BENIGN AND MALIGNANT HYPERTENSION AFTER ADRENAL ENUCLEATION IN THE RAT*

RELATIONSHIP TO SALT INTAKE, RESPONSE TO HYDROCHLOROTHIAZIDE, AND SIMILARITY TO ESSENTIAL HYPERTENSION

By C. E. HALL, PH.D., O. B. HOLLAND,‡ AND O. HALL

(*From The Carter Physiology Laboratory, University of Texas Medical Branch, Galveston, Texas 77550*)

PLATES 1-4

Initially it appeared that hypertension developing in rats after enucleation of the adrenal gland (adrenal-regeneration hypertension) depended upon the provision of a high salt (NaCl) intake (1, 2). The further observation that such hypertensives exhibited saline polydipsia led to the suggestion that whatever the underlying physiological disturbance might be, the critical component was a heightened susceptibility to the pathologic effects of salt excess (3).

An examination of data obtained by Rapp (4), however, indicated that hypertension developed during adrenal regeneration in rats not given supplements of salt, although admittedly the basic diet was rather high in the mineral. Subsequent studies in this laboratory have shown that most rats eating a normal commercial ration containing about 0.5% NaCl ultimately develop hypertension after adrenal enucleation, even if tap or distilled water is imbibed (5).

Under such circumstances the syndrome which evolves differs from that which develops under high salt regimens in the following manner: (*a*) the onset of hypertension is delayed; (*b*) the rate at which it progresses is characteristically slower; (*c*) hypertension is usually milder; and (*d*) necrotizing vascular lesions are rare and confined prinicipally to kidney glomeruli. This is evidence against the fundamental alteration being one which merely sensitizes to the pathological effects of salt excess. There remains, of course, the possibility that during adrenal regeneration the animal is so sensitized to sodium that even normal dietary levels become injurious.

Further investigation of adrenal-regeneration hypertension revealed that addition of hydrochlorothiazide to the 1% saline given to rats to drink prevents, or, if given after it has developed, usually reverses adrenal-regeneration hypertension (6). If one considers the drug as a saluretic, then the antihypertensive effect may be ascribed to diminished sodium retention. Such a belief has been expressed, because either salt restriction or hydrochlorothiazide therapy has quite similar therapeutic benefits in essential hypertension, whereas in renovascular hypertension both are ineffective (7).

35

Saluresis, however, cannot entirely account for the antihypertensive effect of hydro-chlorothiazide (8), since this property is also demonstrated by salt-retaining, non-diuretic thiazides (9, 10). Later studies showed that during very prolonged hydrochlorothiazide treatment, some adrenal-enucleated rats ultimately broke through the suppressive effect and became hypertensive (11). This prompted further investigation.

In a previous study we reported evidence that adrenal-regeneration hypertension tended to develop more rapidly in rats drinking tap water than in those given distilled water (5), presumably because the sodium content of local tap water may sometimes reach 350 parts per million or more (12). However, the conclusion was based upon responses in two experiments, each of which involved a different rat strain, and it seemed worthwhile to eliminate possible differences in strain sensitivity. These experiments were therefore designed to demonstrate: (a) whether there is a consistent significant difference in response to tap and distilled water after adrenal enucleation; (b) whether hydrochlorothiazide would prove better able to control the blood pressure elevation of adrenal-enucleated rats when water rather than saline was being consumed; and (c) whether escape from hydrochlorothiazide suppression would occur in adrenal-enucleated rats drinking water.

EXPERIMENT 1

Materials and Methods

34 immature female rats were divided into five groups. Groups 1, 2, and 3 were uninephro-adrenalectomized and contralaterally adrenal-enucleated, whereas groups 4 and 5 were only uninephroadrenalectomized. Group 1 received 1% NaCl to drink, groups 2 and 4 were given tap water, groups 3 and 5 drank distilled water. Purina laboratory chow was given ad lib.

Blood pressure of unanesthetized rats was periodically measured by tail plethysmography, and pressures above 150 mm Hg were regarded as hypertensive. The animals were individually caged in temperature-controlled quarters. A 24 hr fluid intake of each animal was taken for 3 consecutive days of each week and the average computed from them considered to be representative of the intake of the animals for the week.

The animals were killed with ether on the 82nd day and various organs and tissues taken for histology were placed in neutral 10% formalin. After fixation, organs were removed, trimmed, blotted, and weighed on an analytical balance. Sections were stained with hematoxylin and phloxine.

Results

Fluid Consumption.—Adrenal-enucleated and control rats drank comparable amounts of distilled and tap water from the outset. There was no substantial variation in fluid volume consumption between any of the groups. More saline than either tap or distilled water was drunk. Intake based upon total quantity rather than in proportion to body weight, since growth rate was equivalent among the groups, is illustrated in Text-fig. 1. Analysis of a sample of tap water (which varies in sodium content) in the last week of the experiment revealed it

contained 225 parts per million of sodium. Samples of distilled water contained from 2 to 5 parts per million of the ion.

Blood Pressure.—Two adrenal-enucleated rats drinking saline were hypertensive on the 17th day and all were by the 26th day. Thereafter, there was a general tendency toward exacerbation. On the 17th day the average blood pressure in this group exceeded that in each of the others ($P < 0.05$). By the 26th day the difference was even more significant ($P < 0.01$ to $P > 0.001$), and it increased thereafter. Terminally the pressures ranged from 178 to 238 mm Hg. One severely hypertensive rat died on the 46th day.
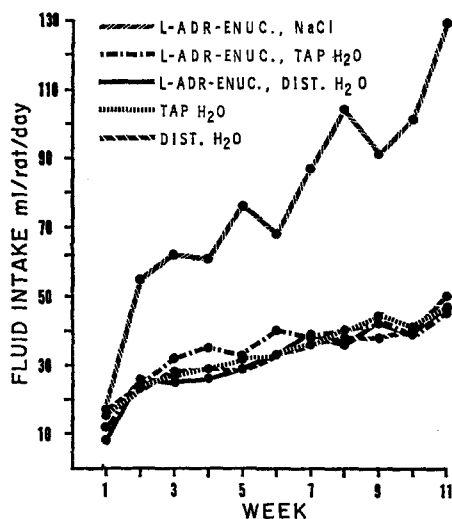
TEXT-FIG. 1. Daily fluid consumption of adrenal-enucleated and control rats offered various liquids. Enucleated rats drank more saline than either tap or distilled water, and no more water than did controls.

Similarly operated rats drinking tap water showed no conspicuous blood pressure change until the 26th day, when one had reached 152 mm Hg; the incidence gradually increased thereafter, but the group average did not reach the hypertensive range until the 49th day, when, at 154 ± 4 mm Hg, it exceeded that of either group of water controls ($P < 0.05$). By the end of the experiment, eight of the nine rats had hypertensive pressures, although the mean reached only 163 mm Hg and the highest individual pressure was 174 mm Hg. The difference in the group average pressure between this group and either of the controls became increasingly greater as the experiment progressed, ultimately reaching $P < 0.001$.

