CHANGING RESPIRATORY IMPORTANCE OF GILLS, LUNGS AND SKIN DURING METAMORPHOSIS IN THE BULLFROG RANA CATESBEIANA

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Abstract. Oxygen uptake ($\dot{M}_{O_2}$) and carbon dioxide excretion ($\dot{M}_{CO_2}$) by the skin, lungs and gills (if present) of *Rana catesbeiana* have been measured at 20 C during 4 developmental stages – strictly water breathing tadpoles, air breathing tadpoles, post-metamorphic bullfrogs and 4-year-old adult bullfrogs.

In aquatic tadpoles, branchial performance is comparable to that of teleost fishes, but a large skin area to body mass ratio, particularly for the tail, plus a thin and highly vascularized skin, presumably facilitates a large (60% of total $\dot{M}_{O_2}$) cutaneous O_2 uptake. As development proceeds, $\dot{M}_{O_2}$ by the gills decreases and the lungs assume importance in O_2 uptake, but the skin remains the major organ of O_2 uptake until metamorphosis is nearly complete. Immediately after metamorphosis, O_2 uptake by the lungs is elevated to 80% of total $\dot{M}_{O_2}$.

Carbon dioxide excretion in both aquatic and air breathing tadpoles was also achieved mostly by the skin (60% of total $\dot{M}_{CO_2}$, R = 0.9). The lungs of air breathing tadpoles excreted less than 2% of total $\dot{M}_{CO_2}$, rising to a maximum of only 20% (R = 0.2) even in adult bullfrogs. The considerable importance of the skin to CO_2 excretion thus rises even further with the degeneration of the gills at metamorphosis, with R for the skin rising from 0.9 before metamorphosis to 7.5 in adults.

Thus, large adjustments in skin and lung gas exchange occur as the larval gills slowly degenerate, and lung ventilation is initiated and increased. Aquatic O_2 uptake is rapidly superceded by the uptake of O_2 from the air, while CO_2 excretion largely remains a function of the aquatic respiratory surfaces throughout the life cycle of the bullfrog.

Aquatic respiration	Metamorphosis
Carbon dioxide	Oxygen consumption
Gill respiration	Skin respiration
Lung respiration	Tadpole

Gas exchange processes in the vertebrates in many respects reach their highest levels of complexity in animals which exchange respiratory gases with both air and water. This complexity arises not only because perfusion and ventilation must be

regulated in more than one gas exchanger, but also because the physico-chemical properties of the two respiratory media are so different. The physiology of vertebrate bimodal breathers has thus attrached much attention (see Johansen, 1970; Singh, 1976; Randall *et al.*, 1981). However, almost all studies have centered upon adults rather than larvae, in part due to the difficulty of working with the much smaller larval forms. Yet, the physiological changes by which strictly water breathing larvae develop into air breathing adult forms can provide important physiological information on the phenomenon of bimodal breathing, as well as give further insights into putative stages in the evolution of air breathing in vertebrates.

Certain physiological aspects of development in bimodal breathers, *e.g.* changes in blood respiratory properties and acid–base balance (see Burggren and Wood, 1981, for references), have received attention. With respect to gas exchange, Whitford and Sherman (1968), for example, have examined O_2 partitioning between water and air in adults and in a single unspecified stage of larval development in the salamander *Ambystoma tigrinum*. However, we know of no systematic study relating both O_2 and CO_2 exchange to development in a bimodal breather.

This study, then, begins the investigation of changes in the processes of oxygen uptake and carbon dioxide elimination during the metamorphosis of a strictly aquatic amphibian larva into an air-breathing adult. The bullfrog, *Rana catesbeiana* was chosen for this study because (1) it is comparatively large at all developmental stages, (2) it undergoes extreme morphological and physiological changes during metamorphosis, (3) it has clearly defined developmental stages and (4) a substantial data base exists in the literature for the respiratory and cardiovascular performance in the adult.

Methods

EXPERIMENTAL ANIMALS

Experiments were performed on a total of 49 *Rana catesbeiana*, ranging from developmental stage IV to adult bullfrogs (see below). Animals were collected in western Massachusetts and maintained in filtered water at $20° \pm 2 °C$ for at least 2 weeks before experimentation. This temperature is well within their normal yearly range of temperatures (1–30 °C) at this location. Tadpoles were fed boiled spinach, while juvenile or adult bullfrogs were fed liver and mealworms.

