

SHORT COMMUNICATIONS

FOETAL AND ADULT BLOOD OXYGEN AFFINITIES  
OF THE VIVIPAROUS SEAPERCH, *EMBIOTOCA*  
*LATERALIS*

BY ROLF L. INGERMANN

*Obstetrics and Gynecology Research, L458, Oregon Health Sciences  
University, Portland, Oregon 97201, U.S.A.*

ROBERT C. TERWILLIGER AND MICHAEL S. ROBERTS  
*Oregon Institute of Marine Biology, University of Oregon, Charleston,  
Oregon 97420, U.S.A.*

*Accepted 13 September 1983*

The high oxygen affinity of foetal *versus* adult blood is an almost universal characteristic of viviparous vertebrates; a notable exception is the cat (Novy & Parer, 1969). There is also likely to be a difference in the oxygen affinities of foetal and adult blood in the teleostean fish, the seaperch *Embiotoca lateralis* Agassiz. Our studies of *E. lateralis* foetal and adult haemoglobins and erythrocytes have shown several mechanisms which are likely to facilitate oxygen transfer to the foetal fish by increasing foetal blood oxygen affinity with respect to that of adult blood. Proposed mechanisms include structurally distinct foetal and adult haemoglobins with foetal haemoglobin having the higher oxygen affinity, lower foetal erythrocyte nucleoside triphosphate (NTP, primarily ATP) concentrations, and possibly, lower mean corpuscular haemoglobin concentrations (MCHC) in foetal cells (Ingermann & Terwilliger, 1981*a,b*, 1982*a*). It is necessary, however, to see if the oxygen affinity of foetal whole blood is greater than that of adult blood as predicted from purified haemoglobin oxygen binding and erythrocyte characteristics. Consequently, we have measured the oxygen affinities of foetal and adult whole bloods under comparable conditions.

Adult *E. lateralis*, standard length greater than or equal to 22 cm, were obtained near Cape Arago on the Oregon coast. Adult blood samples were collected into ice-cold heparinized test tubes. Oxygen dissociation curves were generated on these samples within 4 h. Mid-gestation foetuses obtained in early April, and late-gestation foetuses obtained in late June, were collected into ice-cold 40% sea water (approximately the salinity of ovarian fluid). Each foetus was blotted dry, its caudal peduncle severed, and approximately 3  $\mu$ l blood per fish were collected onto a heparinized glass coverslip. Oxygen equilibria of foetal and adult whole blood samples were determined with a Hem-O-Scan oxygen dissociation analyser (SLM-AMINCO, Urbana, IL) essentially as described by Powers *et al.* (1979). Temperature was maintained at 20°C in the Hem-O-Scan with an external circulator and temperature control. Oxygen dissociation curves were generated in the absence and presence of

Key words: Oxygen affinity, blood, teleost.

5.6% CO<sub>2</sub>. For analysis without CO<sub>2</sub>, the sample was deoxygenated with medical-grade N<sub>2</sub> (verified to contain less than 0.1% O<sub>2</sub> with a Perkin Elmer mass spectrometer, MGA-1100) and reoxygenated with air (21% O<sub>2</sub>). For each analysis with CO<sub>2</sub>, the 100% oxyhaemoglobin absorbance was first established with air before the sample was exposed to CO<sub>2</sub>. The sample was then deoxygenated with 5.6% CO<sub>2</sub> and 94.4% N<sub>2</sub> and reoxygenated with 5.6% CO<sub>2</sub>, 25% O<sub>2</sub> and 69.4% N<sub>2</sub>. Thus, the P<sub>50</sub> (partial pressure of oxygen at which 50% of the haemoglobin is saturated) obtained in the presence of CO<sub>2</sub> represents the half saturation of the blood with oxygen in the presence of CO<sub>2</sub> relative to full saturation with oxygen in the absence of CO<sub>2</sub>.

