Pulmonary diffusing capacity of the bullfrog *(Rana catesbeiana)*

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GLASS, M. L., BURGGREN, W. W. & JOHANSEN, K.: Pulmonary diffusing capacity of the bullfrog (*Rana catesbeiana*). Acta Physiol Scand 1981, 113: 485–490. Received 26 Feb. 1981. ISSN 0001-6772. Department of Zoophysiology, University of Aarhus, Denmark.

Carbon monoxide diffusing capacity of the lungs (D_{LCO}) was measured in bullfrogs, *Rana catesbeiana* (mean body weight 260 g) along with oxygen uptake, pulmonary perfusion and lung volume. The measurements were all performed by methods depending on mass-spectrometry. Pulmonary oxygen uptake, D_{LCO} and perfusion all increased with body temperature. At 20°C O_2-uptake was 0.49 ml STPD · kg^{-1} · min^{-1} at rest and D_{LCO} was 0.022 ml STPD · kg^{-1} · min^{-1} · $Torr^{-1}$. At 30°C the values for O_2-uptake and D_{LCO} approximately doubled. Lung volume was large (90 ml · kg^{-1}) and independent of body temperature. Oxygen uptake and D_{LCO} of the bullfrog were small compared to values for a similar-sized mammal but the ratios of D_{LCO} to O_2-uptake quite similar. Analysis of available data on D_{LCO} and O_2-uptake in ectotherms also suggests a close correlation between O_2-uptake and D_{LCO}.

Key words: Rana catesbeiana, bullfrog, CO-diffusing capacity, lung volume, lung perfusion, O_2-uptake, mass-spectrometry

Bohr suggested in 1909 that an equation similar to Ohm's law in physics could be used to define the diffusive conductance of a gas exchanger. For pulmonary gas exchange the equation states that $\dot{V}_x = D_{Lx} \cdot \overline{\Delta P}_x$, where D_{Lx} = diffusing capacity for the gas x, $\dot{V}_x$ = flux (uptake or elimination) of x through the pulmonary membrane and $\overline{\Delta P}_x$ = mean partial pressure difference for x between alveolar gas and pulmonary capillary blood. Diffusing capacities are most frequently measured using carbon monoxide (Forster & Crandall 1976).

In spite of considerable information on diffusing capacities of mammalian lungs, such data are scarce for ectothermic vertebrates and none are available for pulmonary gas exchange in amphibians. Thus, the present study on bullfrogs (*Rana catesbeiana*) reports values for D_{LCO}, as well as values for lung volume, oxygen uptake and pulmonary perfusion. Experiments were performed at two temperatures (20 and 30°C), since pulmonary gas exchange may be strongly influenced by body temperature.

METHODS

7 bullfrogs (*Rana catesbeiana*) were maintained in shallow water at 20°C during the days before experimentation. All frogs accepted food and their weights ranged from 160 to 310 g (mean 260 g). Both lungs of the frogs were cannulated during anesthesia, which was achieved by immersion in a weak solution of tricaine methansulfonate (MS 222) for about 15 min. The lungs were exposed by short dorsolateral incisions (ca. 5 mm). Polyethylene catheters (PE 160) with side-holes close to the tip, were inserted through the lung wall and positioned about 10 mm into the lung lumen. A small amount of lung tissue was tied around the catheters to prevent blood loss and leakage of lung gas at the points of catheter insertion. The skin incisions were treated with topical antibiotics and sutured to firmly secure the catheters to the skin. The bilaterally placed lung catheters were in a specially constructed T-piece sutured to the dorso-medial surface of the frog. A third, closable catheter was connected to the T and led away from the frog. This catheter served for ventilation of the lungs during the experiment and for gas sampling.

