VENTILATION AND PARTITIONING OF OXYGEN UPTAKE IN THE FROG *RANA PIPIENS*: EFFECTS OF HYPOXIA AND ACTIVITY

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SUMMARY

Pulmonary and cutaneous oxygen uptake ($\dot{M}_{O_2}$) and lung ventilation were measured in frogs floating in water with access to air in respirometers, with and without ventilation of the skin provided by stirring. The frogs were exposed to hypoxia in both water and air, and were variably active.

In inactive frogs floating in unstirred respirometers at 25°C, 23% of total $\dot{M}_{O_2}$ is through the skin. Activity of the animal increases total $\dot{M}_{O_2}$ and also ventilates the skin, so that cutaneous $\dot{M}_{O_2}$ increases with increasing total $\dot{M}_{O_2}$. When the respirometer is stirred, cutaneous $\dot{M}_{O_2}$ increases to 35% of total $\dot{M}_{O_2}$ in resting animals. Activity no longer affects cutaneous $\dot{M}_{O_2}$.

Lung ventilation volume is directly proportional to lung ventilation rate in normoxia. Ventilation rate, and therefore ventilation volume, is proportional to pulmonary $\dot{M}_{O_2}$. Ventilation rate approximately doubles in hypoxia ($P_{O_2} = 52$ mmHg). The pattern of ventilation also changes in hypoxia, from a very irregular pattern in normoxia to one showing regular, large oscillations of lung volume over several ventilation movements.

Increased lung ventilation, enhancing pulmonary $\dot{M}_{O_2}$, is the primary adjustment to increased O_2 demand. Partitioning of $\dot{M}_{O_2}$ shifts towards the lung during both activity and hypoxia. In both cases, however, ventilation of the skin can supplement total $\dot{M}_{O_2}$ by increasing absolute levels of cutaneous $\dot{M}_{O_2}$.

INTRODUCTION

Many adult anurans breathe both water and air, using both skin and lungs. Apnoea, submergence, air exposure, temperature and ambient respiratory gas levels may all affect the relative importance of the lungs and skin in gas exchange.

Regulation of lung ventilation in amphibians has not been extensively studied, in part because of analytical difficulties caused by their intermittent ventilation (Shelton & Boutilier, 1982; Glass & Wood, 1983). Generally, however, lung ventilation in amphibians is regulated in response to both oxygen and carbon dioxide. Either low oxygen or high carbon dioxide in inhaled gas increases the rate of

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lung ventilation (Smyth, 1939; Toews, 1971; Boutilier & Toews, 1977, 1981; McDonald, Boutilier & Toews, 1980; West & Burggren, 1982; Feder, 1983), but different responses to O_2 and CO_2 occur in different amphibians and the control mechanisms remain unclear. Lung ventilation frequency has not been measured simultaneously with oxygen uptake in unrestrained frogs, and few measurements of ventilation volume are available (West & Jones, 1975).

Little attention has been given to skin ventilation of floating or submerged amphibians, although Burggren & Feder (1986) found that experimental ventilation of the skin increased cutaneous oxygen uptake from water by almost 50% in adult *Rana catesbeiana*, probably by disrupting a boundary layer of stagnant water next to the skin. Existing models of cutaneous gas exchange do not incorporate skin ventilation, although boundary layer effects are recognized to be a potential complicating factor of cutaneous gas exchange (Piiper, Gatz & Crawford, 1976; Piiper & Scheid, 1977).

The purposes of these experiments are (1) to establish the effects of activity (and therefore variation in oxygen uptake) and hypoxia on ventilation of both the lungs and skin, and (2) to interpret effects of activity and hypoxia on oxygen uptake partitioning in terms of ventilation and other processes of the lungs and skin.

MATERIALS AND METHODS

Adult *Rana pipiens pipiens* (northern grass frogs) were obtained from a commercial supplier in two groups. One group (37–47 g) was used in the investigation of skin ventilation and activity. The other group (30–45 g) was used for measurement of lung ventilation and effects of hypoxia. All animals were maintained in the laboratory at 22–26 °C for at least 1 week before experimentation. They were housed in aquaria with 5–7 cm of water and a platform above water level, and fed flies, crickets, mealworms or baby voles, as available.

Protocol

All measurements were made at 25 °C using flow-through respirometers in which the animals floated in water and had access either to air or to an air/nitrogen mixture. Oxygen uptake ($\dot{M}_{O_2}$) from gas was considered to be equivalent to pulmonary $\dot{M}_{O_2}$, and $\dot{M}_{O_2}$ from water was considered to be cutaneous $\dot{M}_{O_2}$.

Effects of skin ventilation and activity

The effect of skin ventilation was investigated by comparing pulmonary and cutaneous $\dot{M}_{O_2}$ in experimentally stirred respirometers to $\dot{M}_{O_2}$ values in unstirred respirometers. Activity of the animal, visible through a window in the respirometers, was noted for each measurement of $\dot{M}_{O_2}$. Water entering the respirometer was air saturated. Frogs were acclimated to the respirometer overnight. A total of 17–21 measurements of pulmonary and cutaneous $\dot{M}_{O_2}$, separated by at least 30 min, was made over a 1- to 2-day period on each frog. After obtaining resting $\dot{M}_{O_2}$ measurements, inactive animals were prodded intermittently with a flexible plastic-covered

probe to ensure that some of the measurements were taken during activity. Prodding, when used, was maintained for 30–90 min. The $\dot{M}_{O_2}$ of each frog was measured in separate experiments in both stirred and unstirred respirometers; half of the frogs were first measured in the stirred respirometer, the other half in the unstirred respirometer. The frogs were given 2–3 days to recover between experiments.

Lung ventilation and effects of hypoxia

Lung ventilation and $\dot{M}_{O_2}$ were measured in unstirred respirometers during combined aerial and aquatic normoxia and three levels of hypoxia; 97, 75 and 52 mmHg P_{O_2}. All activity was spontaneous – the frogs were never prodded.

