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Exposure of Guinea Pigs to Intermittent High Oxygen Tension and Its Failure to Depress Erythropoiesis.*

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In recent years the theory has been advanced that the rate of erythropoiesis is regulated by the oxygen tension in the bone marrow. It is well known that exposure to low oxygen tension over a sufficient period of time will produce a marked degree of polycythemia. However, there appears to be little unanimity of opinion among investigators about the effects of high oxygen tensions in man and animals.

Barach and McAlpin[1] found no significant alterations in the erythrocyte counts of patients with polycythemia vera following continuous exposure to 50% oxygen for 15 to 17 days. Reinhard, Moore, Duback and Wade[2] found that subjecting patients with sickle cell anemia to a continuous supply of 70-100% oxygen for 8 to 20 days caused a significant decrease in reticulocytes and erythrocytes. Campbell[3] exposed mice, rats, guinea pigs, rabbits, monkeys, and cats continuously to 40-60% oxygen for 18 to 57 days at normal barometric pressure. In all but the cats he observed a decrease in the number of erythrocytes. In 2 guinea pigs exposed for 57 days there was an average decrease of 35% in the number of erythrocytes and 22% in the amount of hemoglobin. The reports of other investigators who have worked on this problem (Karsner;[4] Archard, Binet and Le Blanc;[5] Binet, Bochet and Bryskier;[6] and Davis[7]) are unconvincing.

The purpose of the present investigation was to determine whether prolonged *intermittent* exposure of guinea pigs to high oxygen tension would cause a depression of erythropoiesis. Two of us[8] have previously shown that intermittent exposure to low oxygen tension produces a marked stimulation of erythropoiesis.

Materials and Methods. Fourteen young adult male guinea pigs weighing 300 to 400 g were used in the first experiment. Four animals served as controls and the remaining 10 were exposed to 80-100% oxygen at normal pressure 6 hours a day 6 days a week for 8 weeks. For the administration of high concentrations of oxygen, a gas-tight cylindrical tank of approximately 16 cu. ft. capacity was used. The tank was just large enough to contain a cage equipped with food and water for the experimental animals. It had 2 glass windows through which the animals could be observed during the exposure, and was equipped with trays of soda lime to absorb carbon dioxide. A constant internal temperature was maintained by the circulation of cool water through a system of coils. Commercially pure oxygen (U.S.P.) was supplied by continuous flow to the tank, and the flow was regulated by a pressure reduction valve. To avoid building up a positive pressure a needle valve connected directly to the wall of the tank, but at the opposite end from the oxygen inflow valve, was used as a constant bleeder. Careful adjustment of this valve served to balance the amount of outgoing air with the incoming oxygen. During

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1 Barach, A. L., and McAlpin, K. R., *Am. J. Med. Sc.*, 1933, **185**, 178.

2 Reinhard, E. H., Moore, C. V., Duback, R., and Wade, L. J., *J. Clin. Invest.*, 1944, **23**, 682.

3 Campbell, J. A., *J. Physiol.*, 1927, **63**, 325.

4 Karsner, H. T., *J. Exp. Med.*, 1916, **23**, 149.

5 Achard, G., Binet, L., and LeBlanc, A., *C. R. Acad. d. Sc.*, 1927, **184**, 771.

6 Binet, L., Bochet, M., and Bryskier, A., *J. de Physiol. et de Path. Gen.*, 1939, **37**, 524.

7 Davis, J. M., *J. Pharm. and Exp. Therap.*, 1943, **79**, 37.

8 Jensen, A. V., and Alt, H. L., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 384.

exposure, oxygen flowed into the tank at a rate of approximately 4.4 liters per minute.

After the experimental animals were sealed in the tank at the beginning of a daily exposure, the tank was flushed out with pure oxygen for 10 to 15 minutes, care being taken not to raise the tank pressure more than 10 mm Hg pressure over normal. The process of flushing required about 900 liters of oxygen. After flushing the oxygen concentration was determined. This initial concentration varied between 80 and 95% oxygen for all exposures. At the end of the 6-hour exposure period another oxygen concentration determination was made. This terminal concentration varied from 87 to 100% for all exposures. The average concentration of oxygen to which the animals were exposed was therefore about 92% which provided a partial pressure of oxygen of 699 mm Hg (4.4 times the normal). All oxygen concentration determinations were made by means of the Analox instrument.† Periodic observations of the experimental animals were made during exposures and at interval periods in an attempt to detect any abnormalities of behavior. The animals were weighed twice a week.

Blood studies were made on the experimental animals at the end of 183 hours exposure (covering 36 days) and at 287 hours (covering 57 days). The controls were studied at the same intervals. The animals were anesthetized with nembutal, and blood was drawn from the femoral artery 16 to 18 hours after periods of exposure to high oxygen tension. Erythrocyte counts were made with pipettes and hemocytometer chambers certified by the U. S. Bureau of Standards. The amount of hemoglobin was determined by the Duffie method[9] with an instrument calibrated by the oxygen capacity technic.

