate (K_m) and maximum velocity of the reaction are unaltered in the presence of postnephrectomy plasma.

We are indebted to Dr. William Wagner, Ciba Laboratories, for the supply of angiotensin used in this investigation.

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Transfer of Experimental Renal Hypertension and Vascular Disease

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ON THE assumption that renal hypertension results from hyperactivity of the renal pressor system, various but unsuccessful attempts have been made to mimic the course of renal hypertension by administering renin preparations to normal animals. Subcutaneous injections of up to 100 Goldblatt units per day or intravenous infusion for periods up to 18 days caused only a temporary and slight rise in pressure[1, 2] but no vascular lesions.[3, 4] These experiments, while supporting the view of those who believed that renin had no primary role in the pathogenesis of hypertension, suggested that some other principle, either destroyed during the extraction of renin or absent in normal kidneys from which renin is usually prepared, might be involved. Whatever the correct interpretation, the problem remained to demonstrate the endocrine nature of renal hypertension.

In reinvestigating this problem we decided to use hypertensive instead of normal animals as kidney donors, to prepare crude instead of fractionated extracts, and to test these extracts in uninephrectomized rats. Kidney donors were made hypertensive by aortic stenosis. The technique is fairly reproducible, involves only one kidney which becomes atrophic, does not form urine (so-called endocrine kidney), and, most important, causes an acute type of hypertensive vascular disease; moreover, possible interfering substances such as the enzymes associated with urine formation are eliminated. By injecting extracts of endocrine kidneys into uninephrectomized rats we had a situation similar to that which exists in rats with aortic stenosis in which one kidney is ischemic and the other intact (Fig. 1).

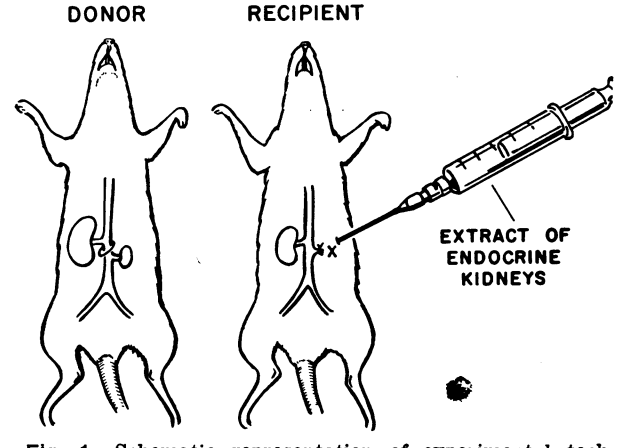

Fig. 1.—Schematic representation of experimental technique.

This presentation will consist first of a description of the hypertensive disease caused by an endocrine kidney and, secondly, of the effects of extracts of endocrine kidneys in uninephrectomized rats.

I. *Course of Hypertensive Vascular Disease Caused by an Endocrine Kidney*

The concept of the endocrine kidney was developed by Selye and Stone.[5] They demonstrated that, by partially constricting the aorta between the origins of the two renal arteries and therefore reducing renal arterial pressure to a level equal to or lower than filtration pressure, the left kidney

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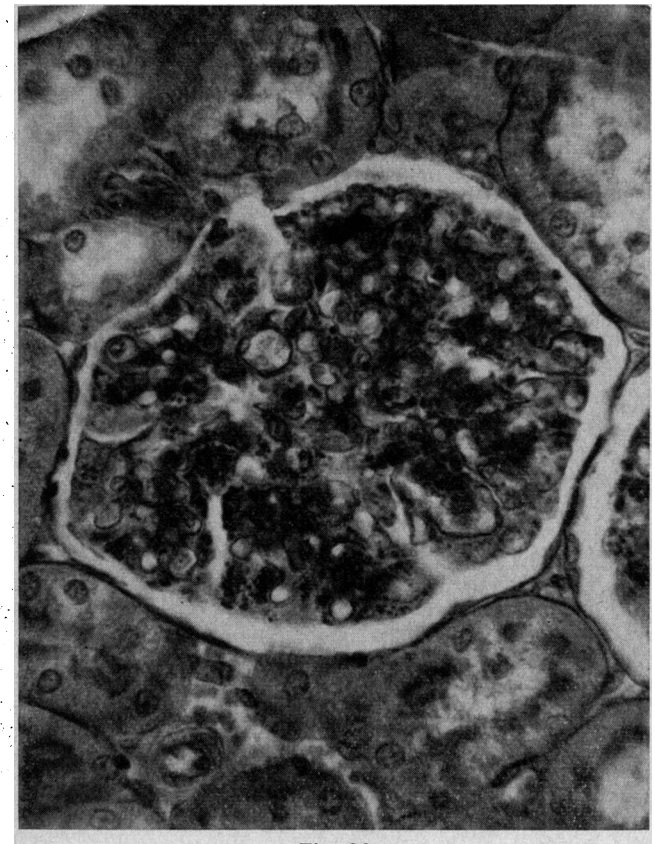

