Lactate efflux is unrelated to intracellular P_{O_2} in a working red muscle in situ

R. J. CONNETT, T. E. J. GAYESKI, AND C. R. HONIG
Department of Physiology, School of Medicine and Dentistry, University of Rochester, Rochester, New York 14642

CONNETT, R. J., T. E. J. GAYESKI, AND C. R. HONIG. *Lactate efflux is unrelated to intracellular P_{O_2} in a working red muscle in situ.* J. Appl. Physiol. 61(2): 402–408, 1986.—Blood flow, lactate extraction, and tissue lactate concentration were measured in an autoperfused pure red muscle (dog gracilis). Muscles were frozen in situ during steady-twitch contraction at frequencies of 1–8 Hz [10–100% of maximum O_2 consumption ($\dot{V}O_{2\,max}$)]. Myoglobin saturation was determined spectrophotometrically with subcellular spatial resolution. Intracellular P_{O_2} (Pt_{O_2}) was calculated from the oxymyoglobin-dissociation curve. Tissue lactate was well correlated with $\dot{V}O_2$ but not with Pt_{O_2}. Lactate efflux increased markedly above a threshold work rate near 50% $\dot{V}O_{2\,max}$. Efflux was neither linearly correlated with tissue lactate nor related to Pt_{O_2}. Pt_{O_2} exceeded the minimum P_{O_2} for maximal $\dot{V}O_2$ in each of 2,000 cells examined in muscles frozen at 1–6 Hz. A small population of anoxic cells was found in three muscles at 8 Hz, but lactate efflux from these muscles was not greater than from six other muscles at 8 Hz. Our conclusions are that *1*) the concept of an anaerobic threshold does not apply to red muscle and *2*) in absence of anoxia neither tissue lactate nor blood lactate can be used to impute muscle O_2 availability or glycolytic rate. A mechanism by which the blood-tissue lactate gradient could support aerobic metabolism is discussed.

anaerobic threshold; lactic acid; exercise; glycolysis; muscle metabolism; oxygen transport; transport of lactate; critical partial pressure oxygen; anoxia; hypoxia; ischemia

THE ARTERIAL LACTATE concentration ($[LA]_a$) increases sharply during voluntary exercise above ~50% of the maximal rate of O_2 consumption ($\dot{V}O_{2\,max}$) (1). The ratio of ventilation to $\dot{V}O_2$ also increases above an apparent threshold work rate (38). These observations have been interpreted to mean that a submaximal work rate exists above which $\dot{V}O_2$ cannot fully meet energy demand, resulting in recruitment of anaerobic glycolysis (1, 38). The term "anaerobic threshold" has been widely used to describe the phenomenon.

The interpretation of blood lactate data is controversial. No direct evidence has been offered for O_2-limited muscle metabolism at the threshold work rate and aerobic glycolysis is known to accompany submaximal exercise (7–9, 18). Evidence has been presented suggesting that factors related to whole-body lactate metabolism rather than muscle lactate efflux govern $[LA]_a$ changes (4, 5, 13, 33, 37). Despite the foregoing, $[LA]_a$ and lactate extraction ($[LA]_{v\text{-}a}$) continue to be used in experimental and clinical science to evaluate tissue O_2 supply.

This report describes the relation between intracellular P_{O_2} and lactate efflux from a pure red muscle autoperfused in situ. A threshold for accelerated lactate efflux was observed at work rates comparable to reported thresholds for increased $[LA]_a$ in humans. No anoxic loci were found, however, even at work rates well above the threshold work rate for accelerated lactate efflux. Moreover, neither the tissue lactate concentration ($[LA]_T$) nor lactate efflux were correlated with intracellular P_{O_2}. The data are consistent with the proposal that aerobic glycolysis and aerobic accumulation of lactate in working muscle increase cytosolic redox in support of oxidative phosphorylation in mitochondria (7–9).

METHODS

General

All experiments were performed on hound-type mongrels weighing 20–25 kg. Animals of either sex were anesthetized with pentobarbital sodium, intubated, and allowed to breathe room air. The gracilis muscle was vascularly isolated and autoperfused as described previously (15, 21). Muscles were wrapped with Saran (an O_2 barrier) and maintained at 37°C with radiant heat. The distal tendon was attached to an isometric force transducer, and the resting tension was adjusted to 300 g. Isometric twitch contraction was induced by stimulating the cut obturator nerve with supramaximal square-wave monophasic pulses, 0.1 ms in duration. Such pulses do not excite sympathetic vasoconstrictor fibers (20). All motor units should have participated in each twitch.

