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SHORT COMMUNICATION

A COMPARISON OF AVIAN AND REPTILIAN CHORIOALLANTOIC VASCULAR DENSITY

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Oviparous amniotes exchange respiratory gases across a specialized vascular membrane, the chorioallantois. Although many investigations into the physiology of amniote eggs, particularly those of the chicken, have been carried out, there is no comparative information about the chorioallantoic membrane. Given the differences in size and phylogeny, it might be expected that significant morphological differences exist in this gas-exchange organ.

A major determinant of the rate of diffusive exchange of gases in animals is the morphology of the vascular bed (Wangensteen and Weibel, 1982). The particular parameters of interest determined by the morphology are the diffusion distance and the exchange area. In most adult tissues there appears to be a general matching between angiogenesis and aerobic metabolic rate (so that as these two parameters increase they effect a decrease in diffusion distance and an increase in exchange area) (Adair *et al.* 1990). This study examines the relationship between metabolic rate and vascular density in the chorioallantoic membrane (as indicated by a vascular density index) in two developing vertebrates of similar size but from different taxonomic classes. Quail (class Aves) and snapping turtle (class Reptilia) embryos have significantly different maximal embryonic oxygen demands (Miller and Packard, 1992; Vleck *et al.* 1979). Therefore, a comparison of chorioallantoic membrane vascular density of these two species allows a direct examination of the relationship between angiogenesis in the respiratory exchanger and metabolic rate. Vascular density measurements were also carried out on chorioallantoic membrane of the domestic chicken as a control and for comparison with quail.

Domestic chicken (*Gallus gallus*) and Japanese quail (*Coturnix coturnix*) eggs were obtained from a local supplier. Eggs were incubated at 37˚C and automatically turned in a small table-top incubator. Chicken and quail eggs were incubated for 15 days before being sampled. These incubation times were chosen because they were comparable to previous studies and represent maximum vascular density (chicken) or the point of

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maximum embryonic oxygen consumption (quail) (Dusseau and Hutchins, 1988; Vleck *et al.* 1979). The eggs analyzed were obtained during two separate experimental runs.

Snapping turtle (*Chelydra serpentina*) eggs were collected in the Valentine National Wildlife Refuge, Nebraska, and transported to George Mason University. Three eggs from six different clutches were incubated in plastic boxes at 30°C in a Percival incubator. Eggs were incubated half buried in moistened Vermiculite (water potential of −150kPa) for 2 weeks. After 2 weeks, eggs were placed in bowls on a large-mesh plastic screen over moistened Vermiculite. Bowls containing a maximum of three eggs were then placed in partly open plastic bags to reduce evaporation. This manipulation was carried out to ensure that the chorioallantoic membrane was subjected to similar gaseous conditions over the entire surface of the egg. Eggs were weighed regularly. At weekly intervals, water was added to the incubation boxes/bowls to replace losses due to egg uptake or evaporation. Eggs were sampled on day 48 of incubation. This represents part of the period of maximum embryonic metabolism in this species at this temperature (Miller and Packard, 1992; and personal observations).

On the day that chorioallantoic membrane samples were taken, egg oxygen consumption was measured using a closed manometric system and the methods described by Birchard (1991), with the following modifications. Measurements were performed in 134ml glass chambers. The pressure changes within the reaction chamber were balanced by the plunger movements of a 1 or a 3ml syringe. All measurements were performed at the incubation temperature of the species being studied. Oxygen consumption values were corrected to $cm^3 O_2\,day^{-1}$ STPD.

The procedures for obtaining the chorioallantoic membrane were similar in all species. In avian eggs, the shell was removed from the blunt end of the egg, a slit was made in the shell membranes, the egg was turned with the blunt end down and the embryo and yolk were gently drawn from the egg. The major blood vessels connecting the embryo with the chorioallantoic membrane were then cut and the shell with attached membranes was immediately submerged in 10% buffered formalin. The same basic procedure was used for turtle eggs but, as no air space exists in snapping turtle eggs, a small round hole was made in an area on the bottom lateral side of the egg and the embryo was drawn out. As in the avian eggs, the chorioallantoic membrane blood vessels were cut and the egg shell and attached membranes were placed immediately in preservative.

The vascular density index was determined following procedures similar to those of Dusseau and Hutchins (1988). After preservation, a section of chorioallantoic membrane from each egg was gently peeled away and floated off the egg shell. The membrane was stained with Methylene Blue for approximately 1min (chicken and quail) or 2min (turtle) to improve the visualization of blood vessels. The membrane was soaked for at least 1h in 0.9% saline to remove excess stain. Membranes were then spread flat on gelatin-coated microscope slides and a plastic coverslip with four concentric circles 4, 5, 6 and 8mm in diameter (72mm total length) etched into it was placed over a section of the membrane. The total number of blood vessels intersecting with the circles was then counted under a dissecting microscope at 25× magnification. Only vessels with a defined vascular wall were counted. All counting was carried out by one individual who was not aware of the species being examined.

Data were analyzed using a Student's *t*-test or a one-way analysis of variance (ANOVA) followed by a Fischer's LSD test. A *P* value less than 0.05 was considered significant.

