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Blood Volume Responses of Rats Adapted to Different Barometric Pressures[1] (38482)

ROBERT R. WOLFE AND STEVEN M. HORVATH

Institute of Environmental Stress, University of California, Santa Barbara, Santa Barbara, California 93106

Rats exposed to altitude have shown an increasing degree of erythropoiesis with a resultant increase in circulating red cell volume while plasma volume has generally decreased or remained constant (1–6). The extent of red cell and plasma volume changes appeared to be dependent upon such factors as the severity and duration of exposure and the age and weight of the rats. However, the independent influence of any one of these factors has not been investigated. In order to evaluate the effect of the severity of exposure on these parameters, animals were adapted to barometric pressures of 480 mm Hg and 380 mmHg (simulated altitudes of 3700 m and 5480 m) for 30 days and their blood volumes were compared with sea level rats of similar weights and age.

Methods. Four groups of male Sprague–Dawley rats[2] were treated as follows: (a) rats born and raised at sea level ($n = 8$) remained at sea level, (b) rats born and raised at sea level until 45 days were adapted to a barometric pressure of 480 mmHg (simulated altitude of 3700 m) for 30 days ($n = 9$), (c) rats born and raised at sea level until 51 days were adapted to a barometric pressure of 380 mmHg (simulated altitude of 5480 m) for 30 days, and (d) rats born and raised at sea level until 51 days had their diets restricted for 30 days so that their growth curve matched that of the rats adapted to 380 mmHg ($n = 8$).

Each rat was housed individually and given food and water *ad lib.* except the rats with food withheld, who were only given water *ad lib.* The rats adapted to altitude were housed in desiccators adapted for use as hypobaric chambers. Each chamber was approximately 8.5 in. in diameter and had a domed roof 9 in. high at the peak. The barometric pressure was maintained at either 480 mmHg or 380 mmHg with an air flow of approximately 3 liters per min. The temperature within the desiccators was maintained at 25°. Every other day the chambers were returned to sea level for 30–45 min. for cleaning and replacement of food and water. All four groups were maintained on a 12-hr light/dark schedule.

Two days prior to the blood volume determinations, polyethylene catheters were surgically implanted (under Nembutal anesthesia) adjacent to the right atrium and in the root of the aorta. The catheters were externalized at the back of the neck. Altitude adapted rats were returned to altitude a few hours after the surgery. All rats gained approximately 5–10 g from the first day after surgery to the second and appeared to be in good health.

Circulating red cell volume was measured using ^{51}Cr labeled red cells (7). Blood from donor rats was used for labeling. When tagged cells were being prepared for injection into altitude adapted rats the hematocrit was adjusted to approximately 60, in sea level animals to approximately 47. All counting was performed in a gamma well counter.

A background sample of 1.0 ml was withdrawn from the awake rat. Immediately after drawing the background sample, 1.0 ml of the tagged red blood cell suspension was injected through the venous catheter. In preliminary studies on three rats, 0.7-ml samples were drawn at 10, 20, and 40 min. The counts per minute were plotted against time on semilog paper. The lines were almost perfectly horizontal, and extrapolation to $T = 0$ resulted in a value almost identical with the

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[2] In conducting this research, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee for Laboratory Animal Facilities and Care, of the Institute of Animal Laboratory Resources, National Academy of Sciences—National Research Council.

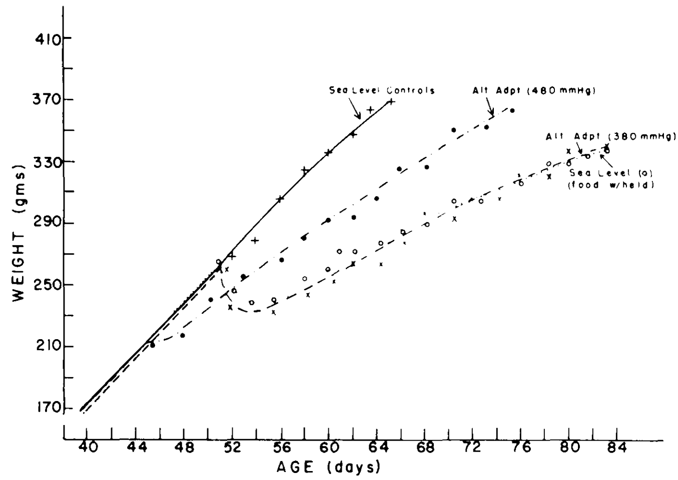

FIG. 1. Growth curves for untreated sea level controls given food and water *ad lib.* (+), rats born and raised at sea level until day 45, when they were adapted to a barometric pressure of 480 mmHg for 30 days (●), rats born and raised at sea level and on day 51 exposed to 380 mmHg for 30 days (×), and sea level rats with their diets restricted from day 51 to day 81 (○).

average values of the three samples. Consequently, in all subsequent blood volume determinations only two samples were withdrawn, at 15 and 30 min, and averaged. Five-tenths milliliter of each sample was utilized for counting. The remainder was used for hemoglobin determinations using the Hycel cyanomethemoglobin method (8) and hematocrit determinations using the microhematocrit technique (corrected for trapped plasma).[3] The reproducibility of the blood volume determinations was further checked by the remeasurement of three of the sea level rats weight-matched with the rats adapted to 380 mmHg 6 days after their initial blood volume determination.

