Relative Contributions of Anaerobic and Aerobic Energy Production during Activity in Amphibia

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Summary. 1. Measurements of oxygen consumption and lactate production during activity were made in three species of amphibians. Oxygen debts and rates of lactate removal were also studied.

2. Maximal values of oxygen consumption are 200 and 500% above resting levels in the plethodontid salamander *Batrachoseps attenuatus* and the frog *Hyla regilla*, respectively (Fig. 1). However, these values are not attained until 5–15 min after the cessation of activity.

3. *Batrachoseps,* a lungless animal, derives 25 times more energy during 2 min of activity from lactate production than from oxygen consumption. *Hyla* is also predominantly anaerobic during activity but *Bufo boreas* is mainly aerobic.

4. An inverse relationship exists between aerobic and anaerobic scopes in amphibians, such that total energetic output during activity is similar even though the component factors may differ by ten-fold.

5. Oxygen debts in *Hyla* and *Batrachoseps* after 2-min activity are repaid in approximately one hour but rates of lactate removal are much lower (Fig. 2); large amounts of lactate persist long after oxygen consumption has returned to resting levels. Oxygen debt, therefore, cannot be used as an estimate of energetic expenditure during activity.

Introduction

The metabolic basis of activity in many vertebrate classes is only now being explored in sufficient detail to permit comparison among groups. The mammals and birds can attain and sustain far higher levels of aerobic metabolism than the lower vertebrates: standard levels of oxygen consumption in the homeotherms are equal to the maximal rates attained during activity by reptiles and fish (Brett, 1972; Bennett and Dawson, 1974). Aerobic metabolism in poikilotherms is supplemented during vigorous activity by anaerobic metabolism with the subsequent accumulation of lactic acid (Black *et al.*, 1961; Heath and Pritchard, 1962; Moberly, 1968; Bennett and Licht, 1972, 1974). Despite the deleterious physiological effects of lactic acid production (Von Buddenbrock, 1938; Secondat, 1950; Bennett, 1973), anaerobiosis may account for nearly all the ATP produced in many poikilotherms during maximal activity (Bennett and Licht, 1972; Bennett and Dawson, 1972). Consequently,

many of the lower vertebrates are prone to rapid exhaustion after short bursts of activity.

The few available data regarding activity metabolism in amphibians (Seymour, 1973; Bennett and Licht, 1974) indicate that physiological systems supporting activity in these vertebrates are more phylogenetically labile and more closely attuned to the behavioral patterns of individual species than in other vertebrate groups, such as lizards (Bennett and Licht, 1972). Slow-moving forms such as the toad, *Bufo boreas*, have high aerobic scopes and low rates of lactate production; in contrast, amphibians engaging in rapid bursts of activity, such as the frog, *Rana pipiens*, have low aerobic scopes, high anaerobic scopes, and exhaust rapidly (Seymour, 1973; Bennett and Licht, 1974). However, the lack of simultaneous measurements of aerobic and anaerobic metabolism in amphibians has prevented quantitative assessment of the relationship between these two modes of energy production. The present study examines the relative contribution of each metabolic system to the total energetic yield during activity and their influence on each other during recovery from activity. Aerobic metabolism was measured as oxygen consumption. Lactic acid production was used as an index of anaerobic energetic expenditure.

Studies were performed on two species of amphibians, the California slender salamander, *Batrachoseps attenuatus* (Family Plethodontidae) and the Pacific treefrog, *Hyla regilla* (Family Hylidae). Both are characterized by rapid movement which is associated with high anaerobic scope and capacity (Bennett and Licht, 1974). A comparison of the activity energetics of these two was of special interest because the salamander is lungless. Additional anaerobic data were collected for a slow-moving species with low anaerobic scope, *Bufo boreas* (Family Bufonidae); these data are compared with aerobic values previously obtained for related species.

Materials and Methods

Batrachoseps attenuatus (averaging 1.55 g), *Bufo boreas halophilus* (33.3 g), and *Hyla regilla* (2.76 g) were collected locally in Northern California and maintained at 20–25° C for at least a week. All animals fasted at least 3 days before experimentation.

