LACTATE PRODUCTION, TISSUE DISTRIBUTION, AND ELIMINATION FOLLOWING EXHAUSTIVE EXERCISE IN LARVAL AND ADULT BULLFROGS RANA CATESBEIANA[1]

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(Accepted 3/25/83)

The time course following exhaustive exercise of changes in lactate in blood, muscle, skin, kidney, urine, and branchial exhaled water in larval *Rana catesbeiana,* and in blood and muscle of adult *R. catesbeiana,* has been investigated. Tissue concentrations of pyruvate, lactate dehydrogenase (LDH) activity, and whole-animal $\dot{V}O_2$ and $\dot{V}CO_2$ were also measured. Resting levels of blood and muscle lactate in tadpoles were not significantly different from those of adults. Muscle lactate concentrations, determined immediately following exhaustive exercise, were the same in tadpoles and adults and increased six times above resting levels in both forms. Blood lactate in adults was maximal within 1 min following exercise in adults but did not peak for 30 min in tadpoles. Blood lactate in tadpoles and adults returned to resting values 4 and 6 h, respectively, following exercise. The major difference between tadpoles and adults was thus the time course of blood lactate changes during recovery from exhaustive exercise. In tadpoles, 7% of total lactate produced during exercise was eliminated via the gills (80% of eliminated lactate), skin (13%), and urine (6%). Prevention of gill ventilation (responsible for less than one-half of total oxygen uptake) resulted in a 140% increase in blood and muscle lactate concentration during the first 2 h after exercise. Gill lactate elimination probably is important to acid-base regulation by tadpoles during recovery from exercise. The LDH activity in both larvae and adults can be summarized as follows: muscle $\gg$ kidney $>$ liver $\gg$ heart $\sim$ lung. Muscle concentrations of pyruvic acid decreased following exercise in tadpoles and adults, even though blood pyruvate increased two times and remained elevated for more than 2 h. Pyruvate concentration of kidney and of whole-body homogenates of tadpoles increased two times and five times, respectively, immediately following exhaustive exercise. Low pyruvate and high LDH activity, conditions favoring reconversion of lactate to pyruvate, were thus most evident within the muscle itself. The rapid decrease in muscle lactate following exercise, which was not paralleled by an increase in blood lactate, suggests that reconversion of lactate to pyruvate may occur in muscle. Oxygen uptake remained significantly elevated after exercise for 10 h in tadpoles and 4 h in adults, partially reflecting O_2 consumed in the metabolism of lactate produced during the exercise bout. The respiratory quotient in both stages remained depressed for several hours after exercise, possibly because of acid-base imbalances incurred during and after exercise.

INTRODUCTION

Aerobic metabolism in ectothermic vertebrates is usually supplemented by anaerobic metabolism during strenuous exercise (Bennett and Licht 1973; Seymour 1973; Turney and Hutchison 1974; Hutchison and Turney 1975; Putnam 1979*b*). The duration of strenuous exercise, that is, stamina, is believed to be limited by the buildup within muscle cells of lactate ions, which are toxic in high concentration. Lactate is formed when stored glycogen is transformed to lactate by the Embden-Meyerhof pathway of glycolysis (Lehninger 1975; Harper, Rodwell, and Mayes 1979).

Interspecific differences in lactic accumulation in anurans during exercise have been correlated with differences in the muscle fraction of the total body mass (Putnam 1979*a*), the use of different muscle groups during activity (Putnam 1979*a*), and methods of predator avoidance (Ben-

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Physiol. Zool. 56(4):597–613. 1983.

 0031-935X/83/5604-8270$02.00

and Licht 1974; Feder and Arnold 1982). Unfortunately, little information is available on rates of lactate production and removal from specific tissues in adult anurans.

Interspecific differences in aerobic/anaerobic metabolism and lactate production related to developmental stage may also occur in anurans. In tadpoles the tail muscle is the major locomotory muscle mass. Upon metamorphosis, the hind limbs become the locomotory muscle mass for both swimming and hopping. Because of morphological and mechanical differences in locomotion between tadpole and adult frogs, lactate production during exercise and its subsequent disappearance from muscle might differ with developmental stage.

Since lactate is an incompletely oxidized end product, its elimination in large quantities would be energetically wasteful. Lactate produced during exercise in mammals is believed to be eliminated from muscle into blood and taken up by the liver, where ample enzymes for gluconeogenesis occur (Cori and Cori 1929; Himwich, Koskoff, and Nahum 1929–1930; Krebs 1964). Although white muscle has high glycolytic capacity, it does not contain the full array of enzymes necessary for conversion of lactate to glucose (Moon and Johnston 1980). However, in the frog about 75% of lactate produced during anaerobiosis is subsequently converted directly into glycogen in the muscle (Meyerhoff 1920; Hill 1924; Bendall and Taylor 1970; Batty and Wardle 1979). Nonetheless, little is known about the fate of lactate released into the blood during exercise in amphibians and particularly in the aquatic larvae, where both gills and skin could serve as possible avenues for lactate elimination should this toxic by-product begin to accumulate in high concentrations. Elimination of lactate via the gills has been suggested for teleost fish (Dridzic and Kiceniuk 1976), but Cushman, Packard, and Boardman (1976) concluded that neither branchial nor cutaneous lactate elimination occurred in gill-bearing larvae of tiger salamanders. Lactate elimination via the kidneys may also occur following exercise in both larval and adult anuran amphibians. In mammals blood lactate remains elevated only briefly following exercise (Yudkin and Cohen 1975), so renal elimination of lactate is quite low. In anurans, blood lactate remains high for several hours following exercise (present study). Such extended periods of elevated blood lactate may allow the kidneys to play a greater role in lactate excretion than they do in endotherms. Certainly renal elimination of lactate occurs in turtles following dives (Jackson and Silverblatt 1974), in dogfish following activity (Piiper, Meyer, and Drees 1972), and in rainbow trout following hypoxic exposure and infusion of lactate (Hunn 1969; Kobayashi and Wood 1980).

This study was thus designed to assess the production and distribution of lactate and the extent and rate of lactate elimination following exhaustive exercise in larval and adult bullfrogs.

MATERIAL AND METHODS

ANIMALS AND MAINTENANCE

Bullfrog tadpoles were collected from ponds in western Massachusetts in the spring and summer and maintained in holding tanks at 23 C. The animals were kept on a 12L:12D photoperiod and supplied ad lib. with native pond plants and boiled lettuce. Tadpoles of stages IV–XVII (Taylor and Kollros 1946) weighing 18 ± 12 g (no. = 475) were used.

Adult bullfrogs (384 ± 16 g, no. = 15) were purchased from suppliers in western Massachusetts and were stored at 23 C for up to 7 days in a large aquarium containing a 2-cm layer of aerated tap water. Frogs were force-fed raw liver after 3 days but not within 24 h prior to experimentation.

EXPERIMENTAL PROTOCOL FOR TADPOLES

1. *Exercise regime.*—Tadpoles were divided into control and exercise groups. All were placed in individual containers and left unfed and undisturbed for at least 24 h prior to the exercise bout.

