this failure *in vivo*. Furacin is relatively insoluble and must be administered as a suspension of large particles which tend to settle out easily. It is not known how much is absorbed or what happens to the compound in the body. No Furacin can be demonstrated in the blood of animals to whom the drug has been given, and only a very small amount in the urine.[9] It is not known how much of the drug is converted to an inactive compound in the body of the guinea pig.

The best chemotherapeutic results in mice infected with Staphylococci, Streptococci, and Salmonella organisms were obtained by feeding 150 to 200 mg per kg daily for 3 days.[5] It was not possible to administer this amount of Furacin to guinea pigs without fatal results, and it was necessary to decrease the dose to about 35 mg per kg daily, which seemed to be the maximum tolerated dose for prolonged therapy. This relatively small amount of Furacin was probably not sufficient to produce a bacteriostatic or bactericidal level in the body of the guinea pig.

It was observed that the toxic effects of Furacin continued to be manifest for many days after the administration of the drug was stopped. On the regimen of 18.75 mg per day, the guinea pigs appeared healthy for 10 days, but then almost all began to show loss of appetite, listlessness, and loss of weight. Deaths started to occur on the 11th day. Even after the drug was withdrawn, some animals continued to decline and die for the next 5 or 6 days, before improvement in the appearance of the group was noted.

Conclusions. 1. Furacin exerts a bacteriostatic and bactericidal action on virulent human tubercle bacilli *in vitro*. In a concentration of 1:20,000 (5 mg %) it prevented growth for 22 days in 4 different types of fluid media. A concentration of 1:5000 (20 mg%) was required for bactericidal effect.

2. Resistance to Furacin was not evident after many transfers of the H37 Rv culture in drug-containing medium.

3. The combination of Furacin and streptomycin in Tween-albumin liquid medium delayed, but did not prevent, the development of streptomycin-resistance by the H37 Rv tubercle bacillus.

4. Furacin, in the maximum tolerated dose of about 35 mg per kg daily for 64 days, did not appreciably affect the course of tuberculosis produced in guinea pigs by the human type H37 Rv culture.

[9] Unpublished results, Eaton Research Laboratories.

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Effects of Adding Carbon Dioxide to Inspired Oxygen on Tolerance to High Altitudes.*

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When CO_2 is added to the inspired air at altitudes around 20,000 feet or to low oxygen mixtures, there is an improvement in the physiological condition which is due to an increase in the alveolar pO_2.[1-4] There is no general agreement, however, on the question

* Work done under contract recommended by Committee on Medical Research between the Office of Scientific Research and Development and the University of Rochester, and under contract with Air Materiel Command, Wright Field.

[1] Fenn, W. O., Rahn, H., and Otis, A. B., *Am. J. Physiol.*, 1946, **146**, 637.

[2] Rahn, H., and Otis, A. B., *Am. J. Physiol.*, 1947, **150**, 202.

[3] Gray, J. S., A.A.F. School of Aviation Medicine, Randolph Field, Project Report 310, August, 1944.

[4] Gibbs, F. A., Gibbs, E. L., Lennox, W. G., and Nims, L. F., *J. Av. Med.*, 1943, **14**, 250.

of whether any advantage is gained by adding CO_2 to pure oxygen at high altitudes. For example, Humm *et al.*[5] and Garasenko[6] seem to find favorable effects. On the other hand, Johnson *et al.*[7] and Himwich *et al.*[8] are unable to find any benefit. The data reported below confirm the latter point of view.

Experiments on Mice. Four series of mice maintained on a stock diet were exposed in an evacuated chamber (a vacuum desiccator) to simulated altitudes in excess of 40,000 feet in various gas mixtures. Before the experiments were begun, the mice were conditioned individually to sit on a horizontal cross bar, ½ inch in diameter, which extended across the experimental chamber 3 inches above the floor. The latter consisted of a double metal grill which was connected to the secondary coil of an inductorium. When a mouse was on the grill, the operator could shock him at will by depressing a key in the primary circuit. After a few trials, the mice learned to seek safety on the cross bar, and would voluntarily take position there whenever they were put into the chamber. It was thus possible to use as an end point in the experiments the altitude at which a mouse was no longer able to maintain himself on the cross bar. In this way there was established a fairly definite criterion which did not depend on the judgment of the experimenter or place too great a strain on the endurance of the mice. After proper conditioning each mouse was exposed to altitude during a number of runs in each of the several gas mixtures.

