water content of the tissue were not a factor in the present investigation since no change was detected with acclimation. Stangenberg (9) reports a significant increase in samples of muscle tissue taken from animals kept at 6°C. These had a higher water content than those at 20°C. If water is a factor, does this mean that metabolism could be increased by artificially increasing water content of a given tissue?

Summary. Total body metabolism of *Rana pipiens* and *Triturus viridescens* was determined in animals acclimated to cold and warm environments. Those animals adjusted to cold had a higher oxygen uptake when measurements were made at an intermediate temperature. Thyroidectomy had no effect on respiratory metabolism of Triturus. Tissue metabolism of skeletal muscle and stomach had higher oxygen consumption when taken from cold acclimated (5°C) animals than when obtained from warm adjusted (25°C). Liver showed no significant changes. Ventricle responded just the opposite from skeletal muscle and stomach. The wet/dry weight ratios of all 4 tissues did not change during acclimation.

1. Freeman, J. A., *Biol. Bull.*, 1950, v99, 416.
2. Suhrman, R., *Biol. Zentralbl.*, 1955, v74, 432.
3. Roberts, J. L., *Proc. XIXth int. Physiol. Congr.*, 1953, p706.
4. Bullock, T. H., *Biol. Rev.*, 1955, v30, 311.
5. Umbreit, W. W., Burris, R. H., and Stauffer, S. F., *Manometric Techniques and Tissue Metaoblism*, 1951, Burgess, Minneapolis.
6. Rogers, C. G., Textbook of Comparative Physiology 1938, McGraw Hill, N. Y.
7. Adams, E. A., Kuder, A., Richards, L., *J. Exp. Zool.*, 1932, v63, 1.
8. Fromm, P. O., *J. Cell. Comp. Physiol.*, 1955, v45, 343.
9. Stangenberg, G., *Arch. ges. Physiol.*, 1955, v260, 320.

Received December 12, 1959. P.S.E.B.M., 1960, v103.

CO_2 Retention in Anesthetized Dogs after Inhibition of Carbonic Anhydrase.* (25550)

STEPHEN M. CAIN† AND ARTHUR B. OTIS
Dept. of Physiology, College of Medicine, University of Florida, Gainesville

Berliner and Orloff(1) pointed out that an immediate effect of inhibiting the carbonic anhydrase of the blood "would be expected to be a diminution of the elimination of CO_2 and the accumulation of CO_2 in the body until a new steady state was reached." Such a steady state would require that elimination of CO_2 equaled its metabolic production. Measurements of CO_2 output 30 minutes or more after administration of carbonic anhydrase inhibitors revealed no such diminution(2,3). Mithoefer(4) made frequent measurements prior to 30 minutes, but found CO_2 output to be below the control measurement only in a dog in which ventilation was held constant. In the experiments reported here, particular attention was paid to the changes in CO_2 output occurring during the first 30 minutes after injection of a carbonic anhydrase inhibitor.

Methods. Dogs anesthetized with sodium pentobarbital were intubated with a tracheal cannula having an inflatable cuff so that an airtight seal was made within the trachea. Expired gas was collected in a Douglas bag from a respiratory valve attached to the cannula. Analyses of CO_2 and O_2 in duplicate samples of expired gas were made with a thermal conductivity type of CO_2 analyzer (Gow-Mac Gas Master) and a Beckman Model C oxygen analyzer. The volume of gas remaining in the Douglas bag was measured by a dry test meter, and corrected for the vol-

* Supported by Contract with School of Aviation Medicine, Brooks Air Force Base, Texas.

† Present address: Dept. of Physiology-Biophysics, School of Avia. Med., Brooks Air Force Base, Texas.

