

## SHORT COMMUNICATION

### EFFECT OF EXPERIMENTAL VENTILATION OF THE SKIN ON CUTANEOUS GAS EXCHANGE IN THE BULLFROG

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The significance of skin ventilation to cutaneous gas exchange in vertebrates has seldom been recognized. Unlike the lungs and gills, the skin lacks a dedicated ventilatory pump, and most analyses have implicitly or explicitly ignored skin ventilation because the skin is in contact with an 'infinite pool' (Piiper & Scheid, 1977) of respiratory medium. For example, models of gas exchangers consider amphibian skin to have an infinite ventilatory conductance (e.g. Piiper & Scheid, 1977) and in experimental studies the respiratory medium is often stirred thoroughly (e.g. Gottlieb & Jackson, 1976; Burggren & Moalli, 1984), which may eliminate any impact of ventilatory inadequacy upon cutaneous gas exchange. Indeed, as discussed by Piiper (1982) and Feder & Burggren (1985), most previous analyses have not accounted for 'stratification' or 'diffusion boundary layers' about the skin, which theoretically may limit cutaneous gas exchange, especially in still water. Skin ventilation may be important in dissipating such boundary layers, and several skin-breathing vertebrates have particular behaviour patterns that might serve this function (reviewed by Feder & Burggren, 1985). However, there is no direct experimental evidence that the movement of respiratory medium across the skin *can* affect cutaneous gas exchange.

To gather such evidence, we measured the rate of cutaneous oxygen uptake in frogs in unstirred and stirred water, the latter resulting in artificial ventilation of the skin. Although our results suggest that skin ventilation may directly affect cutaneous oxygen uptake from water, other responses may potentially confound this conclusion. For example, behavioural responses to the experimental regime (e.g. fright) or physiological responses that should augment cutaneous gas exchange [e.g. cutaneous capillary recruitment (Burggren & Moalli, 1984), decreases in the  $P_{O_2}$  of the blood in cutaneous capillaries (Malvin & Boutilier, 1986), heart rate, and lung ventilatory rate], rather than ventilatory dissipation of the diffusion boundary layer about the skin, might account for the changes we observed in cutaneous  $\dot{M}_{O_2}$ . Accordingly, we performed additional experiments to examine each of these possibilities.

Key words: amphibian, cutaneous gas exchange, skin, ventilation.

Bullfrogs (*Rana catesbeiana*) were placed individually in respirometers containing a magnetic stirring bar; by activating or inactivating the stirring bar, we could control ventilation of the skin. As determined with a calibrated electromagnetic flow meter (Zepeda Instruments, Seattle, WA), activation of the stirring bar produced water movement at  $0.35\text{--}1.80\text{ cm s}^{-1}$  next to the skin. Each respirometer also included an air space about the frog's nares for pulmonary respiration. In all experiments, frogs were confined within a close-fitting plastic screen envelope beforehand. This envelope allowed contact between each frog and the stirred or unstirred water in the respirometer, permitted lung ventilation, ensured that the frog's skin was maximally exposed to the water, and prevented other movements by the frog that could inadvertently produce uncontrolled ventilation of the skin. Animals in their screen containers were placed in the stirred, aerated respirometer several hours before measurements began. Thereafter, animals were exposed to cycles of skin ventilation in which the water was first stirred and then not stirred.

Oxygen uptake was measured with respirometry techniques similar to those described by Burggren & West (1982) for adult bullfrogs. The respirometer consisted of a translucent plastic funnel mounted inverted on top of a translucent plastic cylinder (11.5 cm diameter  $\times$  10.0 cm height). Water (1100 ml) filled this container nearly to the stem of the funnel; the air volume was 175 ml. A layer of mineral oil approximately 1 cm thick was placed on top of the water to minimize  $\text{O}_2$  diffusion between the water and the air space. Before measurements, both the aquatic and aerial compartments were aerated. To measure the rate of oxygen consumption ( $\dot{M}_{\text{O}_2}$ ), both the aerial and aquatic compartments were sampled and the respirometer was sealed. After 20–40 min, the aerial and aquatic compartments were again sampled. The  $\text{P}_{\text{O}_2}$  of each sample was determined with an Instrumentation Laboratories Micro-13 blood gas analyser. The pulmonary  $\dot{M}_{\text{O}_2}$  and cutaneous  $\dot{M}_{\text{O}_2}$  were calculated from the change in  $\text{P}_{\text{O}_2}$  within the aerial and aquatic compartments respectively, the volume of each compartment, the elapsed time, and the animal's mass.

