Respiratory functions of blood of the yak, llama, camel, Dybowski deer, and African elephant

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To understand the gas transport function of the blood, these data are required: the oxygen capacity, the oxygen dissociation curve, the buffering capacity, the carbon dioxide dissociation curve, the Bohr effect (the effect of changes in pH on the oxygen dissociation curve), and the Haldane effect (the effect of changes in oxygen saturation upon the carbon dioxide dissociation curve). The oxygen capacity and the oxygen dissociation curve of the blood are known for many mammals. It is not clear whether the established differences among animals are important for gas exchange. The high oxygen affinity of the blood of the llama suggests an adaptation to its high-altitude home. Blood of the rat, mouse, or cat shows a relatively low affinity for oxygen when compared to larger mammals, suggesting a relationship between blood oxygen affinity and metabolic mass (1). A correlation also exists (2) between the shape of erythrocytes and the oxygen affinity.

To further clarify these relationships we have studied blood from the yak (another high-altitude resident), the camel which has oval erythrocytes and is a relative of the llama, the deer whose erythrocytes become sickle-shaped, and the elephant which, among the mammals, has the largest red cells known. As the second largest living mammal, the elephant will also contribute to the suggested correlation between oxygen affinity and oxygen consumption.

The rare opportunity of studying the blood of these animals led us to carry out some measurements which have no direct bearing on the respiratory function of the blood but which may be important for comparative physiology.

METHODS

General. About 50 ml of venous blood were drawn without anesthesia from each animal. The blood for gas analysis was mixed with 0.1% sodium fluoride, 0.2% potassium oxalate, and powdered heparin (Vetren). It was kept in ice water for a maximum of 4.5 hr before analysis. Blood for electrolyte analysis was prevented from clotting with calcium heparinate and centrifuged within 45 min of sampling. Blood counts, blood smears, and hemato-d analysis were prepared within 20 min of blood withdrawal.

Blood gas analysis. Equilibration, analysis, and calculation of gas content in the blood samples and construction of the oxygen dissociation curves were done as described in detail by Bartels and Harms (3). Because of the rapid sedimentation rate of elephant red blood cells, samples of this blood were hemolyzed anaerobically immediately after equilibration. Hemolysis was accomplished by freezing (in a bath of dry ice and acetone) of the blood in polyvinyl chloride tubing which had previously been rinsed for 1 hr or more with pure CO₂. After thawing the hemolysate was transferred directly to pipettes for im-
mediate introduction into the Van Slyke apparatus. Analysis for oxygen content of similar hemolysates gave results within 0.1 vol% (randomly distributed) of analyses of the same human or goat blood analyzed without hemolysis. Analyses for CO2 content and pH were done without hemolysis, as speedily as possible. The equilibrations were all made at 37 C although the body temperatures of some species (the deer and the yak) may be as much as 2 C higher. With the facilities available, analyses at body temperatures were not possible. If one uses the temperature correction factor for human blood (4), the T50 value is increased by approximately 0.5 mm Hg for the African elephant and the llama, 1.7 mm Hg for the camel, and about 2.6 mm Hg for the yak and the deer. The human temperature factor may not be correct, however, since a smaller value has been found for goat blood (unpublished results).

For measurement of the Bohr effect, blood samples were equilibrated with such gas mixtures that at oxygen saturations of approximately 50%, the pH varied from 7.0 to 7.7. From the pO2 values for 50% saturation (T50) and the measured pH values, the Bohr effect was determined and expressed as Δ log pO2/Δ pH (see Table 3).

The Haldane effect (expressed as the difference in carbon dioxide capacity at a pCO2 of 40 mm Hg between fully oxygenated blood and blood containing no oxygen) was calculated using a carbon dioxide dissociation curve at full oxygen saturation drawn with a nomogram which Henderson published in an appendix to the German edition (5) of his book. This nomogram was constructed from data obtained on human blood. The carbon dioxide contents of the samples which were 50% or less saturated with oxygen at a pCO2 of 40 mm Hg were sketched in. The increase of carbon dioxide content resulting from decreasing oxygen saturation was read off and the Haldane effect for complete desaturation calculated.

