The Effect of Hydrochlorothiazide and Triamterene on the Tissue Content of Sodium and Potassium in the Rat[1] (35776)

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(Introduced by S. Solomon)

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The administration of diuretics on a chronic basis is commonly associated with changes in the electrolyte composition and volume of the extracellular fluid compartment. The most common electrolyte problems encountered with the frequently prescribed thiazide diuretic agents are hypokalemia and hyponatremia. Gifford (1) has estimated that upward of 40% of thiazide-treated patients develop hypokalemia at some time during the course of therapy. Hollander *et al.* (2) reported a significant depression of the exchangeable K^+ in hypertensive patients treated with chlorothiazide for 4 to 8 weeks, although similar effects were not observed by Talso and Carballo (3) following chronic administration of hydrochlorothiazide (HCTZ). These results have been interpreted by Weller (4) to indicate that hypokalemia associated with diuretic therapy may reflect a deviation from the normal intracellular–extracellular K^+ gradient and not a depletion of body stores of K^+.

Recently, diuretic agents have been developed that produce a natriuresis without inducing a concomitant kaluresis. Spironolactone (5) is a "potassium-sparing" diuretic agent because it apparently blocks the renal action of aldosterone (6). Liddle (7) has suggested that elevated circulating levels of aldosterone (8) may be involved in the enhanced loss of K^+ during diuretic therapy. Later, triamterene (TA) was shown to produce a natriuresis without a kaluresis in edematous and normal subjects (9–11). TA has a broader spectrum of effectiveness than spironolactone (12) since it does not depend on the presence of aldosterone for its action (13). TA has been combined with HCTZ to successfully reduce K^+ loss expected following administration of thiazides (14). Tissue levels of electrolytes have not been measured after similar combination therapy. Because of the important relationship between ion content and functions of tissue, the present study was undertaken to determine the effects of HCTZ and TA, individually and in combination, on the levels of Na^+ and K^+ in plasma, kidney, heart, and skeletal muscle of the rat.

Methods. Drug administration and tissue removal. Female albino rats were randomly divided into 4 groups with approximately the same average weight of 250 g. Each group consisted of eight rats. All rats were allowed food and water *ad libitum* during the period of drug administration. The drugs were administered orally to the rats each morning for 7 days. On the morning of the eighth day the rats were anesthetized with pentobarbital (40 mg/kg). The abdomen was opened and the kidneys were exposed. The aorta was clamped superior to the renal artery, the renal artery was perfused with 0.3 *M* sucrose, and the renal vein was clipped. When the renal venous effluent was clear, the kidney was rapidly removed, cleaned, and frozen in liquid nitrogen. The biceps femoris was excised, rinsed in 0.3 *M* sucrose, trimmed of connective tissue and frozen in liquid nitrogen. The inferior vena cava was clamped and the heart was flushed with 0.3 *M* sucrose.

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The blood was readily flushed from the beating heart if the aorta was severed. As soon as the coronary vessels were clear the heart was rapidly removed, trimmed, sectioned, blotted, and frozen in liquid nitrogen. Blood samples were drawn from the abdominal aorta prior to tissue removals.

Tissue preparation. The frozen tissues were pulverized in a stainless steel mortar and pestle which was at the temperature of liquid nitrogen. No attempt was made to separate the functional or anatomical components of either the heart or the kidney. The resulting tissue powders were therefore a homogenous mixture of tissue components which could be easily transferred and extracted. The frozen powders were transferred to tared vials and the wet weight was determined to the nearest 0.1 mg. Samples were dried overnight at 100° and reweighed to determine the tissue water content. The dried tissue powders were then extracted twice for 3 hr with 5 ml of ethyl ether–heptane (1:1) solution. The samples were again dried overnight at 100° and reweighed to determine the fat-extracted dry weight. The dry tissue residues were digested in 1.0 ml of concentrated (70%) nitric acid and diluted to 25 ml with double distilled water. Appropriate dilutions were made with LiCl solutions and the Na^+ and K^+ concentrations were determined with an Instruments Laboratory model 143 flame photometer. The low variability of tissue water content between animals demonstrated the effectiveness of the technique using liquid nitrogen freezing.

Drug preparation. All drugs used in the present study were suspended in 0.025 *M* (2.2 mg/ml) lactic acid to solubilize the triamterene (TA) when present. Sodium chloride (final concentration 0.9%) was added to the drug solution after TA had been dissolved in the lactate. The final mixture was actually a suspension of TA. All drug administrations were made by gastric intubation via a B-D 16-gauge animal feeding needle.

