Blood gases in hibernating and active ground squirrels: \( \text{HbO}_2 \) affinity at 6 and 38 C

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MUSACCHIA, X. J., AND W. A. VOLKERT. Blood gases in hibernating and active ground squirrels: \( \text{HbO}_2 \) affinity at 6 and 38 C. Am. J. Physiol. 221(1): 128–130. 1971.—Venous and arterial blood \( \text{PO}_2 \), \( \text{PCO}_2 \), and pH were measured in hibernating (6 and 13 C) and normothermic (38 C) ground squirrels, Citellus tridecemlineatus. Direct measurements were made using chronic cannulas in the aorta and vena cava. A comparison of active and hibernating animals showed that the venous \( \text{PO}_2 \)'s were markedly reduced in hibernation whereas arterial \( \text{PO}_2 \)'s were not. Both arterial and venous \( \text{PCO}_2 \) levels were reduced in hibernation. The \( \text{HbO}_2 \) dissociation curves determined at 6 and 38 C on blood taken from active winter animals showed a marked shift to the left indicating increased \( \text{HbO}_2 \) affinity at low temperatures. The partial pressure of oxygen at 50% hemoglobin saturation \((P_{50})\) was 6 mm Hg at 6 C and 29 mm Hg at 38 C. In addition, the common observation that the blood in hibernating animals “appears” bright red may be credited to comparably higher percent saturation of the hemoglobin.

Hibernation; blood \( \text{PO}_2 \); blood \( \text{PCO}_2 \); blood pH; \( \text{HbO}_2 \) dissociation

SVIHLA AND BOWMAN (15) using Citellus parryi ablisus, in July, August, and September, reported that the volume percent \( \text{O}_2 \), percent saturation, \( \text{O}_2 \) capacity, and hemoglobin became elevated during dormancy. Popovic (11), in contrast, reported that both arterial and venous \( \text{O}_2 \) content (volume %) were decreased during hibernation in Citellus tridecemlineatus. The conflicting reports may be related, in part, to contrary erythrocyte/blood volume changes reported by these authors. However, in neither case can an evaluation of functional oxygen saturation be made. Popovic (11) does argue for further experiments dealing with hemoglobin and blood \( \text{PO}_2 \) relationships. We concur fully with the idea that the oxygen affinity for hemoglobin at reduced temperatures is essential to an understanding of relationships between blood gas changes and tissue oxygenation during hibernation.

To date, simultaneous direct measurements of blood oxygen and carbon dioxide tensions have not been made in normothermic and hibernating Citellus tridecemlineatus. With the development of a variable-temperature ultramicro blood gas analyzer, carbon dioxide and oxygen tensions and pH can be measured simultaneously from blood samples which are sufficiently small so that little or no insult is incurred.

The present investigation was conducted in order to examine both arterial and venous \( \text{PO}_2 \) and \( \text{PCO}_2 \) in hibernating and nonhibernating ground squirrels; acid-base relationships were also assessed. In addition, the effect of temperature on \( \text{HbO}_2 \) dissociation, comparable to that of blood in active (38 C) and hibernating (6 C) ground squirrels, was also measured.

MATERIAL AND METHODS

The aorta (via the right carotid artery) and vena cava (via the left jugular vein) of the ground squirrel (Citellus tridecemlineatus) were permanently cannulated by modifications of a method described by Popovic and Popovic (12) and previously used in hamsters in this laboratory (17). Blood from active and hibernating animals was taken with minimal disturbance and no anesthesia. A fundamental advantage of our cannulation procedure was the collection of blood without handling the ground squirrel. Both arterial and venous samples were obtained from normothermic animals (38 C) and from hibernating animals at a body temperature of 6 and 13 C. The body temperature was recorded by Tele-thermometer thermistor probes (Yellow Springs Instrument Co., Inc.) immediately after the blood sample was taken. Body temperature recordings were utilized to further substantiate that animals were normothermic (active) or hibernating at the time of blood collection. A polyethylene tube (Clay Adams, PE-10, Intramedic polyethylene tubing) about 2 feet long was attached to the permanently implanted cannulas of active (38 C) animals. These cannulas were exteriorized at the back of the neck. Disturbance by an investigator was minimized, since the animals were accustomed to his presence. Each ground squirrel was undisturbed for approximately 1 hr before samples were taken. Most of the squirrels were laboratory reared and were relatively tame compared to animals born in the wild. Blood was sampled from hibernating animals, without handling, while still in the curled position on their nests. Forma-lab environmental rooms (Forma Scientific, Inc.) were used to accommodate hibernacula. Most of the animals were in their hibernacula (Maryland Plastics, acrylonitrile cages, 10.5 inches long x 8 inches wide x 6 inches high) with cedar wood shavings for approximately 30 days before sampling. The \( \text{PO}_2 \), \( \text{PCO}_2 \), and pH were determined immediately after sampling. Measurements were done with Instrument Laboratory equipment (model 123 ultramicro blood pH/\text{PCO}_2 analyzer and model 125A
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polarographic oxygen analyzer). One 0.15-ml sample was used to measure three parameters, P₀₂, Pco₂, and pH. The oxygen electrode, the CO₂ electrode, and the micro pH capillary electrode were maintained at the body temperature of the animal and measurements were made at 38, 13, or 6°C. Hemoglobin determinations were made by the acuglobin method with only a 0.02-ml blood sample.

