'ACTIVE' REGULATION OF CUTANEOUS GAS EXCHANGE BY CAPILLARY RECRUITMENT IN AMPHIBIANS: EXPERIMENTAL EVIDENCE AND A REVISED MODEL FOR SKIN RESPIRATION

WARREN BURGGREN[1] and RICHARD MOALLI[2]

[1]*Department of Zoology, University of Massachusetts, Amherst, MA 01003-0027, and* [2]*Department of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510, U.S.A.*

Abstract. Oxygen uptake, carbon dioxide elimination, cutaneous and systemic blood flows (measured by microsphere technique) and the number of perfused capillaries in the hind foot web have been measured at 25 °C in unanaesthetized bullfrogs (*Rana catesbeiana*) both while breathing air as they float in water and while resting totally out of water in humidified air. The gas exchange ratio, approximately 1 while breathing with both water and air, fell to 0.5 or lower during 4 h of complete air exposure. A concomitant decrease occurred in both cutaneous blood flow and the proportion of perfused to non-perfused capillaries in the hind foot web. Upon returning to floating at the water surface, cutaneous blood flow and capillary recruitment increased again and the gas exchange ratio increased to above 2 for several hours. These data suggest that a partial inhibition of CO_2 excretion is linked with a decrease in the extent and pattern of blood flow through the skin, which is the major site of CO_2 elimination.

Conventional models for cutaneous CO_2 elimination in amphibians reveal major diffusion limitations but minor, even insignificant, perfusion limitations. Consequently, CO_2 elimination is regarded as highly responsive to changes in blood P_{CO_2}, but nearly insensitive to changes in blood flow. Importantly, however, such models have treated the skin as a single blood compartment (*i.e.*, single 'capillary'), through which blood flow is varied.

We propose a multi-capillary model which incorporates changes in capillary recruitment, and thus changes in the surface area across which CO_2 elimination from the blood can occur. In such a model, changes in the number of perfused capillaries cause major changes in CO_2 elimination. Experimental data on CO_2 elimination agrees well with predicted changes using this new multi-capillary model.

Amphibian	Capillary	*Rana catesbeiana*
Blood flow	Carbon dioxide	Respiratory quotient
Bullfrog	Dual breather	Skin respiration

The significance of the skin to both O_2 uptake and CO_2 elimination in amphibians has long been recognized (see Krogh, 1904; Noble, 1925; Foxon, 1964, for early literature). More recently Gatz *et al.* (1975) and Piiper *et al.* (1976) have measured O_2 uptake and CO_2 elimination across the skin of *Desmognathus fuscus*, a lungless

Accepted for publication 18 November 1983

and therefore exclusively skin breathing salamander, under conditions assumed to be steady state for gas exchange. This animal was examined in particular not so much as a biological curiosity but rather as the most simple system in which to study cutaneous gas exchange in a quantitative fashion. Emerging from these studies was a model describing gas fluxes across the skin. In essence, this model treated the skin as a single large compartment, analogous to a single capillary, with a constant surface area, gas diffusion pathway and diffusive conductance. Variables include the gas partial pressures of the respiratory medium and capillary blood, the latter potentially influenced by changes in blood flow through the compartment. Using this model and physiological values measured *in vivo* under steady state conditions, Gatz *et al.* (1975) and Piiper *et al.* (1976) predicted that cutaneous gas exchange in *Desmognathus* should be highly sensitive to changes in the partial pressure gradients across the skin and not sensitive to all but the most gross changes in blood flow (*i.e.*, 'diffusion' rather than 'perfusion' limited). Subsequently Moalli (1981) carried out a detailed investigation of cutaneous gas exchange in an anuran, *Rana catesbeiana*. Changes in cutaneous blood flow coincident with changes in cutaneous gas exchange were observed during diving and other experimental conditions, but analyses using the single-compartment model of Piiper *et al.* (1976) similarly indicated that cutaneous gas exchange was limited almost completely by the partial pressure gradient across the skin, and not by cutaneous blood flow. This study corroborated earlier suggestions by Gottlieb and Jackson (1976) and Mackenzie and Jackson (1978) that cutaneous gas exchange in bullfrogs was almost entirely diffusion limited.

In light of these and other studies, cutaneous respiration in amphibians has come to be regarded as 'poorly regulated' and 'passive'. Since the system is supposedly diffusion limited, and since the partial pressure of the respiratory medium is comparatively constant over a short period of time, then cutaneous gas exchange purportedly can be changed only by adjustments in the partial pressure of blood in the capillaries. Changes in blood flow, which can be so effective in gas exchange organs which are even partially perfusion limited (*i.e.*, lungs), are thus regarded as ineffectual with respect to skin exchange in amphibians.

