Physiological properties of hemoglobin in the branchiopod crustacean Triops

FRANCIS R. HORNE AND KLAUS W. BEYENBACH

Southwest Texas State University, San Marcos, Texas 78666

HORNE, FRANCIS R., AND KLAUS W. BEYENBACH. Physiological properties of hemoglobin in the branchiopod crustacean Triops. Am. J. Physiol. 220(6): 1875-1881. 1971. Hemoglobin of the freshwater crustacean, Triops longicaudatus, was found to have oxygen equilibrium curves similar to those of the white rat when compared under identical experimental conditions (e.g. pH=7.1; temp = 22-23 C; Hb = 2 mg/100 ml; P50 = 6.8 mm O2 (Triops); P50 = 6.1 mm O2 (rat)). Magnitudes of temperature and Bohr effects, however, were different. Triops hemoglobin was affected least by both parameters. Physiological concentrations of the chloride salts of calcium, magnesium, potassium, and sodium did not affect equilibria, whereas high concentrations of calcium (52 mm) and magnesium (15 mM) increased oxygen affinity (P50 reduced from 8.73 to 6.7 mm O2). Oxygen-carrying capacity of the blood, hemoglobin concentration, and carbon monoxide affinity of hemoglobin were 3.22 vol %, 1.62 g/100 ml, and 282:1 (CO:O2). By comparing respiratory rates of normal and carbon monoxide treated animals, Triops hemoglobin was found not to be essential for survival. Instead, hemoglobin seems only to assist in oxygen delivery to the tissues during periods of oxygen stress. Some individuals in all Triops populations have little, if any, hemoglobin.

Oxygen transport; tadpole shrimp; respiratory pigment

Most research on respiratory pigments has been conducted on vertebrate hemoglobins. Oxygen transport by vertebrate hemoglobins is therefore well known, whereas in the invertebrate animals the functional significance of hemoglobin is poorly understood. In some invertebrates hemoglobin functions only at low oxygen tensions, while in others it seems to operate in oxygen storage (19).

Among the Crustacea hemoglobin is restricted to the entomostracans where it has been reported from representatives of the Branchiopoda, Ostracoda, Copepoda, Branchiura and Cirripedia (6). But only in the Branchiopoda is hemoglobin widely distributed. Of the many species in this taxon known to possess hemoglobin, the cladocerans, Daphnia spp. and Simocephalus vetulus, and the brine shrimp, Artemia salina, are the only ones where hemoglobin actually has been shown to be important in oxygen transport (7, 9, 13). Generally speaking, investigations into the physical and chemical properties of invertebrate hemoglobins are more common in the literature than studies relating to the functional significance of the pigment.

Because branchiopods are often inhabitants of temporary ponds where daily fluctuations in oxygen concentration are extreme, the physiological value of their hemoglobin and the parameters affecting its function are of interest. This is especially true for the notostracan, Triops longicaudatus, which has an unusually high metabolic rate (16) and lives in an environment where oxygen concentrations range from saturation to less than 1 ppm (12). To establish the physiological value of hemoglobin to the tadpole shrimp, Triops, the effects of temperature, hydrogen ion concentration, and specific ions on hemoglobin oxygen equilibria were tested. Oxygen-carrying capacities and carbon monoxide affinity also were studied. And to estimate oxygen demand under different thermal conditions and to verify the physiological requirement for hemoglobin, respiratory rates were taken on normal and carbon monoxide-treated animals.

Materials and Methods

Triops longicaudatus was collected from temporary ponds in Comal, Hays, and Dawson Counties, Texas. Even though there was no reason to assume that the hemoglobins of the various populations were different, in any one experiment only haemolymph of the same population was pooled.

Isolation of hemoglobin. Depending on the experiment, different amounts of haemolymph were collected from Triops according to the method of Horne (10). For an oxygen equilibrium experiment with dilute hemoglobin, 20-50 tadpole shrimp weighing from 184 to 346 mg (wet wt) were sacrificed so that their pooled haemolymph totaled 2 ml. The haemolymph was kept on ice during extraction. Since experiments were run immediately after collection of haemolymph, poisoning the sample with CO was unnecessary. Following collection, the sample was centrifuged at 10,000 X g, and the supernatant hemoglobin solution was diluted with an appropriate buffer.

