sponds to vibration as well as sound. Thus, if snakes cannot "hear" it is not for want of neural information about sounds.

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Bullfrog (Rana catesbeiana) Ventilation: How Does the Frog Breathe?

Abstract. *Two short-term cyclic events, buccal oscillations and ventilatory cycles, occur in the bullfrog's respiration. During ventilation the frog fills the buccal cavity with air, then blows the pulmonary contents through the buccal cavity, and finally closes the nostrils while pumping the buccal contents into the lungs. The pulmonary efflux streams by the buccal contents with minimal mixing, and relatively pure air is pumped into the lungs. Buccal oscillations serve mainly to flush out the buccal cavity between ventilatory cycles.*

The mechanism of lung filling in frogs differs from that of higher vertebrates in that it consists of a compression rather than a suction mechanism (*1*), but considerable disagreement exists about its details. Myographic analysis of respiration in the bullfrog *Rana catesbeiana* (*2*) reveals two kinds of cycles. The first, buccal oscillation cycles, proceed with the nostrils open and the glottis closed; air is alternately forced into and out of the buccal cavity by contractions of the buccal floor. During the less frequent ventilatory cycles, the glottis opens after the buccal floor has been depressed. Pulmonary gas, at a pressure which is always above atmospheric pressure, then enters the buccal space. This inflow induces a rise in buccal pressure and a simultaneous outflow of gas through the open nostrils. The nostrils then close, and a sudden contraction of all of the subbuccal muscles sharply increases the buccal pressure, driving some of the gas from the buccal cavity into the lung. The glottis closes near the peak of the buccal pressure, leaving the lung at a pressure substantially above atmospheric pressure. The buccal floor finally depresses while the nostrils open so that ambient air flows in to refill the buccal space.

Relatively little is known about volumetric relationships, particularly since these vary drastically, even between successive cycles (*2*). The lung of a 300-g frog ordinarily seems to hold 30 to 60 ml of gas, whereas actual tidal volumes are less than the 6- to 15-ml volume of the buccal cavity. Observed tidal volumes were low and varied by a factor of 10 as a function of behavioral parameters (*3*). This suggests that statements (*1*) that pulmonary gas is mixed with the contents of the buccal cavity and that the mixture is subsequently rebreathed into the lungs might be correct, so that the process seems most inefficient.

Yet at least three factors suggest that those buccal gases ultimately shunted into the lungs have not suffered random admixture of pulmonary efflux. (i) Most of the buccal volume lies in a posterior chamber, ventral to the opening of the larynx and surrounded by the petrohyoid muscles. (ii) The glottis is vertical and faces directly forward. Gases escaping from it under pressure might tend to pass in a stream through the distended buccal cavity and to impinge on the internal nares. (iii) The petrohyoid muscles fire during ventilation but not during oscillation cycles. Gas escaping from the lungs may thus pass directly forward, bypassing the posterior portion of the buccal cavity, with its contents remaining relatively unmixed. The unmixed gas would then be driven into the lung during the inflow phase of the ventilation cycle, and the bypassing mechanism would reduce the pumping work required because almost none of the inhaled gas is derived from the previous expiration.

We monitored gas leaving the nostrils of adult bullfrogs (*Rana catesbeiana*) with a respiratory mass spectrometer (*4*). The gas-sampling probe was set in the center of a tube (5 to 15 mm long with an internal diameter of 2 mm) placed opposite the nostrils by a mask (Fig. 1). The mask, cast individually from a quick-set rubber compound, covered the surface of the upper jaw, and curved slightly around the upper lip. Its lateral and dorsal wings were sutured, respectively, to the sides of the face below the eyes, and to the skin of the back. Because the posterior edges were thinned, there was minimal interference with the frog's eyes and ears. The internal surface of the mask was cut back to allow full freedom of nostril movement. Gas concentrations in the buccal efflux and buccal pressure were determined simultaneously (Fig. 2) on unrestrained and unanesthetized animals placed beneath an inverted bar-mesh basket.

In the first experiment the frog was permitted to breathe air. In the second experiment frog and basket were placed into a 4-liter desiccator jar, the contents of which could be rapidly flushed with a mixture of 80 percent argon and 20 percent oxygen or with air. Replacement (99 percent) was achieved in less than 15 seconds for a flow rate of 3 to 4 liter/sec.