Oxygen consumption before, during and after 2 min of stimulated activity at 20° C was measured in a specially-designed closed manometric chamber connected to a Warburg manometer. The chamber consisted of a jar (4.0 cm high × 4.0 cm diameter) containing a closed wire-screen cage (2.5 cm high × 3.5 cm diameter) in which the animal was placed. The floor of the screen cage contained a coiled electrical grid of copper wire connected to an electrical stimulator. A saturated atmosphere was maintained with wet filter paper, and CO_2 was absorbed by another filter paper saturated with 15% KOH. The entire chamber was submerged in a constant temperature water bath regulated at $20 \pm 0.2°$ C. The system responded rapidly to changes in pressure; injections of carbon dioxide were completely ab-

sorbed in 2–3 sec. Therefore, changes in manometric readings were taken to reflect instantaneous changes in oxygen consumption.

Animals were weighed and placed individually in the chamber and allowed to rest undisturbed for several hours to reach thermal equilibrium. The chamber was then sealed and oxygen consumption was measured until steady resting levels were maintained for one hr (this usually occurred after 2 hr). Electrical shocks (5–10 volts) were then delivered through the copper-wire grid; activity was monitored visually. Stimulation was delivered every 5–10 sec and was adjusted to maintain continuous and vigorous activity for 2 min. The animals were judged to be exerting themselves maximally since activity was intense and its level did not increase with further increments in shock intensity. Oxygen consumption was monitored during and after stimulation until post-active oxygen consumption could not be distinguished from initial resting levels.

The anaerobic energetic expenditures of *Batrachoseps*, *Hyla*, and *Bufo* during 2 min of activity at 20° C were determined in a separate series of experiments by stimulation and total lactate analysis of whole body homogenates as described by Bennett and Licht (1974). This stimulation was considered comparable to that in the metabolic chamber, since the amount of activity sustained and the levels of lactic acid formed were similar under both conditions.

Recovery from anaerobic activity, i.e., the rate of disappearance of lactate, in *Hyla* and *Batrachoseps* was determined by stimulating the animals, replacing them in their individual containers in the dark, and allowing them to recover for periods up to 3 hr. These exercised animals were observed visually and remained quiet throughout this recovery period. In some cases, animals were restimulated after the initial activity period.

Results

Values of oxygen consumption (corrected to STP) of *Batrachoseps* and *Hyla* are given in Fig. 1. Increments in oxygen consumption during the 2 min of activity proved too small to measure manometrically, and it was necessary to integrate this time period with the 5 min post-active period to obtain a sufficient decrement in pressure to calculate oxygen consumption. This method probably slightly overestimates oxygen consumption values during the active period since the rate continues to increase in the second measurement period after the cessation of activity (Fig. 1).

Although resting metabolic rates of *Hyla* are identical to those of *Batrachoseps* (0.06 cc O_2/g × hr), the frog utilizes about twice as much oxygen during and immediately after maximal activity as the lungless salamander. Oxygen consumption in both species is not maximal until 5–15 min after activity ceases. This situation suggests that a considerable lag period is necessary before aerobic metabolism can be completely mobilized. Aerobic scope values, not attained until after the completion of activity, are 0.13 cc O_2/g × hr for *Batrachoseps* and 0.34 cc O_2/g × hr for *Hyla*. These represent a three-fold and a six-fold increment, respectively, over resting aerobic metabolic levels.
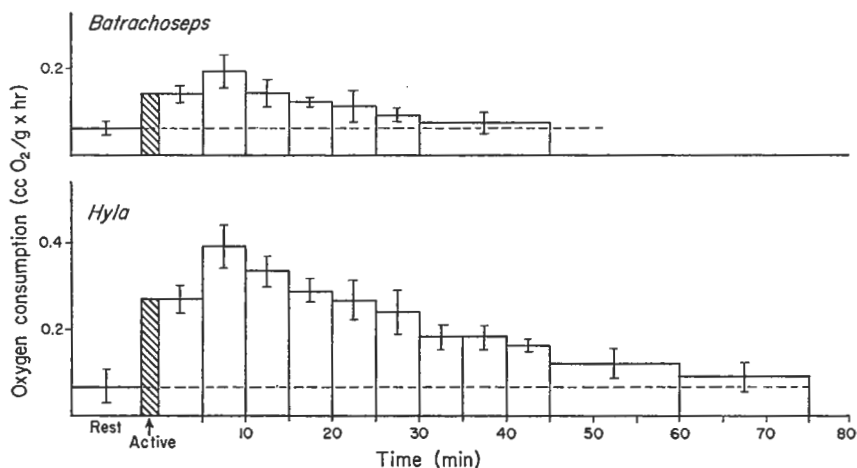
Fig. 1. Oxygen consumption in *Batrachoseps attenuatus* and *Hyla regilla* at rest and during and after 2-min activity at 20° C. Resting levels were maintained for one hr before stimulation. Measurements of oxygen consumption during and after activity were integrated over 5 to 15 min intervals, indicated by the width of the vertical bars. Mean values are reported; vertical lines indicate 95% confidence limits of the means. Four animals of each species were measured