Fig. 2A

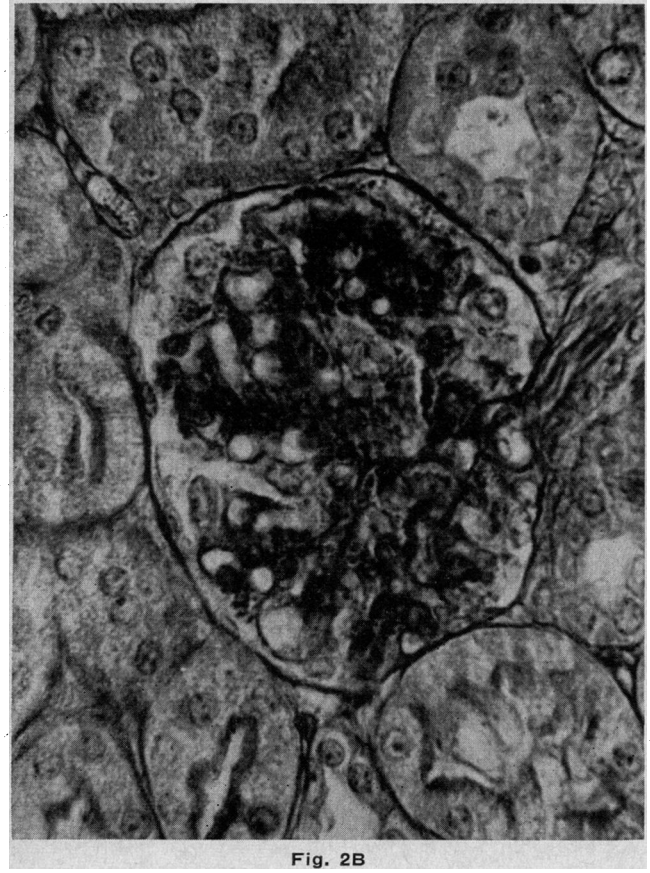

Fig. 2B

Figs. 2A and 2B.—Renal glomeruli from the kidney contralateral to an endocrine kidney (A) and from the sole kidney of a rat treated with extracts of endocrine kidney (B). Glomeruli are enlarged and have lost their delicate pattern owing to partial necrosis (B), thickening and disruption of membranes, increased cellularity (A), and intense deposition of PAS-positive material (× 700).

