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Elimination kinetics of acetylene and Freon 22 in resting and active lungless salamanders

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Abstract. To quantify diffusion limitation in cutaneous gas exchange, the elimination of two inert gases of different diffusivity, Freon 22 (CHClF_2) and acetylene (C_2H_2), was measured simultaneously in exclusively skin-breathing lungless salamanders, *Desmognathus quadramaculatus*. In resting salamanders, elimination of both gases could be described as the sum of three exponential terms. For both the medium and the slow exponential component, the ratio of the respective rate constants (k) for acetylene and Freon averaged 1.77. This value is between the values expected for perfusion limitation (1.00) and diffusion limitation (1.94), indicating combined diffusion and perfusion limitation. In salamanders stimulated to run on a treadmill, the elimination rates and the rate constants increased more for Freon than for acetylene. During spontaneous activity, the increase in elimination of Freon was larger than that of acetylene. These findings suggest an increase in the diffusing capacity of the skin during exercise. Thus the diffusing capacity of salamander skin for gases appears to be variable and to be adjusted to meet the increased O_2 requirement during exercise.

Amphibian; Cutaneous gas exchange; Diffusion; Salamander; Skin breathing

Although skin serves as a gas exchanger in many diverse vertebrates, it is of particular importance in amphibians and is the only avenue for gas exchange in lungless salamanders (Feder and Burggren, 1985a,b). Numerous studies, with mixed results, have examined the extent to which cutaneous exchange of O_2 and CO_2 in amphibians may be limited by diffusion and perfusion. For example, elimination kinetics of Freon 22 in exclusively skin-breathing salamanders indicated that the cutaneous transfer of O_2 and CO_2 must be mainly limited by diffusion (Gatz *et al.*, 1975; Piiper *et al.*, 1976). If cutaneous diffusing capacity is fixed and cutaneous gas exchange is primarily diffusion limited, then the regulation of cutaneous gas exchange ought to be cumbersome. By contrast, more recent studies present evidence for the regulation of cutaneous gas

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exchange and have suggested mechanisms by which regulation might be achieved. For example, exclusively skin-breathing salamanders can increase O_2 uptake considerably during exercise (Withers, 1980; Full, 1985; Feder, 1986), and frogs can vary cutaneous gas conductance during exposure to hypoxia and hypercapnia (Malvin and Hlastala, 1986; Pinder, 1987). These changes are seen as occurring largely through recruitment of previously unperfused cutaneous capillaries (Burggren and Moalli, 1984; Feder and Burggren, 1985b). Indeed, significant variation in capillary recruitment is evident through gross visual inspection (Feder and Burggren, 1985a) or observation of individual capillaries (Poczopko, 1957; Burggren and Moalli, 1984; Burggren and Feder, 1986) and is correlated with changes in the partial pressures of O_2 and CO_2 . However, Malvin and Hlastala (1987) suggest that frogs may achieve large changes in gas exchange even without capillary recruitment and despite the large diffusion limitation, presumably by changes in gross blood flow to the skin.

These recent observations led us to extend earlier inert gas wash-out studies (Gatz *et al.*, 1975; Piiper *et al.*, 1976) under conditions that would more likely reveal changes of cutaneous diffusive conductance (diffusing capacity) if any are present. Two major modifications to the design of the earlier experiments were introduced. First, we observed animals both at rest and during exercise. Second, we simultaneously determined the elimination of two inert gases, Freon 22 ($CHClF_2$) and acetylene (C_2H_2), of differing molecular mass (86.5 and 26, respectively) and diffusivity.

Materials and methods

Desmognathus quadramaculatus (Plethodontidae) were purchased from a commercial supplier (Amphibians of North America, Inc., Memphis, TN). Animals were maintained at 13 °C for several months. Insect larvae or earthworms were available *ad libitum* during this time. Body masses of the experimental animals averaged 23.0 g (SD 3.8 g). The experiments were conducted at room temperature (22 °C).

