Electrolyte Changes in Renal Cortex, Inner Medulla and Papilla in Renal Artery Clamp Hypertension[1] (34623)

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When Goldblatt (1) first produced hypertension experimentally by partially constricting the renal artery, he noted that, in the dog, it was necessary to excise the contralateral kidney or place a constriction on both renal arteries to insure persistent hypertension. In the rat, however, Wilson and Byrom (2) found that constriction of only one renal artery is sufficient to produce chronic hypertension. Certain interesting differences have been noted between rats bearing a clamp on one renal artery and rats similarly prepared but with the opposite kidney excised. In the former preparation, pressor substances appear in the blood initially (3–5) and aldosterone secretion is increased (6). The renin content of the clamped kidney becomes and remains persistently elevated (7–9), while that in the untouched kidney falls (10). Although renin changes persist in the kidneys and the animals continue to be hypertensive, pressor substances can no longer be demonstrated in the blood after 2–3 weeks (5, 11). In rats with a renal artery clamp and the contralateral kidney excised, no rise in pressor substances in the blood (12), no change in renal renin (9), and no increase in aldosterone (6) has been found though severe hypertension develops.

In sheep, Blair-West *et al.* (13) have reported that the blood renin rises promptly following the placement of a clamp on the renal artery. In the animals, which had been previously uninephrectomized, the rise was transient. In animals with an untouched contralateral kidney, a second and marked rise in blood renin occurred concomitantly with the development of severe sodium depletion, a condition which did not occur in the sheep with the contralateral kidney removed.

In the present study, renal tissues from rats with clamp hypertension were examined to determine whether the water, sodium, potassium content, or osmolarity of the clamped kidney differed from its untouched pair or from a control kidney and also whether these values differed in the uninephrectomized rat with an artery clamp from a control uninephrectomized animal. Our interest in obtaining such information was also prompted by a recent report of Ishii *et al.* (14), that the ratio of the sodium content of the renal papilla to that of the cortex was reduced in clamp, postclamp and also in "post-salt" hypertension in the rat.

Methods. Twenty-four pathogen-free male Wistar rats were divided into 4 groups. Group I served as controls, the remaining 3 groups were subjected to the following operations at the beginning of the experimental period when they were 120–150 g in weight; Group II, silver tape clamp on left renal artery; Group III, right nephrectomy; Group IV, clamp on left renal artery and 1 week later, right nephrectomy. The technique employed in clamping the renal artery in the rat was originally outlined by Wilson and Byron (2) and has recently been described in full detail by Byron (20).

All animals received Purina rat chow and water *ad libitum*. At weekly inervals, they were weighed and blood pressures were recorded from the tail by the microphonic manometer method of Friedman and Freed (15). The rats were prewarmed in a vented incubator set at 38–40° and during the readings they were restrained but not anesthesized. For 24 hr prior to sacrifice the rats were placed in individual cages and their water intake was measured. Animals were killed from 8 to

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12 weeks postoperatively. Under pentobarbital anesthesia (50 mg/kg), the right kidney pedicle was clamped and this kidney was excised, stripped of fascia, and sectioned longitudinally, slightly off center. In order to obtain sufficient material to permit valid determination, the papilla and inner medulla, which remained in the larger half, were removed together, then divided into two roughly equal portions by a radial cut through the center of the papilla. In a few instances where the original longitudinal cut passed through the papilla, roughly equal portions were cut from each half kidney. Each sample was placed on a previously tared aluminum foil square and weighed. One portion was subsequently placed in a drying oven (90–110°) for 24 hr to determine water content. The other portion was transferred to a 2-ml volumetric flask to which sufficient boiling water was added to immerse the tissue completely. The flask was then stoppered and placed in a boiling water bath, a modification of the method outlined by Appelboom *et al.* (16). A pair of samples was then taken from the outer cortex. The left kidney was then similarly treated. In order to obtain the sampling of the tissues as rapidly as possible and thus minimize the increase in osmolarity which occurs promptly and increases to as much as 50 mmoles/kg within 10 min, according to Appelboom (16), whole kidney weights were foregone. After the tissues had been sampled, blood was drawn from the aorta. The serum was separated for determination of sodium and potassium by flame photometer (Instrumentation Lab. Inc. with automatic dilutor) and for urea nitrogen by urograph chromatography paper (Warner-Chilcott). After the tissues were heated for 1 hr in the boiling water bath, the flasks were cooled, lithium was added, and made to volume. Determinations of sodium and potassium were made on the supernatant using the above photometer without dilutor and with appropriate standards, and of osmolality using an osmometer.

