The Effect of Hydrochlorothiazide on Water, Cation and Norepinephrine Content of Cardiovascular Tissues of Normotensive Dogs[1] (37474)

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The thiazides have been regarded as basic antihypertensive drugs suitable for chronic oral administration (1–3). The mechanism by which these drugs lower blood pressure is a matter of controversy. Reduction in intravascular volume due to their diuretic action in the initial stage of treatment has been proposed by Freis *et al.* (4). Winer (5) and Tarazi, Dustan and Frohlich (6) have reported similar findings but also continuation of the reduced intravascular volume after prolonged treatment, this however has not been confirmed by Conway and Lauwers (7), and Gifford *et al.* (8).

The studies of Freed, St. George and Rosenman (9) and Tobian (10) have indicated that hypertensive subjects or experimentally induced hypertensive animals have increased water, sodium, potassium and calcium content in their arterial tissues. Since thiazides are known as natriuretics, various groups of investigators after considering the above results have studied the possibility of reducing the arterial tissue water and cation content in normotensive or hypertensive animals by administration of thiazides (11–15).

Thiazides have also been examined for their possible effect on the content of the sympathetic neurotransmitters alone in arterial and myocardial tissues (16–19), but the findings reported were not in agreement.

In the present experiments, normotensive dogs received hydrochlorothiazide for a period of 45 days. At the end of this period, water, cation and norepinephrine content of arterial and myocardial tissues were analyzed in order to clarify the effects of this agent on the vascular tissues of normotensive dogs.

Materials and Methods. Fifteen normotensive adult male mongrel dogs, weighing between 18.7 and 23.6 kg, were used in this experiment. They were kept individually in metabolic cages, and fed Purina Dog Chow supplemented with Dr. Ballard's dog food and had access to tap water *ad libitum*. Six received oral hydrochlorothiazide 3.75 mg/kg/day, in two divided doses, for 45 days, and the remaining others served as control.

Direct femoral blood pressure was measured under light anesthesia with intravenous thiopental (25 mg/kg) using a Grass polygraph every week during the 2 wk control period and during the experiment. Blood volume and hematocrit were measured before and on Day 39 of treatment, 16 to 20 hr after the administration of hydrochlorothiazide. Blood volume was measured with RISA using a 10-min equilibration period on Picker Hemoliter. The results were expressed as milliliters of blood volume per kilogram of body weight. Plasma volume was calculated from the blood volume and hematocrit by the following formula:

$$\text{Plasma vol (ml/kg)} = \frac{\text{Blood vol (ml/kg)} \times (100-\text{HCT})}{100}$$

Plasma cations (Na, K, Mg and Ca) were measured during the control period and Days 14, 20 and 41 of treatment. Sodium and potassium were measured by emission spectrophotometry (Unicam SP 900) using lithium nitrate as internal standard, while the magnesium and calcium were determined by atomic absorption spectrophotometry (Perkin-Elmer 303) using lanthanum chloride

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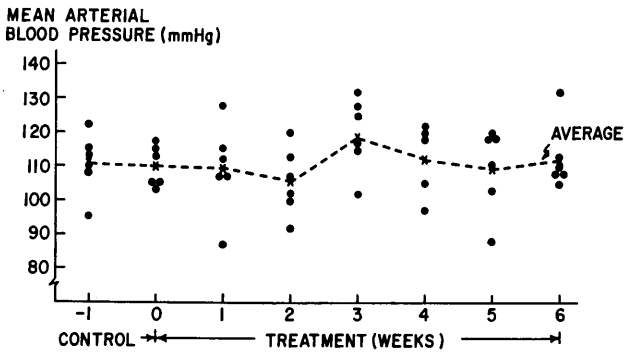

FIG. 1. Effect of hydrochlorothiazide administration on mean arterial blood pressure of normotensive dogs.

as inhibitor of the interference of phosphates.

On the last day of the treatment period, 20 hr after the last administration of hydrochlorothiazide, the animals were anesthetized with pentobarbital (30 mg/kg), and arterial (branches of the mesenteric arteries, carotid artery, and thoracic aorta) and left myocardial tissues were taken for water, sodium, potassium, magnesium, calcium and norepinephrine content determination. Renal juxtaglomerular cell granularity was measured in the control dogs and in four out of the six treated dogs according to the juxtaglomerular index described by Hartroft and Hartroft (20).

