

Skin Breathing in Amphibians

Glenn J. Tattersall

Department of Biological Sciences Brock University

Skin breathing, or cutaneous, gas exchange is an important route of respiration in many aquatic or semiaquatic vertebrates, and is particularly well developed in the amphibians. The skin of amphibians contains a unique vasculature that facilitates oxygen (O₂) uptake and carbon dioxide (CO₂) excretion. Cutaneous gas exchange can fulfill routinely 0% to 100% of O₂ uptake and 20% to 100% of CO₂ excretion (1). Amphibians pay a price for this: They require a relatively thin epidermis and, as a result, suffer from high rates of water loss. Thus, amphibians are, for the most part, tied to an aquatic or semiaquatic life. Furthermore, unlike lungs or gills, the skin lacks a dedicated ventilatory pump and, as such, has been thought to be a poorly regulated respiratory organ, with little scope for change. Research over the past couple of decades has revealed that amphibians may exhibit partial control over the cutaneous vasculature, and that such control is under both neural and possibly hormonal control. For the purposes of this chapter, most information will be taken from literature on ranid frogs (Order Anura), because the majority of research has been done on this group.

AMPHIBIAN CUTANEOUS CIRCULATION

Most amphibians possess a double circulation – the pulmonary and systemic – consisting of a right and left atrium and an undivided ventricle. A similar blood flow distribution pattern exists in reptiles and certain air-breathing fishes (2) (see Chapter 5). Primarily deoxygenated (deoxygenated systemic blood plus oxygenated blood from the skin are mixed) blood returns to the heart via the right atrium, whereas oxygenated blood from the lung only returns to the heart via the left atrium. Both atria connect to the single, undivided ventricle, although considerable flow separation of blood is achieved through the extensive system of trabeculae within the ventricle (3,4). Upon ventricular contraction, deoxygenated blood primarily travels through the spiral valve to the pulmocutaneous artery, which subsequently divides into the pulmonary and cutaneous artery. Oxygenated blood, on the other hand, travels via the aorta to supply blood to the tissues and other parts of

the skin. Thus, the skin generally receives blood from both the systemic and pulmocutaneous circuits (Figure 9.1). Specifically, at least in *Rana catesbeiana* (the American bullfrog), the dorsal skin receives blood primarily from the cutaneous artery, whereas skin surrounding the legs and, to some extent, the ventral surface receives mainly systemic blood (5). The skin circulation is drained by a pair of large cutaneous veins that feed into the subclavian veins and back to the heart. Thus, the skin receives both oxygenated and deoxygenated blood, but returns oxygenated blood to the heart, which is subsequently mixed with deoxygenated blood from the systemic circulation before returning to the right atrium.

A further aspect of import of the amphibian circulation concerns the innervation of the primary circulations to the lung and skin. The cutaneous artery is innervated by adrenergic fibers of sympathetic origin, which cause constriction. The pulmonary arteries themselves are innervated by cholinergic fibers of parasympathetic origin (i.e., the vagus) which cause constriction of the pulmonary artery and dilation of the cutaneous artery (6,7). This reciprocal autonomic innervation of the pulmonary and cutaneous arteries leads to situations in which blood can be directed to the respiratory organ that has the greatest potential for gas exchange. In most cases, this will be the lung, although in cases when the lung is hypoxic, hypercapnic, or poorly ventilated (e.g., during diving), blood flow to the lung will be decreased and blood flow to the skin will at least remain constant, if not increase (8,9).

CUTANEOUS VASCULATURE

In many amphibians, 20% to 95% of the entire body’s respiratory capillaries are in the skin (Figure 9.2) (10). Indeed, in the smaller frogs, skin capillaries can be more abundant than pulmonary capillaries (10). In Czopek’s examination of amphibian skin, he observed that the epidermis is only four to seven cell layers thick. Overall, the dorsal epidermis is 10% to 20% thinner (18–24 μm) than the ventral epidermis (32 μm). Unlike in most vertebrates, the skin capillaries are located inside the epidermis rather than the dermis, making

