Effect of Chlorothiazide on Blood Pressure and Electrolytes of Normotensive and Hypertensive Rats.* (28178)

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When chlorothiazide is given to hypertensive patients there is an initial increase in urine volume and sodium excretion, fall in body weight, reduction in plasma volume and decline in blood pressure(1,2). With prolonged administration, plasma and extracellular fluid volumes, as well as exchangeable sodium, return to normal(3). The following studies were carried out to determine the effect of chlorothiazide on electrolyte and water distribution in plasma and tissues of normotensive (Experiment I) and hypertensive rats (Experiments II, III and IV).

Methods. Male Sprague-Dawley rats were used. In Experiment I, 11 rats were fed for 6 weeks 50 mg chlorothiazide/kg body weight/day mixed with ground Purina Chow, then sacrificed along with 9 control animals not given chlorothiazide.

In Experiment II, rats weighing 104-152 g had figure-of-eight ligatures applied to one kidney, followed in 12 days by contralateral nephrectomies. Four weeks later 27 animals had mean blood pressures greater than 140 mm Hg. Eighteen were given 50 mg chlorothiazide† /kg body weight/day in drinking water for 16 days. Nine received tap water to drink. After removal of drug for 12 days, a second study was done to determine if blood pressures would respond similarly at a later stage and to ascertain the effect upon blood pressure of the chlorothiazide vehicle. Twelve rats received chlorothiazide (100 mg/kg body weight/day) plus vehicle in drinking water for 14 days, then 150 mg/kg body weight/day for 3 days. Eight received only chlorothiazide vehicle (50 mg mannitol and 0.08 mg thimerosal/kg body weight/day) in drinking water for 17 days. All were then sacrificed.

In Experiment III, 13 rats weighing 95-100 g had figure-of-eight ligatures applied to the left kidney and 2 weeks later contralateral nephrectomies. Ten weeks later 7 animals were given for 11 days 10 mg chlorothiazide/kg body weight/day and 6 a placebo tablet, each mixed with ground Purina Chow. All were sacrificed on the twelfth day.

In Experiment IV, rats weighing 95-100 g underwent figure-of-eight ligation of the left kidney, followed in 11 days by contralateral nephrectomy. Four weeks later 2 groups, each containing 10 hypertensive rats with similar systolic blood pressures, were given for 9 days, respectively, 10 mg hydrochlorothiazide/kg body weight/day in drinking water and an equal volume of placebo vehicle solution.‡ Both groups were then given tap water to drink for 12 days. Because of the death of 2 animals, 2 rats from the drug group were exchanged with one from the placebo group to equalize mean systolic blood pressures of treatment and control groups, each now containing 9 rats. They were then given hydrochlorothiazide and placebo solution as previously for 21 days, then tap water for 12 days, then drug and placebo for 13 days. At time of sacrifice there were 7 animals in the drug group and 8 in the placebo group.

Blood pressures, determined under light ether anesthesia(5), and body weights were recorded. All animals were sacrificed by a blow on the head and exsanguinated *via* abdominal aorta. Thigh muscle and thoracic and abdominal aorta were removed, dissected free of fat and connective tissue and analyzed by the method of Lowry and Hastings, except that sodium and potassium were determined by flame photometry(6). Calculations of extracellular and cellular water were based upon extracellular location of chloride.

In Exp. I, carcasses were placed in covered beakers and dried at 105°C for 5 to 7

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days. Water contents were derived as difference in weight. Dry carcasses were ground with mortar and pestle until they could be sifted through No. 16 mesh. Duplicate aliquots underwent 2 cold petrol ether extractions. Difference in dry weight before and after extraction was recorded as neutral fat content. Decanted ether was returned to each vessel and evaporated. Tissue electrolytes were extracted with 0.75 N HNO_3 for 24 hours. Because of interference with analysis of sodium, calcium was extracted by a modification of Bergstrom's technique(7). Sodium and potassium were determined by flame photometry.

Results. Plasma and tissue electrolytes of normotensive rats in Exp. I are shown in Table I. Total water content was significantly higher in skeletal muscle of animals on chlorothiazide. Calculations based on extracellular location of chloride show that this was due to increase in cell water. Heart muscle sodium and potassium rose in chlorothiazide rats. Sodium and potassium content of aortas of chlorothiazide rats in Exp. I also increased. Whole body sodium and potassium contents were unaltered.