**pH Measurement.** Duplicate electrometric measurements were made on each blood sample used for the determination of the Bohr effect. The pH of the red cells was measured as described elsewhere (6).

**Morphology of the blood.** Number of erythrocytes (RBC) was counted in duplicate in the Neubauer chamber after dilution with physiological saline (7). Hematocrit (Vc) determinations were done in duplicate using a microhematocrit centrifuge. Hemoglobin (Hb) concentration was calculated from triplicate measurements of the oxygen capacity. Erythrocyte surface (MS) was measured by planimetry of the magnified red cell image.

Values for mean cell volume (MCV), erythrocyte diameter (MD) (in the camel and yak, two additional diameters are given since we are dealing with elliptical erythrocytes), erythrocyte thickness (MT), and erythrocyte surface area (MSA) were obtained by methods described elsewhere (8).

**Electrolytes.** Plasma and an hemolysate of packed red cells were analyzed for potassium and sodium with a flame photometer (without phosphorus correction), for magnesium according to Orange and Rhein (9), and for chloride (10). Total phosphorus was determined by a method modified from Fiske and Subbarow (11). Blood water was measured by drying at 80 C to constant weight. Plasma proteins were determined according to Kingsley (12).

**Starch block electrophoresis.** The hemolysate of washed erythrocytes was prepared with carbon tetrachloride and

### Table 1. General information about the animals studied

<table>
<thead>
<tr>
<th>Zoological Name</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Birthplace</th>
<th>Estimated Wt., kg</th>
<th>Body Temperature, C</th>
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</thead>
<tbody>
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<td>2</td>
<td>Basel Zoo</td>
<td>250</td>
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</tr>
<tr>
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<td>200</td>
<td>38-39</td>
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<tr>
<td>Camel</td>
<td>Camelus bactrianus</td>
<td>10</td>
<td>Rotterdam</td>
<td>300</td>
<td>37-5</td>
</tr>
<tr>
<td>Dybowski deer</td>
<td>Pseudonax hortulorum</td>
<td>1.5</td>
<td>Basel Zoo</td>
<td>35</td>
<td>38.5-39.5</td>
</tr>
<tr>
<td>African elephant</td>
<td>Loxodonta africana</td>
<td>10.5</td>
<td>Tanganika</td>
<td>2,300</td>
<td>37-38</td>
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</table>
TABLE 2. Gas pressures and contents, O₂ saturation, and pH of blood (calculated from gas analyses and measured with glass electrode), and in hemolysates of red cells (measured with glass electrode)

<table>
<thead>
<tr>
<th>pO₂</th>
<th>pCO₂</th>
<th>CO₂ tot. vol. %</th>
<th>CO₂ tot. vol. %</th>
<th>HbO₂ %</th>
<th>HbO₂ Gasometric</th>
<th>HbO₂ Glass Electrode</th>
<th>pH</th>
<th>T½ mm Hg</th>
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<tr>
<td>295.0</td>
<td>45.3</td>
<td>21.18</td>
<td>48.2</td>
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<td>7.428</td>
<td>7.441</td>
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<tr>
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<td>12.31</td>
<td>54.5</td>
<td>100</td>
<td></td>
<td>7.422</td>
<td>7.422</td>
<td></td>
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<tr>
<td>26.1</td>
<td>44.5</td>
<td>13.45</td>
<td>55.6</td>
<td>100</td>
<td></td>
<td>7.432</td>
<td>7.425</td>
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<td>47.0</td>
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<td>100</td>
<td></td>
<td>7.392</td>
<td>7.392</td>
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</table>

The oxygen pressure necessary for half saturation of the hemoglobin (T½) at the corresponding pH is also given.

separated at a pH of 8.6 using 50 ml and 36 ml for 16 hr at 5 C.

Oxidation of hemoglobin with potassium ferricyanide. This method (13) measures a process closely related to the uptake of oxygen because conversion of the iron in hemoglobin to the ferric state depends upon the dissociation of O₂ (or CO) from hemoglobin (14).