For equilibration with inert gases the animals were placed individually into gas-impermeable plastic bags (volume 1.5 L) filled with 5% Freon 22 and 5% acetylene in air. After 12–24 h, the salamanders were removed from the equilibration gas mixture and quickly (within 4–8 sec) placed into gas-tight chambers (either a 130 ml Plexiglas cylinder or a 360 ml Plexiglas chamber that included a motor-driven treadmill) through which water vapor-saturated air flowed at $50 \text{ ml} \cdot \text{min}^{-1}$. Animals in the cylinders rested upon a metal screen and those in the treadmill chamber upon the fabric treadmill belt, thus allowing all surfaces of the animal exposure to air flow. Salamanders remained in these chambers until the concentrations of Freon and acetylene in the effluent air were both less than 1% of the maximum concentration observed initially (typically 3–5 h). Air exiting from the chambers was sampled continuously at 6 ml/min with a respiratory mass spectrometer (M3, Varian MAT, Bremen, F.R.G.), modified as described by Scheid (1983). Output of the mass spectrometer was displayed continuously on a chart

recorder. The mass spectrometer was calibrated using gas mixtures provided by precision gas mixing pumps (Wösthoff, Bochum, F.R.G.).

Animals in the treadmill chamber were allowed to rest until the acetylene concentration in the effluent air declined to approximately 10% of the maximum value observed at the start of washout. The treadmill was then run at $1.7 \text{ cm} \cdot \text{sec}^{-1}$ until the salamander was exhausted (in 24–33 min). Animals generally walked voluntarily until nearly exhausted, at which time they were prodded with metal rods. Salamanders were considered exhausted when they failed to walk after 3 consecutive prods with a metal rod. The inert gas concentrations from the treadmill chamber were recorded continuously before, during, and after exercise.

Measurement of solubility. Solubility of Freon 22 and acetylene in entire salamanders was determined by equilibrating animals overnight in bags filled with either 5% Freon or 5% acetylene as described above. A sample of the equilibration gas mixture was removed from the bag and stored in a gas-tight syringe. Animals were quickly transferred into Plexiglas cylinders (130 ml), which were immediately sealed and remained sealed for 3 h (time shown sufficient for practically complete equilibration). At this time, the concentration of test gas within the chamber (F_1) was determined with the mass spectrometer, the animal was removed, the chamber was again filled with water-saturated air, and a sample of the equilibration gas mixture previously set aside equal in volume to the experimental animal was added to the chamber. After 15 min to allow mixing the concentration of the test gas within the chamber (F_2) was determined. The tissue-gas partition coefficient (λ) was calculated as follows:

$$\lambda = \frac{1 - V_s/V_o}{F_2/F_1 - V_s/V_o} \quad (1)$$

V_o , chamber volume; V_s , volume of the salamander determined by water level displacement in a graduated cylinder, or corresponding sample of the equilibration gas mixture.

Measurements on 5 animals yielded the following mean values (\pm SE) for λ and solubility $\alpha = \lambda/(R \cdot T)$ (R , gas constant; T , absolute temperature):

acetylene: $\alpha = 61.3 \pm 1.6 \text{ } \mu\text{mol}/(\text{L} \cdot \text{Torr})$; $\lambda = 1.13 \pm 0.03$;

Freon 22: $\alpha = 91.2 \pm 3.3 \text{ } \mu\text{mol}/(\text{L} \cdot \text{Torr})$; $\lambda = 1.68 \pm 0.06$;

acetylene/Freon 22 ratio (for both λ and α): 0.67 ± 0.02 .

Results

Resting, undisturbed animals. A typical recording of the elimination kinetics of the test gases acetylene (Ac) and Freon 22 (Fr) is depicted in fig. 1. The elimination of the test gases was quantitatively characterized by the maximum concentration and the decay kinetics.

(1) The maximum concentration, F_o , was reached about 1.7–3.1 min after transfer

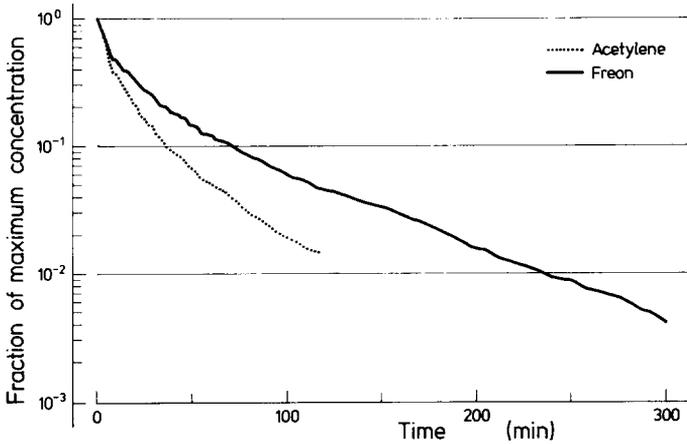


Fig. 1. Wash-out of Freon 22 and acetylene from a 20.2 g salamander. Gas fluxes have been standardized as a percentage of the maximum gas concentrations in the outflow stream, where 100% is equivalent to 94.4 nmol/(g · min) and 128.7 nmol/(g · min) for Freon and acetylene, respectively.

