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SODIUM, POTASSIUM DISTRIBUTION IN KIDNEY DURING
INDUCTION OF EXPERIMENTAL HYPERTENSION IN THE RAT

By

Marlin Hiyam Lutfi Atallah

"Submitted in partial fulfillment for the requirements
of the degree of Master of Science
in the Biology Department of the
American University of Beirut
Beirut, Lebanon" 1962

KIDNEY IN HYPERTENSION

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ACKNOWLEDGMENT

The writer wishes to express a debt of gratitude to Dr. Fred N. White, Professor of Biology at the American University of Beirut, who suggested the problem and whose constant advice and help made this work possible. Thanks also go to the Biology Department for the materials and facilities provided.

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PART I

INTRODUCTION

Several forms of experimental hypertension show a common disturbance in sodium-potassium balance. Sodium retention and potassium depletion are observed in hypertensions caused by potassium deficiency diets, and DOCA or aldosterone administration. The ischemic technique for producing hypertension has not been evaluated as to electrolyte disturbance; however, several workers have demonstrated aldosterone release as a result of kidney ischemia. The present work has been undertaken to determine whether there is a disturbance in sodium-potassium balance of the kidney during renal ischemia produced by a figure-of-eight ligature.

Potassium deficiency in the absence of reduction in renal blood flow causes hypertension. A potassium deficiency as a result of reduction in renal blood flow would indicate a common feature between these two forms of hypertension. If a retention of sodium and a loss of cell potassium could be demonstrated, the various forms of hypertension would be linked by a similar electrolyte disturbance.

Progesterone is a hormone which also may promote sodium retention and potassium depletion. A preliminary investigation has been undertaken to determine whether or not potassium depletion occurs in the kidney under the influence of progesterone.

PART II

HISTORICAL

The basic idea that hypertension may frequently be of renal origin goes back to Richard Bright in the nineteenth century.⁴¹ He observed that cardiac hypertrophy (this would imply hypertension) was caused by primary renal disease, and he even attributed this to some chemical in the blood. This started the debate of whether the kidney was the victim, or the culprit in hypertension, and has led to many experimental investigations.

The following is a review of some of the literature dealing with this problem.

A. Methods of Inducing Experimental Hypertension

The first attempt, at producing experimental hypertension, was in 1879 when Gravitz and Israel demonstrated hypertrophy of the heart, attributed to hypertension, in rabbits as a result of partial nephrectomy.⁴¹

In 1905, Passler and Heinke, using the same procedure, demonstrated a rise in blood pressure in dogs. Katzenstein, in the same year, tried the effect of constricting the main renal arteries in the dog with negative results. Bridgeman and Hirose 1918 repeated his experiment, but the results were unsatisfactory.⁴¹ It remained for Goldblatt and coworkers⁴⁰ in 1934 to establish that the blood pressure in the dog and other animals could be elevated to permanent pathological levels by constricting one main renal artery and removing the other kidney. Other means of causing

experimental hypertension by kidney manipulation are: (1) constriction of both main renal arteries, (2) placing a silk, colloidin, or cloth capsule around the kidney (Page, 1939),¹⁰⁰ (3) removal of both kidneys (Grollman),⁴⁷ and (4) compressing the renal parenchyma with a figure-of-eight loop around the kidney (Grollman).⁴⁵

Ryland produced hypertension in rats by partially occluding the aorta proximal to one or both of the main renal arteries.¹¹⁷

White, and Grollman⁵⁴ obtained hypertension by subjecting rats at the time of their weaning to a choline free or potassium free diet. Hartcroft and Best also demonstrated hypertension in rats after one week choline deficiency in early life.⁵⁸

Friedman, Polley, and Friedman implanted desoxycorticosterone acetate pellets subcutaneously in rats and observed hypertension and cardiac hypertrophy.⁶ Skelton and Hyde also demonstrated the use of adrenal steroids in pellet form and microcrystalline suspension.¹²⁴ Sodium chloride was needed in both these cases. Injections of aldosterone with sodium chloride in their drinking water, also caused hypertension in rats.⁷⁴

Schroeder and Vinton showed a marked hypertension in female rats and a tendency toward it in male rats by keeping the animals on a cadmium free diet and then giving them 5 ppm. of cadmium in their drinking water for 180-240 days after weaning.¹²²

Hypertension can be induced during salt intake. Sapirstein¹³¹ demonstrated this in normal rats drinking two per cent sodium chloride. Meneely et al.⁸⁶ showed in different groups of rats, with different concentrations of sodium chloride in the diet, that the arterial pressure went stepwise in proportion to sodium chloride concentration.

B. Theories of Genesis of Hypertension

1. Renal Hypertension.

This theory, supported by Goldblatt, states that the ischemic kidney releases a pressor substance into the blood stream which causes an elevated blood pressure.

Goldblatt's method involves the application of a silver clamp constricting one main renal artery.³⁷ He observed hypertension in goats, sheep, rabbits, and rats; but in dogs the other kidney had to be removed to keep the blood pressure elevated. In those animals whose blood pressure returned to normal, the accessory circulation was blocked by a fish skin condom around the kidney. Kidney lesions were observed with greater constriction of the arteries. Moritz and Oldt in 1937 showed that the kidney was the only site of arteriolar sclerosis in hypertensive animals as compared with normal individuals.⁹⁰

Hypertension, produced by Page with a cellophane membrane,¹⁰⁰ was also due to renal ischemia. In this case it was caused by the compression of the renal parenchyma, and the development of a thick layer of connective tissue under the membrane.

Goldblatt did not get hypertension in bilaterally nephrectomized dogs, and so concluded that lack of renal excretory function by itself was not sufficient to cause hypertension. The first indirect evidence, that a renal humoral mechanism was present, was demonstrated by occluding both main renal veins as the renal arteries were constricted. This did not bring about the expected hypertension. Any part the liver may play was ruled out by shunting the venous blood in a dog with a renal artery constricted through the liver. This did not decrease or prevent the elevated blood pressure.⁴¹

When a kidney was transplanted to the neck or groin of a bilaterally nephrectomized dog or rabbit, the same pressor effect was noted when its renal artery was constricted. Transplanting an ischemic kidney into such an animal would also increase the blood pressure. Since these kidneys had no nervous connections, it was concluded that a chemical agent of renal origin was responsible for the hypertension.⁴¹

Tigerstedt and Bergman in 1898 had shown that an intravenous injection of a crude extract of a normal kidney, from one rabbit into another, had a pressor effect.⁴¹ This substance was called Renin which, however, had no direct vasoconstrictor properties on the blood vessels. Two groups of workers^{10, 101} in 1940 independently stated that renin was a key substance acting on a substrate in the blood to form a new substance which had the vasoconstrictor properties. This pressor substance was

called hypertensin by Braun-Menendez, Fasciolo, Leloir, and Munoz,¹⁰ and angiotonin by Page and Helmer.¹⁰¹ It has been agreed upon to refer to it as angiotensin.⁸

Taquini, Blaquer and Taquini Jr.¹²⁸ found that, when kidneys of a chronic renal hypertensive dog were transplanted into the neck of a normal dog, the renin content increased before and while the blood pressure of the normal dog was increasing.

Taquini and Fasciolo¹²⁹ in 1946 observed that the renin content, in the blood of hypertensive dogs with renal ischemia, was the same as that found in the controls.

Dexter and Haynes²¹ measuring the amount of renin in the systemic blood of hypertensive patients, observed renin only when the blood pressure was rising abruptly. In 1947, the same workers demonstrated the presence of renin in the blood of dogs at twenty-four hours after the renal artery constriction. This built up to a maximum at one week, but after three months they were unable to demonstrate it.⁵⁹ Kahn, Skeggs, and Shumway²⁸ demonstrated an excess of angiotensin in the blood of dogs during the early phase of renal hypertension. Since, however, repeated injections of renin renders the animal tachyphylactic to it⁷⁹ it could not produce a sustained hypertension. Blackett, Depoorter, Pickering, Sellers, and Wilson⁴ in 1950 were able to maintain hypertension in rabbits for eighteen days with a slow injection of renin; however, the blood pressure decreased as soon as the

injections were stopped. Flasher and Drury²⁶ maintained that in rabbits with early renal hypertension which were rendered tachyphylactic to renin, the blood pressure did not go down.

