Effect of Dietary Acetazolamide on Plasma Electrolytes, Bone Mass, and Renal Mineral Contents in Rats[1] (36410)

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Under certain conditions the carbonic anhydrase inhibitor, acetazolamide, has marked effects on calcium metabolism in the rat. We have shown that the drug inhibits the hypocalcemic response to calcitonin (1). In addition, acetazolamide blocks the hypercalcemic response to either parathyroid hormone or dibutyryl 3′,5′-cyclic AMP (2, 3). This latter finding led to the observation that acetazolamide, when incorporated in the diet at a concentration of 0.5%, inhibits the development of disuse osteoporosis in the rat (Conaway, Waite, and Kenny[2]). Acetazolamide then has potential as an orally effective therapeutic agent for the treatment of disuse osteoporosis in man.

Acetazolamide has been widely used clinically as a diuretic agent and is still used in the treatment of epilepsy and glaucoma. Hence its toxicity in man and experimental animals has been well documented (4, 5). In general its toxicity is low but chronic administration has revealed renal pathology in rats (5, 6) and in man (7). For this reason, we decided to study the effect of various levels of dietary acetazolamide on the renal mineral contents in order to determine if the drug causes any renal toxicity at or below the 0.5% therapeutically effective level. Plasma calcium, sodium, and potassium levels and the defatted dry weights of the humeri bones were also monitored.

Materials and Methods. Female rats (weighing between 150 and 190 g) were obtained from Carworth (Portage, MI) and were maintained on a commercial chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL) in suspended stainless steel wire-mesh cages in rooms with a 12-hr light/12-hr dark cycle. In the experimental groups sodium acetazolamide (Lederle Laboratories,[3] Pearl River, NY) was incorporated in the ground commercial chow at a concentration of 0.05, 0.2, or 0.5%. A total of 60 rats were randomized on the basis of body weight into 6 groups of 10 rats each (a control and acetazolamide-treated group for each of the 3 dietary levels) and fed the control diet (ground chow) for 7 days before switching to the acetazolamide diets. At this point pair-feeding was begun; each control rat received only that amount of food which its experimental pair consumed *ad libitum* the day before. The body weights and food consumption were monitored daily. After 20 days on the exerimental and control diets, heparinized blood was withdrawn by cardiac puncture and the left humerus and both kidneys were removed. The plasma was analyzed for calcium (8) and for sodium and potassium using the IL Model 143 flame photometer[4] (Instrumentation Laboratory Inc., Lexington, MA). The kidneys were dried, ashed, and dissolved in 5 ml of 5 *N* HCl as described by Kenny and Heiskell (9). The solution was analyzed for phosphorus (10);

[1] This investigation was supported in part by U.S. Public Health Service Grant AM 12184 and by research funds from the Space Sciences Research Center, University of Missouri-Columbia.

[2] Conaway, H. H., Waite, L. C., and Kenny, A. D., unpublished data.

[3] Obtained through the courtesy of Dr. Ira Ringler.

[4] As described in the manufacturer's handbook, "Instruction Handbook Model 143 Flame Photometer." Instrumentation Laboratory Inc., Lexington, MA, 1967.

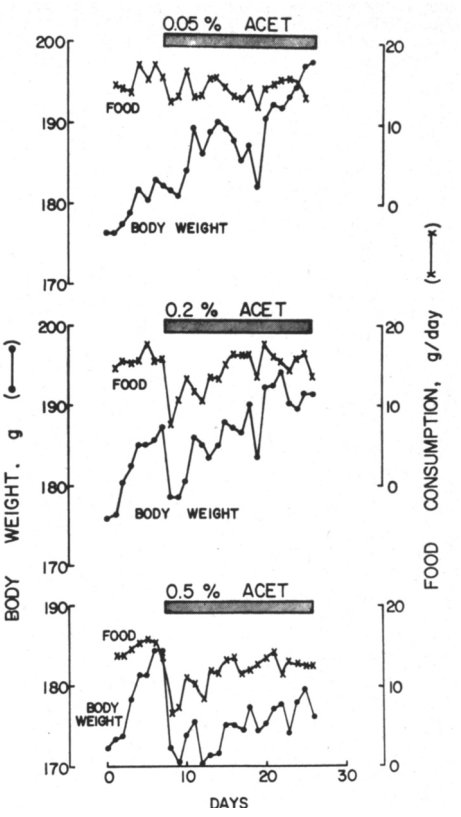

FIG. 1. The effect of 0.05, 0.2 and 0.5% acetazolamide sodium in the diet on food consumption (x—) and body weight (O—) in female Carworth rats. All rats were placed on ground commercial chow for 7 days before being placed on the acetazolamide diet for 19 days.

for calcium, magnesium, and zinc with a Perkin–Elmer 303 atomic absorption spectrophotometer[5] (Perkin–Elmer Corp., Norwalk, CT); and for sodium and potassium by flame photometry. The humeri bones were defatted, dried, and weighed as described by Kenny, Toepel, and Schour (11).