The methods used for tissue water and cation determinations have recently been described by us (21). The tissue norepinephrine was determined by spectrophotofluorometry (Perkin-Elmer 203) and applying the method of Anton and Sayre (22) with modifications (23).

Results. Blood pressure. Mean arterial blood pressure during hydrochlorothiazide treatment when compared to that obtained during control period remained unchanged (Fig. 1).

Blood volume, hematocrit and plasma volume. The mean values of the blood volume, hematocrit and plasma volume of the day before and at the end of treatment are shown in Table I. Blood volume was decreased by 4.1% and plasma volume decreased by 9.6%, however, these changes were not significant. Hematocrit increased by 5.5% but again this change was not significant.

Plasma cations. Table II shows the plasma cation determinations obtained before and during treatment. The major changes consisted of a significant ($p < 0.05$ to $p < 0.01$) decrease in plasma potassium and magnesium during the experiment. Sodium on Day 14 after treatment was at the lowest level than any other time, but this decrease was not significant. Calcium increased slightly (6%).

Cardiovascular tissue water and cations. Table III shows the mean ± SE values of the water and cation content of the control and treated animals. The difference between the two means is expressed as percentage change from the mean of the control value. Water decreased significantly in all the tissues examined, branches of mesenteric arteries

TABLE I. Effect of Hydrochlorothiazide on Blood Volume, Hematocrit and Plasma Volume of Normotensive Dogs.[a]

	Before treatment	Day 39 of treatment	Change (%)
Blood vol (ml/kg)	87.2 ± 2.7	83.6 ± 5.6	−4.1% ns[b]
HCT (%)	45.3 ± 1.1	47.8 ± 1.1	+5.5% ns
Plasma vol (ml/kg)	48.0 ± 1.9	43.4 ± 2.2	−9.6% ns

[a] Each value is the mean of 5 dogs ± standard error.

[b] Not significant.

TABLE II. Effect of Hydrochlorothiazide Administration on Plasma Cations of Normotensive Dogs.[a]

Cations (mEq/liter)	Before treatment	Treatment period (day) 14	20	41
Sodium	143.6 ± 2.8	139.5 ± 1.7 (— 3%)	143.6 ± 1.1 (0%)	144.9 ± 0.9 (+ 1%)
Potassium	5.1 ± 0.2	4.5 ± 0.2 (—12%)[c]	4.4 ± 0.1 (—14%)[b]	3.7 ± 0.1 (—27%)[c]
Magnesium	1.80 ± 0.05	1.64 ± 0.06 (— 9%)[c]	1.68 ± 0.02 (— 7%)[c]	1.45 ± 0.04 (—19%)[c]
Calcium	5.2 ± 0.2	5.4 ± 0.1 (+ 4%)	5.3 ± 0.2 (+ 2%)	5.5 ± 0.2 (+ 6%)

[a] Each value is the mean of six dogs ± standard error. Values in parentheses show the percentage difference from the values before treatment.
[b] $p < 0.05$, [c] $p < 0.01$ by paired t test.

by 3.3% ($p < 0.005$), carotid artery by 2.2% ($p < 0.001$), thoracic aorta by 2.6% ($p < 0.005$), and left myocardium by 1.1% ($p < 0.05$). Potassium content was decreased significantly in branches of mesenteric arteries by 17% ($p < 0.02$), carotid artery by 22% ($p < 0.005$), and thoracic aorta by 24% ($p < 0.001$), however, in the myocardium

TABLE III. Cardiovascular Tissue Water, Cations, and Norepinephrine of Normotensive Dogs Treated with Hydrochlorothiazide (3.75 mg/kg/day po for 6 wk) Compared with Control, Untreated Normotensive Dogs.[a]