Pickering¹⁰⁵ in 1945 showed a fall in blood pressure to normal after removing the single remaining clipped kidney of one week hypertensive rabbits. He could not demonstrate this in eight week hypertensive animals. Pritchard and McQuaid²⁸ in 1954 confirmed these observations. This led to a general belief that there are two phases to renal hypertension; an early one, in which a renal pressor substance is secreted by the kidney and a late phase in which the elevated blood pressure appears to be maintained by some mechanism outside the kidney or what is referred to as an extra renal mechanism.

Kremen and Wakerlin⁷³ 1955 were the first to produce antirenin. By repeated injections of renin subcutaneously or intramuscularly antirenin was developed in the blood, and the blood pressure of a hypertensive dog came back to normal. Helmer⁶⁰ 1955 said that the antirenin, to injected dog renin, appeared in the sera of dogs with renal hypertension at the same time as the blood pressure was decreasing in both acute and chronic cases. He therefore maintained that renin plays an important role in both phases.

In hypertensive rats of two week duration, no fall occurred after total nephrectomy which implies an extra renal mechanism.²⁸

Even though the baroreceptors would be reset at a higher level to maintain a higher blood pressure, they are not necessarily the cause.⁸⁴ An irreversible narrowing of the arterioles might be implicated; however, no such narrowing was observed.²⁸ Bryom and Dodson¹² in 1949 also showed that in chronic hypertension of twelve weeks in rats removal of the clip restores the pressure to normal. This excludes any possibility of narrowing arterioles and the baroreceptor mechanism as the primary cause. Blackett and Sellers⁵ confirmed this in 1951.

Floyer^{27, 28} in 1951 showed that in rats made hypertensive by constriction of one renal artery, the clipped kidney remained structurally normal while the untouched kidney showed marked vascular lesions. Removal of the clip reduced the blood pressure to normal. Removal of the clipped kidney kept the high blood pressure. Restoring the circulation to the kidney resulted in a decrease in pressure. This can be explained in that the kidney checks a pressor substance; the extra renal mechanism.

So the general trends in this theory point to a renal factor which causes an increase in the blood pressure.

2. Renoprival Hypertension.

This theory, according to Grollman, explains hypertension in that the kidney normally secretes a substance which is necessary for normal blood pressure. As soon as it disappears, is destroyed, or neutralized the blood pressure is elevated. Thus interference with kidney function gives rise to hypertension.

Grollman and Rule⁵¹ 1943 demonstrated hypertension in parabiotic rats. Removing both kidneys of one rat caused hypertension while the other rat, with the intact kidneys, kept a normal blood pressure. Ledingham⁷⁸ confirmed this in 1951. Grollman, Muirhead, and Vanatta⁵⁰ 1949, showed that dogs kept alive by peritoneal lavage, after total nephrectomy, developed hypertension and vascular lesions. Kolff and Page,⁷¹ 1954, also produced hypertension by total nephrectomy in dogs and they demonstrated that the blood pressure returned to normal when the blood of the hypertensive animal was perfused through a normal kidney. Braun-Menendez and von Euler¹¹ Floyer, Kolff and Page⁷¹ obtained hypertension after total nephrectomy in rats.

Grollman⁴⁹ showed that the presence of the kidney, even though it is not excreting, is necessary for normal blood pressure. This he did by removing one kidney and transplanting the ureter of the other into the vena cava, thus having all urine coming back into the blood. No elevation in the blood pressure appeared. This was also demonstrated by Kolff, Page and Corcoran in 1954.

White, Sambhi & Grollman¹³⁹ demonstrated that in rats, made hypertensive by low potassium diet, the kidney function remained normal. This shows that hypertension developed without renal ischemia, and without the presence of renal excretory insufficiency.

Grollman and Harrison⁴⁵ prepared a renal extract which,

when given orally to hypertensive subjects, resulted in reductions of the blood pressures. This would support the idea that the absence of this principle causes hypertension. By supplying it the hypertension would be relieved.

Masson, Corcoran, and Page⁹ showed that parabiotic union of a hypertensive with a normal rat resulted in a reduced blood pressure of the hypertensive one. This did not occur if a normal rat was joined to another normal, or if a hypertensive rat was joined to a hypertensive one.

Muirhead, Stirman, Lesch, and Jones⁹¹ transplanted a kidney into the neck of a renoprival hypertensive dog and demonstrated a decline in pressure. Merrill, Murray, and associates in 1956⁸⁹ transplanted a normal kidney from one brother to his hypertensive twin, and obtained a normalization of the blood pressure.

Grollman and Braun-Menendez agree that the depressor effect of the kidney might not be a substance as such but some action of the normal kidney.⁸

Leonard and Heosler⁴⁴ 1951, 52 attributed renoprival hypertension to water overload. Kolff, and Page and Kolff et al.²⁸ observed that overhydration in rats and dogs accelerated the hypertension but that renoprival hypertension did occur in its absence.

Floyer 1951²⁸ tried to see the relationship between renal and renoprival hypertension. He thought that the renal mechanism, which operates in late renal hypertension, might be

the same as that causing an increase in blood pressure after a total nephrectomy. He demonstrated that both are abolished by the presence of a normal kidney in the circulation, and by adrenalectomy, and both are restored by giving salt. Ledingham⁷⁷ observed similar electrolyte compositions in the fluid compartments of both hypertensions. Floyer²⁷ in 1955 showed that the blood pressure after total nephrectomy took forty-eight hours to rise, which is a longer time than after renal constriction. So Floyer deduced that, if the two forms of hypertension have a different mechanism, removal of the only clipped kidney in a hypertensive rat should at first induce a reduction in blood pressure and, then after forty-eight hours, an increase as the renoprival hypertension takes over. This did not occur which means that the renoprival effect was there before.

Kolff and Page⁷² found that antirenin had no effect on renoprival hypertension. Wakerlin⁷³ tried to explain the effect of antirenin on the reduction of blood pressure in the late phase of renal hypertension. He said that renin may act on the blood pressure regulating effect of the kidney, and that antirenin would neutralize it thus removing the interference.

Renal and renoprival hypertension differ in that removal of one kidney does not result in hypertension, while constriction of one renal artery does so in the presence of a normal kidney. Floyer²⁸ 1957 suggested that the renin may be acting on the normal kidney neutralizing its effect. Hoobler states that a

normal kidney causes a decrease in the pressure in both types of hypertension by some protective function.

3. Other explanations.

Braun-Menendez⁹ put forth the renotrophin hypothesis. He hypothesized that the normal kidney size and functional capacity depends upon the substances in the blood it has to eliminate. This he referred to as renotrophin or the renal growth factor. A decrease in the functional renal mass leads to disbalance with more renotrophin which causes hypertension. Since animals are made hypertensive by a reduction in renal mass, he suggested that hypertension was due to the resulting renal growth factor. Page and Sweet in 1937¹⁰⁴ demonstrated that hypophysectomy caused a reduction in blood pressure of hypertensive dogs. Ogden, Page and Anderson⁹⁵ 1944, 46 and Salgado¹¹⁸ 1955 demonstrated the same in rats. The presence of the anterior lobe of the hypophysis seemed to be indispensable, yet previous hypophysectomy did not prevent hypertension. Braun-Menendez explained this on the basis of the renotrophic factor. Hypophysectomy would decrease the rate of producing renotrophin and thus lowers the blood pressure. In a normal animal hypophysectomy would decrease renotrophin and functional renal mass. This is then further reduced by kidney manipulation, thus causing more renotrophin and a higher blood pressure.

Handler and Bernheim⁹ in 1950 showed that protein restriction acts in the same way as a hypophysectomy in that

an animal on a severe low protein diet has no functioning anterior hypophysis.

