

Oxygen-binding characteristics of the haemolymph of the freshwater snail *Bulinus (Physopsis) globosus*

W.J. van Aardt and B.J. Frey

Potchefstroom University for CHE, Potchefstroom

Oxygen dissociation curves were made and the Bohr-effect determined from micro-volume samples of the haemolymph of the freshwater snail *Bulinus (Physopsis) globosus*.

Haemoglobin dissolved in the haemolymph is typically a high oxygen affinity respiratory pigment with a P_{50} value of 4,0 mm Hg measured at 26 °C and a carbon dioxide tension of 3 mm Hg. The eco-physiological implications of the experimental findings are discussed.

S. Afr. J. Zool. 1981, 16: 1–4

Suurstofdissosiasiekrommes is gemaak en die Bohr-effek vasgestel van mikrohoeveelhede hemolimfmonsters afkomstig van 'n varswaterslak *Bulinus (Physopsis) globosus*.

Hemoglobien opgelos in die hemolimf is tipies die van 'n hoë suurstofaffiniteit-pigment met o.a. 'n P_{50} -waarde, van 4,0 mm Hg by 26 °C en 'n koolsuurgasspanning van 3 mm Hg. Die eko-fisiologiese betekenis van die eksperimentele bevindinge word bespreek.

S.-Afr. Tydskr. Dierk. 1981, 16: 1–4

Since the pioneering work by Zaaijer & Wolvekamp (1958) and Jones (1964a, 1964b) respiratory research on the haemoglobin-bearing basommatophorans has been restricted mainly to some physio-chemical measurements made on purified haemoglobin solutions (Figueiredo, Gomez, Heneine, Santos & Hargreaves 1973, Almeida & Neves 1974). No similar investigation has been carried out on the South African basommatophorans containing haemoglobin in solution in their haemolymph. Compared with *Biomphalaria pfeifferi* and *Biomphalaria sudanica*, which usually occur in swampy or stagnant water with very low values of dissolved oxygen (Jones 1964a), *Bulinus (Physopsis) globosus* usually prefers small, slow-running streams with a relatively high oxygen content. It is thus appropriate to compare haemoglobin-oxygen dissociation curves for *B. (P.) africanus* with those obtained for *B. sudanica* (Jones 1964b) and *Planorbis corneus* (Zaaijer & Wolvekamp 1958) to discover whether the haemolymph of *B. (P.) globosus* possesses a high oxygen-affinity haemoglobin or not. Furthermore, particular attention has been paid to the pH, P_{CO_2} and a possible Bohr-effect of the haemolymph samples subjected to oxygenation.

Materials and Methods

The snails were obtained from domesticated stocks reared by the Bilharzia Field Unit of the South African Medical Research Council at Nelspruit, Transvaal. Before starting the experiments the snails were kept in the laboratory for at least two weeks in well-aerated snail aquaria according to the methods described by Van der Schalie & Berry (1973) and Jennings (1976). The temperature of the aquaria was regulated at 26 °C (± 1 °C), which coincides with the optimum temperature of *B. (P.) globosus* (De Kock 1973).

Haemolymph was collected from each snail by mechanical stimulation of the foot surface. During the retraction response haemolymph is extruded, probably by way of the haemal pore (Lever & Bekius 1965). By means of glass capillaries, 30–50 μ l of haemolymph could be collected from the vacant last whorl which acts as a reservoir.

Haemoglobin-oxygen dissociation curves were constructed using the technique of Tucker (1967). For our haemolymph samples from these small snails the Perspex-reaction chamber was reduced to a volume of 240 μ l. The tonometers used were part of the model

W.J. van Aardt*

Department of Zoology, Potchefstroom University for CHE, Potchefstroom 2520

B.J. Frey

Snail Research Unit of the South African Medical Research Council, Potchefstroom University for CHE, Potchefstroom 2520

*To whom correspondence should be addressed

Received 18 March 1980; accepted 16 August 1980

BMS-2 blood-mixing apparatus (Radiometer) consisting of a double pair of open cuvette tonometers with humidifiers and a built-in water bath. About 150–200 μl of haemolymph, collected and pooled from 3–4 full-grown snails were used to construct a single haemoglobin-oxygen dissociation curve. Each point on the curve represents the average values of duplicates from 10 μl samples. No attempt was made to centrifuge or purify the samples.