In Exp. II by 13 weeks all 26 surviving rats had mean blood pressures greater than 140 mm Hg. Blood pressure and body weight data are presented in Fig. 1. Both periods of chlorothiazide administration were accompanied by decreases in blood pressure. During the first, 10 of 12 rats receiving drug had pressure decreases, the mean maximal decrease being 29 mm Hg. In the second period, 7 rats had pressure decreases averaging 28 mm Hg, while 5 had increases averaging 25 mm Hg. Four of 7 having decreases had further decreases after chlorothiazide was increased to 150 mg/kg body weight/day. Two of 5 having increases of blood pressure had decreases when the dose was increased. At

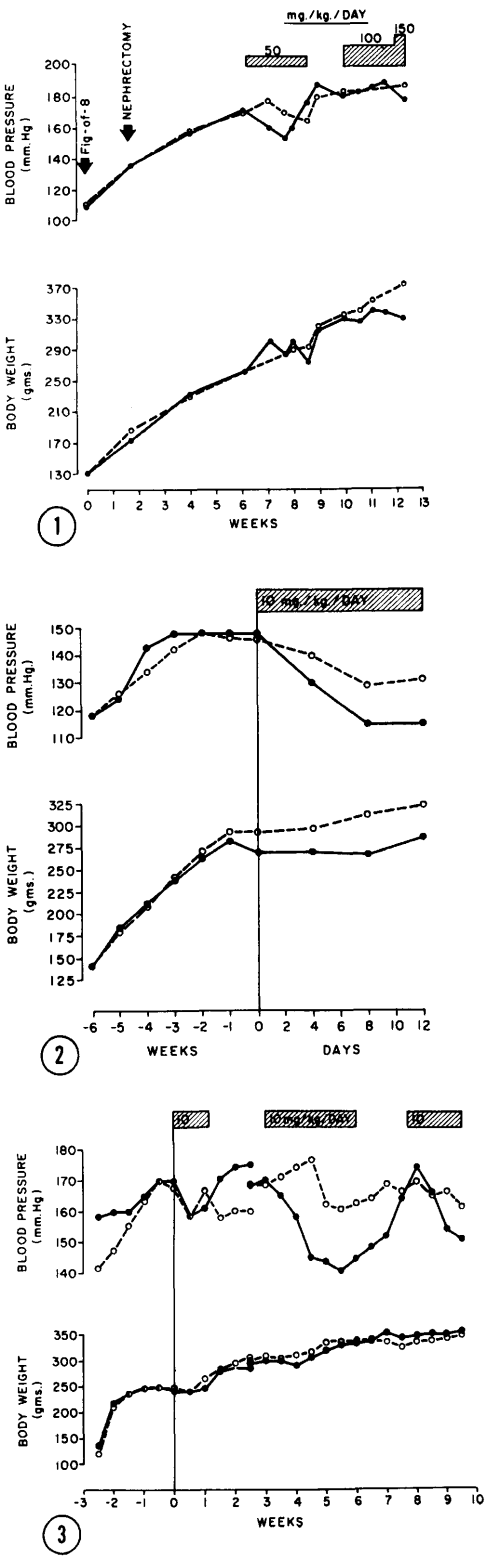

FIG. 1. Mean systolic blood pressures (above) and body weights (below) of rats in Exp II. ● = rats given chlorothiazide and ○ = control group.

FIG. 2. Mean systolic blood pressures (above) and body weights (below) of rats in Exp III. ● = rats given chlorothiazide and ○ = control group.

FIG. 3. Mean systolic blood pressures (above) and body weights (below) of rats in Exp IV. ● = rats given hydrochlorothiazide and ○ = control group.

sacrifice there was no significant difference between blood pressures of treated and untreated groups. In the second drug period half of rats having decreases in blood pressure had decreases in body weight; body weight of other rats increased. Electrolyte values of

TABLE I. Plasma and Tissue Electrolyte Values.

