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EFFECT OF BOUNDARY LAYERS ON CUTANEOUS GAS EXCHANGE

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Summary

Boundary layers may offer significant resistance to cutaneous oxygen uptake by amphibians in water. This hypothesis was tested by measuring resistance to oxygen uptake as a function of water velocity in bullfrogs submerged at 5 °C and by direct measurements of the boundary layer with oxygen microelectrodes.

The oxygen diffusion boundary layer was easily measurable with oxygen microelectrodes. The proportion of the total resistance to oxygen uptake represented by the boundary layer increased from 35 % at a water velocity of $5\,cm\,s^{-1}$ to over 90 % at $0.1\,cm\,s^{-1}$. At water velocities below $1\,cm\,s^{-1}$ oxygen uptake was limited by the resistance of the boundary layer. At $0.1\,cm\,s^{-1}$, the partial pressure of oxygen immediately adjacent to the skin was only 2 kPa (15 mmHg); placing an immobilized frog in still water was tantamount to placing it in anoxic water. Body movements disrupted boundary layers efficiently; even occasional small movements by the animal ($1\,min^{-1}$) were sufficient to maintain oxygen uptake in still water.

Introduction

A layer of oxygen-depleted water, the diffusional boundary layer for oxygen, surrounds aquatic skin-breathers. Because the partial pressure of oxygen (P_{O_2}) at the skin–water interface limits the partial pressure difference for diffusion of oxygen through the skin, the formation of a hypoxic boundary layer ought to be deleterious for cutaneous oxygen uptake. Although many investigators have invoked boundary layers as a potential drawback to cutaneous gas exchange in water and have suggested skin ventilation as a potential regulatory mechanism, oxygen depletion next to the skin of a skin-breather has not yet been measured. Here we quantify the degree of oxygen depletion at the skin–water interface in submerged, skin-breathing frogs as a function of free-stream velocity of the

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surrounding water, and calculate the relative resistances of the boundary layer and the skin to diffusion of oxygen.

When skin-breathing animals extract oxygen across the skin–water interface, both diffusion and bulk flow replenish the oxygen from the surrounding medium. Because diffusion predominates at low flow velocities and over short distances, whereas bulk flow predominates at high flow velocities and over longer distances, the distribution of flow velocities radial to the skin–water interface (i.e. the 'flow profile' of the hydrodynamic boundary layer) will determine the thickness and resistance of the diffusional boundary layer. For an animal in a laminar flow, fluid velocity increases with distance away from the animal and approaches the free-stream velocity (U) asymptotically. The thickness of the hydrodynamic boundary layer is taken to be the distance away from the surface at which velocity reaches some arbitrary fraction of U, usually 0.9 or 0.99 (Barry and Diamond, 1984; Feder and Pinder, 1988; Vogel, 1981). The thickness of the hydrodynamic boundary layer is proportional to $U^{-0.5}$; the diffusional boundary layer is thinner than the hydrodynamic boundary layer but is also proportional to $U^{-0.5}$ [see reviews by Barry and Diamond (1984), Feder and Pinder (1988) or Vogel (1981) for a more detailed treatment]. The resistance of the diffusional boundary layer for oxygen flux ought to be proportional to its thickness and therefore should also be proportional to $U^{-0.5}$. The diffusional boundary layer ought to pose a significant resistance to cutaneous oxygen uptake in still or slowly moving water. Animals should be able to minimize this boundary layer resistance by moving, ventilating the skin, or choosing aquatic microhabitats with moving water (Feder and Burggren, 1985; Feder and Pinder, 1988).

Several investigators have reported findings that are consistent with these expectations. For example, *Telmatobius culeus* (the Lake Titicaca frog), an aquatic skin-breather, bobs its body up and down in hypoxic water (Hutchison *et al.* 1976). *Cryptobranchus alleganiensis* (the hellbender) rocks or sways from side to side more frequently in hypoxic water (Guimond and Hutchison, 1973; Boutilier *et al.* 1980; Boutilier and Toews, 1981). Experimental ventilation of the skin and locomotor movement both increase cutaneous oxygen uptake in ranid frogs (Burggren and Feder, 1986; Pinder and Burggren, 1986).

Direct measurements of the diffusional boundary layer for oxygen have been lacking. With the advent of reliable and rugged oxygen microelectrodes, however, direct measurement of oxygen boundary layers has become possible (e.g. Jørgensen and Revsbech, 1985). In the present study we report the first direct measurements of oxygen boundary layers surrounding skin-breathing vertebrates in water. The physiological significance of these measurements relates to the absolute resistance that the boundary layer poses to cutaneous oxygen uptake (R_{bl}), and its resistance relative to that of the skin itself (R_{skin}) and to that of the skin and boundary layer combined (R_{total}) (Feder and Pinder, 1988). R_{bl} will be inconsequential if water flow velocity (U) is sufficiently rapid or if R_{bl} is small relative to R_{skin} and R_{total}. We chose the bullfrog, *Rana catesbeiana*, as an

experimental subject. In the northern part of their range, bullfrogs overwinter in cold water, often under ice (Pinder *et al.* 1990). While overwintering underwater, frogs cannot ventilate their lungs, nor can they rely for long on anaerobic metabolism (Pinder, 1985; Penney, 1987). Thus, the resistance of oxygen boundary layers may be critically important to bullfrogs during this period.

