A Fetal-Maternal Shift of Blood Oxygen Affinity in an Australian Viviparous Lizard, *Sphenomorphus quoyii* (Reptilia, Scincidae)

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Summary. Compared to adults, the oxygen affinity of blood from fetal *Sphenomorphus quoyii* is very much higher: P_{50} is approximately 70 Torr in adults and 30 Torr in nearly full term embryos (P_{CO2}= 17 Torr, T=34 °C). Following birth, oxygen affinity decreases gradually and adult values are approached after about 15 weeks, with the onset of winter retreat. Electrophoresis revealed a multiple hemoglobin system in both adults and embryos, but there were no apparent differences between them.

Introduction

Among viviparous reptiles, a fetal-maternal shift in blood oxygen affinity has been described previously only in Garter Snakes (Manwell 1955, 1960; Pough 1971, 1977), though it is well known in other viviparous vertebrates (Amoroso 1968; Hogarth 1976). Viviparity is common among Australia's diverse fauna of scincid lizards and the anatomy of various degrees of placental development within the group has been described by Weekes (1933, 1935). Recently the extent to which the embryos of the Eastern Water Skink *(Sphenomorphus quoyii)* are dependent upon placental transfer of water, electrolytes, nutrients and excretory products has been studied extensively (Thomson 1977a, b). She found that yolk provides sufficient nutrient for fetal development, whereas water and electrolytes (particularly Ca, Na and K) move across the placenta to the embryo. Nitrogenous wastes are stored until birth, not excreted via the placenta-maternal route. It seems certain that embryonic gas exchange must occur across the placenta and oviductal walls. Our study of fetal and maternal oxygen equilibria was undertaken in order to add to the emerging understanding of the physiology of viviparity in this species and reptiles in general.

S. quoyii is a medium-sized (snout-vent 100125 mm) viviparous diurnal skink found commonly around rocky streams along the coast and ranges of eastern Australia. Emerging from winter brumation in early spring, ovulation and mating occur in October. Yolky eggs develop within the oviducts, which show little differentiation into structurally distinct placentae. Five to nine young are dropped in January, gestation having taken about three months.

Materials and Methods

Lizards were captured at Narrabeen, N.S.W. in December 1978 and maintained in captivity on a diet of mealworms. They were given shelter sites and, being kept in cages outdoors, had a normal photoperiod and opportunities for sun basking. All females except one were gravid. Four were killed pre-term by an overdose of Nembutal injected intra-peritoneally. The body cavity was then opened to expose the heart and embryo-laden oviducts. In most cases blood was sampled from a nick made in the still-beating left atrium and drawn into heparinized capillary tubes. In the youngest embryos blood was taken from the heart using a fine glass tube drawn to a point. From the smallest embryos only a few microlitres could be obtained. Some embryos were removed intact and weighed so as to obtain an estimate of age (Thompson 1977b). Oxygen equilibria were determined on whole blood within six hours of sampling, using an Aminco Hem-O-Scan (American Instrument Company). Powers et al. (1979) have given a full description of the use of this apparatus. We found the prescribed method of preparing the samples clumsy and often unsatisfactory because of uneven smears or the inclusion of bubbles. Accordingly we devised a modified system in which the sample is held between two layers of silicone copolymer membrane stretched onto an aluminum washer by an O-ring which lodges in a groove machined into the outer edge of the washer. The O-ring is applied with an applicator similar to those in use for securing membranes to many O_2 and CO_2 electrodes (such as those by Radiometer, Copenhagen). In practice 2 µl of blood is placed on a disc of membrane lying over the washer, a second membrane is placed over the top and the membranes and sample are then stretched into place on the washer with an O-ring. This modified sample holder fits snugly into the sample tray of the Hem-O-Scan. Early on, equilibrium curves were determined at P_{CO2}=40 Torr. Later curves were set up at 17 Torr, which is more likely to approximate the range in vivo. Heatwole (1976) tabulated data for thermal preferenda in *S. quoyii.* Animals have been observed active in the field over a range of body

temperatures from 22.3-33.9 °C, whereas the mean voluntary maximum body temperature is 35°C. Accordingly an equilibration temperature of 30°C was chosen. However, lack of a cooling system in the water bath of the Hem-0-Scan, coupled with high ambient temperatures in the laboratory in summer, led to sample compartment temperature above 30°C in some instances. Thus the temperature of measurement ranged from 30-35°C, though it remained stable throughout any single determination.