Animals were exercised in a clear Plexiglas cylinder, either 12 cm long and 7 cm in diameter (tadpoles < 10 g) or 20 cm long and 38 cm in diameter (tadpoles > 10 g). An immersible pump circulated aerated water at 23 C through the cylinder at 8.1 $cm \cdot s^{-1}$. The tadpole swam against this current, and an aluminum screen glued

across the downstream end of the cylinder prevented the tadpole from being flushed out of the cylinder as it approached exhaustion. A large air bubble was maintained at the top of the water-filled cylinder to allow air breathing during the exercise bout.

Almost all tadpoles maintained their position in the current for 3–5 min. After this time, manipulation of the tail with a blunt-end probe inserted through the aluminum screen was used periodically to stimulate swimming for an additional period of time. With manual stimulation, vigorous swimming was maintained for an additional 5 min before complete exhaustion. In about 10% of the tadpoles, up to 10 min of vigorous swimming could be maintained without manual stimulation. *Exhaustion* in all tadpoles was defined as a lack of response to tail pinching with blunt-end forceps inserted through the aluminum screen and a failure of the tadpole to right itself when overturned in the water after removal from the exercise apparatus. Both criteria had to be satisfied for the tadpole to be considered exhausted. Electrical stimulation (Turney and Hutchison 1974; Hutchison and Miller 1979*a*) was avoided, since that technique has been reported to impair aerobic metabolism and elevate lactate production (Hillman et al. 1979).

2. *Tissue sampling and treatment.*— After the exercise bout, tadpoles were returned to their individual containers and left undisturbed until subsequent sampling of blood and other tissues. Individual tadpoles were killed immediately following exercise and at 15, 30, 60, 90, 120, 240, and 300 min into recovery. Each tadpole was quickly stunned by a tap to the head, and a ventral portion of skin was removed to expose the systemic arches. Blood was obtained by inserting a drawn-out heparinized Pasteur pipet into an arch and allowing blood pressure to fill it. This procedure took less than 2 min. Samples of tail muscle also were obtained immediately by skinning the tail, removing a section of muscle, and rinsing it in cold saline. Finally, in tadpoles in which skin and kidney lactate and pyruvate concentrations were to be measured, a PE 50 catheter was tied into a systemic arch and the ventricle cut open. The entire circulation was then flushed with heparinized saline (3–5 ml) until the skin was blanched and red blood cells just stopped appearing in flushed saline.

Blood samples were mixed immediately with 2 vol of 10% cold perchloric acid (PCA), allowed to stand for 5 min, and then centrifuged at 3,000 × *g* for 10 min. The supernatant was stored at 5 C for a maximum of 1 h before assay for lactate (Sigma no. 825-UV) was performed and a maximum of 3 h before pyruvate analysis (Sigma no. 726-UV).

Samples of tail muscle, tail skin, and kidney were ground in 10 times their mass of cold 0.6 M PCA using a cold glass-on-glass tissue grinder. Tissue homogenates were centrifuged at 5,000 × *g* for 10 min. The supernatant was collected and recentrifuged at 12,500 × *g* for 10 min.

Lactate concentration of whole-body homogenates of tadpoles was determined after animal homogenization with 10 times its mass of cold 0.6 M PCA in a Hamilton-Beach blender. The slurry was then ground in a cold, glass-on-glass tissue grinder. Blender homogenization alone was inadequate and led to erratic results. Since lactate concentrations are not uniform throughout the body, poorly ground and extracted body compartments such as skin and muscle can result in underestimation of whole-body lactate. Whole-body homogenates were centrifuged at 5,000 × *g* for 10 min. The supernatant was collected and recentrifuged for 10 min at 12,500 × *g*.

3. *Lactate dehydrogenase activity.*— Tadpoles and adult bullfrogs were killed, and cold, nonheparinized saline was flushed immediately through the systemic arches until no red blood cells were visible in the effluent. Skeletal muscle, liver, kidney, heart, and lung tissue were then excised and rinsed in cold 0.1 M potassium phosphate buffer (pH 7.5). Samples were weighed, then rapidly ground in 0.1 M potassium phosphate buffer at pH 7.5, in a cold glass-on-glass tissue grinder. The dilution factors, obtained through preliminary experiments, were as follows: 1:100 for muscle, 1:10 for liver and kidney, and 1:5 for lung and heart. The lowest dilution possible was 1:5 due to the thick consistency of the homogenate. Samples were

centrifuged at 12,500 × g for 10 min and assayed for LDH (Sigma kit no. 340-UV).

4. *Measurement of lactate excretion.*—A major problem in the study of lactate elimination in aquatic animals has been the collection and concentration of branchial water samples. In larval *Rana* water flowing over both sets of gills leaves the branchial chambers via a single opercular spout on the animal's left side and provides an ideal site for collecting all expired branchial water. The opercular spout of 16 tadpoles (4 ± 1 g body mass) thus was cannulated with a 10 cm length of PE 50 polyethylene tubing (see Burggren and West 1982). Animals were then left for 24 h to recover before an exercise bout. After exercise to exhaustion, cannulated tadpoles were placed in individual containers containing 1 liter of aerated tap water. The opercular cannula was led over the edge of the container and the cannula tip, which emptied into a collecting vessel, was placed 1 cm below the water surface. Branchial water flowing through the opercular cannula was collected over a 2-h period in unexercised, "control" animals and 2 h following exercise in "experimental" tadpoles. Rates of branchial ventilation in control tadpoles were similar to those reported in other studies (Burggren and West 1982; West and Burggren 1982). Collected branchial water was immediately frozen by immersion of the collecting vessel in liquid nitrogen. Frozen samples were then lyophilized with a Thermovac RFS lyophilizer. The lyophilized material from the opercular water was resuspended in 30 ml of distilled water, which produced approximately a 250-fold concentration of the original volume, and assayed for lactate. The accuracy of the lyophilization/reconstitution technique and the present recovery of lactate was determined on eight standards, made by suspending 0.2 mg lactate standard (Sigma no. 826-10) into 500 ml of aerated tap water. A 94% ± 2% yield of lactate was obtained, with no lactate detectable in blank samples.

The cloacae of 20 tadpoles (15 ± 8 g) were cannulated (20 mm PE 100–150) for urine collection. It was not necessary to suture in the cannula as it fit snugly in the cloaca yet could be removed easily without apparent disturbance to the animal. The cannula was air filled when implanted, and air bubbles were extruded from its open end as urine left the cloaca and entered it. Thus urine was not contaminated by container water. No special care was taken to prevent contamination of urine with feces, but all urine samples were clear on visual inspection. Urine was collected simply by removing carefully the urine-filled cannula from the tadpole and emptying it into a test tube. Immediately before an exercise bout, the cannula was emptied and reinserted into the cloaca. Each cannula has sufficient capacity to hold all the urine excreted during the 2-h recovery period following exercise. Urine samples were mixed with 2 vol of 8% cold PCA and allowed to stand for 5 min before being centrifuged at 3,000 × g. The supernatant was assayed for lactate.

Total lactate elimination, that is, the sum of branchial, cutaneous, and renal excretion, was measured on a different group of 10 noncannulated tadpoles (4 ± 1 g). The tadpoles were exercised and allowed to recover for 2 h in individual containers containing 500 ml of aerated tap water. This water was freeze-dried and assayed for lactate as described above. Cutaneous lactate elimination was estimated by subtracting branchial and renal lactate excretion determined in the previous group from total lactate excretion as measured in the noncannulated group.

