Distribution of Carbonic Anhydrase in Alligator. Effect of Acetazolamide on Blood and Aqueous Humor CO_2. (25057)

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In the alligator the carbonic anhydrase inhibitor acetazolamide causes a more acid urine and a tendency toward alkalemia(1,2), an effect opposite to that seen after inhibition of carbonic anhydrase in mammalian kidney (3,4). It has been suggested that "renal carbonic anhydrase is necessary for conservation of chloride by the alligator"(2). As there seems to be no information on distribution of carbonic anhydrase in the reptile, it seemed interesting to see whether the enzyme is present in the reptile kidney and, whether distribution of carbonic anhydrase in tissues of these animals differs from that in the mammal. Qualitative differences in enzyme distribution among different species have been reported: *i.e.* in dogfish, *Squalus acanthias,* lens and kidney contain no carbonic anhydrase(5,6) in contrast to the mammal. It was also of interest to see whether CO_2 content of another carbonic anhydrase dependent secretion, *i.e.* aqueous humor, is changed in the same way as in the mammal following carbonic anhydrase inhibition.

Materials and methods. Ten caimans, (*C. laitrostris,* a South American alligator) averaging 90 g weight and 35 cm length, were fasted 3-4 days before experiments, but had access to water, at room temperature, 25°C ± 1. Experiments were done in April and May. A female alligator (*A. mississipiensis*) weighing 21 kg and measuring 160 cm was maintained in similar fashion to the caimans. Blood was obtained by heart puncture and aqueous humor, 0.7 ml from one eye at a time, was taken from anterior chamber. Acetazolamide, N.N.D., a carbonic anhydrase inhibitor (10% solution, Diamox®) was given intracardially, 50 mg/kg. A micromethod adaptation(7), scaled down 10-fold, of the method of *Philpot* and *Philpot*(8) was utilized in analysis of aliquots for presence of carbonic anhydrase. Tissues were weighed, diluted with double distilled water, and ground in glass homogenizer. The entire kidney, pancreas, and lens were used; gastric mucosa was dissected from the muscularis; the brain was stripped of vessels and cut just anterior to cerebellum; cortical tissue was removed from anterior brain before analysis; the choroid plexus was removed from the anterior brain; the ciliary processes were cut from the underlying connective tissues. After administration of acetazolamide, blood and aqueous humor samples were drawn anaerobically; pH determined (Cambridge pH meter, model R) at cloacal temperature of the reptile and CO_2 determined by micro Van Slyke (Microgasometer, model 600; Kopp-Natelson) method. The content of acetazolamide in plasma, saline washed red cells, and aqueous humor was determined by enzymatic method of *Maren et al.*(7).

Results. The enzyme results are tabulated in Fig. 1 and the inhibitor experiment is shown in Fig. 2. Distribution of carbonic anhydrase was qualitatively similar but quantitatively less than in the mammal(9) with the exception of the lens, where no activity was found. Such activity has, however, been reported in the chicken lens(10). The role of the enzyme in the lens is not understood(11).

The finding of carbonic anhydrase activity in the kidney explains the effects on composition of urine, observed following administration of carbonic anhydrase inhibitor(2). The comparatively long lasting effect (4 days) on alligator kidney, following acetazolamide, is explained by the very slow disappearance of the drug from plasma; the plasma half life was about 40 hours compared to 1½ hours in man. Sequestration of the drug into red cells is similar to that seen in the mammal (12). All of these pharmacological characteristics of acetazolamide are similar to those seen in *Sq. acanthias*(6). The steady state ratio of the acetazolamide concentration in the aqueous over that in plasma was about 0.8 compared to 0.05 in the rabbit(13).

The CO_2 content of reptile blood varies con-

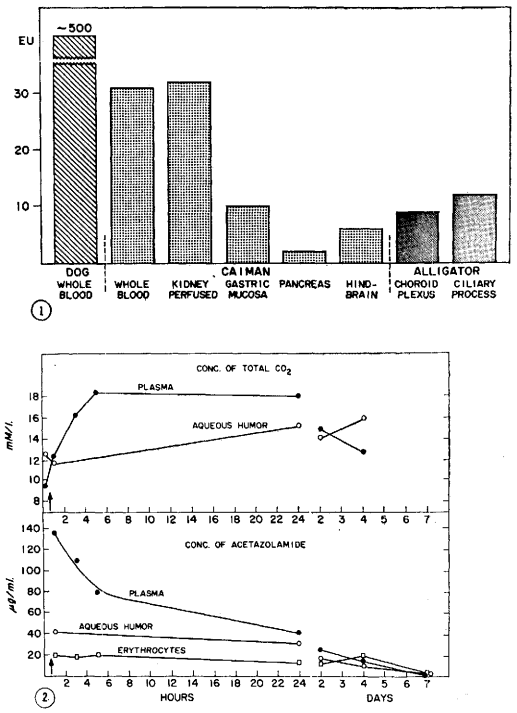

FIG. 1. Distribution of carbonic anhydrase in the caiman and alligator. Abscissa: different tissues. Each column represents avg value of tissues from 4 animals, except for the alligator where the column represents one animal. Ordinate: enzyme units (EU) as defined by Maren *et al.*(7).