The percentage of haemoglobin which is oxygenated, S_{HbO_2} , can be calculated from the following relation (Dejours 1975):

$$S_{\text{HbO}_2} = \frac{C_{\text{HbO}_2}}{C_{\text{HbO}_2} + C_{\text{Hb}}} = \frac{C_{\text{HbO}_2}}{C_{\text{HbO}_2}^{\text{max}}}$$

where C_{HbO_2} , is the relationship between the concentration of HbO_2 and P_{O_2} ($C_{\text{HbO}_2} = f(P_{\text{O}_2})$), and $C_{\text{HbO}_2}^{\text{max}}$ represents all the haemoglobin transformed into oxyhaemoglobin (oxygen-binding capacity) at a P_{O_2} of 128–132 mmHg prevailing at Potchefstroom at the time of the experiment. To determine C_{HbO_2} , the dissolved O_2 in the haemolymph sample (C_{O_2} diss.) and the O_2 bound to the haemoglobin (C_{O_2} bound) must be known. The concentration of oxygen dissolved, in ml, was calculated according to Henry's Law. This could be accomplished by measuring the P_{O_2} of the gas in the tonometers (with a P_{O_2} -electrode fitted into a thermostated cell, Model D616, Radiometer) and using the data for oxygen solubility by Sendroy, Dillon & Van Slyke (1934) and Christoforides & Hedley-Whyte (1969).

To find the O_2 bound to haemoglobin (C_{HbO}) it is first necessary to subtract the concentration of dissolved oxygen from the total O_2 concentration, C_{O_2} tot. The C_{O_2} tot. was measured according to Tucker (1967). The $C_{\text{HbO}_2}^{\text{max}}$ for the domesticated stocks from Nelspruit was found to be 1,70 ml O_2 dl $^{-1}$ tested at a range of 20 mmHg to 132 mmHg at 26 °C. Gas mixtures were obtained commercially or made up in inner tyre tubes. To obtain a gas with a constant percentage of carbon dioxide but variable percentage of oxygen, two streams of gases were mixed in different proportions as described by Tucker (1967). For this, two rotameters (Model 1875-Y, Schutte & Koerting, U.S.A.), each coupled to a sensitive differential pressure regulator (Schutte & Koerting, U.S.A.) were used. The resultant partial pressures for CO_2 and O_2 were routinely checked by means of P_{O_2} – P_{CO_2} -electrodes.

The pH (10–12 μl haemolymph from the tonometers) was measured by means of a micro-electrode unit (Model E5021) connected to an acid-based analyser (Model PM71b) both from Radiometer.

Results and Discussion

The method of Tucker (1967) in determining haemoglobin-oxygen dissociation curves for small blood samples with low haemoglobin concentration was found to be reliable and reproducible. Values of duplicate samples differ less than 2% from each other, starting from the low exposure extreme of a P_{O_2} of 2,5 mmHg. Its only disadvantage is that it is time-consuming compared with the method for small blood samples of Hughes, O'Neill & Van Aardt (1976). The latter method, using 50 to 100 μl of vertebrate blood has the unfortunate disadvantage

that for low haemoglobin concentration blood, such as found for *B. (P.) globosus*, up to 3 ml haemolymph must be used for a curve in order to have sufficient haemoglobin in the cuvette to overcome inaccuracies in finding C_{HbO_2} and $C_{\text{HbO}_2}^{\text{max}}$. In our experiments it was aimed at constructing haemoglobin-oxygen dissociation curves without sample purification and concentration, using as few pooled samples of haemolymph as was practically possible.

The maximal concentration of combined oxygen, $C_{\text{HbO}}^{\text{max}}$ (1,70 ml O_2 dl $^{-1}$) is low compared to mammals and birds, where it normally varies between 15 and 20 ml O_2 dl $^{-1}$ blood. The $C_{\text{HbO}}^{\text{max}}$ -values obtained for *B. (P.) globosus* compare favourably with values of 0,94–2,49 for *Planorbis corneus* (Leitch 1916, Borden 1931, Jones 1964a) and 0,98–2,08 for *Biomphalaria sudanica* (Jones 1964b). In contrast to mammals these values may differ within different populations of the same snail species. It mainly depends on their exposure to normoxic or anoxic water conditions. This value is of course directly related to the haemoglobin concentration in the snail. Preliminary experiments on *B. (P.) africanus* have shown that the haemoglobin concentration of field snails (4,6 gHb dl $^{-1}$ haemolymph) was nearly twice that of laboratory reared snails kept in well aerated water. It is therefore advisable to check ($C_{\text{HbO}_2}^{\text{max}}$) routinely when different batches are to be used to obtain comparable haemoglobin-oxygen dissociation curves. Furthermore, in this experiment, it was decided to measure $C_{\text{HbO}_2}^{\text{max}}$ at fixed values of temperature (26 °C) and P_{CO_2} (zero mmHg) (Dejours 1975).