The frogs were allowed to recover overnight in the respirometer after implantation of electrodes for measurement of lung ventilation (see below). Three measurements of $\dot{M}_{O_2}$, separated by at least 30 min, were taken at each oxygen level. Physiograph records of ventilation were taken during a 10-min period bracketing the $\dot{M}_{O_2}$ measurement. The order of presentation of oxygen levels was from normoxia to progressively greater hypoxia. The animals were returned to normoxia for at least 1 h between hypoxic exposures. Further measurements of $\dot{M}_{O_2}$ were made during these recovery periods, so that more measurements were made during normoxia than at any one level of hypoxia.

Measurement techniques

Flow-through respirometers containing 1·8 l of water and 50–75 ml of gas were used. Oxygen uptake was calculated from the P_{O_2} difference between incurrent and excurrent water and gas, the capacitances of water and gas for oxygen, and the flow rates of water and gas through the respirometers. The flow of water was 50–100 ml min^{-1}, resulting in an incurrent − excurrent P_{O_2} difference of 3–10 mmHg. Gas flow was 5–15 ml min^{-1}, also with an incurrent − excurrent P_{O_2} difference of 3–10 mmHg. The P_{O_2} electrodes were calibrated whenever the measurement of incurrent P_{O_2} departed significantly from 152·0 mmHg in normoxia.

Lung ventilation was measured by detecting changes in impedance between electrodes implanted subcutaneously on either side of the chest, a placement which gives an impedance signal that is linearly related to lung volume (West & Jones, 1975; Brett & Shelton, 1979). The impedance electrodes were thin copper disks 2–3 mm in diameter, soldered to insulated 0·1 mm diameter copper leads. In a tracheally cannulated frog, impedance was highly correlated with the volume of air injected into the lungs ($r = 0·99$, $N = 14$, $P < 0·001$).

The impedance leads were led out of the respirometer through a small tube sealed with wax and connected to a Narco Mark IV Physiograph through Narco impedance converters. Lung ventilation rate (fL) was measured by counting all rapid changes in lung volume over a 10-min period (except those caused by movement of the animal, which were usually easily distinguished from lung ventilation). Ventilation volume was measured by summing all vertical displacements of the trace (reflecting lung

volume changes) over the same 10-min period using a Numonics Corporation electronic planimeter with a precision of 0·25 mm.

Data analysis

To analyse the effect of activity on $\dot{M}_{O_2}$, data from the skin ventilation experiment were separated into measurements taken while the frogs were resting or measurements taken while frogs were highly active, according to the visual observations of activity taken simultaneously with the $\dot{M}_{O_2}$ measurements. Three measurements each of resting and active $\dot{M}_{O_2}$ were averaged to give a single measurement of resting and active $\dot{M}_{O_2}$ per animal under each condition. Where more than three observations were available (the usual case), the three measurements to be used were chosen using a random number generator. Each individual animal thus yielded measurements of active and resting pulmonary, cutaneous and total $\dot{M}_{O_2}$, in both stirred and unstirred respirometers.

Unless otherwise noted, individual measurements were treated as independent observations. The number of measurements available depended on the analysis being made. Pulmonary and cutaneous $\dot{M}_{O_2}$ were always measured together. Movement artifacts, damaged leads, and electrodes that had shifted after implantation caused obvious difficulties in interpreting the traces so that not all recordings of ventilation could be analysed for fL and ventilation volume. Thus, not all measurements of $\dot{M}_{O_2}$ had an associated measurement of fL. Measurement of ventilation volume was attempted only for normoxia because of a change in ventilatory pattern in hypoxia.

Because lung ventilation traces were not calibrated, ventilation is in arbitrary units. To compare results from different animals the ventilation volumes were normalized as follows. The sum of the vertical displacements of the recorded trace (ventilation volume) over a 10-min period was divided by fL for that time period, giving a ratio of volume/ventilation movement (stroke volume), in arbitrary units. All stroke volumes (3–9 measurements per animal) for each individual were averaged, and the measured ventilation volume was then divided by the average stroke volume. This procedure sets the average stroke volume for each animal to 1·0, and therefore allows comparison between different animals.

The product–moment correlation coefficient, r, was used to assess the significance of correlations. Least-squares linear regression slopes are reported; slopes were compared using the analysis of covariance technique given in Sokal & Rohlf (1981). Mean values are given ± standard error of the mean. Differences between means were analysed with pair t-tests. A minimum significance of $P < 0.05$ was used for all significance tests. Statistical significance is noted on figures with asterisks: $* = P < 0.05$, $** = P < 0.01$, $*** = P < 0.001$.

RESULTS

In inactive frogs floating in well-aerated, unstirred water at 25 °C, common natural conditions for these frogs, cutaneous $\dot{M}_{O_2}$ provides 23 % of total $\dot{M}_{O_2}$. Partitioning of

$\dot{M}_{O_2}$ between the skin and lungs is highly variable, however, and is affected by skin ventilation, increases of $\dot{M}_{O_2}$ due to physical activity, and environmental hypoxia.

Effect of skin ventilation

Cutaneous $\dot{M}_{O_2}$ (i.e. $\dot{M}_{O_2}$ from water) increases when the skin is ventilated, whether ventilation is caused by stirring the respirometer or by physical activity of the animal itself. Physical activity has the additional consequence of increasing total $\dot{M}_{O_2}$. Stirring of the respirometer does not affect total $\dot{M}_{O_2}$, either in resting or active animals. When the skin is not ventilated by stirring the respirometer, cutaneous $\dot{M}_{O_2}$ increases with increasing total $\dot{M}_{O_2}$ (Fig. 1A), while in a stirred respirometer cutaneous $\dot{M}_{O_2}$ is high and independent of total $\dot{M}_{O_2}$ (Fig. 1B). This difference is also apparent in individual animals (Table 1); cutaneous $\dot{M}_{O_2}$ increases with increasing total $\dot{M}_{O_2}$ in unstirred respirometers but there is generally no correlation between cutaneous $\dot{M}_{O_2}$ and total $\dot{M}_{O_2}$ in stirred respirometers.