A T cannula was inserted into the femoral vein, which received blood from the gracilis muscle only. Closure of a Blalock clamp proximal to the T cannula allowed collection of the gracilis effluent. Blood flow at rest was measured by timed collection of venous outflow, whereas arterial inflow was measured with an electromagnetic flowmeter. This procedure calibrated the flowmeter in every experiment. During exercise the Blalock clamp was kept open to avoid possible congestion and flow was read from the oscillographic recording. In separate experiments flow so determined agreed within 5% with flow measured by timed collection of effluent during exercise. Part of the venous outflow was collected anaerobically for measurement of blood gases and venous lactate. All samples of stimulated muscle were collected after 3 min

of stimulation. Previous studies have shown that blood flow, $\dot{V}O_2$, tissue phosphocreatine, and lactate efflux are at a steady state after 3 min of stimulation (9, 15). Blood samples were collected 10–15 s before freezing of the muscle (see below).

Blood Samples

Venous samples were collected in 5-ml heparinized plastic cups filled with mineral oil. During stimulation sufficient volume could be collected in 10 s after clearing the dead space even though the Blalock clamp was kept open. An arterial sample was collected simultaneously. Samples for blood gases were immediately removed to iced syringes. Measured aliquots of arterial and venous blood were added to 1 N perchloric acid immediately after collection to prevent blood glycolysis from interfering with the estimation of muscle lactate exchange. Arterial and venous O_2 and CO_2 tensions and arterial pH were measured with an Instrumentation Laboratories gas analyzer, model 213. Hemoglobin concentration and arterial and venous O_2 saturation were measured with an Instrumentation Laboratories CO-oximeter, model 282, programmed for canine blood.

Fast Freezing

Muscles were frozen in situ with a copper block (5 cm^3) cooled to −196°C in liquid N_2. Descent of the piston turned off the stimulator ~10 ms before contact. The block was applied to the muscle at 0.1 kg/cm^2 with an air-driven piston. Muscle samples were excised from the dog while they were held firmly in contact with the copper block by the piston. The entire assembly was then immersed in a bucket of liquid N_2. The samples were separated from the copper block under liquid N_2 and stored in liquid N_2 until analyzed. The initial rate of freezing 500 μm from the surface was ~10 μm/ms (14). A mathematical model indicates that, in muscle so frozen, changes in myoglobin (Mb) saturation due to $\dot{V}O_2$ or O_2 binding during freezing would alter Mb saturation by ~0.1% at $\dot{V}O_{2max}$ (6). Empirical tests that confirm predictions of the model are described in Refs. 15 and 16.

Cryomicrospectroscopy

Myoglobin saturation in individual cells viewed in cross section was determined by reflection cryomicrospectroscopy as described in previous reports (14–16). The method is based on a four-wavelength adaptation of Beer's law. It takes account of scattered light and light reflected from structures such as mitochondria, which do not contain Mb. Myocytes averaged 50 μm in diameter. The measuring diaphragm of the photometer was ≤5 μm^2. The calculated volume from which light was collected was ~100 μm^3 (14). Subcellular spatial resolution permits complete separation of the Mb spectrum from similar spectrum of hemoglobin in adjacent vessels. In skeletal muscle cytochromes have no measurable redox-dependent absorption at the selected wavelengths. The reproducibility of the saturation determination in a population of cells was evaluated in muscles treated with cyanide to eliminate variability caused by O_2 consumption. The standard deviation was <4% (16).

The intracellular P_{O_2} (Pt_{O_2}) in equilibrium with Mb was calculated from Mb saturation and the oxymyoglobin dissociation curve. The P_{50} for canine Mb is 5.3 Torr at 37°C (14); it is unaffected by ionic strength, P_{CO_2}, or pH. The error in calculating Pt_{O_2} that results from error in determining Mb saturation depends on the absolute value of saturation because of the influence of the hyperbolic dissociation curve. Values of saturation in muscles near $\dot{V}O_{2max}$ were typically ~20%. The error in Pt_{O_2} at 10 and 20% saturation was 0.1 and 0.3 Torr, respectively. The minimum Pt_{O_2} that could be distinguished from zero was 0.15 Torr, corresponding to a saturation of 3%.

