was similar in all respects to that of neostigmine. The studies on physostigmine confirm those carried out by Anderson[1] though the latter have been erroneously interpreted by Meyer and Gottlieb[3] as evidence of a direct action of physostigmine on the iris.

The action of physostigmine and neostigmine therefore is one wholly due to inactivation of cholinesterase, and consequent preservation of stimulant quantities of acetylcholine. For the production of acetylcholine, some or all of the ganglion cells must be intact or there must exist some local stimulation of postganglionic fibers.

Conclusion. Neostigmine (prostigmin) does not stimulate the denervated iris muscle of the cat. It causes miosis only when some or all of the ciliary (parasympathetic) ganglion cells are intact or when the postganglionic fibers are stimulated.

[3] Meyer, H. H., and Gottlieb, R., *Die Experimentelle Pharmakologie*, 8th edition, 1933, Berlin, page 189.

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Effect of Intermittent Exposure to Simulated High Altitude in Erythropoiesis in the Guinea Pig.*

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Investigations on the effects of intermittent exposure to high altitudes have been pursued only since 1938 when Armstrong and Heim[1] subjected rabbits to a simulated altitude of 18,000 feet, 4 hours daily for 5 weeks. Their animals failed to show an increase in red blood corpuscles in adaptation to the lowered oxygen tension. They also found[2] that young men exposed intermittently to an altitude of 12,000 feet showed evidence of only slight acclimatization. On the other hand, Stickney and his collaborators[3,4] found that dogs subjected discontinuously to an altitude of 18,000 feet for 16 weeks did exhibit an increase in the amount of hemoglobin and number of red blood corpuscles.

The purpose of this report is to present further data from a different species, the guinea pig. At first our study was incidental to an investigation of the effect of exposure to simulated high altitude on the structure of the brain[5] and we made hemoglobin determinations only. Later the blood study was expanded, though done in conjunction with the neurological study; the same animals were used in both problems.

Material and Methods. Fifty-eight young adult male guinea pigs were used. Twenty-six of these served as controls; the remainder were subjected to discontinuous exposures to air at lowered barometric pressure for 6 hours daily, except Sunday. In the first experiment the simulated altitude was 23,000 feet (307 mm Hg pressure); in the second experiment, 30,000 feet (225 mm Hg pressure).

The conditions of the concurrent neurological experiment required that 3 to 6 animals be sacrificed periodically as the whole series accumulated exposure time. In the first experiment, groups were sacrificed after 100, 200, 300, and 500 hours of exposure. In the

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[1] Armstrong, H. G., and Heim, J. W., *J. Aviat. Med.*, 1938, **9**, 92.

[2] Armstrong, H. G., *Principles and Practice of Aviation Medicine*, Williams and Wilkins Co., Baltimore, 1943.

[3] Stickney, J. C., and Van Liere, E. J., *J. Aviat. Med.*, 1942, **13**, 170.

[4] Stickney, J. C., Northrup, D. W., and Van Liere, E. J., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 151.

[5] Windle, W. F., and Jensen, A. V., *J. Aviat. Med.*, 1945, in press.

TABLE I.
Changes in Mean Hemoglobin Content of the Blood During Discontinuous Exposure to Simulated High Altitude.

Group	No. animals	Altitude feet × 1000	Exposure time, hr	Hb. g/100 cc	σ
Controls	17			14.4	± 0.89
A	3	23	100	19.5	± 0.40
B	3	30	100	18.3	± 1.53
C	4	23	204	25.5	± 2.38
D	4	23	300	26.5	± 1.00
E	4	23	500	25.2	± 0.97
F	6	23 / 30	100 / 100	24.4	± 1.19
G	4	30	200	24.5	± 1.91
H	4	30	250	22.9	± 1.17

second experiment groups were sacrificed at 100, 200, and 250 hours. Six other animals received 100 hours at 23,000 feet followed by 100 hours at 30,000 feet.

Blood studies were made on each animal just before it was killed 17 to 24 hours after the last exposure to reduced oxygen tension. Normal controls for each group were sacrificed at the same time and in the same manner. All studies were made on cardiac blood which was placed in a bottle containing an ammonium potassium oxalate mixture. The hemoglobin was determined by the Hellige-Sahli method, with an instrument calibrated by the oxygen capacity technic. The erythrocyte counts were performed with U. S. Bureau of Standards pipettes and chambers. Hematocrit determinations were made in the Wintrobe tube. Centrifugation was carried out at 2800 revolutions per minute for 30 minutes. After vital staining reticulocytes were estimated on unstained dried smears.