The average pressure attained by enucleated rats drinking distilled water was in any given period numerically equivalent to, and never significantly different from, the average of the group on tap water. However, the increase

TABLE I

Blood Pressure Changes in Control and Adrenal-Enucleated Rats with Time As Related to Fluid Consumed

Group	Rat No.	Experimental day								
		17	26	33	43	49	56	63	71	78
Adrenal enucleated, 1% NaCl	1	154	210	256	250	240	194	204	222	228
	2	124	164	154	166	186	200	176	166	178
	3	124	150	150	158	156	172	246	226	230
	4	144	178	204	230	234	226	216	210	234
	5	120	156	170	184	208	210	264	250	254
	6	176	206	230	242	250	238	216	230	238
	7	136	162	154	188	192	200	—	—	—
Mean ± SEM		140 ± 8	175 ± 9	188 ± 16	203 ± 14	209 ± 13	206 ± 8	220 ± 13	217 ± 12	227 ± 11
Adrenal enucleated, tap water	8	118	122	130	150	152	154	150	152	148
	9	116	124	132	134	136	144	144	144	144
	10	134	132	134	164	168	172	170	172	170
	11	116	146	158	144	142	150	154	158	154
	12	114	152	148	160	168	170	170	180	174
	13	122	132	160	146	154	170	166	170	174
	14	144	144	164	156	166	170	160	170	164
	15	112	118	134	148	150	150	154	158	158
	16	114	126	130	136	152	154	150	164	160
Mean ± SEM		121 ± 4	133 ± 4	143 ± 5	149 ± 3	154 ± 4	159 ± 4	158 ± 3	163 ± 4	161 ± 4
Adrenal enucleated, distilled water	17	114	134	132	144	136	148	156	152	156
	18	110	124	124	130	142	152	156	146	154
	19	138	184	214	226	242	246	254	250	246
	20	110	124	126	134	134	138	144	144	142
	21	112	124	130	146	148	154	150	148	152
	22	114	122	124	126	130	144	134	146	152
	23	116	122	132	142	146	136	140	146	150
Mean ± SEM		116 ± 4	133 ± 9	140 ± 12	150 ± 13	154 ± 15	160 ± 15	162 ± 16	162 ± 15	165 ± 14
Control, tap water	24	112	136	128	128	132	140	150	150	150
	25	120	130	128	128	128	126	126	128	126
	26	124	124	126	134	136	130	138	142	142
	27	122	132	128	124	120	116	120	120	120
	28	118	134	124	126	130	136	130	128	126
	29	114	128	130	122	130	126	130	134	132
Mean ± SEM		120 ± 1	131 ± 2	127 ± 1	127 ± 2	129 ± 2	129 ± 3	132 ± 4	134 ± 4	133 ± 5
Control, distilled water	30	120	126	128	126	124	124	128	130	128
	31	120	128	124	126	124	124	124	124	124
	32	116	124	134	134	138	142	152	164	172
	33	112	126	130	130	124	124	128	130	130
	34	116	128	124	124	126	124	132	134	134
Mean ± SEM		117 ± 1	126 ± 1	128 ± 2	129 ± 1	127 ± 3	128 ± 4	133 ± 5	136 ± 7	138 ± 9

among the former was, until late in the experiment, principally due to the response of a single animal which developed a particularly fulminating hypertension. Because most of the others were normotensive the group average pressure, unlike that of rats on tap water, never significantly exceeded that of either group of controls. Late in the course of the experiment the incidence of hypertension began to increase in this group. Finally, all but one (which was prehypertensive) had attained pressures above 150 mm Hg, although, except

TABLE II

Body and Organ Weights in Adrenal-Enucleated and Control Rats As Related to Blood Pressure and Type of Drinking Fluid Consumed

Group and treatment	Body wt		No. of rats	Terminal B.P. av.	Organ weights			
	Initial	Final			Kidney	Heart	Thymus	Adrenals
	g			mm Hg	mg	mg	mg	mg
1 Adrenal enucleated, 1% saline	51 ± 1*	215 ± 18	6	*227 ± 11*	*2620 ± 195*	*1125 ± 67*	228 ± 36	*45.5 ± 3.0*
2 Adrenal enucleated, tap water	51 ± 1	236 ± 6	9	*161 ± 4*	*2038 ± 141*	781 ± 28	319 ± 18	*47.2 ± 2.2*
3 Adrenal enucleated, distilled water	50 ± 1	228 ± 3	7	165 ± 14	1938 ± 155	801 ± 63	294 ± 10	*47.9 ± 4.2*
4 Control, tap water	50 ± 1	207 ± 19	6	133 ± 5	1541 ± 133	711 ± 61	304 ± 11	54.6 ± 4.7
5 Control, distilled water	45 ± 0.5	219 ± 4	5	138 ± 9	1624 ± 84	719 ± 17	288 ± 23	61.1 ± 4.2

Italic figures differ significantly ($P < 0.05$) from group 4.
* Mean ± SEM.

for the one severely hypertensive rat, the systolic pressures were all below 160 mm Hg.

All control rats had normal arterial pressures until the 63rd day, when one rat in each group was noted to be hypertensive. Curiously, here too the response in the animal on distilled water was the greater and more rapidly progressive. In Table I the data for individual pressures illustrate the variability of the response.

Organ Weights.—The kidneys were greatly enlarged in adrenal-enucleated rats drinking saline, significantly larger than those of any other group, and moderately enlarged in those given tap water as compared with either group of nonenucleated controls ($P < 0.05$). Enucleated rats drinking distilled

water had kidneys which, although slightly larger than those of controls, did not differ significantly from them in weight.

The hearts were also significantly larger in adrenal-enucleated rats drinking saline than in any other group, but although those drinking either type of water had slight cardiac enlargement there was no significant hypertrophy as compared with either control group.

The weight of thymus glands did not significantly vary among the groups. Although the regenerated adrenal glands of enucleated rats were smaller than the hypertrophied glands of unilaterally adrenalectomized rats, there was no significant difference between any of the groups of enucleated rats or between the two groups of controls. The data are given in Table II.

Pathologic Findings.—All adrenal-enucleated rats drinking saline had arteriolar nephrosclerosis of varying severity, three of the six had hyaline changes of the cardiac arteries, two had polyarteritis of mesenteric vessels, and two had focal adrenal cortical necrosis.

None of the tap water group had extrarenal lesions, but kidneys in seven of the nine rats had from one to several glomeruli that showed focal glomerular sclerosis and a scattering of hyaline tubular casts (Figs. 1 and 2).

The one adrenal-enucleated rat in the distilled water group that had developed severe hypertension early in the treatment showed the severest degree of nephrosclerosis (Figs. 3 and 4), cardiac lesions (Fig. 5), and polyarteritis nodosa (Fig. 6). Otherwise the changes were quite similar to those present in the previous group, in that there were no extrarenal lesions, and those in the kidney, although detectable, were neither abundant nor severe (Fig. 7).

No pronounced lesions were seen in either group of water controls, although there were some capillary thickening and hyalinization in occasional glomeruli of the one rat in the distilled water group which had finally attained an arterial pressure above 170 mm Hg (Fig. 8).

EXPERIMENT 2

Materials and Methods

38 immature female rats of the Houston-Cheek strain weighing 60–80 g were uninephro-adrenalectomized, contralaterally adrenal-enucleated, and divided into four groups. Group 1 received 1% NaCl solution to drink, group 2, a solution containing 0.03% hydrochlorothiazide and 1% NaCl, group 3, distilled water, and group 4, 0.03% hydrochlorothiazide in distilled water.

Caging, feeding, blood pressure estimation, and fluid consumption measurements were all carried out in the same way as in the previous experiment, except that measurements of fluid intake were not made in the last 2 wk of the experiment.

The surviving rats were killed with ether on the 112th day. A blood sample was collected by cardiac puncture for measurement of the hematocrit and plasma protein concentration (Goldberg refractometer, model 10401, American Optical Co., Buffalo, N. Y.) and for analysis of the serum Na and K by flame photometry, using an internal lithium standard (Beckman Instruments, Inc., Fullerton, Calif., model 150). Tissues and organs were removed and placed

in neutral 10% formalin for histologic preparation. When fixed, those to be weighed were trimmed, blotted, and weighed on an analytical balance.