Spectral analysis. All spectral properties were taken with a Beckman DU-2 spectrophotometer or Bausch and Lomb Spectronic 505.

Hemoglobin concentrations. The cyanmethemoglobin method (Hycel Company, Houston) was used to determine colori-
metrically hemoglobin concentrations of whole hemolymph. For dilute hemoglobin solutions, the concentration was measured in a Beckman DU-2 spectrophotometer using the extinction coefficient of Benesch et al. (3). It was assumed that *Triops* hemoglobin displayed essentially the same optical properties as that of man.

**Oxygen-carrying capacities and carbon dioxide contents of hemolymph.** Oxygen-carrying capacities and dissolved carbon dioxide were measured manometrically with the Natelson microgasometer according to the methods of Van Slyke and Neill (25).

**Oxygen equilibria.** Oxygen equilibria on whole haemolymph were measured manometrically. A tonometer was used to deoxygenate hemoglobin according to the method of Riggs (22). However, the progress of deoxy- and oxygenation was followed manometrically instead of spectrophotometrically. Oxygen equilibria of diluted hemoglobin (approx. 0.2%) solutions were determined with the Beckman DU-2 spectrophotometer at 540, 560, and 580 nm. Solutions were considered deoxygenated when the $A_{560}$ ratio was 0.70 and less. Percent methemoglobin contamination and pH were measured immediately after each experiment (3). The value of the Hill equation was determined for 50% oxygenation from plots of log $y/1-y$ against log $p$ where $y$ is the fractional oxygenation and $p$ the oxygen pressure.

All oxygen equilibria were run with freshly collected haemolymph because preliminary experiments with frozen and thawed haemolymph samples showed a substantial increase in $P_{50}$. Although methemoglobin contamination was kept at a low level, an oxygen equilibrium with frozen and thawed hemoglobin yielded a $P_{50}$ of 18 mm Hg, which is significantly higher than the $P_{50}$ of freshly collected *Triops* hemoglobin.

**Bohr and temperature effects.** To study the Bohr effect on the oxygen equilibrium, a 0.1 M Na$_2$HPO$_4$ - KH$_2$PO$_4$ buffer in 0.1 M NaCl was used between pH 5.5 and 7.5. Between pH 7.5 and 0.5 a borate-borax buffer, 0.5 M Na$_2$B$_4$O$_7$ and 0.2 M H$_2$BO$_3$ in 0.1 M NaCl was used to adjust the hemoglobin concentration spectrophotometrically to the desired concentration of 2 mg/ml. The temperature was held at 23°C. In the studies where temperature was the variable, the pH was held at 7.39 ± 0.20 and the temperature of the water bath adjusted from 15 to 45°C, at intervals of 5 or 10°C. All equilibrations were no longer than 10 min.

**Effects of ions.** In testing the effects of various ions on oxygen equilibria, 0.1 M Tris buffer containing 298.7 mm sodium, 23.7 mm potassium, 0.57 mm calcium, and 1.05 mm magnesium was used as a control to compare with whole blood, since such a high concentration of salts did not seem to effect the oxygen equilibria. The experiments differed only in the concentration of one salt (as chloride). The effects of elevated sodium, potassium, calcium, and magnesium concentrations on the oxygen equilibria were examined. Ionic contents were measured after each experiment with the model 290-B Perkin-Elmer atomic absorption spectrophotometer.

**Carbon monoxide affinity.** The affinity of oxyhemoglobin for carbon monoxide was measured spectrophotometrically at 560 nm and at a hemoglobin concentration of 2 mg/ml. At this wavelength, maximum changes in absorbance in the visible spectra occurred during the shift from oxy- to carboxyhemoglobin. Initially hemoglobin was equilibrated with oxygen at atmospheric pressure; then small volumes of carbon monoxide (up to 2 ml) were injected into a tonometer with a volume of 205-220 ml by a syringe. Equilibration time was, as in the other equilibrium studies, 10 min, and tonometers were never saturated at more than 60 rpm. The carbon monoxide affinity relative to that of oxygen is given by the relationship $K = (HbCO)/(HbO_2)(CO)$ in which $K$ is the affinity constant of the reaction.