Figure 9.1

Figure 9.2

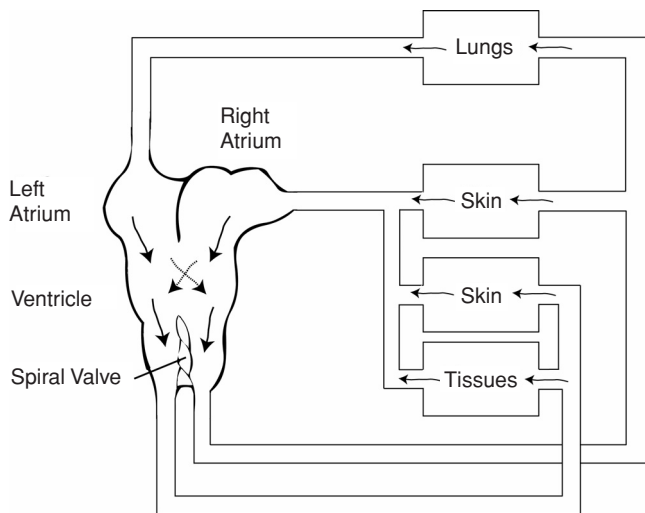


Figure 9.1. Schematic of the generalized circulation in amphibians. Arrows indicate the direction of blood flow. Dotted arrows indicate that a portion of blood can be shunted from one side of the heart to the other within the undivided ventricle.

the barrier to gas exchange much shorter (see Figure 9.2). The values quoted above, however, were from a rather unique amphibian. The epidermis of most frogs is two to three times thicker, although the respiratory capillaries still lie within the

epidermis. This barrier for gas exchange ($18\text{--}60\ \mu\text{m}$) exceeds that of lung epithelium by one to two orders of magnitude, making the diffusive transfer of gases a relatively slow process.

The respiratory capillaries form a vast network of anastomosing vessels (Figures 9.3 and 9.4) lying within the epidermis and forming a virtual sheet for gas exchange (11). These respiratory capillaries are connected to the skin arteries via branching arterioles, and they drain into subcutaneous veins via venules that penetrate the dermis. Very few true (i.e. systemic) capillaries connect the arterioles directly to venules or the venular network. Flow within the respiratory capillaries themselves exhibits a high degree of heterogeneity (12). Furthermore, the number of capillaries perfused can significantly affect gas exchange (13), although the nature of the regulation of these flows is unknown (i.e., whether it occurs at the arteriolar level or within the capillary network itself). Some clue to this may come from studies examining microvascular permeability (14). Although the respiratory capillaries themselves lack smooth muscles, microfilaments within endothelial cells (ECs) have been proposed to be part of a contractile machinery within the frog skin capillaries. These ECs within the capillaries alter their shape and appearance under electrical stimulation (14). Whether they operate in this manner in vivo, respond to neural or hormonal stimulation, or respond to changes in respiratory gases is unknown.

Figure 9.3
Figure 9.4

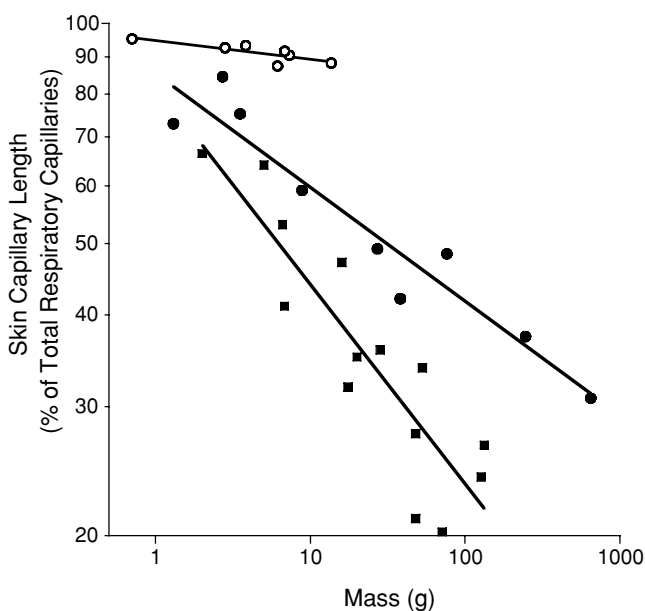


Figure 9.2. The percentage of respiratory capillaries that are cutaneous in origin differs among groups of amphibians. Lungless salamanders (open circles) have greater than 90% of respiratory capillaries in the skin, with the remainder found in the buccal cavity. Lunged salamanders (filled circles) and frogs (squares), on the other hand, have substantial respiratory capillaries in the lungs, buccal cavity, and skin. Note the trend with increasing body size for the skin to play a decreasing role in providing a vascular network for gas exchange. (Values adopted from Szarski H. The structure of respiratory organs in relation to body size in amphibia. *Evolution*. 1964;18:118–126.)

