Immediate Decrease in Respiratory $C^{14}O_2$ Excretion Following Simultaneous Intravenous Administration of $NaHC^{14}O_3$ and Acetazoleamide in the Rat. (26633)

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Speculating on the metabolic sequelae of carbonic anhydrase inhibition, Berliner and Orloff(1) concluded that the effects of carbonic anhydrase inhibitors on CO_2 elimination would be an immediate, though transient, decrease in CO_2 output and the appearance of a difference between alveolar CO_2 tension and that found in arterial blood. Since that time different authors have reported variable effects of the potent carbonic anhydrase inhibitor, acetazoleamide, on output of respiratory CO_2(2-9). The consensus of these various studies is that although acetazoleamide causes an initial hyperventilation that may produce an increased CO_2 output of brief duration, CO_2 output is usually transiently depressed due to inhibition of erythrocyte carbonic anhydrase. The investigation reported here concerns the influence of acetazoleamide on excretion of $C^{14}O_2$ derived from C^{14}-labeled bicarbonate using a device which continuously measures the radioactivity of expired air. Our method allows direct demonstration of Berliner and Orloff's hypothesis in the intact animal, without necessitating recourse to indirect estimates of CO_2 movement based on application of acid-base equations.

Methods. Female Sprague-Dawley rats weighing 65-85 g were starved overnight prior to experiment. Four experimental animals were injected intravenously with 0.1 μc of $NaHC^{14}O_3$ (2.39 mC/mmol in 0.1 cc), followed immediately by acetazoleamide-sodium†, 70 mg/kg (in 0.5 cc/100 g body weight). Nearly-simultaneous injection of $NaHC^{14}O_3$ and acetazoleamide-Na (or saline) was accomplished by means of a double syringe with a 3-way stopcock. This procedure allowed essentially simultaneous administration of the two materials with no variation in $NaHC^{14}O_3$ dosage. Four control animals were injected with $NaHC^{14}O_3$ and saline in the same manner.

Similar animals were pretreated with intraperitoneal acetazoleamide (20 mg/kg) at 30 minutes prior to administration of intraperitoneal $NaHC^{14}O_3$ (5 μc/kg) and their production of respiratory $C^{14}O_2$ likewise studied.

Immediately after injection of the radiotracer each animal was placed in a sealed container attached to a continuous $C^{14}O_2$ monitor. The apparatus, described in detail elsewhere(10), supplies the animal chamber with a constant flow of CO_2-free air. The outflow of the chamber passes through a 4 π G-M counter‡ connected to a scaler§, a rate meter‖ and a graphic recorder¶. The graphic data are correlated with the total counts accumulated on the scaler. After corrections for background and counter efficiency, the per cent of dose recovered per unit of time can be calculated.

Results. The results are presented in Fig. 1 and Table I. When $NaHC^{14}O_3$ and acetazoleamide (70 mg/kg) were given nearly simultaneously *i.v.*, there was an obvious disparity between the curves for treated and control animals (Fig. 1). The difference be-

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† Supplied as the sodium salt (Diamox), by the Lederle Laboratories Division, American Cyanamid Co.

‡ Model 4P5, Instrument and Development Products Co., Chicago, Illinois.

§ Model 162A, Atomic Instrument Co., Cambridge, Massachusetts.

‖ Model 1620A, Nuclear Chicago Corp., Chicago, Illinois.

¶ Recti-riter, Texas Instruments, Inc. Houston, Texas.

TABLE I. Cumulated Per Cent of Injected Dose. Data expressed as mean ± mean deviation.*

Time after $NaHC^{14}O_3$ inj., min.	Intraper. $NaHC^{14}O_3$		Intrav. $NaHC^{14}O_3$	
	Saline control (3)	Acetazoleamide, 20 mg/kg I.P. (4)	Saline control (4)	Acetazoleamide, 70 mg/kg I.V. (4)
5	6.12 ± 1.32	6.80 ± 1.48	13.78 ± 1.80	7.69 ± 0.50
10	21.61 ± 3.46	20.50 ± 0.68	30.92 ± 3.74	23.49 ± 1.48
15	32.04 ± 5.19	31.56 ± 1.95	39.57 ± 5.10	34.46 ± 2.70
30	45.06 ± 7.39	46.96 ± 5.79	50.46 ± 5.95	51.21 ± 5.05

* No. of animals indicated by parentheses.

tween the rate curves at 5 minutes was highly significant ($p<0.01$). Similarly, the difference between the cumulated curves at 10 minutes was significant at $p<0.05$. By 15 minutes the marked changes had subsided.

Discussion. These experiments indicate that the effect of a potent carbonic anhydrase inhibitor on excretion of carbon dioxide can be demonstrated when such an inhibitor, acetazoleamide, and bicarbonate are injected simultaneously intravenously. When the drug is administered 30 minutes prior to injection of the source of tracer $C^{14}O_2$, the inhibitory effect on expired CO_2, although evident, is not significant. The inconclusive result may be due to the large variance of the experimental values (Table I); or may be the consequence of establishment of a new equilibrium among the different forms of carbon dioxide in blood and tissues. The discrepancies between the 30-minute pretreated and the simultaneously treated acetazoleamide animals are consistent with Mithoefer's observations in the dog(8), that the primary effect of acetazoleamide on respiratory CO_2 excretion is manifest very quickly and subsides in 20 minutes.