The procedure was varied in the 4 series, as follows:

Series A. Flushout with 100% oxygen at ground level for 3 minutes; ascent to 30,000 feet in 1½ min.; flushout with experimental mixture for 5 min.

Ascent to 40,000 feet in ½ min.; stay 5 min.

Further ascent in 2000-foot stages, with 5 min. at each altitude, until ceiling was reached.

Temperature varied from 22-25°C.

Three mixtures were used: 100% oxygen (April 29 to May 5), 21.7% CO_2 in O_2 (May 8 to May 20); 20% N in O_2 (May 28 to June 13).

Series B. Flushout with 100% oxygen at ground level for 2 min.; ascent to 30,000 feet in 1½ min.; flushout with test mixture for 3 min.; continuous ascent to ceiling altitude at rate of *ca.* 6,000 ft. per min.; run in constant temperature room at 20-21°C. Three mixtures were used: 100% O_2, and 21.7% CO_2 in oxygen, on alternate days between July 10 and July 28; 10.5% CO_2 in oxygen (August 8 to August 15).

Series C. Procedure as in Series B, except that the chamber was flushed out with the test mixture at 30,000 ft. for 10 min. Two mixtures were used: 10.5% CO_2 in oxygen, and 100% oxygen, on alternate days between August 15 and August 28.

Series D. Same procedure as in Series A, except that temperature was between 20-21°C. Two mixtures were used in random succession: 10.5% CO_2 in oxygen and 100% oxygen.

In a given series, the same mice were used for all the different conditions tested. Some mice were used in more than one series. Each mouse was exposed no oftener than once a day, and usually less frequently. Only one mouse was tested in each run.

The volume of the experimental chamber was approximately 5 liters. Gas flow through the chamber was continuous whenever the altitude was stationary, and amounted to about 9 liters per minute at ground level, 2.5 liters (measured at 760 mm Hg) at 30,000 feet, 1.5 liters (at 760 mm Hg) at 40,000 feet, and 1.0 liter (at 760 mm Hg) at 50,000 feet. Inflow of gas was stopped in order to make an ascent. Altitudes were read with a mercury manometer. In the later experiments, the circuit was checked frequently for leaks, which were found to be negligible.

[5] Humm, F. D., Liberman, A. M., and Nims, L. F., C.M.R. Report No. 335, July 18, 1944.

[6] Garasenko, V. M., *Am. Rev. Sov. Med.*, 1945, **2**, 119.

[7] Johnson, A. E., Eckman, M., Rumsey, C., and Barach, A. L., *J. Av. Med.*, 1942, **13**, 130.

[8] Himwich, H., Fazekas, J., Herrlich, H., Johnson, A. E., and Barach, A. L., *J. Av. Med.*, 1942, **13**, 177.

TABLE I.

Series	Gas mixture	Mean ceiling altitude, 10^3 ft	Standard deviation, 10^3 ft	S.D. of mean, 10^3 ft	No. of mouse runs	No. of mice
A	100% O_2	50.3	2.1	.33	41	6
	21.7% CO_2	50.9	2.4	.39	38	6
	20% N_2	49.5	2.1	.36	35	6
B	100% O_2	48.6	1.3	.19	50	15
	10.5% CO_2	47.4	1.6	.24	45	15
	21.7% CO_2	46.8	1.6	.22	53	15
C	100% O_2	48.6	1.3	.24	28	15
	10.5% CO_2	48.4	1.1	.19	29	15
D	100% O_2	53.4	2.5	.43	34	11
	10.5% CO_2	51.3	2.5	.42	36	11

TABLE II.
Summary of Measurements on Human Subjects Breathing 100% O_2 and 90% O_2-10% CO_2 at 40,000 Feet.

	Min. vol., liters/min. B.T.P.S.		Oximeter, % saturation		Alveolar pCO_2, mm Hg	
Subject	100% O_2	90% O_2-10% CO_2	100% O_2	90% O_2-10% CO_2	100% O_2	90% O_2-10% CO_2
R	14.9	18.2	92.6	91.8	31.9	34.9
S	17.5	21.1	90.6	90.5	37.2	38.1
H	10.3	12.2	92.1	91.8	37.8	37.8
Mean	14.2	17.2	91.8	91.4	35.6	36.9
Δ		+3.0		−0.4		+1.3

Results. Average results for each series are presented in Table I. In none of the series was there any significant increase in altitude ceiling as a result of addition of carbon dioxide to the experimental mixture.

In series A, the substitution of 20% nitrogen for oxygen resulted in a slight but probably real decrease in ceiling.