Materials and methods

Animals

Seven bullfrogs (229±43 g, S.D.) were obtained from commercial suppliers between December and March and maintained in the laboratory for at least 2 weeks at 3–7°C in well-aerated and stirred water in constant darkness before use. The frogs were not fed during acclimation and had access to air. All experiments were done at 5°C.

Experimental procedure

The femoral artery was occlusively cannulated with PE 50 tubing filled with heparinized saline the day before experimentation under MS222 (tricaine methane sulphonate) anaesthesia ($0.5\,g\,l^{-1}$ adjusted to neutral pH). The lungs were emptied, and the frog was submerged in the experimental chamber at 5°C overnight in well-aerated and stirred water with a low concentration of MS222 ($0.1\,g\,l^{-1}$) to sedate the frog. This concentration of anaesthetic leaves cardiovascular reflexes intact.

The flow chamber was a sealable, recirculating design (total volume 3.42 l), in which water velocity was set by a propellor magnetically coupled to an external variable-speed motor (Vogel and LaBarbera, 1978). The frog itself affects water velocity by reducing the cross-sectional area of the chamber. We therefore calibrated flow velocity with a frog present, taking U to be the velocity of water 1–2 cm above the frog. Reference lines were drawn on the chamber wall on either side of the frog (10 cm apart) and the time taken for small, neutrally buoyant particles to travel between them was measured. Ten measurements were taken at each velocity; standard deviations ranged from 8 to 20 % of nominal velocity. Flow over the back of the frog appeared laminar, although eddies were visible in other areas of the chamber.

Oxygen uptake ($\dot{M}_{O_2}$) and arterial P_{O_2} (Pa_{O_2}) were measured as a function of U. To measure $\dot{M}_{O_2}$, the flow chamber was sealed and the decrease of dissolved O_2 was measured continuously with an Orbisphere model 2603 meter and electrode connected to a chart recorder. $\dot{M}_{O_2}$ was calculated from the rate of decrease of dissolved O_2 and the chamber volume. Pa_{O_2} was measured with an Instrumentation Laboratories μ13 meter by drawing blood through the cannula (led to the exterior through a small port in the chamber) past an Instrumentation Laboratories P_{O_2} electrode kept at 5°C. After measurement the blood was returned to the

animal and the cannula flushed with heparinized saline. The P_{O_2} electrode was air-calibrated between measurements and, because blood P_{O_2} readings were usually very low, was equilibrated with N_2 before each measurement.

$\dot{M}_{O_2}$ was measured at six water velocities (5.2±0.3, 2.0±0.1, 0.96±0.03, 0.51±0.02, 0.21±0.01 and 0.1±0.05 cm s^{-1}; mean±S.E.) for 45–60 min each, during which time the P_{O_2} within the chamber decreased by approximately 1.1 kPa. Some measurements were taken while the propellor was stopped and bulk flow halted; free convection was presumably minimal but not entirely absent. Usually, $\dot{M}_{O_2}$ at two velocities was measured before opening the chamber and aerating the water. The chamber P_{O_2} was never allowed to drop below 17 kPa. If the frog started to move during the measurement period the experiment was terminated and more MS222 was added to the chamber (the final concentration of MS222 was always less than 0.2 g l^{-1}). At the end of the M_{O_2} measurements, the frog was removed from the chamber and placed in anaesthetic-free water at 3°C overnight. The $\dot{M}_{O_2}$ of the empty chamber was then measured to control for microbial respiration.

Direct measurements of diffusional boundary layers were made in separate experiments because the chamber could not be sealed during these measurements. The boundary layer was measured with glass microelectrodes (Diamond General model 737, 250 μm diameter tip with a 3–8 μm measuring surface) mounted on a micromanipulator and connected to a Transidyne Chemical Microsensor meter. The microelectrode was calibrated with N_2-equilibrated water at 5°C followed by immersion in the air-equilibrated water in the chamber. The electrode was lowered to the surface of the skin with the micromanipulator to measure the P_{O_2} at the surface of the skin. The point of contact between the microelectrode and skin was observed through a dissecting microscope (27× magnification) set with the angle of view tangential to the curved surface of the skin and perpendicular to the microelectrode. When measurements of complete oxygen boundary layers were required, the microelectrode was withdrawn in increments of 25–200 μm, measured on the micromanipulator scale, until the free-stream P_{O_2} was reached. If frogs started to move at any time during these measurements, more MS222 was added to the water. The water was vigorously aerated between measurements.

To assess the efficacy of body movements in dispersing boundary layers, the respirometry experiment was repeated on four of the frogs under lighter anaesthetic (0.05 g l^{-1} MS222), which tranquillized the frogs and reduced struggling but did not prevent them from moving. Body movements at each U were counted for 10 min near the end of each measurement period.

Statistical analysis

Data are presented as mean±standard error. Two-way analysis of variance (factor A, either flow velocity or ability to move; factor B, individual animal) was used to assess the significance of treatment effects. Paired t-tests were used to compare specific means. The least-squares method was used for linear regression.