The daily single dosages of drugs were as follows: hydrochlorothiazide (HCTZ), 30 mg/kg; TA, 60 mg/kg; HCTZ, 30 mg/kg plus TA, 60 mg/kg. It has been shown that,

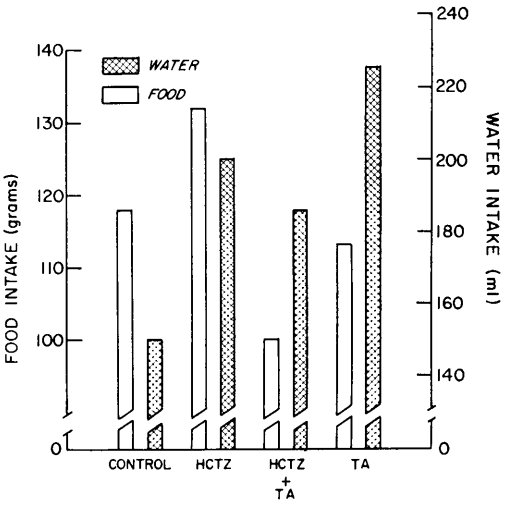

FIG. 1. The effect of hydrochlorothiazide (HCTZ) and triamterene (TA) on total food and water intake over the 7-day treatment period. Each vertical bar represents the average intake per animal in each group of 8 animals.

at these doses in rats HCTZ causes a diuresis, natriuresis, and kaluresis and that TA produces a diuresis, natriuresis and no kaluresis (15). The control group received the saline–lactate solution without drugs. The three drug regimens were suspended in concentrations to allow for an intubation of 2.5 ml of solution/100 g of rat weight.

Statistical analysis. The possible significant difference between means of control and treated groups was calculated by analysis of variance and the Student's *t* test (16).

Results. Groups of rats received daily the drug vehicle (control); hydrochlorothiazide (HCTZ) at 30 mg/kg/day; triamterene (TA) at 60 mg/kg/day; and HCTZ plus TA at the same dosage. Total food intake and water intake for each group are presented in Fig. 1. There was an apparent increase in water consumption in the three diuretic-treated groups. Food intake did not parallel water intake and a notable decrease in food intake was observed when both diuretics were given in combination. Soft feces were also noted in this group.

Data presented in Fig. 2 represent the weight or growth profile of the four groups. Interestingly, TA appears to be a growth factor in this study. However, we were un-

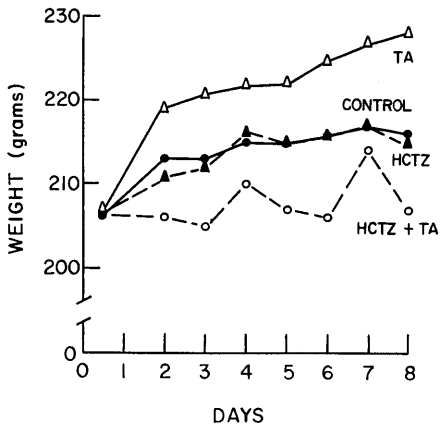

FIG. 2. The effect of hydrochlorothiazide (HCTZ) and triamterene (TA) on the mean weight during the diuretic treatment period.

able in this study to determine whether the enhanced water intake accounted for the greater weight gain. The lack of weight gain in the group treated with HCTZ and TA was anticipated because of loss of fluid and electrolytes in the feces and urine.

Levels of K^+, Na^+, and K^+/Na^+ ratios are illustrated in Table I. In only two tissues were significant changes in K^+ found. The amount of K^+ in kidney was increased following TA administration ($p<0.01$) and K^+ content of skeletal muscle was decreased by the combination of HCTZ and TA ($p<0.001$). The HCTZ administration caused an apparent, although not significant decrease in the K^+ content of all tissues examined except blood. Skeletal muscle appeared to be most sensitive to K^+ loss from diuretic therapy.

It is readily apparent from data presented in Table I that skeletal muscle Na^+ is extremely sensitive to the administration of diuretics. In all three diuretic regimens, significant changes in Na^+ content were observed. Interestingly, HCTZ caused an increase and TA caused a decrease in skeletal muscle Na^+. When given in combination, HCTZ and TA caused a decrease in Na^+ content comparable to that seen with TA alone. A significant depression of Na^+ in the heart was observed in the TA treated group. The Na^+ content of other tissues were not significantly altered by diuretic therapy, although it appears that HCTZ and HCTZ plus TA produce an elevation in the Na^+ content of heart.

