Failure of Chlorothiazide to Influence Tissue Electrolytes in Hypertensive and Non-Hypertensive Nephrectomized Dogs.* (27454)

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The importance of the metabolism of sodium and water in regulation of blood pressure has been repeatedly demonstrated in the study of both human and experimental hypertension(1). Recent observations that saluretic agents are frequently effective in treatment of human hypertension(1) have added considerable impetus to the study of the mechanisms involved. It has been proposed that these agents influence hypertension by reducing circulating blood volume or extracellular fluid volume(2 and 3); and Friedman *et al.*(4) present evidence to indicate that the sodium gradient between the intracellular and extracellular compartments may also be a factor.

It is well established that chlorothiazide acts by altering the electrolyte excretion through the kidney, but little attention has been given to the possibility that it might also have a direct action on the electrolyte in the tissues. To test the possibility that chlorothiazide has a direct action on electrolyte distribution in tissues, nephrectomized dogs were used. These animals are specially suited for such an experiment since the effects of the kidneys are excluded and the renoprival form of hypertension is readily influenced by fluid and electrolytes(5 and 6). While this work was in progress Blackmore(7) reported that bilaterally nephrectomized dogs did not show alterations in serum electrolyte levels within a period of 4 hours when given chlorothiazide.

The first experiment was designed to test the effect of chlorothiazide (Diuril)† on tissue electrolyte concentration in nephrectomized dogs made hypertensive by daily intraperitoneal injection of modified Locke's solution(5). The second experiment was designed to determine whether tissue electrolyte concentration could be altered by chlorothiazide when nephrectomized dogs were given no fluid and electrolyte load.

Methods. Mongrel dogs were fed a full ration of Purina Dog Chow for a minimum of one month before blood pressure determinations were begun. Mean blood pressure was determined 3 times each week by use of a mercury manometer and direct femoral artery puncture. These determinations were continued until a reproducible pressure was obtained. A nephrectomy was then performed through a flank incision. After an interval of a week or more the second nephrectomy was performed by the same technic. The time of the second nephrectomy marked the beginning of the experimental period. This 2-stage technic for bilateral nephrectomy kept operative trauma to a minimum at the beginning of the experimental period.

In the first experiment 2 groups of 5 bilaterally nephrectomized dogs were used. No food or water was given by mouth during the experiment. Blood pressure was determined daily in each dog throughout the experiment. Serum Na, K and Cl were determined every other day. The control group was given modified Locke's solution intraperitoneally at the rate of 100 ml/kg/day beginning the day of the second nephrectomy. The experimental group was treated in the same way as the first except that each animal received 500 mg of chlorothiazide in 20 ml of distilled water intravenously each day of the experiment. Animals of both groups were sacrificed on the sixth day after the second nephrectomy. Gross and microscopic examinations of the tissues were made, and Na, K and Cl concentrations determined on skeletal muscle, left ventricular myocardium, and aorta.

Tissue samples were prepared and analyzed by the method of Lowry and Hastings(8) to obtain a 0.75 nitric acid extract of a known

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amount of dried, defatted tissue. Sodium and potassium were determined on both serum and tissue extract by flame photometry and chloride by the method of Schales and Schales (9). This chloride method was not sufficiently sensitive, giving reproducible but high results, and was replaced by the method of Wilson and Ball(10) in which aliquots of the digested tissue were titrated with 0.05N NH_4SCN using a Rehberg microburette.

Because we had found more variability in all tissue electrolyte concentrations among our animals in the first experiment than is usually reported in the literature, the wet ash method of Wilson and Ball(10) was tested as the initial step for all determinations. Comparison of the 2 methods on normal and nephrectomized dogs gave no indication that one was superior to the other. Since the two methods were in close agreement and since the wet ash technic of Wilson and Ball was quicker, it was used throughout the second experiment. For this method, 3 samples of tissue were taken. Two were used as duplicates for the electrolyte determinations and the third was dried and defatted so that electrolyte concentrations could be calculated in terms of dried, defatted tissue.