To form carboxyhemoglobin in live shrimp, enough water saturated with carbon monoxide was added to oxygen-saturated water to give a final CO:O$_2$ ratio of 1:66. After subjecting to the carbon monoxide solution for at least 30 min, *Triops* hemoglobin was essentially in the carboxy state. Extraction and spectral examination of the blood after 30 min were used to show this change. By comparing the difference between the ratio of absorbance ($A_{570}$ nm (oxy-peak))/$A_{560}$ nm (carboxy-peak)) of the unknown with the total difference of the ratios of oxy- and carboxyhemoglobin, an estimate of percentage carboxyhemoglobin was obtained. Longer periods of exposure to the CO solution did not significantly increase the spectral shift. Because the animals did not die from the carbon monoxide treatment, it is assumed that the CO:O$_2$ was not sufficient to block the cytochromes.

**Respiratory rates.** For small *Triops* (100-200 mg) rates of oxygen consumption were taken either with a Warburg respirometer or a Yellow Springs Instrument oxygen monitor. The Winkler method as employed by Hotovy (16) was used for larger specimens (1,000-2,500 mg). Respiratory rates were taken at temperatures ranging from 23 to 36°C and all specimens were maintained in the laboratory at 21 ± 2°C for at least 48 hr prior to each experiment.

**RESULTS AND DISCUSSION**

**Spectral properties of *Triops* hemoglobin.** The absorption spectra of four hemoglobin derivatives, deoxy-, oxy-, carboxy-, and cyanmethemoglobin were taken on the same sample of hemoglobin (Fig. 1). Absorption spectra maxima are summarized and compared with other crustacean and mammalian hemoglobin in Table 1. Positions of the absorption peaks of *Triops* hemoglobin illustrate the similarity of the crustacean hemoglobins. The span, which is the distance in nanometers between the bands of oxy- and carboxyhemoglobin (1), was five for *Triops* hemoglobin and identical to that of the closely related conchostracan *Ozyius*. Information is not available for the carboxy peaks of the hemoglobin of *Lepadus*, which is the only other genus in the order Notostraca. Hoshi’s study (13) allowed the determination of three cladoceran hemoglobin spans. For *Simocephalus vetulus*, *Daphnia pulex*, and *Moina macrocopa*, the span was 4 nanometers. Thus, the cladoceran, conchostracan, and notostracan hemoglobins exhibit a basic similarity in their spectroscopic properties.

**Carbon monoxide affinity.** Spectral analysis of carboxyhemoglobin had already shown that the hemoglobin of *Triops* combines readily with carbon monoxide. In the carboxy state the pigment was more stable since conversion to
Physiological properties of a crustacean hemoglobin

1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2

Wavelength (nm)

Absorbance

DeoxyHb
OxyHb
COHb
MetHb

FIG. 1. Typical spectra of Triops hemoglobin derivatives.

<table>
<thead>
<tr>
<th>TABLE 1. Spectroscopic data for hemoglobin and its derivatives of Triops, other Branchiopoda, and a mammal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Triops</td>
</tr>
<tr>
<td>Lepidurus</td>
</tr>
<tr>
<td>Daphnia</td>
</tr>
<tr>
<td>Moina</td>
</tr>
<tr>
<td>Simocephalus</td>
</tr>
<tr>
<td>Cyclicus</td>
</tr>
<tr>
<td>Man</td>
</tr>
</tbody>
</table>

λ = nm.

methemoglobin by K3Fe(CN)6 did not proceed with the usual ease. Because hemoglobins are known to differ in their reactions with carbon monoxide, the affinity of Triops hemoglobin was measured. Based on four determinations, and considering the absorption coefficients of oxygen and carbon monoxide, the average affinity of Triops hemoglobin for carbon monoxide was about 282 times greater than for oxygen.