SKIN AS A GAS EXCHANGER: ROLE OF DIFFUSION

As in any respiratory organ, the diffusion of gases occurs across a layer of tissue and into the nearby bloodstream. Across large distances, the molecular movement of respiratory gases, which is governed by Fick's law of diffusion is not a very efficient process¹; therefore the larger the barrier between environmental concentrations of respiratory gases and the internal concentrations, the longer it takes for diffusion to occur. Some, amphibians have anatomical modifications that capitalize on Fick's law of diffusion by increasing the total surface area available for gas exchange. The salamander, *Cryptobranchus* (15), and the Lake Titicaca Frog, *Telmatobius* (16), have marked folds in their skin that allow for greater diffusive exchange area, and these species are thought to make very little use of their lungs for gas exchange (15). In addition, the male hairy frog, *Astylosternus*, develops epidermal "hairs" during its breeding season that may supplement the total gas exchange surface (17).

Gas exchange across the skin of amphibians is believed to occur between the skin and an "infinite pool" of gases from the direct, surrounding environment (18). However, theoretical considerations and empirical evidence show that an external

1 Fick's law of diffusion: $= \frac{dQ_g}{dt} = D_g A \frac{dP_g}{dx}$ where $\frac{dQ_g}{dt}$ is the rate of diffusion of gas (g), D_g is the Krogh diffusion coefficient, A is the surface area, dP_g is the partial pressure gradient, and dx is the diffusion distance.