In the absence of CO<sub>2</sub>, mid-gestation foetal blood showed the lowest P<sub>50</sub>, or highest affinity for oxygen, with a P<sub>50</sub> of  $8.4 \pm 2.4$  Torr (mean  $\pm$  s.d.,  $N = 5$ ), and adult blood the lowest affinity, with a P<sub>50</sub> of  $21.2 \pm 5.2$  Torr ( $N = 3$ ) (Fig. 1). Late-gestation foetal blood had an intermediate value, with a P<sub>50</sub> of  $12.5 \pm 2.5$  Torr ( $N = 8$ ). (Each  $N$  for foetal blood represents a single determination per individual; each  $N$  for adult blood represents a mean of quadruplicate determinations per individual.) The same basic pattern was seen in the presence of 5.6% CO<sub>2</sub>; however, all curves were shifted towards the right (Fig. 2). With CO<sub>2</sub>, mid-gestation foetal blood showed a P<sub>50</sub> of  $67 \pm 13$  Torr ( $N = 3$ ) and late-gestation foetal blood had a P<sub>50</sub> of  $86 \pm 12$  Torr ( $N = 3$ ). In the presence of CO<sub>2</sub>, one adult fish showed a blood P<sub>50</sub> of about 106 Torr; however, samples from two other adults consistently did not exceed 25% oxygen saturation at a P<sub>O<sub>2</sub></sub> of 150 Torr.

The pH of foetal and maternal blood was measured under conditions comparable to those of the Hem-O-Scan. Adult and late-gestation foetal blood samples were first equilibrated with either N<sub>2</sub>, air, N<sub>2</sub> plus CO<sub>2</sub> or air plus CO<sub>2</sub> at 20°C. Blood pH was then measured with a Bio-Rad combination pH microelectrode, calibrated *versus* an Orion glass electrode. Six measurements were made per equilibration gas. Under N<sub>2</sub>, both foetal and adult bloods were at  $\text{pH } 7.7 \pm 0.1$ ; when these samples were reoxygenated with air, their pH values were  $7.7 \pm 0.1$  and  $7.9 \pm 0.1$ , respectively. In the presence of CO<sub>2</sub>, deoxygenated foetal and adult samples showed pH values of  $7.3 \pm 0.1$  and  $7.2 \pm 0.1$ , respectively; reoxygenated values were  $7.2 \pm 0.2$  and  $7.1$ , respectively. One can thus conclude that since the pH values of the bloods were nearly the same, *E. lateralis* foetal blood had a higher oxygen affinity than adult blood when the CO<sub>2</sub> concentration was either zero or high, two very different conditions.

The blood-oxygen binding data above do not necessarily represent the *in vivo* state, which is dependent upon variable physiological parameters such as lactate concentration and CO<sub>2</sub> tension. However, the data do provide direct evidence that the intrinsic oxygen affinity of adult blood is significantly less than that of either mid- or late-gestation foetal blood when measured under comparable conditions. This is consistent with earlier studies which reported indirect evidence that in this teleost the blood oxygen affinity of the foetus is higher than that of the adult (Ingermann & Terwilliger, 1981*a,b*, 1982*a*).

Previous reports have shown that *E. lateralis* mid-gestation and late-gestation foetal bloods do not differ in NTP levels (mol NTP/mol haemoglobin tetramer) and only slightly in MCHC (Ingermann & Terwilliger, 1981*b*, 1982*a*). The haemoglobins of these two foetal stages, however, are structurally and functionally different.