Much of the variation in total $\dot{M}_{O_2}$ is attributable to variable physical activity. This is obvious from comparing 'active' with 'resting' animals in either stirred or unstirred respirometers (Table 2; Fig. 1). Active frogs have a total $\dot{M}_{O_2}$ about double their resting $\dot{M}_{O_2}$ ($P<0.001$ in stirred respirometer, $P<0.01$ in unstirred respirometer). [Note: this difference should not be construed as the aerobic scope, because 'active' $\dot{M}_{O_2}$ is not maximum $\dot{M}_{O_2}$, both because the animals were not prodded to maximal activity and because the procedure used in choosing 'active' $\dot{M}_{O_2}$ did not necessarily choose the highest $\dot{M}_{O_2}$ from each animal – see Fig. 1.]

Activity thus increases both total $\dot{M}_{O_2}$ and cutaneous $\dot{M}_{O_2}$ in the unstirred respirometer, resulting in the significant correlation of cutaneous with total $\dot{M}_{O_2}$ in Fig. 1A. When there is no skin ventilation from either activity or stirring (i.e. resting animals in unstirred respirometers), cutaneous $\dot{M}_{O_2}$ is reduced by 34 % compared to resting animals in stirred respirometers ($P<0.05$). As a result, cutaneous $\dot{M}_{O_2}$ is 35 % of the total $\dot{M}_{O_2}$ in the stirred respirometer but only 23 % of the total $\dot{M}_{O_2}$ in the unstirred respirometer (comparing resting animals). The contribution of cutaneous $\dot{M}_{O_2}$ decreases to 19 % of total $\dot{M}_{O_2}$ in both stirred and unstirred respirometers during activity.

Individual variation in cutaneous $\dot{M}_{O_2}$ is apparent in the slopes of the regression of cutaneous $\dot{M}_{O_2}$ on total $\dot{M}_{O_2}$ ($m = 0.062-0.476$), and from an analysis of variance (ANOVA), comparing measurements of cutaneous $\dot{M}_{O_2}$ of individual animals in stirred respirometers ($P<0.001$). Different types of activity (e.g. bursts of struggling or steady, slow movement) will provide different amounts of skin ventilation per unit $\dot{M}_{O_2}$. Thus, the relationship of cutaneous $\dot{M}_{O_2}$ to total $\dot{M}_{O_2}$ is expected to be variable depending on the activity patterns of individual frogs.

Lung ventilation in normoxia

Lung ventilation frequency (f_L) is directly proportional to pulmonary $\dot{M}_{O_2}$ (Fig. 2). Although there is considerable short-term variability in f_L (Fig. 3A), and probably in $\dot{M}_{O_2}$ because of bursts of activity, f_L and pulmonary $\dot{M}_{O_2}$ are highly correlated when short-term variations are averaged out over longer time periods.
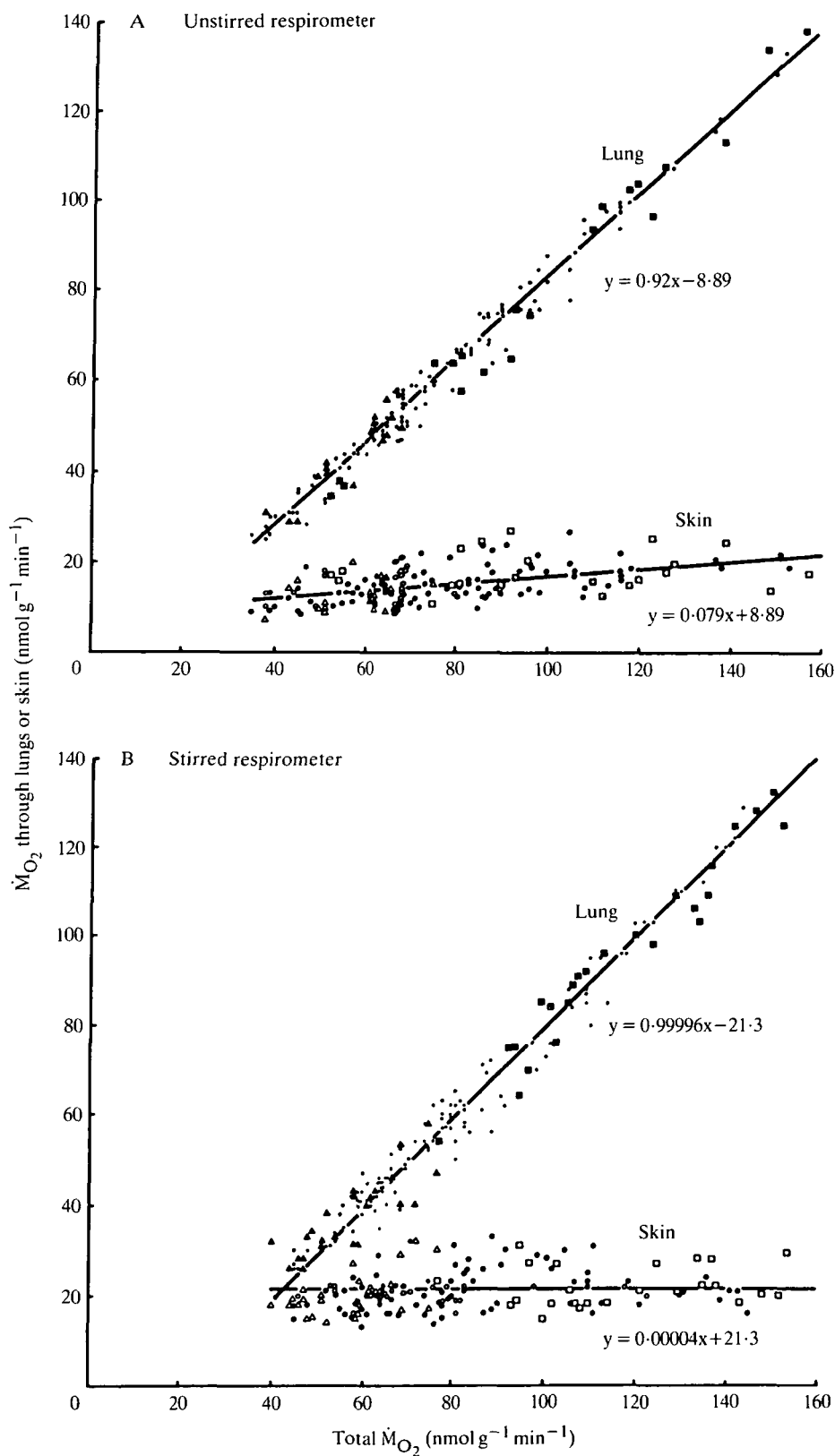
Fig. 1