Our present findings from experiments on gas exchange in non-steady state conditions in the bullfrog, *Rana catesbeiana*, seem quite incompatible with the concept of a strictly diffusion limited cutaneous gas exchange organ. This study reveals that changes in capillary recruitment in the skin, either independent of or accompanied by changes in blood flow, can greatly influence cutaneous gas exchange. We therefore suggest that assuming the skin of amphibians or any other animal to be a single, diffusion-limited compartment under all conditions is inappropriate, and can greatly underestimate the contribution of even quite small adjustments to cutaneous blood flow which involve capillary recruitment.

Materials and methods

Experimental animals. Experiments were performed on a total of 17 *Rana catesbeiana* obtained from animal suppliers. Two age/size categories were used. Juveniles which had metamorphosed 3–6 weeks earlier in the laboratory (mean body weight 14 ± 1 g, n = 5) and 2-3 year old adults (mean body weight 251 ± 64 g, n = 12) were used. All experiments were performed on healthy animals maintained at 25 ± 2 °C for at least two weeks before experimentation.

Measurements of O_2 uptake and CO_2 excretion. Gas exchange in all juvenile frogs and one population of 6 adults (228 ± 70 g) was measured with respirometery techniques indentical to those described in detail by Burggren and West (1982). Animals were allowed 24 h to acclimate to the respirometer before measurements of gas exchange were made. In a second population of 6 adults (273 ± 54 g), gas exchange was measured with respirometery techniques indentical to those described in detail by Gottlieb and Jackson (1976), Mackenzie and Jackson (1978) and Moalli (1981). Venous and arterial cannulations of animals in this second population (see below) were carried out at least 12 h before the experiment, and the animals were given a 12 h acclimation period in the respirometer.

During acclimation to either system, the respirometer was largely filled with water, leaving a ventilated air bubble at the top. Frogs generally floated at the water surface and breathed air with their lungs. All experiments were performed at 25 ± 2 °C.

With both respirometery systems, O_2 and CO_2 exchanges for both the skin and the lungs could be determined individually, and the total O_2 and CO_2 exchanges determined by simple addition of exchange in each respiratory medium. Both respirometery systems could also be drained of water, leaving the bullfrog totally exposed to humidified air. In this condition, only total gas exchange and not its partitioning between lungs and skin could be measured in the air-filled respirometer.

The experimental protocol was very similar for the two groups of animals. In the first group (juveniles plus 6 adults), a series of 5 measurements in the water-filled respirometer was made over a 5 h period between 10 : 00 and 15 : 00 EST. The following day, beginning at approximately 10 : 00, a single measurement was made with the animal floating in water and breathing air. After this measurement, the respirometer was carefully and quietly drained of water, and a series of three gas exchange measurements over a 4 h period of air exposure was immediately begun. After this period, the respirometer was filled with water again, and a series of 4 gas exchange measurements over the next 6 h was made, with the animal floating in water and breathing air. A final gas exchange measurement was made at approximately 10 : 00 the following day. Thus, 'control' gas exchange measurements were made on 3 successive days for each animal, with the experimental period of air exposure occurring on the middle day.

A different protocol was used in the second group, in which injection of radioactive microspheres for determination of blood perfusion rates of various tissues

occurred during the course of the gas exchange measurements (see below). In this second group of 7 frogs, a single gas exchange measurement was made between 9:00 and 11:00 EST, followed by 4 measurements made during a 4 h period of air exposure, and a final measurement made 1 h after the respirometer was refilled with water. Microsphere injection/blood withdrawal occurred immediately before air exposure, at the end of air exposure, and 1 h after a return to water (see below).

Measurement of blood perfusion with microspheres. Radiolabeled microspheres were used to assess blood flow during air exposure and partial submergence in awake and unrestrained bullfrogs using the reference sample technique (Hales, 1974; Heymann *et al.*, 1973; Moalli *et al.*, 1980). Double label experiments permitted comparison of air exposed and partially submerged conditions in the same animal.

(1) Surgical preparation. Adult bullfrogs were anesthetized with tricane methanesulfonate (1.5 mg/100 g body mass, subcutaneously). Small cannulae (PE50) were placed in the musculocutaneous vein and sciatic artery for microsphere injection and reference sampling, respectively. Additionally, bilateral ligation of the cutaneous branches of the pulmocutaneous arteries through small incisions over the temporalis muscle guaranteed that the concentration of microspheres reaching the skin was the same as in the reference sample. Failure to ligate these arteries would have allowed microspheres of pulmocutaneous origin to reach the skin. The concentration of microspheres from this source was not represented by the systemic reference sample due to mixing that occurs in the single anuran ventricle (Moalli *et al.*, 1980; Moalli, 1981). Upon completion of surgery, the cannulae were filled with heparinized Ringer's solution, sealed, and the frogs were allowed to recover overnight.