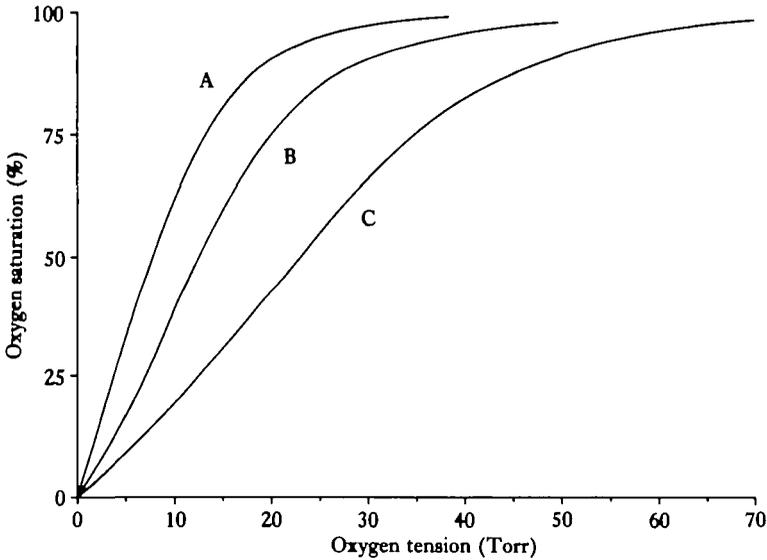


Fig. 1. Oxygen equilibrium curves of mid-gestation foetal (A), late-gestation foetal (B) and adult (C) bloods in the absence of exogenous  $\text{CO}_2$ .

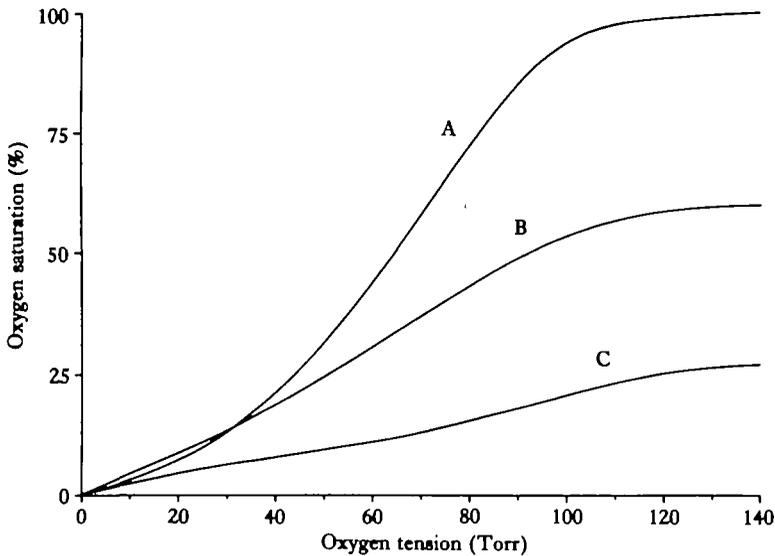


Fig. 2. Oxygen equilibrium curves as in Fig. 1 in the presence of 5.6%  $\text{CO}_2$ .

Mid-gestation foetal haemoglobin, stripped of organic phosphates, has a higher oxygen affinity than late-gestation stripped foetal haemoglobin; the latter haemoglobin appears to be a mixture of foetal and adult haemoglobin structures (Ingermann & Terwilliger, 1981a). Thus, the apparent decrease in foetal blood oxygen affinity with increasing development in *E. lateralis* during the last half of gestation is probably due to the replacement of foetal haemoglobin by adult haemoglobin.

Szabo & Karplus (1976) have theorized that at low haemoglobin to organic phosphate concentrations, the ratio of stripped haemoglobin  $\text{P}_{50}$  in the presence of