Sampling for Mb Spectroscopy

Muscles were fractured into tissue blocks about 1 × 2 × 0.3 cm under liquid N_2 and a freshly cleaved cross section prepared as described previously (15, 16). The tissue block was then transferred to the microscope cold stage at −110°C. At this temperature Mb spectra are stable for >5 h. Observations on a particular block generally required <0.5 h. At least five tissue blocks were examined in each muscle so that heterogeneity on a scale of centimeters was sampled. In each block Mb saturation was determined 200–500 μm beneath the surface. Cell boundaries could be visualized without use of chemical stains. Measurements were made at the center of 10 cell profiles in each block. Consequently, Mb saturation and the corresponding P_{O_2} were determined near the low end of O_2 gradients from sarcolemma to cell interior. Each cell sampled was at least 500 μm from any other, to take account of heterogeneity of O_2 delivery and fiber type on the scale of a small arteriole. It is important that dog gracilis contains equal numbers of interspersed slow- and fast-oxidative red fibers; there are no fast-glycolytic white fibers (27). Thus probability distributions for each muscle were based on a total of 50 widely spaced red muscle cells from five macroscopic regions. The number of muscles examined at each stimulation frequency ranged from 5 at 2 Hz to 12 at 4 Hz. The corresponding number of cells sampled ranged from 250 at 2 Hz to 600 at 4 Hz.

Sampling and Analysis of Tissue Metabolites

When spectroscopy was completed, the fascial layer on the muscle surface was split off and the underlying 0.5 mm of muscle was broken into 1- to 5-mg chips. Chips from several blocks (i.e., macroscopic regions of the same gracilis) were pooled, weighed, and extracted in perchloric acid. In five muscles, reassay based on chips from different tissue blocks yielded results within the error of the assays (±5%). Measurements are expressed as micromoles per gram wet tissue or as concentrations. Tissue metabolites were analyzed on neutralized deproteinized aliquots of the perchloric acid extracts using an automated procedure (9). Muscle lactate content (μmol/g wet wt) was converted to intracellular concentration by cor-

recting for 0.2 ml/g extracellular volume and 0.56 ml/g intracellular volume. Blood concentrations were expressed as millimoles per liter of whole blood. Although there may be some change in total tissue water during stimulation this effect is small (≤15%, see Ref. 9). Even at the highest stimulation rates the increase in blood [lactate] and hence the extracellular correction is small (~×2.5), relative to the increase in tissue [lactate] (~×20). Thus constant conversion factors do not introduce significant error or frequency-dependent variation into the results.

RESULTS

Determinants of Lactate Efflux

Lactate efflux is the product of blood flow and the venoarterial lactate difference ($[LA]_{v-a}$). These variables are plotted as functions of $\dot{V}O_2$ and twitch frequency in Fig. 1. Blood flow was high at rest because the muscles were extrinsically denervated. Nevertheless flow rose significantly at 1 Hz and increased almost linearly with $\dot{V}O_2$, between 1 and 8 Hz. Twitch contraction at 8 Hz evoked 90–100% of $\dot{V}O_{2\,max}$ in various muscles (8, 21). There was no significant change in $[LA]_a$ with twitch frequency and muscle $\dot{V}O_2$. Consequently, $[LA]_{v-a}$ in Fig. 1 reflects changes in the venous concentration. $[LA]_{v-a}$ did not exceed the resting value at 2 Hz or ~30% $\dot{V}O_{2\,max}$. It doubled between 2 and 4 Hz and reached an apparent maximum at 6 Hz.

Correlation of Lactate Efflux with $\dot{V}O_2$ and Pt_{O_2}

The relations among lactate efflux, twitch frequency, and $\dot{V}O_2$ are shown in Fig. 2*A*. Lactate efflux did not change through 2 Hz, but at 4 Hz it rose to half its maximum value. At 4 Hz, $\dot{V}O_2$ was 5 μmol $O_2 \cdot g^{-1} \cdot min^{-1}$ or ~70% $\dot{V}O_{2\,max}$. Thus a threshold work rate exists for accelerated lactate efflux from a fully recruited red muscle devoid of fast glycolytic fibers. This threshold work rate appears to lie at about half aerobic capacity.