Heart rate and lung ventilatory frequency were determined by implanting unipolar electrodes in the chest wall of frogs that were otherwise treated as described above. Electrodes were connected to high-gain preamplifiers in a Narco Biosystems Mark IV recording system, and cardio-respiratory frequencies were calculated from the chart records (Burggren, Feder & Pinder, 1983). To determine arterial  $\text{P}_{\text{O}_2}$ , bullfrogs were first anaesthetized by immersion in tricaine methanesulphonate (1;10 000) buffered to pH 7.0. A systemic artery and a pulmocutaneous artery were non-occlusively cannulated with 30-cm lengths of PE50 tubing through an incision in the chest wall, the cannulae were filled with heparinized Ringer's solution, and the incision was closed. Frogs were then placed in respirometers, allowed to recover until the arterial  $\text{P}_{\text{O}_2}$  stabilized ( $>5$  h), and then exposed to cycles of stirred/unstirred water as described above. Blood  $\text{P}_{\text{O}_2}$  was measured by connecting the cannulae directly to the blood gas analyser, drawing blood past the electrode, and then returning the blood to the animal through the cannula (Burggren & Shelton, 1979). Capillary recruitment was determined according to the method of Burggren & Moalli (1984). Essentially,

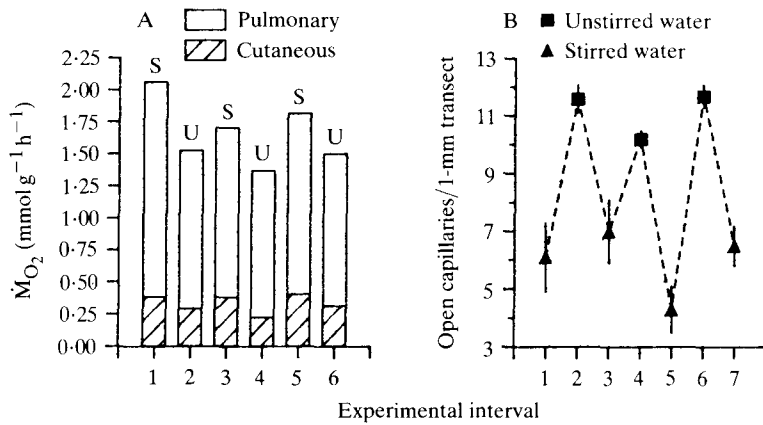


Fig. 1. Effect of experimental ventilation of the skin on oxygen consumption and capillary recruitment in two representative bullfrogs. (A) Effect of skin ventilation on the pulmonary (aerial) and cutaneous (aquatic) oxygen uptake of a 100-g frog. The animal was exposed alternately to stirred (S) and unstirred (U) water about its skin; each bar is designated accordingly. The height of each bar corresponds to the total oxygen consumption. (B) Effect of alternating exposure to stirred and unstirred water on the number of perfused ('open') capillaries along a 1-mm transect in the hind limb web of a representative bullfrog. Each mean  $\pm$  1 standard error (vertical lines) represents the average of five successive 15-s observation periods on a single web 'field' under a single experimental condition (i.e. stirred or unstirred). Intervals between conditions averaged 15 min each.

a frog's hind limb was led out of the water and the hind limb web was spread beneath a dissecting microscope. Repeated observations were made of blood flow through a capillary 'field', defined by those capillaries that crossed a 1-mm transect along the web. For these observations we used a water-filled plastic box instead of a respirometer. Otherwise, animals were exposed to cycles of stirred/unstirred water as described above.

The acclimation and experimental temperature was 25°C. Differences between animals in stirred water and unstirred water were tested for statistical significance with a *t*-test for paired comparisons.