In the second experiment, 2 groups of bilaterally nephrectomized dogs were used. The control group of 9 dogs was given nothing by mouth or parenterally following the second nephrectomy. The experimental group of 4 dogs was treated in the same way except that each animal received 500 mg of chlorothiazide in 20 ml of distilled water intravenously each day of the experiment. All animals were sacrificed on the third day after the second nephrectomy. The relative short time that this experiment was continued after nephrectomy was based on the belief that chlorothiazide should induce changes in 24-48 hours and that the short time period would reduce the influence of uremia on the results. Gross and microscopic examinations of the tissues were made, and Na, K and Cl were determined in serum, skeletal muscle, left ventricular myocardium and aorta as described above.

Tissues of 10 normal dogs were similarly analyzed to provide base-line values of Na, K and Cl in skeletal muscle, left ventricular myocardium and aorta by the methods employed.

Results. In the first experiment the control dogs all developed hypertension and vascular necrosis similar to previously reported results with this technic(5), and the experimental dogs receiving chlorothiazide injections developed a similar degree of hypertension and vascular necrosis. The increase in mean blood pressure averaged 48 mm Hg for the chlorothiazide treated dogs and 43 mm Hg for the untreated ones. Serum electrolyte values at the beginning and end of experiment are listed in Table I. Both the chloride and potassium levels rose similarly in control and experimental animals. The serum sodium was inexplicably lower in the experimental

TABLE I. Tissue Moisture Content and Serum Electrolyte Values in Dogs under Various Types of Treatment.

Experimental procedure	No. of dogs	Muscle moisture	Aorta moisture	Heart moisture	Serum Na	Serum K	Serum Cl
		——% wet fatty tissue——			——— meq/l ———		
Normal	10	74 ± 1.1	71 ± 2.1	78 ± .6			
Nephrectomy	9	72 ± 2.0	70 ± 2.2	77 ± .4	155.1 ± 2 155.1 ± 7	4.6 ± .5 7.7 ±1.2	110.7 ± 4.9 101.8 ±10
Neph. + chlorothiazide	4	70 ± 3.6	72 ± .0	79 ± 2.8	155.9 ±11.5 155.8 ± 6.9	4.2 ± .38 7.7 ± .77	113.3 ± 4.0 105.3 ± 3.5
Neph. + Locke's sol.	5	80 ± 1.8	75 ± .7	80 ± .4	152.2 ± 6.6 150.5 ± 4.5	4.9 ± .46 7.9 ± .32	109.4 ± 3.2 118.1 ± 4.5
Neph. + Locke's sol. + chlorothiazide	5	76 ± 5.6	74 ± 1.3	80 ± .2	140.3 ± 3.7 146.4 ± 3.5	5.1 ± .31 7.8 ± .76	112.4 ± 4.1 117.8 ± 5.6

$$S = \sqrt{\frac{\Sigma N^2}{N-1}}$$

TABLE II. Tissue Electrolyte Concentrations in Tissues of Dogs under Various Types of Treatment. Results are reported in meq/kg of dried, defatted tissue.

Experimental procedure	No. of dogs	Muscle			Aorta			Heart		
		Sodium	Potassium	Chloride	Sodium	Potassium	Chloride	Sodium	Potassium	Chloride
Normal	10	83 ± 8	444 ± 88	91 ± 28	279 ± 18	164 ± 26	253 ± 28	169 ± 21	399 ± 56	145 ± 40
Nephrectomy	9	85 ± 29	478 ± 39	88 ± 23	321 ± 35	177 ± 12	231 ± 24	130 ± 20	417 ± 40	119 ± 19
Neph. + chlorothiazide	4	87 ± 10	430 ± 32	73 ± 6	342 ± 19	187 ± 20	258 ± 30	143 ± 21	419 ± 55	122 ± 17
Neph. + Locke's sol.	5	189 ± 50	420 ± 17	139 ± 11	359 ± 22	217 ± 17	340 ± 25	183 ± 26	446 ± 19	150 ± 25
Neph. + Locke's sol. & chlorothiazide	5	170 ± 86	370 ± 91	176 ± 59	544 ±182	246 ± 86	292 ± 13	202 ± 47	470 ±134	195 ± 27

$$S = \pm\sqrt{\frac{\Sigma N^2}{N-1}}$$

group at the beginning of the experiment and rose toward the level of the controls while the serum sodium of the controls did not change. Differences in serum sodium concentration were not statistically significant, however. The changes in tissue moisture are also recorded in Table I. The injections of modified Locke's solution increased the moisture content of skeletal muscle and aorta over that of the normal dogs. Injection of chlorothiazide in the experimental animals did not alter this accumulation of fluid. There is a drop in mean moisture content in the skeletal muscles of the experimental dogs, but the difference is not significant.