The group means for weight, hematocrit, hemoglobin, red cell volume, plasma volume, and total blood volume were analyzed using a single-factor analysis of variance. Where significance was found, a Newman–Keuls post hoc test was used to determine which groups differed from each other.

Results. The growth curves for the four groups of rats are shown in Fig. 1. The sea level control rats gained weight steadily and weighed an average of 366 g when their blood volumes were measured. Exposure to 480 mmHg retarded growth to some extent. However, the final weight of these rats, when their blood volumes were determined, was not significantly different ($P < 0.05$) from the controls. Growth was severely retarded in the rats adapted to 380 mmHg, and their average weight at the time of the blood volume determinations (320 g) was significantly lower than either of the prior groups ($P < 0.01$). Rats kept at sea level on the restricted diet had a final weight of 322 g, their growth curve being almost identical to that of the rats adapted to 380 mmHg.

In almost all cases samples withdrawn at 15 min resulted in calculated blood volumes within 0.1 and 0.2 ml of those determined from the 30-min sample. Blood volumes performed on the three sea level weight-matched rats were unchanged from the initial values determined 6 days earlier; in all cases they fell within the 0.05 confidence interval.

The hematologic data are summarized in Table I. Rats adapted to 480 mmHg had a higher mean red cell volume, hematocrit and hemoglobin level than sea level controls ($P < 0.01$). Adaption to 380 mmHg resulted in further increases. On the other hand, sea level rats weight-matched with rats adapted to 380 mmHg had a significantly lower ($P < 0.01$) mean red cell volume, hematocrit and hemoglobin level than the untreated controls.

The increase in red cell volume in the rats

[3] Correction factor of 0.95 was determined using ^{51}Cr labeled red blood cells and ^{125}I RISA in our laboratory.

TABLE I. GROUP MEAN DATA FOR CONTROL RATS AND EXPERIMENTAL RATS.*

Group	Weight (g)	Hematocrit (%)	Hemoglobin (g %)	CRCV (ml/100 g)	PV (ml/100 g)	TBV (ml/100 g)
1. Sea level controls (n = 8)	367 ± 12	45.4 ± 0.92	15.46 ± 0.32	2.45 ± 0.05	2.89 ± 0.10	5.34 ± 0.12
2. Adapted to 480 mmHg (n = 9)	360 ± 7[c]	55.3 ± 0.96[a]	18.44 ± 0.53[a]	3.07 ± 0.11[a]	2.52 ± 0.08[e]	5.59 ± 0.14
3. Adapted to 380 mmHg (n = 8)	320 ± 9[e]	65.4 ± 1.4[a, b, d]	21.85 ± 0.44[a, b, d]	5.29 ± 0.23[a, b, d]	2.74 ± 0.10[d]	8.03 ± 0.19[a, b, d]
4. Sea level, weight matched with group 3 (n = 8)	322 ± 3[e]	42.9 ± 1.4	14.60 ± 0.38	1.74 ± 0.07[e]	2.34 ± 0.07[e]	4.08 ± 0.09[e]

* All values ± SEM.
[a] Significantly greater than group 1 ($P < 0.01$).
[b] Significantly greater than group 2 ($P < 0.01$).
[c] Significantly greater than group 3 ($P < 0.01$).
[d] Significantly greater than group 4 ($P < 0.01$)
[e] Significantly less than group 1 ($P < 0.01$).

adapted to 480 mmHg was accompanied by a significant ($P < 0.01$) decrease in plasma volume, resulting in total blood volumes that did not differ significantly ($P < 0.05$) from the untreated controls, 5.34 and 5.99 ml/100 g respectively for controls and for rats adapted to 480 mmHg. Animals adapted 380 mmHg had plasma volumes equivalent to those observed in sea level animals but with the large increase in red cell volume their total blood volume (8.03 ml/100 g) was significantly elevated above all other groups ($P < 0.01$). The blood volume of the rats adapted to 380 mmHg was close to 100% greater than the blood volume of the sea level weight-matched rats, whose total blood volume (4.08 ml/100 g) was significantly lower ($P < 0.01$)than all other groups.

