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FOETAL–MATERNAL BLOOD RESPIRATORY PROPERTIES OF AN OVOVIVIPAROUS SNAKE THE COTTONMOUTH, *AGKISTRODON PISCIVORUS*

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SUMMARY

The respiratory properties of maternal, foetal and juvenile whole blood were studied in the ovoviviparous snake, *Agkistrodon piscivorus*. Haematocrit, haemoglobin concentration, O_2 capacity, Bohr effect and Hill coefficients were not significantly different in foetal and maternal blood and were similar to values previously reported from snakes. A significant difference in foetal–maternal blood P_{50} (foetal 19·5, maternal 48·8) was found. Nucleoside triphosphate (NTP) levels were lower in foetal than in maternal and juvenile snakes. The foetal–maternal difference in P_{50} and NTP levels disappeared soon after birth (juvenile P_{50} 45·5). Starch gel electrophoresis revealed no difference in foetal and maternal haemoglobins. We suggest that the foetal–maternal shift in blood oxygen affinity is modulated directly and/or indirectly by NTP levels.

INTRODUCTION

Within the class Reptilia viviparity (live bearing) appears to have evolved many times (Tinkle & Gibbons, 1977). The transition from oviparity (egg laying) to viviparity is thought to have been gradual. The egg is retained within the oviduct for progressively longer periods with a concomitant decrease in shell calcification, until the stage of ovoviviparity (i.e. the young are born live but within the shell membrane) is reached. From this point the final steps to viviparity are taken. The shell membrane is lost and placentae of varying complexities develop (Amoroso, Heap & Renfree, 1979; Shine & Bull, 1979; Moriss, 1975).

It appears that maternal to foetal nutrient transport would be most difficult in the ovoviviparous form where exchange must occur between oviducal and chorioallantoic blood. This is based on the probable existence of a large diffusion distance between foetal and maternal circulations and a limited surface for exchange. Thus in ovoviviparous species foetal and maternal blood characteristics must be of great importance in oxygen transfer.

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The difference in maternal and foetal blood oxygen affinity appears to occur in almost all live-bearing animals (Ingermann & Terwilleger, 1981; Garlick *et al.* 1979; Pough, 1977; Toews & MacIntyre, 1977; Metcalfe, Bartels & Moll, 1967; Manwell, 1958, 1960), and its influence on such critical factors as foetal weight gain has been clearly demonstrated (Bauer, Jelkmann & Moll, 1981; Hebbel, Berger & Eaton, 1980). The physiological basis for this difference is varied. It may result from a specific foetal haemoglobin, as in man, or be totally phosphate-mediated, as in the guinea pig (for review see Bauer & Jelkmann, 1981).

Although the difference in foetal and maternal O_2 affinity has been studied in the greatest detail, other blood properties, such as the haemoglobin O_2-carrying capacity and Bohr effect, have been suggested to be of major importance in O_2 transfer (Longo, Hill & Power, 1972; Metcalfe *et al.* 1967). Although the above blood respiratory characteristics are well understood in mammals, similar data on reptiles are sparse and incomplete.

Manwell (1960) and Pough (1977) examined the blood respiratory characteristics of the garter snake *Thamnophis sirtalis*, a species with a placenta (Hoffman, 1970), and demonstrated a significant foetal–maternal difference in blood oxygen affinity and haematology. More recently, Grigg & Harlow (1981) observed a significant difference in foetal and maternal blood O_2 affinities in the skink *Sphenomorphus quoyii*, which has a less well-developed placenta. In all of the above cited studies reptiles with placentae were used, and in one study, complete analysis was limited by small blood sample sizes.

We have studied the cottonmouth (*Agkistrodon piscivorus*), a large ovoviviparous snake of the south-eastern United States (Burkett, 1966; Conant, 1975). Insemination occurs in late summer and/or spring, and ova are fertilized in spring. Gestation is approximately 4 months and 1 to 16 offspring are produced (Burkett, 1966). Because this species produces a small number of relatively large offspring, sufficiently large blood samples can be obtained such that standard techniques can be used without pooling blood.

