Blood Volume and Body Haematocrit of Rats Native to a Simulated Altitude of 3500 m

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Abstract. Circulating blood volume (BV) as the sum of circulating red cell volume (RCV) and plasma volume (PV) was estimated in rats native to a simulated altitude of 3500 m ("natives"), in rats born at sea level and later in life transferred to the simulated high altitude ("newcomers"), and in control sea-level rats. RCV per kg body weight (b.w.) was significantly larger in both "newcomers" and "natives" than in controls. PV per kg b.w. was in the "newcomers" insignificantly and in the "natives" significantly smaller than in the controls. BV per kg b.w. in both high altitude groups tended to be larger than in controls but the difference was not significant. Arterial haematocrit (Ahct) in the "newcomers" was significantly higher than in the controls, and in the "natives" significantly higher than in both other groups. Body haematocrit (the ratio of RCV and BV in per cent) was smaller than Ahct in all groups; this was more pronounced in the "newcomers" than in the controls and even more so in the "natives". Apparently the haematocrit in the minute vessels of the organs of animals exposed to chronic hypoxic hypoxia increases much less than might be expected from changes of the Ahct.

An attempt was made to evaluate the possible error of the more commonly used method of estimating BV, when only RCV, or only PV, is measured, and BV and its complementary fraction are calculated from arterial or venous haematocrit. When, in our results, BV was calculated from RCV and Ahct, the absolute values and also the differences between groups were somewhat underestimated. When BV was calculated from PV and Ahct, the BV itself, and particularly the differences between groups, were overestimated quite considerably. It is suggested that the only safe way to estimate BV is to measure RCV and PV separately.

Key words: High altitude — Blood volume — Red cell volume — Plasma volume — Body haematocrit — Rat.

Introduction

Several studies dealing with the effect of exposure to natural or simulated high altitude on blood volume were performed in rats (Fryers, 1952; Anthony and Kreidler, 1961; Tribukait, 1963; Feigen and Johnson, 1964; Johnson and LaRoche, 1968; Pepelko, 1971; Wolfe and Horvath, 1975; Kasalický et al., 1977). All these papers reported a larger circulating red cell volume per unit of body weight in rats exposed to high altitude. Plasma volume has been found to be decreased, not changed or increased. Consequently, the total circulating blood volume per unit of body weight must have been either the same or larger in rats exposed to high altitude, when compared with control sea-level animals. It has been suggested that variations in plasma volume probably are due to varying experimental conditions, particularly due to a different degree of hypoxia and variation in the duration of exposure (Wolfe and Horvath, 1975). To our knowledge, only rats born at sea level and exposed to high altitude later in their life were studied, but never animals born at altitude. The primary purpose of this study was to estimate the circulating red cell volume (RCV), the plasma volume (PV), and the total circulating blood volume (BV) in rats that spent their entire (also prenatal) life in conditions of chronic hypobaric hypoxia, and to compare them with control sea-level animals and with rats born at sea level and later exposed to simulated high altitude.

A common method of estimating blood volume is that either RCV or PV is measured, and BV and its complementary fraction are calculated from arterial or venous haematocrit. When, in our results, BV was calculated from RCV and Ahct, the absolute values and also the differences between groups were somewhat underestimated. When BV was calculated from PV and Ahct, the BV itself, and particularly the differences between groups, were overestimated quite considerably. It is suggested that the only safe way to estimate BV is to measure RCV and PV separately.
Age, body weight, arterial haematocrit (Ahct), circulating red cell volume (RCV), plasma volume (PV) and total circulating blood volume (BV) per kg body weight (b.w.), body haematocrit (Bhct), (Ahct-Bhct) and Bhct/Ahct in sea-level control animals, in rats born at sea level and later in life exposed to simulated altitude ("newcomers"), and in rats born and spending their entire life in a low pressure chamber ("natives") at a simulated altitude of 3500 m (mean ± S.D.; n = number of animals)

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 8)</th>
<th>&quot;Newcomers&quot; (n = 7)</th>
<th>&quot;Natives&quot; (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (days)</strong></td>
<td>85 ± 4</td>
<td>84 ± 2</td>
<td>86 ± 5</td>
</tr>
<tr>
<td><strong>Weight (g)</strong></td>
<td>316 ± 23</td>
<td>283 ± 16*</td>
<td>242 ± 20*</td>
</tr>
<tr>
<td><strong>Ahct</strong></td>
<td>45.9 ± 2.2</td>
<td>54.7 ± 1.4*</td>
<td>64.4 ± 5.2*</td>
</tr>
<tr>
<td><strong>RCV per kg b.w. (ml/kg)</strong></td>
<td>23.5 ± 3.8</td>
<td>28.7 ± 1.2*</td>
<td>32.8 ± 6.0*</td>
</tr>
<tr>
<td><strong>PV per kg b.w. (ml/kg)</strong></td>
<td>30.8 ± 2.2</td>
<td>29.6 ± 1.1</td>
<td>25.9 ± 2.2*</td>
</tr>
<tr>
<td><strong>BV per kg b.w. (ml/kg)</strong></td>
<td>54.2 ± 5.1</td>
<td>58.3 ± 1.7</td>
<td>58.7 ± 6.0</td>
</tr>
<tr>
<td><strong>Bhct</strong></td>
<td>43.1 ± 3.3</td>
<td>49.2 ± 1.3*</td>
<td>55.6 ± 5.2*</td>
</tr>
<tr>
<td><strong>Ahct-Bhct</strong></td>
<td>2.8 ± 2.1</td>
<td>5.5 ± 1.8*</td>
<td>8.8 ± 2.0*</td>
</tr>
<tr>
<td><strong>Bhct/Ahct</strong></td>
<td>0.938 ± 0.044</td>
<td>0.900 ± 0.030</td>
<td>0.863 ± 0.031*</td>
</tr>
</tbody>
</table>