Results

Oxygen boundary layers

The P_{O_2} at the skin–water interface varies considerably over the surface of the frog, but on a microspatial scale (Fig. 1). At 0.5 cm s^{-1} flow velocity, for example, the P_{O_2} at the skin–water interface varied from 3.2 to 7.6 kPa above a 2–3 mm^2 section of skin (Fig. 1A,B). The range of values for this small section was as large as the range of P_{O_2} values along the entire dorsal midline of the frog (Fig. 1C,D); the mean P_{O_2} over the length of the dorsal midline was not significantly different from the mean P_{O_2} measured mid-dorsally. For no region of the skin was the oxygen boundary layer characteristically large or small. Subsequent estimations of boundary layer resistance will therefore assume that average measurements for small patches of skin approximate the boundary layer over the entire skin surface.

In every subject and in every experimental condition, boundary layers significantly reduced the P_{O_2} at the skin–water interface. For example (Fig. 2), at a single point on the dorsal midline of a frog, decreasing U from 5.2 cm s^{-1} to zero decreased the P_{O_2} at the skin–water interface from 14.4 kPa to 2.0 kPa. Thus, even at 5.2 cm s^{-1} the P_{O_2} at the skin–water interface was only 70 % of free-stream P_{O_2}, and fell to 10 % of ambient in still water. Evidently, a skin-breathing animal in normoxic but still water faces a profound hypoxic challenge.

To assess the effect of oxygen consumption by the electrode itself, the P_{O_2} immediately next to a clean rubber stopper was measured as a function of water velocity (Fig. 3). The measured P_{O_2} decreased in this circumstance; however, the decrease was small and would, at worst, underestimate the P_{O_2} next to the skin by 7 % (e.g. the true P_{O_2} next to the skin in still water would be 2.1 kPa instead of the measured 2.0 kPa). This error was subsequently ignored.

Effect of boundary layers on Pa_{O_2} *and* M_{O_2}

Even at U=5 cm s^{-1}, Pa_{O_2} averaged only 2.9 kPa in exclusively skin-breathing bullfrogs (Fig. 3). As U decreased, Pa_{O_2} declined ($P<0.0001$), presumably due to the drop in P_{O_2} at the skin–water interface. In still water, Pa_{O_2} was 0.7 kPa.

Not surprisingly, reduction in U, which decreased both the P_{O_2} at the skin–water interface and Pa_{O_2}, also reduced oxygen uptake in exclusively skin-breathing bullfrogs (Fig. 4). The reduction in $\dot{M}_{O_2}$ was small until U was less than 1 cm s^{-1}, but at 0.1 cm s^{-1} $\dot{M}_{O_2}$ decreased by 58 %, from 0.27 to 0.12 mmol kg^{-1} h^{-1}. Conversely, increases in U, whether due to experimental manipulations or voluntary movements by the frogs, increased $\dot{M}_{O_2}$. If frogs were not immobilized, body movements were sufficient to prevent the decrease in $\dot{M}_{O_2}$ even in still water. The $\dot{M}_{O_2}$ of mobile frogs and immobile frogs did not differ significantly at high ($\geq$1 cm s^{-1}) values of U ($0.10<P<0.25$). At lower values of U (0.2 and 0.1 cm s^{-1}), by contrast, the $\dot{M}_{O_2}$ of mobile frogs was significantly higher than that of immobilized frogs ($P<0.025$ and $P<0.001$, respectively). The movements of mobile frogs were not correlated with U: at 5 cm s^{-1} the frogs moved 5.5±2.2 times in 10 min, and in still water they moved 8.8±3.5 times in 10 min.

