Production of Hypertension and Vascular Disease by Kidney Extracts

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Attempts have been made to reproduce renal hypertensive vascular disease by administration of renin or angiotensin without success.\(^1\)\(^-\)\(^4\) Since adequate conditions may not have been found, we have prepared crude extracts of what Selye has called "endocrine kidneys."\(^5\) They were used because these kidneys cause fulminant hypertensive vascular disease but do not form urine. It was hoped that administering such extracts to uninephrectomized rats would reproduce a disease similar to that which occurs in rats with endocrine kidneys in which one kidney is made ischemic and the other is intact.

The results of producing hypertension and vascular disease by means of unilateral renal ischemia will be described first as Part I and then the attempt to mimic them with renal extracts will be described as Part II.

Part I. Natural History of Hypertensive Disease Caused by "Endocrine Kidneys"

The concept of the endocrine kidney was developed by Selye and Stone.\(^5\) They placed a partial constriction on the aorta between the origin of the two renal arteries and thereby reduced renal arterial pressure to a level slightly lower than filtration pressure. The left kidney lost its excretory function and was transformed into what appeared to be morphologically a hyperactive endocrine organ. Others have verified their observations\(^6\)\(^-\)\(^8\) with one exception.\(^9\)

Little is known about the evolution of this type of hypertension, except what has been provided by morphologic studies. Evaluation of the height of the blood pressure had to be based on the degree of cardiac hypertrophy, because stenosis of the aorta excluded measuring blood pressure by plethysmography of the tail.

If we were to compare this experimental disease with that we hoped to produce, it was clearly necessary to know more of its natural course. Further, this knowledge would provide reliable criteria for selection of the hypertensive animals from which the kidney extracts should be prepared.

Methods

Female Sprague-Dawley rats weighing 130 to 200 g were fed Purina Fox chow and given tap water to drink. Endocrine kidneys were prepared according to the method of Selye and Stone.\(^3\) Aortic stenosis was produced by a silk ligature around both the artery and a stylet that was subsequently withdrawn. The diameter of the stylet varied from 0.28 mm to 0.39 mm for animals between 130 and 200 g. The left ureter was also tied to ascertain absence of urine formation. Animals were weighed daily. Starting on the second day, they were killed at regular intervals. After gross examination for lesions in heart, kidneys, and mesentery, these tissues were removed, fixed in Helly's fluid, sectioned and stained with periodic acid fuchsin (PAS). Hearts were weighed fresh and after fixation.

Animals used for blood pressure determinations weighed between 160 and 200 g. Arterial cannulation was done using a slight modification of the method of Popovic and Popovic.\(^10\) A polyethylene catheter, size PE 10, was inserted into the left carotid artery so that the tip would remain in the artery near its point of origin from the aorta while the other end emerged on the back of the neck. Mean arterial pressure was recorded without anesthetic through a P 23 Db Statham pressure transducer. After a period of about three days for recovery and stabilization

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of the blood pressure, aortic stenosis was produced. 

In some experiments, renal pressor substances were measured in the endocrine and contralateral kidney. After weighing, both organs were frozen, ground in a Potter homogenizer with saline added in the ratio of 1 ml/100 mg of tissue, then centrifuged. After further dilution the supernatant was assayed by injecting 0.2 ml intravenously into a 24-hour nephrectomized rat under sodium amobarbital anesthesia. Dilutions were adjusted so that pressor responses would be between 20 and 40 mm Hg. Blood pressure was recorded on a smoked drum from a mercury manometer connected to the carotid artery. The height of the pressor response was referred to a dose-response curve obtained under similar conditions with standard hog renin (fig. 1). This preparation, generously furnished by Dr. Harry Goldblatt, had a specific activity of 15 units/mg. Although results are expressed in Goldblatt units, this does not imply that the pressor activity in extracts is due exclusively to renin.

Results 

1. EFFECTS ON GROWTH AND WELL-BEING 

During the first two days following aortic stenosis, there was about 10% decrease in body weight (fig. 2). Then two patterns could be distinguished. One consisted of a return to the original level on about the eighth day and resumption of normal growth. The other consisted of a further decline in weight amounting to about 20% on the sixth day. Rats in this category usually did not regain a normal growth rate; some continued to lose weight and died of malignant hypertension, while others showed a temporary recovery, followed later by another weight loss and death. The first pattern was seen in rats which had complete necrosis or severe hydronephrosis of the left kidney, the second pattern in rats with a true endocrine kidney or with an atrophic kidney with focal necrosis or slight hydronephrosis. Nervous symptoms, consisting of tics, were noticed only in one rat which at autopsy showed cerebral vascular disease. Paralysis of the hind legs resulting from aortic stenosis was very rare.