TERMINOLOGY FOR DEVELOPMENTAL STAGES

Experiments were performed on *R. catesbeiana* at 4 different developmental levels, determined by the scheme of Taylor and Kollros (1946). The least developed tadpoles examined were at stages IV–V, in which hind limb buds were small, and

limb paddles had not appeared. The lungs had begun to develop, but were not ventilated, and all gas exchange was thus with the water through the gills and skin. Tadpoles at this level of development we refer to as aquatic tadpoles. The next developmental level examined consisted of stages XVI–XIX, in which the hind limbs were clearly emerging. The lungs were developed, and had to be regularly ventilated to avert drowning at 20 °C. Gills were still evident, and were actively ventilated by prominent buccal movements. *Rana* at this level of development we have termed air breathing tadpoles. At stage XXIII and XXIV, the gross anatomy (particularly the head) had changed such that the animal was clearly recognizable as a small frog, albeit with a regressing tail. The lungs were fully developed, while the gills were totally absorbed. All traces of the opercular spout had disappeared, and the body wall had closed in around the emergent front limbs. Such animals, whose body mass had not changed from the earlier tadpole stages, we have termed post-metamorphic bullfrogs. Finally, sexually mature, 4-year-old adult bullfrogs were examined.

CANNULATION OF TADPOLE OPERCULAR SPOUT

Aquatic or air breathing tadpoles were anaesthetised with MS 222 (1 : 10,000) buffered to pH 7.0 and the opercular spout was cannulated after the method of Gradwell (1970). Cannulae were constructed from 15 cm lengths of PE tubing (o.d. 1.9 mm, i.d. 1.5 mm) fitted with 3-cm tips of soft and flexible silicone rubber tubing (o.d. 1.5 mm, i.d. 1.2 mm). In the 3–6 g tadpoles used, the lumen of the uncannulated opercular spout was approximately 1.0 mm in diameter. When cannulated, the cross-sectional area of the lumen was thus increased approximately by 30%. We assumed this increased diameter would offset any tendency for reduced opercular spout flow caused by the flow resistance of the length of cannula.

Tadpoles were allowed to recover from anaesthesia and were then transferred to the respirometer described below.

MEASUREMENTS OF $\dot{M}_{O_2}$ AND $\dot{M}_{CO_2}$

Experimental procedures varied with developmental stage. In aquatic tadpoles, O_2 and CO_2 exchange by both skin and gills was measured. The most complex experiments were performed in air breathing tadpoles, in which O_2 and CO_2 exchange by the skin, gills and lungs was measured simultaneously. By stage XXIII and beyond to adulthood, the gills have been resorbed, and all aquatic exchange is cutaneous. In these animals, only pulmonary and cutaneous O_2 and CO_2 exchange was measured.

Cannulated tadpoles were placed in the flow-through respirometer depicted in fig. 1. The internal dimensions of the plexiglass chamber (4 cm deep, 3 cm wide,
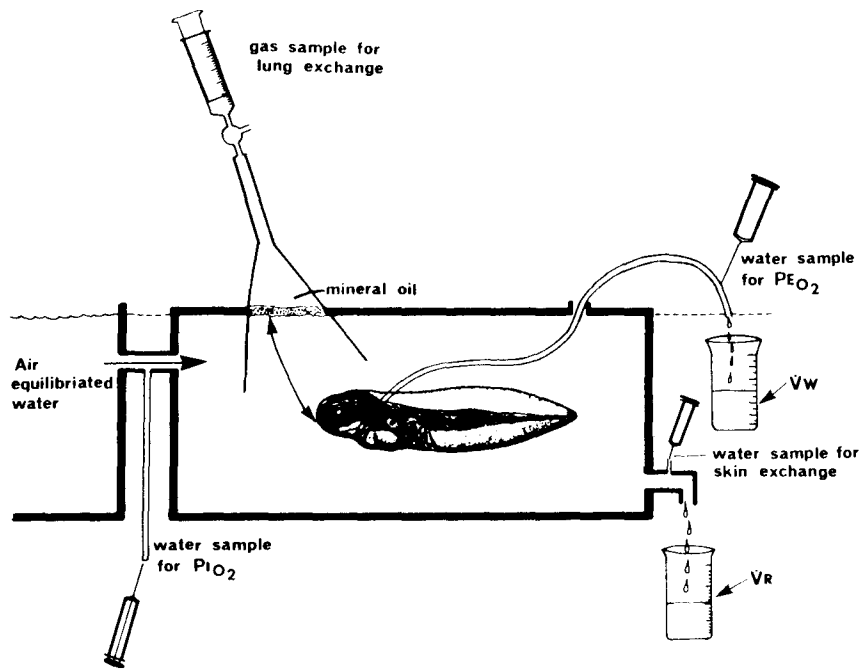
Fig. 1. Diagrammatic representation of the respirometer used for simultaneous determination of $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ by gills, lungs ans skin of the bullfrog tadpole. Water was collected from the opercular spout for determination of gill ventilation ($\dot{V}w$), and from the outflow tube for determination of respirometer water flow ($\dot{V}R$). See text for further details.