Fregly and Gonzales³⁰ show that in treatments that prevent or reduce the activity of the thyroid both renal and sodium chloride induced hypertensions were reduced. Thyroid administration increases the renotrophin and therefore increases blood pressure.

Removal of both adrenals interfered with the development of renal hypertension. Page⁹⁸ 1938 showed that the adrenal cortex plays an important role in experimental hypertension. In the absence of the adrenals, administration of adrenal cortical extract and addition of salt are necessary. Goldblatt showed this in 1937.³⁹ Wilgram and Ingle¹⁵ showed that hypertension and early nephrosclerosis appeared with salt in the absence of adrenal cortex, so they concluded that the adrenal steroids are not pathogenic agents, but enhance the damaging effect of the high sodium load. Friedman, Sombhi and Oppenheimer²² suggest that the action of the adrenal is in sensitizing the blood vessels to the 'pressor' agent from the body. The adrenal cortex secretes, among other things, aldosterone. Aldosterone acts on the renal tubules to promote sodium reabsorption and excretion of potassium.⁶² In patients with aldosteronism an increase in blood pressure is observed.¹¹¹ Administration of aldosterone to animals caused an increase in pressure.¹³⁶ Laragh⁷⁴ and coworkers found that the secretion rate of aldosterone

was higher in hypertensive subjects than in normal ones. In 1951 Deane and Masson⁷⁴ found that injecting renin or producing renal hypertension brought about a hypertrophy in the glomerulosa zone of the adrenal cortex. This is the site of aldosterone production.

Renin is associated with the juxtaglomerular apparatus in the kidney.¹³¹ These cells are sensory receptors in the wall of the afferent arteriole. The amount of extractable renin from the kidney was related to the degree of granulation. Tobian¹³¹ showed that the granularity of the juxtaglomerular cells increases in the clipped kidney of renal hypertension, and decreases in the opposite normal kidney. Administration of DOCA led to a decrease in granularity, and adrenalectomy caused an increase.

Evidence points to an aldosterone stimulating hormone released from the kidney. Aldosterone secretion decreased in nephrectomized hypophysectomized dogs. Hyperaldosteronism was consistently associated with experimental hypertension. Crude extracts of kidney tissue resulted in an increased aldosterone production. The fact that both renin and angiotensin administration caused an increase in aldosterone, and that renin induces hypertrophy in the zona glomerulosa suggest that the aldosterone stimulating hormone of the kidney might be renin itself.²⁰

C. Sodium, Potassium and Extracellular Fluid.

The mammalian kidney normally adjusts the amount of

sodium and extracellular fluid in the body. Retention or loss of sodium, will lead to a retention or loss of water, and therefore, to an increase or a decrease in the extracellular fluid volume. The excretion rate of sodium is affected by the filtration rate, the plasma sodium concentration, the antidiuretic hormone, and the adrenal cortical hormones. Adrenal insufficiency leads to a loss of sodium and water from the body, and therefore to a decreased extracellular fluid volume. Administration of DOCA or aldosterone leads to a sodium and water retention and an increased potassium excretion. In extreme cases this will result in a decreased plasma potassium.¹²⁶

Meneely and Ball⁸⁶ showed that rats altered their fluid intake as to the sodium chloride in the diet. They also showed that at sodium chloride levels of 2.8 - 9.8 per cent hypertension was present with an increase in heart, kidney, and adrenal weights.

Sodium is mainly extracellular and potassium intracellular. Their relationship is of importance. Potassium deficiency or an upset in the normal Na:K ratio results in muscle weakness. Pathological lesions produced by potassium deficiency are augmented by addition of sodium. Changing the concentration of potassium can affect the autonomic nervous system and thus can cause hypertension and hypotension.⁶² The loss of potassium is directly related to the urine volume. When sodium replaces potassium in the cells it has a toxic effect inhibiting many of the enzymatic reactions.⁶²

In 1937 Schrader, Prickett and Salmon¹¹⁹ showed lesions in renal tubular epithelium of rats fed on a low potassium diet. These lesions caused in weanling rats by White, Sambhi and Grollman may have something to do with the hypertension developed in these animals later.

The role of salt in hypertension has been investigated. In 1940 Grollman, Harrison and William⁴⁹ demonstrated that the various sterols are hypertensigenic only if sodium chloride was added. Grollman showed that the main usefulness of the Kempner diet, used for the treatment of hypertension, was in its low sodium content.

Meneely, Tucker, Darby and Auerbach⁸⁸ demonstrated a positive correlation between blood pressure and concentration of sodium in the diet. The administration of sea salt resulted in a greater hypertension than sodium chloride alone, so there may be some other factor involved. Renal tubular cells need an adequate supply of potassium to keep their integrity. Steroids deplete the body of electrolytes and so might damage the kidney. Freed²⁹ and Meneely and Ball⁸⁶ showed that potassium chloride has a protective function when added together with the sodium chloride increasing the survival of the animals. Therefore, inter-relationship between sodium and potassium may affect the development of hypertension and vascular disease.

D. Changes in Water and Electrolytes in Experimental Hypertension.

Experimental hypertension has been linked with changes in tissue electrolytes and fluid compartments.

Grollman in 1953 showed that overloading with saline and water showed no difference in blood pressure when expansion of ECF volume was not very great.⁴⁴ Laramore and Grollman⁷⁵ showed that in the early stages of hypertension, there is little change in total water content of tissues; however, in later stages the water content was greater. Shapiro and Grollman⁵² observed an increase ECF space in hypertensive animals. Grollman suggested that a deviation from the normal electrolyte and water metabolism occurs in hypertension.

Tobian and Binion¹³² showed larger amounts of sodium and water in the renal artery of hypertensive rats than in that from normal rats. There was also an increase in sodium, potassium and water of the aorta in rats made hypertensive by the renal method or by DOCA administration. Haight and Weller⁵⁶ 1961 demonstrated the changes in the aorta. They also showed that the potassium decreased in the aorta as the blood pressure decreased after the administration of reserpine. Tobian and Redleaf¹³⁴ suggested that if the same changes as in the aorta occur in the arterioles, they may be important in the altered peripheral vascular resistance which characterizes hypertension.

Grollman observed that adrenalectomy depletes the cells of their potassium and moves sodium into cells. Nephrectomy accentuates these changes. In various tissues of the

hypertensive animal (blood, brain, heart, liver, gut, muscle, skin and spleen) Grollman demonstrated an increase in sodium and a decrease in potassium. Holley and Holland⁶³ from their experiments concluded that the serum sodium in hypertensive rats was slightly above normal. Kao⁶⁵ showed that in normal rats the serum sodium and potassium were not changed after injections of progesterone or estrogen. Fregly, Yates and Landis³¹ showed no change in serum sodium of some hypertensive animals, and a change in others. The old rats maintained the level of their serum ions within normal limits, while the young rats showed an increase. Greene and Sapirstein⁴³ showed in hypertensive rats (by subtotal nephrectomy) an increase in total body sodium, while the level in the plasma was maintained at the expense of the intracellular compartment.

Ezrow and Sapirstein²³ showed that water excretion is greater in hypertensives than in normotensives so that the hypertensive rats had a larger sodium load. Cottier, Weller and Hoobler¹⁷ also demonstrated that hypertensive patients excreted more water, sodium and chloride than the normal when given sodium chloride. Green et al.⁴² showed that water and sodium excretion is in direct relationship with the blood pressure. There is an increase in most tissues sodium content in hypertensive animals.

Whether the electrolyte changes are the result or the cause of hypertension, or whether there is no causal relationship, has not been determined yet.

PART III

MATERIALS AND METHODS

Albino rats of the Br⁴⁶ strain were used throughout the experiment. Rats were made hypertensive by figure-of-eight ligatures around both kidneys and the administration of one per cent sodium chloride in their drinking water. Systolic blood pressures were measured in the caudal artery by a plethysmographic manometer in the unanesthetized, heated rat.¹³⁸

The following groups of rats were set up and then sacrificed: (An equal number of female and male rats were used for number 1-8. For numbers 9 & 10 young female rats were used).