Lung ventilation volume is directly proportional to fL, at least in normoxia (Fig. 4). Stroke volume does not change with fL; the slope of the regression line for pooled data is 0·996, not significantly different from 1·0. Slopes of regression lines calculated for individual frogs are also not significantly different from 1·0.

In normoxia, ventilation is very irregular (Fig. 3A). Most ventilatory movements consist of an exhalation immediately followed by an inhalation of similar magnitude. Some ventilatory movements, however, are all expiratory or all inspiratory. The period between breaths, during which lung volume remains relatively fixed, is variable.

Effects of hypoxia

Hypoxia both decreases total $\dot{M}_{O_2}$ and increases fL, resulting in a shift of $\dot{M}_{O_2}$ partitioning towards the lung. Lung ventilation frequency relative to $\dot{M}_{O_2}$ (slopes of the regression lines in Fig. 2) increases as ambient P_{O_2} decreases. fL slightly more than doubles for a given $\dot{M}_{O_2}$ when the ambient P_{O_2} is reduced from 150 mmHg to 52 mmHg.

The pattern of lung ventilation is also different during hypoxia, characterized by regular large oscillations in lung volume involving several ventilatory movements (Fig. 3B). The lungs are deflated in one or a few exhalations, then reinflated with a series of several inhalations. Exhalation and inhalation are less frequently coupled into a single ventilatory movement compared to the normoxic pattern.

This increased lung ventilation results in a shift in $\dot{M}_{O_2}$ partitioning towards the lung. Total $\dot{M}_{O_2}$ is reduced in hypoxia (Fig. 5), mostly due to a reduction in cutaneous $\dot{M}_{O_2}$, which decreases 63 % compared to normoxia at an ambient P_{O_2} of 52 mmHg, while pulmonary $\dot{M}_{O_2}$ decreases only 20 % over the same range of ambient P_{O_2}. The proportion of total $\dot{M}_{O_2}$ taken through the lungs therefore increases from 63 % in normoxia to 77 % at 52 mmHg P_{O_2} ($P < 0·01$). These figures are for spontaneously active frogs, with all $\dot{M}_{O_2}$ values from each frog, regardless of activity level, averaged together for each P_{O_2} level. This 'average' activity level results in an $\dot{M}_{O_2}$ in normoxia midway between 'active' and 'resting' $\dot{M}_{O_2}$ values measured in the skin ventilation experiment, and slightly higher than 'standard' or 'resting' $\dot{M}_{O_2}$ values measured by Seymour (1973) and Carey (1979) for *R. pipiens* at 25°C.

Hypoxia and increased $\dot{M}_{O_2}$ resulting from increased activity both increase the proportion of $\dot{M}_{O_2}$ taken up through the lungs (Fig. 6). Thus, pulmonary oxygen uptake is at its most important in active animals and during hypoxia, whereas cutaneous oxygen uptake is at its most important in resting animals in well-aerated water.

Fig. 1. Correlations of pulmonary and cutaneous $\dot{M}_{O_2}$ with total $\dot{M}_{O_2}$ in unstirred (A) and stirred (B) respirometers. Cutaneous $\dot{M}_{O_2}$ is represented by open symbols, pulmonary $\dot{M}_{O_2}$ by closed symbols. Data points selected for further analysis in Table 2 are noted with triangles (resting animals) or squares (active animals). Each point is an individual measurement; in Fig. 1A N (the number of measurements) = 151, n (the number of animals) = 8; and in Fig. 1B $N = 135$, $n = 7$.

Table 1. *Regression lines of cutaneous $\dot{M}_{O_2}$ on total $\dot{M}_{O_2}$ for individuals tested in both stirred and unstirred respirometers*

Animal	Unstirred respirometer					Stirred respirometer				
	m^*	b^*	N^*	r^*	P^*	m	b	N	r	P
1	0·476	−0·0077	17	0·88	<0·001	0·086	0·012	21	0·52	<0·05
2	0·081	0·0048	21	0·77	<0·001	0·017	0·020	20	0·27	NS
3	0·063	0·0095	19	0·81	<0·001					
4	0·062	0·011	20	0·43	<0·1	0·027	0·019	20	0·40	NS
5	0·283	−0·0003	16	0·83	<0·001	0·017	0·018	18	0·15	NS
6	0·117	0·0046	19	0·78	<0·001	0·010	0·019	19	0·11	NS
7	0·083	−0·0103	20	0·73	<0·001	0·033	0·025	17	0·27	NS
8	0·140	−0·0039	19	0·76	<0·001	0·101	0·017	20	0·60	<0·01
Grouped	0·0791	0·00889	151	0·569	≤0·001	−0·00004	0·0213	135	0·063	NS

*Cutaneous $\dot{M}_{O_2} = m$(total $\dot{M}_{O_2}$)$+b$.
N, number of measurements; r, correlation coefficient; P, statistical probability.