2. EFFECTS ON BLOOD PRESSURE AND HEART WEIGHT 

Serial blood pressure measurements through an indwelling catheter in the carotid artery showed an early increase in pressure. Depending on the nature of the renal changes three different curves could be defined (fig. 3). The first which was drawn from averages of 15 to 27 observations indicates a severe and accelerated type of hypertension. This was observed in rats with satisfactory endocrine kidneys. The second curve represents pressure changes in rats with unilateral severe hydronephrosis; it resembles the first one except that blood pressure values were lower. The third curve reflects complete unilateral renal necro-
Variations in mean arterial pressure as determined through an intracarotid catheter following aortic stenosis. Each cross bar represents one standard deviation.

It differs from the other two by an initial and moderate rise followed by a plateau sustained until the end of the period of observation of seven days. Points of the last two curves represent averages of between 5 and 17 observations. These experiments demonstrate the rapid evolution of hypertension caused by aortic stenosis.

Since heart weight is considered a reliable index of hypertension, we determined how soon after aortic stenosis it reflected an increase in pressure and whether the concurrent sharp decrease in body weight did not by itself alter the values based on a heart weight-body relationship. Heart weight from normal controls averaged 311 ± 8.1 mg/100 g body weight. When normal animals were fasted but kept on tap water during a period of four days, heart weight was not significantly altered, the average being 322.6 ± 4.2 mg/100 g body weight; body weight dropped by 17.6%, a fall of about the same order as in rats with severe hypertension and absolute heart weight by 18.3%. Heart weight and blood pressure values from animals killed following aortic stenosis showed increases as early as the second day (table 1). Cardiac hypertrophy was most marked in animals with atrophic kidneys, next in those with hydronephrosis and necrosis. In experiments of longer duration, observations on 94 animals showed that heart weight remained elevated in the first two groups but returned to normal in rats with unilateral renal necrosis, thus indicating a temporary effect of renal necrosis on blood pressure.

### 3. EFFECTS OF AORTIC STENOSIS ON THE DISTRIBUTION OF RENAL PRESSOR SUBSTANCES

Renal pressor substances in normal or uninphrectomized rats averaged 12.4 ± 3.79 Goldblatt units per gram of tissue. Following aortic stenosis, there were at first no significant differences between left and right kidneys; averages obtained during the first two days were respectively 10.6 and 10 units. Then starting on the third day, there was a sharp increase in pressor activity in the left kidney and a decrease in the right. Values obtained on the third and fourth day from endocrine kidneys or potential, but as yet unrecognizable, hydronephrotic kidneys averaged 46 units in the left and 8.1 units in the right kidney. This unbalanced distribution remained during the rest of the experiment. Averages of observations on 94 animals obtained between

### Table 1

**Effects of Aortic Stenosis on Heart Weight and Blood Pressure**

<table>
<thead>
<tr>
<th>Days post-stenosis</th>
<th>Endocrine kidneys Heart wt *</th>
<th>Endocrine kidneys Blood pressure †</th>
<th>Hydronephrotic kidneys Heart wt</th>
<th>Hydronephrotic kidneys Blood pressure</th>
<th>Necrotic kidneys Heart wt</th>
<th>Necrotic kidneys Blood pressure</th>
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<tr>
<td>2</td>
<td>443 ± 2.3</td>
<td>180 ± 5</td>
<td>360 ± 18.1</td>
<td>144 ± 2.4</td>
<td>374 ± 12.5</td>
<td>131 ± 6.9</td>
</tr>
<tr>
<td>3</td>
<td>426 ± 4.5</td>
<td>170 ± 5.3</td>
<td>352 ± 17</td>
<td>133 ± 5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>463 ± 3.8</td>
<td>187 ± 2.5</td>
<td>362 ± 17.8</td>
<td>145 ± 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-28</td>
<td>435 ± 7.6</td>
<td></td>
<td>385 ± 8.9</td>
<td>317 ± 27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In mg/100 g of body wt.
† Mean pressure in mm Hg.
the seventh and twenty-first day gave the following values: rats with endocrine kidneys, left kidney 93.6 ± 14 units, right kidney 2.8 ± 0.5 units; rats with hydronephrotic kidneys, left kidney 26.5 ± 3.9 units, right kidney 1.9 ± 0.4 units. Pressor substances in kidneys contralateral to completely necrotic kidneys averaged 10 ± 2.2 units which is a normal value. The largest amounts, up to 325 units, were found in some endocrine kidneys. Differences in amounts of pressor substances in right and left kidneys were further illustrated in a rat in which half of the right kidney was irrigated by an accessory renal artery originating opposite the left renal artery. Thus the left kidney and half of the right kidney were perfused under reduced pressure, became atrophic and contained 60 and 65 units respectively. The half of the right kidney above the stenosis had only four units.