For experimental purposes frogs were confined in small, transparent and perforated chambers. The chambers were placed in water baths at a depth of about 10 cm. The frogs stayed submerged most of the time, but could easily reach the surface for breathing. All measurements

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took place in a climatic chamber at 20° or 30°C. The frogs were allowed 10 h to acclimate before any measurements were performed. Measurements were obtained on the submerged frog during the first minute following voluntary breathing episodes at the surface and included: Lung volume by He-dilution (cf. West 1974), pulmonary perfusion by acetylene clearance (cf. Butler 1965), CO-diffusing capacity also by clearance technique (cf. Forster & Crandall 1976) and O_2-uptake. The measurements were performed by rebreathing and analysis by mass-spectrometry (Medspect 2 respiratory mass-spectrometer, Searle) and required a known volume of a gas mixture to be introduced into the lungs. This mixture contained: 0.5% $C^{18}O$, 4.4% He, 2% acetylene, 21% O_2 and 72.1% N_2. Helium, O_2 and N_2 were fed from gas cylinders into a Wösthoff gas mixing pump whereas measured volumes of acetylene (99.6%) and $C^{18}O$ (5% in N_2) were added to samples from the gas mixing pump. The outputs of the mass-spectrometer were recorded on two Hewlett-Packard 2-channel recorders (model 7132).

The catheter for introduction of test gases into the lungs fitted into the inlet of the mass-spectrometer. Valves at the sample inlet could be adjusted so that room air or calibration gas was sampled between periods of sampling from the lungs. Syringes containing test gases would also fit into the valves. During measurements the valves were adjusted to provide connection between the test gas mixture in the syringe and alveolar gas. The mass-spectrometer sampled from the lung-syringe gas volume at at minimum rate of 3 ml · min^{-1}. The volume initially injected into the lungs was adjusted to be approximately equal to the volume sampled over a 1 min period. During the measurements small volumes (5 ml) corresponding to about 20% of the lung volume were withdrawn and re-injected repeatedly at a frequency of about 25 breaths · min^{-1} to achieve complete mixing of test gases with alveolar gas (forced rebreathing). Consequently, the forced ventilation of the lungs was about 125 ml · min^{-1}, which corresponds to several times the total lung volume of the bullfrog (see later). Undoubtedly, this ventilation is large compared to normal values for bullfrogs, because voluntary pulmonary ventilation in frogs alternates with periods during which only the buccal cavity is ventilated (Jongh & Gans 1969), and tidal volume may be about 2 ml (Glass et al. 1978). Besides, Tazawa et al. (1979) estimated that the effective ventilation of bullfrog lungs is 12.1 ml BTPS · min^{-1} for a 265 g specimen. However, rebreathing techniques may require hyperventilation to avoid large oscillations of intrapulmonary gas pressures, because errors will be introduced if oscillations exist and are not corrected for in calculations (cf. Adaro et al. 1973). Oscillations were usually small after 10 to 15 s and measurements were concluded after 60 s of rebreathing.

The following calculations were based on the mass-spectrometer analyses:

Changes in fractional He-concentration allowed calculation of gas volume of the "system" (lung + syringe volume) according to the equation:

$$V_i \cdot F^i_{He} = V_{syst.} \cdot F^f_{He} \text{ (cf. West 1974),}$$

where V_i = initial syringe volume, F^i_{He} = initial fractional He-concentration in the syringe, $V_{syst.}$ = system volume and F^f_{He} = final fractional concentration of He in the system after complete mixing of gases.

Oxygen uptake was calculated from system volume (corrected to STPD) and the rate of decrease in fractional concentration of O_2 after nearly complete mixing. The equation for O_2 uptake is:

$$\dot{V}_{O_2} = V_{syst.}(STPD) \cdot \frac{-\Delta F_{O_2}}{\Delta t} \text{ (cf. Crawford et al. 1976).}$$

Pulmonary perfusion was calculated on basis of acetylene clearance during rebreathing. Whereas the dissolution of He in lung tissue and blood is negligible, acetylene is dissolved in the lung tissue and removed by the blood perfusing the lungs (cf. Butler 1965). Several methods are available for calculation of pulmonary perfusion from acetylene clearance and the method here employed follows Triebwasser et al. (1977) and Bonde-Petersen et al. (1980) in requiring that the decrease of fractional concentration of acetylene (F_{ac}) during rebreathing is calculated relative to fractional concentration of the "insoluble" He. Based on relative acetylene concentrations a clearance factor was calculated as:

$$m = \frac{\Delta \ln(\text{rel. } F_{ac}(t))}{\Delta t}.$$

Then pulmonary perfusion was calculated as:

$$\dot{Q}_t = \frac{V^{ac}_{syst.} STPD \cdot 760 \cdot (-m)}{(P_B - P_{H_2O}) \cdot \alpha_{ac}},$$

where P_B = barometic pressure, P_{H_2O} = water vapor pressure (both in Torrs), α_{ac} = Bunsen solubility coefficient for acetylene in blood at body temperature of the frog. According to Schoen (1923) α_{ac} is 1.03 ml · ml^{-1} · atm^{-1} at 20°C and 0.82 ml · ml^{-1} at 30°C. Acetylene is not only present in the gas volume determined by He-dilution, but also in the lung tissue and in the blood contained in the pulmonary capillaries. Calculation of the total acetylene volume of the rebreathing system $V^{ac}_{syst.}$ followed the procedure described by Bonde-Petersen et al. (1980).

