

# SODIUM AND POTASSIUM IN THE WALLS OF ARTERIOLES IN EXPERIMENTAL RENAL HYPERTENSION \*

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In previous studies (1-6) it has been determined that the aortic wall of rats with various forms of experimental hypertension contains an increased amount of sodium, potassium and water per unit of dry weight. The pertinence of these findings to hypertension has always been somewhat in question, since the aorta does not contribute much to the total peripheral resistance. In the normal circulation most of the peripheral resistance is provided by the arterioles, and hypertension is accompanied by a narrowing of these arterioles. Hence an analysis of the arterioles themselves would be expected to give more pertinent information concerning the pathogenesis of hypertension. This was attempted in the present experiment.

The present study was also designed to offset another possible cause of misinterpretation. When an animal becomes hypertensive, the walls of the arterioles become thicker; some of this thickening is probably related to an increased content of acid mucopolysaccharides, which are known to bind sodium (7). In the present experiment a large number of rats was made hypertensive and thickening of the arterioles occurred in all of them. Half the hypertensive rats were then "cured" of their elevated blood pressure by removing the clip which narrowed their renal artery. We were then able to compare the arterioles of hypertensive and normotensive rats, both of which had comparable degrees of arteriolar thickening.

Thus, the present experiment largely overcomes two weaknesses of previous studies. First, electrolytes are estimated in arterioles rather than arteries. Second, there is a similar degree of hypertrophy in both normotensive and hypertensive arterioles.

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## METHODS

A number of Wistar rats was made hypertensive by narrowing one renal artery with a clip and removing the opposite kidney. Seven months after the original operation, the rats with moderate to severe hypertension were culled out and divided into two groups which were evenly matched for arterial pressure. In one of the two groups, an operation was performed on each rat to remove the clip which narrowed the renal artery. On the rats of the other group, a similar sham operation was done but the clip was left intact. Exactly 7 days after either type of operation, the blood pressure was carefully determined on each rat with the microphonic method (8), and a number of mesenteric arterioles was removed for analysis with the aid of a stereomicroscope. The arterioles of completely normal rats of the same age were also analyzed concomitantly. The arterioles were handled in such a way that the lumen was free of blood before excision of the vessel. As little adventitia as possible was included in the tissue sample. The arterioles in the samples varied in outer diameter from about 25 to 140  $\mu$ . By measuring serial cross sections of the arterioles, it was possible to compute the fraction of the total arteriolar sample which was contributed by arteriolar segments of various sizes (Table I). Arteriolar segments with a lumen diameter varying between 24 and 84  $\mu$  contributed 92.5 per cent of the whole arteriolar sample. Maximow and Bloom consider any segment of an artery with an outer diameter of less than 300  $\mu$  as an "arteriole" (9). According to this definition, our entire sample is composed of arterioles. Vessels of this size are small enough to contribute importantly to total peripheral resistance.

The sample of arterioles from each rat was dried *in vacuo* at room temperature and then weighed on a microbalance. The dried samples were extracted for a week in Vycor vessels with 0.1 N nitric acid. At the end of this period the extract was filtered and analyzed for sodium and potassium with a flame photometer and for chloride with an amperometric method (10). Utilizing volumes of fluid and concentrations of sodium that were similar to our unknown extracts, we had an average analytical error of 0.59 per cent with 15 samples of a sodium solution and a maximal error of 1.4 per cent. With 15 samples of a potassium solution we had an average error of 0.67 per cent and a maximal analytical error of 3.0 per cent. With 15 replicates of a chloride

TABLE I

Contribution of various sizes of arteriolar segments to the total arteriolar sample

Various groups of arteriolar segments classified according to the inner diameter of the lumen	Average inner diameter of the group	Average outer diameter of the group	Percentage of the total arteriolar sample contributed by the group
$\mu$	$\mu$	$\mu$	
4-12	8	25	0.1
13-24	19	47	2.0
25-36	30	66	9.0
37-48	41	90	17.9
49-60	54	108	26.0
61-72	66	126	25.9
73-84	78	130	13.7
85-96	89	140	5.4

solution, also similar to our unknown solutions in volume and concentration, we had an average error of 2.0 per cent and a maximal error of 4.8 per cent.