The relationship between blood and muscle lactate concentration and branchial ventilation volume in both resting tadpoles and tadpoles recovering from exercise was determined by adjusting the height of the tip of the opercular cannula (e.g., lowering the tip of the opercular cannula increased branchial ventilation by "siphoning" water over the gills). Tadpoles were divided into three groups. In the first group the cannula tip was adjusted to a level 1 cm below the container water surface, which yielded a branchial water flow of 310–1,670 $\mu l \cdot g^{-1} \cdot min^{-1}$. For the second group, the cannula tip was raised 3 cm above the container water surface, which allowed only 0–110 $\mu l \cdot g^{-1} \cdot min^{-1}$ of water to flow over the gills. In the third group, the tip of the opercular cannula was placed 5–7 cm below the water surface of the container during recovery,

resulting in a branchial water flow of 2,500–4,700 $\mu l \cdot g^{-1} \cdot min^{-1}$ during the 2-h recovery period. Care was taken to keep the container filled to constant height with aerated tap water. Immediately following the 2-h recovery period, blood and muscle samples were obtained from all tadpoles in each group and analyzed for lactate as described above.

EXPERIMENTAL PROTOCOL—FROGS

Cannulae (PE 50) were implanted in the femoral artery of anesthetized (MS 222, tricaine methylsulfonate—1:10,000) adult bullfrogs, which were then allowed 24 h to recovery prior to experimentation. Indwelling cannulation, as opposed to aortic puncture, allowed serial sampling (200 µl per sample) from undisturbed frogs at 23 C immediately following exercise, and at 15, 30, 60, 90, 120, and 240 min after exercise.

Exercise procedures involved inducing frogs to hop "voluntarily" while they were manually stimulated with blunt probe applied to their legs and back. Locomotor activity could usually be maintained for 6 ± 1 min. Failure to right when overturned (Bennett and Licht 1973) was not used as a criterion for exhaustion because animals that were unable to right themselves usually could still hop when turned upright. Samples of femoral arterial blood, as well as a limited number of hind-limb muscle samples, taken before or immediately following exercise, were prepared for lactate analysis with methods described above for tadpoles.

O_2 CONSUMPTION AND CO_2 EXCRETION

Five *Rana catesbeiana* tadpoles (5 ± 1 g) and five adults (175 ± 11 g) were obtained and maintained as described above. A "flow-through" respirometer for tadpoles was constructed from a 250-ml flask, containing 200 ml of aerated tap water covered by 50 ml of air. The top of the flask was sealed with a rubber stopper perforated by two PE 60 polyethylene tubes serving as an inlet and outlet. Humidified air was pumped through the respirometer at a rate of 0.075 $cm^3 \cdot min^{-1}$. Flow rate was adjusted with a Manostat flowmeter. The P_{O_2} of gas entering and leaving the respirometer was measured with a Beckman OM 14 oxygen analyzer, while CO_2 at the inlet and outlet of the respirometer was measured with a Cameron Instrument CO_2 analyzer. Initial tests indicated that gas and water phases took approximately 45 min after a tadpole was placed in the respirometer to come into a P_{O_2} and P_{CO_2} equilibrium.

Adult bullfrogs were placed in a respirometer constructed from a 3.8-liter translucent chamber containing 300 cm^3 of water (about 2 cm deep). The respirometer was immersed in a water bath at 22–23 C. The chamber lid was perforated with PE 60 polyethylene tubing for the exhaust of gas from the respirometer and PE 160 polyethylene tubing to deliver humidified air to the respirometer. Airflow was adjusted to 0.25 $cm^3 \cdot min^{-1}$ with a Manostat flowmeter. Analysis of gas was the same as described for tadpoles. Initial tests indicated that about 30 min was required for P_{O_2} and P_{CO_2} equilibration of the gas and water phase in the system after a bullfrog was placed in it.

Weight-specific O_2 uptake ($\dot{V}_{O_2}$) and CO_2 excretion ($\dot{V}_{CO_2}$) in both tadpoles and adults was calculated in the conventional way on the basis of the gas flow and gas concentration differences across the respirometer and expressed in $\mu l \cdot g^{-1} \cdot h^{-1}$.

Animals were allowed to acclimate to the respirometer for 24 h and were not fed. Subsequent measurements were made on quiescent animals. After determination of resting O_2 uptake and CO_2 excretion, tadpoles and adult bullfrogs were removed from the respirometers and exercised to exhaustion in the apparatus described above. Animals were then immediately returned to the respirometers without further disturbance. The animals were exercised in a fashion identical to that used for animals in which lactate and pyruvate analyses were made to allow a meaningful comparison of results. However, postexercise O_2 uptake and CO_2 release could be measured only after gas equilibrium in the respirometer had been reestablished after return of the animal.

STATISTICAL ANALYSIS

The Tukey multiple-range test of significance (Winer 1971) was used for multiple comparisons of samples taken at

different experimental times. This test categorizes data into different subsets, each subset statistically different from the others at the 0.05 level of significance. Data for tadpoles and adults were treated separately. Direct comparisons of variables for tadpole and adult bullfrog were tested with Student t-test. Data from tadpoles of different stages were pooled since there were no significant differences ($P > .1$) between stages in any given variable.

RESULTS

LACTATE AND EXHAUSTIVE EXERCISE

1. *Tadpoles.*—Immediately following exercise, whole-body lactate concentrations in tadpoles increased approximately five times over resting values and remained significantly greater than resting levels for 2 h following exercise (fig. 1).

Significant elevation of lactate concentration in blood (eight times greater than resting) and muscle (six times greater than resting) occurred immediately following exhaustive exercise. Although lactate concentration in muscle peaked immediately after exercise, blood lactate did not reach maximal concentration (10 times greater than resting) until 30–60 min following exercise. Both muscle and blood lactate fell to values not significantly different from resting 240 min after exercise.

Immediately following exercise there was a significant elevation above resting levels in the lactate concentration in kidney (3.0 times) and skin (2.4 times). However, in both tissues, maximum lactate concentrations (3.6 times resting for skin, 2.5 times resting for kidney) were not attained until 30 min following exercise (fig. 1). Yet, kidney and skin lactate concentrations were significantly higher than blood lactate con-