Results. The growth curves and daily food consumption of the acetazolamide-treated rats are plotted in Fig. 1. Initially all three dietary levels (0.05, 0.2, 0.5%) caused a retardation in growth and a fall in food consumption. By the end of the 20-day period the rats receiving 0.05 and 0.2% acetazolamide had recovered and surpassed their pre-acetazolamide body weights, the 0.05% to a greater extent than the 0.2% acetazolamide-treated rats. The rats receiving 0.5% acetazolamide did not return to pre-acetazolamide levels.

The final plasma Ca, Na, and K levels and the defatted dry weights of the humeri are presented in Table I along with the mean body weights and food consumption at the start of the control feeding period (day 0), the start of the experimental feeding period (day 7), and at the time of sacrifice (day 27). The mean body weights of the control and acetazolamide-treated groups did not differ significantly at any of the time periods or at any of the acetazolamide treatment levels, indicating the effectiveness of the randomization and pair-feeding design. The plasma Ca was significantly ($p < .001$) elevated at the 0.5% acetazolamide level, but not at the 0.05 and 0.2% levels. Neither the plasma Na and K levels nor the bone dry weights were significantly influenced by any of the acetazolamide treatment levels.

The renal contents of Ca, Mg, Na, K, P, and Zn (mg/g of dry kidney) are presented in Table II. None of the parameters measured was affected by the drug at the 0.05 and 0.2% drug levels. At the 0.5% acetazolamide level only one parameter, renal Ca content, was affected; it was significantly ($p < .025$) increased from 0.31 to 0.58 mg/g of dry weight, an increase of 86%.

Discussion. We are reassured by these findings that acetazolamide fed to rats for a 3-week period at levels of 0.05 and 0.2% has no major overt toxicity related to mineral homeostasis or to nephrotoxicity. Although significant effects were noted at the 0.5% level with respect to plasma and renal Ca levels, we do not feel that the changes seen are of major pathological concern. It is quite possible, however, that feeding the drug for longer periods of time might result in more serious disturbances in mineral metabolism. But it is equally possible that doses lower than the 0.5% level known to prevent disuse osteoporosis (Conaway, Waite and Kenny)[2] might be as therapeutically effective. The 0.2% level, which appears to cause no major

[5] As described in the manufacturer's handbook: "Analytical Methods for Atomic Absorption Spectrophotometry." Perkin–Elmer Corporation, Norwalk, CT, 1966.

TABLE I. Effect of 0.05, 0.2, and 0.5% Acetazolamide in the Diet on Body Weight, Food Intake, Bone Defatted Dry Weight, and on Plasma Ca, Na, and K Levels in the Rat.

Acetazolamide in diet (%)	No. of rats	Time[a]	Body wt (mean ± SE; g)	Food intake (g/day)	Plasma levels (mean ± SE) (mg/100 ml) Ca	(mEq/liter) Na[b]	(mEq/liter) K	Humerus fat-free dry wt (mg ± SE)
0	10	0	174 ± 1.7	—				
		7	189 ± 2.3	—				
		27	199 ± 2.7	—	9.6 ± 0.12	134 ± 3.8	4.1 ± 0.58	223 ± 4.1
0.05	10	0	176 ± 1.4	14.9				
		7	182 ± 3.5	15.6				
		27	195 ± 2.8	15.1	9.8 ± 0.12	142 ± 4.6	4.2 ± 0.40	223 ± 3.0
0	9	0	173 ± 2.3	—				
		7	184 ± 3.5	—				
		27	194 ± 3.9	—	10.2 ± 0.16	149 ± 5.0	3.6 ± 0.25	218 ± 4.2
0.2	9	0	176 ± 1.8	14.4				
		7	187 ± 2.4	15.9				
		27	192 ± 2.7	13.7	10.5 ± 0.16	153 ± 5.5	3.0 ± 0.28	214 ± 2.9
0	9	0	174 ± 1.8	—				
		7	184 ± 3.9	—				
		27	182 ± 3.4	—	9.7 ± 0.16	150 ± 5.4	3.6 ± 0.49	202 ± 3.8
0.5	9	0	172 ± 1.4	13.8				
		7	184 ± 2.3	15.4				
		27	175 ± 3.5	12.6	10.6 ± 0.09[c]	143 ± 3.1	2.9 ± 0.26	206 ± 3.6

[a] All rats were fed control (acetazolamide-free) diet *ad libitum* until day 7 and then pair-fed control or acetazolamide diet days 7–27.

[b] Although Na was present in the heparin used, its contribution was considered negligible.