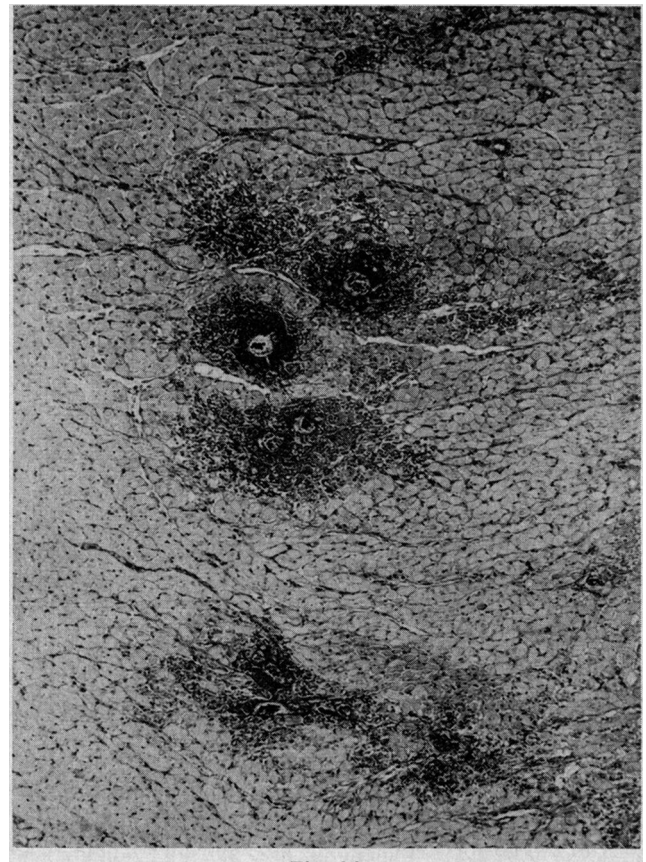

Fig. 3A

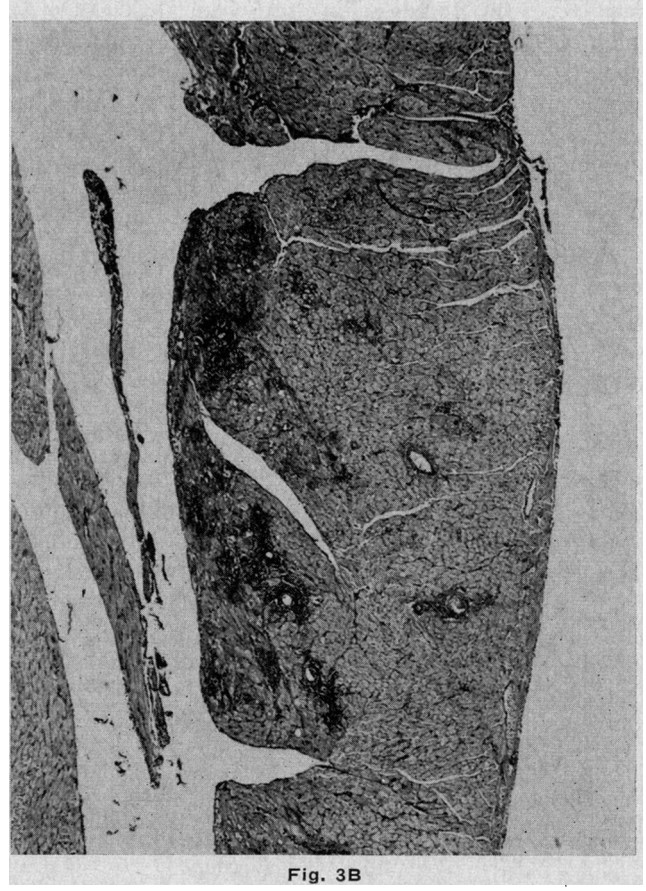

Fig. 3B

Figs. 3A and 3B.—Sections of heart (A—from rat with an endocrine kidney, and B—from rat which received extract of endocrine kidney. Periodic acid-fuchsin stain) showing vasodilation, degenerative vascular lesions and extensive extravasation of PAS-positive material, leading to muscle necrosis (× 80 and × 55, respectively).

loses its excretory function while being transformed into a hyperactive endocrine organ as evidenced by the acute development of vascular disease. Although this situation is identical to that obtained with a Goldblatt kidney, little is known about its evolution, except what has been provided by morphological studies.[6, 7] Even the severity of hypertension had to be based on heart hypertrophy, since blood pressure could not be measured by the routine procedure of tail plethysmography. Therefore, if we were to compare this type of hypertension with what we hoped to produce with kidney extracts, it seemed necessary to know more about its natural course.

Observations were made on over 200 animals. Only data from rats with good endocrine kidneys will be presented. Aortic stenosis caused an immediate and gradual decrease in body weight which on the seventh day amounted to approximately 20% of the original value. Blood pressure recorded from an indwelling arterial catheter increased sharply during the first 24 hours from a control level of 123 ± 9.1 mm. Hg to 153 ± 12.9 mm. Hg. On the seventh day values averaged 188 ± 9.9 mm. Hg. Similarly, there was a significant increase in heart weights from a control value of 311 ± 8.1 mg. per 100 g. of body weight to 444 ± 4.7 and 463 ± 11.8 mg. respectively on the second and seventh day. These observations on blood pressure and heart weight are confirmatory evidence that cardiac hypertrophy is a dependable index of diastolic hypertension. Renal pressor activity remained normal during the first two days, then increased sharply in endocrine kidneys and decreased in contralateral kidneys. On the seventh day post stenosis, pressor activity averaged 94 ± 14 Goldblatt units in endocrine kidneys and 2.8 ± 0.5 Goldblatt units in contralateral kidneys as compared with a normal average of 12.4 ± 3.8 units per gram (g.) of tissue. Values up to 325 units were found in some endocrine kidneys. Reduction of perfusion pressure in the left kidney caused atrophy of the ipsilateral and compensatory hypertrophy of the contralateral kidney. These opposite effects reached their maximum between the tenth and the fifteenth day. At that time left and right kidneys averaged 330 and 938 mg. respectively.