of the animal to the chamber initially containing room air. F_o averaged 0.148% and 0.101% for acetylene and Freon 22, respectively, the ratio being 1.51 ± 0.18 (table 1).

(2) The decay in test gas concentration, starting from F_o , was not linear in the plot of logarithmic concentration against time, *i.e.* not mono-exponential (fig. 1). The change in concentration could be described as sum of three exponentially decaying components (using the conventional technique of repetitive graphical subtraction of logarithmic concentrations):

$$F_t/F_o = A_1 \cdot \exp(-k_1 \cdot t) + A_2 \cdot \exp(-k_2 \cdot t) + A_3 \cdot \exp(-k_3 \cdot t) \quad (2)$$

TABLE 1

Test gas elimination from resting animals. Mean values \pm SE. In parentheses, number of experiments. Analysis according to eq. (2). Amplitudes, A, in fractions. Rate constants, k, in min^{-1} . The maximum, initial concentration, F_o , in fractions of volume.

Variable	Acetylene	Freon 22	Ratio Acetylene Freon 22
F_o	$(1.48 \pm 0.09) \cdot 10^{-3}$ (8)	$(1.01 \pm 0.03) \cdot 10^{-3}$ (8)	1.51 ± 0.18 (8)
A_1	0.29 ± 0.003 (4)	0.32 ± 0.03 (7)	
k_1	0.54 ± 0.10 (4)	0.33 ± 0.07 (7)	
A_2	0.51 ± 0.09 (8)	0.38 ± 0.04 (8)	
k_2	0.093 ± 0.006 (8)	0.053 ± 0.004 (8)	1.78 ± 0.12 (8)
A_3	0.20 ± 0.03 (8)	0.30 ± 0.06 (8)	
k_3	0.027 ± 0.001 (8)	0.016 ± 0.001 (8)	1.76 ± 0.13 (8)

F_t , concentration at time t ; A_n and k_n , amplitude and rate constant of the three components; by definition, $A_1 + A_2 + A_3 = 1.0$.

The mean values of A_n and k_n are presented in table 1. Because the coefficient, k_1 , for the first (rapid) component could not be determined with sufficient accuracy in every case, it should be considered as only approximate. For further analysis (see below), the acetylene/Freon ratios of the rate constants of the medium and slow components, k_2 and k_3 , were of particular importance. They were close to identical, averaging 1.78 and 1.76, respectively.

Spontaneous activity. In many cases, animals in the tubes became spontaneously active for brief episodes. An increase in inert gas elimination accompanied this activity (fig. 2). The relative change (y) in the concentration of eliminated inert gas was defined as $\Delta F_{\text{act}}/F_{\text{rest}}$ (ΔF_{act} , maximum increase of F during episode of spontaneous activity; F_{rest} , linear approximation of resting F at time of maximum F). For 10 randomly chosen episodes of spontaneous activity in 7 animals, the ratio of the relative changes of acetylene and Freon 22,

$$\frac{y_{\text{Ac}}}{y_{\text{Fr}}} = \frac{(\Delta F_{\text{act}}/F_{\text{rest}})_{\text{Ac}}}{(\Delta F_{\text{act}}/F_{\text{rest}})_{\text{Fr}}} \quad (3)$$

averaged 0.89 ± 0.03 (SE).

