Effects of Acetazoleamide, Chlorothiazide, and Dichlorphenamide on Electrolyte Excretion in the Alligator.* (23552)

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Although the theory that potassium and hydrogen compete for renal tubular excretion (1) is of considerable value in explaining some of the functions of carbonic anhydrase in the mammal, it is inadequate to explain the action of this enzyme in the alligator. Inhibition of carbonic anhydrase by acetazoleamide in the mammal leads to acidemia with an increased excretion of both bicarbonate and sodium. In the alligator, inhibition promotes an alkalemia as a result of excessive chloride excretion with little change in sodium loss(2). Diamox (acetazoleamide) enhances the excretion of K in both mammals(1,3) and alligators(4) in spite of the fact that it decreases the urinary H ion in the mammal and increases it in the alligator.

Several experiments were designed to determine if increased K output is directly related to the increased urinary hydrogen ion excretion. It was also of interest to see if prolonged administration of Diamox to alligators could either induce a refractory response to the drug or if the continued daily loss of plasma chloride would result in a gradual decrease in Cl excretion. In the course of these investigations 2 new carbonic anhydrase inhibitors(5,6). Diuril (chlorothiazide), and DCPA (dichlorphenamide) were made available. Their effects on the alligator are reported.

Methods. About 150 different 2 kg alligators were used in these experiments. The animals were fasted for 4 days before any experiment to minimize the effect of a rather considerable "alkaline tide" and were maintained in this condition throughout any given experiment. To ensure a relatively uniform degree of hydration they were kept in a tank of water for 24 hours before the beginning of each experiment. The chemical methods employed for the analysis of the samples have been described(2,7). In the short term experiments urines were collected in an ice bath and frozen immediately in a deepfreeze until the time for analysis. Blood was drawn by intracardiac puncture, heparinized, centrifuged in the cold, and the plasma separated and kept frozen until it could be analyzed. The experiments were done at a temperature of 28° ± 2°C and at an average relative humidity of 60%.

Results. To determine the effect of prolonged injections of Diamox (50 mg/kg), 12 alligators were placed in metabolism cages where they received the drug in aqueous suspension every other day for 30 days. The daily urine volume was measured and one fresh catheterized sample was obtained each day for estimation of the volatile NH_3 and CO_2.

It was desirable to obtain data on carbonic anhydrase inhibition in both "wet" and "dry" alligators because of the fact that there is an increase in excretion of both NH_3 and CO_2 following hydration and a decrease in these ions during dehydration(7). Therefore 6 of the animals were allowed free access to distilled water for a period of one hour each day in order to maintain a moderate degree of dehydration and 6 were overhydrated by the injection of enough water each day to maintain the original weight of the animals. Fig. 1 shows the total meq/kg of various urinary constituents excreted over the 30-day period. In the groups given Diamox there was a considerable increase in K and Cl excretion and no important change in Na in either the "wet" or "dry" experiments. Although an increase in K excretion occurred along with an increase in H ion excretion, there was no evidence of a direct relationship between degree of decrease in urine pH and amount of K in a given sample. While the urine pH increased

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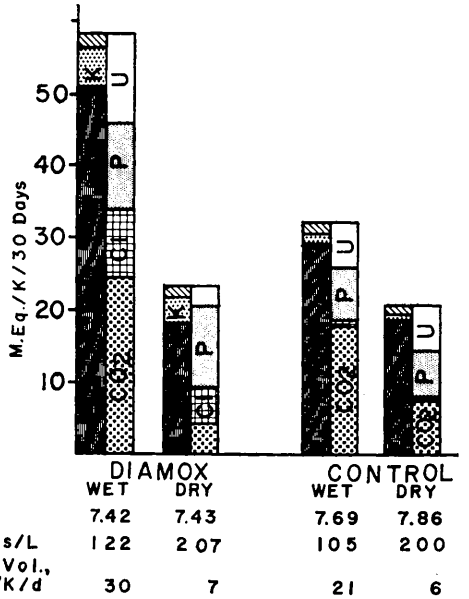

FIG. 1. Effect of intraper. inj. of 50 mg/kg of Diamox every other day for 30 days on urine output. Each bar graph represents total amount excreted for 30 days. Two separate experiments are represented, one over-hydrated and one slightly dehydrated. Six alligators were used in each experiment. Cross-hatched area above the K represents Na.

somewhat in the last days of the experiments, the elevated Cl excretion persisted for the full 30 days in spite of decreased plasma Cl and an increased plasma pH. It was concluded that there was no clear cut evidence of development of a tolerance to the drug and that the chloruretic action of Diamox was largely independent of minor changes in plasma composition.