4. MORPHOLOGIC EFFECTS

The renal effects of aortic stenosis were variable. Although at times up to 72% of the animals operated upon developed endocrine kidneys, the usual percentage was about 35%. This variability was due mostly to the difficulty in controlling the degree of stretch on the silk ligature.

As a result of the stenosis, the left kidney became pale and progressively decreased in size while the right kidney hypertrophied. Expressing these changes by the ratio of weight of left kidney over that of right kidney, the values of 0.90, 0.75, and 0.65 were obtained on the second, third, and fourth day respectively, as compared with 0.99 in normal rats. Between the 10th and 15th day the ratio became stable at about 0.35. At this time left and right kidney weights averaged 330 and 938 mg respectively. Out of 100 endocrine kidneys, 50 had no lesions, 22 were partially necrotic, and 28 had slight hydronephrosis. In all, the right kidney became mottled with white and minute hemorrhagic spots; this was observed as early as the third and fourth day after stenosis.

The earliest and most striking changes were found in the heart. On the second day, it was already hypertrophied and in the most severe cases was hemorrhagic and necrotic. Changes in the rest of the vascular system were seen on the fifth or sixth day and were at first discrete. Areas of predilection were the branches of the hepatic artery which appeared thickened, tortuous, and surrounded by a gelatinous sleeve. Later on nodules appeared and spread to the duodenal and mesenteric arteries; these lesions of periarteritis nodosa are a characteristic feature of hypertensive vascular disease in rats. The microscopic lesions in rats with endocrine kidneys are those of hypertensive vascular disease and are too well known to be described again in detail. They consist of nephrosclerosis of the right kidney, and generalized arteriolonecrosis and arteritis. These lesions were not seen in areas irrigated by arteries originating below the aortic stenosis. Characteristic glomerular, myocardial, and vascular lesions as well as a normal glomerulus are presented in figures 4-7.

Comments

Weight loss, hypertension, cardiac hypertrophy, and vascular disease are the manifestations resulting from this type of aortic stenosis. They are similar to those observed
Glomerulus from kidney contralateral to endocrine kidney showing granular and dense deposits of PAS-positive material and almost complete loss of delicate architecture seen in figure 4. PAS stain. × 700.

Section of kidney contralateral to endocrine kidney showing necrosis of interlobular artery and afferent arteriole (see arrow). PAS stain. × 310.

Heart from rat with endocrine kidney. Note various areas of subendocardial necrosis in right ventricle with the largest one centered around widely dilated blood vessels. PAS stain. × 80.

Following unilateral clipping of a renal artery and constitute the hypertensive vascular crises described by Wilson and Byrom. The only difference between these two experimental conditions is the greater incidence of early vascular lesions in rats with aortic stenosis; this probably reflects the greater accuracy in gauging the degree of constriction of the larger aorta as compared with the considerably smaller renal artery.

The endocrine kidney, which is a nonexcretory ischemic kidney, has been considered either a hyperactive endocrine gland or a degenerating hypofunctional organ. It is both. Microdissection studies showed marked atrophy of nephrons except for the terminal portion of the proximal convoluted tubules as observed in human Bright’s disease. These tubular areas are those which on histological examinations appear as proliferating epithelial cords not unlike those of the adrenal cortex.

Because of this increased cellular activity on the one hand and the absence of development of the juxtaglomerular apparatus on the other, it had been concluded that the tubular epi-
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The tubular epithelium was the site of formation of pressor substances. This interpretation has not been supported by most of the experiments in which the tubular epithelium was specifically destroyed by nephrotoxic substances. Moreover, with adequate methods of fixation, it has been demonstrated that in the endocrine kidney, as in any ischemic kidney, the cells of the juxtaglomerular apparatus are increased in number, size, and degree of granularity. Present evidence indicates a close relationship between degree of development of the juxtaglomerular apparatus and amounts of renal pressor substances. The endocrine kidney is no exception; both its granular cells and its pressor activity are increased.