1. Ligations for forty-eight hours.
2. Ligations for forty-eight hours plus one per cent sodium chloride for ninety-six hours.
3. Ligations for ninety-six hours.
4. Ligations for ninety-six hours plus one per cent sodium chloride.
5. One per cent sodium chloride for ninety-six hours.
6. Ligations for two weeks.
7. Ligations for two weeks plus one per cent sodium chloride.
8. One per cent sodium chloride for two weeks.
9. Daily subcutaneous injections of 0.25 mg. progesterone in 0.5 cc. of corn oil for two weeks.
10. The same as in (9) plus one per cent sodium chloride.

Blood, serum, and whole kidney were analyzed for sodium and potassium using a Baird Flame Photometer. The kidneys were weighed and dried in an oven at eighty degrees centigrade for 24 - 30 hours. They were then homogenized (using a glass homogenizer) in twenty times their dry weight of one normal HNO_3 and left overnight. The next day they were centrifuged and the supernatant analyzed.

The sodium and potassium in the red blood cells per liter of cell water was calculated from the hematocrit and the serum sodium and potassium. Sixty five per cent of the RBC was taken to be water. The other values for the kidney tissue were calculated according to Manery and Hastings⁸³ as follows:

C = Cells, T = Whole tissue, S = Serum, E = Extracellular phase

() = meq or gm. per kg. tissue.

[] = meq per kg. water.

c = gm. per kg. cells.

$\text{Na}_S \times 0.95 = [\text{Na}]_E \text{ meq/kg H}_2\text{O}.$

$\frac{(\text{Na})_T \times 1000}{[\text{Na}]_E} = (\text{H}_2\text{O})_E \text{ gm/kg tissue}.$

$(\text{H}_2\text{O})_T - (\text{H}_2\text{O})_E = (\text{H}_2\text{O})_C \text{ gm/kg tissue}.$

$(\text{H}_2\text{O})_E - 0.99 = (\text{E}) \text{ gm/kg tissue}.$

$1000 - (\text{E}) = (\text{C}) \text{ gm/kg tissue}.$

$\frac{1000 \times (\text{H}_2\text{O})_C}{(\text{C})} = (\text{H}_2\text{O})_C \text{ gm/kg cells}$

$$\begin{aligned} K_S \times 0.95 &= [K]_E \text{ meq/kg H}_2\text{O} \\ [K]_E \times (H_2O)_E &= \sum (K)_E \text{ meq/kg tissue} \\ (K)_T - \sum (K)_E &= \sum (K)_C \text{ meq/kg tissue} \\ \frac{1000 \times \sum (K)_C}{(H_2O)_C} &= K_c \text{ meq/kg cells} \end{aligned}$$

PART IV

RESULTS

The rats, with figure-of-eight ligatures and the intake of one per cent sodium chloride in their drinking water, showed an increase in blood pressure after six months. The blood pressure values are given in Table 1.

Table 1. Systolic Blood Pressures of Normal and Hypertensive Rats.

Normal rat	1	2	3	4	5	6	7	8	9	Average
Sys.B.P.	107.8	108.5	105.7	107.8	106	105	100	100		105 \pm 1.0*
Hyper. rat 6 months after Lig. +NaCl	1	2	3	4	5	6	7	8	9	
Sys.B.P.	151.7	151.6	149.3	151.8	152.2	183	163	141	150	154.8 \pm 3.9*

*Standard error was computed as to Burn¹³

The results of the analyses of the tissues are found in Table 2. We note a decrease in the hematocrit in all the rats with kidney manipulation and in the rats on progesterone and sodium chloride. No such decrease is observed in rats with the injections of progesterone or the intake of sodium chloride alone.

The serum sodium increase in all. However, the values were higher for ligations and sodium chloride than for ligations or sodium alone. They were also higher for progesterone plus sodium chloride than for progesterone. The largest increase was observed in the forty-

Table 2. Analyses of Serum, Blood and Kidneys

State of Animal	Hema-tocrit	Na _S meq/l	K _S meq/l	Na _{WB} meq/l	K _{WB} meq/l	(K) _{RBC} meq/l H ₂ O	Na _T meq/kg Fr. tissue	K _T meq/kg Fr. tissue	Na _K	(H ₂ O) _T	[Na] _E	(H ₂ O) _E	(H ₂ O) _C	(E)	(C)	(H ₂ O) _C	[K] _E	(K) _E	(K) _C	K _C
Normal	41.5	135.2	4.2	83.5	48	169.4	69.6	61.7	1.14	765.8	128.4	541.8	223.9	540.8	459.1	485.1	4.0	2.1	59.6	123.7
(6)	\pm 4.5	\pm 4.4	\pm 0.18	\pm 1.4	\pm 2.6	\pm 9.9	\pm 2.4	\pm 3.0	\pm 0.16	\pm 7.2	\pm 1.3	\pm 20.9	\pm 16.02	\pm 20.9	\pm 15.1	\pm 15.1	\pm 0.2	\pm 0.1	\pm 3.0	\pm 5.6
48 hrs.	36.8	143.6	3.9	88.8	47	146.8	70.5	62.6	1.14	791.8	136.4	514.7	277.1	513.7	486.3	561.2	3.7	1.9	60.7	107.3
(5)	\pm 1.9	\pm 3.2	\pm 0.5	\pm 4.9	\pm 1.5	\pm 9.7	\pm 2.9	\pm 2.6	\pm 0.10	\pm 4.8	\pm 2.9	\pm 8.1	\pm 9.5	\pm 8.06	\pm 8.06	\pm 4.4	\pm 0.5	\pm 0.03	\pm 2.9	\pm 4.5
48 hrs.Lig.	37.4	150.1	3.6	95.8	47.2	181.8	72.8	64.7	1.14	798.5	142.5	510.7	288.2	509.7	490.3	584.1	3.4	1.7	63.0	108.5
+ 96hrs.	\pm 2.5	\pm 3.2	\pm 0.3	\pm 3.6	\pm 6.3	\pm 16.5	\pm 1.2	\pm 2.5	\pm 0.1	\pm 6.8	\pm 3.7	\pm 6.0	\pm 12.5	\pm 6.0	\pm 6.0	\pm 23.9	\pm 0.3	\pm 0.2	\pm 3.5	\pm 5.7
NaCl (6)	35.5	143	4.2	90.8	43.2	146.8	70.6	65.5	1.10	802.7	135.8	519.9	282.9	518.9	481.2	578.1	3.9	2.0	63.3	110.2
Lig.	\pm 2.1	\pm 2.6	\pm 1.5	\pm 6.5	\pm 7.7	\pm 9.7	\pm 1.8	\pm 2.4	\pm 0.03	\pm 4.6	\pm 2.4	\pm 11.9	\pm 12.3	\pm 11.9	\pm 11.9	\pm 11.9	\pm 0.5	\pm 0.8	\pm 1.8	\pm 6.5
(6)	37.4	150.1	3.6	95.8	47.2	181.8	82.0	63.8	1.30	820.3	144.2	575.2	245.1	574.2	425.8	575.6	3.3	2.0	61.8	109.8
Lig.+NaCl	\pm 2.5	\pm 3.2	\pm 0.5	\pm 3.6	\pm 6.3	\pm 16.5	\pm 2.7	\pm 2.5	\pm 0.1	\pm 12.2	\pm 2.7	\pm 16.2	\pm 12.8	\pm 16.2	\pm 16.2	\pm 17.3	\pm 0.4	\pm 0.9	\pm 1.6	\pm 3.1
96 hrs.	41.3	141.8	3.8	87.7	42.0	148	74.5	62.1	1.20	760.2	134.7	546.7	213.5	545.7	454.3	452.1	3.9	2.1	60.1	132.9
NaCl	\pm 2.2	\pm 1.5	\pm 0.4	\pm 5.0	\pm 2.1	\pm 5.5	\pm 4.0	\pm 3.6	\pm 0.1	\pm 4.2	\pm 1.5	\pm 24.5	\pm 20.4	\pm 24.5	\pm 24.5	\pm 78.8	\pm 0.3	\pm 0.1	\pm 2.2	\pm 8.2
96 hrs.	37.7	137.4	3.6	87.3	43.7	171	63.4	72.4	0.88	780.5	130.5	488.0	310.8	487.0	513.0	563.3	3.5	1.7	70.7	126.3
Lig.	\pm 1.8	\pm 3.3	\pm 0.6	\pm 3.0	\pm 2.2	\pm 8.1	\pm 2.7	\pm 2.4	\pm 0.1	\pm 6.0	\pm 3.1	\pm 27.5	\pm 31.1	\pm 27.5	\pm 27.5	\pm 39.5	\pm 0.7	\pm 0.2	\pm 2.4	\pm 8.0
2 weeks	36.6	140	3.7	89.5	47.1	164.1	71.1	72.8	0.99	786.2	133.1	538.3	236.9	537.3	462.7	543.5	3.5	1.9	70.9	135.8
Lig.+NaCl	\pm 1.7	\pm 4.2	\pm 0.1	\pm 2.3	\pm 4.6	\pm 12.7	\pm 1.5	\pm 2.9	\pm 0.04	\pm 3.8	\pm 3.8	\pm 21.3	\pm 18.8	\pm 21.3	\pm 21.3	\pm 24.9	\pm 0.1	\pm 0.1	\pm 2.9	\pm 9.7
2 weeks	40.5	137.9	4.4	82.6	52.3	170.6	77.0	62.2	1.25	773.9	131.0	588.8	185.1	587.8	412.3	443.2	4.2	2.5	59.6	136.8
NaCl	\pm 0.95	\pm 2.3	\pm 0.4	\pm 1.7	\pm 5.6	\pm 18.6	\pm 2.8	\pm 2.0	\pm 0.1	\pm 5.5	\pm 2.1	\pm 27.3	\pm 21.9	\pm 27.3	\pm 27.3	\pm 22.4	\pm 0.4	\pm 0.4	\pm 2.4	\pm 8.3
2 weeks	37.5	143.4	5.3	87.5	47.8	185.5	82.2	59.3	1.40	780.4	136.2	599.2	181.3	598.2	401.8	438.9	5.2	3.1	56.2	133.9
Prog.+NaCl	\pm 0.9	\pm 4.1	\pm 0.5	\pm 0.9	\pm 1.3	\pm 16.8	\pm 2.2	\pm 2.5	\pm 0.1	\pm 15.1	\pm 3.9	\pm 21.7	\pm 26.1	\pm 21.7	\pm 21.7	\pm 47.8	\pm 0.4	\pm 0.2	\pm 2.6	\pm 10.6
2 weeks	40	139.4	4.4	92.4	46.6	173.9	68.6	62.7	1.11	759.5	132.4	514.1	245.5	513.1	486.9	488.9	3.9	2.1	60.6	128.8
Prog.	\pm 1.7	\pm 4.1	\pm 1.4	\pm 2.7	\pm 9.2	\pm 13.6	\pm 2.6	\pm 2.4	\pm 0.1	\pm 4.8	\pm 3.9	\pm 23.5	\pm 30.9	\pm 23.5	\pm 23.5	\pm 30.9	\pm 0.6	\pm 0.5	\pm 2.4	\pm 10.8
2 weeks																				