As gestation, birth and growth of juveniles proceeded in the laboratory population, animals were killed and oxygen equilibrium curves determined in maternal and fetal blood and in blood from juveniles of known age from 5 to 108 days. In May, *S. quoyii* enters a winter brumation and the series of observations was discontinued.

Electrophoresis was carried out on hemolysates from adults 3 and 4 (Table 1) and from two embryos from each using a Gelman Sepratex chamber and Sepraphore III for 35 min at 375 V in a Tris-EDTA-glycine buffer, pH 9.2. Normal human hemolysate was run for comparison.

Details of the animals from which samples were taken are shown in Table 1.

Table 1. Details of the *Sphenomorphus quoyii* from which samples were taken

Date	Animal No.	Snout-vent (mm)	Total weight (g)	Total embryo weight (g)	Number of embryos	Age of embryos (days)
Gravid females (December-January) (Oxygen equilibria were determined on two embryos from each gravid female)						
15 December 1978	Adult 1	120	40.5	8.2	7	50-60
22 December 1978	Adult 2	125	49.6	16.6	9	80-100
3 January 1979	Adult 3	110	39.3	12.4	6	80-100
4 January 1979	Adult 4	111	29.8	6.2	3	80-100
Adult non-gravid females and males (February-April)						
13 February 1979	Adult 5,S	124	39.5	-	-	-
15 March 1979	Adult 6 a	123	48.1	-	-	-
19 April 1979	Adult 7 ~	120	31.3	-	-	-

| *Juveniles (January-May)*				Date of birth	Age from birth (days)
18 January 1979	Juvenile 1	39	1.22	13 January 1979	5
18 January 1979	Juvenile 2	39	1.21	13 January 1979	5
18 January 1979	Juvenile 3	42	1.68	3-7 January 1979	11-15
18 January 1979	Juvenile 4	41	1.31	3-7 January 1979	11-15
13 February 1979	Juvenile 5	45	2.08	3-7 January 1979	38-41
15 March 1979	Juvenile 6	47	2.11	13-14 January 1979	60-61
19 April 1979	Juvenile 7	53	3.09	22 January 1979	87
10 May 1979	Juvenile 8'	44	1.79	22 January 1979	108
10 May 1979	Juvenile 9	54	4.2	22 January 1979	108

[a] Runt

Results

The developing embryos in gravid females (adults 14) weighed respectively 25%, 50%. 46% and 26% of embryo-free body weight and occupied most of the volume of the body cavity. At birth, juveniles weighed a little more than 1 g, and grew to about 4 g by May.

Oxygen equilibria show that the O_2 affinity of embryonic whole blood is much greater than that of blood from adults (Figs. 1, 2). During the postpartum period, blood oxygen affinity decreases gradually until it approximates adult levels before the winter retreat.

Electrophoresis showed no apparent differences between fetal and maternal hemolysates (Fig. 3) and migration was slower than in normal human hemoglobin.