Results. The rats survived and grew satisfactorily with the exception of 2 animals in Group IV, both severely hypertensive, which died during the third and the sixth week postoperatively. With one exception hypertension developed in all rats bearing clamps within 2–8 weeks postoperatively. The one rat which remained normotensive proved, at sacrifice, to have a markedly atrophic kidney on the clamp side. No papillary tissue was found and the animal was excluded from the series. In three of the eight rats with clamped kidneys in Group III, the clamped left kidney appeared smaller than the untouched opposite one. The average papillary and medulla weights in this groups were 24.6 mg on the clamped side *vs.* 36.2 mg on the untouched side; in the control Group I, comparable weights were 35.2 mg on the left *vs.* 35.4 mg on the right. This would suggest that the clamped kidneys were somewhat reduced in overall size and this is in keeping with data presented by Brunner, *et al.* (17) that 3–4 weeks after clamping, the weight of the kidney behind the clamp averaged 296 mg/100 g of body weight as compared to a weight of 449 mg for the opposite kidney and a weight of 349 mg for the kidneys from control rats.

At the time of sacrifice all the clamp rats had had blood pressures in excess of 160 for more than a month (range 4–7 weeks). Terminal blood pressures are shown in Table I. Pressures in the Group II rats with renal artery clamps were significantly greater than in the control Group I at the 1% level, and pressures in the Group IV rats with the left renal artery clamps and right nephrectomies were greater than Group III with right nephrectomies at the 5% level.

Sera. (Table I). There was no significant alteration in the serum sodium concentration in any of the four groups, although in two rats in Group II the values were reduced (131.5 and 130.5 meq/liter). The serum potassium values in the Group II clamp rats and also in the Group IV clamp rats, were significantly lower than in the control rats of Group I at the 1% level. No significant alteration in the urea nitrogen levels were encountered between groups, although modestly elevated values were obtained in 3 rats of Group II (35, 40, and 50 mg/100 ml) and in one rat from Group IV (35 mg/100 ml).

Kidney tissue analyses. (Table I). *Cortex.* No significant differences were noted be-

TABLE I.