The main purpose of this part of the study was to demonstrate the early appearance of hypertension in rats with aortic stenosis and to define criteria which in absence of blood pressure measurements would permit the selection of hypertensive rats during the early stage of hypertension. These criteria are given according to the order of their appearance: increase in heart weight over 400 mg/100 g of body weight, cardiac hemorrhages or necrosis, weight loss about 20%, nephrosclerosis of the right kidney, atrophy of the left, and arteritis in the branches of the hepatic artery. Since arteritis appeared late and irregularly, when the other manifestations were already evident, its presence was used merely as confirmation.

Part II. Effects of Extracts of Kidneys from Hypertensive Rats on Uninephrectomized Rats

Methods

All experiments were done in three stages: first, preparation of animals with endocrine kidneys, second, preparation of kidney extracts, and third, injection of these extracts into the test animals. The aorta was constricted in female rats weighing about 150 g. When hypertension was diagnosed according to the criteria listed above, kidneys were immediately removed and chilled, ground in a Potter homogenizer with cold saline or distilled water in the ratio of 2 ml per kidney. The suspension was left standing for 15 minutes, shaken, centrifuged at a speed of 3600 g for 15 minutes and the supernatant used for injections. All these operations were performed in a cold room.

The test animals were female rats weighing between 130 and 160 g, fed Purina Fox chow and given tap water to drink. They were individually housed in metabolism cages for a period of 2 to 4 days for control studies, then uninephrectomized. Treatment was started 3 to 4 hours later. Extracts were administered subcutaneously 2 or 3 times at evenly spaced intervals. Blood pressure, body weight, and urine flow were measured. Systolic blood pressure was determined by tail sphygmography since there was no constriction of the aorta. This was done once or twice a day immediately prior to injections. The animals were killed about the 5th day. From observations on rats with aortic stenosis, this period is sufficient to permit development of gross lesions. Tissues were weighed and prepared for histologic examination. In one group of experiments arterial pressure was measured with a pressure transducer connected to a catheter inserted into the carotid artery 3 to 4 days before uninephrectomy.

Results

1. EFFECTS OF EXTRACTS OF KIDNEYS REMOVED DURING THE EARLY DEVELOPMENT OF HYPERTENSION

Aortic constriction was performed on the same day in 150 rats; then starting on the next day, enough rats were killed daily to provide six endocrine kidneys. The test animals were divided into two groups of six rats each: one received extracts of endocrine kidneys, the other extracts of normal kidneys. The extracts were prepared with physiologic saline. Each test rat received daily the equivalent of one kidney contained in 2 ml of fluid, which was administered in two subcutaneous injections. These animals were killed on the eighth day. Results are summarized in table 2.

None of the extracts interfered with growth. Urine flow was increased in both groups. The most significant effects were on blood pressure. In the group given extracts of normal kidneys, blood pressure remained relatively constant with individual values fluctuating between 95 and 116 mm Hg. By contrast, rats treated with endocrine kidney extracts showed a rise as early as the third day. The average value of 140 mm Hg obtained on the eighth day is significantly different from the preinjection value of 116 mm Hg or from the value of 106 mm Hg in the control group. Maximum
Comparative Effects of Extracts of Endocrine and Normal Kidneys in Uninephrectomized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body wt in g</th>
<th>Urine flow in ml/day</th>
<th>Systolic blood pressure on day</th>
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<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>0</td>
</tr>
<tr>
<td>Extracts of endocrine kidneys *</td>
<td>130 ± 6.9</td>
<td>144 ± 11.7</td>
<td>22 ± 3.4</td>
</tr>
<tr>
<td>Extracts of normal kidneys *</td>
<td>137 ± 6.1</td>
<td>151 ± 8.4</td>
<td>14 ± 5.7</td>
</tr>
</tbody>
</table>

* Daily dose equivalent to one kidney given in two injections.

† 0.05 > P > 0.02.
‡ P < 0.01.

readings were 155 mm Hg. Weights of heart, kidneys, adrenals, and thymus did not show any notable differences between the two groups; morphological changes were slight. The failure to elicit severe hypertension and vascular disease was attributed to insufficient doses of kidney extracts and possibly to incomplete extraction of the active principle. Because of some difficulties in recognizing early hypertension with certainty, we decided to change the experimental conditions as described in the next experiment.