MATERIALS AND METHODS

Seven pregnant *Agkistrodon piscivorus* (Conant) were obtained from an animal dealer. The animals were maintained in a large galvanized stock tank at 23 °C with a 12: 12 day: night photoperiod. Water was available *ad libitum*. Five females were used in the maternal–foetal blood studies and two were allowed to give birth. Offspring were used for juvenile blood analyses at 5 and 16 weeks postpartum. Blood from one yearling individual was also analysed.

Blood sampling

Animals were anaesthetized with an intraperitoneal injection of ketamine. A mid-ventral incision was made, the dorsal aorta exposed and blood drawn into a heparinized syringe. In pregnant snakes, both oviducts were removed and placed in a large bowl filled with a warm reptilian Ringer solution where individual embryos were separated. Embryonic blood samples were drawn first from the chorioallantoic

artery and then from the dorsal aorta. Blood from all groups was placed on ice immediately after sampling.

Haematology and NTP levels

Haematocrits, haemoglobin concentrations and nucleoside triphosphate (NTP) levels were determined within 5 min of blood sampling. Haematocrit was determined by spinning the blood in a microhaematocrit centrifuge for 5 min. Haemoglobin concentration was determined spectrophotometrically as cyanomethoglobin. Oxygen capacity was measured on blood equilibrated for 12 min with a gas mixture of 25 % O_2, 5·5 % CO_2, balance N_2, in an Astrup microtonometer using the method of Roughton & Scholander (1943). Blood NTP concentration was determined using an enzymatic assay from Sigma Chemical Company (Sigma Technical Bulletin 366-UV).

Oxygen binding curves

Whole blood oxygen dissociation curves were constructed using the mixing method (Edwards & Martin, 1966). Blood was tonometered for 12 min in an Astrup microtonometer at 25 °C with premixed gases of the following composition: 25 % O_2 with either 3·0, 5·5 or 8·0 % CO_2, balance N_2, and 0 % O_2 with either 3·0, 5·5 or 8·0 % CO_2, balance N_2. Following tonometry, ratios of deoxygenated and oxygenated blood calculated to yield 30, 50 and 70 % saturation were drawn into a 1 ml glass syringe which had the dead space filled with mercury. To prevent blood–air contact a small amount of mercury was drawn in behind the blood. Blood volume was measured using a syringe microburette (Micrometric Instrument Co., Cleveland). Blood samples were mixed for 20–30 s and the pH, P_{O_2} and P_{CO_2} were measured in a Radiometer blood micro system, BMS-3 MK II, in conjunction with a digital acid-base analyser, PHM-72 MK II (Radiometer, Copenhagen). No haemolysis was observed following tonometry. Blood samples were tonometered only once to avoid NTP depletion. The Bohr effect was determined as $\Delta \log P_{50}/\Delta pH$ and the Hill coefficient as $\log(Y/100-Y)/\log P_{O_2}$. Oxygen dissociation curves were constructed for blood at pH 7·4 and 25 °C.

Electrophoresis

Starch gel electrophoresis was carried out after the methods of Huehns (1968). Human haemoglobins A and F were run concurrently as controls.

Statistical analysis

Differences between means were evaluated using a Students *t*-test. A P value less than 0·05 was considered significant.