Group	I	II	III	IV
	Control	Left renal artery clamp	Rt. nephrx	Left renal artery clamp; rt. nephrx
No. of rats	6	8	4	3
Terminal blood pressure (mm Hg)	127 ± 4.6[a]	207 ± 5.6[c]	130 ± 4.8	208 ± 26.0[e]
Terminal body wt (g)	367 ± 8.8	354 ±22.2	353 ± 9.3	346 ± 32.4
Serum				
Sodium (meq/liter)	138.4 ± 0.9	137.0 ± 1.5	139.3 ± 0.6	138.3 ± 0.7
Potassium (meq/liter)	6.2 ± 0.3[b]	4.8 ± 0.1[c]	5.6 ± 0.7	4.4 ± 0.03[e]
Urea nitrogen (mg/100 ml)	25 ± 2.0	32 ± 3.4	28 ± 2.4	28 ± 4.4
Kidney cortex	Right / Left	Right / Left	Left	Left
Water (%)	75.5 ± 0.4[b] / 76.0 ± 0.2	78.1 ± 0.6[d] / 72.8 ± 1.9[g]	75.2 ± 0.7	74.5 ± 1.0
Sodium (meq/kg of tissue water)	70 ± 4.0 / 82 ± 6.4[b]	74 ± 2.5 / 74 ± 2.0	75.0 ± 1.6	70 ± 5.7
(meq/100 g of dry wt)	22.8 ± 1.1 / 26.3 ± 2.4[b]	26.5 ± 1.4 / 20.4 ± 1.5[g,i]	23.7 ± 0.2	20.5 ± 2.6
Potassium (meq/kg of tissue water)	93 ± 5.7 / 91 ± 7.4[b]	90 ± 3.0 / 117 ± 3.9[e,f,h]	97 ± 5.6	98 ± 3.8
(meq/100 g of dry wt)	29.1 ± 1.3 / 29.2 ± 1.9[b]	32.1 ± 0.9 / 32.0 ± 1.8	29.3 ± 1.2	29.2 ± 1.2
(mOsm/kg of tissue water)	346 ± 19 / 393 ± 17[b]	386 ±17 / 438 ± 28	424 ± 38	406 ± 27
Papilla and inner medulla				
Water (%)	84.9 ± 0.9 / 85.4 ± 0.6[b]	84.7 ± 0.8 / 84.7 ± 0.8	84.3 ± 1.1	80.7 ± 1.7
Sodium (meq/kg of tissue water)	237 ± 28 / 215 ± 19	170 ±17[d] / 161 ± 15[d]	241 ± 8	179 ± 23[e]
Potassium (meq/kg of tissue water)	55 ± 3 / 51 ± 3	62 ± 5 / 49 ± 7[g]	83 ± 18	70 ± 12
(mOsm/kg of tissue water)	1077 ±157 / 1093 ±168	763 ±53[d,j] / 955 ±117[j]	1356 ±117	1208 ±253

[a] Average ± SE of the means.
[b] Average of 5 rats.
[c] Differs from average of control Group I at 1% level.
[d] Differs from average of control Group I at 5% level.
[e] Differs from average of uninephrx control Group III at 5% level.
[f] Differs from opposite untouched kidneys at 1% level.
[g] Differs from opposite untouched kidneys at 5% level.
[h] Difference between rt. and left kidney greater than difference between rt. and left control Group I kidneys at 1% level.
[i] Difference between rt. and left kidney greater than difference between rt. and left control Group I kidneys at 5% level.
[j] Average of 7 rats.

tween the left and right kidneys of the control Group I rats. In Group II the water content of the kidneys bearing clamps proved widely variable, ranging from 60.7 to 76.8%. Because of this variability, no significant difference from the average water content of kidneys of Group I could be demonstrated. However, in each rat bearing a clamp, the water content of its clamped kidney was less than that of its opposite untouched pair and the average difference between the two proved significant at the 5% level. In the untouched kidney of Group II rats, the water content was less variable and was significantly greater than in the control kidneys from Group I at the 5% level.

The sodium content of the cortices of the clamped and of the untouched kidneys from the Group II rats were similar and did not differ from the values obtained in control rats, when expressed in terms of tissue water. However, when the sodium content was calculated in terms of dry weight, the amount in the kidney bearing a clamp was definitely reduced as compared to its untouched pair, significant at the 5% level, and, furthermore, the difference between the two kidneys of Group II rats was greater than the difference between the two kidneys of Group I animals at the 1% level. The potassium content of the cortex of each clamped kidney was strikingly greater than that of its untouched pair and of the control kidney, significant at the 1% level when expressed in terms of tissue water, but differences were no longer apparent when potassium was calculated in terms of dry weight. No significant differences in osmolarity were noted. The cortices of the 3 rats with a single clamped kidney, Group IV did not exhibit a significant difference in any of the parameters measured from the cortices of control uninephrectomized rats, Group III.