Results

In the first postoperative week there were no deaths in the group given saline to drink, but during this interval one of the saline-hydrochlorothiazide group, two of the group on water, and four of those getting the drug in water died with signs of adrenocortical insufficiency. Thereafter deaths were confined to the first group, all of which succumbed before the experiment ended.

The greatest fluid consumption occurred among the rats drinking saline solution, which individually and collectively exhibited polydipsia throughout the experiment. None was hypertensive by the 13th day of treatment, although half of them were eight days later and all of them were by the 28th day. The group average pressure reached its maximum of 229 ± 8 mm Hg by the 43rd day. After the 51st day deaths began to occur, and the longest survivor succumbed on the 84th day with a pressure of 212 mm Hg.

Incorporation of hydrochlorothiazide in the drinking fluid significantly curtailed saline consumption in each of the measurement periods, and the onset of hypertension was delayed. Elevated blood pressure was first detected on the 53rd day, although 98 days were required for half of the group to become hypertensive and for the group average pressure to reach hypertensive levels. When hypertension appeared it tended to remain mild and progress slowly. The group maximum, 157 ± 10 mm Hg, was reached on the 105th day, and the highest individual pressure observed was 184 mm Hg. When the experiment ended one rat was still normotensive, and another was prehypertensive at 148 mm Hg.

Group 3, which drank distilled water, consumed still less fluid than did the preceding group. Hypertension, however, occurred sooner, appearing on the 28th day, and half of the animals were hypertensive by the 63rd day. The group average ultimately reached 172 mm Hg, and the highest individual pressure was 240 mm Hg. The latter occurred in an animal which attained a pressure of 170 mm Hg within a month after surgery, and which behaved like rat 19 in the first experiment.

Fluid consumption in group 4 was entirely comparable with that of group 3. Hypertension, however, was almost entirely prevented. The first and only hypertensive rat did not appear until the 97th day. In a further 2 wk its pressure had risen from 150 to 158 mm Hg and three other rats had attained prehypertensive levels of 140-146 mm Hg. The remainder were entirely normotensive. The fluid intake levels are compared in Text-fig. 2 and the blood pressures in Text-fig. 3.

There were no differences among the groups in respect to hematocrit, serum protein, or serum sodium concentration. The serum K was significantly lower in the group drinking hydrochlorothiazide in water than in those drinking only
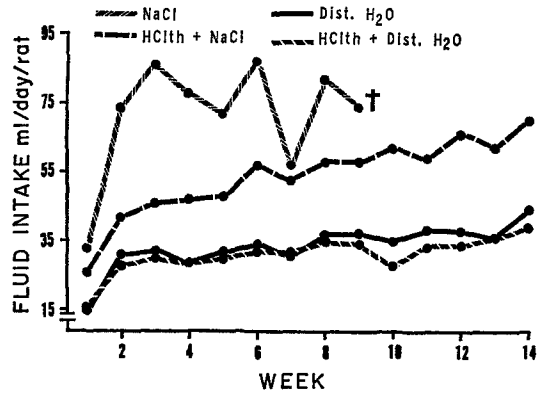
Text-Fig. 2. Fluid consumption of rats drinking water or saline solutions with and without added hydrochlorothiazide. The drug reduced saline, but not water consumption, and more saline than water was drunk whether or not the drug was incorporated in the solution. The high mortality characteristic among enucleated rats drinking saline was prevented by the drug. Although two animals in the saline group lived for an additional 3 wk beyond the last figure tabulated for the group, they were extremely cachectic and the intake was too erratic to plot.
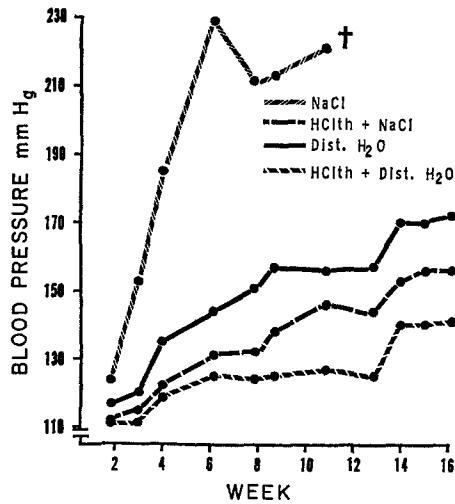
Text-Fig. 3. Blood pressure response after adrenal enucleation as related to fluid consumption. Hydrochlorothiazide more effectively kept blood pressure from rising when water was consumed than when saline was given. Animals given saline and hydrochlorothiazide developed somewhat higher pressures than did those without drug treatment that drank only water. When drug was given with water only one animal became hypertensive. The sharp decline in pressure of those drinking saline after the 7th wk was due to the death in the interim of several of the most hypertensive rats and should not be construed as indicating a general amelioration of the state.

water ($P < 0.05$) or hydrochlorothiazide in saline ($P < 0.025$). The relevant figures are given in Table III.

Organ Weights.—Although all of the enucleated rats on saline had died many weeks before the experiment ended, they had even by then developed significantly larger kidneys, hearts, and adrenal glands than any other group.

Addition of hydrochlorothiazide to saline diminished the degree of kidney enlargement in rats with regenerating adrenal glands, although these organs

TABLE III

Principle Findings in Adrenal Enucleated Rats Drinking Saline Solution with and without Hydrochlorothiazide

Group	No. of rats		Body wt		Ht*	Plasma protein	Serum		Organ weight			
	Ini-tial	Final	Ini-tial	Final			Na	K	Kid-ney	Heart	Thy-mus	Adrenal
			g		*g/100 ml*		*meq/liter*		*mg/100 g body wt*			
1. 1% NaCl‡	8	0	74§ ±3	127‖ ±8	—	—	—	—	1273‖ ±52	732‖ ±60	—	36.5‖ ±1.1
2. 1% NaCl + HCLTH	10	9	77 ±3	252 ±4	49 ±1	7.4 ±0.2	146 ±1.8	4.9 ±0.2	835‖ ±41	318 ±4	88 ±6	18.5 ±0.8
3. Dist. H₂O	10	8	78 ±3	242 ±4	50 ±1	7.5 ±0.1	148 ±1.4	4.8 ±0.2	670 ±27	316 ±9	81 ±6	19.3 ±0.9
4. Dist. H₂O + HCLTH	12	8	76 ±3	249 ±2	46 ±3	7.3 ±0.1	151 ±0.9	4.3‖ ±0.1	703 ±43	303 ±8	97 ±6	19.5 ±0.5

* Hematocrit.

‡ Organ weights in this group based on four longest survivors which died 29–48 days before the experiment ended.

§ Mean ± SEM.

‖ Differs significantly ($P < 0.05$) from group 3.

were larger than those of rats drinking only distilled water or distilled water and the drug ($P < 0.05$). The weights of the hearts, adrenals, and thymus glands were entirely comparable in the three groups surviving the full experimental period. Thymus glands were not removed from the untreated saline group, principally because members had died intercurrently and usually the glands were so atrophied as to be difficult to recognize. They could not in any event be meaningfully compared with those of the other groups, because of the lesser period of treatment that they had undergone. The data are given in Table III.

Histological Changes.—All of the adrenal-enucleated rats given saline to drink had severe necrotizing vascular lesions. All of them had advanced arteriolar nephrosclerosis, marked hyalinization of cardiac arteries, and polyarteritis

nodosa. The majority had focal areas of patchy adrenal cortical necrosis. Cardiac or other extrarenal vascular or degenerative lesions were seen only in members of this group.

Mild to moderate degrees of glomerular hyalinization, tubular cast formation, and arterial hypertrophy were seen in the kidneys of seven of the eight animals that had received saline and hydrochlorothiazide (Figs. 9 and 10), seven of the nine in group 3 given only distilled water (Figs. 11 and 12), and four of the eight that had received distilled water and hydrochlorothiazide (Figs. 13 and 14). Only one of the four had attained a pressure above 150 mm Hg.