In room air oxygen contents of the nasal efflux directly reflected the changes in buccal pressure. The nasal efflux for each ventilatory cycle corresponds to a minimum oxygen value (approximately 1 to 2 percent below ambient). Estimates based on the efflux during the first oscillatory cycle after a ventilation suggest that the nasal efflux contains between 25 and 50 percent of

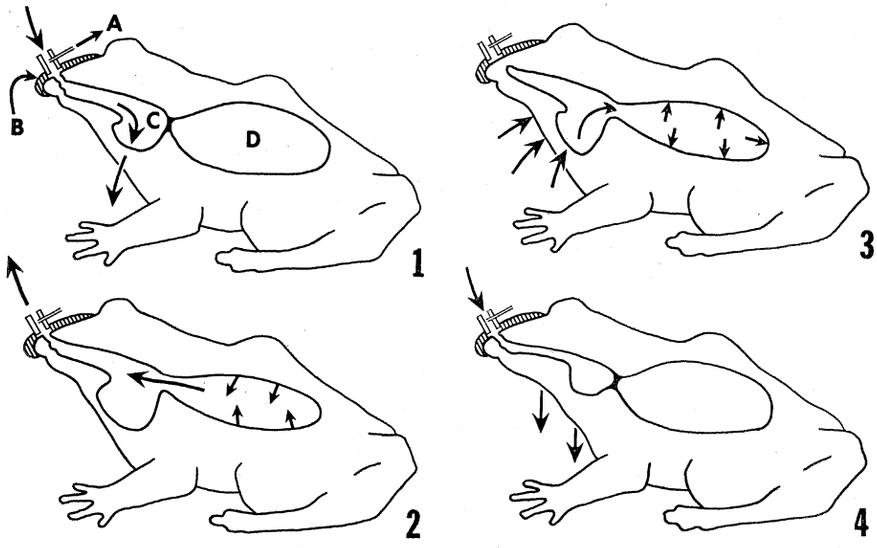

Fig. 1. Successive stages of flow during the ventilatory cycle in *Rana catesbeiana*. The gas sampling probe leading to the respiratory mass spectrometer (*A*) is supported by a plastic collar in the rubber mask (*B*). The arrows indicate the approximate sequence of gas flow into and out of the buccal cavity (*C*) and lung (*D*). Stage 2 shows how the pulmonary efflux apparently bypasses the inhaled gases which rest in the posterior portion of the buccal cavity.

the pulmonary gases. Each efflux during successive oscillatory events (in which the glottis remained closed) showed the pulmonary gas content of the nasal efflux further reduced by 10 to 20 percent. Oscillations of the buccal floor thus serve to flush out gases admixed to the buccal contents; the cycles serve a definite respiratory function. Oxygen concentrations in gas samples aspirated directly from the lungs were within 1 percent by volume of those at the nares.

Switching of the desiccator atmosphere from mixtures of argon and oxygen to mixtures of nitrogen and oxygen created large concentration differences in argon and nitrogen between buccal efflux and ambient air. Instead of involving less than 2 percent oxygen, the concentration difference was as much as 70 percent argon or nitrogen. The best records resulted when the frog engaged in long periods of oscillatory cycles during a change of gas mixture and did not interrupt these with ventilatory cycles. The gas was then flushed from dessicator and buccal cavity but not from lung; the first subsequent ventilatory cycle yielded a maximum concentration difference between efflux and ambient.