Discussion. Results of previous investigations have suggested that the blood volume response of the rat to altitude may be dependent upon the specific altitude in question. Rats exposed to simulated altitudes greater than 5500 m for periods of time ranging from 14 to 50 days have increases in circulating red cell volume (CRCV) of more than 100% (2, 4–6). The consequent increases in total blood volume were of somewhat smaller magnitude due to concomitant decreases of varying degrees in plasma volume. Adaption to lower altitudes has been reported to have much less pronounced effect on blood volume in rats. Feigen and Johnson (3) observed that rats adapted to a natural altitude of 3800 m for either 4–10 days or 30–60 days had red cell, plasma, and total blood volumes which were similar to sea level rats. An increase in CRCV (approximately 25%) was noted only after rats had been kept at altitude 260 days while the plasma volume remained unchanged. Fryers (4) found a slight decrease in total blood volume in rats adapted to a simulated altitude of 2440 m despite a 10% increase in CRCV. In contrast, in a simulated altitude of 4400 m, he observed a 26% increase in total blood volume, primarily attributable to an 82% increase in CRCV.

These earlier studies failed to specifically delineate the effect of the severity of exposure on the blood volume response. Investigators utilized different durations of exposure, and the sea level controls were not age and weight matched with their respective experimental groups. The data of Pepelko (6) indicated that duration of exposure was an important variable in determining the blood volume response to altitude. Garcia (9) and Feigen and Johnson (3) both reported that in sea level rats a decline in CRCV, plasma volume, and total blood volume per unit body weight occurred with advancing age. The older rats were also heavier than the younger ones, and hence the decreased blood volumes might also have been related to the weight of the rats.

In the present study, the extent of increase in CRCV was related to the severity of the hypoxic exposure. Rats adapted to 480 mmHg had a 25% increase in CRCV, as compared to the sea level controls. Since both age and weight were similar, these factors would not account for the differences observed and the effect was simply an anoxic response. Adaption to 380 mmHg resulted in a CRCV which was 72% greater than that observed in the rats adapted to 480 mmHg. The extent of increase in erythropoietic stimulation which occurred at the more severe hypoxia can be better appreciated by comparing the CRCV of the rats adapted to 380 mmHg with that of their weight-matched sea level counterparts. Diet restriction in the absence of hypoxia resulted in a 29% reduction in CRCV, a finding consistent with previous observations of the inhibitory effect on erythropoiesis of food deprivation (1). In comparison to these sea level animals, the rats adapted to 380 mmHg had a 200% increase in CRCV.

Plasma volume responses to reduced barometric pressures were smaller in magnitude than the CRCV responses, but were nonetheless of interest with regards to alterations in the O_2 carrying capacity and rheological properties of the blood. Adaption to 480 mmHg induced a decrease in plasma volume concomitant with the increase in CRCV which resulted in a mean total blood volume which was not different from that of the controls. There was no such decrease in plasma volume in the rats adapted to 380 mmHg. These animals had plasma volumes which were in fact greater than those of their weight-matched sea level controls. Teleologically, the absence of a plasma volume decrease in the rats adapted to 380 mmHg probably helped counteract a potentially detrimental increase in viscosity.

The altitude dependency of the erythropoietic response was presumably due to the release of greater quantities of erythropoietin in the rats adapted to 380 mmHg than in those adapted to 480 mmHg. Several other factors may also have been responsible for some of the dramatic changes seen in our animals. It is possible that the different volume responses at the respective pressures were different functions of time, so at 30 days different phases of the responses were observed. Dehydration, reported to occur in animals adapted to reduced barometric pressure (10, 11) and in the diet-restricted sea level rats, could have been a causative factor in the plasma volume response. Mogharabi and Haines (12) reported that acute dehydration resulting in a 20–25% body weight loss produced an absolute decrease of total body water and blood volume (but not per 100 g body weight). There was a concomitant decrease in plasma volume and due to an increase in hematocrit a larger CRCV. Another possibility arises in that CRCV may have directly influenced plasma volume. In a preliminary study performed in this laboratory, acute isovolumetric hematocrit alterations were produced in two rats. In each case, 16 hr after the change in hematocrit there had been a movement of water (indicated by change in plasma proteins) which tended to minimize that change. The hematocrit which has been acutely elevated from 48.0 to 64.5 was reduced to 55.3, and the hematocrit which had been acutely lowered from 48.5 to 37.3 was increased to 41.5. These and other possibilities remain unanswered, since in most cases the reported effect of food and water deprivation on blood volumes was seen only with drastic alteration in intake, and the fluid shifts observed following acute hematocrit alterations may not accurately reflect the chronic changes observed in this experiment.

Summary. The total blood volume, circulating red cell volume (CRCV), and plasma volume of rats exposed for 30 days to either 480 or 380 mmHg were quite different. At the lower altitude (480 mmHg) total blood volume was similar to that found in sea level controls of a similar weight and age, while

CRCV was increased and plasma volume was decreased. However, at the higher altitude CRCV was increased to a much greater extent, with the result that the total blood volume was approximately 44% greater than at 480 mmHg. The differences in response to the two barometric pressures became even more striking when the rats adapted to 380 mmHg were compared with their age- and weight-matched controls. Diet restrictions in the absence of hypoxia resulted in significant decreases in CRCV, plasma volume, and total blood volume. Consequently, when compared to these animals, rats adapted to 380 mmHg had a 100% greater total blood volume due primarily to a higher CRCV, but also to a higher plasma volume.

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