(2) Blood flow determination. Systemic arterial blood flow was assessed by injecting microspheres into the central venous system by way of the musculocutaneous vein cannula and simultaneously withdrawing an arterial reference sample from the sciatic artery at a controlled rate. The relationship between radioactivity and flow in the reference sample could then be used to relate radioactivity and flow in any tissue by the following equation:

$$\dot{V}_b = \frac{\dot{V}_{reference} \cdot A_{tissue}}{A_{reference}}$$

where $\dot{V}_b$ is blood flow and A is corrected radioactivity. Arterial reference samples were withdrawn by pump (Harvard Apparatus, Inc., model 940). Microspheres were injected 30 sec after withdrawal began, withdrawal continued for 5 min, enough time for complete cardiac ejection and organ entrapment of the microspheres. At the end of the study, frogs were sacrificed by an overdose of anesthetic and the radioactivity in samples of tissues of interest and in the reference sample was analyzed and blood flow calculated as described above (see Moalli *et al.*, 1980).

(3) *Microspheres.* Microspheres (New England Nuclear Corp., mean diameter 35 ± 5 μm) were labeled with either gadolinium 153 or tin 113, both gamma emitting isotopes, and suspended in 0.9% saline with 0.01% Tween 80 added to minimize aggregation (Millard *et al.*, 1977). Meyers *et al.* (1979) have shown that microspheres of this size were completely entrapped in a single pass through the bullfrog circulatory system. The radioactivity of each label in tissue and reference samples was measured in a well-type gamma counter (Packard Autoscintillation Counter) and corrected for background activity and energy spillover.

Capillary recruitment. Changes in numbers of skin capillaries perfused before, during and after air exposure were measured in 6 juvenile bullfrogs (15 ± 2 g). Each animal was placed in a 100 ml wide-mouthed container. The open end was sealed by a rubber bung through which a 10 mm hole was bored. The container was placed on its side and filled to 3/4 capacity with water, leaving a 2 cm high air space from which the animal could breathe. A large hole for air ventilation was bored in the container to prevent O_2 depletion or CO_2 buildup in this gas. A small air stone was placed in the water to maintain its air saturation.

The right or left hind foot of the frog was drawn outside the respirometer through the hole in the rubber bung. Nooses tied in suture silk were slipped over the 2nd and 3rd phalanges, and the web of the foot was expanded and immobilized outside the respirometer by fastening the threads to clamps surrounding the apparatus. A fiber-optic lamp was used to illuminate the web from underneath, and this made individual capillaries readily visible under $60\times$ magnification with a dissecting microscope mounted over the suspended web. Except for the 3-4 min/hour when measurements of capillary flow were being made, both sides of the entire foot emerging from the container were kept covered with moist tissues to prevent any desiccation of the delicate web membranes.

All animals tolerated this procedure well, and soon settled down to a normal posture but for the posteriorly extended rear limb. In this fashion, the frog could float in water and breathe air in the container while changes in perfusion patterns of the capillaries of the web were observed. Animals were allowed 2 h to acclimate to this condition. The ensuing experimental protocol was very similar to that of the gas exchange measurements described above. Two observations of capillary perfusion were made with the animal floating in water, after which the container was drained of water and three more measurements were made over a 2 h period. The container was then carefully refilled with water and three more measurements made over a final 2 h period. After this series of measurements, frogs were removed from the apparatus and phenoxybenzamine, an alpha-antagonist, was injected into the dorsal lymph sac of the frog (1 mg/kg, 20 μl carrier volume). The frogs were immediately placed back into the apparatus, and the entire series of measurements repeated.

Capillary recruitment was determined by observing changes in the numbers of capillaries in which erythrocytes were actually observed to be moving under various

experimental conditions. This was quantified by measuring the number of perfused capillaries that transected a calibration line residing in the microscope eyepiece. Knowing the length of the calibration line under magnification, the number of perfused capillaries at any given time could be calculated for a 1 mm transect. Observations at five randomly chosen regions of the web were averaged to determine a representative value for numbers of capillaries perfused at any given time.

Statistical analyses. All data were analyzed to provide means and standard errors. Analysis of variance (ANOVA) was used to assess treatment effects, and Student's *t*-test for either paired or unpaired observations was used to determine the significance of differences between population means.

Results and discussion

Gas exchange of the whole animal

In water. Figure 1 indicates total O_2 uptake, total CO_2 elimination and the gas exchange ratio of juvenile and the first population of adult bullfrogs while floating in aerated water and voluntarily breathing air. Total O_2 uptake, CO_2 elimination and the gas exchange ratio in juveniles in water were statistically not different ($P > 0.10$, independent *t*-test) on each of the three measurement days. In the non-cannulated group of adult bullfrogs (fig. 1), O_2 uptake and CO_2 elimination were higher and lower on day 2 and day 3, respectively, than the values on day 1. However, the gas exchange ratio was not significantly different from 1 ($P > 0.10$) during the 'control' measurements of all 3 days. Both O_2 uptake and CO_2 elimination measured in the second group of bullfrogs (with chronically implanted arterial and venous cannulae) immediately before air exposure was not significantly different ($P > 0.10$) from that measured before air exposure in the uncannulated first group (fig. 1).

