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Effects of Diuretic Agents on Serum and Tissue Electrolytes in Rats.* (31055)

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The physician's ability to manage patients with hypertensive cardiovascular disease and patients with edema has been aided in part by the synthesis of chlorothiazide(1) and its derivatives(2-5). However, the frequent occurrence of hypokalemia in patients during the course of thiazide administration has disturbed clinicians(6,7). The effect of thiazides upon potassium metabolism has been deduced from balance studies(8), from serial measurements of exchangeable potassium with K^{42} (9,10). and from direct tissue analyses(11, 12). These studies have shown that potassium is lost from the body during the first few days of therapy, potassium balance may then become positive, while hypokalemia persists. Since the mechanism of production of the hypokalemia was obscure, a series of experiments was designed to test the hypothesis that the hypokalemia occurring during thiazide administration did not reflect a deficit of body potassium.

Methods. Eighty Sprague-Dawley rats including equal numbers of males and females weighing from 175-250 g were randomly assigned to one of 4 coded diet programs (with 20 rats in each group): I. Normal rat diet,† II. Normal diet with chlorthalidone‡ added to supply a daily dose of 2.1 mg/kg, III. Normal diet with Hydrochlorothiazide‡ added to supply a daily dose of 1.4 mg/kg, IV. Normal diet with polythiazide§ added to supply a daily dose of 0.028 mg/kg.

These diets were prepared elsewhere and were delivered to our laboratory in coded containers. All animals were individually caged, food intake was estimated by weighing the food containers 3 times weekly, and the weight gain of individual animals was determined by weighing each animal once weekly. These data demonstrated that food intake by the 4 groups of animals was consistent and that all 4 groups gained equal amounts of weight during the period of the study. The animals were permitted distilled water *ad libitum* during the 4-week treatment period. At the end of that time, the animals were anesthetized with ether, a sample of blood was drawn from the abdominal aorta and then both quadriceps femoris muscles were removed for tissue analysis. After the blood had been allowed to clot under oil at room temperature, the samples were centrifuged and the serum removed for analysis.

Serum water was determined by drying aliquots of serum to constant weight at 110°C, chloride by Cotlove titration and sodium and potassium by an internal standard flame photometer. Tissue analyses were perfomed by methods previously described(14) except that an internal standard flame photometer was used for determination of sodium and potas-

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† Diets were obtained from Rosner-Hixson Laboratories, Chicago, Ill.

‡ Chlorthalidone and Hydrochlorothiazide used in this study were supplied by Dr. John D. Sproul, Geigy Pharmaceuticals, Yonkers, N. Y.

§ Polythiazide used in this study was supplied by Dr. Findlay Crowe, Pfizer Laboratories, New York.

sium and a Cotlove titrator was utilized for measuring tissue Cl^-.

From the analytical data obtained, the distribution of extracellular and intracellular water and electrolytes was calculated utilizing the formulae of Manery(15). Standard statistical methods were used for determining significance(16).

TABLE I. Serum and Tissue Content of Water and Electrolytes in Controls and Treated Animals.

Treatment	Serum				Skeletal muscle			
		mEq/l						
	g/l H_2O	Cl^-	Na^+	K^+	H_2O,* g/kg	Cl^-,* mEq/kg	Na^+,* mEq/kg	K^+,* mEq/kg
Control	911 ± 10	105 ± 5	141 ± 4	5.35 ± .56	766 ± 9	14.2 ± 1.9	21.6 ± 1.9	103 ± 4
Chlorpthalidone	914 ± 11	†101 ± 4	140 ± 2	‡4.87 ± .51	765 ±16	13.1 ± 1.5	21.4 ± 2.3	101 ± 6
Hydrochlorothiazide	918 ± 17	‡103 ± 4	141 ± 2	(5.04)± .43	765 ± 6	14.1 ± 1.9	21.9 ± 2.8	102 ± 6
Polythiazide	918 ± 14	†101 ± 5	140 ± 2	5.11 ± .41	765 ± 4	14.4 ± 1.8	21.8 ± 1.6	100 ± 6

Values listed represent mean and standard deviation for each set of determinations.