Table 1. Lactate content of amphibians during rest and after 2 min of manual or electrical stimulation. Mean values are given with standard errors; number of animals is indicated in parentheses

Species	Lactate content (mg/g body wt.)		
	Rest[a]	2 min manual stim.	2 min electrical stim.
Batrachoseps attenuatus	20.3 ± 0.7 (3)	137.1 ± 4.6 (5)	150.3
Hyla regilla	28.2 ± 1.2 (3)	89.5 ± 5.2 (5)	98.4
Bufo boreas	10.7 ± 2.4 (4)	33.9 ± 2.9 (3)	27.5

[a] From Bennett and Licht, 1974.

A measurable oxygen debt persists for 45 min after activity in *Batrachoseps* and 75 min after activity in *Hyla*. The total oxygen debt in *Hyla* (0.165 cc O_2/g) is four times as great as that of *Batrachoseps* (0.041 cc O_2/g).

Considerable interspecific variation is evident in anaerobic energetic expenditure during 2 min of manual stimulation (Table 1). As previously
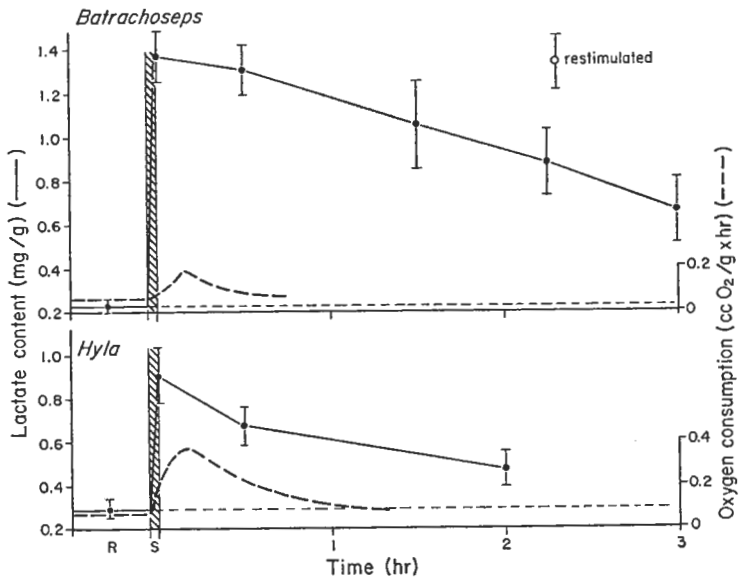
Fig. 2. A comparison of lactate persistence and oxygen debt after activity in *Batrachoseps attenuatus* and *Hyla regilla* after 2-min activity at 20° C. Lactate content is indicated by a solid line and closed circles; a group of restimulated animals is reported as an open circle. Mean values are given for 3 to 5 animals per group; vertical lines report 95% confidence limits of the means. The light dashed line indicates lactate content of resting animals. The dark dashed lines are oxygen consumption, as taken from Fig. 1

observed in these three species (Bennett and Licht, 1974), *Batrachoseps* has the highest levels of lactate production (1.17 mg/g). *Hyla* is intermediate (0.61 mg lactate/g), and *Bufo* sustains very little anaerobiosis (0.23 mg/g). Lactate contents between manually and electrically stimulated animals are in good agreement (Table 1).

The rates of disappearance of the lactate produced during 2 min activity in *Batrachoseps* and *Hyla* are relatively slow (Fig. 2). During the first 30 min after the cessation of activity in *Hyla*, only 38% of the lactate produced is removed, and insignificant amounts ($p = 0.6$) are removed during the same time period in *Batrachoseps*. In *Batrachoseps*, the persistence of lactate is associated with inhibition in the capacity for activity during the recovery period: no springing response can be elicited after 30 min recovery; after 90 min, springing is sustained for only 30 sec; after 135 min recovery, springing is sustained for about 1 min and the lactate content of these restimulated animals is identical to that during the initial activity (1.33 mg/g).