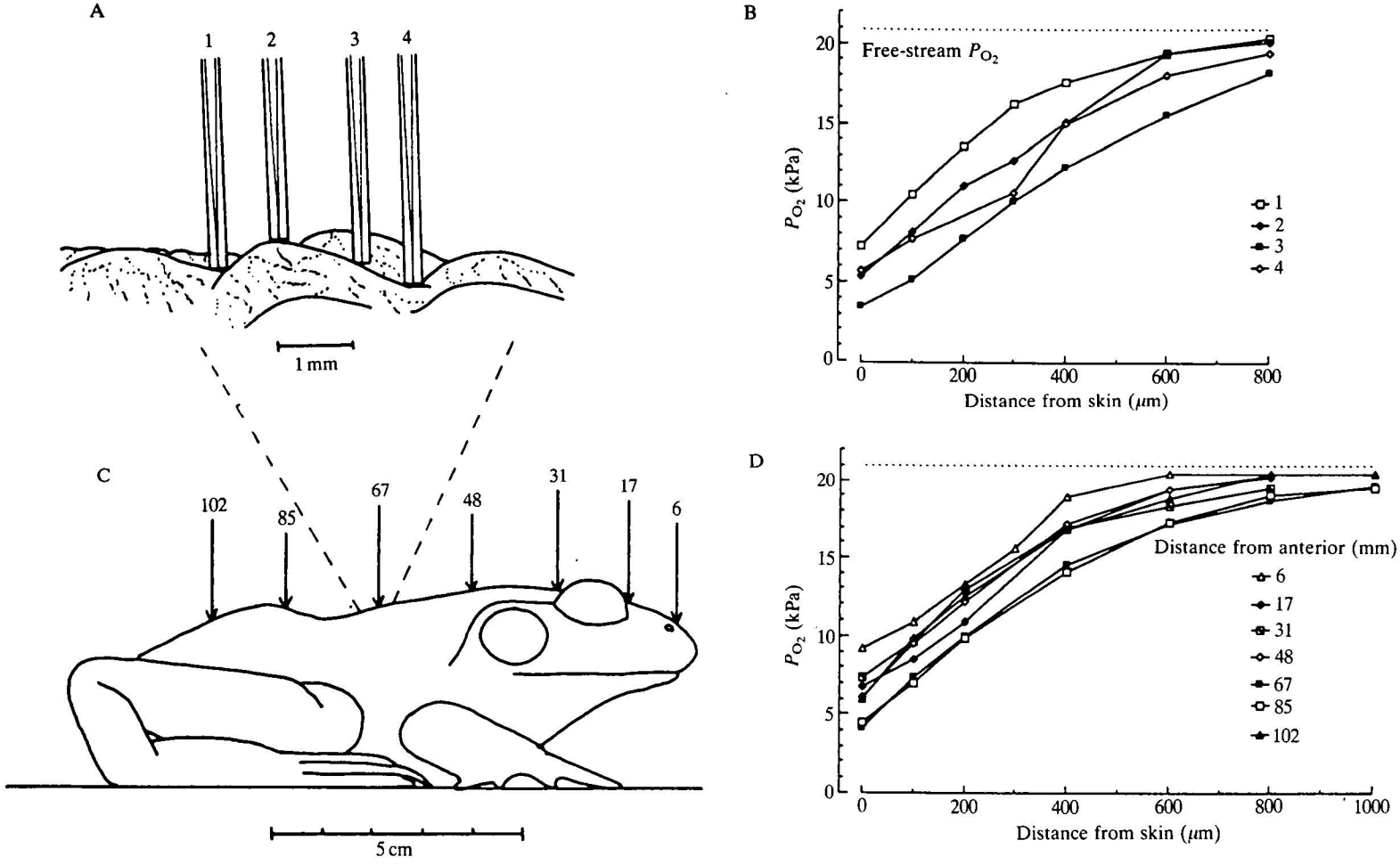

Fig. 1. Oxygen-depleted boundary layers adjacent to skin irregularities (A and B) and over the length of the body (C and D) of a 12 cm bullfrog; water velocity=0.5 cm s^{-1}. The diagrams (A and C) show the positions over which the diffusional boundary layer was measured: A and B positions are labelled arbitrarily; in C and D the labels are the distances from the upstream (anterior) end of the frog (in mm).

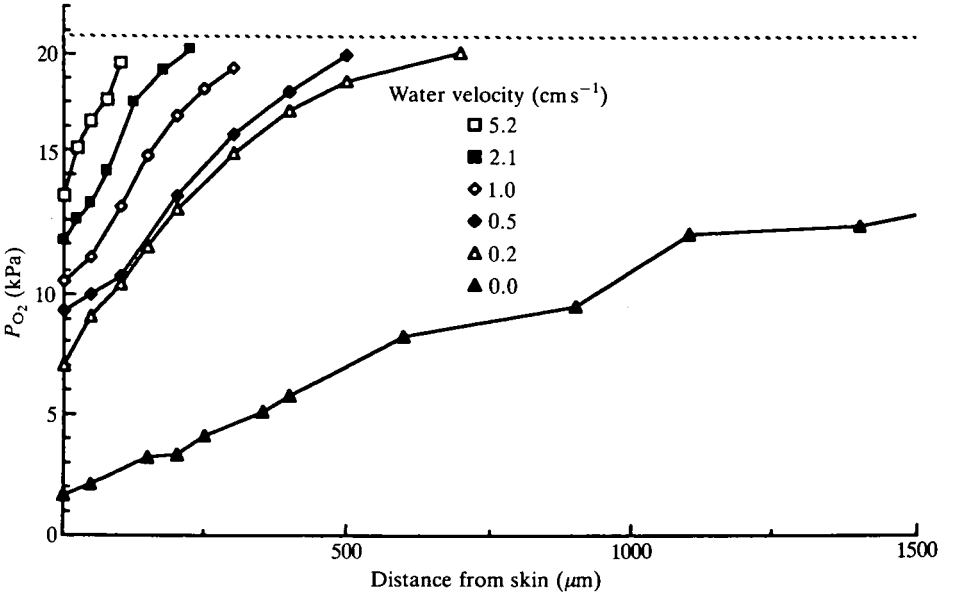

Fig. 2. Oxygen boundary layers measured at a single mid-dorsal point on a bullfrog at various water velocities (U). The region of oxygen depletion in 'still' water was measured after 1 h with no convection. There was probably no steady state under these conditions; the oxygen depletion next to the skin continued to increase gradually and oxygen uptake to decrease until convection was restarted.