It is clear from the results (Fig. 1, Table 1) that the haemoglobin fraction in the haemolymph of *B. (P.) globosus* is typically a high oxygen affinity respiratory pigment found for several invertebrates, including the haemoglobin bearing basommatophoran snails. This low HbO_2 dissociability is always associated with periodic hypoxic conditions of the water medium (Jones 1972) and the ability of the snail to attenuate its oxygen consumption

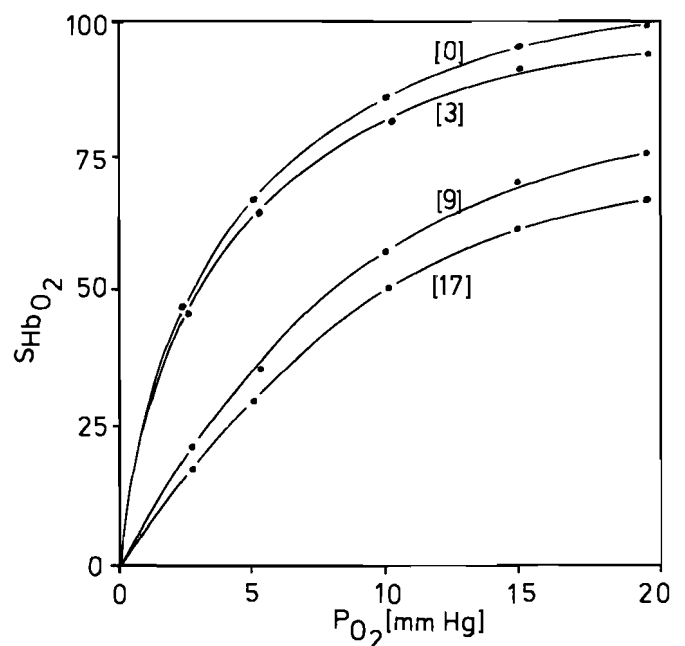


Fig. 1 Haemoglobin-oxygen dissociation curves of *B. (P.) globosus* made at 26 °C. The figures in brackets denote the P_{CO_2} -values (in mmHg) applied to the haemolymph samples during tonometry.

Table 1 The pH, P_{50} and P_{95} values of *B. (P.) globosus* haemolymph samples at different temperatures and carbon dioxide tensions

P_{CO_2} (mmHg)	18 °C				26 °C				32 °C			
	0	3	9	17	0	3	9	17	0	3	9	17
P_{50} (mmHg)	2,0	2,7	4,6	7,2	3,6	4,0	7,8	9,5	7,3	8,4	10,4	11,4
P_{95} (mmHg)	10,6	-	-	-	14,2	-	-	-	19,5 at P_{80}	-	-	-
pH of haemolymph	8,30	8,02	7,65	7,40	8,35	8,10	7,80	7,40	8,55	8,10	7,70	7,50

tion accordingly (Van Aardt & Frey 1979). The P_{50} values determined for *B. (P.) globosus* are practically the same as those found for *B. sudanica* and *P. corneus* (Jones 1972). For these animals we have the novel situation that at relatively low P_{O_2} values (above 20 mmHg) of the water medium the pigment remains fully saturated when it leaves the tissues. Thus a high affinity respiratory pigment can be functionless at high ambient P_{O_2} while it can perform a vital function when ambient P_{O_2} falls below a certain level. For *B. (P.) globosus* this level is below 20 mmHg of the water. The pigment will thus constitute a reserve transport facility rather than a reserve store. One of the most interesting features of the role of a high affinity pigment is the regularity with which its reserve potential is utilized to cope not with general environmental oxygen deficiencies but with highly localized shortages often occasioned by the rhythmic nature of the animal's personal behaviour (Jones 1972). We believe that the respiratory function of the haemoglobin in *B. (P.) globosus* acts in the same manner and can be vital when ambient P_{O_2} suddenly falls below a specific level. At 26 °C and with a haemolymph P_{CO_2} of 3 mmHg this oxygen saturation level for *B. (P.) globosus* starts at 20 mmHg.