[c] $p < .001$, compared with pair-fed controls.

TABLE II. Effect of 0.05, 0.2, and 0.5% Acetazolamide in the Diet on Renal Ca, Mg, Na, K, P, and Zn Contents in the Rat.

Acetazolamide in diet[a] (%)	No. of rats	Renal contents (mean ± SE; mg/g of dry kidney)					
		Ca	Mg	Na	K	P	Zn
0	9	0.30 ± 0.02	0.87 ± 0.01	6.7 ± 0.16	10.9 ± 0.14	14.6 ± 0.41	0.124 ± 0.006
0.05	8–10	0.43 ± 0.13	0.91 ± 0.55	6.6 ± 0.20	11.0 ± 0.09	15.8 ± 0.61	0.128 ± 0.004
0	9	0.34 ± 0.03	0.86 ± 0.02	7.3 ± 0.26	10.5 ± 0.27	15.2 ± 0.78	0.124 ± 0.008
0.2	9–10	0.45 ± 0.05	0.89 ± 0.02	6.6 ± 0.18	10.8 ± 0.18	15.2 ± 0.68	0.110 ± 0.004
0	8–9	0.31 ± 0.01	0.86 ± 0.02	6.8 ± 0.20	10.8 ± 0.26	15.4 ± 0.35	0.131 ± 0.005
0.5	9	0.58 ± 0.10[b]	0.91 ± 0.04	6.8 ± 0.26	11.0 ± 0.20	16.4 ± 0.62	0.124 ± 0.005

[a] Rats were pair-fed diets for 20 days.

[b] $p < .025$, compared with pair-fed controls.

disturbance in mineral metabolism (Tables I and II), would be an obvious choice in future therapeutic studies in rats.

Maren, Mayer, and Wadsworth (5) studied the toxic effects of acetazolamide when given in the diet at doses of 100, 300, and 900 mg/kg of body weight/day to rats for extended periods up to 20 weeks. The levels used in the present study may be converted to these units for the purpose of comparison. Assuming a body weight of around 175 g and a food consumption of around 13 g/day (Table I) then the acetazolamide intake was approximately 37, 149, and 371 mg/kg of body weight/day at the 0.05, 0.2, and 0.5% levels, respectively. Our findings (Fig. 1) are in accord with those of Maren, Mayer, and Wadsworth (5) who found that at the 100 mg/kg/day dose growth was hardly retarded, whereas at the 300 and 900 mg/kg/day doses growth was progressively retarded. Our pair-feeding data support the suggestion made by Maren, Mayer, and Wadsworth (5) that the retardation in growth was mainly due to reduced food intake; the body weights of the experimental and pair-fed controls did not differ significantly (Table I).

The conclusion, from the present study, that acetazolamide results in no major nephrotoxic effect is based on chemical analysis. No histological examinations were carried out. Maren, Mayer, and Wadsworth (5) had found no morphological evidence of nephrotoxicity in rats receiving up to 300 mg/kg/day of acetazolamide. Our data and those of Maren, Mayer, and Wadsworth (5) are at variance with the report of Harrison and Harrison (6) who found that young rats fed 0.25% acetazolamide in diets containing 2 different Ca/P ratios for 3 weeks developed nephrocalcinosis. No simple explanation of this discrepancy can be readily advanced; perhaps it lies in the age of the rats or in the nature of diets used.

The hypercalcemic effect of 0.5% acetazolamide under pair-feeding conditions has been observed by us in another study (Conaway, Waite and Kenny)[2] but has not been reported elsewhere in the literature. The basis for this response is not known.

Summary. Acetazolamide sodium, when incorporated in the diet at concentrations of 0.05 and 0.2% and pair-fed to female Carworth rats for 20 days, had no significant effects on plasma Ca, Na, and K levels, the defatted dry weights of the humerus, or the renal Ca, Mg, K, Zn, and P levels. Growth rate was moderately and progressively inhibited with increasing dosage. At the 0.5% level, which other studies have shown to prevent the development of disuse osteoporosis in rats, only two parameters, plasma Ca and renal Ca, were affected. Plasma Ca was increased from 9.7 to 10.6 mg/100 ml ($p < .001$); renal Ca content was increased from 0.31 to 0.58 mg/g of dry weight of kidney ($p < .025$). It is concluded that acetazolamide fed to female rats for a 3-week period has no major toxicity related to mineral metabolism at dietary levels equal to and below that level which has been shown to prevent disuse os-

teoporosis in rats. These findings further enhance the potential of acetazolamide as an orally effective therapeutic agent for the control of osteoporosis of disuse in man.

The author acknowledges the technical assistance of Richard Lane.

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Received Jan. 12, 1972, P.S.E.B.M., 1972, Vol. 140.