At autopsy pathologic changes were noticed first in the heart, then in the right kidney and finally in hepatic arteries. The heart showed hemorrhages and areas of necrosis as early as the second day. The right kidney became discoloured, mottled with white and flea-bite spots around the third or fourth day. Lesions of arteritis as discrete round nodules in the branches of the hepatic artery occurred after the sixth day. All these lesions which are those of hypertensive vascular disease are too well known to be described again in detail.[8] Representative figures of various lesions (Figs. 2A to 4A) are presented for ulterior comparative purposes.

Besides demonstrating the early appearance of hypertension in rats with aortic stenosis, these observations gave us some criteria which in the absence of blood pressure measurements permitted

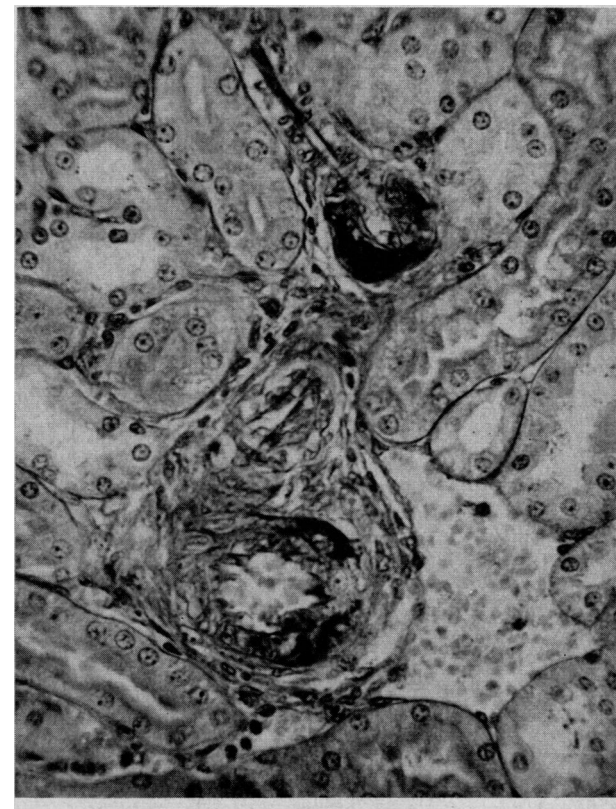

Fig. 4A

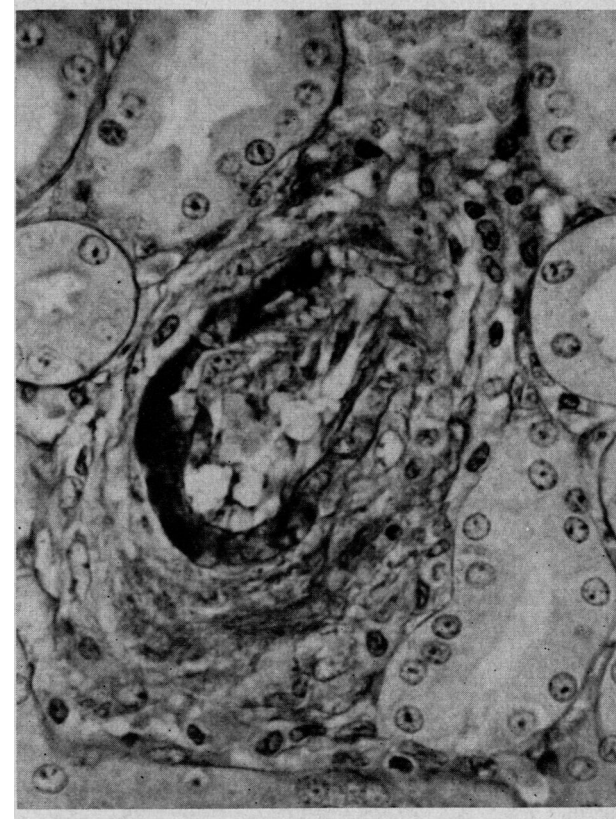

Fig. 4B

Figs. 4A and 4B.—Sections of renal arterioles and arteries (A—from rat with an endocrine kidney, and B—from rat which received extract of endocrine kidney. Periodic acid-fuchsin stain) showing focal medial necrosis (× 430 and × 630, respectively).

selection of endocrine kidneys to be used for the preparation of kidney extracts. These criteria are given according to the order of their appearance: heart weight over 400 mg. per 100 g. of body weight, cardiac hemorrhages or necrosis, weight loss of about 20%, nephrosclerosis of the right kidney and atrophy of the left, and arteritis in the branches of the hepatic artery. Arteritis which occurred only after the sixth day was used solely as confirmatory evidence of hypertension.