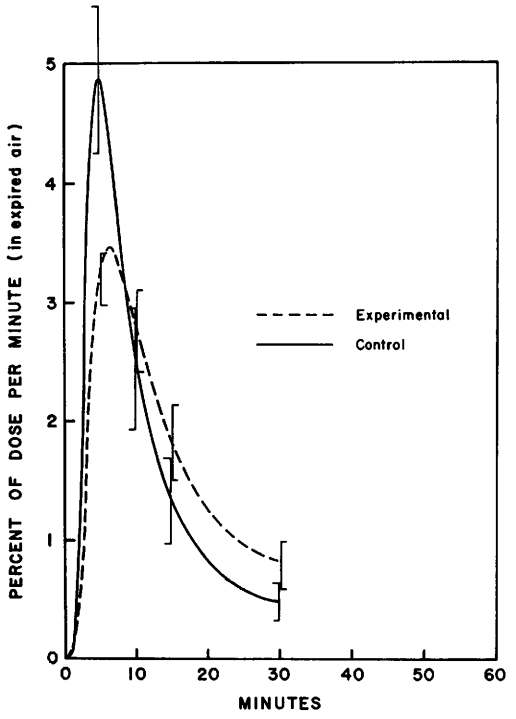

FIG. 1. Rate of excretion of $C^{14}O_2$ in expired air following intravenous injection of $NaHC^{14}O_3$. Experimental animals (broken line) were administered acetazoleamide, 70 mg/kg, simultaneously with bicarbonate. Points on smoothed curves represent means ± standard deviations.

Summary. The effect of acetazoleamide on rate of expiration of carbon dioxide was studied in rats using carbon-14 labeled bicarbonate. A marked transient reduction in expired $C^{14}O_2$ was observed during the first 10 minutes after simultaneous intravenous injection of the drug (70 mg/kg) and $NaHC^{14}O_3$. The rate of appearance of $C^{14}O_2$ was reduced 35 ± 7% in the treated animals 5 minutes after injection. When the animals were pretreated (30 minutes) with acetazoleamide (20 mg/kg) and when the labeled $NaHC^{14}O_3$ entered the blood slowly as a result of intraperitoneal injection, the reduction in rate of $C^{14}O_2$ elimination was not nearly as marked.

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Factors Influencing Experimental Modification of Ehrlich Ascites Tumor Cells.* (26634)

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Previous studies have shown that the dye nigrosin can be used to differentiate unmodified cells, whose membrane is intact (unstained cells), from modified cells whose membrane permeability has been altered experimentally or by death of the cell (stained cells)(1). This differential staining technic has been used to quantitate metabolic data of ascites tumor cells and suspensions of cells obtained from solid tumor as to the proportion of activity due to stained or unstained cells(2). Recently, a modification procedure was described to increase experimentally the permeability of ascites tumor cells by brief exposure of the cells to a hypotonic medium (1). It is of considerable interest that the modification procedure does not result in uniform modification of all cells present, *i.e.*, some cells are apparently resistant to such injury-provoking conditions as hypotonic shock. The following experiments were done to determine whether or not any of the variables involved in experimentally increasing the permeability of Ehrlich ascites tumor cells would influence the resistance and/or lack of resistance of these cells to these experimental conditions.

Material and methods. Preparation of ascites tumor cells. A hypotetraploid subline of Ehrlich ascites tumor cells (EAT cells), carired in Strong A mice, was used in all experiments. The ascites tumor from each animal was collected in 15 ml centrifuge tubes containing 2 to 4 ml of cold buffered saline (9 parts of 0.9% NaCl + 1 part M/15 phosphate buffer). Cold saline was used because it partially prevents coagulation of the ascites fluid. The cells were centrifuged from the ascitic fluid, and twice washed in buffered saline. Centrifugation was carried out at about 125 to 150 $\times$ gravity for 3 minutes, then increased to about 200 $\times$ gravity for an additional 2 to 3 minutes. This centrifugation procedure aided in separating the tumor cells from the red blood cells and white cells; the latter tended to remain in the supernatant fluid. Tumors that were grossly contaminated with erythrocytes were discarded.

Preparation of modified tumor cells (MA cells). The ascites tumor cells were modified according to the following procedure: 0.5 ml of cells (about 20 $\times$ 10^6 cells) in buffered saline were diluted with distilled water to 4.0 ml. The cells were allowed to stand at room temperature for 4 minutes, then 4.0 ml of double strength saline (1.8%) were added to stop the modification. This procedure was altered only in those experiments described in the text that were designed to test the effect of different salt concentrations on the modification procedure.

Staining procedure: Cells were diluted with 0.2% water-soluble nigrosin dissolved in buffered saline. Differential cell counts of stained and unstained cells were made in

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