Table 2. Aerobic and anaerobic scope of amphibians at 20°C

Species	Aerobic scope (cc O_2/g × hr)	Anaerobic scope (mg lactate/g × min)
Bufo cognatus	1.06[a]	—
Bufo boreas	—	0.24[c]
Scaphiopus hammondi	0.72[a]	—
Hyla regilla	0.34	0.80[c]
Geotrypetes seraphinii	0.31[b]	0.65[b]
Rana pipiens	0.27[a]	1.01[c]
Batrachoseps attenuatus	0.13	1.31[c]
Rana catesbeiana	0.12	—

[a] Seymour (1973).
[b] Bennett and Wake (1974).
[c] Bennett and Licht (1974).

Discussion

A marked inverse relationship is now apparent between aerobic and anaerobic scopes in amphibians: a high aerobic scope is accompanied by a low anaerobic scope and *vice versa* (Table 2). The toad *Bufo* exhibits only moderate activity during vigorous stimulation but shows no fatigue (Seymour, 1973; Bennett and Licht, 1974). Comparison of aerobic scope (Seymour, 1973) with our measurements of anaerobiosis indicates that this activity is mainly supported aerobically. In contrast, *Rana*, *Batrachoseps*, and *Hyla* are much more active forms and rely primarily on anaerobic metabolism, fatiguing rapidly (Seymour, 1973; Bennett and Licht, 1974). These observations support the conclusion (Bennett and Licht, 1974) that rapid activity in amphibians can only occur at the expense of extensive anaerobiosis. The resultant lactate and its persistence may have serious consequences for further activity as indicated by the reduced responsiveness in *Batrachoseps* following vigorous activity. Even relatively aerobic amphibians, such as toads, seem to regulate routine behavior (e.g., burrowing) to avoid anaerobic metabolism (Seymour, 1973); they may resort to anaerobiosis only during escape stress.

Observations on maximal oxygen uptake in *Hyla* and *Batrachoseps* reveal certain deficiencies in current concepts regarding aerobic scope. In this original formulation of metabolic scope, Fry (1947) proposed that the maximal increment in oxygen consumption could be used as one index of the capacity for activity. However, in the two amphibians studied, maximal oxygen consumption and, hence, aerobic scope do not occur until 5–15 min after the cessation of activity. Thus, the scope does not represent the immediate source of energy for activity but rather appears to represent a part of the recovery phenomenon.

Table 3. Predicted ATP production from aerobic and anaerobic sources during activity in amphibians at 20°C

Species	30 sec activity				2 min activity			
	Aerobic ATP production (μM ATP/g)	Anaerobic ATP production (μM ATP/g)	Total ATP production (μM ATP/g)	Ratio of anaerobic to aerobic ATP	Aerobic ATP (μM ATP/g)	Anaerobic ATP (μM ATP/g)	Total ATP (μM ATP/g)	Ratio of anaerobic to aerobic ATP
Batrachoseps attenuatus	0.20	10.89	11.09	55.0	0.79	19.50	20.29	25.0
Hyla regilla	0.49	6.65	7.14	13.5	1.97	10.24	12.21	5.2
Bufo boreas	2.55[a]	1.97	4.52	0.8	10.22[a]	3.87	14.09	0.4

[a] Data from Seymour (1973) for *Bufo cognatus* of similar size.

Both *Hyla* and *Batrachoseps* are highly dependent on cutaneous respiration. Plethodontid salamanders such as *Batrachoseps* must rely almost exclusively on cutaneous oxygen uptake (Whitford and Hutchison, 1965) and cutaneous respiration in three species of *Hyla* investigated accounted for up to two-thirds of total oxygen consumption during rest (Hutchison *et al.*, 1968). The more rapid rise in oxygen consumption during activity in *Hyla* (300% vs. 100% in *Batrachoseps*) may reflect the contribution of the functional lung in this species. The lung component might also account for the greater maximal increment in oxygen consumption attained by *Hyla* (500% vs. 200% in *Batrachoseps*). The delayed aerobic scopes in this frog and salamander contrast with the more immediate development of maximal oxygen consumption in the caecilian, *Geotrypetes seraphinii*, measured with identical technique and apparatus (Bennett and Wake, 1974). Morphological evidence indicates that caecilians rely primarily on pulmonary ventilation for oxygen uptake (M. Wake, pers. comm.). Oxygen consumption also increases rapidly during a 2-min burst of activity in the lizard, *Dipsosaurus dorsalis* (Bennett and Dawson, 1972); oxygen exchange across the saurian integument is low if present at all. These comparative observations suggest that delayed development of aerobic scope is related to the dependence on cutaneous respiration.