The administration of HCTZ caused a significant decrease in the K^+/Na^+ ratios of skeletal muscle and TA caused a significant increase. TA reversed the depressed K^+/Na^+ ratio caused by HCTZ. TA also produced a significant increase in the K^+/Na^+ ratio in the heart. Apparent changes in this ratio in heart after HCTZ and HCTZ plus TA were similar to that seen in skeletal muscle.

In both groups of animals given TA there was a significant increase in the water content of the kidney (Table II). The administration of HCTZ plus TA was also associated with a significant increase in the tissue water content of the heart. Skeletal muscle appears resistant to changes in tissue water although there was an apparent, though not significant, decrease in the concentration of Na^+ plus K^+ in the tissue water following the administration of TA alone or in combination. There is no evidence that diuretic agents used in the current investigation produced a tissue dehydration.

Discussion. In the present study it is obvious from the data that the plasma Na^+ and K^+ levels do not adequately describe the disposition of these two ions in the rat during diuretic treatment. It is also readily apparent from data in Table I that skeletal muscle is particularly sensitive to ionic changes following the administration of hydrochlorothiazide (HCTZ) and triamterene (TA). The significant increase (21%) in the skeletal muscle content of Na^+ caused by HCTZ is in agreement with the earlier report of Tobian *et al.* (17) who showed a similar increase (17%) with chlorothiazide treatment. Although not significant, the apparent depression (4.1%) in K^+ content of skeletal muscle in our studies compares favorably with that observed by others who treated rats with thiazides (17, 18). The mean differences in Na^+ and K^+ content following HCTZ treatment demonstrate an apparent increase of 18.1 μEq Na^+ (Table I) and a decrease of 16.6 μEq of K^+ (Table I)/g of dry weight which suggest an equal exchange of these two cations. This exchange would be expected to be intracellular and could not be explained by

TABLE I. Effect of Hydrochlorothiazide (HCTZ) and Triamterene (TA) on Tissue K^+, Na^+, and K^+/Na^+ Ratios.[a]

	Plasma (μEq/liter)		Kidney (μEq/g of dry wt)			Skeletal muscle (μEq/g of dry wt)			Heart (μEq/g of dry wt)		
	K^+	Na^+	K^+	Na^+	K^+/Na^+	K^+	Na^+	K^+/Na^+	K^+	Na^+	K^+/Na^+
Control	4.5 ±0.1	141.4 ±1.4	280.4 ± 6.8	188.6 ±12.8	1.57 ±0.14	407.0 ± 8.5	85.4 ±3.1	4.79 ±0.12	322.0 ±4.0	78.8 ± 5.9	4.26 ±0.33
HCTZ	4.6 ±0.1	140.7 ±1.0	267.5 ± 6.2	166.3 ± 8.4	1.63 ±0.08	390.4 ± 8.0	103.5[b] ±4.8	3.83[b] ±0.20	306.5 ±8.8	94.8 ±11.3	3.65 ±0.52
HCTZ+TA	4.4 ±0.1	139.7 ±1.2	281.8 ±16.4	189.4 ±12.9	1.52 ±0.11	353.0[b] ±18.5	67.3[b] ±3.2	5.31 ±0.38	328.6 ±5.0	93.0 ±10.4	3.81 ±0.39
TA	4.3 ±0.1	139.1 ±1.0	336.2[b] ±12.0	217.8 ±16.1	1.61 ±0.15	388.4 ±11.6	73.3[b] ±4.0	5.38[b] ±0.24	313.5 ±6.6	59.6[b] ± 5.1	5.51[b] ±0.38

[a] Values are expressed as μEq/liter of plasma or μEq/g of fat-extracted dry weight for tissues. Each value is the mean of 8 observations ± standard error of the mean. Each observation represents one sample of tissue per rat. Ratios were calculated from individual observations.

[b] Significantly different from controls at the 5% level.

TABLE II. Tissue Water Content and Total Levels of Tissue Sodium and Potassium (amount/ml of tissue water).

Tissue	Control		HCTZ		HCTZ + TA		TA	
	Tissue water[a]	μEq/ml[b]	Tissue water	μEq/ml	Tissue water	μEq/ml	Tissue water	μEq/ml
Kidney	0.744	157.6	0.750	141.9	0.761[c]	145.2	0.785[c]	144.7
Skeletal muscle	0.738	176.3	0.742	168.8	0.740	153.4	0.740	160.9
Heart	0.730	150.5	0.737	147.2	0.742[c]	149.5	0.735	134.4

[a] Values expressed as ml of H_2O/g of wet weight.
[b] μEq of sodium plus potassium per ml of tissue water.
[c] Significantly different from controls at the 5% level.

tissue hydration (Table II). As noted earlier, HCTZ did not significantly alter the K^+ content of any tissue studied. However, the observed trend to depress the K^+ content in each tissue examined might suggest a depletion of the total body stores of this ion following HCTZ administration.