Blood was drawn at the beginning, at 15 days, and at 30 days of the long term experiment on Diamox. There were no significant changes in K or indeed in any of the ions in the plasma other than Cl and CO_2. By the 15th day there was a 11 meq/l fall in plasma Cl and a reciprocal rise of 11 meq/l in plasma bicarbonate. The 50% increase in bicarbonate is evidence of an alkalemia which is about as marked as the acidemia reported to be the result of repeated injections of Diamox to mammals.

After Diuril and DCPA were made available it was decided to determine if the observed effects were unique for inhibition of carbonic anhydrase by Diamox or if they were the same regardless of the means of inhibition. A pilot experiment was performed by injecting aqueous suspensions of each drug in doses of 0.1, 1, 10, 25, and 50 mg/kg to get a crude estimate of the minimal effective dose. Using the results from the preliminary experiment groups of alligators were given 1 mg/kg (the marginal dose) and other groups received 50 mg/kg (the dose for maximal effect).

The experiments were repeated on large numbers of alligators until the average daily urine flow for any one group varied by no more than 20% from the average of the other groups. Although control of the urine volumes reduced the random variation in total electrolyte excretion, it was not always possible to reproduce the same osmotic pressures. If one uses the daily output of salts as an index of the activity of a compound, a high osmotic pressure is associated with a high excretion of Na, K, Cl, CO_2 and NH_3, which tends to exaggerate the effects. If, however, the Na and K are compared with the NH_3, and the Cl is compared with the CO_2, the effect of any particular compound on the excretion pattern is immediately apparent. A graph was therefore employed (Fig. 2) which illustrates the *ratio* of Na and K to NH_3 and of Cl to CO_2. It is evident that any increase in Na and K must be at the expense of NH_3 and that any increase in Cl must be at the expense of CO_2. The first 3 columns show the maximum range of variation which we have found for the 40 controls whose average appears in column II. It would be unwise to attach much significance to any change fall-

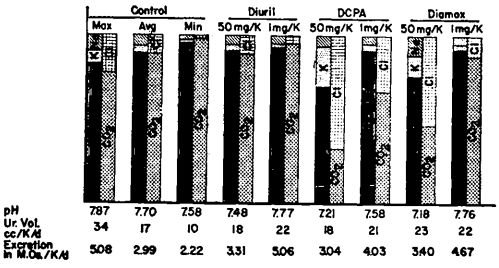

FIG. 2. Ratio of Na and K to NH_3 and of Cl to CO_2 in alligator urine. Each bar graph represents the avg for a total collection of 3 consecutive days. Each alligator received 13 cc of water/kg. There were 40 alligators in the control group and 15 or more in each of the other groups.

ing within the range of variation of the controls.

Diamox and DCPA were both effective in the larger dose in inhibiting CO_2 output and in producing a chloruresis whereas Diuril proved to be inactive. The inactivity of Diuril is in keeping with the observation by Moyer *et al.*(8) that its saluretic properties in mammals were not associated with carbonic anhydrase inhibition. Diamox is certainly less active than DCPA and at a level of 1 mg/kg the significance of the change in Cl in the Diamox group is highly questionable. One surprising feature of the action of DCPA is its ability to cause a much greater increase in K excretion than that found for Diamox. In the larger doses, the greatest K loss in any alligator receiving Diamox was below that found for the least change in any alligator injected with DCPA. On the other hand a slight and possibly significant rise in Na follows the injection of large doses of Diamox but not DCPA. Whereas 1 mg kg of DCPA was enough to cause a 3-fold increase in Cl excretion, it was insufficient to change the K excretion which makes it appear that depression of CO_2 production and enhancement of Cl excretion is not necessarily associated directly with K loss.