The contribution of aerobic and anaerobic factors to the total energetic mobilization during activity can be estimated by converting oxygen consumption and lactate generation to ATP production according to the following equations (Bennett and Licht, 1972):

1.0 mg lactate formed = 0.0167 millimoles ATP;

1.0 cc O_2 (STPD) consumed = 0.290 millimoles ATP.

The theoretical ratio between lactate and ATP production has been experimentally substantiated by Cerretelli *et al.* (1972); the aerobic ratio assumes tight-coupling and complete phosphorylation. These calculations assume that oxygen consumption and lactate production represent the sole, or at least, the principal pathways of ATP production: the existence of other anaerobic endproducts of activity metabolism has not been demonstrated in amphibians. The relative amounts of ATP produced during 30 sec and 2 min of activity are given in Table 3. Aerobic measurements are probably exaggerated because of the integrating properties of the sampling procedure (see Results section).

The ratio of anaerobic to aerobic energy production is very high in the lungless *Batrachoseps:* over fifty times more energy is derived anaerobically than aerobically during the first 30 sec of activity. *Hyla* also obtains the major proportion of its energy for activity from anaerobic sources, but it has a somewhat higher aerobic scope. In contrast, anaerobiosis contributes less than half of the energetic production in *Bufo.* Even though the aerobic and anaerobic scopes may differ by a ten-fold factor among species, the total energetic outputs differ by only a factor of two or three.

Comparisons of oxygen debt to the formation and elimination of lactate demonstrate the inadequacy of oxygen debt as a measure of energy metabolism during activity. Consequently, measurements of oxygen consumption alone cannot account for the total metabolism of amphibians. Oxygen debt, the increment in oxygen consumption above resting levels after a bout of activity, is commonly considered to represent the energy required to rephosphorylate creatine and replenish depleted oxygen stores (alactacid debt) and to eliminate lactate (lactacid debt) (Karpovich, 1959). A comparison of oxygen debt and lactate elimination in *Batrachoseps* (Fig. 2) shows that 90% of the oxygen debt has been repaid 30 min after activity; however, there has been no significant decrement in lactate content in that time period. The ATP production connected with the entire oxygen debt accounts for only half that associated with lactate formation during the 2-min activity period. The inadequacy of oxygen debt as a measure of activity metabolism is further demonstrated by a comparison of oxygen debt and energetic expenditure during 2-min of activity in *Batrachoseps* and *Hyla* (Fig. 1 and Table 3). The frog has an oxygen debt four times greater than that of the salamander; the latter, however, utilizes almost twice as much energy during activity. Oxygen debt cannot, therefore, reflect total energetic expenditure and is probably related primarily to aerobic aspects of recovery (alactacid debt).

The difficulties associated with the concept of oxygen debt in mammals involve accounting for an actual debt many times greater than the theoretical debt based on all oxygen-requiring sources; a major factor

seems to be the uncoupling of phosphorylation and oxidation caused by a rise in body temperature (Brooks *et al.*, 1971a, b). The situation in *Batrachoseps* is, however, completely reversed: oxygen consumption after activity does not account for the theoretical oxygen debt. The cost of activity in these animals appears to be the sum of oxygen consumed during activity (a relatively small quantity), the oxygen debt, plus the lactate production.

Earlier studies on resting oxygen consumption led to the conclusion that the probable ultimate limit to energy production in amphibians is the ability to obtain oxygen (Whitford and Hutchison, 1967; Hutchison *et al.*, 1968). This assertion should be reconsidered in view of the present results. Much of the maximal metabolic rate attained during activity comes from anaerobic sources which are not measured by an analysis of aerobic scope and/or oxygen debt. These anaerobic processes create physiological conditions that limit further energy production.

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