activity. However, in the case of serum S-1 it would appear as though the activity resided almost entirely in the α_2 component. It was also of interest that this preparation contained some material that combined with ovalbumin although the patient failed to show any clinical history of egg sensitivity. Some of the fractions were tested for diphtheria antitoxin activity, typhoid agglutinins, and blood group specific antibodies. The antitoxin and typhoid agglutinins were always present when gamma globulin predominated and the blood group agglutinins were always strongest in the T-2 fractions, as would be expected from previous work. Of the four patients not sensitive to egg white, only one produced a serum fraction which combined with ovalbumin (the BG-2 fraction of S-1 serum). All the M-1 serum fractions failed to combine with ovalbumin, but it was later found that this individual was sensitive only to the ovomucin component of egg white. Although some of the apparent discrepancies in the relation of PK titers to the relative concentration of serum components may be the result of error inherent in the method of titration, there is also a possibility that inhibiting substances may occur in serum. Since these serums all came from individuals who had no previous history of treatment, one would not expect to find classical "neutralizing" antibodies to be present.

References

1. CAMPBELL, D. H., *et al. J. Allergy* **21**, 519 (1950).
2. COOKE, R. A., *et al. Ibid.* **22**, 211 (1951).
3. LOVELESS, MARY H., and CANN, J. R. *Science* **117**, 105 (1953).
4. MILLER, H., and CAMPBELL, D. H. *Ann. Allergy* **5**, 236 (1947).
5. CANN, J. R., BROWN, R. A., and KIRKWOOD, J. G. *J. Am. Chem. Soc.* **71**, 1609 (1949).
6. LONGSWORTH, L. G., and MACINNES, A. *Ibid.* **62**, 705 (1940).
7. LANNI, F., and CAMPBELL, D. H. *Stanford Med. Bull.* **6**, 97 (1948).

Manuscript received November 20, 1953.

The Effect of Carbonic Anhydrase Inhibition on the Composition of Urine and Plasma of the Alligator

Thomas Hernandez and Roland A. Coulson

Department of Biochemistry, Louisiana State University School of Medicine, New Orleans

The administration of a potent carbonic anhydrase inhibitor (6063 or Diamox 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide)[1] causes a marked increase in excretion of Na, K, water, and bicarbonate, a decrease in titratable acidity, and an increase in urine pH in the dog, man, rat, and freshwater catfish (*1–3*). According to modern theory, carbonic anhydrase seems to be essential in mammals for acid secretion by the renal tubules, the H ions competing with Na and K for excretion (*4*). Carbonic anhydrase enables these animals to produce an acid urine and so conserve base.

The electrolytes normally present in the plasma of the alligator (*A. mississippiensis*) (*5*) occur in approximately the same concentrations as those of the human yet the composition of alligator urine is quite dissimilar to that of mammals. Although the major share of the urinary nitrogen is excreted in the form of uric acid (*6*), enough ammonia is produced to maintain the urinary level at about 70 meq/l. In well-hydrated fasting alligators this ammonia is normally excreted with an almost equivalent amount of bicarbonate which is presumably produced through the influence of carbonic anhydrase in the nephron. Of the total osmotic pressure of the urine, which never exceeds that of the plasma, about two-thirds is due to the NH_4 and HCO_3 ions. As the alligator normally produces an alkaline urine (pH 7.80) due to the large amounts of NH_4 and HCO_3 ions, the proof of the existence of a base conservation mechanism in this animal is difficult to obtain.

It was felt that the injection of a carbonic anhydrase inhibitor into the alligator should change his urinary excretion pattern considerably. If carbonic anhydrase is necessary, directly or indirectly, for the production of both NH_4 and HCO_3 ions then the inhibition of carbonic anhydrase would lead to a great decrease in both these components in the urine. On the other hand, if carbonic anhydrase inhibition prevents the formation of bicarbonate in the tubule without affecting ammonia production the ammonia would then be excreted with a different anion.

In a series of experiments several alligators with an average weight of about 1.5 kg were placed in a tank of water for 24 hr to hydrate them. Some were then removed from the tank, injected with 50 mg of 6063 per kilo, and kept dry under the same condition as the controls for the next 4 days. Urine was obtained by catheterization at frequent intervals and was analyzed immediately or quick-frozen until the analyses were done. Sodium and potassium were determined by flame photometry, chlorides by a modification of the method of Schales and Schales (*7*), ammonia by the method of Conway (*8*), CO_2 content by the Van Slyke manometric method, and phosphorus by the method of Kuttner and Cohen (*9*). A Beckman pH meter with the glass electrode designed for blood analyses was used for all pH determinations. Figure 1 shows the effect of 6063 on the experimental alligators compared with controls during the first 24 hr.

It is apparent that carbonic anhydrase inhibition leads to a great decrease in CO_2 excretion without effecting a corresponding reduction in urinary ammonia. The chloride ion has been substituted almost quantitatively for the bicarbonate. This exchange, which is almost the exact reverse of that found in mammalian experiments, results in a decreased urinary pH and a mild alkalemia. By the 3rd day there was a rise of about 7 meq/l in plasma bicarbonate and a very slight and possibly insignificant fall in plasma chloride. No change in the other plasma electrolytes occurred.

[1] We wish to thank Lederle Laboratories Division of the American Cyanamid Company for a generous supply of Diamox.