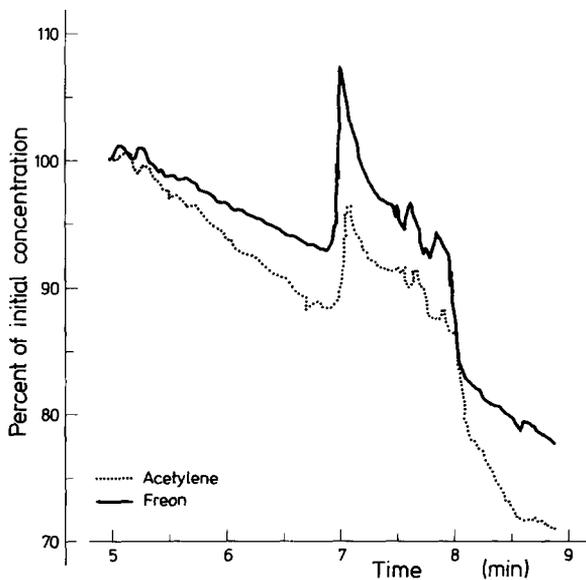


Fig. 2. A typical change in inert gas elimination associated with spontaneous activity in a 'resting' salamander. Values refer to the concentration of inert gases in the outflow stream.

Changes during forced exercise. Unfortunately, an analysis of inert gas washout in the same manner as in resting animals was impossible because the salamanders could not walk steadily for sufficient time to eliminate inert gases completely and to distinguish and quantify the several exponential components. Thus the exercise was done during a washout experiment (fig. 3). Exercise was begun 44–62 min after the start of the washout. Animals exercised for 24–33 min before exhausting. Because the elimination rates of acetylene and Freon were different at rest, the extent of washout reached just before exercise was different for the two gases, as was the instantaneous effective *k* value (*i.e.* relative logarithmic slope of the elimination curve). Accordingly the elimination curve for resting conditions was extrapolated to the time of exercise (values marked with

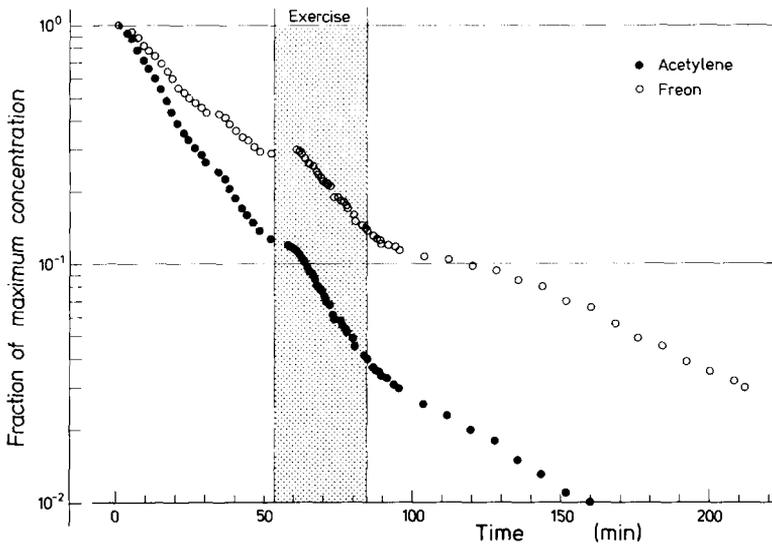


Fig. 3. Washout of acetylene and Freon 22 from a 23.7 g salamander before, during, and after a 29 min exercise period. Gas fluxes have been standardized as a percentage of the maximum gas concentrations in the outflow stream, where 100% is equivalent to 63.6 nmol/(g · min) and 78.6 nmol/(g · min) for Freon and acetylene, respectively.

TABLE 2

Test gas elimination from animals on treadmill. Mean values ± SE (8 experiments). Effective rate constants, *k*, in min⁻¹. (E) refers to exercise, (R) to resting value extrapolated for time of exercise.

Variable	Acetylene	Freon 22	Ratio $\frac{\text{Acetylene}}{\text{Freon 22}}$
<i>k</i> (E)	0.038 ± 0.003	0.032 ± 0.002	
<i>k</i> (R)	0.029 ± 0.001	0.020 ± 0.001	
<i>k</i> (E)/ <i>k</i> (R)	1.32 ± 0.11	1.61 ± 0.11	0.85 ± 0.10

(R) in table 2) to provide a standard for comparison of the actual values, marked with (E), measured during exercise (table 2).

The slope, k , increased during exercise on the average by a factor of 1.32 for acetylene and 1.61 for Freon 22. The Ac/Fr ratio for the change, 0.85, was smaller than but not significantly different from unity.