TA caused a significant decrease in Na^+ content of skeletal muscle without significantly affecting K^+ or tissue water content. Therefore it might be expected that the effects of HCTZ and TA on skeletal muscle Na^+ would be obliterated when both drugs are given. Instead, when both diuretic agents were given, skeletal muscle sodium was decreased similarly to that seen with TA alone. The significant decrease in skeletal muscle K^+ may have resulted from an additive effect of apparent decreases seen with the diuretics given alone. Due to the mild diarrhea observed in this group, it could be argued that K^+ loss in the feces could have contributed to the decreased K^+ in skeletal muscle. The decrease in both Na^+ and K^+ content in skeletal muscle could not be accounted for by a change in tissue water. The addition of TA to HCTZ treatment did not prevent the expected K^+ loss from skeletal muscle (17) but did prevent the decrease in K^+/Na^+ ratio seen with HCTZ alone.

HCTZ and/or TA did not significantly affect K^+ content of the heart, however triamterene significantly decreased the Na^+ content of heart resulting in an increase in the K^+/Na^+ ratio but not in total cation content. The magnitude and direction of Na^+ changes in the heart suggest that the HCTZ effect may predominate in this tissue following combination treatment. This interpretation is reinforced by the similarity between the cardiac K^+/Na^+ ratios of the HCTZ and the HCTZ plus TA groups (Table I).

After the administration of TA alone there was a significant increase (19%) in the content of K^+ in the kidney. Since there was an apparent increase (15%) in Na^+ and a significant increase (6%) in water content, the increase in K^+ may reflect a specific action of TA on K^+ retention in the kidney. An increase in either interstitial water or a retention of urine in the kidney would have favored Na^+ accumulation rather than K^+.

As mentioned earlier, HCTZ and TA have been shown to produce a Na^+ diuresis even in Na^+-depleted rats and concurrent with this natriuresis, HCTZ caused an increase and TA a decrease in the urinary excretion of K^+ (15). Diuretic agents that deplete body Na^+ are known to increase aldosterone secretion (8). Aldosterone has been shown to increase the Na^+ content of the rat diaphragm (19) and inhibit Na^+ flux in erythrocytes *in vitro* (20, 21). The administration of aldosterone to nephrectomized rats apparently caused a direct effect on tissue Na^+ (22, 23). Other investigators have also observed effects of aldosterone on tissue electrolytes, but these effects were not distinguished from an action of aldosterone on the kidney to cause Na^+ retention (24, 25).

In our study, it might be expected that the administration of diuretic agents resulted in a compensatory increase in aldosterone secretion. The increase in skeletal muscle Na^+

following the administration of HCTZ could therefore have resulted from a direct action of aldosterone and not the diuretic action. The decrease in skeletal muscle Na^+ after TA alone or with HCTZ would not be expected to result from a direct action of aldosterone.

TA has been shown to antagonize the renal action of aldosterone in that it reverses the pattern of Na^+ and K^+ excretion (26). However, this action is not dependent on the presence of aldosterone (12, 15, 27). In an *in vitro* study, Daniel (28) was unable to establish an unequivocal action of TA on the transport of Na^+ and K^+ by uterine and aortic smooth muscle. The observed decrease in Na^+ content in cardiac and skeletal muscle after TA in the present study may have resulted from a direct action on the muscle since plasma levels were not significantly reduced. In fact, plasma levels of Na^+ and K^+ did not reflect any of the observed changes in tissue levels of these cations.

Summary. Hydrochlorothiazide (HCTZ), triamterene (TA), HCTZ plus TA or saline was administered orally to female albino rats once daily for 7 days. Plasma, kidney, heart, and skeletal muscle (biceps femoris) were analyzed for Na^+, K^+, and water content. Skeletal muscle was found to be the most sensitive, of the tissues examined, to alteration in ionic content following the administration of these diuretic agents. The Na^+ content of skeletal muscle was significantly increased with HCTZ and was significantly decreased following TA alone or TA plus HCTZ. The increase observed following HCTZ was unexpected in view of the natriuretic action of this agent. Treatment with TA alone caused a significant depression of Na^+ content of the heart. A significant decrease in K^+ was observed in skeletal muscle following TA plus HCTZ and an increase in kidney following TA alone. It is apparent from this study that HCTZ and TA can affect the tissue content of Na^+ and/or K^+ without an apparent effect on plasma levels of these ions. A possible interaction of these diuretics with aldosterone to produce these ionic changes is discussed.

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