The hemoglobin-oxygen saturation curves for whole blood were done on samples taken from active winter animals (38°C). Dissociation curves were determined on samples at 38°C and 6°C at a pH of 7.40 ± 0.04 by a method previously described (17). These temperatures were selected since they represent body temperatures of normothermic and hibernating ground squirrels.

RESULTS

The blood gas and blood pH data for both hibernating and active normothermic ground squirrels are presented in Table 1. The hemoglobin concentrations in the hibernating animals decreased 20% from 15.8 ± 1.1 mg/100 ml in the normothermic animals to 12.6 ± 1.7 mg/100 ml in hibernating squirrels.

Venous PO₂ is markedly reduced in hibernating animals, and arterial Par values show a great deal of variability and are not significantly changed (Table 1). Despite the considerable variation in the values for arterial Par, in all cases the levels were sufficient to saturate the hemoglobin 100% at both 6 and 13°C (Fig. 1).

**TABLE 1. Blood gases and pH values in ground squirrel**

<table>
<thead>
<tr>
<th>Body Temp. °C</th>
<th>No. of Animals</th>
<th>P₀₂, mm Hg</th>
<th>Pco₂, mm Hg</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Venous blood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>10</td>
<td>97.9 ± 2.9</td>
<td>51.9 ± 4.63</td>
<td>7.39 ± 0.03</td>
</tr>
<tr>
<td>(25.0-31.0)</td>
<td></td>
<td>(46.0-54.0)</td>
<td></td>
<td>(7.35-7.42)</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>11.0</td>
<td>43.0</td>
<td>7.41</td>
</tr>
<tr>
<td>10.5</td>
<td></td>
<td>39.0</td>
<td></td>
<td>7.47</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>6.3 ± 1.2</td>
<td>36.7 ± 2.8</td>
<td>7.42 ± 0.06</td>
</tr>
<tr>
<td>(4.0-8.0)</td>
<td></td>
<td>(33.0-41.0)</td>
<td></td>
<td>(7.33-7.48)</td>
</tr>
<tr>
<td>P &lt; .001†</td>
<td></td>
<td>P &lt; .001</td>
<td>P - NS</td>
<td></td>
</tr>
<tr>
<td><strong>Arterial blood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>16</td>
<td>64.9 ± 5.8</td>
<td>47.7 ± 5.0</td>
<td>7.40 ± 0.03</td>
</tr>
<tr>
<td>(30.0-78.0)</td>
<td></td>
<td>(40.0-52.0)</td>
<td></td>
<td>(7.34-7.44)</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>48.0</td>
<td>35.0</td>
<td>7.47</td>
</tr>
<tr>
<td>67.0</td>
<td></td>
<td>41.0</td>
<td></td>
<td>7.38</td>
</tr>
<tr>
<td>90.0</td>
<td></td>
<td>33.0</td>
<td></td>
<td>7.41</td>
</tr>
<tr>
<td>80.0</td>
<td></td>
<td>37.0</td>
<td></td>
<td>7.46</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>87.7 ± 22.3</td>
<td>32.9 ± 2.3</td>
<td>7.44 ± 0.03</td>
</tr>
<tr>
<td>(33.0-120.0)</td>
<td></td>
<td>(29.0-37.0)</td>
<td></td>
<td>(7.40-7.36)</td>
</tr>
<tr>
<td>P = NS</td>
<td></td>
<td>P &lt; .001</td>
<td>P &lt; .01</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± sd, ranges are in parentheses. Individual values are cited where only 2 or 4 specimens were available. * 38°C = active (normothermic) animals; 13 and 6°C = hibernating animals. † Statistical differences between 38 and 6°C animals.

Venous Pco₂ showed a fall in the hibernating animals, and concomitantly the arterial Pco₂ values were lowered during hibernation (Table 1).