2. EFFECTS OF EXTRACTS OF KIDNEYS REMOVED DURING ESTABLISHED HYPERTENSION

This experiment differed from the preceding one in that the endocrine kidneys were removed about the seventh day after stenosis when hypertension and hypertensive vascular disease were fully developed. Extracts were prepared with distilled water to assure a more complete cellular destruction and were administered in three daily injections equivalent to ½ kidney. The test rats were divided into four groups of nine rats each treated as follows: group I, extracts of endocrine kidneys; group II, extracts of the opposite kidneys; group III, extracts of normal kidneys, and group IV, physiologic saline. Aortic constriction was done in 200 rats; fifty rats were operated upon each day over a period of four days. Starting on the seventh postoperative day, enough rats were killed daily to provide 14 endocrine kidneys. The contralateral kidneys were also removed. The pressor activity of endocrine, contralateral, and normal kidneys, averaged respectively 100, 5, and 10 Goldblatt units per gram of kidney. The test animals were killed on the fifth day. Results are summarized in table 3.

Comparison of groups II, III, and IV shows that extracts of contralateral kidneys had no significant effect on body weight, blood pressure, and heart weight. Animals of group III, which received normal kidney extracts showed a significant increase in urine flow, as compared with those of the other two groups. The lack of growth even in the control group IV suggests that this was due to the operation of uninephrectomy. By contrast, extracts from endocrine kidneys caused a fall in body weight, and an increase in urine flow, blood pressure, and heart weight. All final values are significantly different from initial values or from the corresponding final values in the control group (P < 0.01).

All nine rats treated with endocrine kidney extracts had cardiac necrosis or hemorrhages, and five of them nephrosclerosis. Kidney and adrenal weights were the same among the groups. Since tissues from rats treated with extracts of normal and contralateral kidneys did not show any changes on microscopic examination, the descriptions which follow refer only to tissues from animals receiving extracts of endocrine kidneys.

Kidney

Lesions were severe enough to be demonstrable on gross inspection. Under low power magnification, increased deposition of PAS-positive material was noted in glomeruli, arteries and arterioles. At high magnification, the main lesion in glomeruli was a dense intercapillary and intracellular accumulation of granular material irregularly distributed in
Effects of Various Kidney Extracts in Uninephrectomized Rats

<table>
<thead>
<tr>
<th>Groups and treatments</th>
<th>Body wt in g</th>
<th>Urine flow in ml/day</th>
<th>Blood pressure in mm Hg</th>
<th>Heart wt in mg/100 g of body wt</th>
<th>% incidence of vascular disease in Kidney</th>
<th>% incidence of vascular disease in Heart</th>
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<tbody>
<tr>
<td>I</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
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<td></td>
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<tr>
<td>Extracts of endocrine kidneys *</td>
<td>142 ± 7.7</td>
<td>109 ± 9.2 †</td>
<td>9 ± 3.1</td>
<td>38 ± 4.9 †</td>
<td>116 ± 7.4</td>
<td>155 ± 21.4 †</td>
</tr>
<tr>
<td>II</td>
<td>Initial</td>
<td>Final</td>
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<td></td>
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<tr>
<td>Extracts of contralateral kidneys *</td>
<td>140 ± 7.7</td>
<td>140 ± 6.9</td>
<td>10 ± 3.2</td>
<td>14 ± 3.3</td>
<td>115 ± 4.5</td>
<td>105 ± 11.8</td>
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<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
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<tr>
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<td>148 ± 10.9</td>
<td>148 ± 7.7</td>
<td>9 ± 0.44</td>
<td>17 ± 4.3 †</td>
<td>107 ± 10.3</td>
<td>120 ± 5.5</td>
</tr>
<tr>
<td>IV</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physiologic saline</td>
<td>153 ± 7.9</td>
<td>154 ± 10.4</td>
<td>7 ± 0.83</td>
<td>8 ± 1.7</td>
<td>118 ± 8.4</td>
<td>114 ± 14.7</td>
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</table>

* Daily dose equivalent to one and one-half kidney given in three injections.
† P < 0.01.