10 cm long) allowed movement back and forth or up and down in the chamber, but prevented tadpoles from turning through more than $45°$. The free end of the cannula in the opercular spout was led out through a 4-mm hole in the lid of the chamber, and positioned exactly at the water level in the respirometer. Since all water perfusing the gills passed through the opercular spout, the volume of water leaving the chamber *via* this cannula represented branchial water flow, $\dot{V}w$. Also, the gas partial pressures of water sampled from this cannula represented the partial pressures of exhaled branchial water, *i.e.* PE_{O_2} and PE_{CO_2}. Gill ventilation frequency (fG) was counted by direct observation through the respirometer wall.

A flow of air-saturated water (7–12 ml/min) was constantly maintained through the respirometer. Water entered the respirometer near the tadpole's head, flowing posteriorly over the animal and exiting beyond the tail (fig. 1). Knowing the change in P_{O_2} and P_{CO_2} of water passing through the respirometer, and the rate of water flow, $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ by the skin alone could be calculated (see below), since all water ventilating the gills excited via the spout cannula rather than into the respirometer. Crucial to this calculation was the assumption that the partial pressures of water inspired at the mouth were identical to those of water entering the respirometer. This was tested in two ways. Firstly, dye injected into the incoming water stream

revealed a laminar water flow through the chamber, so that water flowing past the mouth of the tadpole was not contaminated with water from more posterior regions of the respirometer which had flowed over the skin. Secondly, water samples from a cannula inserted into the respirometer at the level of the mouth had the same P_{O_2} and P_{CO_2} as water sampled from the inlet tube.

For air breathing tadpoles beyond stage XVI, there was in addition a small inverted glass funnel fastened into the lid of the respirometer (fig. 1). Attached to the funnel stem was a syringe, by which known volumes of air could be injected part way down the funnel mouth. The tadpoles were thus able to raise their head into the funnel to ventilate their lungs at will. The area of the total air water interface in the funnel was about 3 cm^2. A 1-2 mm thick layer of mineral oil was placed on the water in the funnel to prevent the exchange of respiratory gases between the water and gas phases. Behavior related to air breathing, as well as air breathing frequency, was identical when the mineral oil layer was absent or present. Ten minutes after injection of a known volume (usually 4.0 ml) of air into the funnel, the gas was withdrawn and its new volume and its P_{O_2} and P_{CO_2} recorded (see below). From these data, $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ attributable to the lungs could be determined by calculating the rate of O_2 depletion and CO_2 addition.

In the case of stage XXII XXIV animals and adults, which of course had no opercular cannula, individuals were placed in a functionally similar respirometer composed of a glass chamber with a volume of either 100 ml (tadpole) or 4000 ml (adult). The lid of each chamber was constructed from an inverted glass funnel into which the animal could rise to breathe air. Air in the funnel (25 ml for adults), was separated from the water by a layer of mineral oil, and was renewed periodically as with the tadpole chambers. A constant flow of water was maintained through the respirometer so the $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ of skin and lungs could be measured simultaneously.

All animals were allowed at least 18 hours to acclimate to the respirometer before measurements were begun. During this period, a constant flow of air equilibrated water was maintained through the chamber, and the funnel was continuously ventilated with a stream of moist air. The walls of all the respirometers were covered with a semi-opaque material to shield the movements of the investigators.

CALCULATION OF $\dot{M}_{O_2}$ AND $\dot{M}_{CO_2}$ FROM GAS AND WATER PARTIAL PRESSURE

The P_{O_2} and P_{CO_2} of gas and water samples was measured with an Instrumentation Laboratory Micro-13 blood gas analyzer regulated to 20.0 ± 0.1 C. Problems of measuring CO_2 and O_2 partial pressures in the study of gas exchange in small bimodal breathers have been addressed by Burggren (1979). The use of expanded scales on the electrometers, careful selection of electrodes and membrane material, and, for P_{CO_2}, calibration in 0.2 0.5 mm Hg P_{CO_2} increments contributed greatly toward accuracy. $\dot{M}_{O_2}$ of the skin and gills (if applicable) was calculated from the

difference in O_2 concentration of water ($\Delta P_{O_2} \times O_2$ solubility at $20°$) before and after flowing over the gills or skin. Conversion of P_{CO_2} to CO_2 content using a solubility factor for distilled water can be problematic if the water used contains carbonates (Dejours, Armand and Verriest, 1968), as the tap water in Amherst does. To circumvent these complications, a Natelson microgasometer was used to determine the total CO_2 concentration of tap water samples at various P_{CO_2}'s, allowing construction of a P_{CO_2}–CO_2 concentration curve for water at $20\,°C$. By using P_{CO_2} differences between inspired and expired water to determine the actual increase in CO_2 concentration, $\dot{M}_{CO_2}$ of the skin or gills could be readily calculated.