Hemoglobin-oxygen dissociation curves at 6 and 38°C were determined in whole blood near a pH of 7.40 (Fig. 1). This pH value was selected particularly since it was representative of blood pH, in vivo, at these temperatures. The partial pressure of oxygen at 50% saturation of the hemoglobin (P₉₀) was found to be 6 mm Hg at 6°C and 29 mm Hg at 38°C.

DISCUSSION

Our results, and those of others (11, 14, 15), show that there is a significant change in hemoglobin concentration during hibernation. Despite this change in total protein content, the squirrel in hibernation apparently is able to respond with a maintained acid-base balance similar to that in normothermic animals. These acid-base values reflected in and measured as pH and Pco₂ (Table 1) compare favorably with those discussed elsewhere (8, 10) in the same species.

Quantitation of the predictable shift to the left of the HbO₂ dissociation curve due to low temperatures required experiments such as those herein reported. The relative positions of the HbO₂ curve at 6°C in Fig. 1 show that the oxygen affinity for hemoglobin is markedly increased at low temperatures. The marked reduction in venous P₀₂ during hibernation indicated furthermore the effectiveness of HbO₂ dissociation in this species under conditions of hibernation. A venous P₀₂ of 6.5 ± 1.2 in the hibernating ground squirrel corresponds to approximately 50% saturation of hemoglobin on the HbO₂ dissociation curve at 6°C. Using the average values reported by Popovic (11) for arterial and venous oxygen content (in volume %) for hibernating Citellus tridecemlineatus, the average saturation of the
venous hemoglobin can be calculated to be 54%. Assuming that in the animals used by Popovic the arterial blood of the hibernating animal is 100% saturated, then the latter value (54%) is in agreement with that value (30%) calculated by using the herein reported venous Po2 and HbO2 dissociation curve at 6°C.

The Po2 for the HbO2 dissociation curve for the 38°C ground squirrel was comparable to that reported by Hall (7) for the same species at 37°C and was lower than that which could be predicted on the basis of body weight (13). Based on body weight, the Po2 has been found to be lower in hamsters (13, 16, 17) and other burrowing animals (5, 7, 13). Hall (7) viewed the low Po2’s for the HbO2 dissociation curves, generally exhibited by burrowing animals, as a characteristic adaptation to hypoxic environments.

It is of note that in the normothermic state both the ground squirrel (Table 1) and hamster (17) exhibit arterial and venous Po2 values which are generally lower than those reported for man and other mammals (3, 4, 9). The average arterial Po2 in both ground squirrel and hamster is sufficient to saturate the hemoglobin to only 88% of its capacity. This low arterial oxygen tension under normoxic (21% O2) ambient conditions tends to raise a question concerning the tissue Po2 requirements. It seems reasonable to interpret these findings as well as other evidence (5) to indicate that these animals have a high tolerance to a hypoxic environment. It is probable, therefore, that the ground squirrel is adapted to function normally with lower blood oxygen levels.

Clausen and Erslund (6) reported that the temperature affinity coefficient, K50, for the European hedgehog was lower than that reported for other mammals. The K50 defined by the equation

$$\log P_1 - \log P_2 = K_{50}(T_2 - T_1)$$

where P1 and P2 are the P50 values for the HbO2 curves at temperatures T1 and T2, respectively. The low K50 (0.017) for hemoglobin in the European hedgehog was suggested as a possible adaptation to hibernation. The K50 values for the ground squirrel were 0.0215 and 0.0220 for the hamster (17). These values are similar to those reported for other mammalian hemoglobins (1, 2, 6). Although the hamster and ground squirrel are hibernators, their hemoglobin K50 values are similar to other mammals and do not exhibit an adaptation to hibernation comparable to that of the European hedgehog.

Arterial Po2 in hibernating ground squirrels (6 and 13°C) was variable but always sufficiently high to completely saturate the hemoglobin; this can be seen in the position of the HbO2 dissociation curves at low temperature. Venous oxygen levels, on the other hand, were substantially reduced in hibernation and can be attributed to the increased HbO2 affinity at low body temperatures. With a high HbO2 affinity at lower temperatures, the capability of the blood to supply oxygen to surrounding tissues is reduced. Thus, we maintain that the hibernating ground squirrel may be expected to have an average blood Po2 well below that exhibited by the normothermic animal.

Our experiments may, in part, answer the questions concerning the observation that innumerable investigators have made; that is, that the blood in hibernating animals appears bright red and therefore is highly oxygenated. This has been seen during surgical procedures or blood sampling in animals during hibernation. Thus our results may be offered as evidence that indeed the hemoglobin in the arterial trunk is highly saturated and accounts for the bright red appearance of the blood during hibernation; however, the average blood Po2 is lower than in normothermia.

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REFERENCES