DISCUSSION

Both experiments demonstrated that adrenal-enucleated rats on high salt intake rapidly developed malignant hypertension, and that those with a normal level of consumption slowly contracted benign hypertension. There were occasional exceptions, such as rat 19 in the first experiment and another in the second experiment which rapidly developed malignant hypertension despite a normal salt intake, but this does not invalidate the generalization; it does, however, indicate that adrenal regeneration entrains physiologic changes which are potentially capable of causing malignant hypertension even without salt excess being imposed. Except in particularly susceptible animals these changes are seemingly held in check by control mechanisms which are either overwhelmed or rendered inoperative under conditions of high salt consumption. Whether such individual instances are to be ascribed to an unusually high genetic susceptibility to hypertension, which is known to vary greatly (13), or to the fact that additional but unknown factors are sometimes contributary, remains uncertain. It is noteworthy that a control animal on tap water and another on distilled developed hypertension in the first experiment. Whether such isolated responses are referable to abnormal function of the remaining kidney, which has been held accountable for the blood pressure elevation that occasionally occurs in unilaterally nephrectomized rats (14), or to the spontaneous onset of cryptogenic hypertension known to occur in rats with both kidneys (15, 16) cannot be decided. One may only speculate upon whether these particular animals would have proved to be especially susceptible to adrenal-regeneration hypertension had the adrenal glands been enucleated, and thus have responded inordinately as did one such rat on distilled water in each of the experiments.

The first experiment revealed that hypertension developed sooner when enucleated rats drank tap water than when they drank distilled water, and it seems reasonable to attribute this to the higher salt content of tap water. Parenthetically, the salt content is known to be lower locally in the winter, when this experiment was conducted, than it is in the summer, so that the difference in efficacy we observed was probably minimal. Although distilled water merely delayed the onset, with the result that in the later stages of the experiment the incidence of hypertension was substantially the same in both

groups that drank water, nonetheless only the rats on tap water had enlarged kidneys, probably as a result of the longer duration of their affliction. The slight rise in blood pressure when water was given proved unable to cause significant cardiac hypertrophy.

In the second experiment the ability of saline to protect enucleated rats against postoperative adrenal insufficiency was impaired slightly by hydrochlorothiazide, although animals so treated fared better than did those given distilled water. The combination of drug and distilled water proved most inimical to postoperative recovery, although rats that survived a week had no difficulty thereafter. Consumption of saline, but not of water, was considerably reduced by the drug. A similar response seen when hydrochlorothiazide is injected (17) suggests that some metabolic effect, perhaps involving sodium distribution, rather than a mere aversion to the taste of the drug in solution is responsible for this behavior.

Although hydrochlorothiazide proved able to forestall the onset of hypertension in saline-treated enucleated rats during the interval that it had previously been found to do so (6), nevertheless, despite continued ingestion of the drug, hypertension eventually supervened in all but two animals, one of which had reached prehypertensive levels by the time the experiment was concluded. The drug also held pressures down to levels which prevented the otherwise inevitable death and reduced the attendant cardiovascular lesions. When water was given postoperatively instead of the drug–saline solution, hypertension began sooner and reached higher levels, although the group average blood pressures differed significantly only at the last determination ($P <$ 0.02). The drug proved to be most effective when water was consumed, although even here there was evidence that hypertension could not be prevented indefinitely, and glomerular lesions, although scarce, were detectable in animals so treated.

The antihypertensive effect of hydrochlorothiazide did not appear to be due entirely to the saluretic properties, insofar as serum sodium concentrations, which were the same in the three full-term groups despite the considerable difference in blood pressures, could be used as a criterion. If the drug produced a proportionate loss of sodium and water, thus contracting the plasma volume, it did so without affecting the serum protein concentration or hematocrit in the two groups. The theory, that hydrochlorothiazide exerts its effect upon blood pressure primarily by altering the concentration of potassium in vascular smooth muscle, received some indirect support from the fact that serum potassium was significantly depressed only in the group in which hypertension was all but completely prevented, and where serum potassium was not depressed by the drug, hypertension was present. The drug caused rats given saline to respond essentially in the same manner as enucleated rats drinking water, with benign hypertension.

The occurrence of minimal glomerular lesions in two rats of the hydrochloro-

thiazide water group which had never at any time shown hypertensive pressures was unexpected. It may be either that blood pressure is higher in renal than in systemic arteries, or that in mild hypertension occasional hypertensive episodes are missed. The latter is a distinct possibility (18).

These studies do not support the thesis that adrenal-regeneration hypertension depends upon an increased susceptibility to the pathologic effect of salt excess, even though abundant experimental data indicate that such an increased sensitivity does occur (2-4, 13, 17, 19, 20) which might also be inferred from the fact that adrenal-enucleated rats develop hypertension even when they drink no more saline than controls that remain normotensive (19) and a higher blood pressure than controls when a stock diet somewhat high in sodium chloride is employed (4). They also lend no support to the notion that the fully regenerated adrenal gland is incapable of causing hypertension (20), since in the rats that drank water or were protected by hydrochlorothiazide, hypertension did not begin until well after the time required for complete regeneration. Other evidence has been adduced to show that the fully regenerated gland is, in fact, quite capable of inducing hypertensive disease (11).

When normal levels of salt are ingested, the adrenal-enucleated rat develops the experimental counterpart of benign essential hypertension. The postoperative rise in blood pressure is delayed, and slow to progress once it has begun. It is of slight magnitude as a rule and does not obviously impair health. There are few vascular changes other than relatively minor renal lesions and cardiac hypertrophy is not prominent. Under conditions of excess dietary salt, the syndrome evolves in a manner quite comparable to the malignant phase of essential hypertension, with cardiac hypertrophy, necrotizing vascular lesions, severe nephrosclerosis, terminal weight loss, and high mortality. Salt excess accelerates the onset, exacerbates the progress, and enhances severity of the hypertensive state. As in clinical essential hypertension, there is a favorable response to hydrochlorothiazide.

It might be instructive to explore further the idea that essential hypertension might, in fact, be caused by a type of adrenal dysfunction comparable in effect to that which enucleation experimentally induces.

Attempts to implicate adrenal glands in the genesis of essential hypertension have been typically characterized by efforts to demonstrate signs of cortical hyperfunction, either in terms of the levels of the hormones or their metabolites in blood or urine, by seeking to demonstrate changes in sodium or potassium concentrations in blood, urine, or tissues compatible with such a state, or by examination of the adrenal glands themselves for evidence of hyperfunction Hypersecretion was a logical presupposition for several reasons, chief among them being that hypertension accompanies adrenal cortical hyperfunction of the type seen in Cushing's syndrome or primary aldosteronism, and that several different adrenal steroids will experimentally induce the state (21-24). However,

it has not been a conspicuously successful line of inquiry, and persuasive evidence has not been adduced to indicate that essential hypertension is of adrenal origin.

Adrenal regeneration is accompanied by high blood pressure. Here, however, all the evidence, both direct and indirect, points to an essentially normal or slightly subnormal level of hormone secretion by the largely reconstituted glands (25–29). Furthermore, as shown in this and other studies (4, 19), there is no frank distortion of serum sodium or potassium levels and there are minimal vascular changes. The findings are thus quite analogous to those which have usually been made in essential hypertension.