Exp No.	Group	Plasma Na (meq/l)	Plasma K (meq/l)	Plasma Cl (meq/l)	Skeletal muscle Na (meq/kg)	Skeletal muscle K (meq/kg)	Skeletal muscle Cl (meq/kg)	Skeletal muscle H_2O g/kg
I	Chlorothiazide							
	Mean	144.3	5.24	106.9	42.8	102.6	9.9	785
	± SE of mean	1.54	.43	1.99	2.5	2.3	.3	4.6
	No. of rats	10	9	10	11	11	11	11
	Control							
	Mean	139.3	6.1	107.3	40.0	110.6	10.3	762
	± SE of mean	2.85	.54	1.2	5.1	5.1	.3	6.0
	No. of rats	9	7	8	9	9	9	8
	P between	.2–.1	.3–.2	.9–.8	.7–.6	.2–.1	.2–.3	.01–.001
II	Chlorothiazide							
	Mean	150		104.2	27.8	114.4	12.9	770
	± SE of mean	.8		1.0	.8	1.4	.6	2.4
	No. of rats	9		9	12	12	12	12
	Control							
	Mean	152		107.0	29.4	112.3	12.4	769
	± SE of mean	3.6		1.8	1.0	1.9	.4	1.1
	No. of rats	6		8	8	8	8	8
	P between	.6–.5		.2–.1	.3–.2	.4–.3	.6–.5	.9–.8
III	Chlorothiazide							
	Mean	147.2	4.2	96.5	21.6	116.9	10.9	763
	± SE of mean	2.5	.01	1.5	.5	.9	.3	.4
	No. of rats	7	7	7	7	7	7	7
	Control							
	Mean	148.0	5.4	100.8	23.2	120.1	11.4	762
	± SE of mean	1.2	.2	.8	1.3	1.4	.4	.8
	No. of rats	6	6	6	6	3	6	6
	P between	.8–.7	<.001	.05–.02	.3–.2	.1–.05	.4–.3	.3–.2
IV	Hydrochlorothiazide							
	Mean	145.7	4.6	99.7	23.1	109.2	11.5	763
	± SE of mean	.9	.2	.8	1.2	1.9	.3	2.2
	No. of rats	8	8	8	8	8	8	8
	Control							
	Mean	144.8	4.7	101.5	22.4	109.7	11.2	782
	± SE of mean	1.1	.2	1.0	.9	2.4	.5	5.1
	No. of rats	7	6	7	7	7	7	7
	P between	.6–.5	.8–.7	.2–.1	.8–.7	.9–.8	.8–.7	.01–.001

Exp No.	Group	Heart muscle Na (meq/kg)	Heart muscle K (meq/kg)	Heart muscle Cl (meq/kg)	Heart muscle H_2O g/kg	Aorta Na (meq/kg)	Aorta K (meq/kg)	Aorta Cl (meq/kg)	Aorta H_2O g/kg	Whole rat Na (meq/kg)	Whole rat K (meq/kg)	Whole rat H_2O g/kg
I	Chlorothiazide											
	Mean	72.2	98.9	26.7	772	130.6	55.1	63.4	617	69.45	80.0	
	± SE of mean	2.1	4.2	.6	5.2	5.9	.65	1.2	1.5	2.03	1.53	
	No. of rats	11	11	11	11	11	11	11	11	11	11	
	Control											
	Mean	58.7	80.0	26.5	771	110.9	47.8	66.6	632	70.10	81.11	
	± SE of mean	2.7	1.5	.4	2.8	4.2	2.7	2.8	8.1	3.52	3.92	
	No. of rats	9	9	9	9	9	9	9	9	9	9	
	P between	<.001	<.001	.7–.8	.9–.8	.02–.01	.02–.01	.2–.1	.3–.2	.8–.7	.9–.8	

TABLE I (continued)