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**a** P < 0.05 against controls
**b** P < 0.001 against controls
**c** P < 0.01 against controls
**d** P < 0.05 against "newcomers"
**e** P < 0.01 against "newcomers"
**f** P < 0.001 against "newcomers"

### Methods

Rats of Wistar strain were used. A group of female and male rats mated in the low pressure chamber was kept at a reduced pressure corresponding to an altitude of 3500 ± 200 m, the animals of another group were mated outside the chamber. Male animals born in the low pressure chamber stayed there until the day of the measurements; this group is called "natives". Male rats born outside the chamber were divided, at the age of 30–35 days, into a group which remained outside the chamber at sea level (controls) and a group which was placed into the chamber and stayed there until the day of the measurements, together with the "natives". This group (called "newcomers") lived at simulated altitude for 7–8 weeks. The low pressure chamber was kept at altitude days and nights with the exception of necessary breaks for cleaning and feeding; this happened two or three times a week for about half an hour. Control rats stayed in the same room where the low pressure chamber was placed in order to keep the unspecific conditions, particularly the noise of the pumps, comparable in all groups. Ventilation of the chamber prevented any measurable accumulation of CO₂ or water vapor. Temperature inside and outside the chamber was similar. All rats had free access to food and water.

All rats were studied during general anaesthesia with pentobarbi-
tal (45 mm per kg body weight i.p.). First the right atrium and aorta were cannulated with polyethylene cannulas, a plastic tracheal cannula was inserted, and the rats were allowed to breathe a mixture of about 12 °O₂ in N₂. This was done in order to ensure conditions similar to those in our previous experiments dealing with high altitude adaptation (see Turek et al., 1975). After the animals had been breathing this mixture for 30 min, rat erythrocytes labelled with ⁵¹Cr (about 1 μCi) and human albumin labelled with ¹²⁵I (about 4 μCi), both suspended in 0.3 ml saline, were injected through the venous cannula. Blood from donor rats was labelled early in the morning on the day of the measurements. Donors from all groups were used, and rats of each particular group always received erythrocytes from donor rats of the same group. The erythrocytes were labelled according to instructions of the Radiochemical Centre, Amersham, England. 3 ml blood were mixed with 1 ml ACD-1 (acid citrate dextrose) solution, about 200 μCi ⁵¹Cr sodium chromate were added, and the mixture was incubated for 30 min; then ascorbic acid (50 mg in 5 ml saline) was added, the mixture was centrifuged and washed with 5 ml saline three times; finally the erythrocytes were suspended in 1 ml saline. The suspension was mixed with ¹²⁵I-serum albumin (Amersham), activity about 50 μCi per ml, in a ratio of 3:1.

Ten minutes after the injection of labelled erythrocytes and plasma, 0.3 ml arterial blood was sampled; this equilibration time is sufficient according to Rakušan and Rajhathy (1972), and to our own preliminary experiments.

At the same time blood for the estimation of the haematocrit in a microcentrifuge was taken from the arterial cannula. Haematocrit values were corrected for plasma trapping by multiplying by a factor of 0.967, the mean value obtained from 10 measurements in preliminary experiments. Blood (0.3 ml) for the measurements of the radioactivity was digested in 4.7 ml 6 N KOH for 24 h and the activity of ⁵¹Cr and ¹²⁵I was determined in a well scintillation counter (Philips). From the administered activity, the activity in the blood sample and the corrected arterial haematocrit (Ahct), RCV and PB are calculated. The volumes of the added erythrocytes and the albumin were subtracted. The sum of RCV and PV equals the total circulating BV, assuming that the volume of leucocytes and thrombocytes is negligible. The ratio of RCV and BV in per cent corresponds to the body haematocrit (Bhct).

Statistical evaluations were done according to Snedecor and Cochran (1967). For the evaluation of differences between groups the t-test was used. P < 0.05 was assumed as a criterion of significance.

### Results

The results are shown in Table 1. The rats of all three groups are of the same age, but rats exposed to chronic hypoxic hypoxia grew more slowly, particularly the "natives", as seen from the weights. Arterial haematocrit is larger in "newcomers" than in controls and much larger in "natives". Contrary to previous studies (Turek et al., 1973, 1975), the values in Table 1 were corrected for plasma trapping but this difference is minor. Because of the difference in body weight (b.w.), RCV, PV and BV are expressed per kg b.w. to make them comparable. RCV per kg b.w. in both "newcomers" and "natives" is larger than in control animals, the difference between "newcomers" and "natives" not