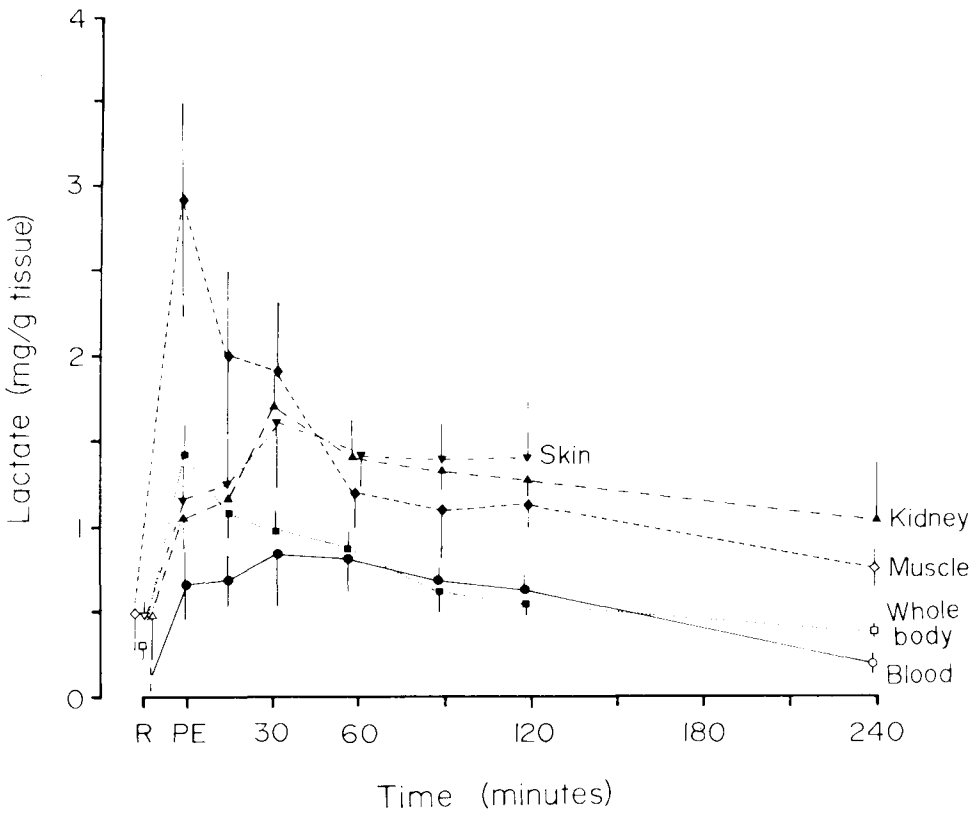

FIG. 1.—Lactate concentrations in whole-body homogenates and selected tissues of the bullfrog tadpole at rest and during recovery from exhaustive exercise. Mean values ± 1 SD are indicated. R = resting conditions, PE = immediately postexercise. Solid symbols for each data set indicate values significantly higher ($P < .05$) than resting values, while open symbols indicate no significant difference ($P > .1$). The number of tadpoles contributing to every point in each data set are as follows: whole body, 9; kidney, 6; skin, 6; blood, 15; muscle, 18.

centrations at all times, including the resting state. Kidney lactate concentrations remained significantly greater than resting values for 5 h following exercise, whereas skin lactate concentrations had returned to resting levels approximately 3 h following exercise.

Liver and lung homogenates obtained from tadpoles and adult bullfrogs before and 1 min following exercise showed no significant change in lactate concentrations and were very low, with respect to all other tissues examined, in lactate concentration.

In resting tadpoles, the bulk of lactate elimination occurred via the skin, while the kidney essentially played no role in lactate elimination (table 1). Branchial water and urine collected for 2 h following exhaustive exercise, as well as water surrounding uncannulated tadpoles recovering for 2 h from exercise, all showed significant increases ($P < .05$) in lactate over resting levels. About 7% of the whole-body lactate produced during exercise was eliminated over the 2-h recovery period. Of this total lactate eliminated, 79% was lost through the gills, 15% through the skin, and 6% through the urine. The amount of lactate lost through the skin appeared to change only slightly between resting and recovery (table 1), even though blood and skin lactate concentrations showed large changes (fig. 1).

No significant differences ($P < .01$) in lactate concentrations in blood and muscle 2 h following exhaustive exercise occurred between tadpoles with opercular cannulae adjusted to give head pressures near zero (i.e., normal ventilatory load) and those with lowered catheter tip to provide enhanced water flow (subambient head pressure). However, lactate concentrations in both blood and muscle were 140% higher ($P < .05$) in tadpoles with positive head pressures, that is, little or no water flow over their gills, compared with these other two groups (table 2). All unexercised tadpoles with a positive head pressure at the opercular spout had significantly ($P < .05$) higher blood and muscle lactate concentrations after a 2-h test period than unexercised animals with subambient or near zero head pressures (table 3).

2. *Bullfrogs.*—Unlike tadpoles, blood lactate concentrations in adult bullfrogs peaked immediately following exercise, at levels almost 13 times greater than resting levels (fig. 2). Blood lactate was still significantly greater than resting concentrations 6 h after exercise.

Mean muscle lactate concentration was 0.8 ± 0.5 mg/g tissue at rest, rising to 3.1 ± 0.8 mg/g tissue immediately following

TABLE 1

LACTATE EXCRETED FROM GILLS, SKIN, AND KIDNEY OF BULLFROG TADPOLES DURING A 2-h PERIOD OF REST AND A 2-h PERIOD IMMEDIATELY FOLLOWING EXHAUSTIVE EXERCISE

	LACTATE EXCRETED DURING A 2-h PERIOD (μg/g body mass)		TOTAL LACTATE EXCRETION (%)	
	Rest	Postexercise	Rest	Postexercise
Total lactate excreted	22.5 ± 7.5 (5)	90.0 ± 7.5 (6)	100	100
Lactate excreted from gills	3.1 ± 2.3 (8)	71.3 ± 6.40 (8)	14	79
Lactate excreted from skin	19.4	13.0	86	15
Lactate excreted in urine	.02 ± .1 (14)	5.8 ± 2.7 (14)	.1	6
Urine flow (μl/g/h)	6.0 ± 2.4 (6)	185.0 ± 146.0 (14)	. . .	. . .

NOTE.—Data reported are mean values ± 1 SD. Number of animals is indicated in parentheses. Estimates for skin lactate excretion were made by substracting branchial and urine lactate excreted in one group of tadpoles from total lactate excreted by another group (see Material and methods).

exercise. Repeated muscle samples during the time course of recovery were not obtained, so no data are available for estimation of blood-muscle lactate gradients or the time course of muscle lactate decline in adult bullfrogs. The lactate gradient from blood to muscle immediately postexercise was 1.5 times greater in tadpoles than in adults. Whole-body lactate levels were not determined on bullfrogs.

There were no significant differences ($P < .10$) in lactate concentration in the blood of adults at rest compared with that of tadpoles at rest. Immediately following exercise and for 15 min into the recovery period, blood lactate concentrations were significantly higher ($P < .05$) in frogs than in tadpoles. Blood lactate concentrations in tadpoles returned to resting levels at least 2 h earlier than in adult bullfrogs (fig. 1 and 2). Lactate concentration in the muscle of the adult bullfrogs was not significantly different ($P < .1$) from tadpoles either at rest or within 1 min following exhaustive exercise.

PYRUVATE AND EXHAUSTIVE EXERCISE

1. *Tadpoles.*—Pyruvate concentrations equaled or exceeded lactate concentrations at rest in all tissues examined in tadpoles. Concentrations of pyruvate in whole-body homogenates were significantly elevated following exercise to exhaustion. This elevation was followed by a decline in whole-body pyruvate concentration, with a return to resting levels 4 h after exercise (fig. 3). Blood pyruvate more than doubled immediately after exercise, followed by a slow

TABLE 2

BLOOD AND MUSCLE LACTATE CONCENTRATIONS 2 h FOLLOWING EXERCISE TO EXHAUSTION IN BULLFROG TADPOLES WITH ASSISTED, UNOPPOSED, OR OBSTRUCTED GILL VENTILATION

	Blood Lactate (mg %)	Muscle Lactate (mg/g)
Unopposed or assisted gill ventilation (310–1,670 μl H_2O/g/min or 2,500–4,700 μl H_2O/g/min)[a]	69 ± 15 (9)	1.13 ± .16 (9)
Obstructed gill ventilation (0–110 μl H_2O/g/min)	98 ± 9 (8)	1.57 ± .27 (8)

NOTE. Mean values ± 1 SD are indicated. Sample sizes are indicated in parentheses.