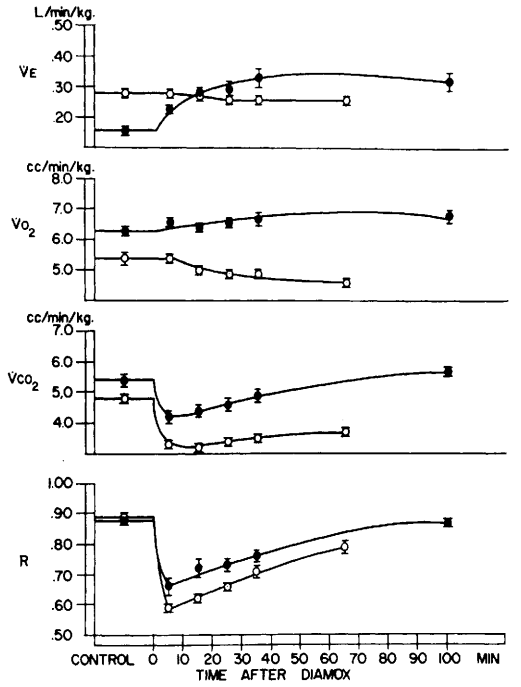

FIG. 1. Ventilation ($\dot{V}_E$), O_2 uptake ($\dot{V}O_2$), CO_2 output ($\dot{V}CO_2$), and respiratory exchange ratio (R) of dogs which ventilated spontaneously (●) and of dogs in which ventilation was controlled (○). Each point for the former is a mean of 6 animals and for the latter, 7 animals. Vertical bars represent ± 1 stand. error of mean.

umes taken for analysis. Expired minute volume of ventilation, O_2 uptake, CO_2 output, and respiratory exchange ratio were calculated from these data. Two series of experiments were conducted. The 6 dogs studied in Series A ventilated spontaneously. Collections of expired gas were made at least twice during the control period, at the end of which 50 mg/kg of acetazolamide‡ were injected intravenously. Five-minute collections of expired gas were completed at 5, 15, 25, 35, and 100 minutes after injection. In Series B, 7 dogs were used. Ventilation was kept constant with an intermittent positive pressure respirator supplied from a compressed air line. The dogs were not curarized, but were moderately hyperventilated in order to reduce voluntary respiratory efforts. Analyses and collections of expired gas were the same as for Series A dogs, except that the final collection was made 65 minutes after injection of actazolamide.

‡ The drug was injected as the sodium salt and contained 1.6 moles of NaOH for each mole of acetazolamide.

Results. Because there was little variation in the control measurements of individual dogs, and because collections of expired gas during the control periods of different dogs were not made at set intervals, all control measurements were pooled into a single average for each series (Fig. 1). After injection of acetazolamide, the ventilation of series A dogs increased within 5 minutes and was double the control value by 30 minutes. Because ventilation was artificially regulated in Series B dogs, there was little change from the control level.

O_2 uptake of Series A dogs tended to increase, but the greatest individual increase amounted to only 18% above the control. The dogs of Series B, on the other hand, showed a general trend toward a decrease in O_2 uptake after the drug. Much of the decrease was accounted for by one dog, in which O_2 uptake decreased 40% by the end of the experimental period. The other dogs in Series B showed much less change.

CO_2 elimination dropped sharply, on the average, in both series of dogs after injection of acetazolamide, although one individual in Series A showed no reduction. During the first 5 minutes, mean values for CO_2 elimination were 78% of control value in Series A and 69% of control value in Series B. Both of these changes were highly significant ($P < 0.01$). Following the initial drop, CO_2 elimination gradually rose. After about 60 minutes, the dogs of Series A reached control level of CO_2 output, but Series B dogs had not returned to control level by the end of the experimental period.

The respiratory exchange ratio decreased sharply in both series of animals after injection of acetazolamide, and then gradually returned toward normal. On the average, the dogs in which ventilation was held constant dropped to a lower ratio and were slower to return toward the control ratio. The indications were that both series would return to the control ratio, but during the experiment only Series A dogs actually reached it at 100 minutes.