II. *Effects of Extracts of Kidneys from Hypertensive Rats*

Having established that endocrine kidneys were suitable for our purpose, we decided to study their effects in uninephrectomized rats. Information obtained from preliminary experiments as to adequate dosage and duration of treatment led us to successful reproduction of hypertension and vascular disease. We will present a general outline of the method and describe a typical experiment.

After aortic stenosis was performed, rats were kept under observation and killed around the seventh day post stenosis. When hypertension was diagnosed according to the criteria listed above, kidneys were immediately removed, chilled, ground with cold distilled water in the ratio of 2 ml. per kidney and centrifuged. The supernatant was then injected subcutaneously into test animals which had been uninephrectomized three to four hours earlier. The daily dose of extract administered in three injections was equivalent to one and a half kidneys.

In one experimental series, test animals were divided into four groups treated as follows: group 1, extracts of endocrine kidneys; group 2, extracts of kidneys contralateral to endocrine kidneys; group 3, extracts of normal kidneys. The group 4, used as control, received injections of physiologic saline. The pressor activity of endocrine, contralateral and normal kidneys averaged respectively 100, 5 and 10 Goldblatt units per g. of tissue. Animals were sacrificed on the fifth day. From the results summarized in Table I, it is evident that extracts of endocrine kidneys had the most significant effects on body weight, urine flow, blood pressure and heart weight. Loss of weight amounted to 23% of the original value; urine flow increased about four times; arterial pressure measured by tail plethysmography rose from 116 to 155 mm. Hg and heart weight was greater than 400 mg. per 100 g. of body weight. On gross and microscopic examination, only rats treated with endocrine kidneys showed pathologic changes. All nine rats had cardiac necroses and five of them nephrosclerosis. Comparison of tissue sections taken from rats with endocrine kidneys (Figs. 2A to 4A) and from rats treated with extracts of endocrine kidneys (Figs. 2B to 4B) demonstrates the similarity of renal, vascular and cardiac lesions in these two conditions.

If we were to compare the evolution and severity of hypertension in rats treated with kidney extracts with that which occurs naturally in rats with endocrine kidneys, it was necessary to measure blood pressure by the same method. Accordingly we repeated the latter experiment in rats with an indwelling arterial catheter, as was done in rats with endocrine kidneys. Determinations were made twice a day prior to injections, to avoid measurement of any acute pressor effect from the previous injection. Results are presented in Fig. 5 together with those obtained in rats with endocrine kidneys. There is an obvious similarity between the blood pressure curves of rats receiving extracts of endocrine kidneys and that of rats with aortic stenosis. The other extracts were inactive. Thus, these results confirm those obtained by tail plethysmography.

TABLE I.—EFFECTS OF VARIOUS KIDNEY EXTRACTS IN UNINEPHRECTOMIZED RATS

Groups and treatments	*Body weight (g.)*	*Urine flow (ml. per day)*	*Blood pressure (mm. Hg)*		*Heart weight (mg. per 100 g. of body weight)*	*% incidence of vascular disease in*	
			Initial	*Final*		*Kidney*	*Heart*
I Extracts of endocrine kidneys	109 ± 9.2*	38 ± 4.9*	116 ± 7.4	155 ± 21.4*	440 ± 23.7*	55	100
II Extracts of contralateral kidneys	140 ± 6.9	14 ± 3.3	115 ± 4.5	105 ± 11.8	290 ± 15.5	0	0
III Extracts of normal kidneys	148 ± 7.7	17 ± 4.3*	107 ± 10.3	120 ± 5.5	333 ± 23.3	0	0
IV Physiologic saline	154 ± 10.4	8 ± 1.7	118 ± 8.4	114 ± 14.7	332 ± 28.6	0	0

*P < 0.01.