\[
K = \frac{(50\% \text{ HbCO}) \times (210.4 \text{ ml } O_2)}{(\text{absorption coefficient of } O_2 \text{ in water } 0.03102)} = \frac{(50\% \text{ HbO}_2) \times (1.0 \text{ ml } CO)}{(\text{absorption coefficient of } CO \text{ in water } 0.02319)}
\]

The absorption coefficients of the gases in water were used because the hemoglobin is reacting with the gas concentration that is dissolved, not the concentration in air. For an invertebrate hemoglobin, such a value is relatively high and comparable to the affinity of horse hemoglobin (K = 280); other invertebrate hemoglobins usually have low affinities, c.g. K = 150 for Arenicola; 40 for Tubifex and Planorbis (21).

Hemoglobin concentration, oxygen-carrying capacity, and carbon dioxide content. In Triops collected throughout the year from local temporary ponds and from ponds in Wyoming, haemolymph hemoglobin concentrations averaged 1.64 ± (sd) 1.08 g/100 ml (n = 6) and 2.04 ± 0.34 g% (n = 10) for the Texas and Wyoming populations. At the former hemoglobin concentration, oxygen values for whole, freshly collected blood of Texas Triops averaged 0.93 vol%, whereas an aliquot of the same sample, but saturated with oxygen, had an oxygen carrying capacity of 3.22%. The figures for unsaturated blood are low because deoxygenation was occurring during extraction of haemolymph under oil. Since the presence of hemoglobin increases the oxygen-carrying capacity by about fivefold and since the molecule can be deoxygenated in vivo, hemoglobin would seem of physiological value to Triops (Table 2).

Age, size, and stage of molt were not observed to affect hemoglobin concentration. Some animals in all of the Triops populations studied had only a small amount of hemoglobin. These individuals were similar to remaining members of the...
population in other features. Neither temperature nor oxygen concentration is known to influence hemoglobin concentration in Triops, although such a response was found by Fox and Phaer (8) in Daphnia.

The carbon dioxide concentration of Triops blood was not unusually high; though as in other crustaceans, it was greater in the hemolymph than in the external media. Values of 15.97 and 17.50 vol% were measured in oxygen-saturated and freshly collected hemolymph, respectively. The effects of carbon dioxide and pH on oxygen equilibria will be discussed later.

Effect of dilution on oxygen equilibria. Oxygen equilibria are subject to a number of variables which include temperature, hydrogen ion concentration, the presence of salts, and ionic strength. Moreover, observations made on diluted hemoglobin cannot always be compared quantitatively with those of whole blood (4). Because oxygen equilibria are influenced by the above mentioned parameters, equilibria of diluted Triops hemoglobin (2 mg/ml) were compared with oxygen equilibria of a mammalian hemoglobin (Rattus norvegicus) under approximately identical experimental conditions. Furthermore, oxygen equilibria on whole Triops haemolymph were determined to show that diluted Triops hemoglobin solutions responded like that of whole blood. In addition to the obvious visual changes that occurred upon oxygenation, a P50 at 6.9 mm Hg for whole Triops haemolymph under physiological conditions was measured. The P50 lies well within the range of the P50 values of diluted hemoglobin oxygen equilibria (Table 3). These data suggest that our results on diluted haemolymph would be similar to those of whole haemolymph and the true in vivo responses of Triops hemoglobin. Dilutions below 1 mg/ml, however, lowered P50 values considerably. As a result, in all oxygen equilibrium studies hemoglobin concentrations were never lower than 1.5 mg/ml.

Depending on pH, values of n (heme-interaction estimates) ranged from 1.4 to 2.0 for Triops hemoglobin and were not significantly different from those of rat hemoglobin, except at pH 8.12 when the hemoglobin of the rat had a value of n = 0.83 (Figs. 2, 3). Triops never exhibited a value of n lower than one. This difference can be associated with the small Bohr effect of Triops hemoglobin. Oxygen equilibria of both rat and Triops showed a decrease in the value of n with lower temperatures (Figs. 5, 6). Again, as the sigmoid shapes of the oxygen equilibrium curves in Figs. 2 and 5 suggested, the calculated values of n confirmed that positive heme interactions exist in the oxygenation of Triops hemoglobin.