Discussion. A decrease in CO_2 output when O_2 uptake is steady or rising and when ventilation is increasing can only mean an interference with the elimination of CO_2. The depression of CO_2 output in Series A dogs when ventilation was increased, and the greater depression in the dogs in which ventilation was controlled, show not only a retention of CO_2 relative to the ventilation, but also an absolute retention of metabolically produced CO_2. Mithoefer(4) reported similar experiments on one dog and found no reduction in CO_2 output, although alveolar ventilation increased 2-fold. Of the 6 dogs in Series A, only one was able to maintain CO_2 output at control level after acetazolamide. The other 5 dogs all had a decrease in CO_2 output in spite of increasing the total ventilation as much as 2-fold.

The CO_2 which was retained was the difference between rate of CO_2 production by the tissues and rate of CO_2 elimination at the lung. By assuming that metabolic production of CO_2 continued in the same proportion to O_2 uptake after acetazolamide as before, it was possible to estimate the volume of CO_2 retained. This was done by extending a line from control CO_2 output in Fig. 1 parallel to O_2 uptake. The area between the extrapolated line and the CO_2 output curve at any time was the volume of CO_2 retained at that time. For Series A mean volume of retained CO_2 at 60 minutes was 23 cc/kg, and for Series B, at 65 minutes, 79 cc/kg.

By assuming that the retained CO_2 would increase the CO_2 stores of the body just as any CO_2 loading, it was possible to estimate the rise in mean tissue P_{CO_2}. Before this could be done, 2 corrections had to be applied to volume of CO_2 retained. The first was for amount of CO_2 bound by the NaOH injected with the acetazolamide. The second correction was to account for the CO_2 which would remain in the blood. The remainder of the retained CO_2 presumably was taken up by the tissues. When the volume of CO_2 retained in the tissues was divided by the slope of the dissociation curve for the tissues of the dog as determined by Farhi and Rahn(5), the rise in mean tissue P_{CO_2} was 10 mm Hg for Series A and 47 mm Hg for Series B.

Because the only difference in treatment of the 2 groups of dogs was maintaining ventilation constant in Series B, the increased ventilation of Series A evidently was an important factor in compensating for inhibition of carbonic anhydrase. If the gradient for P_{CO_2} from the tissues to the alveolar gas is considered, it is clear that it can be increased either by raising the P_{CO_2} of the tissues or by lowering the alveolar P_{CO_2}. Series A dogs increased the gradient by both means. There was a greater rise in mean tissue P_{CO_2} of Series B dogs who could not lower alveolar P_{CO_2} by increasing ventilation. The conclusion reached is that when carbonic anhydrase is inhibited, an increase in the gradient for P_{CO_2} from the tissues to the lungs is essential for reestablishment of a steady state of CO_2 elimination.

Summary. CO_2 elimination of anesthetized dogs was temporarily reduced after injection of carbonic anhydrase inhibitor. Reduction was greater for dogs in which ventilation was held constant than for dogs ventilating spontaneously. Results indicate that increased gradient for P_{CO_2} from tissues to the alveolar gas is essential for reestablishing a steady state of CO_2 elimination when carbonic anhydrase is inhibited. The required increase in this gradient can be produced by means of augmented ventilation, by increased tissue P_{CO_2}, or by combination of these 2 factors.

The authors wish to thank Richard F. Leeds and L. Paul Travis for technical assistance.

Acetazolamide (Diamox) was furnished by Lederle Labs., Am. Cyanamid Co.

1. Berliner, R. W., Orloff, J., *Pharm. Rev.*, 1956, v8, 137.

2. Tomashefski, J. F., Chinn, H. I., Clark, R. T., *Am. J. Physiol.*, 1954, v177, 451.

3. Carter, E. T., Clark, R. T., *J. Appl. Physiol.*, 1958, v13, 42.

4. Mithoefer, J. C., *ibid.*, 1959, v14, 109.

5. Farhi, L., Rahn, H., *Studies in Respiratory Physiology*, Wright Air Development Center Tech. Rep. 55-357, 1955, p268.

Received December 14, 1959. P.S.E.B.M., 1960, v103.