Effects of Acetazolamide on Tissue Cations (33681)

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This study is part of a larger one to determine the basic mechanism of the teratogenic effect of acetazolamide and other related carbonic anhydrase inhibitors (1, 2). Acetazolamide causes renal loss of Na^+, K^+, and HCO_3^- and it was suspected that potassium depletion, noted in all experimental animals on the teratogenic diet, was due to the chronic administration of acetazolamide, and possibly was a primary agent of the teratogenesis.

Other investigators, Talso, *et al.* (3), Lim *et al.* (4), Grollman and Dahl (5) showed that thiazide compounds produce a potassium depletion or redistribution phenomenon, whereas Weller and Haight (6) observed the converse, that is, no potassium depletion or redistribution. Teratogenic effects were not observed with furosemide (2); presumably this also applies to the thiazides. Very recently, digital anomalies following theophylline were reported (7).

Previous evidence of Maren *et al.* (8) suggested that conservation mechanisms for potassium are more sluggish in the dog than that observed for sodium and that about 15% of total body potassium of 40 meq/kg may be lost over a 3-day period. However, K^+ loss was shown to be self limiting, and on a chronic (16 months) administration there was no loss beyond the initial deficit.

Despite large amounts of electrolyte work, tissue concentrations of Na^+ and K^+ in chronic carbonic anhydrase inhibition have not hitherto been studied. Therefore, this study was set up to determine the extent of tissue and plasma potassium depletion following acetazolamide.

Materials and Methods. Sexually mature male and female rats, weighing approximately 200 g, were obtained from Albino Farms and mated in our laboratory. Vaginal smears were obtained every morning from each female and checked for spermatozoa. If these were seen that day was taken to be day zero of gestation. Pregnant rats were placed in separate cages, given a diet of Rockland Farms rat chow and assigned either to the control or treated group. The treated group was given Rockland chow with the addition of 0.6% acetazolamide. Nonpregnant rats used in a corollary experiment were fed the same diet as the pregnant control rats. The renal response following carbonic anhydrase inhibition was then determined for 3 days following the onset of the diet.

The female rats and food were weighed daily to determine the amount of food and drug ingested in any 24-hr period, and to note the effect of this drug upon body weight. At the end of 20 days on diet the females were sacrificed and the fetuses were removed for separate study.

In a separate set of experiments, pregnant females were given subcutaneous injections of 500 mg/kg acetazolamide as the sodium salt at 9:00 a.m. on the tenth day of gestation and 3:00 a.m. on the eleventh day. These rats were then autopsied 11 hr after the last injection. In the same study, nonpregnant rats received injections of 500 mg/kg for three consecutive days starting at 4:00 p.m. on day 1 and 9:00 a.m. and 4:00 p.m. on days 2 and 3. These animals were autopsied 16 hr after the last dose. The renal response to acetazolamide was monitored throughout these experiments also.

Blood for electrolytes was obtained by cardiac puncture. This served also to exsanguinate the animal. The kidney, heart, liver, lung, and tibialis muscle were removed, freed of all visible fat, and placed in previously weighed flasks. The tissue was then weighed and digested in 5–10 ml of hot concentrated nitric acid depending on tissue weight. These flasks were then placed on a hot plate set at 300°F for 2 min. Aliquots of the digest were