The Hill coefficient calculated for oxygen dissociation curves at 26 °C and 18 °C with haemolymph exposed to a P_{CO_2} of 3 mmHg varies between 1,71 – 1,83, indicating that the oxygen-binding sites are not independent of each other. It is generally known that high affinity haemoglobin, which forms macromolecular aggregates, as it is the case for these bassomatophorans, has weak haem-haem interactions (Manwell 1960).

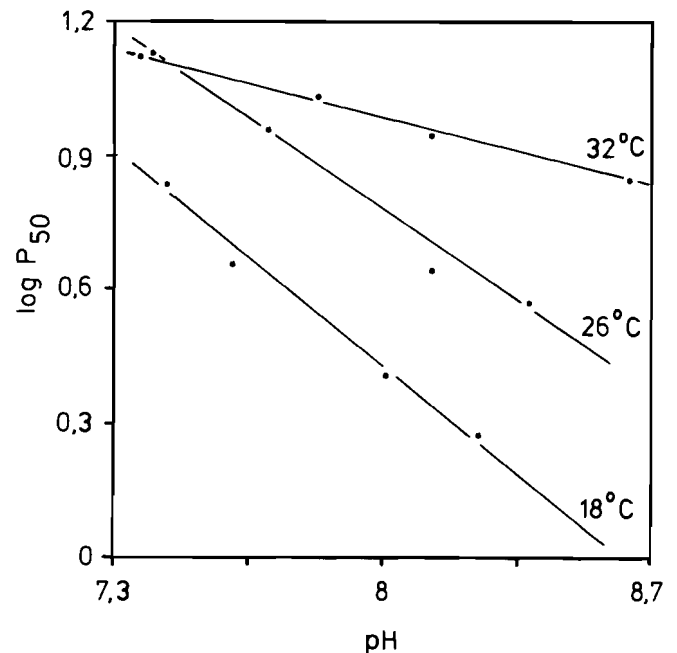
The acid sensitiveness, or Bohr-effect, caused by carbon dioxide is well marked, especially when high experimental P_{CO_2} values of 9 and 17 mmHg are encountered (Table 1, Fig. 2). These high P_{CO_2} values are rarely found in the snail's habitat because of the high solubility of carbon dioxide in the water. Therefore aquatic animals cannot build up a high tension of this gas. It was found that the P_{CO_2} values in the haemolymph of *B. (P.) globosus* did not exceed 3 mmHg at 26 °C (Van Aardt & Van Eeden 1976).

At a P_{CO_2} of 3 mmHg the Bohr-effect was small and very similar to curves made when no carbon dioxide was purged through the tonometer (Fig. 1, Table 1).

The pH of the haemolymph samples in the tonometers decreases with increasing P_{CO_2} (Table 1). This is most probably the result of the relatively high concentrations of CO_2 purged through the samples and the inability of the haemolymph (bicarbonate content: 14,0 mmol litre⁻¹,

Van Aardt & Van Eeden 1976) to buffer this highly water soluble gas.

Figueiredo *et al.* (1973) could not demonstrate a Bohr-effect in purified blood for *Biomphalaria glabrata* at a pH between 6,7 – 7,6. Contrary to this, Jones (1964b) and Zaaijer & Wolvekamp (1958) found a Bohr-effect for *B. sudanica* (measured at 26 °C) and *P. corneus* (measured at 20 °C) respectively at a P_{CO_2} of 10 and 20 mmHg. Unfortunately Figueiredo *et al.* (1973) did not state the temperature and P_{CO_2} conditions for their experiments.

**Fig. 2** The acid sensitivity or Bohr-effect for haemolymph samples of *B. (P.) globosus* measured at different temperatures.

At 32 °C the Bohr-shift is much less pronounced (Fig. 2). Consequently at this temperature the amount of oxygen released to the tissues could be very small. This may be a contributing factor to preventing *B. (P.) globosus* from colonizing water with a temperature above 30 °C if they cannot gain access to the water surface to replenish their mantle cavities with air.

Acknowledgements

We wish to thank the S.A. Medical Council for financial assistance and the staff of the Snail Research Unit of the S.A. Medical Research Council, at Potchefstroom, for providing the aquarium facilities.

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