[a] There was no significant difference ($P > .10$) in lactate concentration when "assisted" (310–1,670 μl H_2O/g/min) was compared with that concentration in "unopposed" (2,500–4,700 μl H_2O/g/min) ventilation. Data from these two conditions were therefore pooled.

TABLE 3

BLOOD AND MUSCLE LACTATE CONCENTRATIONS IN RESTING BULLFROG TADPOLES AFTER A 2-h PERIOD WITH ASSISTED OR UNOPPOSED GILL VENTILATION OR WITH OBSTRUCTED GILL VENTILATION

	Blood Lactate (mg%)	Muscle Lactate (mg/g)
Assisted or unopposed gill ventilation (20–410 μl H_2O/g/min)[a]	6 ± 1 (7)	.52 ± .01 (7)
Obstructed gill ventilation (0 ml H_2O/h)	14 ± 6 (5)	.88 ± .22 (5)

NOTE. Mean values ± 1 SD are reported. Sample sizes are indicated in parentheses.

[a] There was no significant difference ($P > .10$) between lactate concentrations when "assisted" was compared with "opposed" ventilation. Data from these two conditions were therefore pooled.

decline that reached resting levels after 2 h of recovery (fig. 3). Pyruvate in tadpole muscle increased significantly above resting levels immediately following exercise but then decreased significantly below resting concentrations 15 min into recovery and stayed depressed for more than 1 h (fig. 3). Pyruvate concentrations in the kidney of tadpoles increased significantly immediately after exercise, dipped briefly back to resting levels, but then increased again 2 h following exercise (fig. 3).

Pyruvate concentrations measured immediately postexercise and compared among various tissues can best be summarized by muscle > kidney ≫ blood.

2. *Adults.*—Blood pyruvate more than doubled in adults immediately following exercise, with a recovery time course quite similar to that in the blood of tadpoles (fig. 3). Muscle pyruvate concentration decreased by nearly one-half immediately following exercise, without the transient rise observed in tadpoles. Serial muscle samples were not obtained after exercise in adults.

As in tadpoles, concentrations of pyruvate in muscle greatly exceeded those in blood of adults immediately following exhaustive exercise.

LDH ACTIVITY IN TISSUES

Muscle had the highest LDH activity in any of the tissues tested in both tadpoles and adults (table 4). The LDH activity was significantly higher ($P < .001$) in the muscle of adult bullfrogs than in tadpoles. Both kidney and liver of the tadpoles had similar LDH activities, which was about 25% of that in muscle (table 4) and significantly greater activity than the same organs in adults. The LDH activity in the liver and kidney of adult bullfrogs was only 3% and 6%, respectively, that of muscle. No LDH activity was found in the lungs of tadpoles or heart of frogs. Activity of LDH in the tissues of both tadpoles and adults can thus be summarized as follows: muscle ≫ kidney > liver ≫ heart ~ lungs.

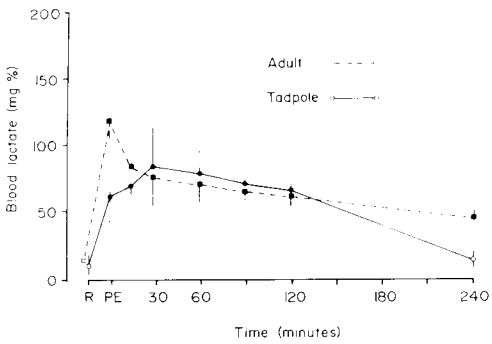

FIG. 2.—Blood lactate concentrations in tadpoles and adults at rest and during recovery from exhaustive exercise. Mean values ± 1 SD are indicated. *R* = resting conditions, *PE* = immediately postexercise. Solid symbols indicate values significantly higher ($P < .05$) than resting values, while open symbols indicate no significant difference ($P > .1$). Each circle represents data obtained from 15 tadpoles. Each square represents data obtained from five frogs.

O_2 CONSUMPTION, CO_2 EXCRETION, AND EXERCISE

Resting weight-specific $\dot{V}O_2$ and $\dot{V}CO_2$ in adult bullfrogs and tadpoles were not significantly different in spite of large differences in body mass. The respiratory exchange ratio, *R*, of $\dot{V}CO_2$:$\dot{V}O_2$ at rest was approximately 1 in both tadpoles and adults.

1. *Tadpoles.*—Oxygen uptake in tadpoles 45 min following exercise (the earliest recovery time for which reliable measurements could be obtained; see Material and methods), was 1.5 times resting values (fig. 4). With the exception of a brief fall 4 h into recovery, $\dot{V}O_2$ was elevated above resting values for at least 10 h following exercise.

Carbon dioxide elimination following exercise was not significantly different at any time from that in resting tadpoles. Consequently, *R* was depressed to 0.5–0.7 for about 16 h until $\dot{V}O_2$ returned to resting values (fig. 4).

2. *Adults.*—Weight-specific $\dot{V}O_2$ in adult bullfrogs 30 min following exercise (which was the earliest recovery time for which reliable measurements could be obtained; see Material and methods), was elevated four times above resting values and did not return to values significantly different from resting values for 4 h. A second significant elevation in $\dot{V}O_2$ occurred in all frogs 13–14 h following exercise, which corresponded to 0:00–1:00 EST.

Carbon dioxide release in adults increased approximately five times above resting values 30 min following exercise (fig. 4). By 1 h after exercise, $\dot{V}CO_2$ had declined to values less than twice that of