SKIN SURFACE AREA

Freshly killed tadpoles were weighed, and the tail severed from the body at the level of the cloacal vent. The trunk and tail were then weighed separately, and the skin carefully removed from each piece. Skin surface area of each section was determined by cutting the skin into pieces which would lay flat without distortion, and then tracing their outline onto a uniform density graph paper, which was subsequently weighed.

All data were analyzed to provide means and standard errors, and the differences between population means were assessed with Student's 't' test, using a significance limit of $P < 0.05$.

Results

BRANCHIAL VENTILATION AND GAS EXCHANGE

Values for gill ventilation and branchial gas exchange in both aquatic and air breathing tadpoles are presented in table 1. $\dot{M}_{O_2}$ by the gills of 'aquatic' tadpoles represented approximately 41% of total $\dot{M}_{O_2}$, with a similar percentage of total CO_2 excretion occurring via the gills. Expired P_{O_2} was low at 49 mm Hg, while O_2 utilization by the gills was thus relatively high, at about 64%. Gill ventilation frequency was above 90 beats/min at $20\,°C$, generating a total branchial water flow of nearly 0.3 ml/g/min.

Although the gills were still present and actively ventilated in air breathing tadpoles, significant changes in gill performance had developed. The frequency of gill ventilation (fG) remained unchanged, but stroke volume (VS) decreased such that a significant fall in $\dot{V}w$ occurred in air breathing tadpoles. Both P_{CO_2} and P_{O_2} were significantly higher in expired water, compared with the earlier stages. Oxygen utilization was not significantly different but the O_2 uptake by the gills, and its contribution to total $\dot{M}_{O_2}$ by the gills was considerably greater in the more advanced tadpole stages.

TABLE 1

Branchial performances in bullfrog tadpoles of two developmental levels. Mean values ± standard error are given. An asterix in the second column indicates a significant difference ($P < 0.05$) from the earlier developmental level.

	Aquatic tadpole (Stage IV-V)	Air breathing tadpole (Stage XVI-XIX)
Number of animals	6	8
$\dot{V}w$ (ml/g/min)	0.27 ± 0.03	0.15 ± 0.01
f_G (cycles/min)	93 ± 6	96 ± 5
Vs (ml/g)	0.003 ± 0.000	0.002 ± 0.000
$P_{I_{O_2}}$ (mm Hg)	139 ± 2	146 ± 0*
$P_{E_{O_2}}$ (mm Hg)	49 ± 4	60 ± 2*
O_2 utilization (%)	64 ± 3	59 ± 1
$P_{I_{CO_2}}$ (mm Hg)	0.8 ± 0.1	0.7 ± 0.0
$P_{E_{CO_2}}$ (mm Hg)	2.7 ± 0.2	3.7 ± 0.3*
$\dot{M}_{O_2}$ (gills) (μmol/g/h)	2.3 ± 0.2	1.2 ± 0.1*
$\dot{M}_{O_2}$ (gills) (% of $\dot{M}_{O_2}$ total)	41 ± 4	17 ± 2*
$\dot{M}_{CO_2}$ (gills) (μmol/g/h)	1.7 ± 0.2	3.0 ± 0.2*
$\dot{M}_{CO_2}$ (gills) (% of $\dot{M}_{CO_2}$ total)	42 ± 4	44 ± 4

Further experiments were designed to study aspects of diffusion/ventilation limitations in branchial gas exchange, and to determine whether cannulation of the opercular spout might be impairing branchial gas exchange. An increase in hydrostatic pressure head across the gills was induced by lowering the level of the opercular cannula tip, which remained in air outside the respirometer (fig. 1), 1-2 cm below the level of water in the respirometer. This produced a significant rise in $\dot{V}w$ of over 150% (table 2). Gill ventilation frequency (f_G) fell significantly, while Vs tripled. Not surprisingly, $P_{E_{CO_2}}$ fell, $P_{E_{O_2}}$ rose, and the O_2 utilization decreased sharply. Importantly however, $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ of the gills were not significantly changed, nor were their percentage contributions to total gas exchange. These data indicate that water flow over the gills of cannulated tadpoles under normal conditions (i.e. no artificial pressure head) was not limiting to either branchial O_2 uptake or CO_2 excretion.