* Expressed as g/kg or mEq/kg of fresh fat-free tissue. † $p < .001$. ‡ $p < .01$. ( ) $p < .05$.

Results. The results are tabulated in Tables I and II.

Serum chloride: A statistically significant decrease in mean serum chloride concentration occurred in all 3 groups of treated animals when compared with the controls ($p < .001$ for chlorthalidone and polythiazide and $p < .01$ for hydrochlorothiazide).

Serum sodium: Minor changes in serum sodium concentration were observed in the treated groups of animals; however, these changes were not statistically significant.

Serum potassium: The mean value for serum potassium was lower in each of the treated groups of rats than in the controls; and these differences were statistically significant in the group of animals receiving chlorthalidone ($p < .01$) and those receiving hydrochlorthiazide ($p < .05$).

Serum water: The mean serum water content was higher in each of the 3 treated groups than in the control animals; however, these changes were not significant at the 0.05 level.

Tissue water and electrolytes: The mean values for the tissue contents of water chloride, sodium, and potassium in the treated animals did not differ significantly from those of the controls ($p > 0.05$).

Distribution of water and electrolytes: Changes in the volumes of extracellular and intracellular water and in intracellular sodium and potassium concentrations were observed; however, these changes were not significant at the 0.05 level. The mean value for the ratio $K^I:K^E$ (intracellular potassium to extracellular potassium concentration) was higher in the treated animals than in the controls; however, this increase was significant only in the group receiving chlorthalidone ($p < .05$).

Discussion. In the present study, 3 commonly used oral diuretics have been evaluated as to their effects on serum and tissue levels of potassium in rats. The doses administered were comparable to those prescribed on a mg/kg basis for humans. That the doses administered were biochemically effective is suggested by the fact that all 3 groups of treated animals developed a significant decrease in serum chloride concentration. Values for serum potassium were lower in the treated animals than in the controls; and the lowering

TABLE II. Mass-Phase Data for Rat Skeletal Muscle—Controls and Treated Animals.

Treatment	E.C.W.	I.C.W.	I.C. Na^+	I.C. K^+	I.C. Na^+ + I.C. K^+	Ratio K^I/K^E
Control	121 ± 16	645 ± 18	6 ± 3	159 ± 8	165 ± 8	28 ± 3
Chlorphthalidone	119 ± 14	650 ± 17	6 ± 4	155 ± 10	161 ± 9	(31) ± 4
Hydrochlorothiazide	123 ± 17	643 ± 19	7 ± 3	158 ± 12	164 ±13	30 ± 3
Polythiazide	127 ± 19	638 ± 19	5 ± 3	156 ± 11	162 ± 9	30 ± 3

( ) $p < .05$.

The values listed represent mean and standard deviation for each set of determinations.

E.C.W. = Extracellular water, g/kg of fresh fat-free tissue. I.C.W. = Intracellular water, g/kg of fresh fat-free tissue. I.C. Na^+ = Intracellular sodium, mEq/kg of intracellular water. I.C. K^+ = Intracellular potassium, mEq/kg of intracellular water. I.C. Na^+ + I.C. K^+ = Sum of intracellular Na^+ and K^+, mEq/kg of intracellular water. Ratio K^I/K^E = Ratio of intracellular K^+, mEq/kg intracellular water, to extracellular K^+, mEq/kg extracellular water.

of serum potassium was statistically significant in the groups receiving chlorthalidone and hydrochlorthiazide. No significant changes in serum sodium concentration occurred in the treated groups and no significant changes were noted in the tissue content of water, chloride, sodium or potassium. Furthermore, no significant changes in the distribution of water or in intracellular concentration of sodium and potassium were observed. Thus, the decrease in serum potassium concentration observed in the treated animals occurred without a significant decrease in intracellular potassium concentration. This observation supports the hypothesis based upon previous data, that the hypokalemia associated with thiazide administration is due to redistribution of potassium rather than depletion of this ion(10-13).