The relationship between hyperplastic or adenomatous transformation of the adrenal cortex on the one hand and so-called "essential" hypertension on the other has been vigorously debated. Some investigators have found a significant positive correlation between the two, while others have not. It is evident that some patients so diagnosed are subsequently found to have either Cushing's syndrome (30) or some form of aldosteronism (31). Such cases commonly, although not invariably, are found to have cortical adenomas or hyperplasia, frequently although not universally associated with some biochemical evidence of the condition, such as increased excretion of cortical hormones, their metabolites, or hypokaliemia. Removal of the adenomas or partial resection of the adrenal glands has usually, although not always, led to normalization or at least amelioration of the hypertensive state. The frequent inability to demonstrate abnormalities of adrenal mass or structure to account for the hypercorticism of patients with hypertension of known adrenal origin is well documented (32, 33).

Hypertension that accompanies adrenal regeneration is also abolished by removal of the regenerating glands (34). This, unfortunately, does not prove they were the source of an abnormal hormone secretion which caused the hypertensive state, for adrenalectomy will also abolish hypertension of renal origin (35). The fact that small doses of corticosterone will prevent both regeneration and the hypertension which otherwise ensues after enucleation (22) is a more convincing argument than such is, in fact, the case. Failure to demonstrate an abnormal type of adrenal secretion by regenerating adrenal glands may simply reflect ignorance as to exactly the degree of aberration compatible with maintenance of a normal blood pressure. There are changes, both qualitative and quantitative (25–29), and the fact that they have been reported as being insignificant reflects the conviction of the investigator.

Recently Birmingham et al. (36) have presented evidence to indicate that during regeneration steroid biogenesis by the zona glomerulosa is impaired, whereas the responsiveness of the fasciculata reticularis to ACTH is increased. They suggest that an imbalance in corticoid secretion in a direction opposite to that proposed by Skelton might underlie the hypertensive response.

More disquieting is the failure to find hypokaliemia during adrenal-regeneration hypertension. In this context it must be borne in mind that a considerable reduction in total body potassium occurs before a change in serum concentration develops, a fact used to explain those normokaliemic cases of clinical hypertension cured by removal of an adrenal adenoma and presumably due to hyperaldosteronism. Quite obviously adrenal cortical hypersecretion and hypertension may be associated or independently manifested, and when they are associated a cause and effect relationship may or may not exist between them. This being so, any hypothesis that attempts to implicate adrenal hormones in the genesis of hypertension, except when it is caused by their injection, is susceptible of strong counterargument.

Masson and Corcoran (37), however, have shown that ligation of the adrenal pedicle which does not lead to detectable changes in glandular morphology is quite as effective as adrenal enucleation in causing hypertension in rats. The experimental evidence would thus indicate that adrenal enucleation or ligation of the adrenal pedicle are merely interventions, and very probably not the only ones, which share in common the ability to disturb adrenal function in such a way as to evoke a pattern of steroid secretion favorable to the development of high blood pressure. Both operations might depend for their effect upon a combination of glandular stimulation in the face of a reduced blood supply. Both would initially reduce cortical hormone secretion and thus lead to an increased level of ACTH secretion and stimulation of the cortical cells. Pedicle ligation has the obvious effect of reducing blood supply. Adrenal enucleation is followed by intense hemorrhage into the gland, which doubtless impairs circulation. The peculiar nodular type of reconstruction which then begins might not be ideally suited to circulation dynamics. A deficient blood flow through the regenerated gland, which might well alter steroidogenesis, has, in fact, been reported (25, 29). Whatever the sequence of events leading to a deranged type of cortical secretion might be—and in no other way can hypertension induced by surgical intervention be as easily accounted for—the adrenals, in this case, are the victims of circumstance. Quite conceivably there might be cases of human essential hypertension due to a similar type of hormonal imbalance brought about either by impaired adrenal blood supply, in which case the glands themselves would be normal, or by intrinsic glandular changes involving cell permeability characteristics, or alterations in enzyme concentration or ratios, despite a normal blood supply. Evidence has been adduced that essential hypertension might, in fact, be due to a subtle alteration in the metabolism of adrenal steroids (38) rather than to an appreciable change in total output of one or more.

Investigations concerning the possibility that essential hypertension might have an adrenal basis have not produced conclusive evidence to the contrary. There are good reasons why the sequelae to adrenal enucleation have not been considered significantly relevant to essential hypertension. First, the pathologic

changes in the experimental condition, elicited as they were under high salt intake, have always been those of malignant hypertension; and second, the adrenal glands of human hypertensives show none of the bizarre structural characteristics of the regenerated gland. Since under conditions of normal salt consumption the consequence of adrenal enucleation is usually benign hypertension, the first objection is no longer valid. Furthermore, hypertension induced by pedicle ligation, which presumably has the same etiology as that consequent to adrenal enucleation, reveals no abnormality in adrenal structure, secretory pattern, or electrolyte metabolism to account for it. If essential hypertension had a similar basis, it too would lack these same definitive features; which, since the disorder is diagnosed by exclusion (39), appears to be the case. Clearly, however, until the nature of either the clinical or the experimental condition can be precisely delineated, the question of whether they are entities of a similar or entirely different etiology will remain unanswered.

SUMMARY

Adrenal-enucleated, mononephrectomized rats given a high salt diet rapidly develop malignant hypertension, characterized by the presence of necrotizing vascular lesions in a number of organs and tissues. If a normal salt intake is provided, or if hydrochlorothiazide is given together with a high salt diet, there is, instead, the delayed onset of benign hypertension which either stabilizes or increases in intensity extremely slowly. Such animals display few, if any, pathologic vascular changes other than occasional focal glomerular hyalinization, show insignificant cardiac enlargement, and do not exhibit alterations in the serum sodium or potassium. Occasional animals behave atypically and develop malignant hypertension despite normal salt consumption, demonstrating that in susceptible rats excess salt is not essential to this disorder. Hydrochlorothiazide given to rats that imbibed distilled water postoperatively prevented hypertension entirely for 97 days, when one of eight rats developed mild hypertension and some others reached what is regarded as a prehypertensive range.

It is concluded that adrenal regeneration provides a physiological milieu favorable to the development of benign hypertension, which is not, as a rule, manifest until regeneration is complete. Salt excess converts the response into one in which malignant hypertension begins during regeneration and worsens rapidly thereafter until death. The course and findings are compared with those of the benign and malignant phases of clinical essential hypertension, and the implications of the similarities are discussed.

REFERENCES

1. Skelton, F. R. 1956. Adrenal-regeneration hypertension and factors affecting its development. *Arch. Internal Med.* **98**:449.
2. Skelton, F. R. 1959. Adrenal regeneration and adrenal-regeneration hypertension. *Physiol. Rev.* **39**:162.