The results of the determinations of electrolytes in the tissues are given in Table II. Comparison of the level of Na, K and Cl in the tissues of the normal dogs with the levels in the controls (nephrectomized dogs given modified Locke's solution) shows the latter to have an increase in Na and Cl in skeletal muscle, a questionable increase in the aorta, but no increase in the heart. Potassium was questionably increased in the aorta, but not in the heart or skeletal muscle. Hence it is seen that bilateral nephrectomy followed only by modified Locke's solution induces specific alterations in electrolyte distribution in the tissues studied. Using the values obtained in these controls as a basis for comparison with the experimental dogs treated with chlorothiazide, it is found that no further significant change in tissue electrolytes resulted from the chlorothiazide injections.

In the second experiment neither control nor experimental dogs developed hypertension or vascular necrosis. The changes in serum Na, K and Cl are reported in Table I. During the experiment the serum Na remained unchanged in both control and experimental groups. In the 2 groups serum potassium rose and serum chloride fell; the amount of change was the same in each group. The moisture of the heart, skeletal muscle and aorta is also recorded in Table I. No identifiable change in moisture from that of the normal animals occurred and no differences were found between control and experimental groups. The tissue electrolytes did not change from normal in either the control group that was only

nephrectomized or the experimental group that was nephrectomized and then given chlorothiazide intravenously (Table II).

Discussion. Under conditions of the first experiment, in which control dogs were nephrectomized and given modified Locke's solution and the experimental dogs were treated similarly but received intravenous chlorothiazide in addition, both groups became hypertensive and developed similar vascular necrosis. From these results it appears that chlorothiazide is unable to control hypertension and vascular necrosis when the kidneys are absent and intraperitoneal Locke's solution is given. Furthermore, no effect of chlorothiazide on serum or tissue electrolyte concentrations or on tissue moisture was identified when the values obtained on nephrectomized dogs given modified Locke's solution were compared with those obtained on dogs treated in the same manner but given chlorothiazide in addition. The average value for moisture in the skeletal muscle of the chlorothiazide treated dogs is lower than that in the controls, but the difference is not statistically significant.

Under the conditions of the first experiment both control and experimental dogs were given a large load of fluid and electrolyte in the absence of the kidneys. This treatment resulted in a large amount of fluid in the peritoneal cavity and obvious subcutaneous edema. It also resulted in increased moisture content in the skeletal muscle, questionable change in aorta, but no change in the heart. Since the skeletal muscle in both groups of animals contained an increased amount of sodium and chloride, the possibility was considered that this electrolyte increase was only a reflection of the increased fluid content. However, if one calculates the expected Na and Cl concentrations in the skeletal muscle from serum concentrations at time of sacrifice and amount of moisture in the skeletal muscle, the value obtained is still well below that observed. Therefore, the increase in Na and Cl in the skeletal muscle apparently includes an increase in intracellular Na and Cl over and above that expected from the increased extracellular fluid. The aorta tends to follow the same pattern, but the observed change is not nearly as definite as in the skeletal muscle. This observed increase in Na and Cl above that expected by increased extracellular fluid would fit with the observations of Friedman (4) that an increase in the ratio of Na inside the cell to Na outside the cell is characteristic of the hypertensive state. It is further noted that in these experiments a shift in Na was found only in the 2 groups of dogs that developed hypertension.

Since both control and experimental animals in the first experiment were treated with large amounts of fluid, it is possible that changes induced by chlorothiazide were obscured by the amount of fluid and electrolyte given. To check this possibility the second experiment was performed on nephrectomized dogs without giving a fluid and electrolyte load. The absence of any observable effect of chlorothiazide on the dogs in the second experiment adds evidence that chlorothiazide has no direct effect on tissue electrolyte.

Summary. From these observations, no evidence was obtained to indicate that chlorothiazide influences the levels of blood pressure, tissue moisture, tissue electrolyte or serum electrolyte in the absence of the kidneys.

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