Both weight-specific O_2 uptake and CO_2 elimination in juveniles was about 3 × higher than adults. However, the overall gas exchange ratio was about 1 for both size/age groups. About 30% and 20% of O_2 uptake was via the skin of juvenile and adult bullfrogs, respectively, while about 70% and 80%, respectively, of CO_2 elimination was via the skin. These data, which reveal the skin to be much more important to CO_2 exchange than the lungs, are consistent with studies on bullfrogs at comparable temperatures by Mackenzie and Jackson (1978), Moalli (1981), and Burggren and West (1982).

Effects of air exposure on gas exchange. Upon total air exposure, bullfrogs tended to be more active within the respirometers. Not surprisingly, then, total O_2 uptake rose in both juveniles and adults (fig. 1). However, total CO_2 elimination did not similarly increase and, as a consequence, the overall gas exchange ratio fell progressively to a value of 0.5–0.6 after 4 h of air exposure. This phenomenon

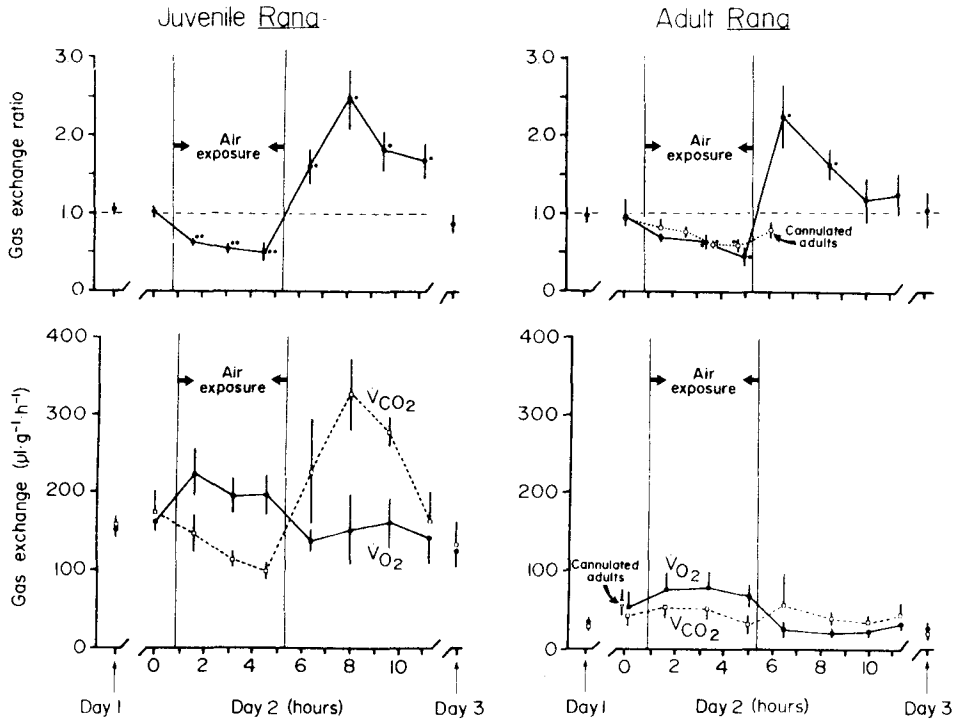

Fig. 1. Oxygen uptake, carbon dioxide elimination and the gas exchange ratio of *Rana catesbeiana* at 25 °C. Data are from 3 successive days for juveniles and uncannulated adults and for 1 day in adults cannulated for microsphere studies. Experimental protocol involved allowing the frog to float in the respirometer while freely breathing air, with an intervening 4 h period of complete air exposure (see text for details). Mean values ± 1 SE are shown and n = 5, 6 and 6 for juveniles, uncannulated adults and cannulated adults, respectively. Asterisks beside data points for the gas exchange ratio indicate significant differences (*, $P < 0.05$; **, $P < 0.001$) by independent *t*-test from control values before air exposure.

occurred consistently in juveniles, non-cannulated adults and cannulated adults, and was statistically significant ($P > 0.05$, ANOVA) in all three cases.

Given that complete emersion from water must be regarded for bullfrogs as a natural and rather innocuous occurrence, it is highly unlikely that stress-induced quantitative changes in metabolism, resulting in a major change in the respiratory quotient of the tissues, developed during air exposure. In fact, when the bullfrogs were returned to water after about 4 h of air exposure, a large and significant ($P < 0.05$) increase in total CO_2 elimination above that at hour zero began to develop. In juvenile and the uncannulated adults this increased CO_2 elimination was maintained for at least 4 h after the return to water, even though O_2 uptake quickly fell to the control levels evident before air exposure. The net result was that the overall gas exchange ratio in this 'recovery' period rose significantly above

control values to greater than 2, and did not return to values not significantly different from control levels for several hours (fig. 1).