	Group	Branches of mesenteric art.	Carotid artery	Thoracic aorta	Left myocardium
Water	Control	71.3 ± 0.4	70.0 ± 0.2	72.5 ± 0.3	79.6 ± 0.3
	Treated	69.0 ± 0.7	68.5 ± 0.2	70.6 ± 0.4	78.7 ± 0.3
	% change	—3.3[c]	—2.2[c]	—2.6[c]	—1.1[b]
Sodium	Control	323.8 ± 7.0	312.3 ± 13.1	286.5 ± 7.5	218.1 ± 9.8
	Treated	353.4 ± 14.2	303.7 ± 16.1	278.7 ± 15.3	206.9 ± 14.0
	% change	+9	—3	—3	—5
Potassium	Control	157.4 ± 7.1	124.8 ± 4.6	154.5 ± 4.6	297.1 ± 13.3
	Treated	130.9 ± 5.5	96.9 ± 7.0	116.9 ± 4.5	310.0 ± 13.0
	% change	—17[b]	—22[c]	—24[c]	+4
Magnesium	Control	31.0 ± 0.7	22.9 ± 0.6	25.0 ± 0.5	79.1 ± 2.4
	Treated	29.0 ± 1.3	23.0 ± 0.6	23.4 ± 0.7	84.1 ± 3.1
	% change	—7	+0.3	—7	+6
Calcium	Control	21.0 ± 0.8	17.9 ± 0.8	21.3 ± 0.4	8.8 ± 0.6
	Treated	20.9 ± 0.5	19.5 ± 1.1	21.5 ± 1.0	10.6 ± 0.5
	% change	—0.3	+9	+0.7	+22[b]
Norepinephrine	Control	7.18 ± 0.69	1.98 ± 0.16	1.56 ± 0.20	0.47 ± 0.04
	Treated	4.42 ± 0.25	1.15 ± 0.07	1.21 ± 0.10	0.38 ± 0.07
	% change	—38[c]	—42[c]	—22	—19

[a] Values are the means ± standard error. Control $N = 9$; Treated $N = 6$. Water is expressed as % wet weight. Cations are expressed as μEq/g dry weight. Norepinephrine is expressed as μg/g wet weight.
[b] $p < 0.05$.
[c] $p < 0.01$.

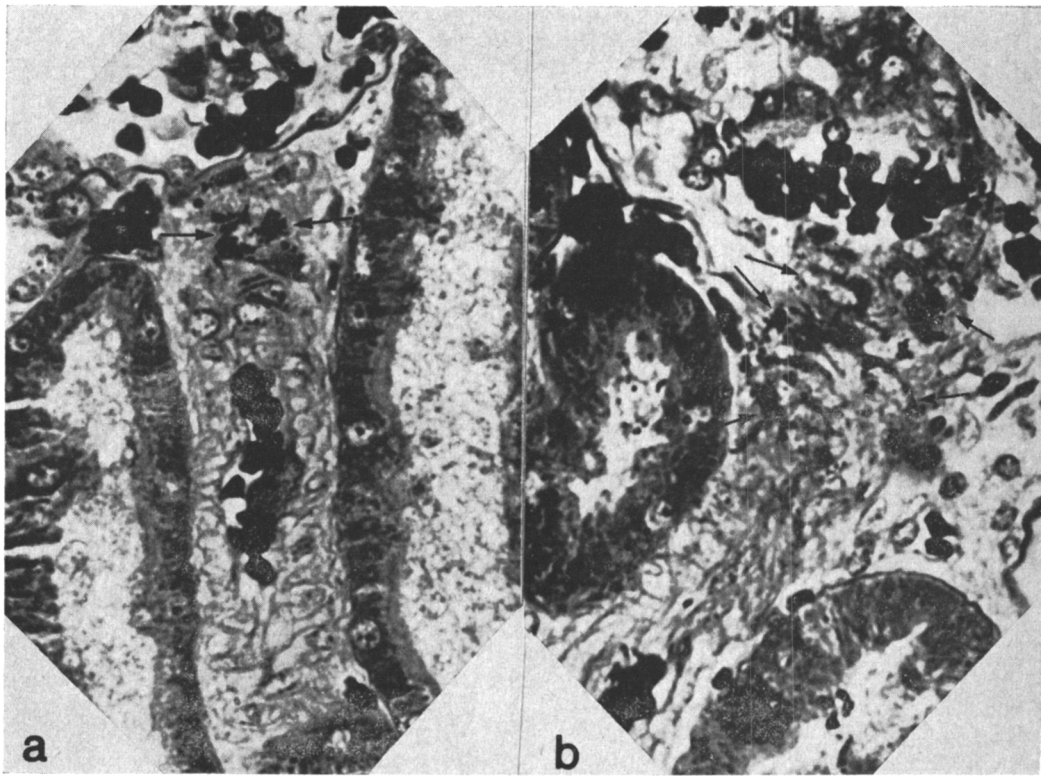

FIG. 2. The afferent arterioles, oblique sections, showing juxtaglomerular granulated cells (arrows) in (a) a control dog, and (b) a dog treated with hydrochlorothiazide. Ethylviolet, Biecbrich Scarlet-Orange G method (×700).

it was increased by 4%, but this change was not significant. Sodium, magnesium and calcium did not change significantly in any of the tissues examined except in the myocardium where the calcium was significantly increased by 22% ($p < 0.05$).