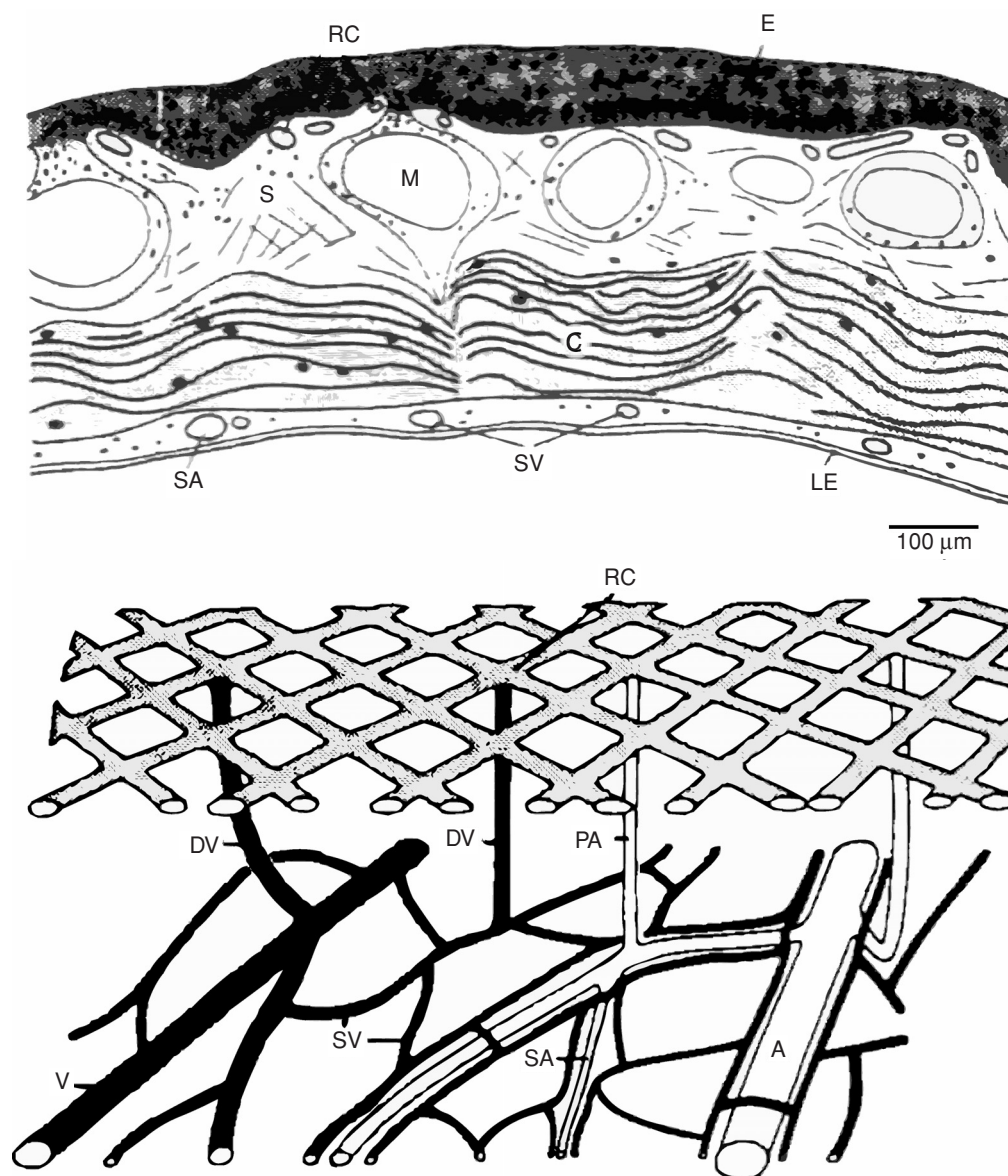


Figure 9.3. Organization of skin microcirculation in amphibians. *Top*: Cross-sectional schematic of frog skin, showing the relative placement of blood vessels within and beneath the epidermis. *Bottom*: Schematic of the network of blood vessels within the skin. Mucous gland (M), Stratum Compactum (C), Stratum Spongiosum (S), Skin arteries (A), branching arterioles (SA), subepidermal or respiratory capillary network (RC), arteriolar branches (PA), venules (DV), subcutaneous veins (V), subcutaneous venular plexus (SV), epidermis (E), lymph endothelium (LE). (Reproduced with permission from Olesen SP, De Saint-Aubain ML, Bundgaard M. Permeabilities of single arterioles and venules in the frog-skin – a functional and morphological study. *Microvasc Res.* 1984;28:1–22.)

boundary layer of poorly mixed gases contributes to limiting cutaneous gas exchange (19,20). These diffusion-boundary layers result from decreased fluid velocity close to solid surfaces. The decreased fluid velocity prevents bulk flow from occurring close to the solid surface, resulting in a stagnant layer of fluid, through which diffusion is the only means of gas movement. The resulting diffusion-boundary layer poses a limitation to gas exchange (especially O_2) primarily because the partial pressure of O_2 at the skin–fluid medium interface

is low, and thus presents a relatively small partial pressure gradient (i.e., driving pressure) across the skin (20). Because it is the partial pressure gradient that ultimately determines the rate of diffusion of a gas across any surface, low values at the skin–water interface result in decreased rates of uptake and subsequent limitations to cutaneous gas exchange.

Amphibians have had to develop methods for coping with these diffusion boundary layers (namely, the hypoxic boundary layers). Increased water velocity acts to minimize or disrupt

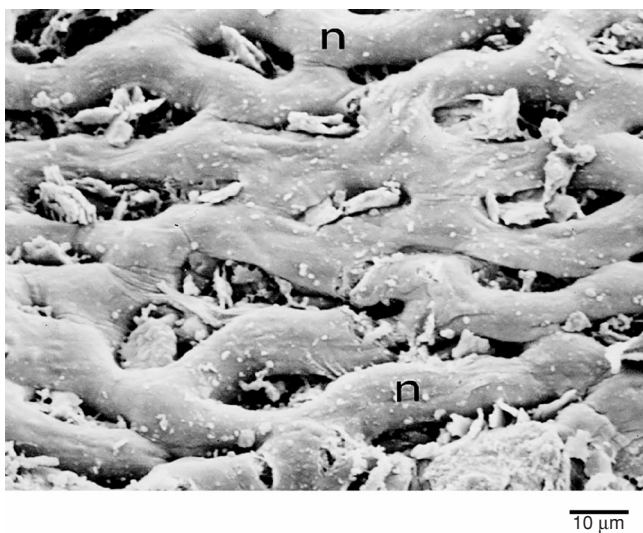


Figure 9.4. Scanning electron micrograph of the respiratory capillary network of the tree frog, *Chiromantis petersi*. Note the relatively flat anastomosing capillaries that form the respiratory exchange network. The black bar denotes 10 μm . (Reproduced with permission from Maina JN. Is the sheet-flow design a ‘frozen core’ (a Bauplan) of the gas exchangers? Comparative functional morphology of the respiratory microvascular systems: illustration of the geometry and rationalization of the fractal properties. *Comp Biochem Physiol A Mol Integr Physiol*. 2000;126:491–515.)

the boundary layer surrounding the amphibian skin; thus, increased activity or movement could be one method of alleviating boundary layers (21,22). The primarily skin-breathing Lake Titicaca frog, the exclusively aquatic salamander *Cryptobranchius*, and cold-submerged (and thus skin-breathing) bullfrogs all show behaviors described as “bobbing” or “rocking” when confronted with ambient hypoxia (15,16,23), suggesting an integrated detection of O_2 . These behaviors act to disrupt the hypoxic boundary layers at the skin (18) and promote increased O_2 diffusion gradients and therefore O_2 uptake across the respiratory surface (24). Increased locomotor activity has been observed in response to hypoxia in frogs (23,25), and has been interpreted as a means of disrupting and minimizing hypoxic boundary layers by increasing skin ventilation (9,23). This detection of low O_2 has been suggested by behavioral studies of overwintering amphibians that demonstrate a moderate ability to detect and avoid hypoxic environments (26). This is further supported by data showing that these same submerged, skin-breathing frogs exhibit a decline in preferred body temperature while in hypoxic environments, suggesting a sensory mechanism that modifies behavior (27).