RESULTS

Body weights and snout-vent lengths of maternal, foetal, juvenile and one yearling snake are shown in Table 1. Foetal snakes were developmentally staged according to

Table 1. *Haematology and NTP concentrations for cottonmouths*

	Mean body weight (g)	Mean snout-vent length (mm)		Haematocrit (vol %)	Hb (g %)	Oxygen capacity (vol %)	NTP (μmol g^{-1} Hb)
Foetal	10·1	190	X	23·8	4·85	7·1	29·0*
			S.E.	2·2	0·37	1·0	1·9
			N	10	13	4	9
Maternal	288	695	X	23·1	6·23	7·6	65·1
			S.E.	1·1	0·34	0·4	7·1
			N	5	5	4	5
5 weeks	16·3	247	X	20·3	6·0	7·2	66·6
			N	2	2	2	2
16 weeks	24·2	292	X	23·4	6·22	7·2	58·3
			N	2	2	2	2
1 year (approx.)	96·8	445	X	38	10·2	–	50·4
			N	1	1	–	1

* Significantly different from maternal concentration $P < 0{\cdot}001$.

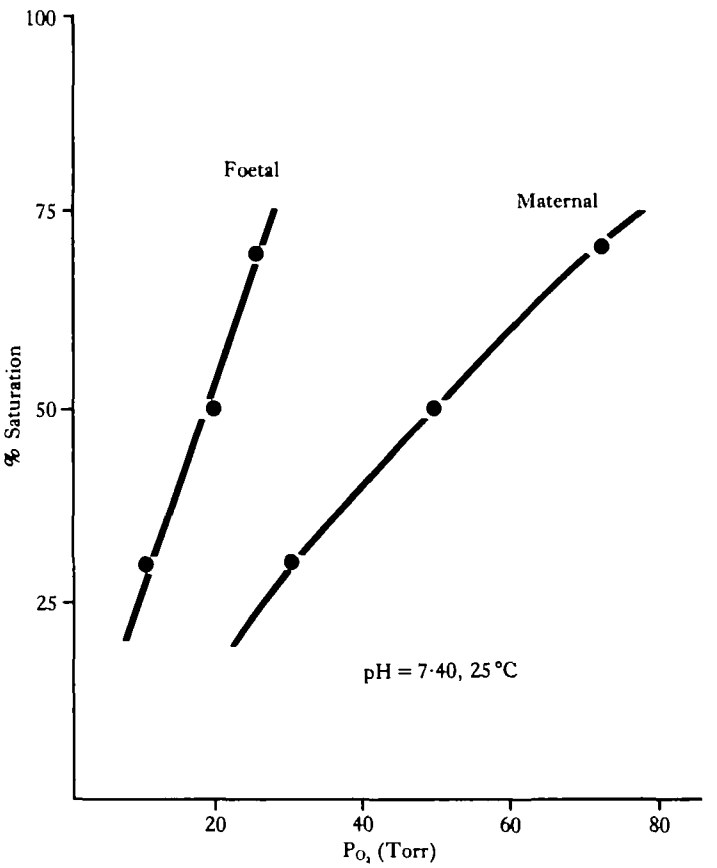

Fig. 1. Whole blood haemoglobin oxygen dissociation curves for pregnant female and foetal (near time of parturition) cottonmouths (*Agkistrodon piscivorus*).