3. Crane, W. A. J. 1960. Hypertension associated with regenerating adrenal cortical tissue in the rat. *J. Pathol. Bacteriol.* **80:**1229.
4. Rapp, J. R. 1964. Electrolyte and juxtaglomerular changes in adrenal-regeneration hypertension. *Am. J. Physiol.* **206:**93.
5. Hall, C. E., O. B. Holland, and O. Hall. 1966. The sodium chloride requirement in adrenal-regeneration hypertension. *Arch. Pathol.* **81:**247.
6. Hall, C. E., O. B. Holland, and O. Hall. 1966. Prevention and reversal of adrenal-regeneration hypertension with hydrochlorothiazide. *Texas Rep. Biol. Med.* **24:** 73.
7. Tobian, L., and K. Coffee. 1964. Effect of thiazide drugs on renovascular hypertension in contrast to their effect on essential hypertension. *Proc. Soc. Exptl. Biol. Med.* **115:**196.
8. Fregly, M. J., and J. F. Gennaro, Jr. 1965. Effect of thiazides on metacorticoid hypertension and thyroid activity of rats. *Can. J. Physiol. Pharmacol.* **43:**521.
9. Rubin, A. A., F. F. Roth, R. M. Taylor, and H. Rosenkilde. 1962. Pharmacology of diazoxide, an antihypertensive, nondiuretic benzothiadiazine. *J. Pharmacol. Exptl. Therap.* **136:**344.
10. Freed, S. D., S. St. George, and D. Beatty. 1963. Mechanism of antihypertensive action of thiazides. *Proc. Soc. Exptl. Biol. Med.* **112:**735.
11. Hall, C. E., O. B. Holland, and O. Hall. 1967. Evolution of adrenal-regeneration hypertension in rats with actively regenerating or fully regenerated glands. *Lab. Invest.* **16:**488.
12. Editorial. 1961. *Time.* **57:**40.
13. Skelton, F. R. 1955. Development of hypertension and cardiovascular-renal lesions during adrenal regeneration. *Proc. Soc. Exptl. Biol. Med.* **90:**342.
14. Renzi, A. A., J. J. Chart, and R. Gaunt. 1959. Sulfonamide compounds with high diuretic activity. *Toxicol. Appl. Pharmacol.* **1:**406.
15. Dahl, L. K., M. Heine, and L. Tassinari. 1965. Effects of chronic excess salt ingestion. Further demonstration that genetic factors influence the development of hypertension: evidence from experimental hypertension due to cortisone and to adrenal regeneration. *J. Exptl. Med.* **122:**533.
16. Grollman, A., R. T. Harrison, and J. R. Williams, Jr. 1943. The mechanism of experimental renal hypertension in the rat. The relative significance of pressor and anti-pressor fractions. *Am. J. Physiol.* **139:**293.
17. Smirk, F. H., and W. H. Hall. 1958. Inherited hypertension in rats. *Nature.* **182:** 727.
18. Aoki, K. 1963. Experimental studies on the relationship between endocrine organs and hypertension in spontaneously hypertensive rats. *Japan Heart J.* **4:**443.
19. Koletsky, S. 1958. Hypertensive vascular disease produced by salt. *Lab. Invest.* **7:**377.
20. Chappel, C. I., K. Voith, and G. Rona. 1962. Further studies on the role of the adrenal gland in adrenal regeneration hypertension. *Can. J. Biochem. Physiol.* **40:**1567.
21. Hall, C. E., and O. Hall. 1953. The potency of certain desoxycorticosterone esters with respect to the production of hypertension and cardiovascular lesions. *Endocrinology.* **52:**157.

22. Skelton, F. R. 1958. Production and inhibition of hypertensive disease in the rat by corticosterone. *Endocrinology.* **62**:365.

23. Friedman, S. M., C. L. Friedman, and M. Nakashima. 1952. The hypertensive effect of compound F acetate (17-OH-Corticosterone-21-Acetate) in the rat. *Endocrinology.* **51**:401.

24. Hall, C. E., and O. Hall. 1965. Hypertension and hypersalimentation 1. Aldosterone hypertension. *Lab. Invest.* **14**:285.

25. Brogi, M. P., and C. Pellegrino. 1959. The secretion of corticosterone and aldosterone by the rat adrenal cortex regenerating after enucleation. *J. Physiol. (London)* **146**:165.

26. Fortier, C., and J. De Groot. 1959. Adenohypophysial corticotrophin and plasma free corticosteroids during regeneration of the enucleated rat adrenal gland. *Am. J. Physiol.* **196**:589.

27. Macchi, I. A., and L. C. Wyman. 1963. Qualitative characterization of corticoids produced by mesenteric adrenal autografts in the rat. *Endocrinology.* **73**: 20.

28. Masson, G. M. C., S. B. Koritz, and F. G. Peron. 1958. Corticosteroid formation in regenerating rat adrenals. *Endocrinology.* **62**:229.

29. Kolthoff, N. J., I. A. Macchi, and L. C. Wyman. 1963. Isotopic determinations of blood volume in intact and regenerated rat adrenals during cold stress. *Endocrinology.* **73**:27.

30. Raker, J. W., O. Cope, and I. P. Ackerman. 1964. Surgical experience with the treatment of hypertension of Cushing's syndrome. *Am. J. Surg.* **107**:153.

31. Conn, J. W., R. F. Knopf, and R. M. Nesbit. 1964. Clinical characteristics of primary aldosteronism from an analysis of 145 cases. *Am. J. Surg.* **107**:159.

32. Nichols, J. 1965. Adrenal cortex in tumors and hypertension. *J. Am. Med. Assoc.* **194**:893.

33. Sherwin, R. P. 1964. Present status of the pathology of the adrenal gland in hypertension. *Am. J. Surg.* **107**:136.

34. Masson, G. M. C. 1960. An attempt to mimic adrenal regeneration hypertension. *Arch. Intern. Pharmacodyn.* **126**:277.

35. Collins, D. A., and E. H. Wood. 1938. Experimental renal hypertension and adrenalectomy. *Am. J. Physiol.* **123**:224.

36. Birmingham, M. K., G. Rochefort, and H. Traikov. 1965. Steroid fractions from untreated normal and regenerated adrenal glands of male and female rats. *Endocrinology.* **76**:819.

37. Masson, G. M. C., and A. C. Corcoran. 1958. Comparative effects of adrenal enucleation and adrenal pedicle ligation on blood pressure of rats. *J. Lab. Clin. Med.* **51**:786.

38. Kornel, L., and R. Takeda. 1965. Is essential hypertension a deficiency of enzymes of corticosteroid metabolism? Proceedings of the Endocrine Society, 47th Meeting. New York. 102.

39. Editorial. Recent advances in hypertension. 1965. *Am. J. Med.* **39**:616.

EXPLANATION OF PLATES

PLATE 1

FIG. 1. Low power magnification of renal cortex and outer medulla of an adrenal-enucleated rat given tap water to drink. The blood pressure had reached 164 mm Hg and hypertension had existed for 1 month. Many tubules are dilated and hyaline casts are visible at upper left. Hematoxylin and phloxine. × 65.

FIG. 2. High power of a glomerulus (at 12 o'clock position in Fig. 1) in the same kidney. There are proliferation and hyalinization of the capsular epithelium and shrinkage of glomerular tuft. Hematoxylin and phloxine. × 250.

FIG. 3. Low power section from kidney of an adrenal-enucleated rat given distilled water. First hypertensive pressure of 184 mm Hg was detected 26 days after surgery and the animal had been hypertensive for at least 52 days, reaching 254 mm Hg. Compare severity with Fig. 1. Cortical surface is at bottom. Nephrosclerosis is severe. Hematoxylin and phloxine. × 40.