Bohr effect. That the affinity of hemoglobin for oxygen is influenced by pH is well known. Generally, increasing acidity decreases oxygen affinity (Bohr effect) and in some instances the partial pressure at which saturation occurs

<table>
<thead>
<tr>
<th>Hemoglobin Conc, mg/ml</th>
<th>P50, mm Hg</th>
<th>pH 7.42</th>
<th>Determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.93*</td>
<td>5.0</td>
<td>7.42</td>
<td>Spectral</td>
</tr>
<tr>
<td>1.87*</td>
<td>6.6</td>
<td>7.42</td>
<td>Spectral</td>
</tr>
<tr>
<td>17.0*</td>
<td>6.9</td>
<td>7.1</td>
<td>Manometric</td>
</tr>
</tbody>
</table>

* Mean of duplicate samples.

(fig. 2. Effect of pH on oxygen equilibrium curve of Triops hemoglobin. (Typical curve, n = 3). ○ T: 23 C; Hb: 1.96 mg/ml; % met-Hb: 2.6; P50: 3.7; n = 1.8. □ T: 23 C; Hb: 1.98 mg/ml; % met-Hb: 0; P50: 5.0; n = 2.0. △ T: 23 C; Hb: 2.02 mg/ml; % met-Hb: 0; P50: 6.8; n = 1.5. ▼ T: 23 C; Hb: 2.07 mg/ml; % met-Hb: 6.7; P50: 9.1; n = 1.9. ▲ T: 22.5 C; Hb: 2.04 mg/ml; % met-Hb: 24; P50: 10.8; n = 1.4.

(fig. 3. Effect of pH on oxygen equilibrium curve of rat hemoglobin. (Typical curve, n = 4). ○ T: 22 C; Hb: 2.05 mg/ml; % met-Hb: 4.8; P50: 1.2; n = 0.83. □ T: 22 C; Hb: 1.83 mg/ml; % met-Hb: 8.2; P50: 6.1; n = 2.4. △ T: 22 C; Hb: 1.77 mg/ml; % met-Hb: 3.8; P50: 9.9; n = 1.9. ▼ T: 22 C; Hb: 1.91 mg/ml; % met-Hb: 8.5; P50: 18.5; n = 1.8.

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rat at the same temperature. A small Bohr effect might be advantageous for shrimp living in ephemeral ponds where CO2 may reach high levels. High internal CO2 tensions would have slight effects on the respiratory function of hemoglobin.

Most invertebrate hemoglobins studied show no Bohr effect (19), but quantitative measurements on the cladoceran Ceriodaphnia laticaudata and Daphnia magna suggested a slight shift with change in pH (5). In the same paper Fox (5) reported half-saturation values of 0.8 and 3.1 mm Hg (pH = 7.7; 17°C) for Ceriodaphnia and Daphnia, while Hoshi et al. (14) found Moina to have a P50 at 3.5 mm Hg (pH = 7.2; 28°C). Clearly the cladoceran values are similar to one another, but considerably lower than those for Triops. In the clam shrimp, C. cylindricus, a sigmoid curve was noted, but the P50 for the equilibrium curve was 0.035 mm Hg (2). Such high-oxygen affinities are usually associated with the hyperbolic oxygen equilibrium curves of muscle hemoglobin. Perhaps the high-oxygen affinities of the crustacean hemoglobins assure oxygen loading during hypoxic conditions and may contribute to survival when the oxygen concentration of the water decreases.

Temperature effect. As expected, a rise in temperature also shifts the oxygen equilibrium curve to the right, indicating a lowering of affinity (Fig. 5, 6). In this respect rat and Triops hemoglobin exhibit the same behavior; but again, Triops hemoglobin demonstrates a larger Root effect with an increase in temperature. At 45°C and with 14.7% methemoglobin contamination, a P50 of 18.9 mm Hg could be measured for rat hemoglobin, while at 40°C methemoglobin contamination was already so large for Triops (58.5%) that the P50 of 17.8 mm Hg at this temperature was doubtful. Hemoglobin in Triops is quite obviously only slightly functional at such high temperatures. Such enhanced rates of methemoglobin formation may be influential in thermal death.