Lig. = Figure-of-eight ligatures
 Prog. = Progesterone
 NaCl = 1% NaCl in drinking water

Standard error computed as to Burn (13).

eight and ninety-six hour ligations and sodium chloride. The serum potassium increased slightly in the progesterone and sodium chloride rats.

The sodium values in whole blood increased in all except the two week sodium chloride rats. Again, the largest increase was in the forty-eight and ninety-six hour ligations and sodium chloride.

There is a marked decrease in the cellular potassium K_C in the forty-eight and ninety-six hour manipulated kidneys. No such decrease is observed in the red blood cell potassium content. In the manipulated kidneys, there is an increase in the kidney water content, which is found to be cellular. There is a decrease in the extracellular phase and an increase in the intracellular phase.

The assumption that the water increment, in the forty-eight and ninety-six hour manipulated kidneys, is primarily intracellular seems valid on the basis that the total sodium in these kidneys remained the same. Since the extracellular sodium is in equilibrium with the serum sodium, if the water increment were extracellular, the total sodium would have to increase in proportion. It follows that intracellular dilution has occurred.

PART V

DISCUSSION AND CONCLUSIONS

Experimental hypertension is associated with an increased extracellular fluid volume of the body.^{44, 52, 141} Such an increase is indicated in the results by the reduced hematocrits in those rats with ligated kidneys.

The results also show an increase in the kidney sodium and water of the figure-of-eight ligated rats. The water increase is calculated to be in the intracellular space together with a decrease in the intracellular potassium. These changes are most marked in the shorter term ligations. In these early ligations the changes appear without the sodium chloride loading; however, sodium chloride by itself did not cause potassium depletion or an increase in the intracellular water.

No cellular potassium depletion was observed in the progesterone injected rats. This may be due to the dosage level used. Higher levels might be needed to effect the changes.

Renin production is known to be at its maximum in the early ischemic kidney.^{21, 28, 41} This would fit in with the time of maximum changes observed. Angiotonin and crude kidney extracts have been shown to increase aldosterone secretion^{34, 74}. Renin itself caused a hypertrophy in the zona glomerulosa and has been described as the possible aldosterone stimulating hormone of the kidney.²⁰ Aldosterone acts on the renal tubules to promote sodium chloride reabsorption and potassium excretion.⁶³

Aldosterone, DOCA, and potassium deficiency diets cause an intracellular potassium depletion, and a retention of water as the result of sodium retention^{143, 132, 134}. These changes are observed in the figure-of-eight ligated kidneys.

Injections of aldosterone increase the blood pressure¹³⁶ probably by aldosterone's effect on the electrolyte balance⁷⁴. Ligating the kidneys might cause hypertension by effecting these changes. The electrolyte disturbance of the kidney observed in aldosterone DOCA, and potassium deficiency hypertensions is also found in hypertension produced by renal ischemia due to figure-of-eight ligature.

In all of these forms of experimental hypertension potassium depletion appears to be a common underlying feature, whether induced by reduction in renal blood flow, operating through the renin-angiotonin-aldosterone mechanism, aldosterone or DOCA administration, or simple potassium deficiency. The fact that intracellular potassium deficiency appears in ischemic kidneys offers a ground for a unified concept for experimental hypertension. The mechanism by which altered sodium-potassium balance produces hypertension remains obscure.

PART VI

BIBLIOGRAPHY

1. Anderson, E., Page, E.W., Li, C.H., and Ogden E.,
Restoration of Renal Hypertension in
Hypophysectomized Rats by Administration of
Adrenocorticotropic Hormones. Amer. J. Physiol.
141: 393, 1944.
2. Biron, P., Koiv, E., Nowaczynski, W., Brouillet, J. and Genest, J.,
The Effects of Intravenous Infusions of Valine -
5, Angiotensin II and Other Pressor Agents on
Urinary Electrolytes and Corticosteroids Including
Aldosterone. J. Clin. Invest. 40: 338, 1961.
3. Bitman, J., Cecil, H.C., Hawk, H.W., and Syke, T.F., Effect of
Estrogen and Progesterone on Water and Electrolyte
Content of Rabbit Uteri. Am. J. Physiol. 197: 93,
1959.
4. Blackett, R.B., Depoorter, A., Pickering, G.W., Sellers, A.L.,
and Wilson, G.M., Hypertension Produced in the
Rabbit by Long Continued Infusions of Renin.
Clin. Sc. 9:223, 1950.
5. _____, and Sellers, A.L., The Effect of Removing the
Constriction of the Renal Artery in Acute and
Chronic Hypertension in the Rabbit. Clin. Sc.
10: 177, 1951.
6. Bohr, D.F., Conference on Electrolyte and Adrenal Factors in
Human and Experimental Renal Hypertension. Circulat.
17: 771, 1958.
7. Bradley, S.E., Physiology of Essential Hypertension. Am. J. Med.
4: 398, 1948.
8. Braun-Menendez, E., Conference on Mechanisms of Protective Action
of Kidney and Relation of Renoprival to Chronic
Renal Hypertension. Circulat. 17: 719, 1958.