BIMODAL GAS EXCHANGE AND O_2 AND CO_2 PARTITIONING

Total $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$, as well as the individual contribution of gills, lungs and skin were determined in the four developmental stages (table 3). Total $\dot{M}_{O_2}$ was approximately 6-7 μmol/g/h in the first three developmental levels examined, but fell to only 1 μmol/g/h in the adult bullfrogs, which were 15 times greater in body weight. Total $\dot{M}_{CO_2}$ was slightly smaller than $\dot{M}_{O_2}$ at all four developmental levels, yielding an overall respiratory exchange quotient of 0.74-0.98.

TABLE 2

Branchial performance and gas exchange in aquatic tadpoles (stages XVI-XIX) during normal gill ventilation and with a 1 cm H_2O pressure head assisting gill ventilation. Mean values $\pm$ 1 standard error are given. Asterix in second column indicates significant difference from values in first column (*. $P < 0.05$; **. $P < 0.01$)

	Normal gill ventilation	Assisted gill ventilation
Number of animals	8	7
$\dot{V}w$ (ml/min/g)	0.26 $\pm$ 0.02	0.64 $\pm$ 0.10*
f_G(beats/min)	95 $\pm$ 1	71 $\pm$ 2*
Vs (ml/g)	0.003 $\pm$ 0.000	0.009 $\pm$ 0.001*
PI_{O_2} (mm Hg)	137 $\pm$ 3	137 $\pm$ 4
PE_{O_2} (mm Hg)	48 $\pm$ 4	85 $\pm$ 4**
O_2 utilization (%)	64 $\pm$ 3	38 $\pm$ 3**
PI_{CO_2} (mm Hg)	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1
PE_{CO_2} (mm Hg)	3.3 $\pm$ 0.4	1.7 $\pm$ 0.3
$\dot{M}_{O_2}$ (total) (μmol/g/h)	6.5 $\pm$ 0.3	7.5 $\pm$ 1.4
$\dot{M}_{O_2}$ (gills) (μmol/g/h)	2.3 $\pm$ 0.2	2.8 $\pm$ 0.2
$\dot{M}_{O_2}$ (gills) (% of $\dot{M}_{O_2}$ total)	35 $\pm$ 3	40 $\pm$ 2
$\dot{M}_{O_2}$ (total) (μmol/g/h)	4.6 $\pm$ 0.2	5.3 $\pm$ 0.3
$\dot{M}_{CO_2}$ (gills) (μmol/g/h)	1.7 $\pm$ 0.1	1.9 $\pm$ 0.2
$\dot{M}_{CO_2}$ (gills) (% of $\dot{M}_{CO_2}$ total)	39 $\pm$ 2	34 $\pm$ 3

TABLE 3

$\dot{M}_{O_2}$, $\dot{M}_{CO_2}$, and R of the gills, skin and lungs of selected developmental levels of *Rana catesbeiana*. Mean values $\pm$ 1 standard error are given. $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ are expressed in μmol gas/g/h.