During the dissection it was apparent that the arterioles of both the actively hypertensive and previously hypertensive rats were thicker than those of normal rats. The arterioles were always dissected as uniformly as possible, so that the total length of the arterioles in a given sample was about the same for each rat. Since the length of vessels in a sample was approximately constant, the total dry weight of the arteriolar sample gives a reflection of the amount of arteriolar thickening. Table II shows the average of the total dry weights of the samples for the three different groups. The samples from both the continuously hypertensive group and the previously hypertensive group weighed about 53 per cent more than those of the normal group ( $p < 0.00001$ ). However, the samples from the group with continuing hypertension had virtually the same increase in weight as the samples from the group with "cured" hypertension ( $p > 0.5$ ). Thus the arterioles of the rats with

TABLE II

Average dry weight of arteriolar samples from the various groups of rats \*

	mg
25 normal rats	$2.83 \pm 0.16$
30 rats with "cured" hypertension	$4.27 \pm 0.21$
25 rats with continuing hypertension	$4.37 \pm 0.27$

\*  $\pm$  Indicates standard error of the mean.

"cured" hypertension seemed to be just as thick as those of the rats with continuing hypertension.

## RESULTS

The results of the analyses are shown in Table III. The rats with continuing hypertension had a 16 per cent greater content of sodium in their arterioles than those with "cured" hypertension, a highly significant difference ( $p < 0.0001$ ). The hypertensive rats also had 2.6 per cent more potassium in their arterioles than the rats with "cured" hypertension. This difference was not significant. The chloride content of the arterioles in the group with continuing hypertension was 9 per cent greater than that of the group with "cured" hypertension. The  $p$  value of this difference was 0.08, and therefore of borderline significance.

The rats with "cured" hypertension had a slightly but significantly higher mean blood pressure than the completely normal rats ( $p = 0.02$ ; see Table III), hence they were probably not completely "cured." Moreover, they had a definite thickening of the arterioles (Table II). A combination of these two factors probably accounts

TABLE III

Average electrolyte levels in the arteriolar wall of various normotensive and hypertensive rats \*

	25 Normal rats (A)	p Value of the difference between A and B	30 Rats with "cured" hypertension (B)	p Value of the difference between B and C	25 Rats with continuing hypertension (C)
Na (mEq/100 g dry tissue)	$22.1 \pm 0.4$	0.03	$23.5 \pm 0.5$	0.00006	$27.3 \pm 0.7$
K (mEq/100 g dry tissue)	$21.2 \pm 0.3$	$>0.5$	$21.2 \pm 0.3$	0.3	$21.7 \pm 0.4$
Cl (mEq/100 g dry tissue)	$21.5 \pm 0.8$	0.5	$22.3 \pm 0.7$	0.08	$24.3 \pm 0.9$
Na + K (mEq/100 g dry tissue)	$43.3 \pm 0.6$	0.14	$44.6 \pm 0.7$	0.0004	$49.0 \pm 1.0$
Arterial pressure† before clip removal (mm Hg)	126 (101-140)		197 (160-236)		202 (158-250)
Arterial pressure† one week after clip removal or sham operation (mm Hg)	125 (100-141)	0.02	132 (111-152)		197 (157-245)

\*  $\pm$  Indicates the standard error of the mean.

† Mean and range.

for the increased amount of sodium and chloride in the arterioles of rats with "cured" hypertension when compared with those of completely normal rats. The potassium in the arterioles was the same for these two groups.

The concentrations of sodium, potassium and chloride in the serum are given in Table IV. These concentrations are not significantly different in any of the three groups.

#### DISCUSSION

Although the rats with continuing hypertension have more sodium in their arterioles, it is hard to be sure what fraction of it is intracellular and what fraction is extracellular. The chloride content in the arterioles of the hypertensive rats is also increased about 2 mEq per 100 g of dry arteriole. If one assumes that all of this increment of chloride is extracellular, it should be accompanied by 2.45 mEq of extracellular sodium per 100 g of dry arteriole. This calculation, allowing for Donnan factors, assumes that the concentration of interstitial sodium is 0.95 that of serum sodium and that the concentration of interstitial chloride is  $1/0.95$  that of the serum chloride (11). The actual difference in the sodium content of the arterioles from the hypertensive group compared with the "cured" group was 3.79 mEq per 100 g. If only 2.45 mEq per 100 g were extracellular, this would then leave 1.34 mEq per 100 g that is intracellular. Hence, with these assumptions concerning chloride, the hypertensive arteriole would have a definite increase of intracellular sodium. Furthermore, one has no real basis for assuming that the increment of chloride in the hypertensive arterioles is indeed all extracellular. Such an assumption might be on somewhat firmer ground with regard to skeletal muscle. However, a recent investigation of the smooth muscle cells in the taenia coli of guinea pigs indicates that there are 74 mEq of chloride and 73 mEq of sodium per L intracellular water (12). The smooth muscle cells in the walls of arterioles may also normally contain an appreciable amount of intracellular chloride and may contain even more in the presence of hypertension. Hence, the data presented here would indicate that at least some of the increment of sodium in the hypertensive arterioles is located intracellularly, and it is possible that almost all of it is intracellular.