Activity of glucose-6-phosphate dehydrogenase. A method modified from Motulsky (15) was used.

**Observations and Discussion**

Data on the respiratory function of the blood can be interpreted with full validity only when the gas pressures and contents in arterial and mixed venous blood are known. It remains to be seen if such values can be obtained in these animals without narcosis.

**Oxygen dissociation curves.** In Tables 2 and 3 and Fig. 1, the most important data are presented. Among the mammals which live at high altitude, only the llama, glama and llama vicuna have previously been studied (16, 17). The high oxygen affinity of the blood in these animals has been likened to that of fetal blood (17) and clearly aids in the uptake of oxygen by blood in the lungs at high altitude. It has not been demonstrated, however, whether this increased oxygen affinity is an adaptation to high altitude or if these animals, because of previous high blood oxygen affinity, have been able to survive and succeed biologically in places of great height. The blood of the camel, which belongs to the same family as the llama, has a half-saturation pressure of 24.1 mm Hg, relatively close to that measured in the llama. In contrast, the yak, an animal which lives in altitudes equally high as the llama, has a lower blood oxygen affinity, resembling that of humans (a T½ value of 26 mm Hg) and its relative, the domestic cow. This association of evidence suggests that the shape of the oxygen dissociation curve of llama blood is a family characteristic which enables the species to live in high places rather than an adaptation to high-altitude living. When the shape of the curves is considered (Fig. 1), the steepness of the upper part of the yak blood curve is a feature which would encourage oxygen uptake in the lungs under lowered oxygen partial pressure.

The lowest oxygen affinity (expressed as the T½ value)
The loss of the blood (I oxygen tension as in human drepanocytosis, but to alkalosis of the blood at high altitude. Some confirmation of this remarkable finding has been given by Meschia et al. (16), who found an oxygen capacity of only about 13.5% in llamas at sea level. In the single animal which they studied at high altitude they found a decrease of oxygen capacity. We found a high oxygen capacity in llamas. and highest in the deer (35 kg). Although our other subjects were of intermediate weight and had intermediate $T_{50}$ values, there was no regular correlation among them between $T_{50}$ and body weight. However, the general rule of increasing oxygen affinity with increasing body size finds general support from this work.

**Oxygen capacity.** We found a high oxygen capacity in llamas and in agreement with Hall and co-workers (28) and the general rule of increasing oxygen affinity with increasing body size finds general support from this work. **Oxygen capacity.** We found a high oxygen capacity in llamas and in agreement with Hall and co-workers (28) and the general rule of increasing oxygen affinity with increasing body size finds general support from this work. **Oxygen capacity.** We found a high oxygen capacity in llamas and in agreement with Hall and co-workers (28) and the general rule of increasing oxygen affinity with increasing body size finds general support from this work. **Oxygen capacity.** We found a high oxygen capacity in llamas and in agreement with Hall and co-workers (28) and the general rule of increasing oxygen affinity with increasing body size finds general support from this work. **Oxygen capacity.** We found a high oxygen capacity in llamas and in agreement with Hall and co-workers (28) and the general rule of increasing oxygen affinity with increasing body size finds general support from this work. **Oxygen capacity.** We found a high oxygen capacity in llamas and in agreement with Hall and co-workers (28) and the general rule of increasing oxygen affinity with increasing body size finds general support from this work.
vol % in a llama at Morococha (altitude 15,000 ft). However, their animal was pregnant.

The oxygen capacities of yak, camel, and deer blood lie in the area of 20–22 vol %. Only the African elephant has an appreciably lower value with 16 vol %. Despite the reservation mentioned above, we believe this finding to be valid since three values showed only small variation. Lang and Undritz (20) showed that the circus elephant frequently has an anemia which can be corrected by the administration of iron. The animal which we studied had been treated in this way, so the low blood oxygen capacity can be regarded as physiological.