FIG. 2. Distribution of acetazolamide in plasma, erythrocytes and aqueous humor following 50 mg/kg intracardially, to the alligator, and effect of this inj. on CO_2 content of plasma and aqueous humor.

siderably with season(14) and body temperature(15). It is also changed by food intake and excitement(16,17). Our values for plasma CO_2 (Fig. 2) are comparatively low. The sharp rise in plasma total CO_2 (Fig. 2) following acetazolamide has been seen in another species, the dogfish(5,6). This rise of plasma CO_2 is probably due to inhibition of both blood and kidney carbonic anhydrase in the alligator, but in the dogfish only to inhibition of the blood-gill system.

Total CO_2 of aqueous humor was higher than that of the plasma (Fig. 2), also seen in some mammals(18). Following acetazolamide, not much of a change was seen in the aqueous total CO_2, in spite of the rise in plasma, causing ratio of total CO_2 in the aqueous humor to that in plasma to fall considerably. The drop of this ratio is also seen in some mammals(19) but in those cases (*cf.* rabbit) the change is caused by more rapid drop of aqueous humor CO_2 as compared to that of plasma.

It is not possible from these experiments to tell whether acetazolamide changes the local production of CO_2 into the aqueous humor of the alligator, since such an effect could be masked by the simultaneous change of plasma CO_2.

Summary. Carbonic anhydrase was found in the caiman (*C. laitrostris*) erythrocytes, kidney, gastric mucosa, pancreas and hindbrain. No activity, however, was found in the lens. The enzyme was also found in the alligator (*A. mississipiensis*) choroid plexus and ciliary processes. Carbonic anhydrase inhibition in the alligator caused a long-lasting rise of plasma total CO_2 but no certain change of anterior aqueous total CO_2 was observed.

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Received May 28, 1959. P.S.E.B.M., 1959, v101.

Device to Control Constriction of Main Renal Artery for Production of Hypertension in Small Animals.* (25058)

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Various methods have been employed for production of experimental renal hypertension in the rat, but most of them have drawbacks. For small animals, the production and application of the original silver clamp devised for constriction of the main renal artery in the dog and other large animals(1) are impractical. The simplest method is the constriction of the main renal artery by a silver clip, or band(2-4). This method lacks adequate means of controlling degree of constriction of the artery. To this end, an instrument has been constructed with which this constriction can be made uniform and which facilitates production of acute and chronic hypertension in a high percentage of rats of equal weight. The instrument was fashioned from old type surgical needle holder. Through one jaw of instrument (Fig. 1, A) a screw (B) is inserted, the end of which comes in contact with inner surface of opposite jaw (C). Adjustment of this screw keeps the jaws apart to any desired extent, and turning of screw in one or the other direction controls the space left between the jaws, the width of which can be definitely determined. This is done by closing the jaws of forceps on blades of a mechanic's feeler gauge, graduated in fractions of an inch, in such a way that, when the screw touches the opposite jaw, the desired gauge blade can be slipped snugly into the space. By testing the effect of the forceps on a gauge blade of next size, larger and smaller, a check on correct adjustment can be made. The jaws of the instrument must be rigid toward the tip, and the surfaces which oppose one another must be machined perfectly flat and smooth. The silver clip (Fig. 1, D & E) for constriction of main renal artery is made from a strip of annealed, rolled, silver ribbon 2 mm wide and 0.127 mm thick. This strip is divided into sections measuring 6 mm in length, and the edges are filed round and smooth. The clip is then bent into a U-shape, over an ordinary pin, and the 2 ends are bent slightly outward (Fig. 1, E), to facilitate application.

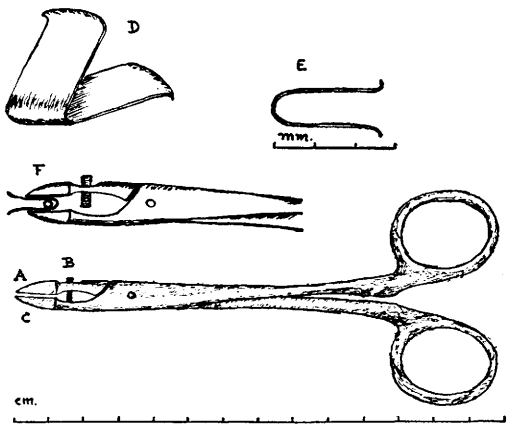

FIG. 1.

Application of clip. Through a costo-lumbar incision, with kidney retracted toward the abdomen, the renal pedicle is exposed, the artery is dissected clean, and the clip is slipped around it, near the aorta. The free edges of the clip are then approximated by means of a hemostat, to enclose the artery. With the jaws of instrument previously adjusted to desired gauge, about half of the "U" of the clip, including the rounded end, in which the ar-

* Supported by Grants U.S.P.H.S., and L. D. Beaumont Fn.

† I wish to thank Dr. Erwin Haas for advice and Louise Fingers for technical assistance.