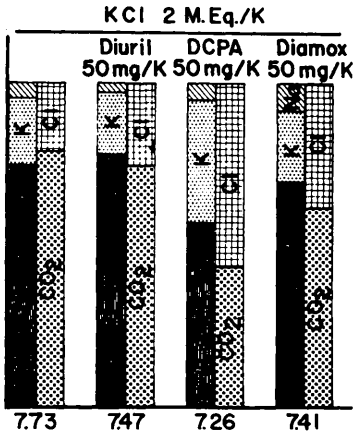

FIG. 3. Effect of Diamox, Diuril and DCPA on urinary ratio of Na and K to NH_3 and Cl to CO_2 in alligators primed with 13 cc of isotonic KCl/kg. There were 15 alligators in each group. Each bar graph represents the avg for a total collection of 3 consecutive days.

On the theory that all 3 compounds could influence K excretion if the plasma level was elevated, the high dose experiments were repeated on alligators primed with 2 meq/kg of KCl. The results are shown in Fig. 3. Increasing the plasma K level did not alter the relative effectiveness of the three compounds. Diuril was still inactive and DCPA was the most active as before.

Discussion. The decrease in CO_2 production caused by Diamox and DCPA is most probably the result of a direct inhibitory effect of these compounds on the tubular production of CO_2 by carbonic anhydrase. A decrease in renal CO_2 production in the alligator inevitably produces an increase in Cl excretion. The existence of a direct relationship between K excretion and carbonic anhydrase inhibition in the alligator is by no means certain. Neither is there any evidence of a positive correlation between the changes in urinary H^+ concentration and the quantity of K appearing in the urine. In view of the above it would appear that the differences in the effects of Diamox and DCPA are an indication that the actions of carbonic anhydrase are more complex than was suspected, or that the individual effects are not necessarily associated with carbonic anhydrase inhibition but with unknown reactions associated with differences in their respective molecular structures.

That something more than simple carbonic anhydrase inhibition is involved is illustrated in Table I which compares the effects of the 3 compounds on dogs(9,10) and alligators. It would appear that the knowledge that a compound will inhibit the carbonic anhydrase in horse erythrocytes provides little help in predicting the action of the compound on intact animals.

Summary. Prolonged administration of acetazoleamide to alligators leads to the excretion of considerable amounts of chloride which produces an alkalemia. A continued K excretion also occurs which has little effect on the plasma level, presumably due to a removal of K from the cells in an effort to compensate for the loss. No evidence of the development of a refractory state to acetazole-

TABLE I. Comparison of Effects of 3 Different Carbonic Anhydrase Inhibitors on Urine Electrolytes of the Dog and the Alligator.

Compound	Dog					Alligator				
	Na	Cl	K	CO_2	H	Na	Cl	K	CO_2	H
Chlorothiazide	++	++	+	+	—	0	+?	0	—?	+?
Dichlorphenamide	++	+	+	+	—	0	+++	++	——	+
Acetazoleamide	++	0?	++	++	——	+?	+++	+	——	+

Direction of the change is indicated as follows: +++, great increase; +, increase; ——, great decrease; —, decrease; 0, little or no change.

amide after 30 days of injections was noted nor was there any sign that the decreased plasma Cl prevented the excretion of Cl. The degree of K loss was not directly correlated with hydrogen ion excretion. Two new carbonic anhydrase inhibitors, chlorothiazide and dichlorphenamide were tested. Dichlorphenamide was more active in promoting K and Cl loss at low dose levels than acetazoleamide. Chlorothiazide showed no significant activity. Since the effects noted after the inhibition of carbonic anhydrase by acetazoleamide and dichlorphenamide are not the same, it is probable that something more than simple inhibition is involved.

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Blood Epinephrine Levels and Automatic Reinfusion of Blood During Hemorrhagic Shock in Dogs.* (23553)

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Vasoconstriction is a prominent feature of the early stages of hemorrhagic shock in dogs. Most authorities agree that this is initially a protective mechanism mediated through the carotid sinus to maintain a blood supply to vital organs. However, if this vasoconstriction is of adequate severity and duration it is possible for the ischemia to produce irreversible tissue damage(1). This concept is supported by the fact that several investigators have produced shock by the intravenous infusion of epinephrine(2), and that adrenergic blocking agents protect dogs against hemorrhagic shock(3). Various procedures for producing hemorrhagic shock in dogs have been standardized by Wiggers(4) and other investigators. When dogs are bled to a mean arterial pressure in the range of 40-60 mm Hg by means of an arterial pressure compensator (5) spontaneous reinfusion will commence in about one hour and continue until the animal

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