Resistance of the skin and boundary layer

In frog skin, gas flux is proportional to the product of the diffusing capacity of the gas exchanger (D_{O_2}) and the P_{O_2} difference between the free stream and the blood perfusing the skin (ΔP_{O_2}). Because free-stream P_{O_2} was fixed during experimentation and both $\dot{M}_{O_2}$ and Pa_{O_2} were measured, D_{O_2} can be calculated as $\dot{M}_{O_2}/\Delta P_{O_2}$. Mainly because of the large decrease in $\dot{M}_{O_2}$, cutaneous D_{O_2} decreased sharply at low U in immobilized frogs, from 16.4 μmol kg^{-1} h^{-1} kPa^{-1} at 5 cm s^{-1} to 6.6 μmol kg^{-1} h^{-1} kPa^{-1} at 0.1 cm s^{-1} (Fig. 5). The inverse of D_{O_2} is the resistance to oxygen uptake across the skin and boundary layer (R_{total}); R_{total} increased 2.5 times when U was decreased from 5 to 0.1 cm s^{-1}.

In mobile frogs, D_{O_2} at high flow was not significantly different from that of immobilized frogs (22.0 *vs* 16.4 μmol kg^{-1} h^{-1} kPa^{-1} at U=5 cm s^{-1}, $0.05<P<0.10$) and did not decrease with flow (D_{O_2} was 21.8 μmol kg^{-1} h^{-1} kPa^{-1} at 0.1 cm s^{-1}). D_{O_2} in mobile animals was significantly higher than that of immobilized animals when $U \leq 0.5$ cm s^{-1} ($P<0.05$ at U=0.5, $P<0.025$ at U=0.2 and $P<0.0001$ at U=0.1 cm s^{-1}).

R_{bl} was calculated from R_{total} according to the formula:

$$R_{bl} = R_{total} \frac{\Delta P_{O_2 bl}}{\Delta P_{O_2 total}},$$

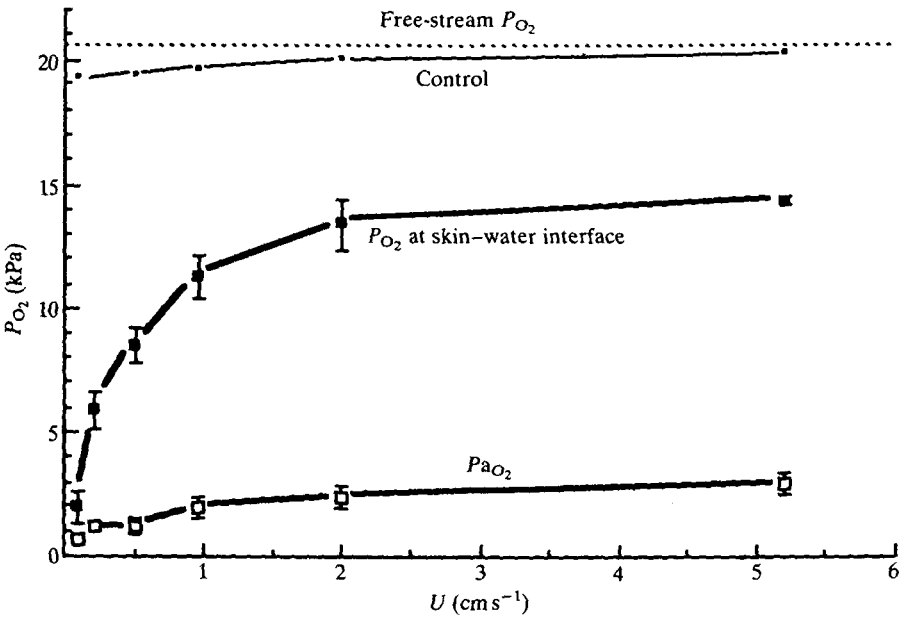

Fig. 3. P_{O_2} measurements at the skin–water interface and in arterial blood as a function of U. There was always a large ΔP_{O_2} between the free stream and blood, as expected in a diffusion-limited system. Even at $U=5\,\mathrm{cm\,s^{-1}}$ the P_{O_2} next to the skin was almost 6.7 kPa below free-stream P_{O_2}. At $U=0.1\,\mathrm{cm\,s^{-1}}$ the P_{O_2} next to the skin was only 2.0 kPa and almost the entire ΔP_{O_2} between the free stream and blood was in the boundary layer. The line labelled Control is the P_{O_2} measured immediately next to a clean rubber stopper; the slight decrease below free-stream P_{O_2} at low U can be ascribed to oxygen uptake by the electrode itself combined with flow restriction between the stopper and electrode tip.

where ΔP_{O_2bl} is the P_{O_2} difference between the free stream and the skin–water interface (Feder and Pinder, 1988) and ΔP_{O_2total} is the P_{O_2} difference between the free stream and blood. Regression of R_{bl} against U after logarithmic transformation yields the relationship $R_{bl}=0.042U^{-0.484}$ ($r^2=0.97$), in which the exponent -0.484 ± 0.04 is not significantly different from the exponent -0.5 expected from the relationship of boundary layer thickness to U. These data are plotted against $U^{-0.5}$ in Fig. 6.