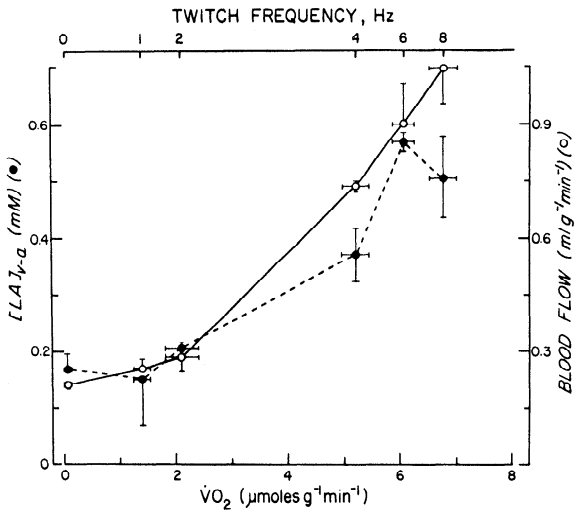

FIG. 1. Average blood flow, (*open circles*), and $[LA]_{v-a}$, (*closed circles*) plotted against average $\dot{V}O_2$ at rest and at 1, 2, 4, 6, and 8 Hz. *Horizontal error bars* show SE for $\dot{V}O_2$ for following number of measurements: rest, 11; 1 Hz, 9; 2 Hz, 7; 4 Hz, 17; 6 Hz, 7; and 8 Hz, 8. *Vertical error bars* show SE for flow and $[LA]_{v-a}$ with following number of measurements: rest, 76; 1 Hz, 16; 2 Hz, 6; 4 Hz, 20; 6 Hz, 5; and 8 Hz, 23. *Error bars* at rest lie within *symbols*.

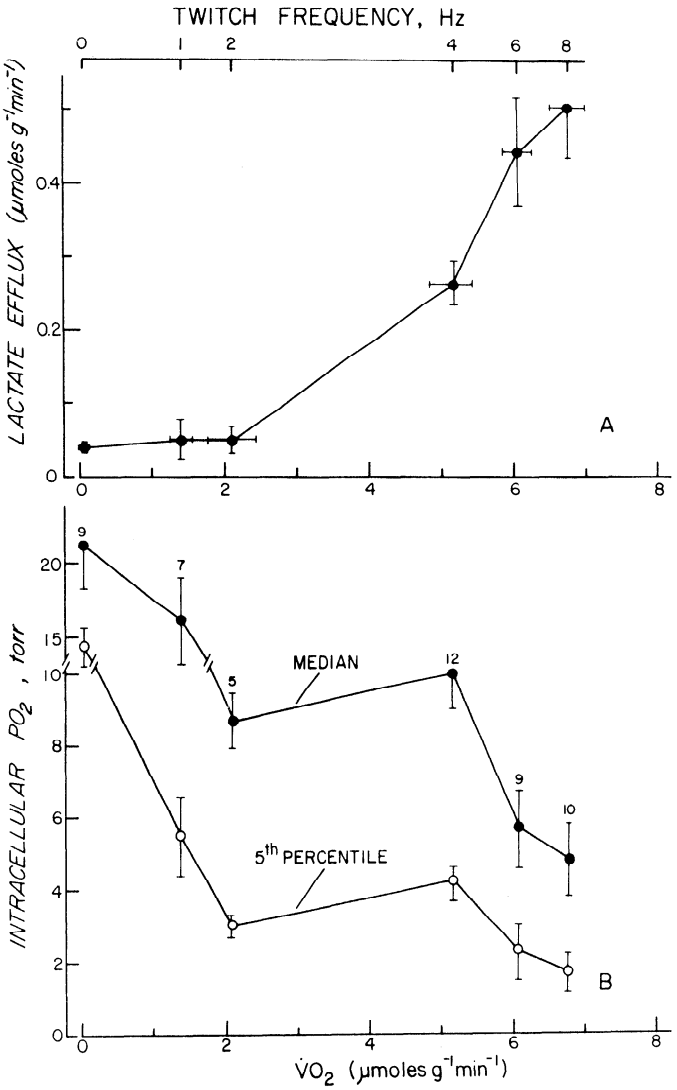

FIG. 2. *A*: product of flow and $[LA]_{v-a}$ was obtained for each muscle. Average efflux at each frequency is plotted as a function of $\dot{V}O_2$. Number of muscles is given in legend for Fig. 1. *B*: median intracellular P_{O_2} (*closed circles*) and P_{O_2} at the lowest 5th percentile of probability distributions (*open circles*) are related to $\dot{V}O_2$, twitch frequency, and lactate efflux. *Bars* and *numerals* in *B* denote SE and number of muscles. Note change in scale of ordinate at 10 Torr.