Calculation of CO-diffusing capacity is based on the equation $\dot{V}_{CO} = D_{CO} \cdot \overline{\Delta P}_{CO}$, in which $\overline{\Delta P}_{CO}$ is considered equal to P_ACO assuming that pulmonary capillary P_{CO} is negligible (Forster et al. 1954). In the rebreathing method the formal calculation of CO-diffusing capacity is very similar to the procedure employed in calculation of pulmonary perfusion (cf. Crawford et al. 1976). The slope of ln (rel. F_{CO}) versus time (m for CO) was calculated and CO-diffusing capacity obtained as:

$$D_{LCO} = \frac{V_{syst.} STPD \cdot (-m)}{(P_B - P_{H_2O})}.$$

RESULTS

Table 1 presents resting values for D_{LCO} and other respiratory variables in *Rana catesbeiana* at 20° and 30°C. Increases of oxygen and CO-diffusing capacity with temperature were clearly highly significant (paired t-test), whereas the increase of pulmonary perfusion with temperature was marginal.

Table. Rana catesbeiana: Values for respiratory variables at rest at 20° and 30°C

Mean body weight: 260 g. $\bar{X} \pm SE$. $N = 7$. A paired t-test gives P-values for the null hypothesis that mean values at 20° and 30°C are identical

Temperature, °C	20	30	P
Lung volume, ml · kg^{-1}	84 15	96 23	NS
Oxygen uptake, ml STPD · kg^{-1} · min^{-1}	0.49 0.05	1.11 0.15	<0.01
Pulmonary perfusion, ml · kg^{-1} · min^{-1}	25 3	35 4	<0.05
CO-diffusing capacity, ml STPD · kg^{-1} · min^{-1} Torr^{-1}	0.022 0.004	0.044 0.008	<0.01

Lung volume was the same at 20° and 30°C. With slight increase of oxygen uptake due to moderate activity CO-diffusing capacity also tended to increase slightly, but this aspect was not systematically studied, since activity levels could not be quantified.

DISCUSSION

Relationship between temperature and D_{LCO}

In order to discuss increases of D_{LCO} with temperature it is necessary to explain the composite nature of diffusing capacity. In the original definition of diffusing capacity Bohr (1909) assumed that all resistance to diffusion was within the pulmonary membrane between alveolar gas and pulmonary capillary blood. Later, it was recognized that additional resistance is located within the pulmonary capillaries (Kruhøffer 1954, Roughton & Forster 1957). This is expressed in an equation by Roughton & Forster (1957), in which resistances are added in series:

$$1/D = 1/D_M + 1/\theta \cdot V_c,$$

where $1/D$ = total resistance to diffusion (reciprocal of diffusing capacity), $1/D_M$ = membrane resistance, V_c = pulmonary capillary blood volume, and θ = the rate at which a gas (CO or O_2) diffuses in and reacts with a unit volume of capillary blood at a tension difference of 1 Torr (ml · ml^{-1} · min^{-1} · Torr^{-1}).