Papilla and inner medulla. No significant differences were observed between the right and left kidneys of the control Group I rats in any of the determinations. In the Group II rats no significant differences were noted between the clamped and the contralateral untouched kidneys with respect to water, sodium, or osmolarity. The potassium content of the kidney bearing a clamp was reduced compared to its untouched pair ($p<0.05$). The reduction was noted in the rats sacrificed 8–10 weeks postoperatively but not in those sacrificed 11 and 12 weeks postoperatively. Although the sodium content of the clamped and the untouched kidney papilla and inner medulla were the same, both were reduced significantly at the 5% level when compared to similar tissues from control rats. In the 3 rats of Group IV similarly, the sodium content of the papilla and inner medulla proved significantly less at the 5% level than that in the 4 uninephrectomized control Group III rats.

Discussion. The changes found in the present study in the cortices of the kidneys of rats bearing a renal artery clamp and an intact contralateral kidney are in keeping with the concept that the untouched kidney is volume expanded while the kidney bearing the clamp is relatively volume depleted. These volume changes would appear to involve chiefly the extracellular phase, since the postassium content per unit of dry weight of both kidneys is the same and similar to that in control kidneys. It seems possible that the persistently high content of renin in the clamped kidney as well as the strikingly lowered renin in the untouched contralateral one is related to these changes in fluid content, since Ziegler and Gross (18) have reported that the blood renin rises promptly following a decrease in intravascular volume, though in their acute experiments the renin content of the kidney did not change. It should perhaps be emphasized that the data of Brunner *et al.* (17) make clear that although the clamped kidney is somewhat smaller than a normal kidney and the untouched kidney somewhat larger than a normal kidney, the total renal renin as well as the renin per gram of tissue is increased markedly in the clamped kidney and decreased in the opposite untouched kidney as compared to a control kidney.

The changes seen in these clamped kidneys do not seem to be an inevitable result of the artery clamping with its attendant decrease in pressure since the few animals, in which contralateral kidney had been removed, did not exhibit significant changes in water, sodium, or potassium. This is in keeping with

what Regoli *et al.* (8) pointed out with respect to renin, *i.e.*, that the changes in the clamped kidney can develop only in the presence of the other intact kidney, which in this current study showed evidence of overhydration.

It is of interest that the papilla and inner medulla of the clamped kidney do not share in the above cortical changes in water content and showed no difference from the contralateral intact kidney except in potassium content, which in this instance was reduced. The reason for this is not obvious. The fact that it was apparent only in animals sacrificed 8–10 weeks after placement of the clamp and not seen in the rats sacrificed after this time, suggests the change is a short-term one for which the organism subsequently compensates.

The lowered papillary and inner medullary sodium content of the hyertensive animals might conceivably reflect a washout phenomenon induced by the raised arterial pressure since it is known that the medulla does not autoregulate (19). Ishii *et al.* (14) have proposed this theory to explain a reduced papillary content of sodium and urea, which they observed in "post-salt" hypertension, in hypertension produced by clipping, and in hypertension which persisted after removal of a clipped kidney. They correlated the washout of the papilla with a decrease in papillary lipid granules.

The findings in the current study question the liklihood of the decreased sodium in the papilla and inner medulla being exclusively the result of washout for several reasons. In the first place the fluid intake of the clamped rats was not uniformly increased. Figure 1 plots the sodium content of the papilla and inner medulla against the fluid intake, expressed as percentage of the average intake of the control Group I rats. As shown, several of the Group II rats had a markedly increased water intake but it is also evident that others with equally low values for sodium in the papilla and inner medulla had intakes no greater than the control animals. Secondly, it seems likely that the urine flow from the volume-depleted clamped kidneys was, if anything, reduced and yet the sodium

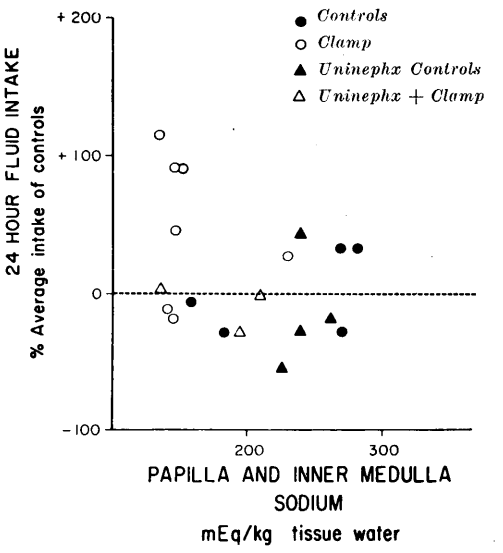

FIG. 1. Sodium content of the papilla and inner medulla related to fluid intake.

levels were reduced as much in this kidney as in its untouched pair.