|	Aquatic tadpole (Stage IV-V)	Air breathing tadpole (Stage XVI-XIX)	Post-metamorphic tadpole (Stage XXIII-XXIV)	Adult bullfrog
Number of animals	6	8	9	6
Body weight	3.6 $\pm$ 1.03	5.3 $\pm$ 1.2	4.3 $\pm$ 1.2	228.2 $\pm$ 70.3
$\dot{M}_{O_2}$ (skin)	3.4 $\pm$ 0.4	4.6 $\pm$ 0.4	1.7 $\pm$ 0.3	0.2 $\pm$ 0.0
$\dot{M}_{O_2}$ (gills)	2.3 $\pm$ 0.2	1.2 $\pm$ 1.2	(gills absent)	(gills absent)
$\dot{M}_{O_2}$ (lungs)	(not ventilated)	1.2 $\pm$ 0.4	4.3 $\pm$ 0.3	1.1 $\pm$ 0.1
$\dot{M}_{O_2}$ (total)	5.9 $\pm$ 0.3	6.9 $\pm$ 0.5	7.0 $\pm$ 0.5	1.1 $\pm$ 0.1
$\dot{M}_{CO_2}$ (skin)	2.6 $\pm$ 0.3	4.2 $\pm$ 0.6	4.9 $\pm$ 0.6	0.9 $\pm$ 0.1
$\dot{M}_{CO_2}$ (gills)	1.7 $\pm$ 0.2	3.0 $\pm$ 0.2	(gills absent)	(gills absent)
$\dot{M}_{CO_2}$ (lungs)	(not ventilated)	0.2 $\pm$ 0.0	0.6 $\pm$ 0.1	0.2 $\pm$ 0.1
$\dot{M}_{CO_2}$ (total)	4.3 $\pm$ 0.2	7.3 $\pm$ 0.7	5.4 $\pm$ 0.6	1.1 $\pm$ 0.1
R (skin)	0.9 $\pm$ 0.2	0.9 $\pm$ 0.1	3.2 $\pm$ 0.7	7.6 $\pm$ 1.5
R (gills)	0.7 $\pm$ 0.1	2.7 $\pm$ 0.3	(gills absent)	(gills absent)
R (lungs)	(not ventilated)	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.0
R (total)	0.7 $\pm$ 0.2	1.0 $\pm$ 0.2	0.9 $\pm$ 0.2	1.0 $\pm$ 0.2
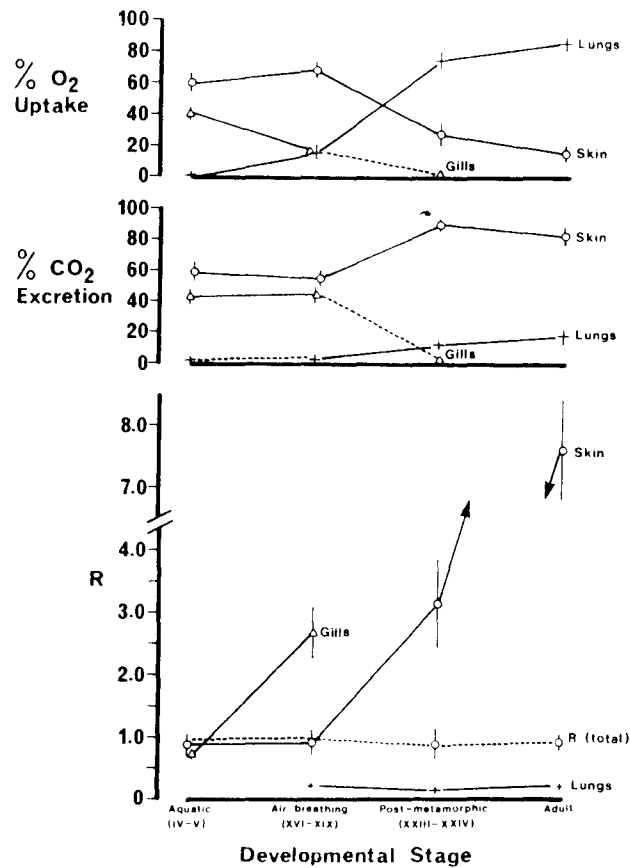
Fig. 2. Changes in the gas exchange ratios (R) and in gas exchange partitioning between gills, lungs and skin during metamorphosis at 20 C in the bullfrog, *Rana catesbeiana*. Number of animals used were, aquatic, 6; air breathing, 8; post-metamorphic, 5; and adult, 6.

Oxygen and CO_2 partitioning between gas exchange organs changed dramatically with development (table 3). Changes in the percentage contribution to total $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ by a particular exchange organ are depicted in fig. 2. In aquatic tadpoles, the skin actually contributed more to total $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ than did the gills, which accounted for only 40"ₒ of the exchange of these two gases. The importance of the skin to total gas exchange was fully retained in the next developmental level examined. As the lungs began to supplement gas exchange and the gills began to degenerate in air-breathing tadpoles, the fall in O_2 uptake by the gills was offset by the rising importance of the lungs as O_2 uptake organs. Interestingly, however, the gills continued to serve an important role in CO_2 excretion, while the lungs made little contribution. Consequently, R for the gills was nearly 3, while R for the lungs was only 0.2.

With the disappearance of the gills and the enlargement and elaboration of the

TABLE 4

Relationship between mass and surface area of the trunk and tail of air-breathing tadpoles
(Stages XVI-XIX)

	Trunk	Tail
Number of animals		20
Total body mass (g)		4.48 ± 0.31
Total skin mass (g)		0.74 ± 0.1
Total surface area skin (cm²)		15.09 ± 0.85
Surface area		
$\dfrac{\text{Total surface area}}{\text{Total body mass}}$ (cm²/g)		3.49 ± 0.19
	Trunk	Tail
Mass (g)	3.35 ± 0.22	1.12 ± 0.10**
Surface area (cm²)	8.07 ± 0.34	6.37 ± 0.43**
$\dfrac{\text{Surface area}}{\text{Mass}}$ (cm²/g)	2.50 ± 0.10	5.24 ± 0.52**

** Indicates significant difference from trunk values, at a significance level of $P < 0.01$.

lungs, $\dot{M}_{O_2}$ by the skin of post-metamorphic frogs fell from above 60% to less than 30%, with total $\dot{M}_{O_2}$ being partitioned entirely between skin and lungs. However, pulmonary CO_2 excretion had hardly risen above levels evident in the tadpole, so R for the lungs remained very low. With the disappearance of the gills and the continuing small pulmonary excretion of CO_2, the skin became almost the sole site of CO_2 excretion (R(skin) rose above 3).