These data indicate that an inhibition of CO_2 elimination relative to O_2 uptake, rather than a qualitative change in metabolism, is correlated with air exposure in both juvenile and adult bullfrogs. That is, CO_2 arising from metabolic processes is sequestered in the body during air exposure, and then released over a period of hours upon a return to water.

Mackenzie and Jackson (1978) demonstrated that adult *Rana catesbeiana* at constant temperature in water but breathing air could increase pulmonary CO_2 elimination relative to that achieved via the skin, presumably through increases in lung ventilation. However, this increase in pulmonary relative to cutaneous CO_2 elimination only occurred in response to a large increase in total CO_2 production by tissues. For example, an increase in CO_2 loss via the lung from 50% up to 75% of total CO_2 elimination was correlated with a 3-fold increase in total CO_2 production. In the present experiments, with the same species at similar temperatures, CO_2 elimination remained the same or even fell during air exposure, which would suggest that the skin should remain as the major site of CO_2 elimination during air exposure. That, in fact, a major reduction in the overall gas exchange ratio during air exposure occurred strongly implies that (1) the role of the skin in CO_2 elimination was reduced, and (2) any increase in CO_2 loss from the lungs did not compensate for the reduction in cutaneous CO_2 elimination.

How could an uncompensated reduction in cutaneous CO_2 elimination be explained? In accordance with the generally held notion of a largely diffusion limited cutaneous CO_2 elimination in bullfrogs, a reduction in movement of CO_2 across the skin could be caused by a reduction in capillary blood P_{CO_2} (and thus a reduction in the P_{CO_2} gradient across the skin) or by a very gross restriction of cutaneous blood flow. Given that as much as 30–50% of the CO_2 produced during 4 h of air exposure is sequestered in the body tissues, a fall in capillary blood P_{CO_2} during this period was considered most unlikely. Experiments were thus designed to determine if a reduction in the relative proportion of blood flow to the skin accompanied air exposure and the attendant decrease in CO_2 elimination.

Changes in cutaneous perfusion during air exposure

Blood flow. Changes in blood flow to skin, muscle and viscera measured before and during air exposure in 4 adult bullfrogs are presented in table 1. No significant change ($P > 0.10$) in blood flow to visceral tissue or muscle occurred upon air exposure. However, blood flow to the skin decreased from 0.038 ml/min/g tissue to 0.026 ml/min/g tissue. This 32% reduction in cutaneous blood flow was significant at the $P = 0.05$ level (one-tailed t-test for paired observations).

Capillary recruitment. Changes in the proportion of capillaries perfused in the web of the hind foot of 6 juveniles before, during and after a 2 h period of air exposure are indicated in fig. 2. Approximately 7 capillaries/1 mm transect along the web were perfused in bullfrogs floating in water and breathing air. Within 30

TABLE 1

Blood flow (ml/min/g tissue) to selected tissues of 7 unanesthetised *Rana catesbeiana* (mean weight 270 ± 54 g). Bullfrogs were either floating at the surface breathing air ('in water') or completely air exposed ('in air'). Mean values ± 1 SE are given. Significance values are from a one-tailed *t*-test. N.S. = not significant.

	Number of tissue samples	In water	In air	Significance level
Liver	12	0.128 ± 0.027	0.132 ± 0.027	N.S.
Stomach/small intestine	8	0.265 ± 0.167	0.195 ± 0.067	N.S.
Spleen	4	1.538 ± 0.615	6.599 ± 2.764	N.S.
Brain	4	0.043 ± 0.015	0.035 ± 0.017	N.S.
Kidney	8	1.201 ± 0.295	0.973 ± 0.125	N.S.
Skeletal muscle	24	0.030 ± 0.004	0.037 ± 0.007	N.S.
Buccal cavity/epithelium	8	0.044 ± 0.024	0.026 ± 0.012	N.S.
Flank skin	4	0.040 ± 0.008	0.026 ± 0.003	$P < 0.05$

min of air exposure (and within 5 10 min in some individuals), the number of perfused capillaries dropped by nearly 1/2 to about 4 capillaries/1 mm transect. Subsequent return to the initial conditions in water resulted in an increase in the number of perfused capillaries back to control levels. These changes in capillary perfusion were highly significant ($P < 0.001$, ANOVA and independent *t*-test – see legend of fig. 2).