Thirdly, Koletsky *et al.* (11) have shown that the pressure distal to a clamp remains markedly lower than the pressure in the brachial artery over a period of many months, so that it can be assumed that the clamped kidney is not subjected to an increased pressure. Fourthly, were the reduction in sodium the result of a washout phenomenon, one might expect an indiscriminate lowering of sodium potassium and osmolarity. In the untouched kidney the reduction in osmolarity does fit with this concept but the fact that the sodium was equally reduced in the clamped kidney where no significant lowering in osmolarity was noted, makes it impossible to explain the lowered sodium here on the theory of increased washout. The decrease in medullary sodium content is inversely related to the systemic blood pressure as shown in Fig. 2 in which terminal blood pressure is plotted against the sodium content. It is also possible that the decrease in medullary and papillary content is related to an increased aldosterone output. No measurements of aldosterone were made in this study but the relatively lower serum potassium, which both groups of clamp rats exhibited, suggests that aldosterone secretion may have

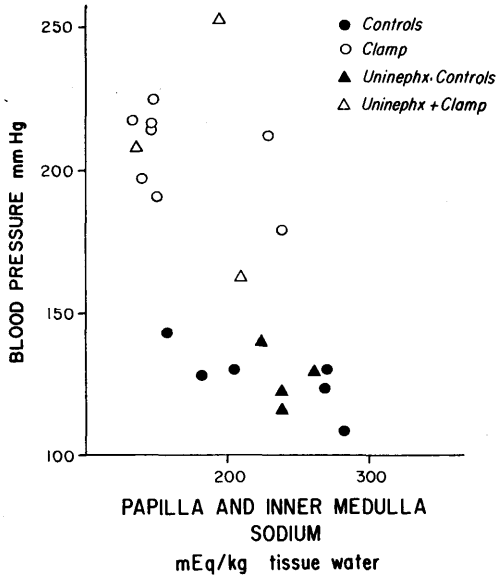

FIG. 2. Final blood pressure related to sodium content.

been increased. If so, our findings differ in part from those of Singer *et al.* (6), who found aldosterone to be increased only in the rats with an intact as well as a clamped kidney.

Conclusion. Chronic hypertension was produced in rats by renal artery clamps and the renal tissues (cortex and a combined sampling of papilla and inner medulla) were examined for water, sodium, potassium content, and osmolarity.

In hypertensive rats with a contralateral untouched kidney, the cortex on the side bearing the clamp showed evidence of volume depletion compared to the opposite intact side, *i.e.*, significance tests showed a decreased water content, an unchanged sodium, and increased potassium content per kilo of tissue water; a decreased sodium and an unchanged potassium content when expressed per unit of dry weight. In the three hypertensive rats in which the contralateral kidney was excised after placement of the renal artery clamps, no significant difference was noted from the cortex of control uninephrectomized animals.

Unlike the cortex, tissue from the inner medulla and papilla did not exhibit a difference between the clamped and the untouched kidney with respect to water or sodium content. The potassium content of the clamped kidney was reduced as compared to its opposite untouched kidney (5% level) and the osmolarity of the untouched kidney was reduced as compared to controls (5% level).

Although both kidneys of clamp bearing rats contained equal amounts of sodium in the papilla and inner medulla, both contained significantly less than control rats (5% level), as did similar tissue from uninephrectomized rats with a clamped kidney (5% level). The findings suggest that the reduction is not due exclusively to washout and is independent of perfusion pressure.

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