FIG. 4. High power of preceding section showing an almost completely hyalinized glomerulus. An artery at the top shows endarterial proliferation, destruction of inner elastic membrane, and distortion of lumen. Tubules are dilated. Hematoxylin and phloxine. × 250.
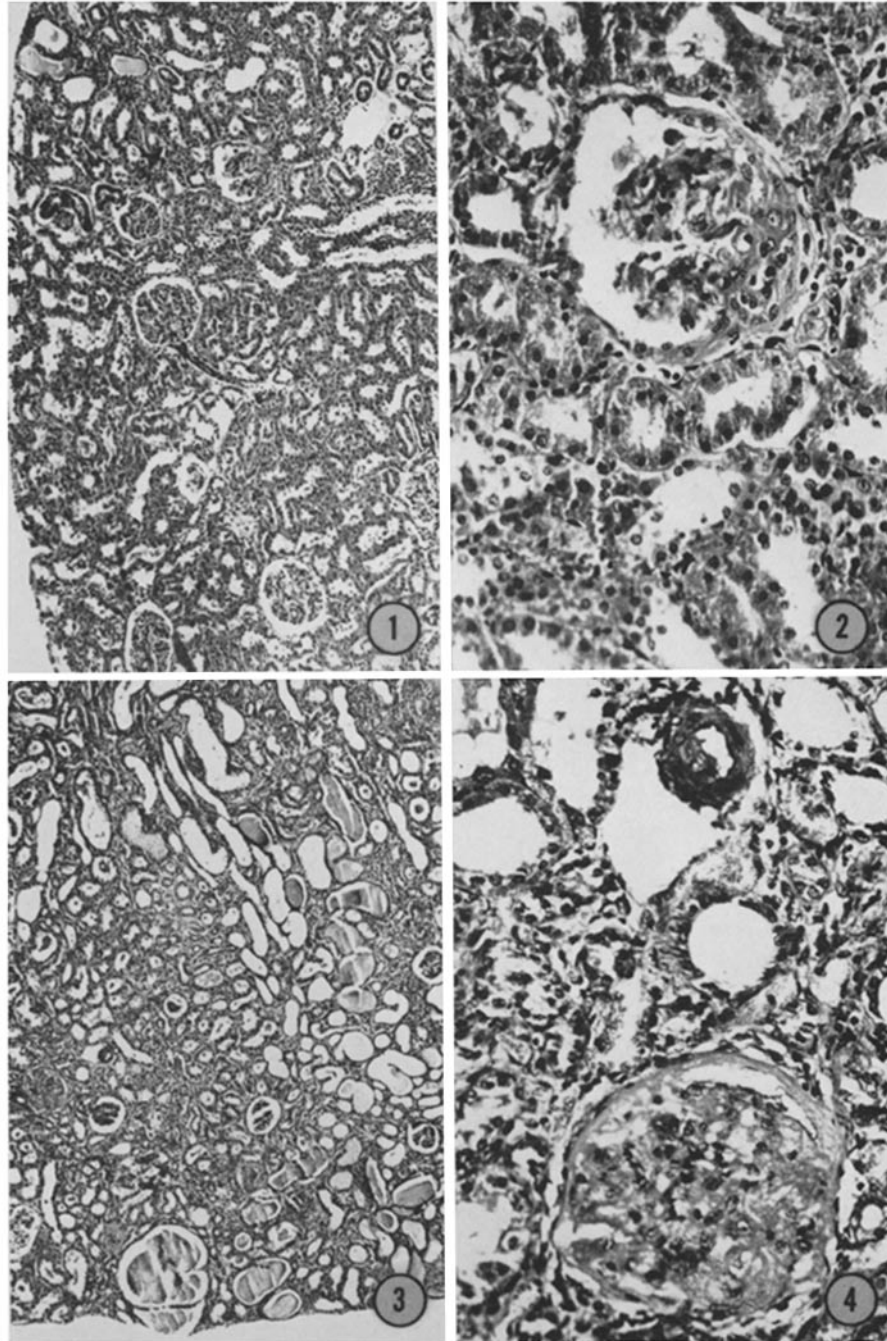
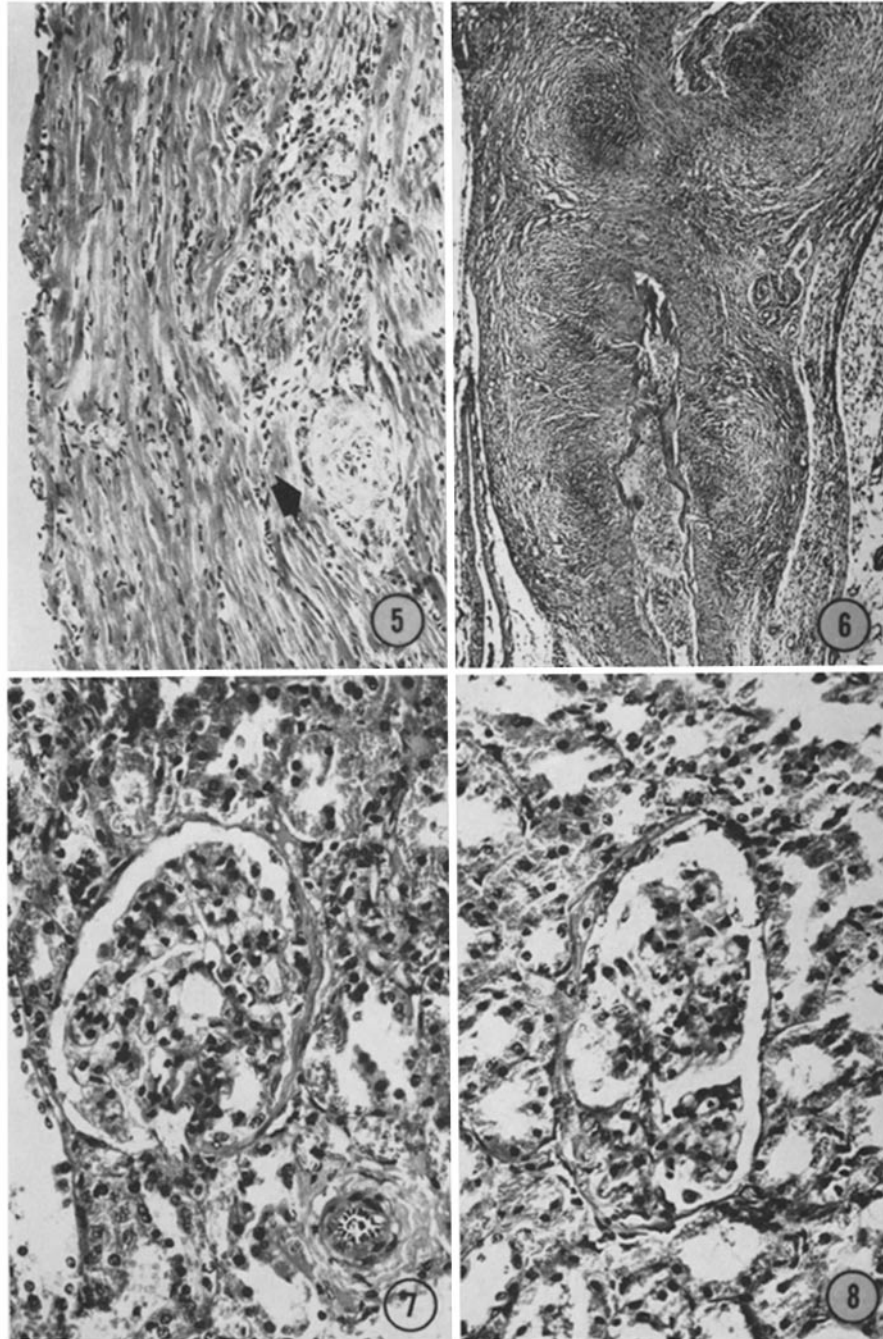(C. E. Hall et al.: Hypertension after adrenal enucleation in the rat)

FIG. 5. Section of heart from the above animal. An artery (arrow) shows medial hypertrophy and a reduced, somewhat eccentric lumen. There is sclerosis of myocardial fibers and some connective tissue replacement of degenerated fibers. Hematoxylin and phloxine. × 128.

FIG. 6. Severe polyarteritis nodosa of mesenteric arteries in the same animal, with intimal fibrinoid necrosis of vessel at the bottom. Hematoxylin and phloxine. × 40.

FIG. 7. Mild glomerular alteration typical of enucleated rats given distilled water. The blood pressure had reached 150 mm Hg at the terminal reading; although prehypertensive pressures 140–148 mm Hg had been in effect for 5 wk prior thereto. There are thickening and hyalinization of the visceral and parietal epithelium and the tuft adheres to the capsule at its right border. There is some thickening of glomerular capillaries. The lower artery shows hypertrophy. Hematoxylin and phloxine. × 250.