Figure 2*B* summarizes the relations among $\dot{V}O_2$ and twitch frequency (lower and upper abscissae) and intracellular P_{O_2}. The P_{O_2} at the lowest 5th percentile and P_{O_2} at the 50th percentile were determined for each muscle individually. Then the average P_{O_2} at these probabilities was determined for the population of muscles frozen at each frequency. The data points in Fig. 2*B* represent these averages. The 5th percentile was chosen as the smallest fraction of muscle mass that could have a detectable effect on muscle performance. No anoxic loci whatsoever were found among the 1,650 cells sampled at 1–6 Hz. Even at 8 Hz and near-maximal $\dot{V}O_2$ anoxic loci were found in only 3 of 9 muscles and in each of these accounted for less than 15% of cells examined.

The critical P_{O_2} for energy turnover ($P_{O_{2\,crit}}$) may be defined as the intracellular P_{O_2} at which $\dot{V}_{O_2}$ falls by 10%. $P_{O_{2\,crit}}$ for dog gracilis in vivo is ~0.5 Torr at $\dot{V}_{O_{2\,max}}$ (10). This corresponds to an apparent K_m for O_2 at $\dot{V}_{O_{2\,max}}$ in vivo of ~0.06 Torr. At 8 Hz and near $\dot{V}_{O_{2\,max}}$ the average P_{O_2} at the lowest 5th percentile was 3–4 times $P_{O_{2\,crit}}$, and median P_{O_2} generally exceeded $P_{O_{2\,crit}}$ by at least an order of magnitude. $P_{O_{2\,crit}}$ is <0.5 Torr at 1–6 Hz because $\dot{V}_{O_2}$ is less than maximal at those frequencies.

We are now prepared to relate P_{O_2} in Fig. 2*B* to lactate efflux in Fig. 2*A*. The largest drop in P_{O_2} occurred between rest and 2 Hz (~2 μmol $O_2 \cdot g^{-1} \cdot min^{-1}$). This ΔP_{O_2} was not accompanied by change in lactate efflux. Median P_{O_2} did not decrease further as $\dot{V}_{O_2}$ rose to ~5 μmol $\cdot g^{-1} \cdot min^{-1}$ at 4 Hz. P_{O_2} in the lower tails of probability distributions actually rose between 2 and 4 Hz. Nevertheless the threshold for accelerated lactate efflux lies between 2 and 4 Hz; lactate efflux increased more than 10-fold in this range. A further 2-fold rise in efflux occurred between 4 and 6 Hz. However, P_{O_2} at 6 Hz exceeded $P_{O_{2\,crit}}$ at each of the 450 locations sampled; note in Fig. 2*B* that the 5th percentile P_{O_2} was about the same at 2 Hz as at 6 Hz. The small rise in efflux between 6 and 8 Hz is not statistically significant despite a fall in P_{O_2} and the appearance of anoxic loci in some muscles. Clearly, lactate efflux did not furnish information about intracellular P_{O_2}.

Succinic dehydrogenase activity and aerobic capacity differ significantly among individual gracilis muscles (7, 10). Consequently, the population means shown in Fig. 2 might obscure a relation between lactate efflux and P_{O_2} in muscles working at or above aerobic capacity. To evaluate this possibility measurements at 8 Hz are considered on a muscle-by-muscle basis in Table 1. The 10 muscles in Table 1 are ranked from lowest to highest P_{O_2} according to the percentage of anoxic cells and/or the P_{O_2} at the lowest 5th percentile. *Muscle 126* with 14% anoxic loci had the same lactate efflux as *muscle 140*. The minimum P_{O_2} in *muscle 140* was 2.4 Torr or about 5 times $P_{O_{2\,crit}}$ (10). *Muscle 118* with 6% anoxic loci had a smaller lactate efflux than *muscles 122* and *124*, in which the minimum P_{O_2} was four times $P_{O_{2\,crit}}$.