It is of interest to compare the present data

TABLE IV  
*Mean concentrations of sodium, potassium and chloride in the serum of various normotensive and hypertensive rats\**

	25 Normal rats	30 Rats with "cured" hypertension	25 Rats with continuing hypertension
Sodium	142.4	142.5	142.7
Potassium	3.6	3.7	3.7
Chloride	104.8	104.8	105.1

\* Concentrations are in mEq/L. The p value of the differences between the groups was in every case greater than 0.3.

with those previously obtained using the wall of the aorta. In several forms of experimental hypertension, both sodium and potassium were significantly elevated in the aortic wall (2-6). In the present study the sodium content was significantly increased but potassium was not. In the previous studies on the rat aorta, the chloride content was either not increased at all or increased only slightly and to a lesser degree than the increase in sodium (2-6). The present results would agree better with the latter pattern of change.

Recent studies have indicated that intracellular osmolality is usually similar to that in the extracellular compartment (13). In previous studies with the wall of the rat aorta or the human renal artery, the total cation content (Na + K) strongly correlated with the total water content (1, 3). In the present study (Table III) the total cation content (Na + K) is 10 per cent greater in the arterioles from hypertensive rats compared with the rats with "cured" hypertension ( $p = 0.0004$ ). The total cation content is 13 per cent greater in the hypertensive arterioles compared with the arterioles from completely normal rats ( $p < 0.00001$ ). If the total cation content has its usual relationship to tissue water in hypertensive arterioles, the data would suggest that the water content per unit of solids is about 10 per cent greater in these hypertensive arterioles than in the normotensive ones. However, such an assumption cannot be made with absolute safety. If, indeed, there were an increase of water in relation to solids in the walls of the hypertensive arterioles, the extra water could encroach somewhat on the lumen and increase resistance to flow (1). Further speculations concerning the cations and water in arterial wall have been recently considered elsewhere (14).

We allowed our rats with "cured" hypertension only 7 days to achieve their cure. Admittedly, their blood pressure was almost down to normal by this time, but it was slightly elevated in about 25 per cent of the rats with "cured" hypertension. Moreover, Floyer has shown that rats with a clip removed from the renal artery retain some evidence of their hypertensive diathesis for 1 to 2 weeks, even though their blood pressure comes down to near normal in a few hours (15). If the lone remaining kidney in his rats was excised 1 to 2 weeks after the clip had been detached from its renal artery, these rats developed renoprival hypertension at a decidedly accelerated rate, indicating that some hypertensive trait lingered on. In the present study, we are comparing hypertensive rats with rats whose hypertensive diathesis is only partially "cured." The difference in electrolyte content might have been even greater if the comparison had been made with rats that were given enough time after removal of the clip to achieve a complete "cure." This lack of a complete cure in the present experiment may partially account for the differences between the rats with "cured" hypertension and the rats that were completely normal.

#### SUMMARY

Fifty-five rats were made hypertensive by narrowing the renal artery of their one remaining kidney. Seven months later these hypertensive rats were divided into two evenly matched groups. The rats in one group were "cured" of their hypertension by releasing the arterial constriction. The rats in the other group had a comparable sham operation. One week after either procedure, a sample of arteriolar tissue from vessels of 0.025 to 0.14 mm outer diameter was obtained. Arterioles from the rats with continuing hypertension had 16 per cent more sodium per unit of dry weight than those from the rats "cured" of hypertension ( $p < 0.0001$ ). The potassium in the arteriolar wall was similar in the two groups and a difference in chloride content was of borderline significance ( $p = 0.08$ ). At least part of the increment of sodium in the hypertensive arterioles appears to be located intracellularly. A 10 per cent increase in the total cation content (Na + K) in the hypertensive arterioles ( $p = 0.0004$ ) may indicate that these vessels have about 10 per cent more water per unit of dry weight than the ar-

terioles from the rats with "cured" hypertension. The arterioles from the rats with either continuing or "cured" hypertension had undergone about the same degree of thickening; hence the difference in sodium content is probably not related to this. The extra sodium in a hypertensive arteriole may contribute to its narrowed lumen.

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