Table 2. *Partitioning of $\dot{M}_{O_2}$ in resting and active animals*

	Stirred respirometer			Unstirred respirometer		
	$\dot{M}_{O_2}$ (water)	$\dot{M}_{O_2}$ (air)	$\dot{M}_{O_2}$ (total)	$\dot{M}_{O_2}$ (water)	$\dot{M}_{O_2}$ (air)	$\dot{M}_{O_2}$ (total)
Resting	20.3 ± 1.9	37.7 ± 2.7	58.6 ± 2.87	13.3 ± 1.2	45.1 ± 8.4	58.0 ± 3.4
Active	22.3 ± 1.5	94.4 ± 6.1	117 ± 6.2	18.4 ± 1.6	82.4 ± 11.3	100 ± 11.4

Data are from the same set as Fig. 1A,B and Table 1, separated on the basis of observed activity as described in Materials and Methods, data analysis.

All $\dot{M}_{O_2}$ data are presented as $\bar{x} \pm$ s.e. Units are nmol $O_2 g^{-1} min^{-1}$. $N = 7$.
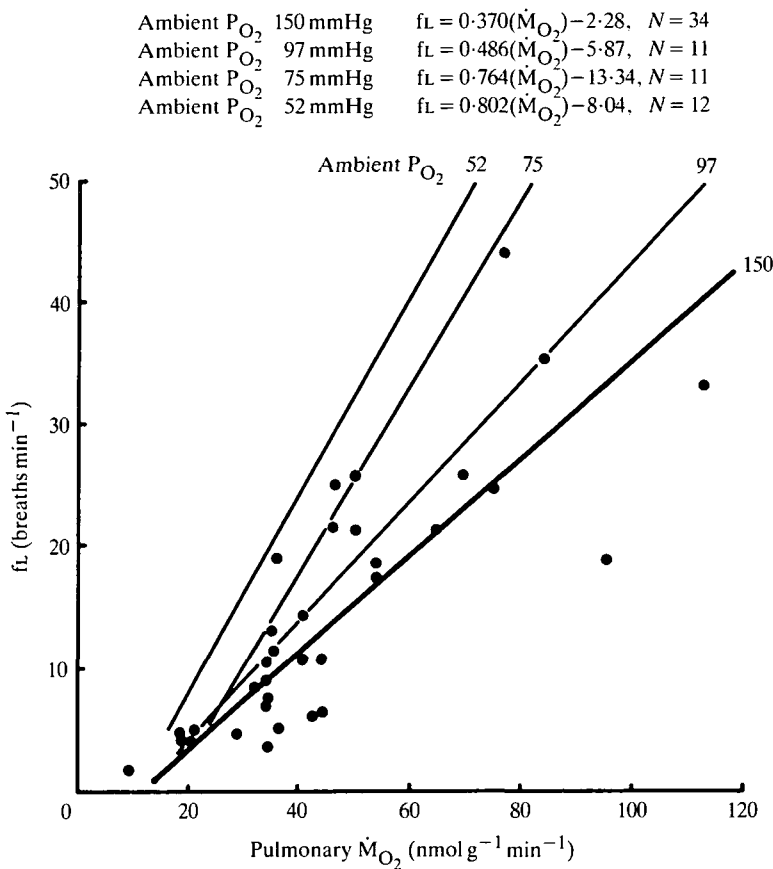

Fig. 2. Changes in ventilation frequency (f_L) with hypoxia. The data points and heavy regression line show the relationship between f_L and pulmonary $\dot{M}_{O_2}$ in normoxia. The other regression lines are for the three levels of hypoxia; for clarity, individual data points are not shown. The slope of each of the regression lines for hypoxic frogs is significantly different from that in normoxia ($P < 0.001$), although they are not significantly different from each other. n (number of animals) $= 7$ for all lines; N (the number of measurements) is noted next to the appropriate regression line equation at the top of the figure.
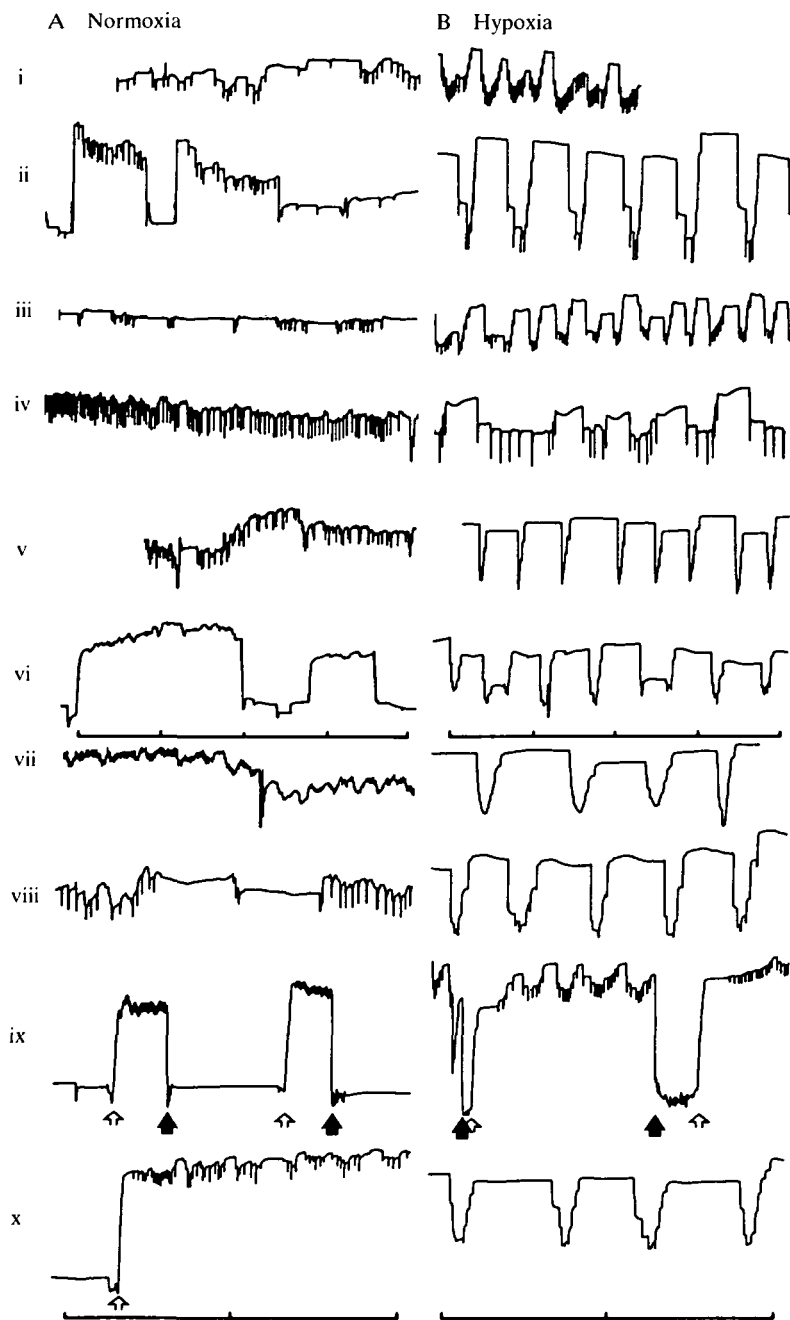
Fig. 3. Change in ventilatory pattern in hypoxia. The ventilation patterns in 10 frogs were recorded in normoxia (A) and in hypoxia (B) (ambient $P_{O_2} = 52$ mmHg). Adjacent traces are from the same animal. Seven of these animals (iv–x) were used in the full set of experiments of lung ventilation and hypoxia, the other three (i–iii) were used in preliminary experiments and did not provide data for further analysis. Solid arrows indicate the start of a dive, open arrows indicate the end of a dive. Time marker in minutes in all traces.