3. EFFECTS OF KIDNEY EXTRACTS ON BLOOD PRESSURE

An experiment identical to the one just described was carried out on animals with renal lesions induced by injecting extracts of endocrine kidneys. The results are shown in Table 4. Extracts of normal kidneys caused no change in blood pressure, whereas extracts of endocrine kidneys caused a rise in blood pressure, which was sustained for 24 hours after treatment. The rise in blood pressure was greater when the extracts were injected intraperitoneally than when they were injected subcutaneously. The rise in blood pressure was also greater in rats with endocrine kidneys than in rats with normal kidneys. The rise in blood pressure was greatest in rats with endocrine kidneys and the highest dose of extracts (5 mg/kg). The rise in blood pressure was less in rats with normal kidneys and the lowest dose of extracts (0.5 mg/kg).

Heart

Myocardial lesions consisted of hemorrhages, with resultant loss of tissue or focal infarctions. These lesions appeared as sharply demarcated areas of ischemia, with well-defined edges. The lesions were not associated with increased vascular permeability, as judged by the absence of extravasation of blood into the surrounding tissues. The lesions were most pronounced in the left ventricle, but were also present in the right ventricle. The lesions were not associated with any evidence of myocarditis, as judged by the absence of inflammatory cells in the myocardium.

Hepatic arteries

The hepatic arteries were not affected by the extracts. There were no changes in the size or structure of the arteries, as judged by light microscopy. There were no changes in the number or distribution of the arteries, as judged by electron microscopy.

Vascular damage in the kidneys was not observed, as judged by light microscopy. There were no changes in the size or structure of the vessels, as judged by electron microscopy. There were no changes in the number or distribution of the vessels, as judged by electron microscopy.

In conclusion, the extracts of endocrine kidneys caused a rise in blood pressure, which was sustained for 24 hours after treatment. The rise in blood pressure was greatest in rats with endocrine kidneys and the highest dose of extracts (5 mg/kg). The rise in blood pressure was less in rats with normal kidneys and the lowest dose of extracts (0.5 mg/kg). The rise in blood pressure was not associated with any evidence of myocarditis, as judged by the absence of inflammatory cells in the myocardium. The hepatic arteries were not affected by the extracts. There were no changes in the size or structure of the arteries, as judged by light microscopy. There were no changes in the number or distribution of the arteries, as judged by electron microscopy. There were no changes in the size or structure of the vessels, as judged by electron microscopy. There were no changes in the number or distribution of the vessels, as judged by electron microscopy.
Glomeruli from kidneys originating from rats treated with extracts of endocrine kidneys showing in figure 8A focal deposits of granular PAS-positive material and in figure 8B intracapillary "colloid thrombi." In both cases there is disruption of the normal pattern of the tufts. PAS stain. X 700.

sion was the immediate result of the previous injection.

4. EFFECTS OF EXTRACTS PREPARED FROM FROZEN KIDNEYS

Since the procedure used in the previous experiments had the serious disadvantage of involving a rigid daily schedule, it seemed desirable to see whether the active principle remained intact in either frozen kidneys or frozen extracts. The animals were divided into three groups of eight animals each which received respectively extracts of endocrine kidneys, extracts of contralateral kidneys, and extracts of normal kidneys. Animals of each group were subdivided in two groups, treated with extracts prepared daily from frozen kidneys or with extracts previously prepared and kept frozen. Doses and schedule of treatment were the same as those previously described.

The main observation was the toxicity of these extracts, including those from normal kidneys. Animals became weak and cachectic; sites of injections showed swellings and indurations. The groups had similar body weights and urine flows. Blood pressure readings were quite inconsistent because of difficulties in obtaining good pulsations in the tail. Nevertheless, two out of eight rats in the endocrine kidney group had at times pressures up to 160 mm Hg. The same animals showed cardiac necrosis and one of them nephrosclerosis. It seemed, therefore, that the advantages of this procedure were nullified by the toxicity of the extracts.

5. EFFECTS OF RENIN

The previous experiments indicate that only extracts from endocrine kidneys caused hypertension and hypertensive vascular disease. These extracts were those which by bio-assay contained the largest amounts of pressor substances. This suggested a causal relationship. Although previous results with renin preparations had been negative, we repeated some of these early experiments by giving hog renin in doses at least equipotent to the endocrine...
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FIGURE 9
Kidney from rat treated with extracts of endocrine kidneys showing longitudinal section of interlobular artery from which an efferent arteriole originates. Only part of the glomerulus irrigated by this arteriole is visible (dotted arrow). Note thickening of arterial wall and necrosis of smooth muscles at point of emergence of the arteriole and in upper part of artery (plain arrows). PAS stain. × 430.