Increased diffusing capacity (decrease of diffusion resistance) may then result from increases of D_M, V_c and θ. Increase of temperature will affect the Krogh diffusion konstant, K, which is related to D_M ($D_M = A \cdot K/E$), where A = surface area of the pulmonary membrane and E = thickness of the membrane. However, increase of K with temperature is only about 1% per °C (Bartels 1971). Pulmonary perfusion increased with temperature in *Rana catesbeiana;* this increase may possibly, but not necessarily, be accompanied by an increase in pulmonary capillary blood volume (V_c). The rate of uptake of CO by whole blood (θ_{CO}) depends on rates of diffusion within the capillary compartments (plasma, red cell membrane and the intra-erythrocyte compartment) and also on the reaction speed between CO and hemoglobin (Roughton & Forster 1957). I is known from studies on mammals that θ_{CO} increases with temperature (Holland 1969, Lawson 1971), and this increase is sufficient to account for 50–60% of the temperature effects on diffusing capacity of isolated perfused mammalian lungs (Power et al. 1971). It is not possible to offer similar estimates for *Rana catesbeiana*, because information is not available for solving the equation of Roughton and Forster. An increase of CO-diffusing capacity with temperature has earlier been reported for reptiles (Glass et al. 1981). Therefore, it is tempting to speculate that in ectothermic vertebrates the diffusing capacities for O_2 as well as CO increase with temperature, meeting increased demands for O_2 at higher temperatures.

Oxygen uptake, lung volume and perfusion of the lungs

The pulmonary O_2-uptake at rest for *Rana catesbeiana* was slightly lower than the total O_2-uptake expected for a 260 g amphibian based on allometric relations (Hemmingsen 1960). This may reflect that 9 to 23% of total O_2-uptake in *Rana catesbeiana* is cutaneous (Gottlieb & Jackson 1976). When this fraction is corrected for the O_2-uptake is of the expected magnitude. The values of this study are somewhat lower than those measured by Tazawa et al. (1979). This refutes the possible objection to the present study that measurements of O_2-uptake and other respiratory variables should be performed over longer periods rather than during brief test periods such as employed.

It is more problematic to compare the present values for pulmonary perfusion in the bullfrog with those obtained in other studies, because variations in pulmonary blood flow may occur as a consequence of intermittent breathing (Meyers et al.

...ble ... *Rana catesbeiana* compared to a 260 g mammal

Values for the bullfrog at 30°C. Values for a 260 g mammal are based on allometric equations by Stahl (1967) and Takezawa (1980)

	Bull-frog	Mam-mal	Bullfrog/ mammal
Lung volume, $ml \cdot kg^{-1}$	96	17	5.6
Pulmonary O_2-uptake, $ml\ STPD \cdot kg^{-1} \cdot min^{-1}$	1.11	16.0	0.07
Pulmonary perfusion, $ml \cdot kg^{-1} \cdot min^{-1}$	35	253	0.14
CO-diffusing capacity, $ml\ STPD \cdot kg^{-1} \cdot min^{-1} \cdot Torr^{-1}$	0.044	0.54	0.08
CO-diffusing cap./ O_2-uptake, $Torr^{-1}$	0.040	0.034	1.20

1979). Though measured in submerged frogs, all measurements were made immediately following a series of breaths, and the values for pulmonary perfusion are probably closer to those existing during breathing than during diving. The values for pulmonary perfusion are in agreement with those obtained by Tazawa et al. (1979) on restrained bullfrogs.

The large lung volume calculated for *Rana catesbeiana* to about 90 $ml \cdot kg^{-1}$ may serve as an O_2-store during shallow dives. Prior to diving the arterial P_{O_2} of the bullfrog may be as high as 95 Torr (Lenfant & Johansen 1967). Assuming a slightly higher intrapulmonary P_{O_2} and a lung volume of 90 $ml \cdot kg^{-1}$, the volume of O_2 stored in the lungs will be about 11 ml $STPD \cdot kg^{-1}$. When this is compared to a resting O_2-uptake of 0.49 $ml\ STPD \cdot kg^{-1} \cdot min^{-1}$ at 20°C (Table 1) the bullfrog dives with an intrapulmonary O_2-store sufficing to fill 20 min of O_2-requirements if the store could be fully used.

Rana catesbeiana compared to an endotherm

The dependence of D_{LCO} on body temperature implies that interspecies comparisons preferably should be based on data obtained at comparable body temperatures. This requirement may not be easy to meet in all comparisons, because the range of normal body temperatures may be specific to a species or to a taxonomic group. Thus, the normal and preferred body temperatures of amphibians are generally lower than normal body temperatures of endothermic vertebrates (cf. Bartholomew 1972). Another requirement is that body weights should be similar. Some data exist on the allometric relations for D_{LCO} in mammals (cf. Takezawa et al. 1980), but such information is non-existent for other vertebrate classes. Thirdly, the data should be obtained on resting animals, because activity may be accompanied by increased diffusing capacity (cf. Forster & Crandall 1976). These requirements also apply to interspecies comparisons for other respiratory variables (O_2-uptake, pulmonary perfusion).