Exp No.	Group	Heart muscle Na (meq/kg)	Heart muscle K (meq/kg)	Heart muscle Cl (meq/kg)	Heart muscle H_2O g/kg	Aorta Na (meq/kg)	Aorta K (meq/kg)	Aorta Cl (meq/kg)	Aorta H_2O g/kg	Whole rat Na (meq/kg)	Whole rat K (meq/kg)	Whole rat H_2O g/kg
II	Chlorothiazide											
	Mean					123.6	42.8		693			
	± SE of mean					3.7	1.3		6.0			
	No. of rats					12	12		12			
	Control											
	Mean					122.6	46.0		776			
	± SE of mean					3.4	1.2		6.0			
	No. of rats					8	8		8			
	P between					.9–.8	.1–.05		<.001			
III	Chlorothiazide											
	Mean	43.2	86.0	26.2	768	102.0	46.5	65.6	636			
	± SE of mean	.9	1.4	.3	2.6	1.9	1.1	1.1	9.1			
	No. of rats	7	7	7	7	7	7	7	7			
	Control											
	Mean	47.3	86.7	28.4	774	109.7	53.8	71.8	612			
	± SE of mean	1.8	2.2	.5	1.5	2.7	3.4	2.9	9.2			
	No. of rats	6	3	6	6	3	3	6	6			
	P between	.1–.05	.8–.7	.01–.001	.1–.05	.1–.05	.05–.02	.1–.05	.1–.05			
IV	Hydrochlorothiazide											
	Mean	42.6	82.8	28.1	760	105.8	41.0	70.3	580			
	± SE of mean	.5	.9	.4	2.8	1.2	.9	1.8	12.3			
	No. of rats	8	8	8	8	8	8	8	8			
	Control											
	Mean	42.7	83.1	28.4	768	110.0	41.6	64.8	583			
	± SE of mean	.3	.7	.4	3.3	2.3	.9	1.5	10.5			
	No. of rats	7	7	7	7	7	7	7	7			
	P between	.9–.8	.9–.8	.6–.5	.1–.05	.2–.1	.8–.7	.05–.02	.9–.8			

Tissue electrolyte values based on fat-free wet wt, except whole rat Na and K on basis of fat-free dry wt.

plasma and tissues of rats in Exp. II are shown in Table I. No statistically significant differences were present in animals receiving drug as compared to control animals except for aorta water content. This value was abnormally high in the control group, as compared to control rats in Experiments III and IV ($p<0.001$) and is felt to reflect a difference in technique in stripping aorta of adventitia.

As shown in Fig. 2, mean systolic blood pressure of rats in Exp. III receiving chlorothiazide fell from 148 to 115 mm Hg, or 16 mm Hg lower than that of control animals ($0.02<p<0.05$). Lowering of mean pressure in the control group appears to have been the natural course of this experimental renal hypertension. Mean weight of the control group was greater than that of those receiving drug during this 12 day period. Electrolyte values are shown in Table I. In the chlorothiazide group potassium was significantly lower in plasma and aorta. Chloride content of plasma and heart muscle also was reduced.

Systolic blood pressure and body weights of animals in Exp. IV are shown in Fig. 3. During the second and third periods of hydrochlorothiazide administration, mean blood pressures of animals on drug were 10 to 20 mm Hg lower than those of control rats; however the difference was not statistically significant at time of sacrifice. Mean body weights of the 2 groups were not greatly different. Electrolyte values are given in Table I. No significant alterations in sodium or potassium contents of plasma or tissues were found, but skeletal muscle water content was lower in animals on hydrochlorothiazide.

Discussion. Several investigators have observed the short-term effects of thiazides. Chlorothiazide does not cause acute altera-

tions in plasma electrolytes in nephrectomized animals(8). Electrolytes of normotensive rats given chlorothiazide for 4½ days were studied by Beavers(9). A decrease in sodium and potassium was found in aorta, but not in skeletal muscle. In nephrectomized hypertensive and non-hypertensive dogs Orbison found no effect of chlorothiazide on electrolyte concentrations in serum, skeletal or heart muscle, or aorta at 3 or 6 days, nor was hypertension ameliorated(10). Chlorothiazide appears to have no direct effect on tissue electrolytes. That acute diuresis and natriuresis is not necessary for vasodepressor action was shown by Armstrong who found that rats made hypertensive by drinking 2% sodium chloride solution showed decreases in blood pressure, urine volume and sodium excretion over a 3 day period when given chlorothiazide, while control animals showed increases in urine volume and sodium excretion(11).

The long-term antihypertensive action of thiazides also does not appear related to initial natriuresis. With continued administration of chlorothiazide to hypertensive patients exchangeable body sodium returns to normal (3). Similarly, oral administration of chlorothiazide to animals for 5 months results in no diminution in total body exchangeable sodium(12). Tobian *et al.*, found no change in sodium content of aorta, mesenteric arterioles, heart, or kidney of normotensive rats given chlorothiazide for 9 weeks(13). Skeletal muscle actually contained more sodium. In our experiments there is similarly no consistent deviation in tissue sodium.