FIG. 1

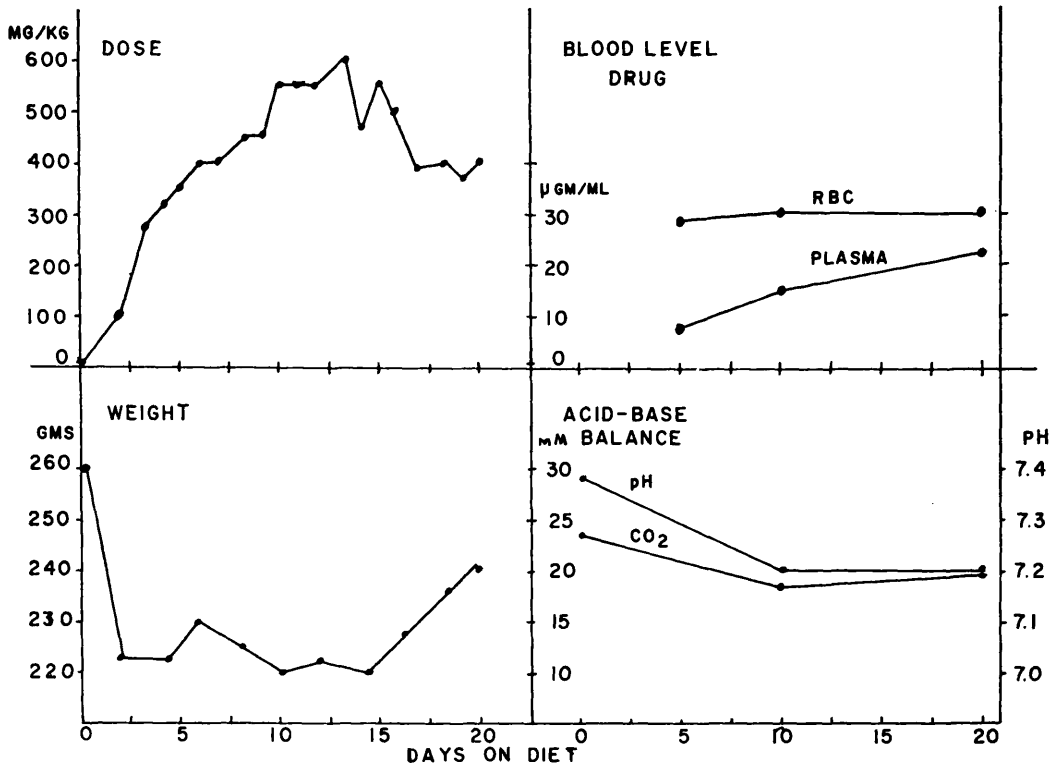

FIG. 1. Metabolic effects of 0.6% acetazolamide diet on pregnant rats. $n = 4$.

then diluted 1:200 for sodium and potassium determinations.

Chemical methods were as follows: sodium and potassium analyses were done on an Eppendorf flame photometer, CO_2 in plasma was measured manometrically in the Kopp-Natelson microgasometer, and pH was determined in a Metrohm pH meter with an electrode which holds an anaerobic sample at 37°. All determinations were made in duplicate, the average was recorded and the standard error of the mean was used to define the difference observed between the treated and control group. Data are recorded as meq/kg of wet weight of fresh tissue.

Results. Figure 1 shows the relationship of drug intake to body weight. The calculated dose based on food consumption was 400–500 mg/kg per day. This gave a constant red cell level of 30 μg/ml and a plasma concentration rising from 6 to 24 μg/ml. On this diet all treated rats, pregnant and nonpregnant, exhibited initially a weight loss of 20–40 g (Fig. 1). After 5 days this weight loss became stabilized and no further loss was observed. Food intake was also initially decreased. All controls, pregnant and nonpregnant, gained on the average 60 and 120 g respectively.

The renal response to acetazolamide in terms of potassium excretion was complete by the end of day 2 and somewhat earlier in terms of sodium output (Table I). The total net deficit for this 3-day period was 2.6 meq or 20–25% of the total body potassium of approximately 10 meq. This deficit would tacitly assume equal food intake in control and treated group. But since the treated rats ate about half that of the controls, the actual deficit is somewhat greater.

After 20 days on the 0.6% diet, pregnant rats showed a marked depletion of potassium in the muscle and plasma with a paradoxical increase in liver potassium. The heart and

TABLE I. Urinary Excretion of Cations Following 0.6% Acetazolamide in Diet of Rats ($n = 7$).

Day	Na (meq/24 hr ± SE)	K (meq/24 hr ± SE)
1	2.78 ± 0.35	2.84 ± 0.41
2	2.00 ± 0.25	2.62 ± 0.37
3	1.00 ± 0.25	1.92 ± 0.29
Control ($n = 5$; mean of 3 days)	1.28 ± 0.43	1.59 ± 0.16
Net deficit[a]	1.94	2.6

[a] Net deficit = total 3-day output—daily control × 3.

kidneys also show some evidence of potassium depletion. Mean plasma potassium was 3.1 meq/liter and total CO_2 was decreased to 16 m*M*, (Table II). In the potassium-deficient tissues sodium concentration was increased.