Models and evaluation

When 3 exponential components were discerned experimentally, 3 compartments were needed for a descriptive model. For the arrangement (interconnections) of the compartments, many configurations are compatible with the experimental data. The circulatory system in plethodontids is such that the cutaneous (arterialized) venous blood and the (O_2 poor) venous blood from the tissues mix in the venous system, the undivided heart and the arterial system (Gatz, 1973). The arteries distribute the mixed blood to both the skin and the other tissues. This arrangement is compatible with the three-compartment model shown in fig. 4. The fast component is associated with the skin, the medium and slow components with body tissues with relatively high and low specific perfusion. The arrangement is a combined parallel-serial model. It is derived from the serial model used by Gatz *et al.* (1975) by subdividing their single body tissue compartment into two compartments (2 and 3).

A complete tissue/blood equilibration is assumed for all compartments (see Discussion). Thus only the gas transfer between compartment 1 and the chamber atmosphere may be diffusion-limited. For estimation of the extent of diffusion limitation, k_2 and k_3 are considered comparatively for both test gases, acetylene and Freon 22. The overall pattern of gas elimination from such a model is complex, but the rate constants associated with the single compartments are determined by volume and blood flow of

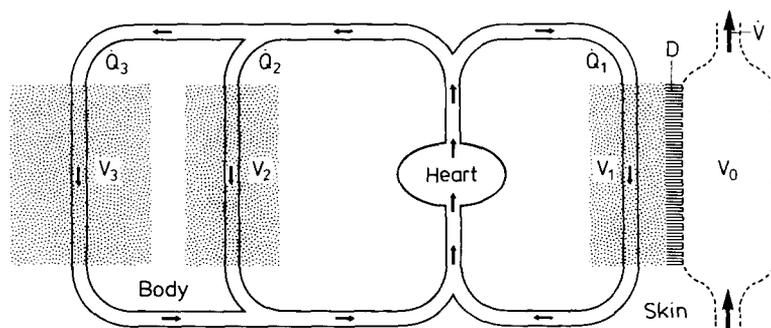


Fig. 4. The analog model, devised on the basis of the arrangement of the circulatory system, for analysis of gas transfer. The animal chamber (gas volume V_0 , air flow rate \dot{V}) is shown in broken line. Tissue compartments 1 (skin), 2 and 3 (internal tissues), of volumes V_1 , V_2 and V_3 , and blood flow, \dot{Q}_1 , \dot{Q}_2 and \dot{Q}_3 (the \dot{Q} and \dot{V} values have not been estimated, but rate constant k_n is approximately proportional to \dot{Q}_n/V_n). D , skin diffusing capacity (diffusive conductance).

the compartment and by skin blood flow and skin diffusing capacity (*cf.* Scheid and Piiper, 1986).

The principal aim of the analysis is to determine to what extent inert gas elimination is limited by diffusion. For this purpose, the expected rate constant ratios k_{Ac}/k_{Fr} for the medium and slow components are derived for the limiting cases of exclusive perfusion and exclusive diffusion limitation, and the experimental values are compared with the predictions.

Perfusion and diffusion limitation. If gas elimination of a model, like that shown in fig. 4, is limited by diffusion only, the rate constant k should be proportional to the diffusion coefficient (d) in tissue:

$$k_{Ac}/k_{Fr} \text{ (diff.lim.)} = d_{Ac}/d_{Fr} \quad (4)$$

The d_{Ac}/d_{Fr} ratio for salamander skin and tissues is not known. Kawashiro *et al.* (1975) estimated this ratio at 1.94 for rat abdominal muscle at 37 °C. This value approximates the prediction from molecular weight according to Graham's law, which is 1.82.

If diffusion is not limiting, the elimination rate constant is determined by (1) the blood/tissue solubility ratio (β/α) and (2) by blood flow and its arrangement in the model. Since the factor (2) is identical for both gases, the following is obtained:

$$k_{Ac}/k_{Fr} \text{ (perf.lim.)} = (\beta_{Ac}/\alpha_{Ac})/(\beta_{Fr}/\alpha_{Fr}) \quad (5)$$

Because solubility, β , has not been measured in these animals' blood, it is assumed that the β/α ratio for acetylene and Freon 22 is equal, meaning that the solubility ratio of eq. (5) equals unity. The indirect evidence in agreement with this assumption is presented in the Appendix. Thus, for perfusion-limited gas elimination, $k_{Ac}/k_{Fr} \text{ (perf.lim.)} = 1.0$.