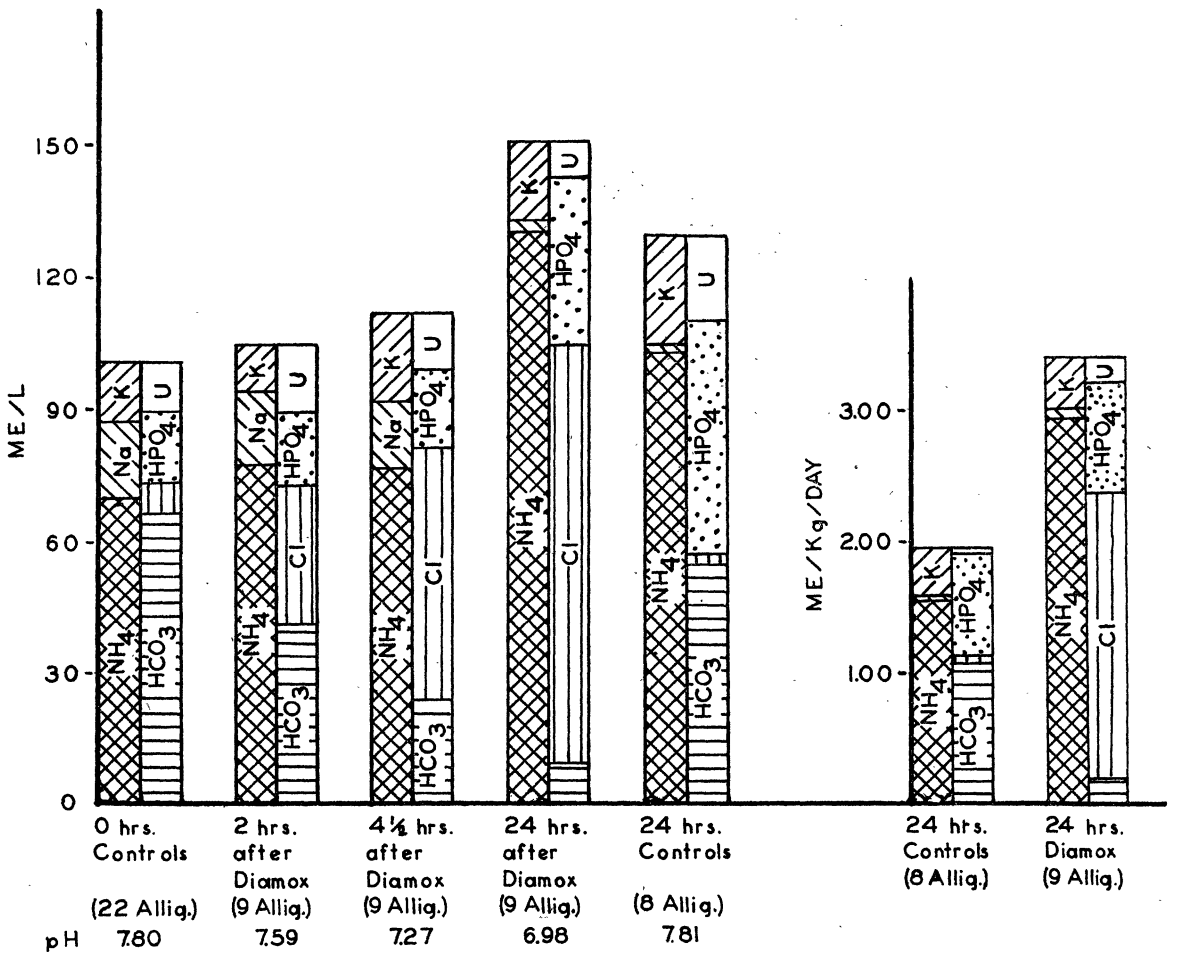

FIG. 1. The effect of 6063 on the urinary excretion pattern of the alligator. The average valence of HPO_4, used in determining the meq/l, was calculated from the observed urinary pH values. U represents undetermined anions.

Neither sodium nor potassium is excreted in increased amounts, which may account for the fact that 6063 has only a feeble diuretic action in the alligator. The dehydration due to 24 hr of water deprivation and the slight diuresis caused the urinary osmolar concentration of the experimentals to rise to approximately that of the plasma. No significant changes occurred in any of the other urinary constituents.

The changes in urinary composition due to a single injection of 6063 are apparent for at least 4 days. Repeated injections show progressively less effect; although the chloride excretion remains high the pH and the CO_2 content of the urine slowly rise. Reversion of the composition of urine electrolytes to the normal pattern after continuous administration of 6063 to dogs has also been noted by Maren (*2*).

References

1. BERLINER, R. W., KENNEDY, T. J., JR., and ORLOFF, J. *Am. J. Med.* **11**, 274 (1951).
2. MAREN, T. H. *Trans. N. Y. Acad. Sci.* **15**, 53 (Ser. II) (1952).
3. HEINEMANN, H. O., and HOLDER, J. E. *The Bulletin—Mount Desert Island Biol. Lab.* **4**, 42 (1953).
4. SMITH, H. W. *The Physiology of the Kidney.* New York: Oxford Univ. Press, 1937.
5. COULSON, R. A., HERNANDEZ, T., and BRAZDA, F. G. *Proc. Soc. Exptl. Biol. Med.* **73**, 203 (1950).
6. Unpublished observations.
7. SCHALES, O., and SCHALES, S. S. *J. Biol. Chem.* **140**, 879 (1941).
8. CONWAY, E. J. *Micro-diffusion Analysis and Volumetric Error.* New York: Van Nostrand Co., 1940.
9. KUTTNER, T., and COHEN, H. E. *J. Biol. Chem.* **75**, 517 (1927).

Manuscript received December 4, 1953.