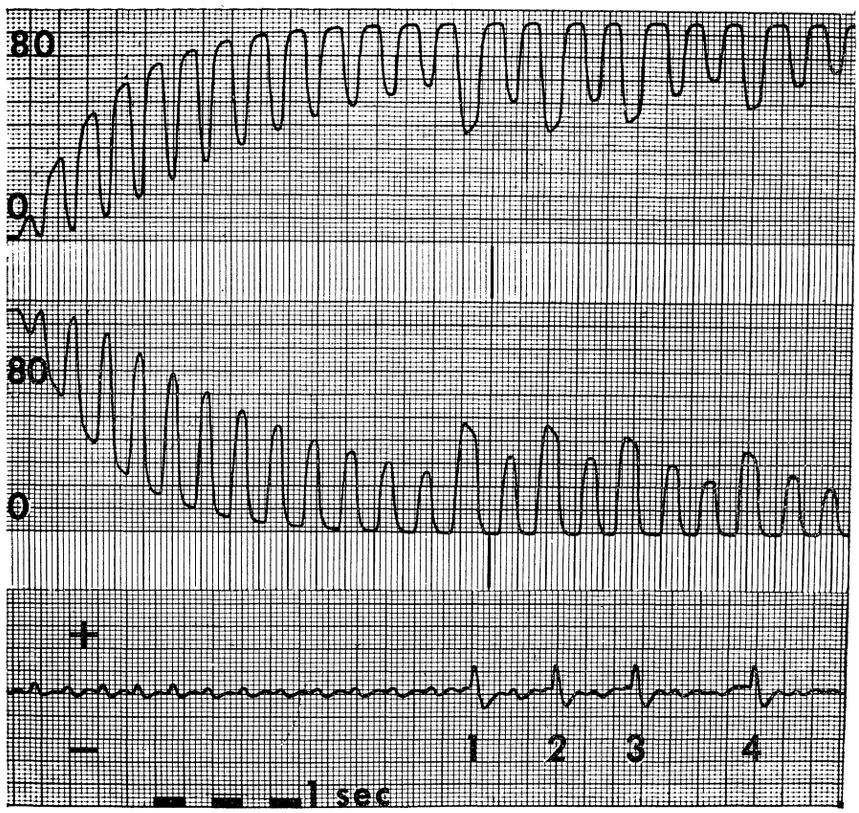

Fig. 2. Changes in expired argon and nitrogen composition upon switching breathing mixture from 80 percent argon and 20 percent oxygen to air. Top, nitrogen; center, argon; bottom, pressure in atmospheres. Record of buccal pressure shows oscillatory cycles and four ventilatory cycles (labeled 1 through 4). Note that shapes of gas concentration curves for the oscillatory and ventilatory expirations are different. The pressure record has been horizontally displaced to compensate for the difference in time delays.

The different rates of gas concentration observed during ventilatory and oscillatory outflow support the concept that the gas passes across the buccal cavity in a coherent stream. During ventilatory cycles the concentration of argon in the nasal efflux rises sharply and then decays more gradually (Fig. 2). In contrast there is a more gradual rise and a more rapid decay during buccal oscillations. It seems as though the most concentrated gas is the last to reach the nostrils. The most recently inhaled gas always occupies the anteriormost portion of the buccal cavity; it is also the least mixed and the first exhaled, so that the deepest portions of the cavity have the highest concentrations, and flushing out of the buccal contents is consequently slow.

One may estimate the percentage of buccal gas admixed to the gas leaving the nostrils, and thus determine the percentage of the pulmonary efflux that stays admixed to the buccal contents. Assuming that equal changes in buccal pressure reflect equal tidal volumes, one may utilize successive flushing cycles to obtain the constants for a series of simultaneous equations relating lung concentration, buccal concentration at time of efflux, efflux concentration, and admixing percentage. One can estimate from the peak concentrations during efflux that the fraction of pulmonary

gas admixed to buccal contents varies from 24 to 75 percent (*5*). Calculations based upon a simple mixing system give comparable results.

However, the percentage of admixture of the gas leaving the nostrils is presumably less important than is the percentage of admixture to the gas being shifted into the lungs. Gas concentration during a single ventilatory efflux indicates that the coherent stream of pulmonary gas must bypass pockets of air in the buccal cavity. The gradual reduction of efflux concentration after the rapid attainment of a concentration peak could only result from the mixing between portions of such pockets and the slowing air stream. Furthermore, occasional high-velocity exhalations result in maximum concentrations of pulmonary gas in the nostril efflux of much lower concentrations. The gas introduced into the lung (diluting its argon or nitrogen concentration) must then have been in the buccal cavity at the time of the maximum efflux. The introduced gas must consequently have had a significantly lower concentration than that leaving the nostrils during the previous cycle; that is, it could not have been mixed with lung efflux during the period of high outflow.