The second experiment was similar to the first except that the increase in partial pressure of oxygen was obtained principally by raising the barometric pressure in the tank. Six guinea pigs were subjected to 60 to 70% oxygen at 2 atmospheres pressure, which provided a partial pressure of oxygen of 998 mm Hg (6.3 times the normal). This was accomplished by first reducing the atmospheric pressure in the exposure chamber slowly to 400 mm Hg pressure below normal by means of an evacuation pump, and then gradually building up the pressure in the chamber with pure oxygen to one atmosphere over normal, and maintaining it there in the same manner as in the first experiment. The animals were exposed 6 hours a day 6 days a week, and received 17 exposures over a period of 20 days with a total exposure time of 106 hours. The number of erythrocytes and amount of hemoglobin were then determined.

Results. In the first experiment there was no significant change in the erythrocyte count and hemoglobin concentration after exposure to 80-100% oxygen at 1 atmosphere pressure. At the end of 183 hours of intermittent exposure covering a period of 36 days, the experimental animals showed a mean erythrocyte count of 5.29 (±.625) million per cu mm and a mean hemoglobin value of 12.9 (±.908) g per 100 cc. The control animals had a mean erythrocyte count of 5.11 (±.294) million per cu mm and a mean hemoglobin of 13.22 (±.511) g per 100 cc. The experiment continued with 9 experimental animals, one having died during the process of blood extraction presumably from an overdose of anesthetic. Blood was again taken from the animals at the end of 287 hours of intermittent exposure covering 57 days. The experimental animals then had a mean erythrocyte count of 5.22 (±.547) million per cu mm and a mean hemoglobin of 13.11 (±1.29) g per 100 cc. The controls had a mean erythrocyte count of 5.22 (±.646) million per cu mm and a mean hemoglobin value of 13.35 (±.983) g per 100 cc.

In the second experiment we also observed no change in erythropoiesis. After 106 hours of intermittent exposure to 50-60% oxygen tension at 2 atmospheres pressure the mean erythrocyte count for 6 animals was 4.81 (±.527) million per cu mm and the mean value for the amount of hemoglobin was 13.1 (±.538) g per 100 cc.

† Instrument produced by Oxygen Equipment and Service Co., Chicago, Ill.

[9] Duffie, D. H., *J. A. M. A.*, 1944, **126**, 95.

During both experiments the guinea pigs exhibited no adverse symptoms. They all ate readily, gained weight, and were normally active while in the tank and when they were put back into the storage cage with their controls during the interval period. No differences between the normal and the experimental animals could be observed.

Comment. Using the intermittent exposure method, it is apparent that high oxygen tension does not cause a depression of erythropoiesis in guinea pigs in contradistinction to the marked stimulation of erythropoiesis produced by low oxygen tension. With the continuous exposure method Campbell[3] observed significant reductions in the erythrocyte count and hemoglobin values in various species of animals. Because of the importance of this observation we exposed 6 guinea pigs continuously to 50-60% oxygen. Three animals died of pneumonia during the experiment. After 30 days, the remaining 3 animals had an average decrease of 21% in the erythrocytes and 13% in the amount of hemoglobin. Whereas these changes are not as great as those reported by Campbell, they further suggest that long continued exposure to oxygen may depress erythropoiesis in a normal animal.

The interesting experiment of Reinhard[2] *et al.* shows conclusively that erythropoiesis is depressed in patients with sickle cell anemia exposed to high oxygen tension. As such an effect was not obtained in patients with polycythemia vera (Barach and McAlpin)[1] it might be inferred that the hemopoietic equilibrium in sickle cell anemia is especially sensitive to increased oxygen pressures. The effect of prolonged high oxygen tension on the rate of erythropoiesis in animals and normal human beings warrants further investigation.

Summary. Guinea pigs were intermittently exposed to 80-100% oxygen at atmospheric pressure for 57 days, and to 60-70% oxygen at 2 atmospheres pressure for 20 days. The periods of exposure covered 6 hours a day 6 days a week. There was no significant diminution in the erythrocyte counts and hemoglobin values in any of these animals.

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The Effect of *Lithospermum* on the Mouse Estrous Cycle.*

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Introduction. Based on a report[1] that certain American Indians have employed the ingestion of the herb *Lithospermum ruderale* as a conception preventative, Cranston[2] performed a series of experiments on mice and found that a fluid extract of *Lithospermum* when mixed with normal diet would rapidly induce a suppression of the estrous cycles as well as a lowered birth incidence in breeding females. From inferential evidence Cranston concluded that the active factor in the herb operates directly on the pituitary gland, suppressing the formation or release of gonadotropic hormone.

Since this type of "anti-hormonal" action

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1 Train, P., Hendricks, J. R., and Archer, W. A., *Medicinal Uses of Plants by Indian Tribes of Nevada*, Part II, p. 102. Issued by the Division of Plant Exploration and Introduction, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D.C., 1941.

2 Cranston, E. M., *J. Pharm. and Exp. Therap.*, 1945, **83**, 130.