Experimentally, Pinder and Feder (23) demonstrated that increased fluid velocity decreased the actual size of the hypoxic boundary layer around the skin. At high velocities ($5.2 \text{ cm} \cdot \text{s}^{-1}$), hypoxic boundary layers were approximately $200 \mu\text{m}$, whereas at low velocities ($0.2 \text{ cm} \cdot \text{s}^{-1}$) they were nearly four times as large, and increased to several millimeters when no water movement was present (23). Increasing the flow of water over the skin also results in a reduced hind limb capillary recruit-

ment, suggesting that local external hypoxia at the skin surface can serve to regulate the open probability of many capillaries. How this autoregulation of blood occurs in amphibian skin is unknown. Furthermore, when boundary layers are disturbed, the overall cutaneous O_2 uptake rises in frogs (21), suggesting either a limitation of surface PO_2 to gas exchange, a reflex increase in localized capillary blood flow or recruitment, or both.

SKIN AS A GAS EXCHANGER: REGULATION OF CUTANEOUS RESPIRATION

Because the skin lacks a dedicated ventilatory pump characteristic of gills (buccal and opercular muscles) and lungs (intercostal and diaphragmatic muscles), it often has been considered a poorly regulated respiratory organ (21), incapable of matching external medium flow with internal blood perfusion. Ample evidence suggests, however, that cutaneous blood flow can be altered in physiologically meaningful ways (reviewed by (28) and is most strongly affected when amphibians are removed from water; in other words, cutaneous blood flow decreases when the skin is exposed to air, presumably to minimize water loss (13,29). It is not normally the overall rate of blood flow that changes in individual capillaries, but rather the total number of capillaries through which blood flows appears to be altered (13).

The simplest and most compelling evidence that amphibians can, to a large extent, regulate cutaneous gas exchange comes from evidence of obligately skin-breathing amphibians. Lungless salamanders are capable of maintaining a constant rate of O_2 uptake in hypoxic environments as low as 8% O_2 ($\sim 60 \text{ mm Hg}$) (30). Furthermore, numerous species of frogs that overwinter under the ice are functionally and exclusively skin breathers for up to 6 months of the year. These species show a capacity to maintain constant O_2 uptake down to 5% O_2 ($\sim 40 \text{ mm Hg}$) (24,26). Some obligately skin-breathing amphibians are capable of elevating O_2 uptake three to five times if made to exercise (31), suggesting that the skin is not 100% limited by its diffusing capacity, and also that there exists a degree of scope and regulation available that is presumably derived from vasculature adjustments and changes in the partial pressure gradients.

Recent work on frog skin suggests that some similarities may exist between the cutaneous artery circulation and the circulation to the lung. In mammals, a well-established relationship exists between alveolar O_2 levels and blood flow, whereby poorly ventilated alveoli receive little blood (referred to as hypoxic pulmonary vasoconstriction). Based on experiments in which local hypoxia is created over certain regions of the skin, changes in cutaneous perfusion have been observed to occur (28). Within 1 to 1.5 minutes of regional and localized hypoxia, red cell flux velocity decreases by 50%, and the temporal heterogeneity of blood flow within capillaries increases by 30% (32). This response to hypoxia is not likely centrally driven, because the areas of skin exposed were too small to elicit changes in arterial PO_2 , but is rather an autoregulatory

response brought about by the cutaneous vasculature. This suggests that the O₂ sensor is located within the skin, although where and how it regulates capillary flow is unknown.

Simple and definitive evidence exists, however, for some neural involvement in skin blood flow, at least in the regulation of global changes in skin blood flow. Descending influences from the brain are required for the vasodilatory response of the frog hind web, as evidenced by the fact that hind web capillaries of pithed frogs do not vasodilate under normal stimuli (33). Furthermore, α -adrenergic blockade in bullfrogs increases the number of perfused capillaries (29) or, at the very least, prevents the normal “closure” of capillaries upon air exposure. Moreover, nonthermal vasodilation in frog hind web capillaries is blocked by guanylate cyclase inhibitors, suggesting that nitric oxide (NO) and Ca²⁺ signaling also are implicated in the responses of smooth muscles (presumably only in the arterioles or precapillary junctions) (34). However, no definitive studies have attempted to integrate and compare the various roles of circulating versus neural influences.