The entire experiment was repeated for each bullfrog after the injection into the dorsal lymph sac of phenoxybenzamine (1 mg/kg), an alpha antagonist which blocks skin vasoconstriction in higher vertebrates. Phenoxybenzamine had no effect on the number of perfused capillaries in the foot web when the bullfrog

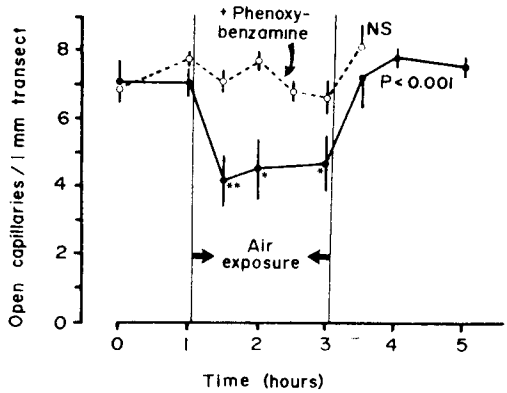

Fig. 2. Numbers of perfused capillaries in the hind foot web of unanesthetised *R. catesbeiana* before, during and after complete air exposure. Data are also shown before and after the injection of phenoxybenzamine, an alpha-adrenergic blocker (see text). Means ± 1 SE (n = 5) and significance levels of ANOVA are given. Convention for asterisks indicating significance levels between particular means as in the legend to fig. 1.

was floating in water. However, alpha receptor blockade totally eliminated the derecruitment associated with air exposure, since there were no significant differences ($P > 0.10$, ANOVA) in capillary perfusion throughout the course of the experiment. These data show that the reduction in proportion of capillaries perfused which occurs during air exposure in the bullfrog is an 'active' response mediated by constriction of vascular smooth muscle.

Cutaneous CO_2 elimination – diffusion or perfusion limited?

Carbon dioxide elimination across the skin of amphibians is conventionally analyzed by treating the skin as a single perfused compartment of fixed dimensions, analogous to a gas exchange organ containing a single large capillary across whose wall gas transfer occurs. In such a model, the diffusive transfer of a gas from blood to respiratory medium, $\dot{V}$, is satisfactorily described by

$$\dot{V} = \frac{A \cdot d \cdot \alpha}{L} \cdot (Pg - Pm) \quad (1)$$

where d is the gas diffusion coefficient, A is the surface area across which gas transfer occurs, α is the gas solubility, L is the diffusion barrier thickness, and Pg and Pm are the partial pressures of capillary blood and the respiratory medium, respectively. In this single compartment analysis, A, d, α and L are assumed constant for a given experimental condition (Gatz *et al.*, 1975; Piiper *et al.*, 1976) and are reduced to D, the diffusive conductance (corresponding to the diffusing capacity of pulmonary physiology).

Eq. (1) and others quantifying gas delivery to the respiratory membranes by blood convection can be used to derive indices of both diffusion and perfusion limitations (see Piiper *et al.*, 1962 and 1976; Piiper, 1965; Piiper and Scheid, 1977 and Moalli, 1981 for details). Using equations and published constants of D = 0.007 mM/h/mm Hg and α = 0.7 mM/l/mm Hg (Piiper and Scheid, 1977) and a cutaneous blood flow estimated from microsphere studies of 79 ml/h, Moalli (1981) calculated that CO_2 elimination in the skin of adult *Rana catesbeiana* at 20 °C was 88% diffusion limited and only 6% perfusion limited. Using our own measured blood flows of 2.28 ml/g/h during control periods and 1.54 ml/g/h during air exposure (table 1), and the above mentioned constants, then the same single capillary analysis indicates that CO_2 elimination in bullfrogs floating in water and breathing air in the present experiment is 94% diffusion limited and only 3% perfusion limited. The diffusion limitation actually rises to 96% during air exposure. Under such highly diffusion-limited conditions, the 32% reduction in cutaneous blood flow through skin treated as a single compartment would cause no significant reduction in CO_2 elimination, and would therefore not account for the observed fall in the total gas exchange ratio during air exposure.

Unfortunately, treatment of the skin as a single perfused compartment in which D, the diffusive conductance, is constant is inappropriate when capillary recruitment occurs, irrespective of whether changes in total skin blood flow also occur.

The surface area term, A, in eq. (1) in its strictest sense is a complex function of the total surface area across which the partial pressure gradient is applied, *i.e.*, the surface area of the walls of all perfused capillaries. The surface area of the gas exchanger epithelium, though commonly used in calculation of gas exchange, is clearly inaccurate when, through the closing of capillaries, there are no longer perfused blood vessels underlying portions of that epithelial sheet. If capillary recruitment occurs, then A and therefore D necessarily change, and the mass transfer of gas across the skin also changes. Figure 3 illustrates changes in the diffusive conductance, D, which would occur in a system with three capillaries arranged in parallel. Each capillary, which is of identical size, surface area and construction, can be either non-perfused or perfused at a rate identical to the other two perfused capillaries. If perfusion stops in one of the three capillaries (fig. 3b) then D is reduced by 33% because of the equivalent decrease in surface area across which gas exchange may occur. If the partial pressure gradient across the capillary wall remains constant, the gas transfer $\dot{V}$ will also fall by 33%.