The total  $\dot{M}_{O_2}$  of six frogs in stirred water ranged between 1.19 and 2.51 mmol O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> ( $\bar{x}$  = 1.60, s.d. = 0.50), with cutaneous  $\dot{M}_{O_2}$  accounting for 13.5–39.0% ( $\bar{x}$  = 25.4, s.d. = 8.7) of the total  $\dot{M}_{O_2}$ . Thus, whether due to variation in mass (68–100 g) or random behavioural differences, the experimental subjects differed substantially in total  $\dot{M}_{O_2}$ . Because our interest was in variation *within* subjects due to experimental ventilation rather than variation *among* frogs, we express the following results as percentage change from values for each frog in stirred water.

Cessation of ventilation of the skin was associated with a decline in cutaneous  $\dot{M}_{O_2}$  in all six frogs. Fig. 1A depicts the alternating increase and decrease in cutaneous  $\dot{M}_{O_2}$  in an individual bullfrog as the skin was exposed sequentially to stirred and unstirred water. The decline in unstirred water averaged 31% of the cutaneous  $\dot{M}_{O_2}$  in stirred water, and was highly significant ( $N$  = 6;  $P$  < 0.001). Unexpectedly, pulmonary  $\dot{M}_{O_2}$  decreased when stirring was halted in five frogs, and averaged 80%

of 'stirred' levels. This decline was not significant ( $0.05 < P < 0.1$ ) due to inclusion of the sixth frog, in which pulmonary  $\dot{M}_{O_2}$  increased when the skin was not ventilated. However, the total  $\dot{M}_{O_2}$  declined significantly ( $P < 0.05$ ) to 76% of 'stirred' levels when stirring halted. Stirring did not affect the proportion of total  $\dot{M}_{O_2}$  due to cutaneous oxygen uptake ( $0.3 < P < 0.2$ ).

Nearly every time we stopped stirring the water about the frogs' skin additional capillaries were recruited in the hind limb web, which was beneath the microscope in air (Fig. 1B). We observed 10 different fields of web skin (in five animals) for 20 cycles of stirred/unstirred water. The number of perfused capillaries ranged between 2.0 and 8.6 per mm in the limb webs of frogs in stirred water. This number increased significantly ( $N = 10$ ;  $P < 0.01$ ) when stirring ceased (mean increase = 29%; s.d. = 5%), and increased in 19 out of 20 cycles. Systemic arterial blood and pulmocutaneous arterial blood were sampled regularly in five frogs during a 35- to 60-min exposure to unstirred water and during 30–200 min exposures to stirred water before and after the unstirred period. The systemic arterial  $P_{O_2}$  averaged 77 Torr (s.d. = 10), the pulmocutaneous arterial  $P_{O_2}$  averaged 66 Torr (s.d. = 13), and neither  $P_{O_2}$  changed as a function of stirring. Heart rate and lung ventilatory frequency were recorded in four bullfrogs exposed to 20-min cycles of stirred/unstirred water. Lung ventilation showed no significant change between stirred and unstirred water ( $N = 7$  cycles;  $0.4 < P < 0.5$ ). Heart rate increased significantly ( $N = 10$  cycles;  $P < 0.001$ ) but only very slightly (by  $2.1 \text{ beats min}^{-1}$ , or a 4% increase) in stirred water.

We hypothesized initially that the formation of a diffusion boundary layer can pose a significant barrier to cutaneous gas exchange and that ventilation of the skin can increase cutaneous gas exchange by dissipating this barrier. The 31% difference in cutaneous  $\dot{M}_{O_2}$  observed between frogs in stirred and unstirred water is consistent with this hypothesis. Although this change could also be due to factors other than dissipation of a boundary layer, none of our additional observations favour an alternative explanation. One such alternative explanation is that the physical disturbance of the flowing water or stirring bar stressed the animals and thereby increased their metabolic rate when the water was stirred. However, the total  $\dot{M}_{O_2}$  that we observed is consistent with many previous determinations for bullfrogs (reviewed by Burggren & Moalli, 1984), and possible indicators of stress such as heart rate, ventilatory rate and blood gas levels differed little or not at all between stirred and unstirred conditions. Another alternative explanation is that physiological changes in blood gases, capillary recruitment or blood flow to the skin account for the decrease in  $\dot{M}_{O_2}$  in unstirred water. Arterial  $P_{O_2}$  values did not change in unstirred water, which is inconsistent with this alternative explanation. Likewise, additional cutaneous capillaries were recruited in the hind limb webs of frogs when stirring was ceased, which should have increased (rather than decreased) cutaneous  $\dot{M}_{O_2}$ . The limitations of our measurements to date are that we have examined only a small portion of the entire capillary bed, we have not measured blood flow to the skin, nor have we determined the relative proportions of systemic