Simple calculations serve to demonstrate that the decreases in serum potassium concentration observed in these studies were of a magnitude which would induce only minimal changes in the apparent tissue content of potassium and that such minimal changes (reflecting only a decrease in extracellular potassium) could not be detected by the techniques utilized in this study.

Binding or complexing of potassium ions by elements of connective tissue or other constituents of the extracellular phase with a resultant decrease in apparent serum potassium concentration cannot be ruled out as a possible cause for the hypokalemia observed in these studies.

Summary and conclusions. Hydrochlorothiazide, polythiazide and chlorthalidone were added to the diets of 3 groups of Sprague-Dawley rats. After 4 weeks of subsistence on this diet, these animals together with matched controls were sacrificed and analyses of serum and tissue electrolytes were done. The serum chloride concentration was significantly lower in each experimental group. Serum potassium concentration was decreased in all 3 groups, and was significantly lowered in the groups receiving chlorthalidone and hydrochlorthiazide. Changes in serum sodium concentration were not statistically significant. Tissue analyses revealed no significant alterations in water or electrolyte content. These studies suggest that the hypokalemia associated with oral diuretic therapy results from a redistribution of potassium stores rather than a frank depletion of this ion in rats.

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Attempts to Find Creatine Phosphokinase and 5′-Nucleotidase Activity In Canine Prostatic Fluid.* (31056)

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A partially purified acid phosphatase from human prostate has been found to transfer phosphate from β-glycerylphosphate and phosphocreatine to carbon 6 of glucose as well as to hydrolyze organic phosphates(1). Newton and Rothschild(2) identified phosphocreatine in bull spermatozoa and Yanagisawa(3) demonstrated this phosphamide and its related transferase, creatine phosphokinase, in sea urchin sperm. No report has appeared on the examination of prostatic fluid for these substances although the absence of creatine and creatinine in canine prostatic secretion has been reported(4).

Of relative pertinence is the 5′-nucleotidase activity demonstrated in human semen(5) and purified from the seminal vesicle secretion of the bull(6). The optimal pH of this enzyme was 8.5, similar to that of alkaline phosphatase. A confusing factor is that purified acid phosphomonoesterase has some hydrolytic activity against 5′-nucleotides(7).

The overlap in phosphatase activities and lack of knowledge on creatine phosphokinase suggested an exploration of canine prostatic fluid for these enzymes. The dog with a cystopreputiostomy fistula provides a particularly suitable opportunity for such an investigation since it yields an ample supply of prostatic fluid without vesicular or testicular contamination(8). Furthermore, purified fractions of canine prostatic enzymes are available for comparison with whole prostatic fluid (9); and canine prostatic fluid is suited for studies of phosphate release through any mechanism since at the most only a trace of endogenous orthophosphate has been detected in this fluid(4).

Materials and methods. Creatine phosphokinase catalyzes the transfer of a phosphate group from phosphocreatine to adenosine diphosphate (ADP). The reaction may be followed in either direction by determining the appearance or disappearance of creatine. Neither the internal anhydride, creatinine, nor phosphocreatine give a colored product with the diacetyl reagent. However, it is possible that under acid conditions a small amount of creatine could be released from phosphocreatine by hydrolysis(10). Since the enzyme reaction was to be terminated with 10% trichloroacetic acid (TCA), an estimation of non-enzymic hydrolysis of phosphocreatine was obtained in the presence of TCA. The satisfactory application of the diacetyl procedure for determination of creatine in the presence of prostatic fluid and its components was evaluated by addition of varying amounts of exogenous creatine to prostatic fluid.

The details of the incubation system are as follows: 5 ml of a mixture containing 6 ml 0.4 M glycine-NaOH buffer with 0.1 M magnesium sulfate at pH 9.0 were mixed with 2 ml 0.005 M ATP and 100 μg creatine in 0.1 ml water(11). The enzyme source was 0-3.0 ml canine prostatic fluid or 10-60 mg of a purified canine prostatic fluid fraction. The purified fractions were the insoluble, non-dialyzable acid phosphatase (C-material); the insoluble, non-dialyzable acid phosphatase produced through a preliminary gel filtration

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