Now, for each individual capillary, eq. (1) is perfectly valid if dimensions of D for a single capillary are used. If it is assumed that gas transfer within *each capillary* is diffusion limited, then blood flow in capillary 1 and 3 can actually increase to accomodate blood flow lost through capillary 2, and gas transfer will still be reduced by 33% (fig. 3b). Thus, in a model in which the smallest functional unit is diffusion limited, changes in perfusion are important only if they include changes in the number of capillaries perfused, but then in such a case gas transfer will be greatly affected.

Clearly, the value for the diffusive conductance must be carefully considered for the analysis of gas transfer in a gas exchange organ where capillary recruitment can occur (*i.e.*, every gas exchange organ that has been examined so far). In the gas transfer equation for the single compartment of the single capillary model, A represents the total surface area across which the partial pressure gradient is applied.

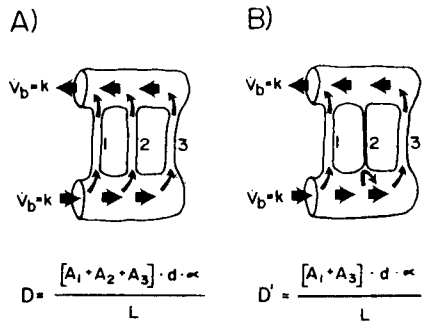

Fig. 3. Changes in calculation of the diffusive conductance, D, in a multi-capillary model in which numbers of capillaries perfused can vary. Blood flow ($\dot{V}_b$) is constant and equal into and out of the capillary network in both (A) and (B). In (A), all three capillaries are perfused, and the surface area, A, is the sum of $A_1 + A_2 + A_3$. In (B), only 2 capillaries are perfused. $A = A_1 + A_3$ and D' is consequently significantly smaller than D. See text for detailed explanation.

We can thus let D_{total} denote the diffusive conductance for the single capillary model. In a multi-capillary model, however, surface area may be variable, so A_{total} should be reduced to its constituent parts. In this type of analysis, A should be more restrictively defined as the surface area of the wall of a single capillary, which in the simplest case will be identical for every capillary. A diffusive conductance identical for each capillary can thus be generated, and defined as D_{cap}. The diffusive conductance when every capillary is perfused accordingly can be defined as:

$$D_{total} = n_{total} \cdot D_{cap} \tag{2}$$

where n = total number of capillaries in the mode. Gas transfer is thus decided by eq. (3) when every capillary is perfused:

$$\dot{V} = n_{total} \cdot D_{cap} \ (P_g - P_m) \tag{3}$$

However, when changes in the number of perfused capillaries occur, a term expressing the fraction of capillaries open at any given time must be introduced to equation (3). Therefore:

$$\dot{V} = \frac{n_{open}}{n_{total}} \cdot D_{cap} \ (P_g - P_m) \tag{4}$$

where n_{open} = number of perfused capillaries.

Equation (4), though theoretically most appropriate, is of limited utility since D_{cap}, n_{open} and n_{total} cannot be measured practically in a gas exchange organ. However, if D_{total}, which has been estimated in a number of studies for resting amphibians in air saturated water (see Gatz *et al.*, 1975; Piiper *et al.*, 1976; Moalli, 1981) is substituted into eq. (4), then n_{open}/n_{total} can become the ratio of open to total capillaries measured in any arbitrarily sized segment of the gas exchange organ that is deemed representative. Under conditions of constant capillary dimension and partial pressure gradient, it becomes apparent that gas transfer across a skin composed of diffusion-limited capillary units arranged in parallel will vary directly with the ratio of n_{open} to n_{total}.

Application of a multiple capillary model

In the present experiments it could not be determined if all capillaries were being perfused in the sample area of the hind foot web during the initial control period. However, if it is assumed that (1) the web is representative of the body skin in general, and (2) that 7 capillaries/1 mm transect represents total capillary recruitment, then reduction of open capillaries by about 1/3 to 4.5 during air exposure should reduce CO_2 elimination under steady state conditions by a fraction of 4.5/7 (from eq. (4) and substitution of D_{total}) or to 64% of its level under control conditions. In fact, CO_2 production by the tissues was probably not constant throughout the

experiment (O_2 uptake varied greatly - fig. 1), but the overall gas exchange ratio did decrease from 1.0 to about 0.55 upon air exposure. This reduction is reasonably well correlated with the observed reduction in fraction of perfused capillaries. Also, the 32% decrease in total skin blood flow measured during air exposure is entirely consistent with the notion of decreased numbers of perfused capillaries.

Finally, why did total skin perfusion and the number of perfused capillaries decrease during air exposure? Adult *Rana catesbeiana* are very susceptible to water loss during air exposure, with one day of exposure to dry air sufficient to induce critical dehydration (unpublished). One response that would reduce dehydration during air exposure would be to reduce evaporative water loss from the skin. By reducing the delivery of blood plasma and thus body water to the skin, a decrease in dehydration could be effected. Certainly in the salamander genus *Aneides* skin capillary recruitment decreases as the relative humidity of the ambient air decreases (Brown, 1972). During air exposure in *Rana catesbeiana*, vascular adjustments to decrease skin blood flow and potentially decrease dehydration appear to be more important than maintaining CO_2 elimination and acid–base status. Chronic acid–base disturbance is well tolerated in anurans (Boutilier *et al.*, 1979), and a hypercapnic acidosis may well be a natural consequence during winter hibernation.