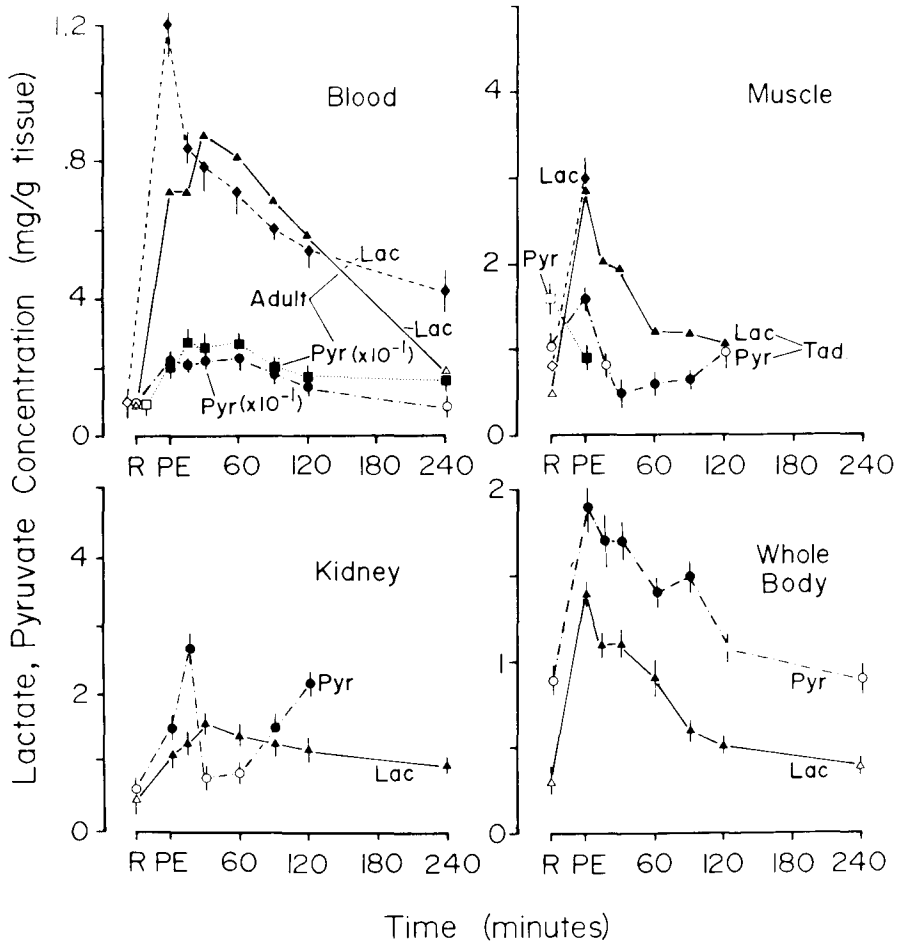

FIG. 3.—Lactate and pyruvate concentrations in various tissues of larval and adult bullfrogs (*Rana catesbeiana*) at rest and during recovery from exhaustive exercise. Mean values ± 1 SD are indicated. *R* = resting conditions, *PE* = immediately postexercise. Solid symbols for each data set indicate values significantly different ($P < .05$) from resting values, while open symbols indicate no significant difference ($P > .10$). The number of tadpoles contributing to every point in each data set are as follows: blood, 15; muscle, 18; kidney, 6; whole body, 9. Data from five animals are included in each mean for adults.

TABLE 4

LDH ACTIVITY IN MUSCLE, KIDNEY, LIVER, HEART, AND LUNG OF LARVAL AND ADULT *Rana catesbeiana* (IU/g tissue)

	Tadpole	Frog
Muscle	2,154 ± 46 (4)	3,968 ± 32 (4)
Kidney	533 ± 99 (4)	230 ± 32 (4)
Liver	522 ± 23 (4)	115 ± 12 (4)
Heart	268 ± 14 (2)	Not detectable
Lung	Not detectable	115 ± 12 (4)

NOTE.—Mean values ± 1 SD are given, with number of animals contributing to each mean indicated in parentheses.

controls, while 2 h following exhaustive exercise, $\dot{V}CO_2$ was not significantly greater than resting values. The respiratory exchange ratio R approximated 1.0 both at rest and from 30 min to 1 h following exercise. However, a depression in R to 0.50–0.70 developed after 1 h and persisted for about 16 h (fig. 4).

Maximal $\dot{V}O_2$ and $\dot{V}CO_2$ 30–60 min following exercise in adult bullfrogs was three to four times that in tadpoles, although it must be reemphasized that data were not obtained during the early minutes of recovery.

DISCUSSION

LACTATE PRODUCTION, TISSUE DISTRIBUTION, AND ROUTES OF ELIMINATION

Blood lactate concentrations are unreliable for predicting lactate concentrations

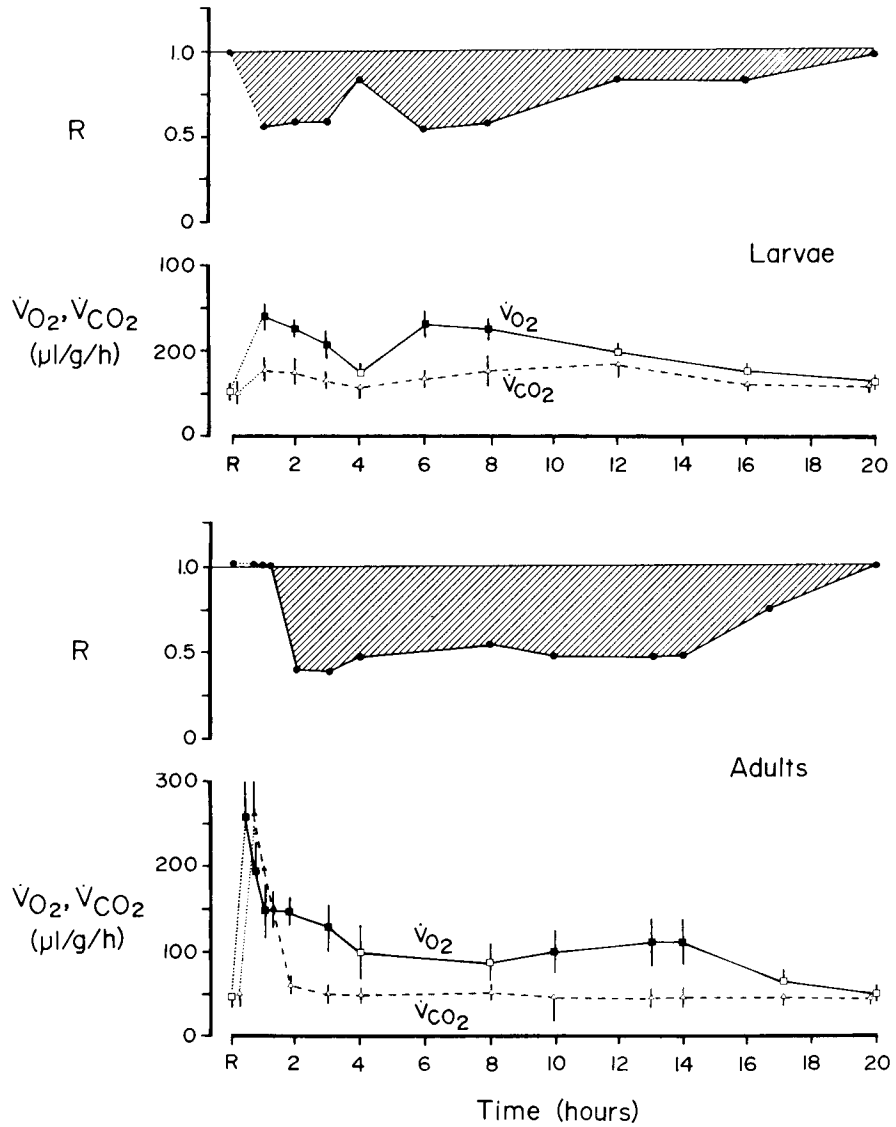

FIG. 4.—Oxygen consumption, carbon dioxide excretion, and the respiratory quotient at rest and during recovery from exhaustive exercise in larval and adult bullfrogs (*Rana catesbeiana*). Mean values ± 1 SD are given. Solid symbols indicate values significantly different ($P < .05$) from resting values, while open symbols indicate no significant difference ($P > .10$) from resting values. No. = 5 for all points for both tadpoles and frogs.

of the whole animal and rates of lactate disappearance, since blood lactate concentration is a complex function of many variables. In bullfrog tadpoles these may include the quantity of lactate produced by muscles and the rate of lactate removal by the liver, gills, skin, and kidney. The delayed peak in the blood lactate relative to whole-body lactate following exercise in bullfrog tadpoles (fig. 1), which is similar to that reported for exercised lizards (Bennett and Licht 1973), indicates only a limited value for blood lactate as a precise indicator of whole-body lactate. However, both blood and whole-body lactate concentrations return to resting levels at approximately the same time, so blood lactate in tadpoles may nonetheless provide an indication of the return to resting state.