When examining cutaneous respiration, it also is useful to consider the association between perfusion limitation and diffusion limitation. At low blood perfusion rates, O₂ uptake is limited by the blood flow through the capillaries and should be theoretically directly proportional to the rate of blood, or hemoglobin, flow. At higher perfusion rates, the epidermal barrier limits the exchange of O₂, due to a relatively stable diffusive conductance. In general, O₂ is mainly diffusion-limited across amphibian skin (35). Increasing blood flow does little to alter cutaneous respiration; plus, most of the parameters determining diffusive conductance are usually difficult to adjust in response to acute challenges (35). Such parameters include cutaneous surface area, capillary radius, distance between capillaries, thickness of the skin, and the diffusion constant of O₂. Therefore, it seems reasonable to suggest that, for significant blood flow regulation of O₂ uptake to be possible, the overall O₂ requirement of the animal must be relatively low, allowing for regulation to occur within the range of blood flows that accommodate the metabolic demand.

CUTANEOUS BREATHING: SPECIAL CIRCUMSTANCES IN NATURE

Many temperate amphibians become obligate skin-breathers for up to 6 months of the year when they overwinter under the ice of frozen lakes. Not only are they capable of movement and behavioral thermoregulation (27), but they also enter into a state of reversible metabolic suppression (36,37). Because submerged frogs are capable of regulating constant levels of O₂ uptake down to extremely hypoxic levels, it is possible that they are utilizing alterations in skin perfusion or arterial gases to achieve these results. When exposed to ambient hypoxia or the hypoxemia associated with diving, O₂ delivery to certain tissues is drastically altered. Although perfusion of muscle or other tissues can decrease, the skin must continue to be perfused at an elevated rate to maintain O₂ uptake (38,39),

because pulmonary O₂ uptake is no longer possible. Studies on lungless salamanders suggest that these skin-breathing amphibians are primarily diffusion-limited for gas exchange (13,40,41). As a result, increasing cutaneous blood flow should be an ineffective means of increasing O₂ uptake when required. However, most of these experiments were conducted at higher temperatures, at which skin perfusion is likely already at its maximum; therefore, extrapolating to much lower temperatures may lead to erroneous conclusions about the effectiveness of cutaneous gas exchange in overwintering frogs (24,29). For example, cold-submerged bullfrogs can regulate cutaneous O₂ uptake either through rocking or small movements (i.e., spontaneously ventilating the skin) that disrupt hypoxic boundary layers or by the recruitment of otherwise underperfused capillaries in the skin (24).

Early work also suggested that cutaneous CO₂ conductance does not change with temperature in amphibians (42, 43). These results, however, came from frogs at higher temperatures in air. It appears that the constant cutaneous CO₂ conductance prediction does not fit the scenario at these low temperatures in submerged frogs. Tattersall and Boutilier (44) showed that arterial Pco₂ remains constant in submerged frogs between 0°C and 7°C, despite an incredible increase in whole animal metabolism (and thus CO₂ excretion), suggesting that CO₂ conductance increases at higher temperatures in submerged frogs, at least up to a critical point. This is quite opposite to the conclusion reached by Mackenzie and Jackson (42) when observing air-breathing frogs at higher temperatures. This is likely to be achieved by the recruitment of blood flow through otherwise underperfused capillaries, thus presenting a greater functional surface area for the exchange of CO₂ to the environment (13). The probable reasons why cutaneous CO₂ conductance changes with temperature in cold-submerged frogs are (a) because their total metabolic requirements are so much lower, and (b) these metabolic demands must be met solely through the skin. Air-breathing frogs at higher temperatures would have sufficient control of O₂ uptake and CO₂ excretion through the combined contributions of the lungs and the skin, but the skin itself will be governed by other external influences, namely that of water conservation. Because any increase in cutaneous conductance for respiratory gases is bound to accelerate the evaporation of water from the skin, air-breathing amphibians likely keep cutaneous conductance at a minimum across a wide temperature range through reductions in cutaneous blood flow, thus minimizing their water loss.

KEY POINTS

- The amphibian skin is a relatively simple, yet not entirely understood respiratory organ, particularly with reference to the regulation of its circulation and the role of the endothelium.

- The amphibian skin serves numerous functions, including respiration, osmoregulation, thermoregulation, protection, reproduction, and communication; as such, it is little wonder that compromises in the efficiency of any one particular system are made to accommodate the function of another. Thus, the amphibian’s skin is adaptive in the sense of its flexibility in serving multiple needs (e.g., thermal, hydric, and respiratory), even though it is not optimal for any one particular function.
- The regulation of cutaneous respiration is primarily determined by the diffusive properties of the skin and, ultimately, the boundary layer around it. Capillary recruitment, coupled with voluntary ventilation of the skin, which disrupts the boundary layer, combine to provide amphibians with a unique way of regulating respiration.