Acknowledgements

This study was supported in part by NSF grant PCM 80-03752 (WWB). R. M. is currently a Research Trainee in Lung Pharmacology and Pathophysiology supported by Training Grant HL-07410 from U.S.P.H.S. Christine Herbert rendered technical assistance in the microsphere studies, while Juan Markin made useful suggestions during the preparation of the manuscript.

References

Boutilier, R. G., D. J. Randall, G. Shelton and D. P. Toews (1979). Acid–base relationships in the blood of the toad, *Bufo marinus*. III. The effects of burrowing. *J. Exp. Biol.* 82: 357–365.

Brown, A. G. (1972). Responses to problems of water and electrolyte balance by salamanders (Genus *Aneides*) from different habitats. Ph.D. Dissertation. University of California, Berkeley.

Burggren, W. W. and N. H. West (1982). Changing respiratory importance of gills, lungs and skin during metamorphosis in the bullfrog *Rana catesbeiana*. *Respir. Physiol.* 47: 151–164.

Foxon, G. E. H. (1964). Blood and respiration. In: Physiology of the Amphibia, edited by J. A. Moore. New York, Academic Press, pp. 151–209.

Gatz, R. N., E. C. Crawford and J. Piiper (1975). Kinetics of inert gas equilibration in an exclusively skin-breathing salamander, *Desmognathus fuscus*. *Respir. Physiol.* 24: 15–29.

Gottlieb, G. and D. C. Jackson (1976). Importance of pulmonary ventilation in respiratory control in the bullfrog. *Am. J. Physiol.* 230: 608–613.

Hales, J. R. S. (1974). Radioactive microsphere techniques for studies in the circulation. *Clin. Exp. Pharmacol. Physiol.* Suppl. 1: 31–36.

Heymann, M.A., R.K. Creasy and A.M. Rudolph (1973). Quantitation of blood flow patterns in the foetal lamb *in utero*. In: Fetal and Neonatal Physiology, edited by K.S. Comline, Proc. of the Sir Joseph Barcroft Centenary Symposium. London, Cambridge University Press, pp. 129–135.

Krogh, A. (1904). Some experiments on the cutaneous respiration of vertebrate animals. *Scand. Arch. Physiol.* 16: 348–357.

MacKenzie, J.A. and D.C. Jackson (1978). The effect of temperature on cutaneous CO_2 loss and conductance in the bullfrog. *Respir. Physiol.* 32: 313–323.

Meyers, R.S., R. Moalli, D.C. Jackson and R.W. Millard (1979). Microsphere studies of bullfrog central vascular shunts during diving and breathing in air. *J. Exp. Zool.* 208: 423–430.

Millard, R.W., H. Baig and S.F. Vatner (1977). Cardiovascular effects of radioactive microsphere suspensions and Tween-80 solutions. *Am. J. Physiol.* 232: H331-H334.

Moalli, R., R.S. Meyers, D.C. Jackson and R.W. Millard (1980). Skin circulation in the frog, *Rana catesbeiana*: Distribution and dynamics. *Respir. Physiol.* 40: 137–148.

Moalli, R. (1981). The effect of temperature on skin blood flow, gas exchange and acid base balance in the bullfrog. Ph.D. Dissertation, Brown University.

Noble, G.K. (1925). The integumentary, pulmonary, and cardiac modifications correlated with increased cutaneous respiration in the Amphibia: A solution of the "hairy frog" problem. *J. Morphol. Physiol.* 40: 341–416.

Piiper, J., R.E. Canfield and H. Rahn (1962). Absorption of various inert gases from subcutaneous gas pockets in rats. *J. Appl. Physiol.* 17: 268–274.

Piiper, J. (1965). Physiological equilibria of gas cavities in the body. In: Handbook of Physiology. Sect. 3. Respiration, Vol. II, edited by W.O. Fenn and H. Rahn. Washington, DC, American Physiological Society, pp. 1205–1218.

Piiper, J., R.N. Gatz and E.C. Crawford (1976). Gas transport characteristics in an exclusively skin-breathing salamander, *Desmognathus fuscus* (plethodontidae). In: Respiration of the amphibious vertebrates, edited by G.M. Hughes. New York, Academic Press.

Piiper, J. and P. Scheid (1977). Comparative physiology of respiration: Functional analysis of gas exchange organs in vertebrates. International Review of Physiology: Respiration Physiology II. Vol. 14, edited by J.G. Widdicombe. Baltimore, University Park Press, pp. 219–253.