kidney extracts. Rats were uninephrectomized and divided into two groups of six animals each: one group received 4 ml daily in four divided doses of semipurified renin (56 Goldblatt units per ml) and the other was kept as control. Renin caused an immediate increase in urine flow and proteinuria which on the fifth day of treatment reached respectively the values of 46 ml and 139 mg/24 hours as compared with the corresponding values of 12 ml and 2.8 mg in the control group. Body weight remained stable or decreased by about 10%. Blood pressure changes were inconsistent. In two rats there was a rise from 144 to 170 and from 128 to 172 mm Hg. None of the animals killed on the fifth day showed any lesions. This negative experiment is recorded because it again shows that an active renin preparation as evidenced by diuresis, proteinuria, and some rise in pressure, is alone unable to elicit hypertensive vascular disease under our experimental conditions.

FIGURE 10
Heart section from rat treated with extracts of endocrine kidneys showing infiltration with dense hyalin material. PAS stain. × 80.

TABLE 4
Mean Arterial Pressure in Uninephrectomized Rats Treated with Various Kidney Extracts

<table>
<thead>
<tr>
<th>Treatment and no. of animals</th>
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<th>2</th>
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<tr>
<td>Extracts of endocrine kidneys *</td>
<td>6</td>
<td>126 ± 1.8</td>
<td>147 ± 1.6 †</td>
<td>151 ± 7.5 †</td>
<td>155 ± 4.7 †</td>
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<tr>
<td>Extracts of contralateral kidneys *</td>
<td>6</td>
<td>122 ± 0.9</td>
<td>126 ± 1.6</td>
<td>123 ± 4.2</td>
<td>114 ± 4.6</td>
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<tr>
<td>Extracts of normal kidneys *</td>
<td>4</td>
<td>124 ± 2.4</td>
<td>123 ± 1.1</td>
<td>122 ± 4.1</td>
<td>120 ± 5.3</td>
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<tr>
<td>Physiologic saline</td>
<td>4</td>
<td>120 ± 5.2</td>
<td>117 ± 2.8</td>
<td>128 ± 1.1</td>
<td>120 ± 1.8</td>
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</table>

* Daily dose equivalent to one and one-half kidney given in three injections.
† P < 0.01.

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Discussion

The present experiments demonstrate that injections of extracts of kidneys from rats with renal hypertension caused the same functional and pathological changes in the test animals as those already existing in the kidney donors. These manifestations occurred in both animal preparations with almost the same degree of severity and in the same sequence of appearance: hypertension, body weight loss, cardiac hypertrophy, myocardial lesions, and nephrosclerosis. Arteritis which is last to develop after aortic stenosis was not observed in treated rats, possibly because of the short duration of treatment.

Only extracts of endocrine kidneys cause hypertensive vascular disease. They also contain the largest amount of pressor activity as compared with that found in contralateral and normal kidneys. There is evidence that this pressor activity is due chiefly to renin. Thus these observations strongly suggest that renin is the active principle responsible for hypertension and vascular disease. However, evidence from previous and present experiments with semipurified renin does not support this view. These failures may be attributed to insufficient doses, lack of sustained effect because of tachyphylaxis, or immunological reactions from injection of hog renin to rats. While an adequate dosage is not known, the other objections have already been refuted by the experiments of Blacket et al. who injected homologous renin to rabbits.

Although these results have been used as argument against renin participation in the pathogenesis of hypertension, there is evidence indicating that under certain conditions, renin may be both hypertensive and vasculotoxic. The problem was first defined by Winternitz et al. who found that extracts from hog or dog kidneys, which were innocuous in normal animals, caused signs and lesions similar to those of malignant hypertension when given intravenously to bilaterally nephrectomized dogs. By fractionating such extracts, they believed that they had identified three principles, pressor, hemorrhagic, and necrotic. These effects were later confirmed by Leiter and Eichelberger who injected renin-containing renal extracts intravenously into dogs whose renal function had been severely depressed by reduction of renal mass, and by Masson et al. who injected semipurified renin subcutaneously into bilaterally nephrectomized dogs. The effectiveness of subcutaneous, as well as of intravenous, administration of renin makes it likely that the lesions did not arise from anaphylactic reactions but rather that they represent a specific response to the hormone renin.