Results. The data from both experiments are presented in two tables. Table I shows the average hemoglobin values for each group of experimental animals and 17 controls. The table indicates that following 100 hours of exposure to 23,000 feet (A) or 30,000 feet (B) the amount of hemoglobin was essentially the same, about 19 g per 100 cc. Within the limits of experimental error both values were equal. After 200 hours exposure to either altitude, (C and G) there was no further increase of significance. Even after 500 hours (E) the amount of hemoglobin did not increase over that found after 200 hours, about 25 g per 100 cc. After 100 hours at either altitude the animals were about one-half acclimatized; after 200 hours at either altitude, or a combination of the two altitudes (F) the animals were fully acclimatized.

In Table II all data on the blood picture are summarized. The figures represent average values for each group. Each group is designated by a letter corresponding to those in Table I. Average values for 9 normal controls are presented for purposes of comparison. The groups have been placed in the table, so far as

TABLE II.
Average Changes in Blood Values Obtained During Discontinuous Exposure to Simulated High Altitude.

Group	No. animals	Altitude, feet × 1000	Exposure time, hr	R.B.C., mil.	Hb., g/100 cc	Hemat., %	Retic., %	Spleen wt., g/kg
Control	9			5.07	13.7	43	0.9	1.67
B	3	30	100	6.51	18.3	60	1.3	2.39
F	6	23 / 30	100 / 100	7.70	24.4	76	1.1	3.26*
G	4	30	200	9.04	24.5	79	1.4	2.09
H	4	30	250	8.15	22.9	72	3.2	2.25
E	4	23	500	8.19	25.2	72	2.5	3.49

* Avg of 4 animals.

possible, in the order of severity of exposure to lowered oxygen tension.

An increase in number of red blood corpuscles and in hematocrit and hemoglobin values proceeds up to 200 hours of exposure time after which there is a leveling off. Neither 250 hours at 30,000 feet or 500 hours at 23,000 feet caused an increase over the values obtained for 200 hours at 30,000 feet. A greater degree of reticulocytosis was obtained following the more severe experimental conditions (H and E). Enlargement of the spleen occurred.

The maximum number of red blood cells encountered in any animal was 10.04 million. This animal had a hemoglobin value of 26.0 g per 100 cc and an hematocrit of 87%. The animal belonged in Group G. Several other animals from this and other groups also showed increases of almost 100% over the mean control values for hemoglobin, red corpuscles and hematocrit. These values are similar to maximal values seen in patients with polycythemia vera.

Comment. The results obtained in this study demonstrate that an extraordinary degree of polycythemia may be produced by intermittent exposure to simulated high altitude. Among the 32 experimental animals subjected to varied grades of severity of experimental conditions there was no single animal in which a notable increase in amount of hemoglobin and red blood corpuscles did not occur. It should be borne in mind that the animals were exposed to low oxygen tensions for only 6 hours a day and 6 days a week. The degree of anoxia used was much greater than man can tolerate; *e.g.*, man cannot ascend to altitudes over 18,000 feet for more than a few minutes at a time without experiencing impairment of psychological and physiological functions, unless he is breathing air enriched with oxygen. Armstrong[2] states that at an altitude of 25,000 feet death may occur in man at any time after 20 to 30 minutes. At 30,000 feet the guinea pigs behaved lethargically and exhibited a moderate hyperpnea when active, but they were otherwise apparently normal. So long as they remained quietly huddled in their usual position no physiological distress was observed. They could even make a small amount of movement without deleterious effects. With greater exertion, however, they fell upon their sides and remained there with legs outstretched and apparently in a coma for 30 to 60 seconds, afterwards righting themselves and remaining quiet. It appears, therefore, that the critical altitude for the guinea pig lies at about 30,000 feet. Its "ceiling" is something more than 5,000 feet higher than that of man.

The enlargement of the spleens in these animals is probably related to the polycythemia. Our results on this point support the continuous exposure experiments of Van Liere[6] on guinea pigs, and the experiments of Van Liere and Stickney[7] on dogs.

Conclusions. Guinea pigs exposed to simulated altitudes of 23,000 and 30,000 feet 6 hours a day and 6 days per week became fully acclimatized after 200 hours, as shown by maximal increase in the values for red corpuscles, hemoglobin, and hematocrit. Reticulocytosis and enlargement of the spleen were also demonstrated.

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[6] Van Liere, E. J., *Am. J. Physiol.*, 1936, **116**, 290.

[7] Van Liere, E. J., and Stickney, J. C., *J. Aviat. Med.*, 1943, **14**, 194.