Cardiovascular tissue norepinephrine. Norepinephrine decreased in all the tissues analyzed but only significantly in the branches of mesenteric arteries by 38% ($p < 0.01$) and carotid artery by 42% ($p < 0.005$) (Table III).

Juxtaglomerular index (JGI). The mean JGI ± SE in four of the six treated dogs examined was 32.0 ± 5.6, and this value was significantly higher ($p < 0.005$) than in control dogs, which was 11.5 ± 1.0 (Fig. 2).

Discussion. In the present study it was shown that hydrochlorothiazide (3.75 mg/kg/day) given orally to normotensive dogs over a period of 6 wk did not have any significant effect on blood pressure, blood and plasma volumes, and hematocrit. Similar findings regarding blood pressure were reported by Freis *et al.* (4, 24) and Hollander, Chobanian and Wilkins (25) in normotensive subjects receiving chlorothiazide. In the studies of Freis *et al.* (24) the plasma volume, extrapolated from the values of the hematocrit, was decreased. However, these results could be due to the shorter duration of treatment (6 days) used than in the present study.

It is possible that this small although not significant decrease of plasma volume reported here, could be reflected in the increased juxtaglomerular index. However, other possibilities, such as the natriuresis and/or the potassium loss could be of importance in order to explain the increased JGI in hydrochlorothiazide-treated dogs. Such hypergranularity of the juxtaglomerular cells may

well support an increase in renin secretion. This suggestion is supported by the findings of an increase in plasma renin activity and juxtaglomerular index in normotensive rats treated with hydrochlorothiazide and sodium restriction (26).

Our findings of plasma cations are in agreement with those reported by others. The slight decrease in plasma sodium reported here in the first 2 wk of treatment is a well-known phenomenon (1, 27).

Although the significant and persistent decrease in plasma potassium reported here is a finding that has been reported many times (2, 27), the significant and persistent decrease in plasma magnesium has been reported, to our knowledge, only by Smith, Kyriakopoulos and Hammarsten (28) and Seller *et al.* (29).

The unchanged sodium content in the arterial tissues is in agreement with other studies (11, 14). Arterial tissue magnesium and calcium have not been studied in relation to the mechanism of action of thiazides except the study of Zsotér, Hart and Radde (14) who have reported no change in calcium content in iliac artery of dogs treated with hydrochlorothiazide for 6 to 8 wk. Our results did show the same trend in the arteries examined.

The most striking results in our study were the significant decrease in the water and potassium content of arterial tissues. This is in contrast to the previous studies done by Tobian *et al.* (11), Weller and Haight (13) and Zsotér, Hart and Radde (14). However, simultaneously with these changes in water and potassium contents there was a marked decrease in the norepinephrine content, significantly in carotid and mesenteric arteries. This may be of importance despite the fact that the blood pressure remained unchanged during the treatment. It will not be possible to extrapolate these changes observed in normotensive dogs to the explanation of the mechanism of the antihypertensive effect of thiazide drugs until a similar study is carried in dogs with experimental renal hypertension. Since thiazide diuretics given acutely or chronically have been reported to depress the vascular response to norepinephrine (14, 30, 31), it can be speculated that this change could be the consequence of the decrease of water and potassium content.

Summary. Hydrochlorothiazide 3.75 mg/kg/day given orally to six normotensive dogs for 45 days had no significant effect on blood pressure, blood and plasma volumes, and hematocrit. Significant decrease in plasma magnesium and potassium persisted throughout the treatment. There was no significant change in plasma sodium and calcium. Of the four cations (Na, K, Mg and Ca) analyzed only potassium was significantly decreased in the arterial tissues examined (mesenteric arteries, carotid artery and thoracic aorta). Calcium was significantly increased only in the left myocardium. These changes were accompanied by significant decrease in the water content of the same tissues. Norepinephrine content was also decreased in all these tissues, but significantly only in the mesenteric arteries and in the carotid artery. The juxtaglomerular index in four dogs examined was significantly increased.

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