"Standard bicarbonate" content of the blood. This value was about the same for all the animals except the deer. The lower value seen in the deer could have resulted from the great difficulty experienced in obtaining blood. The great motor activity before the puncture was accomplished would probably lower the carbon dioxide binding capacity (and increase the oxygen capacity).

The Bohr effect. This is of about the same size in the camel and the yak, smallest in the African elephant, and largest in the deer. The correlation between the magnitude of the Bohr effect and body weight which Riggs (21) found in hemoglobin solutions does not appear in our results (P = 0.5). Furthermore, a quantitative estimation of the Bohr effect with regard to its efficiency in the delivery of oxygen to the tissue requires, in addition to knowledge of the Bohr effect, an understanding of its interaction with the oxygen capacity and the oxygen dissociation curve (5). One sees (Table 3), for example, that with practically identical Bohr effects in the camel and the yak, the blood of the yak can deliver considerably more oxygen by acidification of 0.1 pH unit, largely because of a steeper oxygen dissociation curve. The blood of the deer has functionally the highest potential because of both a high oxygen capacity and a moderately steep oxygen dissociation curve. Therefore, the single value Δ log pO₂/Δ pH tells us relatively little. In general, one can say that with the same Bohr effect, its efficiency in promoting oxygen delivery increases with increasing oxygen capacity and increasing steepness of the oxygen dissociation curve.

The Haldane effect. The values obtained in the llama, yak, deer, and African elephant lie near values which have been found in humans (approximately 6 vol %). Camel blood contains 10 vol % more CO₂ at a pCO₂ of 40 mm Hg when it is completely reduced than when it is completely saturated with oxygen.

Morphology of the red blood cells. See Table 4. The number of erythrocytes varied between 3.2 (African elephant) and 10.8 (llama) million/mm³. The erythrocyte volume varied in inverse ratio to the number of erythrocytes: 120 μ³ in the African elephant and 25 μ³ in the llama. In previously studied mammalian erythrocytes the mean corpuscular hemoglobin concentration is remarkably constant at values between 29 and 35 % (22). In the family Cameliidae (the camel and the llama), we found 50 % higher concentrations. (Wintrobe (23) gives a hemoglobin concentration of 40 % in llama erythrocytes on the basis of colorimetric measurements.) The oxygen binding capacity can also be related to the erythrocyte volume (O₂/Vc ratio) (24). Again, 50 % more oxygen can be taken up per unit volume of cells in the Camelidae than in other mammals. The belief that we are dealing with a higher hemoglobin concentration in the erythrocytes is further supported by the lower cell water content (Table 5) in these animals. These findings are especially remarkable since Perutz (25) has given 34 % as the highest possible hemoglobin concentration in the erythrocytes.

The thickness of the red cells is greatest in the yak and least in the Tylopoda. Our observations give no support to the finding (26) that with increasing thickness of the red cells there is increasing oxygen affinity.

Electrolytes. Only the phosphate concentrations in the cells and plasma of the camel, African elephant, and deer have been previously published to our knowledge (22). A connection between the oxygen affinity and the intra- or extracellular electrolyte cannot be established from our studies.

Starch block electrophoresis. Hemoglobin travels with increasing speed in the following order: camel, yak, deer, and African elephant. Llama hemoglobin travels slowly at a speed similar to camel hemoglobin, but a strict simultaneous comparison of its migration speed with the other species was not carried out. Only in the yak does the pigment separate into two equally large components.

Oxidation speed. See Fig. 2. The speed is greatest for camel and yak blood and least in the blood from the African elephant. In the deer and camel the oxidation behaves, as in the human, like a monomolecular reaction. Yak, llama, and African elephant hemoglobin each
behave like a mixture of two hemoglobins with different reaction speeds.

Activity of glucose 6-phosphate dehydrogenase. In comparison to human erythrocytes which are taken as 100%, the activity in the erythrocytes of the llama is greater than 200%, camel 165%, African elephant 55%, deer 40%, and yak 0%. It is noteworthy that the yak erythrocytes are the thickest, while erythrocytes from the llama

REFERENCES