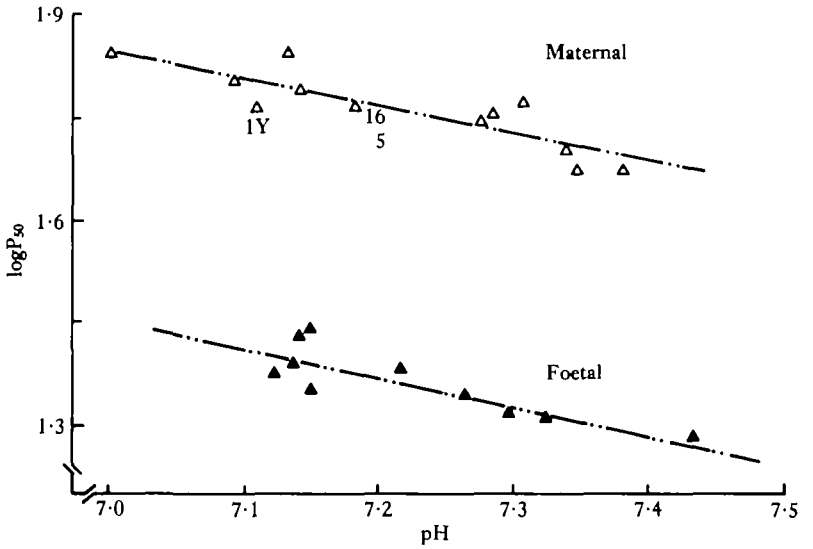

Fig. 2. Bohr factor plots determined on whole blood from pregnant female, juvenile and foetal (near time of parturition) cottonmouths (*Agkistrodon piscivorus*). 1Y, 5 and 16 refer to points from juveniles of ages 1 year, 5 weeks, and 16 weeks respectively.

Zehr (1962), and all were between stages 36 and 37, indicating sampling had occurred close to the time of parturition.

Blood respiratory characteristics of all groups are shown in Tables 1 and 2 and in Figs 1 and 2. Foetal and maternal haematocrit, haemoglobin concentration, blood oxygen-carrying capacity, Bohr effect and Hill coefficients were not significantly different. The P_{50} and blood NTP concentrations of foetal blood were significantly lower than those of maternal blood.

No statistical comparisons including 5- and 16-week-old, as well as yearling, groups

Table 2. *Blood oxygen-binding characteristics for cottonmouths*

		P_{50} (Torr)	$\Delta\log P_{50}/\Delta pH$	n
Foetal	X	19·5*	−0·41	1·62
	S.E.	0·4	0·03	0·26
	N	10	10	10
Maternal	X	48·8	−0·41	1·93
	S.E.	0·9	0·03	0·06
	N	5	5	5
5 weeks	X	45·5	†	–
	N	2		
16 weeks	X	47·0	†	–
	N	2		
1 year (approx.)	X	43·7	†	–
	N	1		

* Significantly different from maternal value $P < 0{\cdot}001$.
† Assumed equal to maternal – see text.

were done because of the small sample sizes. The small number of individuals also precluded a separate Bohr effect determination. For analysis of P_{50} this factor was assumed to be equal to that of maternal snakes. This was done on the basis that the NTP levels were similar (Table 1) and that the points determined fell along the maternal $\Delta\log P_{50}/\Delta pH$ curve (Fig. 2). Blood parameters measured in these three groups were similar to those of maternal snakes (Tables 1, 2).

Starch gel electrophoresis indicated no difference between foetal and maternal haemoglobin.

DISCUSSION

Haematology

Haematocrit, haemoglobin concentration and blood oxygen capacities for foetal and maternal cottonmouths show no significant differences (Table 1). That pregnancy does not appear to influence maternal haematology in reptiles is supported by the similarity of these values with those reported for other reptiles (see Pough, 1979 for review) and a recent study which compared haematological parameters in pregnant and non-pregnant garter snakes (*Thamnophis sirtalis*) (Birchard, Black, Schuett & Black, 1984). Unlike a previous study on the garter snake (Pough, 1977), we did not observe any ontogenetic changes in haematology.

NTP levels

Nucleoside triphosphates in reptiles are composed primarily of ATP (Bartlett, 1980; Isaaks & Harkness, 1980; Rapoport & Guest, 1941). The NTP levels found in adult, yearling and juvenile cottonmouths are similar to those reported for other adult snakes while those of foetuses appear somewhat lower (Seymour, Dobson & Baldwin, 1981; Bartlett, 1980; Johansen & Lykkeboe, 1979). Marked ontogenetic increases in NTP concentrations have been observed in the lizard *Iguana* but not in the rat snake, both oviparous squamates (Bartlett, 1978).