If alterations in sodium content do not correlate with blood pressure-lowering effect accompanying prolonged administration of thiazides, it is possible that changes in potassium might. Freed has stressed the correlation between arterial potassium and level of blood pressure as well as importance of the Na:K ratio of arterial tissue(14). However, exchangeable body potassium, although reduced in the first 2 weeks of treatment with thiazides, subsequently is repleted(15,16). Tobian *et al.*(13) found no change in potassium content of aorta, mesenteric arterioles, heart, or kidney of rats given chlorothiazide for 9 weeks, although skeletal muscle potassium was lower. In our normotensive animals given chlorothiazide for 6 weeks heart and aorta potassium increased. In hypertensive rats no consistent changes were evident in tissue potassium of those given thiazide. Only in Exp. III were alterations found, consisting of lowering of plasma and aorta potassium.

Changes in water distribution due to chlorothiazide administration have also been reported. In hypertensive patients treated with chlorothiazide for 3 or more weeks, body weight falls, total body water declines, but extracellular fluid volume remains normal, suggesting depletion of intracellular water(3, 17). However, in chlorothiazide treated rats there was no change in water content of tissues(13). We likewise found no consistent deviations in water content. In normal rats given chlorothiazide, total water was increased in skeletal muscle, due to calculated intracellular water being increased. In hypertensive rats only in Exp. IV was skeletal muscle water altered, being decreased in the hydrochlorothiazide group.

The mechanism of the antihypertensive action resulting from prolonged administration of thiazide drugs remains unknown. The acute diuresis, natriuresis and kaliuresis occurring in the first days of treatment with thiazides apparently is subsequently corrected for by the kidney, so that changes in tissue electrolytes do not occur.

Summary. Chlorothiazide was given to normal rats for 6 weeks and to renal hypertensive rats for 11 to 17 days. Lowering of elevated blood pressure by this agent was variable in duration and degree. Plasma, skeletal and heart muscle, aorta, and in one experiment the whole animal, were analyzed for sodium, potassium, chloride and water. No consistent alterations were found in electrolyte or water contents of plasma or tissues of normotensive or hypertensive rats given thiazide drugs.

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Antigenic Relationship of Streptococcal Diphosphopyridine Nucleotidases.* (28179)

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Antibodies capable of specifically inhibiting the enzymatic activity of streptococcal diphosphopyridine nucleotidase (DPNase) are found in the sera of humans and experimental animals(1,2). These occur with other streptococcal antibodies and increase in the serum in response to streptococcal infection(1). Bernhard and Stollerman(2) suggest that the anti-streptococcal DPNase antibody (ASDA) titration be used in conjunction with anti-streptolysin O titrations to increase the accuracy of diagnosis of antecedent streptococcal infections. These observations imply an antigenic identity of streptococcal DPNase regardless of source. This investigation tests the validity of the assay by determining the antigenic relationship of a number of DPNases from various groups and types of hemolytic streptococci.

Materials and methods. The following strains of hemolytic streptococci were employed in this study: *Streptococcus pyogenes* Group A, types 4-366, 4-3799, 12, 12-350, 12-H8, 12-Witcher, 14, 19, and 28; Group C strains C74, H46a, and 2927; and Group G strain 2986. The medium was that of Todd and Hewitt(3). On occasion, streptococcal lesion extract (SLE) was used as DPNase source prepared by the method of Schwab, Watson, and Cromartie(4). DPNase activity was determined by the method of Carlson, *et al.*(5) using the supernatant fluids from 24-hour cultures which had been centrifuged at 3,000 rev/min for 15 minutes. The unit of DPNase activity was defined as the amount of enzyme that destroys 0.01 micromole of diphosphopyridine nucleotide‡ (DPN) in 7½ minutes at 37°C.

ASDA determinations were done by the method of Kellner, Freeman, and Carlson(1). One unit of ASDA activity is the amount of antibody neutralizing 100 units of DPNase. In cross-neutralization assays, pre-titrated antisera were used at a concentration of approximately one ASDA unit; this permitted the titration of a single enzyme against several pre-titrated antisera reducing the error in the assay.

Immunization of American Dutch rabbits consisted of injecting 3 ml aliquots of the enzyme source, Todd-Hewitt culture filtrate or SLE, on alternate days. The first 3 injections were given intravenously followed by the intradermal route. When a significant Arthus

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