DISCUSSION

Lung ventilation in normoxia

The predominant adjustment by *Rana pipiens* to increased O_2 demand in these experiments is to increase pulmonary ventilation and $\dot{M}_{O_2}$. Lung ventilation is proportional to $\dot{M}_{O_2}$ in mammals (Dejours, 1981; Piiper, 1982) and reptiles (Jackson, 1978; Glass & Wood, 1983). Although this is thought to be true in amphibians as well (Withers & Hillman, 1983; Gottlieb & Jackson, 1976), there are few measurements in amphibians which actually show this relationship. If ventilation is measured over a longer period, as in this study, then analytical problems associated with short-term variability in ventilation pattern (Shelton & Boutilier, 1982) are decreased, and it becomes evident that lung ventilation rate (f_L) is indeed directly proportional to $\dot{M}_{O_2}$ in *R. pipiens* (Fig. 2).
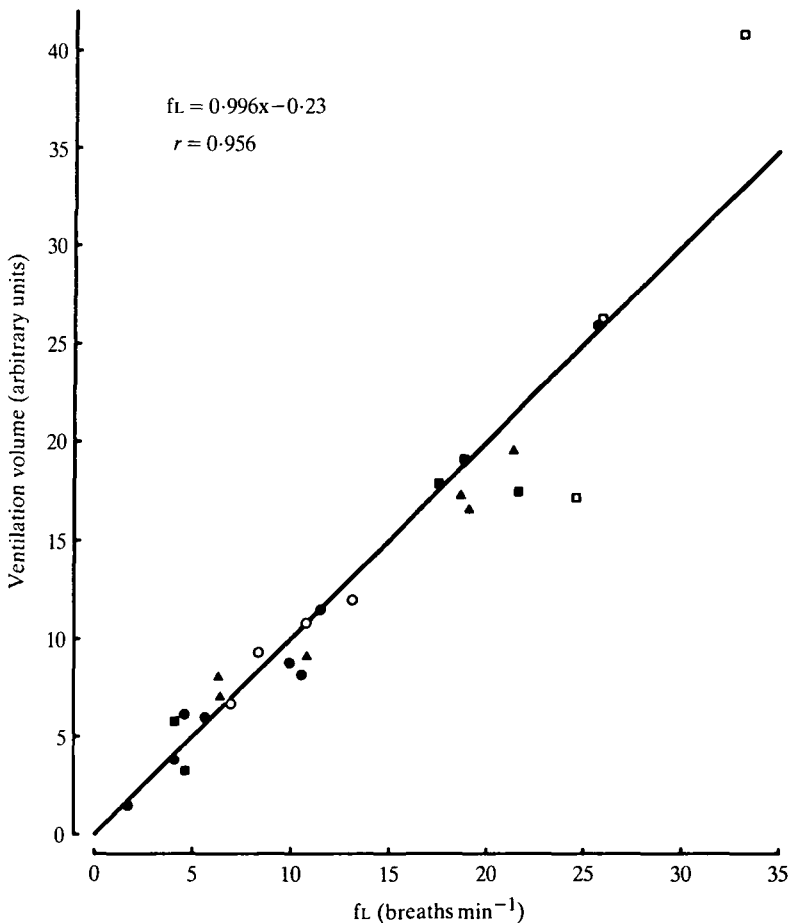