Because of the pronounced microspatial variation in ΔP_{O_2bl} (Fig. 1), we recalculated R_{bl} from R_{total} with different assumptions. At infinite U, boundary layer thickness is zero; thus the y-intercept of R_{total} against $U^{-0.5}$ must equal R_{skin}. R_{skin} decreases by approximately 50 % in severe hypoxia (Pinder, 1987), as exists immediately adjacent to the skin at low U. We therefore assumed a linear decrease in R_{skin} from its value at $U=\infty$ (at which $R_{skin}=R_{total}$) to 50 % of this value at $U=0$. For each U, R_{bl} can be calculated as the difference between R_{total} and R_{skin}. Fig. 6 shows the results of this second calculation, which differ only slightly from the first

calculation. In either case, R_{bl} accounts for the vast majority of R_{total} at low U: 93% according to the calculation using microelectrode data and 85% in the second calculation.

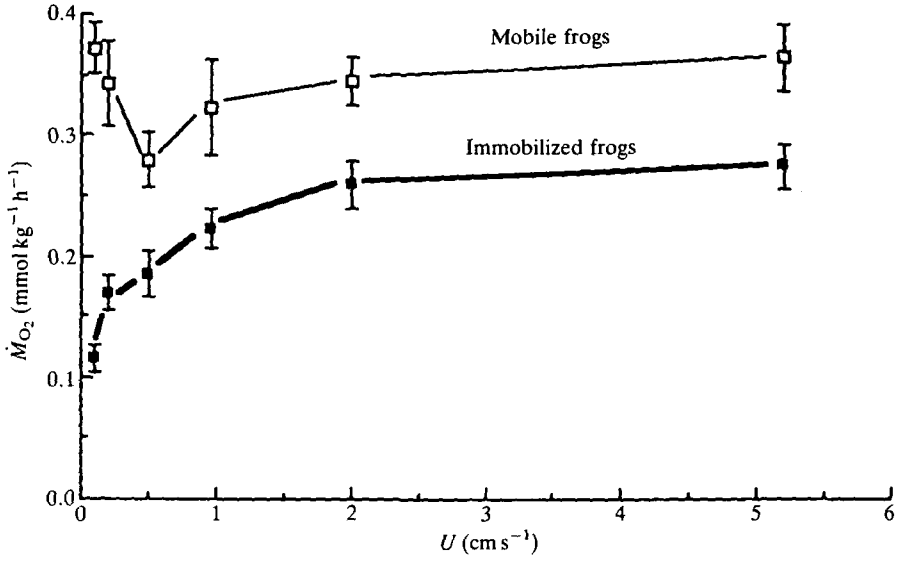

Fig. 4. $\dot{M}_{O_2}$ as a function of U in mobile and immobilized frogs. $\dot{M}_{O_2}$ was significantly reduced below U=1 cm s^{-1} in immobilized frogs, but not in frogs that were able to move.

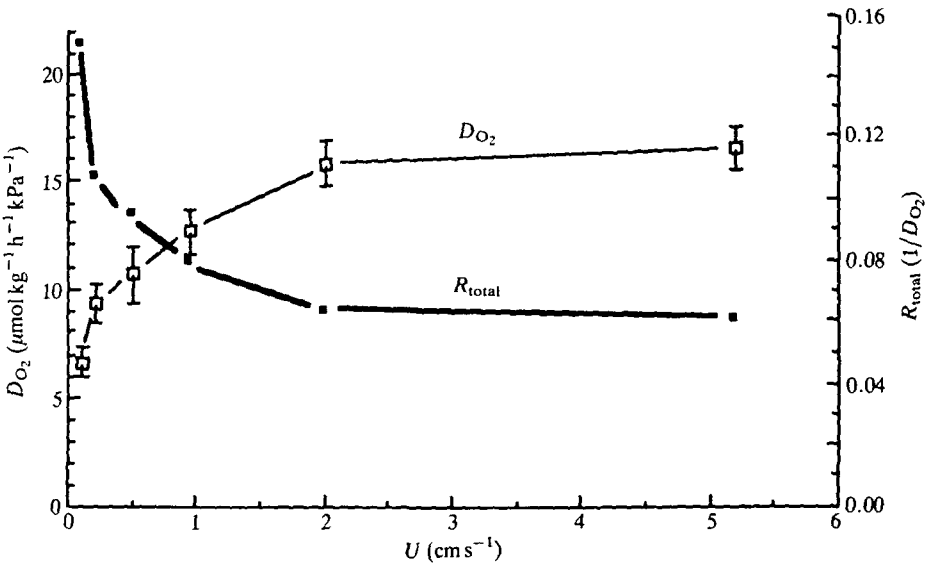

Fig. 5. Calculated cutaneous diffusing capacity ($D_{O_2}=\dot{M}_{O_2}/\Delta P_{O_2}$) and total resistance to O_2 uptake ($R_{total}=1/D_{O_2}$) as functions of U. R_{total} increases 2.5-fold between U=5 and 0.1 cm s^{-1}.