Although both rat and Triops hemoglobins react similarly to temperature, the degree of their response is different (Fig. 7). A plot of log P50 vs. temperature shows that the hemoglobin of Triops was less sensitive to changes in temperature (15–35°C) than that of the rat. A comparison of the magnitude of their temperature effects gave slope values of 0.91 and 1.25 for Triops and rat, respectively. For poikilothermic crustaceans to survive thermal extremes, as they often must, a slight temperature effect and small Bohr effect therefore would be adaptive. It should be mentioned here that Triops has been observed to escape high temperatures by remaining at the bottom of the pond where they burrow into the mud.

Effect of salts on oxygen equilibrium curve. The effects of salts and specific ions on the oxygen equilibrium curve of Triops are particularly important since the pigment is free in the haemolymph rather than in the more homeosmotic environment of the RBC. As the environmental salinity increases substantially, blood salt concentrations also elevate (11). Such a response is typical for freshwater crustaceans (20). Also, the presence of salts generally lowers the P50 of hemoglobin. Consequently, the response of Triops hemoglobin to the chloride salts of sodium, potassium, magnesium, and calcium was tested.
Hemoglobin concentrations ranging from 1.75 to 2.23 mg/ml showed no significant changes in $P_{50}$ when sodium, potassium, magnesium, and calcium levels were raised appropriately 19-, 12-, 50-, and fourfold of physiological concentrations (Table 4). All $P_{50}$ values were well within the standard deviation of the control ($P_{50} = 8.73 \pm 1.13$) except for magnesium and calcium concentrations of 15.4 and 52 mM, respectively. These ions seemed to increase the oxygen affinity slightly. Whether such a decrease in $P_{50}$ is due to a dissociation of $Triops$ hemoglobin into subunits is not known.

Kirschner and Tanford (17) found that magnesium and calcium were more effective than sodium as dissociation agents and that these salts were indistinguishable in their effects on human hemoglobin. Their results on oxygen affinity of hemoglobin are in agreement with those for $Triops$ where at high magnesium and calcium concentrations, the $P_{50}$ values were 6.7 and 6.6 mm Hg, respectively. Both calcium and magnesium also increased the oxygen affinity of dialyzed hemocyanin of the crayfish, Procambarus simulans (18). However, the magnesium and calcium concentrations that elicited an increased oxygen affinity of $Triops$ hemoglobin were respectively 25 and 68 times greater than those under normal physiological conditions. Like calcium and magnesium, in physiological concentrations, monovalent ions apparently had no effect on oxygen equilibria. Since in the experiments with varying salt concentrations all of the oxygen equilibria had $P_{50}$ values close to that of whole blood, it can be concluded that $Triops$ hemoglobin is relatively insensitive to internal fluctuations of the salts and ions investigated.

**Respiratory rates of normal and CO-treated $Triops$.** At comparable temperatures $Triops$ consumes oxygen at greater rates than do other crustaceans. For instance, four tropical crabs were reported by Scholander et al. (23) to use about 100 $\mu$l O$_2$/g per hr, while 5–8 times as much oxygen was utilized by $Triops$ (Fig. 8; Table 5). For the European notostracan, $T. cancriformis$, earlier workers in Germany and Russia have noted high respiratory rates (16, 24), which are similar to those reported here for the American species.

The metabolic rate of $Triops$ decreases with an increase in body size (Fig. 8). For $Triops$ the constant $b$ (slope of a plot of the log of oxygen consumption vs. log of weight), which expresses the change in metabolic rate with a change in body size, is 0.80. Most crustaceans have similar values.