Fig. 4. Normalized ventilation volume as a function of ventilation frequency, f_L. Different symbols are used for different animals. Number of measurements = 26, number of animals = 5.
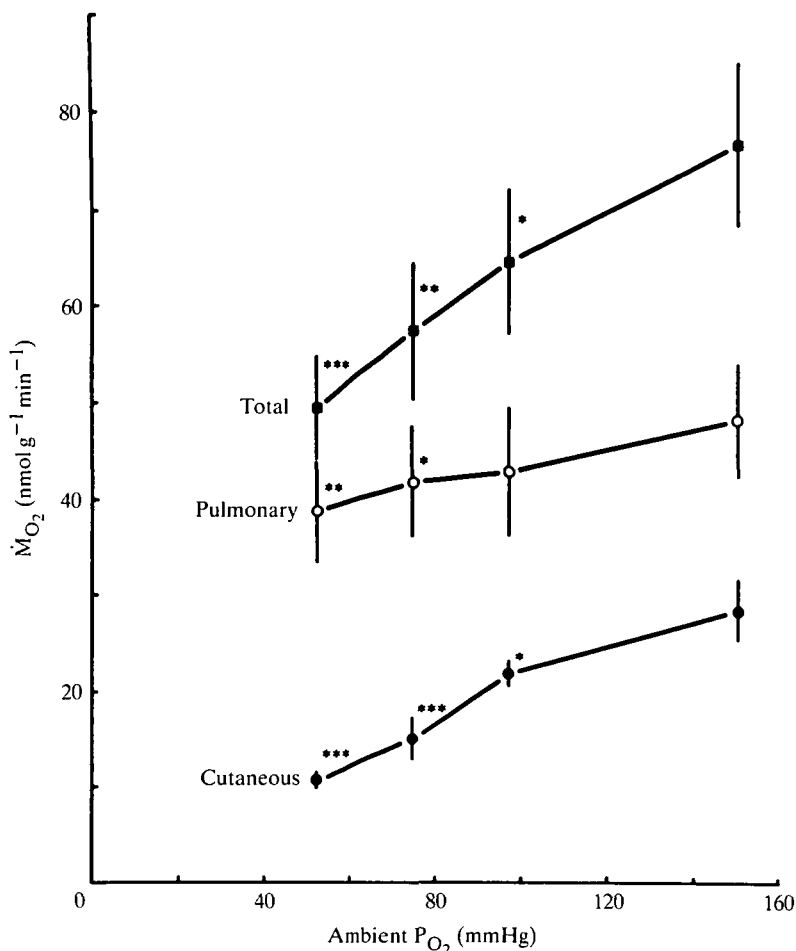
Fig. 5. Partitioning of $\dot{M}_{O_2}$ in hypoxia. All $\dot{M}_{O_2}$ measurements for each animal at each oxygen level were averaged to give a single $\dot{M}_{O_2}$ measurement per animal at each oxygen level. Asterisks denote differences of hypoxic measurements from normoxic measurements (see Materials and Methods). Number of animals = 7.

Pulmonary ventilation volume has not been measured in relation to $\dot{M}_{O_2}$ in any amphibian, although alveolar ventilation has been calculated for *R. pipiens* (Withers & Hillman, 1983). Tidal volume in *R. pipiens* (the stroke volume of the buccal pump), although highly variable from breath to breath, does not change with fL, so ventilation volume increases in direct proportion with fL. Thus, the air convection requirement (the volume of air ventilated for a given $\dot{M}_{O_2}$) remains constant, as it does in mammals and reptiles during exercise (Jackson, 1978; Dejours, 1981).

Skin ventilation

Cutaneous $\dot{M}_{O_2}$ in *R. pipiens* decreases when the skin is not ventilated (Fig. 1; Table 2), as found by Burggren & Feder (1986) in *Rana catesbeiana*. Unlike the experiments of Burggren & Feder (1986), in which the frogs were immobilized, the
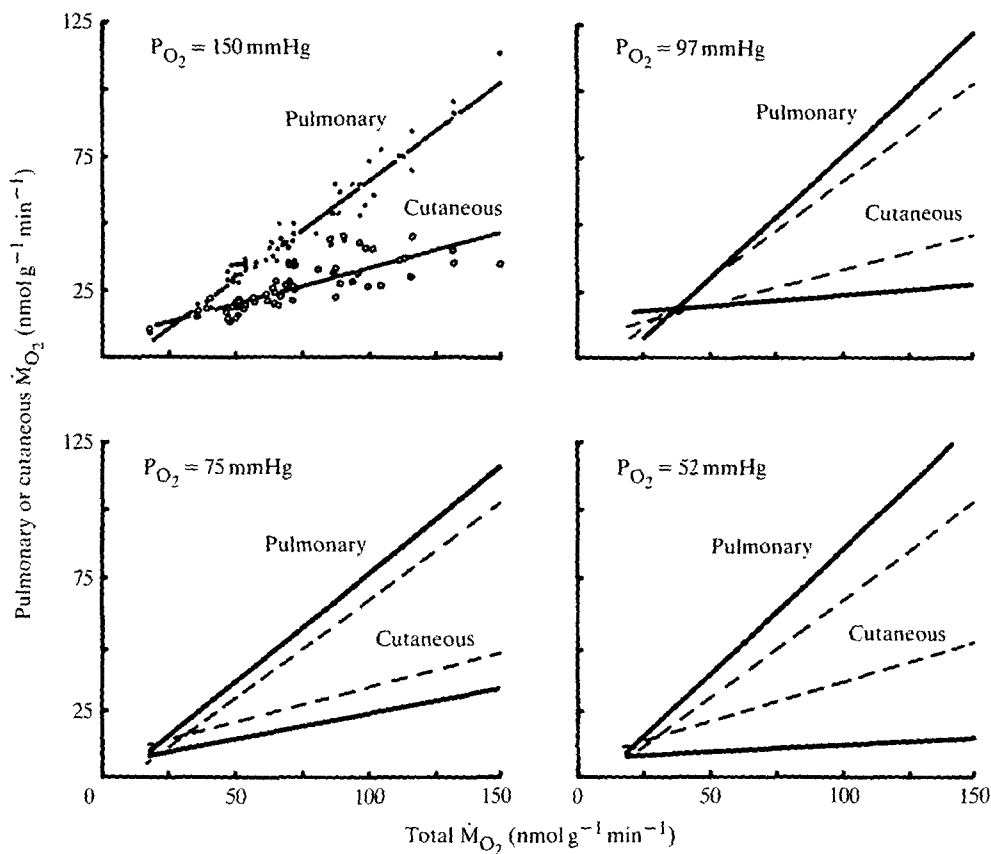
Fig. 6. Interactions of hypoxia and activity with partitioning of $\dot{M}_{O_2}$. Individual measurements are plotted for the relationship in normoxia ($P_{O_2} = 150\,\text{mmHg}$). For comparison, the regression lines for normoxia are plotted as dashed lines on each of the graphs for the three levels of hypoxia. Number of animals = 7.

frogs in the present study were free to move. Thus the skin could be ventilated either by voluntary locomotor movements or, during periods of inactivity, by experimental stirring. Movement within the respirometer (swimming, diving and struggling) simultaneously increases both the total $\dot{M}_{O_2}$ and skin ventilation. Thus cutaneous $\dot{M}_{O_2}$ (dependent on skin ventilation) increases with increasing total $\dot{M}_{O_2}$ (dependent on the amount of movement). The dependence of cutaneous $\dot{M}_{O_2}$ on total $\dot{M}_{O_2}$ must be largely due to skin ventilation, rather than some other change associated with activity, because in stirred respirometers cutaneous $\dot{M}_{O_2}$ is high and increases little with activity.