Since the muscle-to-blood lactate gradient is large immediately following exercise in tadpoles, the 30-min lag in maximal blood lactate concentrations indicates either restricted muscle perfusion or active retention of lactate in the muscle. A delay in elevation of blood lactate following exercise was not seen in adult bullfrogs, in which blood lactate peaked immediately. The higher bicarbonate concentrations in the blood of adults compared with those in tadpoles (Erasmus, Howell, and Rahn 1970; Just, Gatz, and Crawford 1973; D. Quinn, unpublished) may be important in buffering protons associated with the more rapid release of lactic acid from the muscle immediately following exercise.

Bullfrog tadpoles have been reported to have low anaerobic capacities (total lactate produced during exercise to exhaustion, as defined by Bennett and Licht [1973, 1974]), compared with adult forms of other amphibians (Bennett and Licht 1973, 1974; Seymour 1973; Turney and Hutchison 1974; Putnam 1979*b;* Hutchison and Miller 1979*a,* 1979*b*). Yet bullfrog tadpoles are immediately very active when stimulated, a useful characteristic for an animal which is heavily preyed on (Bennett and Licht 1974). A high anaerobic scope would appear advantageous in predator avoidance, since initiation of anaerobic enzyme sequences is rapid and anaerobiosis has a lower thermal dependence compared with aerobiosis (Bennett and Licht 1973). High lactate levels immediately following exercise in the present study indicate that our bullfrog tadpoles heavily exploit anaerobiosis during exercise. Bennett and Licht (1974) reported lactate production during exercise in bullfrog tadpoles that was only one-half of that recorded in the present study. These authors reported lethargic swimming in bullfrog tadpoles after just 10 s, but tadpoles in our study swam voluntarily for 3–5 min, and manual stimulation could evoke a further 5 min of swimming before complete exhaustion. Ten minutes of continuous activity before exhaustion is typical of adult anurans such as *Rana pipiens* and *Hyla regilla* (Bennett and Licht 1974).

Whole-body lactate concentration of bullfrog tadpoles, both at rest and following exercise, was similar in our experiments to values obtained for adult *R. catesbeiana* (Hutchison and Miller 1979*b*) and other adult amphibians (Bennett and Licht 1973, 1974; Seymour 1973; Turney and Hutchison 1974; Hutchison and Miller 1979*a,* 1979*b;* Putnam, 1979*b*). Hutchison and Miller (1979*a*) indicated that 4 h were required for whole-body lactate of adult bullfrogs to return to control levels, whereas other adult amphibians, such as *R. pipiens,* required 6–8 h to recover after electrically stimulated exercise (Hutchison and Turney 1975). Whole-body lactate concentrations in bullfrog tadpoles in the present study had returned to resting levels within 2 h following exercise (fig. 1). It appears that bullfrog tadpoles are more effective than adult bullfrogs in reducing whole-body lactate concentrations to control levels following exercise.

A small but significant amount of lactic acid produced during exhaustive exercise in the bullfrog tadpole is eliminated as lactate anion during the first 2 h of recovery. This contrasts with data obtained for tiger salamanders (*A. tigrinium*) by Cushman et al. (1976), who were unable to detect lactate in the water surrounding animals recovering from activity. This discrepancy may be due to species differences in methodology. In the present experiments, lactate concentrations in the collected branchial effluent and in water surrounding the animal were far too low to measure conventionally unless the samples were

concentrated by lyophilization. The absolute amount of lactate eliminated over a 2-h recovery period following exercise by bullfrog tadpoles is small relative to the total body pool but may influence blood lactate concentrations. Since 79% of the lactate eliminated from bullfrog tadpoles was lactate lost from the blood via the gills, and since much of the lactate produced during exercise apparently may never leave muscle where it is produced, lactate eliminated from the gills may represent a significant fraction of lactate entering the blood. It is not known if branchial lactate elimination varied during the 2-h recovery period, but most lactate may have been excreted during the first hour when the lactate gradient from blood to water was highest. This corresponds with the greatest fluctations in blood pH (D. Quinn, unpublished).

That the gills represent a major site of lactate elimination in bullfrog tadpoles is not surprising, since their surface area is large and the branchial blood-water diffusion distance is only 2–4 μ, compared with 20–40 μ in the skin (Burggren and Mwalukomo 1983). Also, systematic venous blood in tadpoles passes first through the gills, then on to the skin and kidneys, so the diffusion gradient for lactate would be greatest at the gills. The importance of branchial elimination of lactate during recovery from exercise in the tadpole was further demonstrated by the 140% increase in both muscle and blood lactate resulting from obstruction of gill ventilation during the recovery period (table 3). Some of this increase in lactate could result from continued anaerobiosis in the recovery period if oxygen uptake was severely limited, but most of the oxygen uptake occurs via the skin and lungs of bullfrog tadpoles (Burggren and West 1982), so interruption of branchial ventilation may not interfere greatly with a complete return to aerobiosis. Lactate elevation during obstructed gill ventilation could also be due to added acid-base disturbances of aerobiosis resulting from diminished release of lactate and H^+ ions normally leaving the gills. This metabolic acidosis may enhance lactate release from muscle (Mainwood and Worsely-Brown 1975). Finally, lactate conversion to another compound that can easily be excreted via the gills cannot be ruled out. Lactate conversion to ethanol for excretion may occur in goldfish (Shoubridge and Hochachka 1980), and lactate conversion to an unidentified chemical form for excretion apparently occurs in toadfish (Trant 1981).

Lactate elimination in lower vertebrates is very small compared with lactate retained in the body during exercise or experimental dives (Hunn 1969; Jackson and Silverblatt 1974; Kobayashi and Wood 1980), even though urine lactate concentrations may increase significantly. The increase in urine flow following exercise in bullfrog tadpoles agrees with data obtained on rainbow trout by Hoffman and Butler (1979). The small quantity of lactate in the urine is unusual considering the comparatively large concentration in the kidney following exercise. Since kidney lactate concentrations were higher than blood levels during the early recovery period, active transport rather than simple diffusion from blood into kidney tissue appears to be involved. Active uptake of lactate occurs in the proximal tubules of kidneys in the garter snake *Thamnophis* (Brand and Stansbury 1980*a*, 1980*b*, 1981). Lactate may be oxidized or converted to glucose in the kidney, at least in mammals (Dies et al. 1981). Such processes could account for high kidney but correspondingly low urine lactate concentrations, although renal uptake and metabolism of lactate have not been demonstrated in the kidneys of any amphibian.