organic phosphate to stripped haemoglobin  $P_{50}$  in the absence of organic phosphate is a function of the relative binding constants of organic phosphate to deoxy- and oxyhaemoglobin. Ratios of  $P_{50}$  of mid-gestation foetal, late-gestation foetal and adult *E. lateralis* stripped haemoglobins in the presence of 1 mM-ATP to  $P_{50}$  in its absence are very similar; e.g. at pH 7.4, ratios were found to be 1.3, 1.3 and 1.4, respectively (Ingermann & Terwilliger, 1981a). This strongly suggests that at a given concentration of ATP and haemoglobin, ATP binds and affects *E. lateralis* haemoglobins similarly. The ratio of  $P_{50}$  of blood to  $P_{50}$  of stripped haemoglobin at pH 7.4 (approximate intraerythrocytic pH) for mid- and late-gestation fetuses was about 1.5–1.6. The low oxygen affinity of blood relative to the affinity of stripped haemoglobin was probably caused by the presence of appreciable intraerythrocytic ATP (Ingermann & Terwilliger, 1981b). Since ATP exerts a similar effect on these haemoglobins, an adult blood  $P_{50}$  of about 16 Torr would be predicted if the structural and functional differences in haemoglobins were the only difference between foetal and adult bloods. However, the actual measured adult blood  $P_{50}$  value was 21 Torr. This implies that the differences in haemoglobin oxygen affinities alone could not account for the high adult blood  $P_{50}$  and that the ATP concentration, or some other factor in the blood or within erythrocytes, decreased the oxygen affinity of adult blood more than that of foetal blood. A likely explanation is the differences in mean corpuscular NTP concentrations; these concentrations are about 4.1, 4.4 and 11 mM for mid-gestation foetal, late-gestation foetal and adult erythrocytes, respectively (calculated as  $NTP/Hb_4 \times MCHC$ , data from Ingermann & Terwilliger, 1981b, 1982a). Additionally, the differences between adult and foetal MCHCs may contribute to the maternal-foetal blood oxygen affinity difference by the mechanism previously discussed (Ingermann & Terwilliger, 1982a). In most viviparous animals which have been studied, facilitation of foetal oxygen uptake is based either upon structurally different adult and foetal haemoglobins or upon different concentrations of intraerythrocytic organic phosphates. *E. lateralis* appears to incorporate both strategies, and possibly a third – different MCHCs – to facilitate foetal oxygen uptake.

Webb & Brett (1972) found that ovarian fluid  $P_{O_2}$  decreases and foetal oxygen consumption per unit weight increases as foetal development approaches term in the seaperch, *Rhacochilus vacca*, a close relative of *E. lateralis*. Therefore, it might be expected that as the *E. lateralis* foetus approaches the end of gestation and encounters decreased ovarian oxygen tensions, blood oxygen affinity should increase to ensure oxygen loading. We found, however, that the late-gestation foetal blood had a significantly lower blood oxygen affinity than that of the mid-gestation foetus. The physiological significance of this finding is not clear, but it is not without precedent. Decreases in foetal blood oxygen affinity with increased development have been reported in man and the viviparous lizard, *Sphenomorphus quoyii* (Bard & Teasdale, 1979; Grigg & Harlow, 1981).

We found in this study that  $CO_2$  had a dramatic effect on the extent of oxygenation of whole blood. This is consistent with our earlier studies which showed that both *E. lateralis* mid-gestation foetal and adult stripped haemoglobins demonstrate a Root effect which is accentuated by ATP (Ingermann & Terwilliger, 1982b). The shift of the blood oxygen dissociation curve by  $CO_2$  (primarily a pH phenomenon, Root & Irving, 1943) was probably related to the marked sensitivity of these haemoglobins to

low pH. The pronounced shift of the adult curve was likely to be related as well to the high ATP concentrations within the adult erythrocytes. The significance of this finding, if any, to maternal-foetal oxygen transfer is unclear. However, it is possible that at physiological oxygen tensions, a Root effect may be operative in the foetus as well as in the adult.

We are grateful to Dr Robert Koler for use of the Hem-O-Scan analyser and to Mrs Marilyn Jones for her technical assistance. We also appreciate the help of Dr Michael Stock with mass spectrometric analysis of equilibration gases. RLI was supported by an Institutional National Research Service Award, No. HD 07084; this work was also supported in part by NSF Grant No. PCM 8207548 to RCT.

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