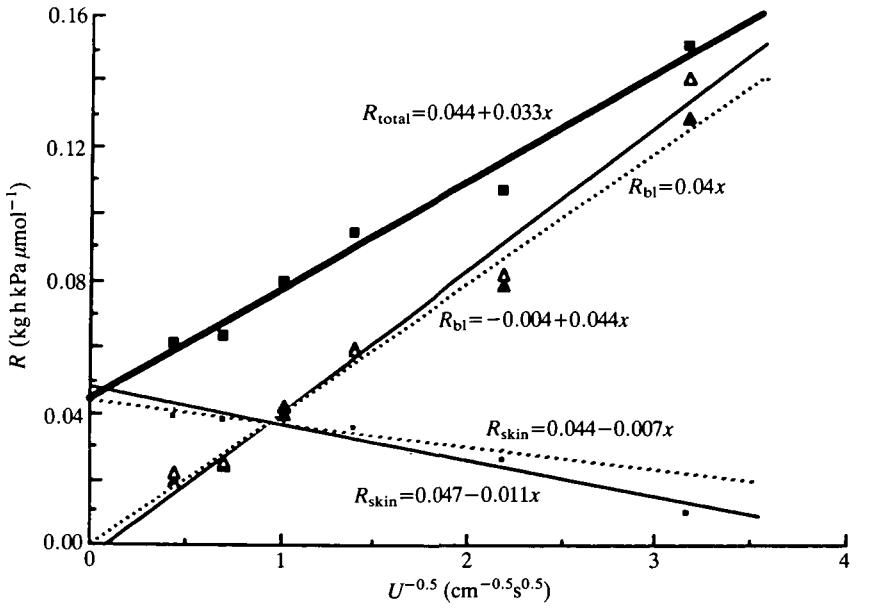

Fig. 6.-Components of resistance to oxygen uptake linearized by plotting against $U^{-0.5}$. R_{total} (solid squares and heavy line) was calculated as in Fig. 5. R_{skin} and R_{bl} were calculated from R_{total} in two ways: (1) from microelectrode measurements of ΔP_{O_2} across the boundary layer and skin ($R_{bl}=R_{total}\times\Delta P_{O_2bl}/\Delta P_{O_2total}$) (light solid lines; open triangles for R_{bl} and dots for R_{skin}) or (2) from assumptions about R_{skin} (dashed lines; solid triangles for R_{bl}, no symbols for R_{skin}). (See text for calculation of line for R_{skin}.) Both ways of calculating R_{bl} and R_{skin} gave similar results.

Discussion

Hydrodynamics of boundary layers

In accordance with hydrodynamic theory (Vogel, 1981), the resistance of the diffusion boundary layer was proportional to $U^{-0.5}$, with an empirically derived proportionality constant of 0.044 (when resistance is in kg h kPa μmol^{-1} and U is in cm s^{-1}; see Fig. 6). The proportionality constant, and thus the resistance of the boundary layer at any given U, is a complex function of the density and viscosity of the respiratory medium, the diffusion constant of the diffusing gas, geometric attributes of the animal (size, shape, texture, orientation to flow and body position) and the nature of the flow of fluid past the animal (laminar *vs* turbulent). As a result, the proportionality constant can be more easily measured than accurately predicted. The physical properties of density, viscosity, solubility and diffusivity can be treated relatively precisely in theory. From differences in these parameters between air and water, for example, boundary layers will seldom affect gas exchange through amphibian skin in air, in particular because the diffusion constant of O_2 in air is approximately 20 000 times that in water (Feder and Burggren, 1985).

The geometric characteristics of animals, which affect the diffusion boundary

layer by affecting patterns of flow around the animal, are much more difficult to treat theoretically. Boundary layer theory is best developed for flat plates, to which frogs bear little resemblance. At the Reynolds numbers for flows used in this study (80–400, calculated for a 12 cm frog submerged in water at 5 °C at U from 0.1 to 5 cm s^{-1}, according to Vogel, 1981, p. 66), the body of the frog would shed a wake of eddies, and eddies should also form behind the limbs and eyes. Vortices and eddies should reduce the thickness of both the hydrodynamic and the diffusion boundary layers.

These disturbances in the nominally laminar flow of water past the frog may explain why the P_{O_2} next to the skin did not decrease systematically with distance from the front of the frog (Fig. 1), as expected in theory from the increase in boundary layer thickness with distance from the leading edge of an object (Feder and Pinder, 1988). The reduction of P_{O_2} next to the skin at the most anterior measurement position (6 mm from the anterior of the animal) was slightly smaller than at more posterior points, but P_{O_2} adjacent to the skin varied greatly from the second measurement point back. The posterior-most measurement point, 102 mm from the anterior, also seemed to show a slightly smaller reduction of P_{O_2} than points 85 and 67 mm from the anterior; 102 mm is posterior to the point at which flow separation and eddy formation should occur (Vogel, 1981). Skin texture would also obscure a relationship between distance from leading edge and reduction of P_{O_2}. Although the irregularities in bullfrog skin are too small to cause turbulence or eddies, they must certainly alter the spacing of streamlines within the boundary layer, thus affecting local velocity and oxygen gradients (Vogel, 1981). In fact, the variability of boundary layer measurements at various positions on a 4 mm long bump on the dorsal skin of a bullfrog was as great as the variability of boundary layers over the frog as a whole (Fig. 1).

Finally, the experimental chamber itself affects the measurement of boundary layers: its walls also have hydrodynamic boundary layers and the flow across the chamber is not perfectly uniform. Wall effects and turbulence reduce the thickness and resistance of the boundary layer (Vogel, 1981). In an unbounded volume with perfectly laminar flow, the boundary layer should pose an even greater resistance to gas exchange than shown here.