The large contribution of the skin to total $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ led us to the measurement of skin surface area (table 4). Total skin surface area was about 3.5 cm²/g body mass. Particular attention was paid to the flat, broad tail which is over half the body length but only 25% of body mass. Thus, the skin surface area/mass ratio of the tail was more than twice that of the trunk.

Discussion

GILL PERFORMANCE IN BULLFROG TADPOLES

Rates of gill ventilation and O_2 utilization in 'aquatic' bullfrog tadpoles are similar to those evident in a variety of teleost fishes at the same temperature and inspired oxygen level (see Shelton, 1970; Itazawa and Takeda, 1978; Burggren and Randall, 1978; Burggren and Cameron, 1980). Unfortunately, nothing is known of the gill morphometrics, blood or water flow patterns, or gas diffusion gradients of the anuran tadpole gills, obviating further comparison.

Branchial $\dot{M}_{O_2}$ declined sharply with advancement from stages-IV-V to XVI-XIX and the initiation of air breathing. Gross gill degeneration has certainly begun by

stage XXIII, and a decrease in gill surface area appears to begin in the immediately preceding developmental stages, though this has yet to be quantified. Significant decreases in both $\dot{V}_G$ and in branchial surface area thus account for the decreasing O_2 uptake role of the gills with development (see below for a discussion of changes in branchial $\dot{M}_{CO_2}$).

Artificially induced increases in gill ventilation in 'aquatic' tadpoles increased PE_{O_2} and decreased % O_2 utilization and PE_{CO_2}, responses entirely compatible with an increased branchial water flow. The absence of any significant change in $\dot{M}_{O_2}$ or $\dot{M}_{CO_2}$ of the gills indicates that cannulation of the opercular spout does not obstruct normal branchial gas exchange. It further suggests that branchial O_2 uptake and CO_2 excretion in these tadpoles was not diffusion limited, since the elevation of PE_{CO_2}, and decrease in PE_{O_2} probably reflect an increase in the partial pressure gradients across at least part of the branchial respiratory membranes.

DEVELOPMENTAL CHANGES IN GAS EXCHANGE SITES

Aquatic bullfrog tadpoles rely heavily on cutaneous exchange of both CO_2 and O_2, like many adult and neotenic amphibians (Foxon, 1964; Shield and Bentley, 1973; Feder, 1976; Eddy and McDonald, 1978, among others). The tail of *R. catesbeiana* tadpoles accounts for approximately 40% of the total skin surface area but only 25% of the total body mass, and thus probably makes a disproportionately high contribution to cutaneous gas exchange, compared to the skin of the trunk. A similarly large tail surface area has been reported for the adult crested newt, *Triturus cristatus*, which respires largely via its skin at rest (Eddy and McDonald, 1978).

Aquatic tadpoles of stages III–V have well developed internal gills, consisting of 4 pairs of branchial arches (arches III, IV, V and VI). The skin is perfused in series with the gills, but lies downstream from them. It is thus paradoxical that the majority of both O_2 uptake and CO_2 excretion occurs cutaneously (table 3, fig. 2), since in a gill without significant diffusion limitations, efferent branchial blood destined for the skin should approach oxygen saturation, and be low in carbon dioxide. Although unsubstantiated, significant blood shunts may exist in the tadpole gill, such that a proportion of 'venous' blood entering the gills may simply never be exposed to branchial water flow. Efferent branchial blood going out to perfuse the skin would thus not be fully O_2 saturated nor have a low CO_2 content, a situation that occurs in the larval salamander *Ambystoma* (Burggren and Wood, 1981). Some proportion of the large $\dot{M}_{O_2}$ (skin) of aquatic tadpoles could also be accounted for if the actual O_2 requirement by the skin is greater than the O_2 supplied to it by convective blood flow. O_2 would thus diffuse inwards from the water along local partial pressure gradients, and CO_2 would similarly diffuse outwards. A large cutaneous $\dot{M}_{O_2}$ has been used to explain a high O_2 uptake by the skin of the eel and other marine fishes (Kirsch and Nonnotte, 1977).