In Table 2 values for respiratory variables in *Rana catesbeiana* at 30°C are compared to those for a similar-sized mammal at rest. Values for a 260 g mammal are calculated from interspecies relations for respiratory variables as a function of body weight in mammals. Carbon monoxide diffusing capacity and lung volume (FRC) for a 260 g mammal are calculated from Takezawa et al. (1980). Oxygen uptake and pulmonary blood flow are calculated from Stahl (1967).

A comparison between *Rana catesbeiana* and mammals on this basis confirms that O_2-uptake of an ectotherm is an order of magnitude less than that of a similar-sized mammal (Hemmingsen 1960). This is also the case for CO-diffusing capacity. Consequently, the ratio of CO-diffusing capacity to O_2-uptake is quite similar in the bullfrog and in a 260 g mammal (Table 2). The ratio between pulmonary perfusion and O_2-uptake was somewhat greater in the frog than in the mammal. This may be related to the fact that the O_2-capacity of blood is smaller in the frog (Lenfant & Johansen 1967) and that the O_2-saturation of systemic arterial blood is lower in the frog due to mixing of pulmonary venous and systemic venous blood in the ventricle (Meyers et al. 1979).

Diffusing capacity for CO:
The bullfrog compared to other ectotherms

Data for pulmonary D_{LCO} in ectotherms are scarce (Crawford et al. 1976, Gatz et al. 1979, Glass et al. 1981), and the situation is complicated by different methods for analysis of experimental data (see Glass et al. 1981 for discussion of the problem). Despite difficulties in conforming to strict requirements for interspecies comparison for D_{LCO}, a consistent and fairly linear relationship ($r=0.96$) can be derived if data for D_{LCO} in *Rana catesbeiana* and in reptiles are plotted relative to O_2-uptakes (Fig. 1).

The values for D_{LCO} in *Rana catesbeiana* may not

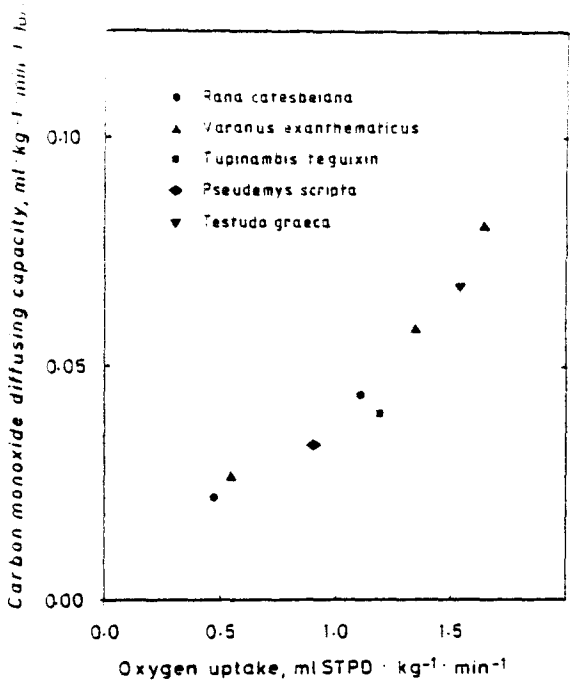

Fig. 1. Carbon monoxide diffusing capacity relative to O_2-uptake in various ectotherms. *Rana catesbeiana*, 260 g, 20° and 30°C: this study. *Pseudemys scripta*, 1 550 g, and *Testudo graeca*, 980 g, 20°–23°C: Crawford et al. (1976). *Tupinambis teguixin*, 2 200 g, 25°–27°C, and *Varanus exanthematicus*, 2 200, 17°–19°C, 25°–27°C and 35°–37°C: Glass et al. (1981).

represent values for amphibians in general, because *Rana* relies more heavily on pulmonary oxygen uptake than is the case for many other amphibians. Urodeles may largely depend on extrapulmonary gas exchange (skin or external gills) throughout their entire life cycle (Lenfant & Johansen 1967). In contrast, adult bullfrogs predominantly use lungs for oxygen uptake at 20–30°C and have a correspondingly high pulmonary diffusing capacity.

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