In series B, the performance was definitely worse in both carbon dioxide mixtures than in 100% oxygen, and probably worse in the higher than in the lower concentration of carbon dioxide. On the other hand, in series C where the mice spent 10 minutes at 30,000 feet in the experimental mixture, instead of only 3 minutes as in series B, approximately the same altitudes were reached in 10.5% carbon dioxide as in pure oxygen. In series D, which resembles series A in that 5-minute stops were made at 2,000-foot intervals above 40,000 feet, performance was definitely better in 100% oxygen.

In view of these findings, it may be concluded definitely that mice gain no benefit, in terms of altitude ceiling, from the substitution of carbon dioxide for oxygen with the patterns employed in these experiments. In some cases, a definite harmful effect (decreased ceiling with added carbon dioxide) has been demonstrated.

Differences in the average ceiling altitude attained in a given gas mixture in the several series may be due to:

(1) Differences in altitude tolerance of individual mice; (2) Slight temperature difference between series A and the other series;

(3) Variations in the adaptation of circulatory and other mechanisms resulting from different patterns (rates) of ascent.[9]

Experiments on Human Subjects. Each of 3 male subjects was taken to a simulated altitude of 40,000 feet wearing an ear oximeter (Millikan compensated circuit) and inspiring 100% oxygen through a gas meter. Measurements of the ventilation and oximeter readings were made during a 15-minute

[9] Hiestand, W. A., and Miller, H. R., *Am. J. Physiol.*, 1944, **142**, 310.

period, and near the end of this interval an end-expiratory alveolar sample was secured. The inspired gas was then changed to a mixture of 10% CO_2 and 90% O_2, and measurements were continued for another 15 minutes at the end of which another alveolar sample was taken.

Results are summarized in Table II. Substitution of the CO_2-O_2 mixture for pure oxygen produced a rise of 1.3 mm in the alveolar pCO_2 (with lowering of the alveolar pO_2 by the same amount) and a drop of 0.4% in the saturation as determined from the oximeter. The change in % saturation is within the error of the instrument, but the expected change for the change in alveolar gas tensions is about 1%. The minute volume of ventilation was increased by 3 liters per minute or about 21%. These data indicate that no favorable physiological changes occurred from the addition of 10% CO_2 (9.4 mm) to the inspired gas at 40,000 feet. One cannot argue from this that it would not be advantageous to add CO_2 under some conditions, but it must be kept in mind that any rise in alveolar pCO_2 is always accompanied by an equivalent drop in alveolar pO_2 when nitrogen is absent. Probably the only condition in which substitution of a CO_2-O_2 mixture for pure O_2 might be advantageous is that of severe hypocapnia accompanied by a relatively mild hypoxia. In such a case the improvement due to the decreased hypocapnia might be greater than the impairment due to the increased anoxia.[10]

Summary. 1. The addition of 10.5% or 21.7% carbon dioxide to oxygen does not improve the altitude ceiling of mice.

2. Mice taken to altitude in 100% oxygen, with continuous ascent from 30,000 feet at a rate of about 6,000 feet per minute, collapse at a lower ceiling than mice which ascend from 40,000 feet in 2,000-foot steps with 5-minute stops at each stage.

3. In experiments on human subjects no favorable physiological effects were observed when 10% CO_2 was added to the inspired oxygen at 40,000 ft.

[10] Otis, A. B., Rahn, H., Epstein, M. A., and Fenn, W. O., *Am. J. Physiol.*, 1946, **146**, 207.

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Simplified Indirect Complement-Fixation Test Applied to Newcastle Disease Immune Avian Serum.

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The investigation in this laboratory of an avian disease presented a problem of accurate specific diagnosis. Because antigens suitable for the performance of the customary agglutination and precipitin tests were not available, attention was turned to the complement-fixation reaction. The development of the *indirect* method by Rice[1,2,3] made this approach more feasible since this technic apparently overcomes the difficulties which have prevented the use of the regular, or *direct*, method on avian serums.[4]

It also seemed worthwhile to attempt to simplify the *indirect* technic by using the common "serum or antigen-dilution" method and the observation of 100% hemolysis rather than the more detailed quantitative method for the standardization of all test com-

[1] Rice, C. E., *J. Immunol.*, 1947, **59**, 365.

[2] Rice, C. E., *Canadian J. Comp. Med.*, 1948, **12**, 130.

[3] Rice, C. E., *J. Immunol.*, 1948, **60**, 11.

[4] Rice, C. E., *Canadian J. Comp. Med.*, 1947, **11**, 236.