9. _____, Evidence for Renotrophin as a Causal Factor in Renal Hypertension. Circulat. 17: 696, 1958.
10. _____, Fasciolo, J. C., Leloir, L.F., and Munoz, J.M., The Substance Causing Renal Hypertension. J. Physiol. 98: 293, 1940.
11. _____, and von Euler, U.S., Hypertension after Bilateral Nephrectomy in the Rat. Nature 160: 905, 1947.
12. Bryom, F.B. and Dodson, L.F., The Mechanism of the Vicious Circle in Chronic Hypertension. Clin. Sc. 8:1, 1949.
13. Burn, H.J., The Background of Therapeutics. Oxford University Press, London. 1948, p.327.
14. Coke, E.C., Mechanism of Renal Hypertension. Special Publications of the New York Acad. of Sc. Vol.III p.99, 1946.
15. Conn, J.W., Adrenal Factors in Hypertension. Circulat. 17: 743, 1958.
16. Conway, J., Vascular Reactivity in Experimental Hypertension Measured after Hexamethonium. Circulat. 17: 807, 1958.
17. Cottier, P.T., Weller, J.M., and Hoobler, S.W., Effect of an Intravenous Sodium Chloride Load on Renal Hemodynamics and Electrolyte Excretion in Essential Hypertension. Circulat. 17: 750, 1958.
18. Dahl, L. and Heine, M., The Enhanced Hypertensinogenic Effect of Sea Salt over Sodium Chloride. Amer. J. Cardiol. 8: 726, 1961.
19. Daniel, P.M., Pritchard, M.M.L. and Ward-McQuaid, J.N., Total Nephrectomy in Rabbits with Chronic Hypertension. Clin. Sc. 13: 247, 1954.

20. Davis, J.O., Hartcroft, P.M., Titus, E.O., Carpenter, C.J., Ayers, C., and Spigel, N., The Role of Renin-Angiotensin System in the Control of Aldosterone Secretion. J. Clin. Invest. 41: 378, 1962.
21. Dexter, L., Mechanism of Human Hypertension. Am. J. Med. 4: 279, 1948.
22. Dustan, H.P. and Masson, G.M.C., Contributions of the Adrenal Cortex to Renal and Renoprival Hypertension. Circulat. 17: 765, 1958.
23. Ezrow, L. and Sapirstein, L., Excretion of Sodium and Water in Rats made Hypertensive by Subtotal Nephrectomy. Amer. J. Physiol. 194: 436, 1958.
24. Farnsworth, E.B., and Baker, M.H., Tubular Reabsorption of Chloride in Hypertensive and in Normal Individuals. Proc. Soc. Exper. Biol. and Med. 52: 74, 1943.
25. Findley, M. and Tobian, L., Discussion of Reports on Electrolyte and Adrenal Factors in Hypertension. Circulat. 17: 768, 1958.
26. Flasher, and Drury, Relationship of Renin to Early Renal Hypertension in the Rabbit. Amer. J. Physiol. 162: 385, 1950.
27. Floyer, M.A., Further Studies on the Mechanism of Experimental Hypertension in the Rat. Clin. Sc. 14: 163, 1955.
28. _____, Role of the Kidney in Experimental Hypertension. Brit. M. Bull. 13: 29, 1957.
29. Freed, C.S., The Role of Potassium and its Relation to Sodium in the Regulation of Blood Pressure. Amer. J. Cardiol. 8: 741, 1961.

30. Fregly, M.J. and Gonzalez, J.V., Activity of Thyroid Gland During Development of Renal Hypertension in Rats. Amer. J. Cardiol. 8: 695, 1961.
31. _____, Yates, J. E., and Landis, E.M., Serum Sodium Concentration of Hypertensive Rats, Relation to Sodium Chloride Intake, Blood Pressure and Age. Proc. Soc. Exper. Biol. and Med. 90: 695, 1955.
32. Friedman, S.M., Friedman, C.C., and Nakashime, M., Sodium Gradient and Renoprival Hypertension in the Rat. Circulat. Res. 7: 917, 1959.
33. _____, Hinke, J.A.M., and Hardwick, D.F., Sodium Tolerance in Experimental Hypertension. Circulat. Res. 3: 297, 1955.
34. Ganong, W.F., Mulrow, P.J., Boryczka, A., and Cera, G., Evidence for a Direct Effect of Angiotensin - II on Adrenal Cortex of the Dog. Proc. Soc. Exper. Biol. and Med. 109: 381, 1962.
35. Goldblatt, H., Conference on the Role of the Renal Pressor System in Maintenance of Experimental Renal Hypertension. Circulat. 17: 683, 1958.
36. _____, Experimental Renal Hypertension. Am. J. Med. 4: 100, 1948.
37. _____, Experimental Renal Hypertension. Mechanism of Production and Maintenance. Circulat. 17: 642, 1958.
38. _____, The Renal Origin of Hypertension. Physiol. Rev. 27: 100, 1947.
39. _____, Studies on Experimental Hypertension V. The Pathogenesis of Experimental Hypertension Due to Renal Ischemia. Ann. Int. Med. 11: 69, 1937.

40. _____, Lynch, J., Hanzal, R.F. and Summerville, W.W.,
Studies on Experimental Hypertension. I. The
Production of Persistent Elevation of Systolic
Blood Pressure by Means of Renal Ischemia. J.
Exper. Med. 59: 347, 1934.
41. Goldring, W., Experimental Hypertension, Special Publ. of New
York Acad. of Sc. III: 1-22, 1946.
42. Green, D.M., Wedell, H.G., Wald, M.H. and Learned, B., The
Relation of Water and Sodium Excretion to
Blood Pressure in Human Subjects. Circulat.
6: 919, 1952.
43. Greene, R.G. and Sapirstein, L.A., Total Body Sodium,
Potassium and Nitrogen in Rats made Hypertensive
by Subtotal Nephrectomy. Amer. J. Physiol.
169: 343, 1952.
44. Grollman, A., Effect of Increasing the Extracellular Fluid
Volume on the Arterial Blood Pressure of the
Normal Hypertensive, and Nephrectomized Dog.
Amer. J. Physiol. 173: 364, 1953.
45. _____, Experimental Chronic Hypertension. Its
Mechanism and Amelioration by Use of Various
Blood Pressure Reducing Substances. Special
Publ. of New York Acad. Sc. Vol. III p.99
1946.
46. _____, Experimental Chronic Hypertension in the
Rabbit. Amer. J. Physiol. 142: 666, 1944.
47. _____, Experimental Hypertension in the Dog.
Amer. J. Physiol. 147: 647, 1946.
48. _____, The Role of Salt in Health and Disease.
Amer. J. Cardiol. 8: 593, 1961.
49. _____, Harrison, T.R., and William, J. R. Jr.,
The Mechanism of Experimental Renal Hypertension
in the Rat: The Relative Significance of Pressor
and Anti-Pressor Factors. Amer. J. Physiol.
139: 293, 1943.