Hill-plots of the oxygen equilibria show a distinct nonlinearity (Fig. 4) which cannot be attributed to a technical artifact because determinations of oxygen equilibria for human blood produced linear Hill-plots (e. g. Fig. 4).
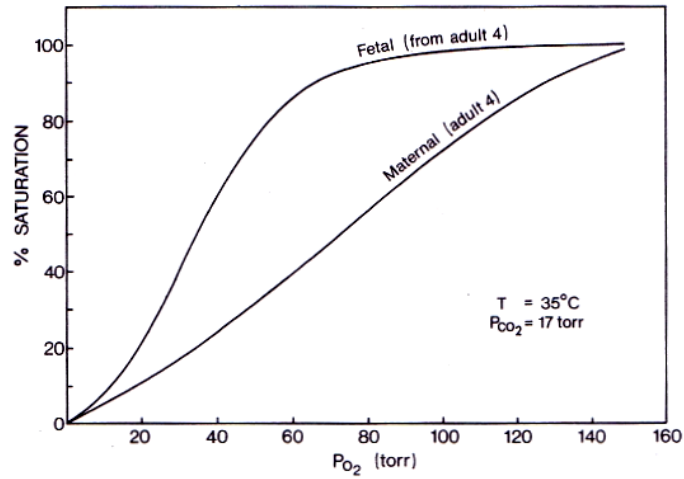
Fig. 1. Representative oxygen equilibrium curves of whole blood from a gravid female (adult 4) and one of her embryos (T=34 °C, P_{CO_2}=17 Torr)
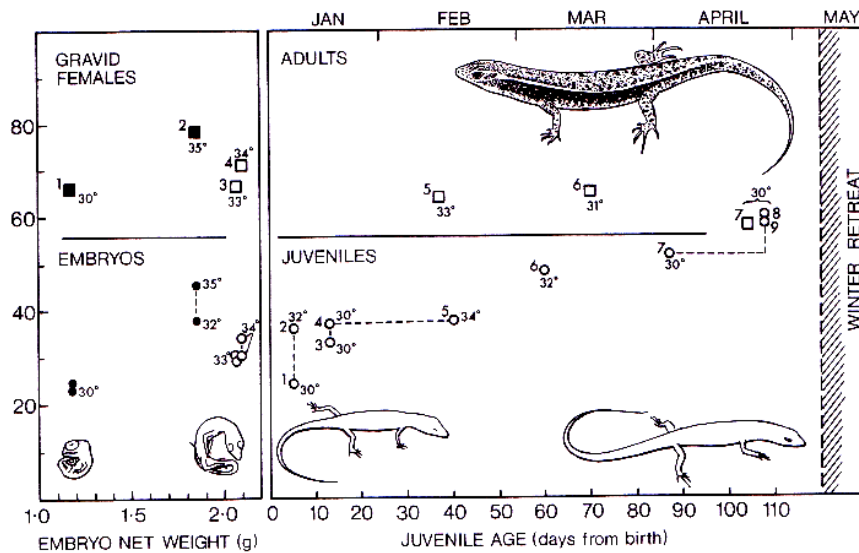
Fig. 2. Oxygen affinity (as P_{50}, Tort) of whole blood from pregnant and non-pregnant adult *S. quoyii*, embryos and juveniles. Each point represents a different individual and dotted lines join data from siblings. Square symbols denote adults, circles are embryos or juveniles. Closed symbols denote P_{CO_2}=40 Tort, open symbols denote P_{CO_2}=17 Torr. Superscript numbers at each data point are the animal numbers and relate to information in Table 1. Measurement temperatures are indicated also.
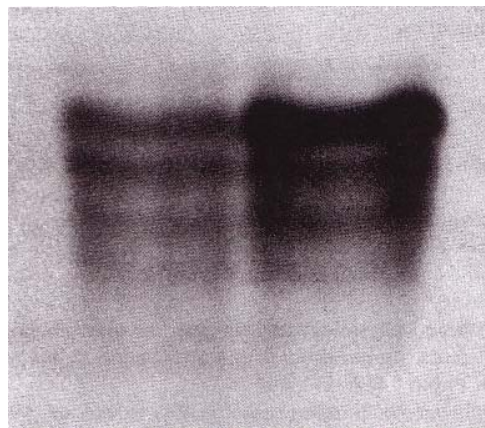
Fig. 3. Electropherogram of hemoglobin from a pregnant female (adult 4), right, and one of her embryos. Sephraphore III (Gelman) Tris-EDTA-glycine buffer pH 9.2, 375 V for 35 min
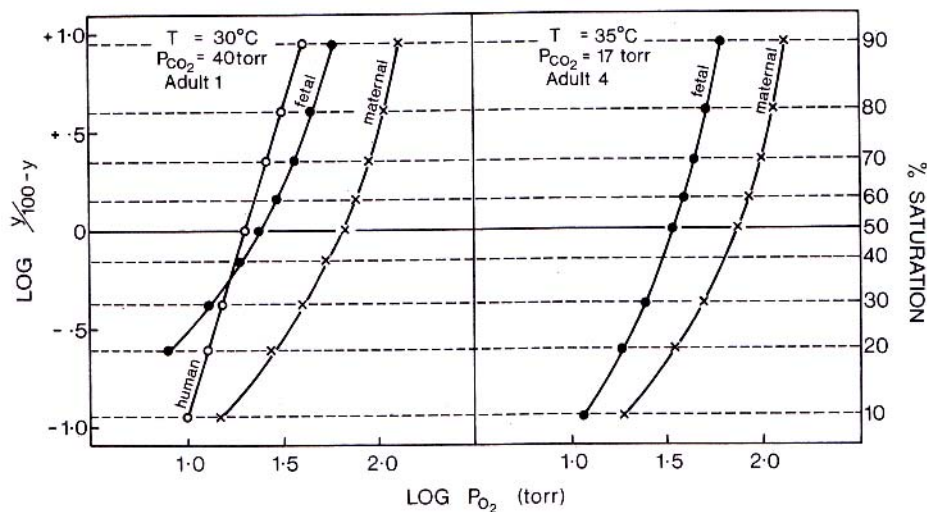
Fig. 4. Hill-plots of O_2 equilibria from two pregnant females and their embryos. Note the non-linearity, compared to a plot for human blood established by the same technique.