Lactate concentrations in the skin were higher than those in the blood at all times, indicating either active uptake or production of lactate in skin. The skin of the tadpole, like the kidney, is relatively unimportant to lactate excretion. Since the skin of amphibians is highly active metabolically (Motais and Garcia-Romeu 1972), lactate may be actively taken up and utilized by the skin as an energy source.

Muscle lactate concentrations at rest and following exercise in adult *R. catesbeiana* were in agreement with those obtained following exercise in adult *R. pipiens* (Putnam 1979*b*). After exercise, the concentration of lactate in hind-limb muscles of adults was not significantly greater than in tail muscle of tadpoles following exer-

cise. Tadpoles with both hind limbs and tail had similar lactate concentrations following exercise in both muscle masses, that is, tail and hind limb. The use of different muscle groups during activity appears to correlate with differences in lactic acid accumulation.

POSSIBLE FATES OF MUSCLE-SEQUESTERED LACTATE

There are several possible fates for lactate produced in the muscle during activity in larval or adult bullfrogs. Lactate may be flushed from the muscle and eventually removed, although less than 1/10 of lactate so produced is eliminated in tadpoles. The large remainder may be aerobically oxidized to CO_2 and water or reconverted to glucose (Lehninger 1975; Harper et al. 1979).

Gluconeogenesis does not occur simply by reversal of glycolysis, because many of the enzymes involved catalyze irreversible reactions. One of these, fructose diphosphate, is present and highly active in amphibian striated muscle (Salas et al. 1964). However, the other two "one-way" enzymes (phosphoenolpyruvate carboxylase and pyruvate carboxylase) are not highly active, and lactate conversion to glucose or glycogen has been considered unlikely in vertebrates (Krebs and Woodford 1965). Yet evidence suggests that glycogen can be formed from lactate or pyruvate in the muscle of mammals and lower vertebrates. For example, Batty and Wardle (1979) found that intramuscular injections of ^{14}C lactate in exhausted plaice resulted in an uptake of the lactate by recovering muscle cells. Substantial portions of the labeled lactate were converted to glycogen. Hermanssen and Vaage (1977) measured arteriovenous differences in glucose in human calf muscle; glucose uptake during recovery accounted for less than 5% of the glycogen synthesized following exercise. Similar results have been obtained with frog and rabbit muscle (Meyerhoff 1920; Hill 1924; Bendall and Taylor 1970).

Changes in blood lactate after exercise in both tadpoles and adult bullfrogs clearly do not parallel changes in muscle lactate (fig. 1). Blood lactate concentrations did not peak until 30 min following exercise, even though the muscle-blood gradient was maximal prior to this point. These data indicate that lactate may be sequestered in the muscle and metabolized to some other compound.

If lactate produced during exhaustive exercise in the muscle of the bullfrog were to be reconverted to pyruvate as a substrate for gluconeogenesis, then high LDH activity coupled with a fall in muscle pyruvate concentrations would create optimal conditions for this reaction at the site of lactate production itself (Opie and Newsholme 1967). The highest LDH activity was found in muscle of both tadpoles and adult bullfrogs, with comparatively little or no LDH activity in the liver, kidney, lung, or heart (table 1). Pyruvate concentrations in tadpole muscle were significantly lower than resting levels from 15 through 90 min in the recovery period, while pyruvate concentrations in kidney, blood, skin, and whole body were all elevated over resting levels. Because favorable conditions for reconversion of lactate to pyruvate (reduced pyruvate, high LDH) were thus found in muscle, and since muscle comprises approximately 30% of body mass in anurans (Putnam 1979*b*), it is unlikely that the liver, kidney, lung, or heart plays a major role in the clearing of lactate produced in the muscle during exercise in the bullfrog. Lactate release to the blood, conversion of lactate to glucose at some remote site, and transport of glucose back to the muscle for metabolism are all eliminated by conversion of lactate directly to glucose within the muscle where it is produced.

The reasons for the large fall in muscle pyruvate immediately after exercise in *Rana* are not clear, but it may result from a combination of intramuscular conversion of pyruvate to glucose and flushing of pyruvate from muscle into the blood, where pyruvate concentration in fact rises following exercise. Lactate is not similarly flushed from the muscle in the early phases of recovery, but lactate released from muscle of lower vertebrates may be independent of perfusion rate (Wardle 1978) and dependent on extracellular biocarbonate concentration (Mainwood and Worsely-Brown 1975).

We cannot conclude whether pyruvate produced by the action of LDH is oxidized

aerobically or converted back to glucose. However, if a significant portion of muscle lactate were oxidized to CO_2 and water, the CO_2 excretion in addition to O_2 uptake of tadpoles or bullfrogs would be expected to increase or stay at elevated, postexercise levels while muscle lactate decreases. This is clearly not the case for either developmental state.

GAS EXCHANGE AND EXERCISE

Resting $\dot{V}CO_2$ and $\dot{V}O_2$ in tadpoles and adults generally agree with cited values for *R. catesbeiana* (see Prosser 1973; Burggren and West 1982; Burggren, Feder, and Pinder 1983) and are similar to data reported for other resting anurans (Emilio 1974; Turney and Hutchison 1974). Oxygen consumption rates of approximately 700–1,200 $\mu l\ O_2 \cdot g^{-1} \cdot h^{-1}$ following activity in adult anurans are high compared with those of other amphibians (Hillman 1976). A comparison of our data with Hillman's (1976) is not possible owing to differences in exercise protocol, though our data for adult bullfrogs are similar to those generated in similar experiments by Seymour (1973).

The very steep decline in the $\dot{V}O_2$ slope from 30 to 60 min following manually stimulated exercise (fig. 4) indicates that aerobic contributions to energy production may be very important in exercising bullfrogs, as reported for anurans (by Hillman et al. 1979). However, studies that have employed electrical stimulation to elicit activity (Turney and Hutchison 1974; Hutchison and Miller 1979*a*, 1979*b*) have failed to reveal a similarly large aerobic component. The slight but significant elevation in the $\dot{V}O_2$ of bullfrogs at 13 and 14 h following exercise (fig. 4) occurred between 12:00 and 1:00 A.M. EST and may reflect circadian variations in $\dot{V}O_2$ (Turney and Hutchison 1974).

Oxygen consumption following exercise is a poor indicator of lactate-dependent oxygen debt in amphibians, because $\dot{V}O_2$ remains elevated after lactate levels have returned to normal (Bennett and Licht 1973; present study). Possibly oxygen is being used to replenish blood and muscle stores of O_2 and to regenerate ATP and NAD. Alternatively, or in addition, hormonal stimulation during exhaustive exercise may have actually elevated cellular metabolic rates (although $\dot{V}CO_2$ of tadpoles was not elevated).

In both tadpoles and adult bullfrogs, CO_2 excretion fell back to resting levels earlier than O_2 uptake (fig. 4), resulting in an R value well below 1 for many hours after exercise. The initial release of metabolic acids into the blood during exercise apparently acidifies blood bicarbonate, causing the early release of large amounts of CO_2 (D. Quinn, unpublished). This constitutes a "CO_2 debt" (Burggren and Cameron 1980), requiring subsequent replenishment of CO_2 to resting levels. If CO_2 in these animals was not being retained to restore preexercise CO_2 levels, then R during recovery might be expected to be considerably higher, particularly if oxidation of pyruvate resulted in generation of CO_2.

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