In partial contrast to these findings, the heart and kidneys of nonpregnant rats given acetazolamide showed no sign of potassium deficiency. Marked effects, however, were noted in plasma and muscle. Mean plasma levels of K^+ were 3.1 meq/liter and total CO_2 was 18 m*M*, (Table II). There was no elevation in liver K^+.

It appears from the data shown in Table II that normal pregnant rats maintain some degree of metabolic acidosis, as total CO_2 is reduced to 22 m*M* with a venous blood pH of 7.36; whereas the normal nonpregnant rat in this study had a total CO_2 of 26.7 m*M* and a venous blood pH of 7.44. There is also some K^+ "depletion" in the normal preg-

TABLE II. Effects of 0.6% Acetazolamide in Diet on Tissue Cations in Pregnant and Nonpregnant Rats.[a]

		Na^+ (meq/kg ± SE)	K^+ (meq/kg ± SE)	CO_2 (m*M*)	pH	*p* value for K^+
			Pregnant ($n = 20$)			
Muscle	C[b]	20.40 ± 1.12	89.14 ± 1.13			
	T	24.40 ± 1.20	73.68 ± 1.24			.005
Heart	C	28.27 ± 0.50	60.42 ± 3.03			
	T	38.00 ± 0.20	50.98 ± 1.65			.08
Kidney	C	49.26 ± 0.12	53.50 ± 1.54			
	T	64.50 ± 0.61	46.20 ± 0.90			.08
Liver	C	20.79 ± 1.55	59.80 ± 1.58			
	T	27.20 ± 1.18	68.60 ± 1.05			.006
Fetus	C	55.00 ± 1.80	38.80 ± 1.30			
	T	56.81 ± 1.20	41.00 ± 1.25			>.5
Plasma	C	143	4.43 ± 0.47	22.63	7.36	
	T	142	3.15 ± 1.25	16.03	7.18	
			Nonpregnant ($n = 20$)			
Muscle	C	21.53 ± 0.82	96.69 ± 2.0			
	T	26.53 ± 1.94	80.67 ± 2.0			.005
Heart	C	30.68 ± 1.32	73.38 ± 4.32			
	T	36.23 ± 2.90	71.80 ± 4.12			.65
Kidney	C	42.80 ± 1.33	54.60 ± 1.50			
	T	55.34 ± 1.86	55.33 ± 2.12			.55
Liver	C	20.33 ± 2.78	62.00 ± 2.34			
	T	23.00 ± 1.66	63.64 ± 1.86			—
Plasma	C	146	4.80 ± 0.67	26.76	7.44	
	T	142	3.10 ± 1.05	18.62	7.16	

[a] All tissues recorded as meq/kg of wet wt. at autopsy following 20 days 0.6% acetazolamide.
[b] C = control; T = treated.

TABLE III. Effects of Subcutaneous Acetazolamide on Tissue and Urinary Potassium.

		Urine K^+ (meq excreted in 28 hr ± SE)	Plasma K^+ (meq/liter ± SE)	(meq/kg of wet wt. ± SE)	
				Muscle K^+	Liver K^+
Pregnant[a] ($n = 6$)	C[c]	1.85 ± 0.37	3.8 ± 0.68	88.0 ± 1.13	59.0 ± 1.58
	T	3.49 ± 0.28	2.9 ± 1.27	78.0 ± 1.47	71.0 ± 2.86
Net loss		1.64			
		(meq excreted in 72 hr)			
Nonpregnant[b] ($n = 6$)	C	5.10 ± 0.56	4.60 ± 0.47	93.0 ± 1.8	62.0 ± 2.30
	T	6.40 ± 2.1	3.10 ± 1.10	91.2 ± 1.6	70.4 ± 1.45
Net loss		1.30			

[a] Received 500 mg/kg at 9:00 a.m. and 3:00 a.m. of pregnancy days 10–11. Sacrificed 11 hr after last injection.

[b] Received 500 mg/kg at 4:00 p.m. and 9:00 a.m. for 3 consecutive days. Sacrificed 16 hr after last injection.

[c] C = control; T = treated.

nant rat. The effect of carbonic anhydrase inhibition appears to be to increase the severity of the preexisting acidosis and to produce greater potassium depletion.