The mean experimental value for the ratio k_{Ac}/k_{Fr} for the medium and slow components, 1.77 (table 1), is intermediate to the values for perfusion limitation (1.0) and diffusion limitation (1.94), indicating that inert gas washout appears to conform to a model with combined diffusion and perfusion limitation, but with diffusion limitation predominating. With exclusive diffusion limitation in the skin, the elimination would be monoexponential.

Body-to-environment conductance. At the start of elimination, the body partial pressures for both Freon 22 and acetylene should be equal because the animals had been equilibrated at the same partial pressure of both gases. Accordingly, the maximum gas concentration at the beginning of elimination, F_o , should be proportional to the effective conductance.

If perfusion were limiting to gas transfer, the F_o ratio should equal the β ratio:

$$F_o(Ac)/F_o(Fr) \text{ (perf.lim.)} = \beta_{Ac}/\beta_{Fr} \quad (6)$$

If diffusion were limiting, the F_o ratio should be proportional to the Krogh diffusion constant ratio, $(d \cdot \alpha)_{Ac}/(d \cdot \alpha)_{Fr}$:

$$F_o(Ac)/F_o(Fr) \text{ (diff.lim.)} = (d \cdot \alpha)_{Ac}/(d \cdot \alpha)_{Fr} \quad (7)$$

The mean experimental $F_o(Ac)/F_o(Fr)$ ratio, 1.51 (table 1) is close to the $(d \cdot \alpha)_{Ac}/(d \cdot \alpha)_{Fr}$ ratio, for which the range 1.36 (= 1.82 · 0.75) to 1.63 (= 1.94 · 0.84) is predicted from the d_{Ac}/d_{Fr} estimates (1.82 and 1.94) and the α_{Ac}/α_{Fr} estimates (0.75 and 0.84) (see preceding text and Appendix), but very different from the β_{Ac}/β_{Fr} ratio (0.75, see Appendix). The experimental value, 1.51, is thus in the range expected for diffusion limitation. Accordingly, the initial elimination of acetylene and Freon 22 from the salamanders appears to be predominantly diffusion-limited. This result is in accordance with expectations since initially inert gas is eliminated from the skin itself, with no (or little) contribution by blood flow, whereas the gas eliminated later in washout stems from deeper regions of the body and requires blood flow for transport.

Changes during exercise. The whole elimination curve could not be determined in steady state exercise, and only a limited portion of the elimination curve could be recorded. This circumstance precludes an unequivocal interpretation. The increased test gas concentrations and higher elimination rate constants (k) during exercise may be due to two changes expected to occur during exercise: an increase of skin diffusive conductance and/or increased transport of gases to the skin via increased blood flow from muscle. Distinction between these two possible explanations may lie in the differential behavior of the two test gases, acetylene and Freon.

The larger increase of k for Fr as compared to Ac (table 2) is expected when diffusing capacity increases, whereas the reverse would occur with increased skin blood flow. This asymmetry is due to the smaller diffusivity of Fr in connection with the perfusion-diffusion model. According to this model, diffusion limitation is determined by the ratio diffusive/perfusive conductance; the smaller the ratio the higher the extent of diffusion limitation. Thus, if the increase in overall gas transfer conductance occurs at the level of the skin, this increase is due to increased diffusing capacity and not to increased skin blood flow.

If the $(\beta_{Ac}/\alpha_{Ac})/(\beta_{Fr}/\alpha_{Fr})$ ratio of eq. (5) is assumed to be unity, perfusion-limited elimination should occur at equal rates for Ac and Fr. Thus, with increased blood flow to the initially underperfused compartment, the output rate and the subsequent decay rate (k) should increase, but *pari passu* for both test gases. Although other explanations, e.g. diffusion limitation and its changes in tissue, may also be possible, an increase of skin-diffusing capacity during exercise appears the most likely explanation of the experimental findings.

Discussion

Validity of analysis. The present analysis is based on a highly simplified model and several assumptions. These include the following:

(1) The simple model used to explain the experimental data is an analog model. The 'compartments' probably have no anatomical identity, and are an approximation of a larger number of functional compartments that no doubt exist in the animal.

(2) Part of the diffusion resistance may reside in the internal organs and not in the skin. However, elimination of inert gases from mammalian tissues (*e.g.* muscle) is usually considered to be limited by perfusion only (Lassen *et al.*, 1964).