The vascular density of chicken chorioallantoic membrane determined in this study (Table 1) was very similar to that reported previously using the same technique (Dusseau and Hutchins, 1988). However, these values are somewhat less than those reported by Strick *et al.* (1991) using a different technique. The gross morphology of quail chorioallantoic membrane was not obviously different from that of chicken. However, quail chorioallantoic membrane vascular density was significantly less than that of chicken (Table 1). The chorioallantoic membrane of turtles had a different appearence from that of both avian species (Fig. 1). The blood vessels were fewer in number and, in general, those present had somewhat larger diameters than the vessels seen in both chicken and quail. The vascular density of turtle eggs was significantly less than that of both avian species (Table 1). The difference between avian and reptilian vascular density is consistent with observations on muscle capillarity in adult vertebrates (Hudlicka *et al.* 1992).

The rates of oxygen consumption of quail and turtle eggs at their respective temperatures and incubation times (Table 1) were similar to those reported previously (Vleck *et al.* 1979; Miller and Packard, 1992). Quail eggs consumed 5.03 times as much oxygen just prior to hatching as snapping turtle eggs.

The observed differences between quail and turtle chorioallantoic membrane vascular density are correlated with the differences in gas exchange rates per unit membrane area. Using egg shell surface area to approximate chorioallantoic membrane area we find the exchange area for a quail egg is 22.2cm^2 and a turtle egg 24.1cm^2 (area estimates were made using the allometric equation of Paganelli *et al.* 1974, for a 10g quail egg and calculating the exchange area for a turtle egg assuming a spherical shape and an average egg diameter of 2.77cm). In quail, 5–6 times as much oxygen must be exchanged across a unit area of chorioallantoic membrane as in snapping turtle. Thus, the diffusive capacity of the air–blood barrier must be significantly increased in this species. These data suggest that, in part, this is accomplished by increasing chorioallantoic membrane vascular surface area. The vascular density of quail chorioallantoic membrane was 2.13 times greater than that of snapping turtle. This difference is not proportional to the difference in oxygen flux rate, indicating that the quail must have other morphological and/or

Table 1. *Vascular density and oxygen consumption in reptilian and avian eggs*

	N	Vascular density index	*N*	Oxygen consumption ($cm^3\,O_2\,day^{-1}$)
Snapping turtle	6*	110.5±13.7	6*	22.2±3.7
Quail	13	235.7±20.3	12	111.7±12.0
Chicken	12	276.4±31.6		ND

*Six clutches where three eggs were sampled per clutch.
ND, not determined.
Values are mean ±S.D.

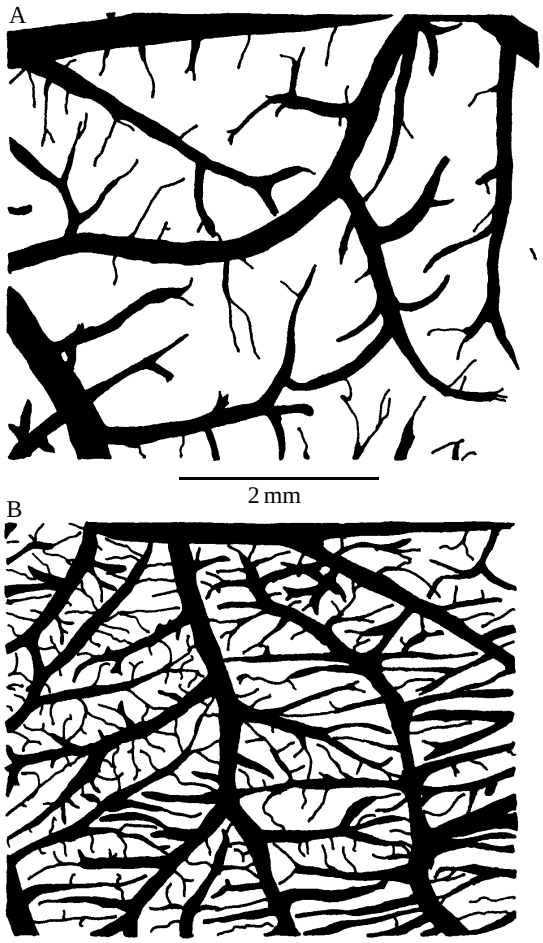

Fig. 1. *Camera lucida* drawings of the chorioallantoic membranes of snapping turtle (A) and quail (B).

physiological modifications to increase the rate of diffusive flux (Wangensteen and Weibel, 1982).

The difference between chicken and quail vascular density may be explained in a similar manner. In both species, the partial pressure of oxygen outside the chorioallantoic membrane is approximately equal prior to 'pipping' (Rahn *et al.* 1974). If the diffusion coefficient and thickness of the membranes are assumed to be similar, then the major determinant of differences in gas exchange capacity is the area of the respiratory surface. A comparable estimate for the chorioallantoic membrane gas exchange rates per unit area can be obtained by examining the ratio of maximal chorioallantoic membrane oxygen exchange rate (equivalent to pre-pipping rates of oxygen consumption) to egg surface area. Using existing allometric equations to predict shell surface area and pre-pipping rate of oxygen consumption (Paganelli *et al.* 1974; Rahn and Paganelli, 1990), it was found that quail eggs have a proportionally greater total surface area available for exchange.

That is, the amount of oxygen exchanged across a unit area of chorioallantoic membrane is about 14% lower in quail than in chicken, which is similar to the observed difference in vascular density (14.7%). Such a decrease is consistent with a matching of vascular growth and chorioallantoic membrane exchange capacity. These results support current hypotheses that angiogenesis occurs as a homeostatic response to changes in metabolic need (Adair *et al.* 1990)

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