Significance of boundary layers to gas exchange

The above caveats notwithstanding, boundary layers had a large and readily demonstrable effect on gas exchange (Fig. 7). The proportion of the total resistance due to the boundary layer (R_{rel}) is 35 % even at 5 cm s^{-1}, a flow velocity that must be much higher than that expected in the still water of a pond or lake, and rises to over 90 % at a velocity of 0.1 cm s^{-1}. At velocities below about 1 cm s^{-1}, the boundary layer limits O_2 uptake even in well-aerated water (Fig. 4). R_{skin} undergoes a compensatory decrease as R_{bl} increases at low U, but R_{bl} so dominates R_{total} below 1 cm s^{-1} that this change in R_{skin} is of little consequence to gas exchange.

Thus, ventilation of the skin is potentially a major means of regulating oxygen

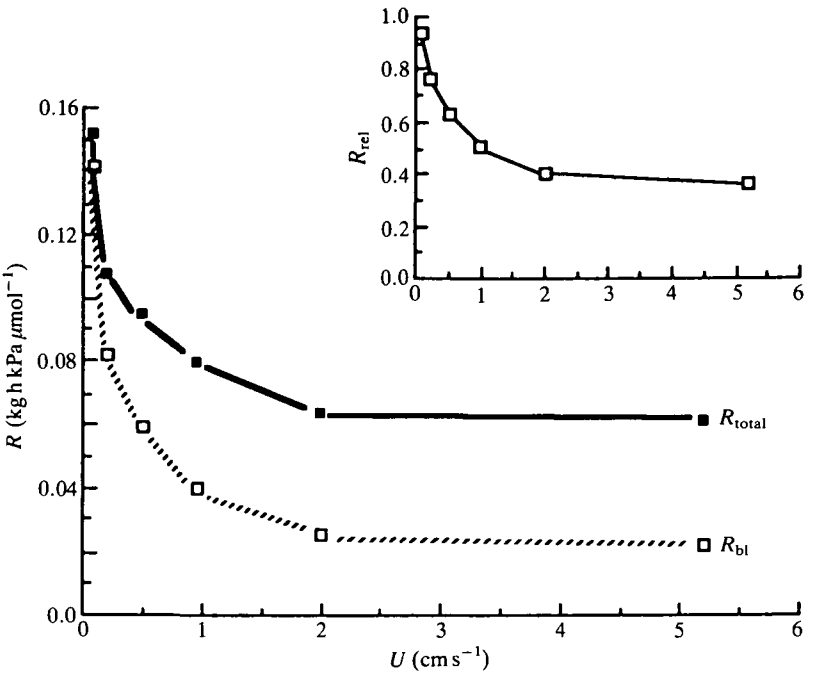

Fig. 7. Components of resistance to oxygen uptake and the proportion of the total resistance within the boundary layer (R_{rel}) as functions of U.

uptake in submerged, overwintering frogs. Two general mechanisms of ventilation are available: convection of the medium around the frog or movement of the frog through the medium. Body movement is effective in dissipating the boundary layer and maintaining $\dot{M}_{O_2}$ at modest metabolic cost. As Fig. 6 shows, $\dot{M}_{O_2}$ can be maintained at U=0.1 cm s^{-1} with only one movement per minute. The respiratory benefit of locomotion is likely to be much greater when animals are not confined to a small chamber; if unconfined, a frog might exceed 5 cm s^{-1} with a single swimming stroke, leaving the bulk of its boundary layer behind (Pinder and Burggren, 1986). Whether hypoxaemia *per se* induced body movement is unclear because frogs moved occasionally even at high U, although they seemed to move more forcefully at low U. Clearly, frequency of movement by itself is an inadequate measure of ventilatory effort, particularly when 'ventilatory' movements are indistinguishable from attempts to escape from the chamber. Nonetheless, the efficacy of such movements at enhancing gas exchange is incontrovertible and, as mentioned earlier, several amphibian species exhibit apparent skin ventilatory movements, especially during hypoxia.

Although body movement is effective in disrupting boundary layers, it increases metabolic rate and may attract predators, so that using convection of the medium itself for skin ventilation may be advantageous. Bullfrogs overwinter in 'still' waters in lakes and ponds, but these waters are likely to have heterogeneous convection patterns within them, especially around inflow and outflow points.

Whether bullfrogs or other amphibians select resting positions in areas of increased convection is unknown.

Boundary layers are ubiquitous; they are potentially important to any aquatic animal that exchanges gases through its outer surface. Whether they are physiologically significant depends on the relative resistance of the boundary layer compared with other resistances to gas exchange. For example, a given boundary layer resistance will be more significant to salamanders than to frogs because salamanders have a much higher cutaneous diffusing capacity (lower R_{skin}) than frogs (Piiper *et al.* 1976; Pinder, 1987). Boundary layers are likely to be more important to species in stagnant water than in fast-flowing streams. Bullfrogs during winter and other bottom-dwelling animals may be long-term inhabitants of a boundary layer, that of the pond bottom itself. Not only are oxygen diffusion boundary layers ubiquitous, but boundary layers exist for anything exchanged between a fluid and a solid: for bullfrogs this includes CO_2, ions and water itself. Movement undoubtedly benefits oxygen uptake, but may also increase water and ion fluxes with as yet unknown costs.

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