(3) Many important values essential for the analysis and interpretation are based on assumptions. In particular, accurate values of the solubilities of acetylene and Freon 22 in blood are decisive in estimating diffusion and perfusion limitation. Although the values we estimated are reasonable extrapolations from data for mammals at 37 °C, direct measurements on salamander tissue would be welcome.

Due to these assumptions and uncertainties, the present analysis is only semi-quantitative.

Variable skin gas conductance. The simultaneous use of two test gases of differing diffusivities confirm the conclusions of similar experiments in *Desmognathus fuscus*, which used only one inert gas (Freon 22) (Gatz *et al.*, 1975; Piiper *et al.*, 1976): the exchange of Freon 22 and acetylene is limited by both perfusion and diffusion in resting salamanders, diffusion limitation being more important.

The considerable increases in conductance, both during locomotion on the treadmill and during spontaneous but brief activity, are findings that were unexpected from the viewpoint of the previous experiments. An increase in diffusion conductance was not expected because the morphometric diffusive conductance, calculated from the total surface area and thickness of the epithelium, yielded a value only slightly higher than the value derived from inert gas wash-out (Piiper *et al.*, 1976). However, data had to be compiled from several studies for these previous calculations, and values for diffusion coefficients determined in other animal tissues had to be used. The recruitment of additional cutaneous capillaries (Burggren and Moalli, 1984; Feder and Burggren, 1985b) or the shunting of blood from deep capillaries to more superficial capillaries are possible explanations for the increased cutaneous conductance.

Oxygen and carbon dioxide. In the present study inert gases served as model gases for the respiratory gases, O₂ and CO₂. However, important differences are expected between inert gases and the respiratory gases. Since O₂ and CO₂ have a highly enhanced total solubility in blood (high β) by virtue of chemical combination, their transfer should be much more diffusion limited than that of inert gases (Piiper and Scheid, 1981). An increase in diffusive conductance would therefore be especially suitable for enhancing O₂ exchange, and may in part account for the 2-fold increase in O₂ uptake observed during treadmill exercise in *Desmognathus quadramaculatus* (Full *et al.*, 1987).

Conclusions. The inert gas transport between body tissues and environment in the lungless salamander *D. quadramaculatus* is limited by both diffusion and perfusion, whereas the exchange and transport of O₂ and CO₂ are expected to be predominantly diffusion limited. In exercise the cutaneous diffusing conductance appears to increase and allow increased O₂ uptake.

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Appendix: solubility values for blood and tissue

The blood/gas partition coefficient, λ , has been found to equal 0.973 for acetylene and 0.942 for Freon 22 in mammalian blood at 37 °C (Hlastala *et al.*, 1982), yielding $\beta_{Ac}/\beta_{Fr} = 1.03$. Robertson *et al.* (1982) determined λ for dog blood in the temperature range 32–43 °C. Analysis of their data on the basis of the relationship (T, absolute temperature):

$$\frac{d\lambda/\lambda}{d(1/T)} = a \quad (A1)$$

yields the following values for the temperature coefficient a : $a_{Ac} = 1640$ K (degrees Kelvin), $a_{Fr} = 3236$ K. These values and the relationship $\beta = \lambda/(R \cdot T)$ (R, gas constant) yield the following prediction for dog blood: $(\beta_{Ac}/\beta_{Fr})_{22^\circ} = 0.75$.

For the solubility in tissue, α , values were determined directly by the equilibration technique (see Methods). In addition, α can be estimated from the integration of gas elimination from resting salamanders and its extrapolation to infinity. The average values of table 1 yield the following values: $\alpha_{Ac} = 55.0$ $\mu\text{mol}/(\text{L} \cdot \text{Torr})$, $\alpha_{Fr} = 65.7$ $\mu\text{mol}/(\text{L} \cdot \text{Torr})$, $\alpha_{Ac}/\alpha_{Fr} = 0.84$.

Combination of the two sets of α values with the β values yields the following values for the $(\alpha_{Ac}/\alpha_{Fr})/(\beta_{Ac}/\beta_{Fr})$ ratio: 0.90 and 1.12. Since both estimates of α have certain methodological advantages but also problems, it was preferred to use the arithmetic mean, $(\alpha_{Ac}/\alpha_{Fr})/(\beta_{Ac}/\beta_{Fr}) = 1.0$.

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