Blood oxygen binding characteristics

Bohr factors show no foetal–maternal difference (Table 2, Fig. 2) and are similar to values previously reported from snakes (Pough, 1979; Johansen & Lykkeboe, 1979). The lack of a significant difference in the foetal and maternal Bohr effects is in contrast to what would be expected given the large difference in NTP concentrations (Benesch, Benesch & Yu, 1969). This is probably due to the pH dependence of the effects of non-diffusable anions on the magnitude of the difference between plasma and intracellular pH (Wood, 1980).

The Hill coefficient (n) for foetal blood was slightly but not significantly lower than that of maternal blood (Table 2). Decreased cooperativity has been observed previously with lower NTP levels (Wood *et al.* 1978; Johansen & Lykkeboe, 1979). This has been suggested to be adaptive at higher blood oxygen affinities as greater oxygen delivery occurs at a higher partial pressure of oxygen than with a more sigmoid curve (Bellingham, 1972). Whether saturation-dependent changes in n occur as has been observed by Grigg & Harlow (1981) is difficult to ascertain, given our use three-point dissociation curves but may be indicated by Fig. 1.

Blood oxygen affinity data show a marked foetal–maternal shift which disappears rapidly after birth (Table 2). The magnitude of the foetal–maternal shift (29·3 Torr at 50 % saturation) is large compared to that of mammals (Metcalfe *et al.* 1967) but similar to that of other live-bearing reptiles and non-mammalian vertebrates (Grigg & Harlow, 1981; Ingermann & Terwilleger, 1981; Garlick *et al.* 1979; Pough, 1977; Toews & MacIntyre, 1977).

The rapid ontogenetic decrease in blood oxygen affinity to adult levels following birth is in contrast to several other snake species (Pough, 1977). In the garter snake (*Thamnophis sirtalis*) Pough (1977) determined that part of the change appeared to correlate with a change in haemoglobin type. In this study, adult and foetal haemoglobins appear to be the same, which probably accounts for the different temporal pattern seen. The changes we observed are similar to those in many mammals where the foetal–maternal shift in blood O_2 affinity is modulated by 2,3-DPG levels (Bauer & Jelkmann, 1981). We propose that a similar mechanism employing NTPs is used by the cottonmouth.

Intracellular blood NTP levels may alter haemoglobin oxygen affinity in two ways. First, NTPs act as direct modulators of haemoglobin oxygen affinity by binding to deoxyhaemoglobin and stabilizing it in a low affinity state (see Imai, 1982 for review). The second mechanism is through changes in intracellular anion concentrations. NTPs represent non-diffusible anions which affect the Donnan distribution of ions across the cell membrane. Thus, a decreased intracellular NTP concentration results in a higher intracellular pH relative to a given plasma pH (Wood & Johansen, 1972). This higher pH through the Bohr effect results in a higher blood oxygen affinity.

Non-diffusible anions probably play a prominent role in determining the magnitude of the foetal–maternal shift in our system. Since greater than equimolar amounts of phosphate *versus* haemoglobin are present (foetal = 1·8, maternal = 4·2) all haemoglobin binding sites could be full. However, given our lack of knowledge of intracellular conditions (particularly as to the portion of measured NTPs available to haemoglobin) and the significance of pH and other ions (e.g. Cl, Mg) on the binding constants of NTPs for haemoglobin (see Imai, 1982 for review), we do not feel that direct modulation can be ruled out.

These data appear similar to observed blood oxygen affinity responses to hyperoxia or hypoxia in avian embryos or fish (Ingermann, Stock, Metcalfe & Shih, 1983; Black & Snyder, 1980; Powers, 1980; Wood & Johansen, 1972). It has been suggested that hypoxia acts directly on metabolic pathways, lowering the level of aerobic metabolism and thus intracellular ATP. Thus, during times of hypoxia, automatic adaptive modulation of blood oxygen affinity occurs. We suggest that cottonmouth foetuses may exist in a state of hypoxia relative to adults and we propose that a similar mechanism is being employed. Thus in the evolution of viviparity, a previously available mechanism for modulating haemoglobin oxygen affinity may have been used to help enable this transition.

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