50. _____, Muirhead, E.E., and Vanatta, J., Role of the Kidney in Pathogenesis of Hypertension as Determined by a Study of the Effects of Bilateral Nephrectomy and Other Experimental Procedure on the Blood Pressure of the Dog. Amer. J. Physiol. 157: 21, 1949.
51. _____, and Rule, C., Effect of Nephrectomy on Blood Pressure in Parabiosis. Amer. J. Physiol. 138: 587, 1943.
52. _____, and Shapiro, A.P., The Volume of the Extracellular Fluid in Experimental and Human Hypertension. J. Clin. Invest. 32: 312, 1953.
53. _____, Turner, L., Levitch, M. and Hill, D., Hemodynamics of Bilaterally Nephrectomized Dog Subjected to Intermittent Peritoneal Lavage. Amer. J. Physiol. 165: 167, 1951.
54. _____, and White, F.N., Induction of Renal Hypertension in Rats and Dogs by Potassium or Choline Deficiency. Amer. J. Physiol. 193: 144, 1958.
55. Hall, C.E., and Hall, O., Some Observations on Free Role of Added Supplement of Sodium and Lithium in the Pathophysiology of Corticoid and Metacorticoid Hypertension in the Rat. Amer. J. Cardiol. 8: 719, 1961.
56. Haight, A.S. and Weller, J.M., Plasma and Tissue Electrolytes of Rats Given Excess Sodium Chloride in Food. Proc. Soc. Exper. Biol. and Med. 108: 209, 1961.
57. Handler, P., and Bernheim, F., Influence of Dietary Factors on Hypertension Induced by Chloride Deficiency. Amer. J. Physiol. 162: 289, 1950.
58. Hartcroft, W.S., and Best, C.H., Hypertension in Rats Following Choline Deficiency. Brit. M.J. 1: 423, 1949.

59. Haynes, F.W., and Dexter, L., Renin, Hypertensinogen and Hypertensinase Concentrations of Blood of Dogs During Development of Hypertension by Constriction of the Renal Artery. Amer. J. Physiol. 150: 196, 1947.
60. Helmer, O.M., Studies on Renin Antibodies. Circulat. 17: 648, 1958.
61. Hollander, W., Chobanian, V., Burrows, B.A., and Bickerman, V., Body Fluids and Electrolyte Composition in Arterial Hypertension I. Studies in Essential of Renal and Malignant Hypertension. J. Clin. Invest. 40: 408, 1961.
62. Holley, H.L., and Carlson, W.W., Potassium in Health and Disease. Modern Medical Monographs, 1955.
63. _____, Eliot, H.C. Jr., and Holland, C.M. Jr., Serum Sodium Values in Essential Hypertension Proc. Soc. Exper. Biol. and Med. 77: 561, 1951.
64. Holman, P. V. and Page, I.N., The Cardiac Output in Arterial Hypertension. Am. Heart J. 16: 321, 1938.
65. Kao, C.Y., Contents and Distribution of Potassium, Sodium and Chloride in Uterine Smooth Muscle. Amer. J. Physiol. 201: 717, 1961.
66. Kark, R.M., Some aspects of Nutrition and the Kidney. Am. J. Med. 25: 713, 1958.
67. Katz, L.N., Friedman, M., Rodbard, S. and Weinstein, W., Observations on the Genesis of Renal Hypertension. Am. Heart J. 17: 334, 1939.
68. Koletsky, S., Pathogenesis of Experimental Hypertension Induced By Salt. Am. J. Cardiol. 8: 576, 1961.

69. Kolff, W.J., Reduction of Experimental Renal Hypertension by Kidney Perfusion. Circulat. 17: 702, 1958.
70. _____, and Page, I. H., Blood Pressure Reducing Function of the Kidney, Reduction of Renoprival Hypertension by Kidney Perfusion. Amer. J. Physiol. 178: 75, 1954.
71. _____, _____, Persistence of Experimental Renal Hypertension after Total Nephrectomy in Dogs. Amer. J. Physiol. 181: 575, 1955.
72. _____, _____, Renoprival Hypertension and Antirenin. Amer. J. Physiol. 181: 575, 1955.
73. Kremen, S.H., and Wakerlin, G.E., Renin and Antirenin in Treatment of Long Term Experimental Renal Hypertension in the Dog. Proc. Soc. Exper. Biol. and Med. 90: 99, 1955.
74. Laragh, J.H., Aldosterone and Arterial Hypertension. Med. Clin. N. Amer. 45: 321, 1961.
75. Laramore, D., and Grollman, A., Water and Electrolyte Content of Tissues in Normotensive and Hypertensive Rats. Amer. J. Physiol. 161: 278, 1950.
76. Lassen, H.C.A., and Husfeldt, E., Kidney Function and Blood Pressure. J. Clin. Invest. 13: 263, 1934.
77. Ledingham, J.M., Disturbances in Water and Electrolyte Metabolism in Experimental Hypertension. Brit. M. Bull. 13: 33, 1957.
78. _____, The Nature of the Hypertension Occurring in the Nephrectomized Paraboitic Rat. Clin. Sc. 10: 423, 1951.
79. Leloir, L.F., Renin and Renal Hypertension, Special Publ. of New York Acad. of Sc. Vol. III p.60, 1946.

80. Lemley-Stone, J., Darby, W.J., and Meneely, G.R., Effect of Dietary Sodium-Potassium Ratio on the Body Content of Sodium and Potassium in Rats. Amer. J. Cardiol. 8: 748, 1961.
81. Lever, A.F., and Peart, W.S., Renin and Angiotensin Like Activity in Renal Lymph. J. Physiol. 160: 548, 1962.
82. Mallov, S., Effect of Hypertension and Sodium Chloride on Vascular Reactivity in Vitro. Amer. J. Cardiol. 8: 542, 1961.
83. Manery, J.F., and Hastings, B.A., The Distribution of Electrolytes in Mammalian Tissues. J. Biol. Chem. 127: 657, 1939.
84. McCubbin, J.W., Carotid Sinus Participation in Experimental Renal Hypertension. Circulat. 17: 791, 1958.
85. McQueen, E.G., Vascular Reactivity in Experimental Renal and Renoprival Hypertension. Clin. Sc. 15: 523, 1956.
86. Meneely, G.R. and Ball, C.C.T., Experimental Epidemiology of Chronic Sodium Chloride Toxicity and the Protective Effect of Potassium Chloride. Am. J. Med. 25: 713, 1958.
87. _____, Lemley-Stone, J., and Darby, W.J., Changes in Blood Pressure and Body Sodium of Rats Fed Sodium and Potassium Chloride. Amer. J. Cardiol. 8: 527, 1961.
88. _____, Tucker, R.G., Darby, W.J. and Auerbach, S.H., Chronic Sodium Chloride Toxicity in the Albino Rat II. Occurrence of Hypertension and a Syndrome of Edema and Renal Failure. J. Exper. Med. 98: 71, 1953.

89. Merril, J.P., Munoz, J.R., Harrison, J. H., and Guild, W.R., Successful Homotransplantation of the Human Kidney Between Identical Twins. J.A.M.A. 160: 277, 1956.
90. Moritz, A.R. and Oldt, M.R., Arteriolar Sclerosis in Hypertensive and non Hypertensive Individuals. Amer. J. Path. 13: 679, 1937.
91. Muirhead, E.E., Stirman, J.A., Lesch, W., and Jones, F., The Reduction of Post Nephrectomy Hypertension by Renal Homotransplant. J. Surg. Gynec. & Obst. 103: 673, 1956.
92. Ogden E., The Extrarenal Sequel to Experimental Renal Hypertension. Bull. N.Y. Acad Med. 23: 643, 1947.
93. _____, Brown, L.T. and Page, E.W., Increased Sensitivity of Arterial Muscle in the Prehypertensive Phase of Experimental Renal Hypertension. Amer.J. Physiol. 129: 560, 1940.
94. _____, Collings, W.D., and Sapirstein, L.A., A Change of Mechanism in the Course of Hypertension of Renal Origin. Special Publ. of N.Y. Acad. Sc. Vol. III p.153, 1946.
95. _____, Page, E.W., and Anderson, E., Effect of Posterior Hypophysectomy on Renal Hypertension. Amer. J. Physiol. 141: 389, 1944.
96. Oster, K.A., and Martinez, O., Water Metabolism in Hypertensive Rats. J. Exper. Med. 78: 477, 1943.
97. Page, I.H., Demonstration of Liberation of Renin into Blood Stream from Kidneys made Hypertensive by Cellophane Perinephritis. Amer. J. Physiol. 130: 22, 1940.