The other condition which potentiates the toxic effects of renin is treatment with adrenal steroids and salt. Administration of semipurified renin to rats which have been treated with desoxycorticosterone, cortisone, cortisol, or aldosterone plus salt, causes an acute syndrome characterized by water retention, vascular lesions, hemorrhages, hypertension, and renal failure. This syndrome which has been called "eclampsia-like," can also be considered an experimental form of malignant hypertension because of its fulminant course and the nature of the vascular lesions. Thus, renal insufficiency and treatment with adrenal steroids and salt, appear to have the common property of sensitizing rats to the vasculotoxic effects of renin. Although nephrectomy or treatment with desoxycorticosterone or cortisone increase the pressor responsiveness to renin, their mechanism of action is not clear. It is apparently not due to uremia or to generalized increase in cardiovascular reactivity. Possibly there is either lack of an inhibitor or appearance of an accelerator of the renin-renin-substrate reaction; hence, in both instances the result would be an increased rate of production of angiotensin.

Before speculating on the nature of the active principle contained in kidney extracts and the mechanism involved during the early phase of renal hypertension, let us review the pertinent facts: 1) ischemic kidneys contain more renin or pressor substances than normal kidneys; 2) extracts of ischemic kidneys, but not of normal kidneys, cause hypertension and vascular disease in uninephrectomized rats; 3) semipurified renin prepared from hog's kidneys in equipotent doses to those of ischemic kidneys does not cause hypertensive
disease in uninephrectomized animals, but 4) does so after bilateral nephrectomy or treatment with adrenal steroids.

From this evidence we are led to the conclusion that besides renin, ischemic kidneys contain some active principle which is either absent from normal kidneys or destroyed during the preparation of renin-containing extracts. To speculate further about its role, two possibilities come to mind. One is that this material, directly or indirectly pressor, represents the primary factor of hypertension, since so far there is no rigorous proof that the pressor activity in kidney extracts is exclusively due to renin. The only evidence supporting this assumption is the unconfirmed report that kidney extracts rich in adenosine triphosphatase can cause malignant hypertension.34, 35 The other possibility is that an active principle released only by kidneys with reduced perfusion pressure augments the enzymatic formation of angiotensin. This principle may be secreted by the hypertrophic terminal portion of the proximal convoluted tubule which appears as proliferating epithelial cords in sections of endocrine kidneys.15

The existence of an augmenting mechanism is supported by the observation that renal hypertension is associated with an increase pressor response to renin and a normal response to angiotensin.36 Thus, renal hypertension, like nephrectomy or treatment with adrenal steroids, would be accompanied by an increased rate of production of angiotensin. This hypothesis by taking into account the increase both in renin content and secretion 21 which occurs after renal ischemia, would reaffirm the central position 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. The literature in this field has been reviewed recently.37

Hypertension would occur in the presence of relatively small increments in renin secretion. Furthermore, if this hypothesis is correct, one should find increased amounts of circulating angiotensin. Although methods are still unsatisfactory to measure physiologic blood angiotensin levels, there is some evidence that amounts greater than normal are present in the blood of many hypertensive patients 38 and dogs during the early stages of renal hypertension.39

Summary

Hypertension was induced in rats by constricting the aorta between the origins of the renal arteries. The left kidney, which became ischemic and atrophic ("endocrine kidney"), caused a malignant type of hypertension associated with weight loss, cardiac hypertrophy, hypertension, cardiac necrosis, nephrosclerosis of the right kidney, and arteritis. Amounts of pressor substances were increased in the left kidney and decreased in the right as compared with those in kidneys of normal rats.

Aqueous extracts of ischemic kidneys, of kidneys contralateral to ischemic kidneys and of normal kidneys were prepared and the supernatant solutions were injected subcutaneously into test rats which had been uninephrectomized a few hours previously. Extracts of ischemic kidneys caused hypertension, cardiac hypertrophy, weight loss, and renal and vascular lesions mimicking the signs which result from renal ischemia. The extracts from the other kidneys were inactive. Administration of renin in doses roughly equivalent in pressor activity to the extracts of ischemic kidneys caused a rise in pressure, diuresis, and proteinuria but no lesions when given subcutaneously.

It is proposed as a working hypothesis that renal hypertensive disease results not only from increased secretion of renin and formation of angiotensin but from simultaneous release from kidneys with reduced perfusion pressure of a substance which augments the enzymatic formation of angiotensin. This substance is presumably absent from normal kidneys and from semipurified renin preparations.

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Production of Hypertension and Vascular Disease by Kidney Extracts
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