Discussion

An increased whole blood oxygen affinity in the fetus, compared to the mother, is well known among mammals and has been described also in vertebrates as taxonomically diverse as Garter Snakes, *Thamnophis sirtalis* (Pough 1971, 1977), a viviparous Caecilian (Toews and MacIntyre 1977; Garlick et al. 1979) and a shark, *Squalus suckleyi* (Manwell 1958). The functional interpretation placed on higher fetal oxygen affinity is the facilitation of loading oxygen across the placenta from the maternal blood. In reptiles such as *S. quoyii,* where the placenta is little developed, it may be that fetal O_2-loading is accomplished both directly from the maternal circulation, and also indirectly from the ambient oviductal and extraoviductal environment. This is reminiscent of the situation in many low-oxygen environments where oviparous reptiles lay eggs. Many Squamata, the Tuatara, Chelonia and Crocodilia bury eggs in soil or sand or in mounds of earth and grass where ambient O_2 is likely to be quite low. One might expect, therefore, to find high blood oxygen affinity in embryos in such circumstances and this is known to be so in at least some oviparous reptiles (McCutcheon 1947; Pough 1969). Ontogenetic changes in blood oxygen affinity are seen also in Amphibia (Riggs 1951; Hazard and Hutchison 1978) and in birds (Bauman and Bauman 1978). Undoubtedly the existence of substantial differences in blood O_2 affinity between embryo and adult preceded the evolution of viviparity. Therefore the high oxygen affinity in the viviparous Garter Snakes (Pough 1971, 1977) and *S. quoyii* (this paper) should probably not be seen as special adaptations to viviparity. Instead it is more likely that a widespread ability of embryonic reptiles to increase blood oxygen affinity in response to low ambient oxygen is a feature which pre-adapted some of them for viviparity.

As to the mechanism by which the fetal-maternal shift occurs in *S. quoyii,* the lack of any apparent difference between the electropherograms from maternal and fetal hemolysates (Fig. 3) suggests that modulation may be effected by an allosteric co-factor such as one of the red cell organic phosphates (Grigg and Gruca 1979), as in the case of a viviparous caecilian (Garlick et al. 1979). No investigation has yet been undertaken to identify the major red cell organic phosphates in S. *quoyii.* The impossibility of pH determination on the few microlitres of blood from embryos precluded any full study of the likelihood of pH changes being involved.

The oxygen affinity of adult skinks was quite low. However, Pough (1969) reported similarly low values for O_2 affinity in *Diposaurus dorsalis, Gerrhonotus multicarinatus, Sceloporus occidentalis* and *Uma notata* ranging from 25-45 g. A negative correlation between P_{50} and body weight has been reported for reptiles by Bennett (1973). The high P_{50} values seen in *S. quoyii* fit that relationship very well. Such low oxygen affinities pose questions about functional aspects of gas exchange, as arterial saturation could occur only at very high O_2 tensions. Full resolution of these questions awaits data on patterns of gas exchange in small reptiles.

Non-linearity of Hill-plots has been described previously in a lemur (Bonaventura et al. 1974) and the Amazonian Manatee (Farmer et al. 1979) and interpreted as an indication of α and β chain

heterogeneity. Steepening values of n_H with increasing oxygen saturation have been described also in two frogs, *Rana temporaria* and R. *catesbeiana* (Lykkeboe and Johansen 1978). These authors suggested that the functional significance of the steepening may be to improve O_2 loading properties in a blood that has a low oxygen affinity around 50% saturation and lower. Certainly *S. quoyii* has a low oxygen affinity at low saturations and a similar functional interpretation may be possible.

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