and pulmocutaneous arterial supply to the skin. Nonetheless, all of our results indicate ventilatory dissipation of a cutaneous diffusion boundary layer.

An unexpected finding was that changes in the pulmonary  $\dot{M}_{O_2}$  paralleled changes in the cutaneous  $\dot{M}_{O_2}$ . Also, capillary recruitment in the hind limb web in air varied when the ventilatory regime about the remainder of the frogs' skin in water was changed. These patterns suggest (but do not confirm) that responses to skin ventilation are mediated centrally and affect all of the respiratory capillary beds simultaneously.

Most previous studies of cutaneous gas exchange have stressed that it is diffusion-limited (Piper & Scheid, 1977; Feder & Burggren, 1985) and that the skin is a 'passive' and 'poorly regulated' respiratory organ (as reviewed by Burggren & Moalli, 1984). Our results suggest that ventilation of the skin can contribute to the regulation of cutaneous gas exchange by dissipation of a diffusion boundary layer. Whether skin-breathing vertebrates intentionally ventilate their skins, the efficacy of skin ventilation as a regulatory mechanism, and the value of skin ventilation in nature remain open questions at this time, although anecdotal evidence suggests that voluntary skin ventilation occurs and is important (Feder & Burggren, 1985).

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#### REFERENCES

- BURGGREN, W. W., FEDER, M. E. & PINDER, A. (1983). Temperature and the balance between aerial and aquatic respiration in larvae of *Rana berlandieri* and *Rana catesbeiana*. *Physiol. Zool.* **56**, 263–273.
- BURGGREN, W. W. & MOALLI, R. (1984). "Active" regulation of cutaneous gas exchange by capillary recruitment in amphibians: experimental evidence and a revised model for skin respiration. *Respir. Physiol.* **55**, 379–392.
- BURGGREN, W. W. & SHELTON, G. (1979). Gas exchange and transport during intermittent breathing in chelonian reptiles. *J. exp. Biol.* **82**, 75–92.
- BURGGREN, W. W. & WEST, N. H. (1982). Changing respiratory importance of gills, lungs and skin during metamorphosis in the bullfrog, *Rana catesbeiana*. *Respir. Physiol.* **47**, 151–164.
- FEDER, M. E. & BURGGREN, W. W. (1985). Cutaneous gas exchange in vertebrates: design, patterns, control, and implications. *Biol. Rev.* **60**, 1–45.
- GOTTLIEB, G. & JACKSON, D. C. (1976). Importance of pulmonary ventilation in respiratory control in the bullfrog. *Am. J. Physiol.* **230**, 608–613.
- MALVIN, G. M. & BOUTILIER, R. G. (1986). Ventilation–perfusion relationships in Amphibia. In *Comparative Physiology and Biochemistry—Current Topics and Trends*, vol. A. Berlin: Springer-Verlag. (in press).
- PIPER, J. (1982). A model for evaluating diffusion limitation in gas-exchange organs of vertebrates. In *A Companion to Animal Physiology* (ed. C. R. Taylor, K. Johansen & L. Bolis), pp. 49–64. Cambridge: Cambridge University Press.
- PIPER, J. & SCHEID, P. (1977). Comparative physiology of respiration: functional analysis of gas exchange organs in vertebrates. In *International Review of Physiology, Respiratory Physiology II*, vol. 14 (ed. J. G. Widdicombe), pp. 219–253. Baltimore: University Park Press.