Although skin ventilation is a major factor affecting cutaneous $\dot{M}_{O_2}$ of frogs in water, it is not the only one. Two out of seven frogs tested in both stirred and unstirred respirometers showed significant correlations of cutaneous $\dot{M}_{O_2}$ with total $\dot{M}_{O_2}$ even in a stirred respirometer. Also, cutaneous $\dot{M}_{O_2}$ in resting animals is significantly lower than in active animals in both stirred and unstirred respirometers (although there is much less difference between them in the stirred respirometer than

in the unstirred respirometer). An increase in the P_{O_2} gradient across the skin at higher $\dot{M}_{O_2}$ values, as suggested by Gottlieb & Jackson (1976), could account for at least some of the correlation. An increase in cutaneous diffusing capacity, as demonstrated in *Rana catesbeiana* during submergence in hypoxic water (A. Pinder, in preparation) or with changes in skin ventilation (Burggren & Feder, 1986), could also be a factor.

Effects of hypoxia

Lung ventilation

Frogs exposed to combined aerial and aquatic hypoxia increase lung ventilation, increase the proportion of $\dot{M}_{O_2}$ from the lungs, and decrease total $\dot{M}_{O_2}$. Increased fL during hypoxia in amphibians has been noted previously in adult *Bufo marinus* (Boutilier & Toews, 1977) and tadpoles of *Rana berlandieri* and *R. catesbeiana* (Feder, 1983; West & Burggren, 1982). Withers & Hillman (1983) also found an increase in ventilation in hypoxia when they calculated ventilation volume in adult *R. pipiens* exposed to aerial hypoxia. The fL of *R. pipiens* in the present study more than doubled at a P_{O_2} of 52 mmHg compared to the rate in normoxia (Fig. 2). An increase in ventilation during hypoxia should decrease the difference between alveolar P_{O_2} and ambient P_{O_2}, thereby partially offsetting a decrease in ambient P_{O_2}.

Although fL increased during hypoxia, the most dramatic and immediately noticeable effect is a change in ventilation from an extremely irregular pattern in normoxia to a more regular hypoxic pattern consisting of large oscillations in lung volume over the course of several buccal movements (compare Fig. 3A with Fig. 3B). This pattern of ventilation during hypoxia in *R. pipiens* is similar to the lung inflation cycles described by Boutilier & Toews (1977) in *Bufo marinus* during hypoxia. The effect of this change in ventilation pattern, which consists largely of an increase in depth and a decrease in frequency of ventilation, may be to reduce functional dead space by reducing mixing of inhaled and exhaled gas within the buccal cavity (West & Jones, 1975; de Jongh & Gans, 1969).

Partitioning of $\dot{M}_{O_2}$

Lung hyperventilation affects $\dot{M}_{O_2}$ partitioning during hypoxia, although ventilation is not the only reason for changes in partitioning. Most of the decrease in total $\dot{M}_{O_2}$ seen in hypoxia is in cutaneous $\dot{M}_{O_2}$. Pulmonary $\dot{M}_{O_2}$ changed little (Fig. 5), probably because of lung hyperventilation; as a result, the pulmonary contribution to total $\dot{M}_{O_2}$ increases. An increase in the proportion of $\dot{M}_{O_2}$ taken through the actively ventilated gas exchange organ has also been observed in studies of $\dot{M}_{O_2}$ partitioning between skin and gills of anuran larvae during aquatic hypoxia (Feder, 1983; Wassersug & Feder, 1983). In these studies, partitioning between skin and gills (the lungs were found to be of lesser importance) shifted towards the gills during hypoxia, largely because gill ventilation was increased to compensate for a decreased ambient P_{O_2}.

In addition to differences in ventilation mechanisms, lungs and skin differ in that cutaneous gas exchange is primarily diffusion limited, whereas pulmonary gas exchange is much less so (Piiper & Scheid, 1977; Burggren & Moalli, 1984; Feder & Burggren, 1985). One result of the diffusion limitation of the skin is that the decrease in ambient P_{O_2} during hypoxia is probably reflected in a decrease in the P_{O_2} gradient across the skin, and therefore a decreased cutaneous $\dot{M}_{O_2}$. The gradient across the skin may also be affected by a Bohr shift of haemoglobin–oxygen affinity due to lung hyperventilation and decreased blood P_{CO_2}.

Hypoxia and activity interact in their effects on cutaneous $\dot{M}_{O_2}$ and the partitioning of $\dot{M}_{O_2}$ (Fig. 6). Cutaneous $\dot{M}_{O_2}$ is reduced in hypoxia for the reasons stated above. In addition, cutaneous $\dot{M}_{O_2}$ is affected by skin ventilation at all ambient P_{O_2} values, as it is in normoxia. As a result of these two effects, the maximum cutaneous $\dot{M}_{O_2}$ (at high activity levels and therefore high $\dot{M}_{O_2}$) decreases with hypoxia, and the slope of the regression line of cutaneous $\dot{M}_{O_2}$ on total $\dot{M}_{O_2}$ is concomitantly reduced.

Partitioning of $\dot{M}_{O_2}$ is thus extremely variable, and the lungs can potentially account for anything between zero (in the case of a submerged frog) and 100 % (in a frog in hypoxic water with access to air) of the total $\dot{M}_{O_2}$. A complete understanding of gas exchange partitioning must include knowledge of internal conditions such as haemoglobin–oxygen affinity, partial pressures of blood gases, acid–base status and distribution of blood flow to lungs, skin and body. These, in turn, will be related to ventilation of lungs and skin, ambient P_{O_2} and total oxygen consumption of the animal.

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