Future Goals

- To determine what aspects of the cutaneous vascular bed are responsible for regulating gas exchange under conditions related to potential ventilation:perfusion heterogeneities and in conditions relevant to what amphibians experience in nature (i.e., diving, overwintering).
- To determine how the skin appears to detect both external Po₂ and internal Po₂ and to respond with appropriate physiological adjustments. In light of recent research into putative cellular O₂ sensors, the amphibian skin may become a tractable and interesting model for future exploration.

REFERENCES

1

Feder ME, Burggren WW. Skin breathing in vertebrates. *Sci Am.* 1985;253:126–142.

2

Withers PC. *Comparative Animal Physiology*. Fort Worth: Saunders College; 1992: xxii, 949, [111].

3

Tazawa H, Mochizuki M, Piiper J. Respiratory Gas Transport by the incompletely separated double circulation in the bullfrog, *Rana-catesbeiana*. *Respir Physiol.* 1979;36:77–95.

4

Shelton G, Boutilier RG. Apnoea in amphibians and reptiles. *J Exp Biol.* 1982;100:245–273.

5

Olesen SP, De Saint-Aubain ML, Bundgaard M. Permeabilities of single arterioles and venules in the frog-skin – a functional and morphological-study. *Microvasc Res.* 1984;28:1–22.

6

Smith DG. The innervation of the cutaneous artery of the toad, *Bufo marinus*. *Gen Pharmacol.* 1976;7:405–409.

7

de Saint-Aubain ML. The morphology of amphibian skin vascularization before and after metamorphosis. *Zoomorphology.* 1982;100:55–63.

8

West NH, Burggren WW. Control of pulmonary and cutaneous blood flow in the toad, *Bufo marinus*. *Am J Physiol.* 1984; 247:R884–R894.

9

Pinder AW, Storey KB, Ultsch GR. Estivation and Hibernation. In: Feder ME, Burggren WW, eds. *Environmental Physiology of the Amphibians*. Chicago: The University of Chicago Press; 1992: 250–274.

10

Czopek J. Distribution of capillaries in the respiratory surfaces in two species of *Batrachophrynus* (Amphibian, Anura, Leptodactylidae). *Zoologica Poloniae.* 1983;30:211–226.

11

Maina JN. Is the sheet-flow design a ‘frozen core’ (a Bauplan) of the gas exchangers? Comparative functional morphology of the respiratory microvascular systems: illustration of the geometry and rationalization of the fractal properties. *Comp Biochem Physiol A Mol Integr Physiol.* 2000;126:491–515.

12

Malvin G. Microcirculatory effects of hypoxic and hypercapnic vasoconstriction in frog skin. *Am J Physiol.* 1993;264:R435–R439.

13

Feder ME, Burggren WW. Cutaneous gas exchange in vertebrates: design, patterns, control and implications. *Biol Rev.* 1985;60:1–45.

14

Tymk K, Weigelt H, Schafer D. Are endothelial-cells rich in filaments involved in the phenomenon of electrically induced stoppages of flow in frog capillaries. *Int J Microcirc Clin Exp.* 1985; 4:121–130.

15

Boutilier RG, Toews DP. Respiratory, circulatory and acid-base adjustments to hypercapnia in a strictly aquatic and predominantly skin-breathing urodele, *Cryptobranchus alleganiensis*. *Respir Physiol.* 1981;46:177–192.

16

Hutchison VH, Haines HB, Engbretson G. Aquatic life at high altitude: respiratory adaptations in the Lake Titicaca frog, *Telmatobius culeus*. *Respir Physiol.* 1976;27:115–129.

17

Noble GK. The integumentary, pulmonary, and cardiac modifications correlated with increased cutaneous respiration in the amphibia: a solution of the ‘hairy’ frog problem. *J Morph Physiol.* 1925;40:341–416.

18

Feder ME, Pinder AW. Ventilation and its effect on “infinite pool” exchangers. *Am Zoologist.* 1988;28:973–983.

19

Vogel S. *Life in Moving Fluids: The Physical Biology of Flow*. Princeton NJ: Princeton University Press; 1994:xiii,467.

20

Booth DT, Feder ME. Formation of hypoxic boundary layers and their biological implications in a skin-breathing aquatic salamander, *Desmognathus quadramaculatus*. *Physiol Zool.* 1991; 64:1307–1321.

21

Burggren WW, Feder ME. Effect of experimental ventilation of the skin on cutaneous gas exchange in the bullfrog. *J Exp Biol.* 1986;121:445–449.