Two crustaceans with respiratory rates similar to $Triops$ are the brine shrimp, Artemia salina, and the cladoceran, Moina macrocopa, which also have a plasma hemoglobin. Since the brine shrimp are active swimmers and have a high

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### Table 4. Effect of salts and their concentrations on oxygen affinity of hemoglobin

<table>
<thead>
<tr>
<th>Experimental Solution</th>
<th>No.</th>
<th>Na, mM</th>
<th>K, mM</th>
<th>Ca, mM</th>
<th>Mg, mM</th>
<th>$P_{50}$, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole $Triops$ haemolymph</td>
<td>2</td>
<td>64.5</td>
<td>3.57</td>
<td>0.76</td>
<td>0.6</td>
<td>6.9*</td>
</tr>
<tr>
<td>Standard</td>
<td>5</td>
<td>298.7</td>
<td>23.7</td>
<td>0.57</td>
<td>1.05</td>
<td>8.73±1.13</td>
</tr>
<tr>
<td>High Na</td>
<td>2</td>
<td>710.9</td>
<td>23.7</td>
<td>0.57</td>
<td>1.05</td>
<td>7.9</td>
</tr>
<tr>
<td>High K</td>
<td>1</td>
<td>298.7</td>
<td>69.2</td>
<td>0.57</td>
<td>1.05</td>
<td>9.3</td>
</tr>
<tr>
<td>High Ca</td>
<td>1</td>
<td>298.7</td>
<td>23.7</td>
<td>31.0</td>
<td>1.05</td>
<td>8.6</td>
</tr>
<tr>
<td>High Ca</td>
<td>2</td>
<td>298.7</td>
<td>23.7</td>
<td>52.0</td>
<td>1.05</td>
<td>6.6†</td>
</tr>
<tr>
<td>High Mg</td>
<td>1</td>
<td>298.7</td>
<td>23.7</td>
<td>0.57</td>
<td>4.1</td>
<td>7.7</td>
</tr>
<tr>
<td>High Mg</td>
<td>2</td>
<td>298.7</td>
<td>23.7</td>
<td>0.57</td>
<td>15.4</td>
<td>6.7†</td>
</tr>
</tbody>
</table>

*All oxygen equilibria were measured spectrophotometrically except the one on whole $Triops$ haemolymph. †Significantly different from standard.

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**Fig. 7.** Temperature effect on $P_{50}$ of rat and $Triops$ hemoglobin.

**Fig. 8.** Effects of body size on $Triops$ oxygen consumption. Vertical bars represent 1 SD about mean of 10 $Triops$ with weights falling within a 50-mg range.

### Table 5. Influence of carbon monoxide treatment on $Triops$ respiration at different temperatures

<table>
<thead>
<tr>
<th>Animal</th>
<th>Temperature, °C</th>
<th>$P_{50}$, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon monoxide treated for 30 min</td>
<td>21°</td>
<td>648±207</td>
</tr>
<tr>
<td>Normal</td>
<td>26°</td>
<td>07±172/354±163/968±125</td>
</tr>
<tr>
<td>(O$_2$: CO = 66:1)</td>
<td>31°</td>
<td>(4)</td>
</tr>
<tr>
<td>Normal</td>
<td>35°</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>36°</td>
<td>(5)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate number of observations.

* Large specimens 1.0–2.5 g; measured by method of Hotovy (16).
† Laboratory reared shrimp, 0.1–0.2 g; Warburg respirometer.
‡ Mean $\mu$l O$_2$/g per hr ± sd.
surface-to-volume ratio, high metabolic rates are not especially surprising. By poisoning the brine shrimp hemoglobin, but not the cytochromes, with carbon monoxide, Gilchrist (9) was able to show that the respiratory pigment was used in oxygen delivery to the tissues and that it was not essential for survival. Similar results also were obtained by Hoshi and Shimada (15) with the respiratory rates of the cladoceran, *Daphnia*.

In our study we used the same type of experiment as did Gilchrist (9) to show that formation of carboxyhemoglobin did not consistently reduce respiratory rates in *Triops*, as it did in *Artemia* (Table 5). Significant differences were noted for specimens larger than 1 g, but the smaller laboratory reared shrimp (0.1–0.2 g) seemed unaffected by the carbon monoxide. Perhaps the smaller surface-to-volume ratio of the larger shrimp places a premium on an oxygen carrier, whereas the smaller *Triops* may rely more on diffusion pressures and efficient circulation. From these data, however, it appears that the physiological role of *Triops* hemoglobin is like that of the brine shrimp.

**REFERENCES**