DISCUSSION

These observations demonstrate that injections of extracts of endocrine kidneys, but not of contralateral and normal kidneys, mimic the early manifestations of renal hypertension. The fact that extracts of endocrine kidneys as compared with other extracts contain the largest amounts of pressor activity mostly if not entirely due to renin[9, 10] further suggests that renin may be the primary factor in the causation of hypertension, especially the one with a rapid evolution and vascular disease. But evidence from experiments with semi-purified renin does not support its pathogenetic role.[1, 2]

There are, however, observations that under certain conditions renin may be both hypertensive

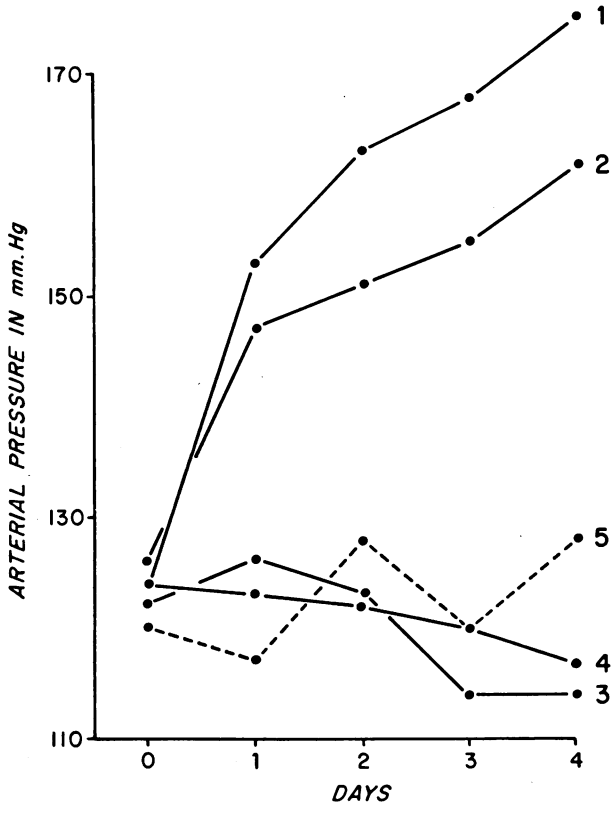

Fig. 5.—Mean arterial pressures obtained through an indwelling arterial catheter from rats with an endocrine kidney (1), from rats which received extracts of endocrine kidneys (2), of kidneys contralateral to endocrine kidneys (3), and of normal kidneys (4). Curve 5 is based on rats which received saline injections.

and vasculotoxic. The problem was first defined by Winternitz *et al.*[11] and later confirmed by Leiter and Eichelberger,[12] who found that kidney extracts caused hypertensive vascular disease when given intravenously to dogs whose renal function had been completely or severely depressed. We observed the same acute and severe effects with semipurified renin administered subcutaneously to bilaterally nephrectomized dogs.[13] The other condition which potentiates the toxic effects of renin is realized by treatment with adrenal steroids plus salt. Administration of semipurified renin to uninephrectomized rats pretreated with desoxycorticosterone, cortisone, cortisol or aldosterone plus salt causes an acute syndrome characterized by water retention, hypertension and vascular disease.[14-16] These manifestations are similar to those of malignant hypertension.

Thus, renal insufficiency and treatment with adrenal steroids have the common property of sensitizing animals to renin. Both conditions increase the height and duration of the pressor response to renin.[17] This effect may account for the development of vascular disease. However, the mechanism of this sensitization is not clear. It is not due to uremia nor to a generalized increase in vascular reactivity. Possibly there is either lack of an inhibitor or appearance of an accelerator of the enzymatic reactions responsible for the formation of angiotensin II;[18, 19] hence, in both instances the net effect would be an increased rate of production of angiotensin. It may be that other factors, such as alterations in the electrolyte composition of the vessel walls, are also contributing to the increase in vascular response and to the damaging effects of increased arterial pressure.

In summary, it has been demonstrated that (1) only extracts of ischemic kidneys mimic the manifestations of renal hypertension, (2) such extracts contain large amounts of renin, (3) renin does not cause hypertensive disease in normal animals but (4) does so after bilateral nephrectomy or treatment with adrenal steroids. On this evidence we are proposing that renal hypertension is caused by the release of renin and of another renal factor which either activates the enzymatic formation of angiotensin or potentiates the peripheral effects of angiotensin. The existence of an activating mechanism is supported by the observation that renal hypertension is associated with an increased response to renin and a normal response to angiotensin.[20] Thus renal hypertension, like nephrectomy or treatment with adrenal steroid, would be accompanied by an increased production of angiotensin. Recent data show that angiotensin is present in amounts larger than normal in the blood of many hypertensive patients[21] and of dogs with a Goldblatt kidney.[22] This hypothesis, by taking into account the increase both in renin content and secretion which occurs after renal ischemia,[9, 10, 23] would reaffirm the central role of the renal pressor system in the genesis of hypertension, while explaining some of the inconsistencies which in the past have led to opposite conclusions.

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