22

Pinder AW, Burggren WW. Ventilation and partitioning of oxygen uptake in the frog *Rana pipiens*: effects of hypoxia and activity. *J Exp Biol.* 1986;126:453–468.

23

Pinder AW, Feder ME. Effect of boundary layers on cutaneous gas exchange. *J Exp Biol.* 1990;143:67–80.

24

Pinder AW. Cutaneous diffusing capacity increases during hypoxia in cold submerged bullfrogs (*Rana catesbeiana*). *Respir Physiol.* 1987;70:85–95.

25

Bradford DF. Winterkill, oxygen relations and energy metabolism of a submerged dormant amphibian, *Rana muscosa*. *Ecology.* 1983;64:1171–1183.

26

Tattersall GJ, Boutilier RG. Behavioural oxy-regulation by cold-submerged frogs in heterogeneous oxygen environments. *Can J Zool.* 1999;77:843–850.

27

Tattersall GJ, Boutilier RG. Balancing hypoxia and hypothermia in cold-submerged frogs. *J Exp Biol.* 1997;200:1031–1038.

28

Malvin GM. Cardiovascular regulation of cutaneous gas exchange. In: Heatwole H, ed. *Amphibian Biology*. Chipping Norton, NSW: Surrey Beatty & Sons; 1994:147–167.

29

Burggren WW, Moalli R. 'Active' regulation of cutaneous gas exchange by capillary recruitment in amphibians: experimental evidence and a revised model for skin respiration. *Respir Physiol*. 1984;55:379–392.

30

Sheafor EA, Wood SC, Tattersall GJ. The effect of graded hypoxia on the metabolic rate and buccal activity of a lungless salamander (*Desmognathus fuscus*). *J Exp Biol*. 2000;203:3785–3793.

31

Tattersall GJ, Boutilier RG. Does behavioural hypothermia promote post-exercise recovery in cold- submerged frogs? *J Exp Biol*. 1999;202:609–622.

32

Malvin GM, Macias S, Sanchez M, Parkrapid A. Hypoxia-induced hemoconcentration in the toad *Bufo woodhousei*: role of spleen and lymph heart. *FASEB J*. 1992;6:1529A.

33

Poczopko P. Further investigations on the cutaneous vasomotor reflexes in the edible frog in connexion with the problem of regulation of the cutaneous respiration in frogs. *Zoologica Poloniae*. 1957;8:161–175.

34

Miura M, Okada J. Non-thermal vasodilatation by radio frequency burst-type electromagnetic field radiation in the frog. *J Physiol*. 1991;435:257–73.

35

Pinder CF. Gas exchange in isolated perfused frog skin as a function of perfusion rate. *Respir Physiol*. 1991;85:1–14.

36

Donohoe PH. Factors effecting metabolic rate reduction during hibernation in the frog, *Rana temporaria*. Department of Zoology. Cambridge: University of Cambridge; 1997:113.

37

Donohoe PH, Boutilier RG. The protective effects of metabolic rate depression in hypoxic cold submerged frogs. *Respir Physiol*. 1998;111:325–336.

38

Poczopko P. Respiratory exchange in *Rana esculenta* L. in different respiratory media. *Zoologica Poloniae*. 1959;10:45–55.

39

Armentrout D, Rose FL. Some physiological responses to anoxia in the great plains toad, *Bufo cognatus*. *Comp Biochem Physiol*. 1971;39:447–455.

40

Gatz RN, Crawford EC, Piiper J. Kinetics of inert gas equilibration in an exclusively skin-breathing salamander, *Desmognathus fuscus*. *Respir Physiol*. 1975;47:151–164.

41

Piiper J, Gatz RN, Crawford EC. Gas transport characteristics in an exclusively skin-breathing salamander, *Desmognathus fuscus* (plethodontidae). In: Hughes GM, ed. *Respiration of the Amphibious Vertebrates*. London: Academic Press; 1976:339–356.

42

Mackenzie JA, Jackson DC. The effect of temperature on cutaneous CO₂ loss and conductance in the bullfrog. *Respir. Physiol*. 1978;32:313–323.

43

Moalli R, Meyers RS, Ultsch GR, Jackson DC. Acid-base balance and temperature in a predominantly skin-breathing salamander, *Cryptobranchus alleganiensis*. *Respir Physiol*. 1981;43:1–11.

44

Tattersall GJ, Boutilier RG. Constant set points for pH and P(CO₂) in cold-submerged skin-breathing frogs. *Respir Physiol*. 1999;118:49–59.

45

Szarski H. The structure of respiratory organs in relation to body size in amphibia. *Evolution*. 1964;18:118–126.