At stages XXIII-XXIV, the skin was still the single most important site for both $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ (R (skin) = 0.9), although infrequent air breaths occur at this stage (West and Burggren, 1982). A progressive shift of O_2 uptake away from the gills and towards the lungs clearly began as (1) the gills visibly began to involute and decrease in surface area, and (2) $\dot{V}_G$ fell sharply. That the gills of 'air breathing' tadpoles continued to operate primarily as a site for CO_2 excretion (R (gills) = 2.27) can be largely explained on the basis of the progressive switch to aerial respiration and the substantial rise in blood P_{CO_2} that occurs in *R. catesbeiana* at this time (Erasmus *et al.*, 1970/1971). The O_2 capacitance of air at 20 °C is 28 times greater than water, but the CO_2 capacitance of air and water is approximately the same. Ventilation in the tadpole is apparently matched to O_2 delivery, and thus, the transition from a large water flow over the gills to a low flow of air through the lungs in a metamorphosing animal will still provide adequate O_2 uptake, but initially will be inadequate for CO_2 excretion at former levels. This will lead to a rise in blood and tissue CO_2. In *R. catesbeiana* tadpoles, in fact, the P_{CO_2} gradient from blood to water rises approximately 3 times concomitant with the development of air breathing (Erasmus *et al.*, 1970/1971). If the P_{CO_2} gradient from afferent branchial blood to water rises high enough in *Rana*, branchial CO_2 excretion at former levels will be resumed even with the reduction in gill surface area and $\dot{V}_G$. A large increase in cutaneous $\dot{M}_{CO_2}$ may not occur simultaneously simply because the first opportunity for CO_2 excretion along this enhanced P_{CO_2} gradient occurs when blood enters the gills. A similar argument would account for the very small CO_2 excretion of the lungs, which are perfused in parallel to the skin.

As metamorphosis nears completion (stages XXII–XXIV), the gills entirely disappear, and the lungs and much of the skin are jointly perfused in parallel by blood from the pulmocutaneous artery, derived from gill arch VI. The lungs, which are now frequently ventilated with an O_2 rich respiratory medium, increasingly assume the major O_2 uptake role (fig. 2). The decrease in contribution of the skin to total $\dot{M}_{O_2}$ probably arises in part from the decreasing surface area to mass ratio of the animal as the tail degenerates. Additionally, the thickness of the skin and its extent of vascularization may change with development.

Exchange of CO_2 and O_2 in the terminal adult stage is sharply partitioned between lungs and skin, as reflected in the respectively very low and very high R values for these gas exchange organs (fig. 2). While different ventilation–perfusion relationships of the skin and lungs may contribute to these differences, a major factor in the large cutaneous CO_2 excretion may be that the P_{CO_2} gradient from pulmocutaneous blood to lung gas will be considerably smaller than from pulmocutaneous blood to aerated water, thus favoring cutaneous over pulmonary CO_2 excretion. A smaller O_2 gradient from pulmonary capillary blood to lung gas probably does not limit pulmonary oxygen uptake, however, since full O_2 saturation of pulmonary venous blood in lung capillaries will occur at lung gas P_{O_2}'s as low as 85 mm Hg (Tazawa *et al.*, 1979). A large cutaneous CO_2 excretion together with a large pulmonary O_2 uptake are common features in the terminal forms of many amphibians (Dolk and

Postma, 1927; Hutchinson, Whitford and Kohl, 1968; Shelton, 1976). Gottlieb and Jackson (1976) have reported a partition of CO_2 excretion similar to that of the present study in adult bullfrogs with low metabolic rates. The lungs of adult bullfrogs apparently assume a progressively greater importance in CO_2 excretion with increasing metabolic rate (Gottlieb and Jackson, 1976) or temperature (Mackenzie and Jackson, 1978).

Finally, in many respects the changes in gas exchange processes during the metamorphosis of *Rana catesbeiana* resemble putative respiratory developments in the emergence of proto-amphibians, as they progressively exploited aerial respiration (Packard, 1976). In both instances, for example, the gas exchange functions of the gills are progressively replaced by pulmonary exchange. In both instances the high O_2 capacitance of air allows sufficient O_2 uptake with a comparatively small ventilatory flow of air, but results in a gradual rise in blood P_{CO_2} due to this relative hypoventilation. In light of these similarities, the sharp partitioning of $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ between skin and lungs, which occurs in many varied developmental stages in *Rana*, may also resemble events in the evolution of moist-skinned amphibian ancestors.

Acknowledgements

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