FIG. 8. Glomerular lesion rather similar to preceding, but from a rat with intact adrenals and drinking distilled water. Terminal pressure was 172 mm Hg and hypertension had existed for 3 wk. Hematoxylin and phloxine. × 250.

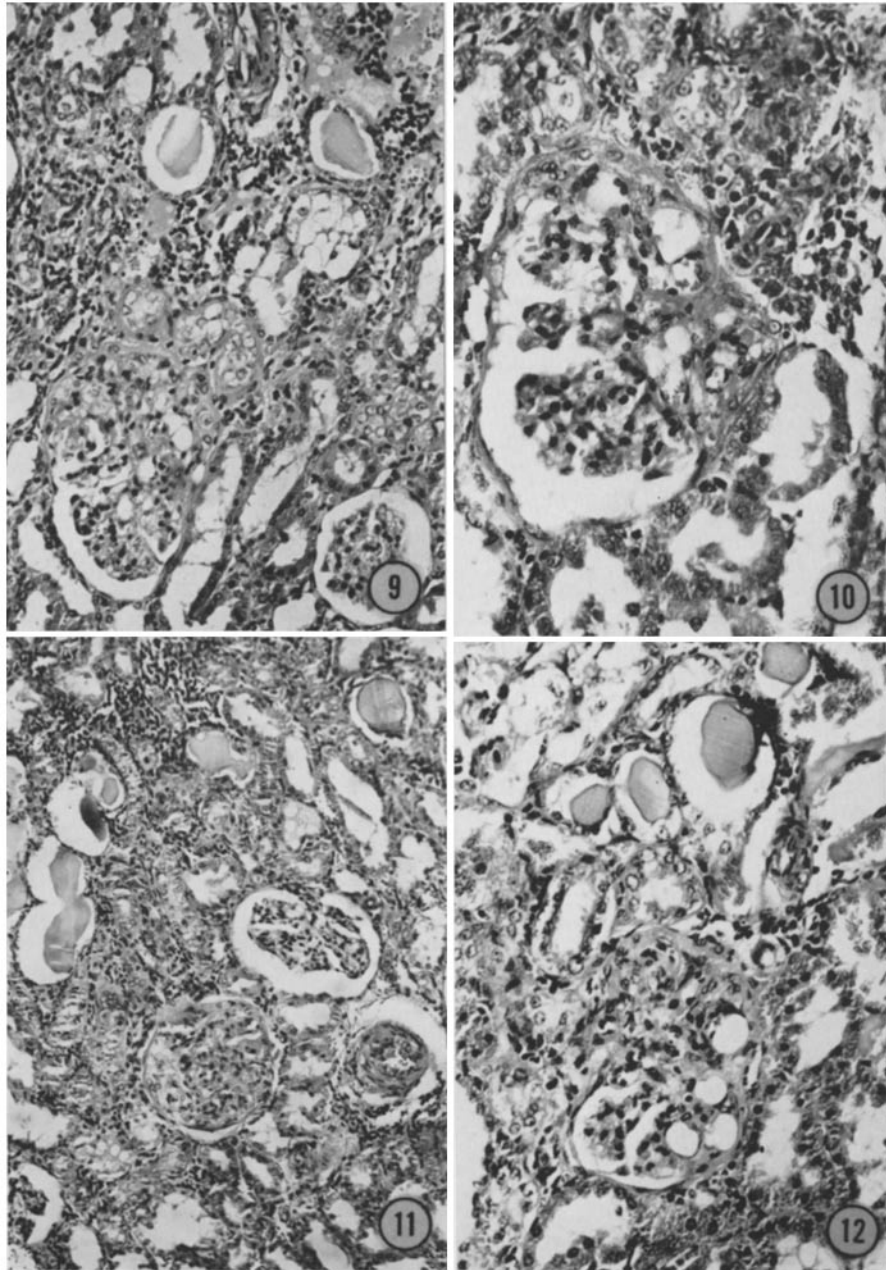(C. E. Hall et al.: Hypertension after adrenal enucleation in the rat)

FIG. 9. Section of kidney from a rat that received saline and hydrochlorothiazide. Hypertension had existed for 44 days although pressure reached only 162 mm Hg. There are glomerular hyalinization and adhesion to the capsule, tubular atrophy, dilation and cast formation, and some round-cell infiltration. Hematoxylin and phloxine. × 160.

FIG. 10. High magnification of glomerulus from another rat of the same group as the above, the blood pressure of which had reached 154 mm Hg. Hyperplasia of capsular epithelium, adhesion of tuft to capsule, and hyalinization, particularly at the vascular pole, are evident. Hematoxylin and phloxine. × 250.

FIG. 11. Extensive hyalinization of a glomerulus, tubular atrophy, and cast formation in the kidney of an adrenal-enucleated rat that drank only distilled water. It had been hypertensive from the 4th postoperative wk and attained a pressure of 240 mm Hg. Hematoxylin and phloxine. × 128.

FIG. 12. Section from the kidney of another rat from the above group. A hyalinized glomerulus with a rather moth-eaten appearance occupies the center, and is rimmed on one side by cast-filled tubules. The highest pressure exhibited was 158 mm Hg and hypertension had been present for 5 wk. Hematoxylin and phloxine. × 206.
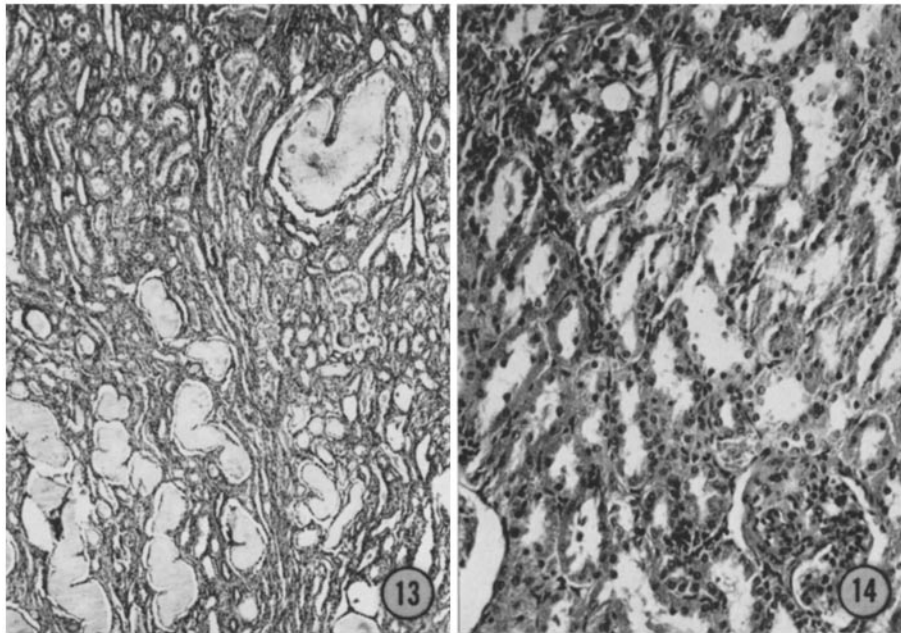
(C. E. Hall et al.: Hypertension after adrenal enucleation in the rat)

PLATE 4

FIG. 13. Section of kidney medulla from an adrenal-enucleated rat that drank distilled water and hydrochlorothiazide. The highest pressure recorded was 146 mm Hg and pressures above 140 mm Hg had been present for only 2 wk. Tubular casts greatly distorting the lumens are noteworthy. Hematoxylin and phloxine. × 250.

FIG. 14. Two glomeruli at 11 and 5 o'clock positions show extensive hyalinization and adhesions. Tubular morphology is normal. All recorded pressures were at or below 132 mm Hg. Hematoxylin and phloxine. × 160.

(C. E. Hall et al.: Hypertension after adrenal enucleation in the rat)