Fetuses removed from these mothers, although grossly underweight, were spared changes in whole body electrolyte concentration (Table II). This was previously reported by Serrano (9) in dogs and Stewart and Welt (10) in rats.

Table III shows the results of injection of acetazolamide on days 10 and 11 of pregnancy along with similarly spaced injections in the nonpregnant rat. No significant muscle depletion was observed in the nonpregnant animal whereas depletion of the muscle did occur in the pregnant rat. Plasma potassium was reduced in both experimental groups. In both groups there was a significant increase in potassium in the liver, although the differences were somewhat more striking in the pregnant rat (Table III).

Total urinary depletion of K^+ in the nonpregnant and pregnant ranged from 1.3 to 1.6 meq, respectively. This resulted in a loss of 15–20% of the total body potassium of the nonpregnant rat or 20–25% in the pregnant animal (Table III).

The plasma acidosis induced by acetazolamide is essentially metabolic in character, with little respiratory component. This is the usual situation in the free-breathing mammal, but does not rule out elevation of pCO_2 at tissue sites (11).

Discussion. The formation of hydrogen ion in the distal tubule is slowed by carbonic anhydrase inhibition. The normal exchange of hydrogen ion for the sodium ion in the renal tubule is slowed and potassium is left for exchange. Since proximal renal reabsorption of sodium is impaired, a larger sodium load is presented to the distal tubule. The net result is then an enhancement of potassium secretion. As the acidosis becomes more severe the net effect of carbonic anhydrase inhibition is overruled by enhancement of proximal HCO_3^- reabsorption and further ion loss is prevented (11). The initial loss of potassium is manifested in a reduced plasma level and muscle level of the nonpregnant rat (p value $<.005$). The effect of pregnancy may potentiate this effect as additional potassium must be distributed over the placenta to maintain a satisfactory embryonic environment. This additional need for potassium further reduces the maternal reserve and depletion is now noticed not only in the musculature but the heart and kidney as well.

Tissue analysis of pregnant rats on day 20 of the 0.6% acetazolamide diet showed a partial recompensation of sodium for potassium in those tissues showing potassium depletion. It was shown by Muntwyler and Griffen (12) that a rise occurs in intracellular sodium

and amounts to two-thirds of the fall in intracellular potassium in potassium deficient rats. That is, two sodium ions were exchanged for every three potassium ions. However, if electrical neutrality is to be maintained, some cation other than Na^+ must enter the cell or the anion content of the cell must decrease. The systemic extracellular alkalosis observed in other potassium deficient pregnant rats suggests that H^+ may enter the cells to replace lost K^+. Thus, it is clear that some of or all of the HCO_3^- lost in the urine represents HCO_3^- from an intracellular site.

The increase in sodium concentration within the liver along with the rise in potassium appears paradoxical, however this can be explained possibly by the recent work of Bartelheimer *et al.* (13). They showed that the liver of potassium-deficient rats exhibited a slight increase in intracellular potassium and a depletion in the extracellular compartment. However, in their analysis of skeletal muscle, potassium depletion was evident in the intracellular as well as the extracellular compartment.

A recent report by Wilson *et al.* (2) showed that teratogenic activity occurred in 36% of all fetuses from mothers on the 0.6% acetazolamide diet or following subcutaneous injections of acetazolamide on days 10 and 11 of gestation. These defects were attributed to the inhibition of maternal carbonic anhydrase, and the possibility was raised that, perhaps potassium depletion, secondary to inhibition of renal enzyme in the mother, or a combination of related effects, may furnish a lead to the localization or mechanism of action of acetazolamide as a teratogen.

Summary. Evidence has been presented that potassium depletion occurs in the skeletal muscle, heart, kidney, and plasma of pregnant rats and in the skeletal muscle and plasma of the nonpregnant rat following chronic administration of 0.6% acetazolamide in the diet for 20 days. A similar pattern of depletion followed two injections of drug spaced 18 hr apart. Sodium recompensation was observed in both pregnant and nonpregnant rat tissue that was partially depleted of potassium. A rise in liver potassium was observed in the pregnant and nonpregnant animal following the injection of 500 mg/kg of acetazolamide on days 10 and 11 of gestation. A similar rise was observed only in the pregnant rat on the 0.6% diet for 20 days.

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