Our results support the concepts that (i) the buccal oscillations serve to flush out the buccal chamber between ventilatory cycles and are definitely respiratory, (ii) the pulmonary gas crosses the buccal cavity in a coherent stream, and (iii) the gas entering the lungs during ventilation is positioned in the posterior portion of the buccal chamber ventral to the glottis and hence subject to minimum admixture during outflow from the lungs.

Recent reports by Bishop and Foxon (*6*) and McMahon (*7*) suggest that the filling of the buccal cavity with air by a hyoid movement and the exhalation of the pulmonary contents through this buccal chamber occur in lung fish and may consequently be phylogenetically old. Frogs have apparently retained this basic pattern but have increased its efficiency by reducing mixing.

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Early Experiences Accelerate Maturation of the 24-Hour Adrenocortical Rhythm

Abstract. *Rats reared under 12-hour alternating periods of light and dark were killed at times corresponding to the times at which the maximum or minimum plasma corticosterone concentrations occur in mature animals. The characteristic 24-hour adrenocortical rhythm was first observed in rats 21 to 25 days old. In rats handled or stimulated with electric shock, the rhythm developed as early as 16 days.*

Although circadian rhythms in mature organisms have been observed in physiological systems virtually wherever they have been sought, frequently they cannot be detected in newborn animals. We first sought to determine when, in the life of the laboratory rat, the light-synchronized 24-hour rhythm in plasma corticosterone concentrations could first be detected. Allen and Kendall (*1*) have reported that the adrenocortical rhythm becomes evident at 30 to 32 days of age. Our data indicate that it develops about 10 days earlier. It was also determined that maturation of the adrenocortical rhythm could be influenced by stimulation of the infant rat by handling or electric shock, stimuli which alter subsequent behavioral and physiological processes and resistance to disease (*2*).

Pregnant Charles River (CD) rats were individually housed in transparent plastic cages containing nesting materials, food, and water. These cages were located in modified office-type cabinets

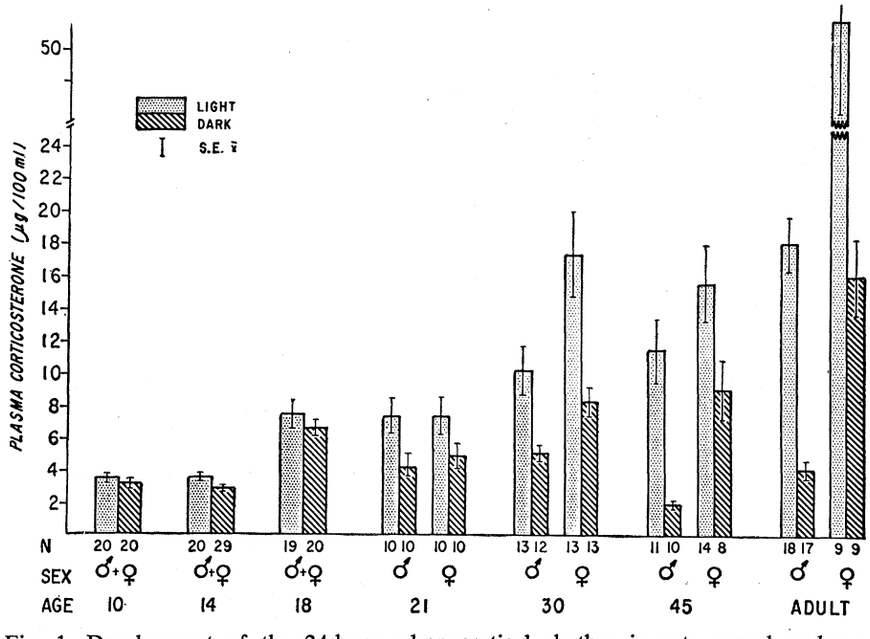

Fig. 1. Development of the 24-hour adrenocortical rhythm in rats reared under a 12-hour light-dark cycle. "Light" values were obtained 2 hours before the onset of the period of darkness, and "dark" values were obtained 2 hours before the onset of the light period. The values for adult animals were obtained from a previous study (*5*).