108. Peters, J.P., Body Water. Ch. V. and VI. The Exchange of Fluids in man. Charles Thomas. Illinois, 1935.
109. Pickering, G.W., The Role of the Kidney in Acute and Chronic Hypertension Following Renal Artery Constriction in the Rabbit. Clin. Sc. 5: 229, 1945.
110. _____, and Prinzmetal, M., Experimental Hypertension of Renal Origin in the Rabbit. Clin. Sc. 3: 357, 1938.
111. Playoust, M.R., and Blackett, R.B., Primary Aldosteronism with Severe Hypertension Relieved by Adrenalectomy. Med. J. Aust. 48: 249, 1961.
112. Regolli, D., Brunner, H., Peters, G., and Gross, F., Changes in Renin Content in Kidneys of Renal Hypertensive Rats. Proc. Soc. Exper. Biol. and Med. 109: 142, 1962.
113. Remington, J. W., Some Physiological Aspects of the Blood Pressure Lowering Effect of Tissue Extracts in the Hypertensive Animal. Special Publ. of N.Y. Acad. of Sc. Vol.III p.139, 1946.
114. Rodbard, S. and Katz, L.N., The Depressor Effect of Tissue Implants in Hypertensive Dogs. Am. Heart J. 26: 114, 1943.
115. _____, _____, The Role of Renal Metabolism in Hypertension and Uremia. J. Exp. Med. 73: 357, 1941.
116. Rondell, D.A., McVaugh, R.B. and Bohr, D.F., Evidence for Humoral Factors in Renoprival Hypertension. Circulat. 17: 708, 1958.
117. Rytand, D.A., The Renal Factor in Arterial Hypertension with Coarction of the Aorta. J. Clin. Invest. 17: 391, 1958.

98. _____, The Effect of Bilateral Adrenalectomy on Arterial Blood Pressure of Dogs with Experimental Hypertension. Amer. J. Physiol. 122: 352, 1938.
99. _____, The Problem of the Occurrence of Vasoconstrictor Substances in Peripheral Blood in Hypertensive Patients and Dogs After Injection of Renin. Special Publ. of N.Y. Acad. of Sc. Vol. III p.77, 1946.
100. _____, Production of Persistent Arterial Hypertension by Cellophane Nephritis. J.A.M.A. 113: 2046, 1939.
101. _____, and Helmer, O.M., A Crystalline Pressor Substance Resulting from the Reaction Between Renin and Renin Activator. J. Exper. Med. 71: 29, 1940.
102. _____, and Lewis, L.A., Arterial Pressure and Serum Protein Pattern in Adrenalectomized Hypertensive and Adrenalectomized Dogs Amer. J. Physiol. 164: 61, 1956.
103. _____, Ogden, E., and Anderson, E., Influence of Steroids on Restoration of Hypertension in Hypophysectomized Rats. Amer. J. Physiol. 147: 471, 1946.
104. _____, and Sweet, J.E., Effect of Hypophysectomy on Arterial Pressure of Dogs with Experimental Hypertension. Amer. J. Physiol. 120: 238, 1937.
105. Pasqualino, A., and Bourne, G.H., Histochemical studies of Kidneys and Adrenals of Rats with Partial Constriction of One Renal Artery. Acta Anatomica 41: 1, 1960.
106. Perera, G.A., Diagnosis and Natural History of Hypertensive Vascular Disease. Am. J. Med. 4: 416, 1948.
107. _____, and Blood, D., The Relationship of Sodium Chloride to Hypertension. J. Clin. Invest. 26: 1109, 1947.

118. Salgado, E., Influence of Hypophysectomy upon the Established Hypertensive Disease Induced by Desoxycorticosterone. J. Lab. and Clin. Med. 45: 865, 1955.
119. Schrader, C.A., Prickett, C.O. and Salmon, W.D., Symptomatology and Pathology of Potassium and Magnesium Deficiency in the Rat. J. of Nutr. 14: 85, 1937.
120. Schroeder, H.A., Low Salt Diets and Arterial Hypertension. Am. J. Med. 4: 578, 1948.
121. _____, Mechanism of Hypertension Charles Thomas. Illinois 1957. 379 p.
122. _____, and Vinton, W.H. Jr., Hypertension Induced in Rats by Small Doses of Cadmium. Amer. J. Physiol. 202: 515, 1962.
123. Shorr, E., Participation of Hepatorenal Vasotropic Factor in Experimental Renal Hypertension. Am. J. Med. 4: 120, 1948.
124. Skelton, F.R., and Hyde, P.M., Plasma Corticosterone Levels and Salt Intake in Experimental Hypertension in the Rat. Am. J. Cardiol. 8: 700, 1961.
125. Smith, H.W. Hypertension and Urologic Disease. Am. J. Med. 4: 724, 1948.
126. _____, The Kidney; Structure and Function in Health and Disease. N.Y. Oxford University Press. 1951.
127. Smithwick, R.H., Surgical Treatment of Hypertension. Am. J. Med. 4: 744, 1948.
128. Taquini, A.C., Blaquer, D., and Taquini, A.C. Jr., Studies on the Renal Humoral Mechanism of Chronic Experimental Hypertension. Circulat. 17: 672, 1958.

129. _____, and Fasciolo, J.C., Renin in Essential Hypertension. Am. Heart J. 32: 357, 1946.
130. Tobian, L., Effect of Salt on the Kidney and the Juxtaglomerular Apparatus. Am. J. Cardiol. 8: 684, 1961.
131. _____, Interrelationship of Electrolytes, Juxtaglomerular Cells and Hypertension. Physiol. Rev. 40: 280, 1960.
132. _____, and Binion, J., Artery Wall Electrolytes in Renal and Desoxycorticosterone Hypertension. J. Clin. Invest. 33: 1407, 1954.
133. _____, _____, Tissue Electrolytes in Renal and Desoxycorticosterone Hypertension. J. Clin. Invest. 32: 608, 1953.
134. _____, and Redleaf, P.O., Ionic Composition of the Aorta in Renal and Adrenal Hypertension. Amer. J. Physiol. 192: 325, 1958.
135. Vander, A.J., Potassium Secretion and Reabsorption in Distal Nephron. Amer. J. Physiol. 201: 505, 1961.
136. Venning, E.H., Dyrenfurth, I., Dossetor, J.B., and Beck, J.C., Essential Hypertension and Aldosterone. Circulat. 23: 168, 1961.
137. Wiggers, C.J., The Dynamics of Hypertension. Am. Heart J. 16: 515, 1938.
138. Williams, J.R. Jr., Harrison, T.R., and Grollman, A., A Simple Method for Determining the Systolic Blood Pressure of the Unanesthetized Rat. J. Clin. Invest. 18: 373, 1939.
139. White, F.N., Sambhi, M.P., and Grollman, A., Renal Function in Hypertensive Cardiovascular Disease of Rat. Am. J. Physiol. 198: 221, 1960.

140. White, F.N., A Method for the Determination of the Systolic Blood Pressure of the Unanesthetized Unheated Rat. Texas Reports on Biology and Medicine 16: 268, 1960.
141. Yamanchi, H., Bigleir, E., and Hopper J. Jr., Blood Volume and Aldosterone Function in Hypertension and Primary Aldosteronism. Proc. Soc. Exper. Biol. and Med. 107: 728, 1961.
142. Zweifach, B.W. and Shorr E., Factors Regulating Blood Pressure 2nd, 3rd, and 4th Conferences. Publ. Josiah Macy Jr. Foundation, N.Y. 1949, 50.
- *143. _____, Sodium, Potassium and Hypertension. Nutrition Reviews. 15: 284, 1957.
- *144. _____, Electrolyte Disturbances in Potassium Deficiency. Nutr. Rev. 13: 148, 1955.
- *145. _____, Potassium Deficiency and the Kidney. Nutr. Rev. 19: 242, 1961.

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