TABLE 1. *Intracellular P_{O_2} measurement of muscles*

Muscle No.	% Anoxic Cells	Min	5th Percentile, Torr	25th Percentile, Torr	$[LA]_T$, mM	Lactate Efflux, μmol $\cdot g^{-1} \cdot min^{-1}$
126	14	0	0	0.9		0.28
118	6	0	0	0.4	35.5	0.61
240	2	0	0.3	0.7	37.8	
300	0	0.2	0.4	2.9	18.8	
122	0	0.3	0.7	1.4	17.9	0.78
288	0	1.3	1.3	4.0	26.2	
125	0	0.2	2.9	4.9	37.4	
140	0	2.4	2.9	7.0		0.25
291	0	2.9	3.0	4.5	19.9	0.54
124	0	3.8	4.7	7.0		0.88

Lower tail of probability distribution for each muscle frozen at 8 Hz is related to tissue lactate concentration ($[LA]_T$) and lactate efflux for that particular muscle.

Thus there was no correlation between lactate efflux and $P_{t_{O_2}}$ in individual muscles working near $\dot{V}_{O_{2\,max}}$.

Relations Among $\dot{V}_{O_2}$, Lactate $[LA]_T$, and Lactate Efflux

Figure 3 demonstrates a shallow but continuous rise in $[LA]_T$ with $\dot{V}_{O_2}$ through 6 Hz or ~85% $\dot{V}_{O_{2\,max}}$. The increase in $[LA]_T$ was statistically significant at the lowest work rate sampled (1 Hz, ~10% $\dot{V}_{O_{2\,max}}$). The small increase in $\dot{V}_{O_2}$ between 6 and 8 Hz was accompanied by a doubling of $[LA]_T$.

The correlation between $[LA]_T$ and lactate efflux is shown in Fig. 4. Efflux did not change through 2 Hz despite a significant rise in $[LA]_T$. Between 2 and 6 Hz efflux increased 10-fold for a 3-fold rise in $[LA]_T$. Thus there appears to be a threshold $[LA]_T$ above which efflux increases sharply. $[LA]_T$ increased from ~12 to 24 mM between 6 and 8 Hz without a statistically significant increase in efflux, suggesting a saturable process.

DISCUSSION

The principal findings we report are *1*) Lactate efflux from a vascularly isolated pure red muscle increased

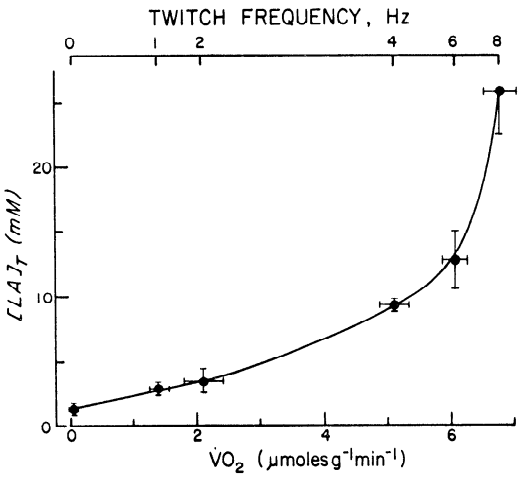

FIG. 3. Relation between $\dot{V}_{O_2}$ and tissue lactate concentration ($[LA]_T$). Error bars indicate 1 SE. Sample size for $\dot{V}_{O_2}$ is given in legend for Fig. 1. Sample sizes for $[LA]_T$ are: rest, 13; 1 Hz, 9; 2 Hz, 5; 4 Hz, 9; 6 Hz, 6; and 8 Hz, 6.

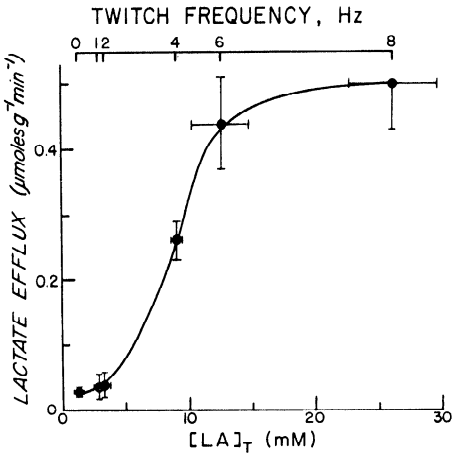

FIG. 4. Relation between tissue lactate concentration ($[LA]_T$) and lactate efflux for same muscles considered in Figs. 1–3. *Upper ordinate* indicates twitch frequency corresponding to each *data point*.

markedly above a threshold work rate; *2*) at this threshold, $\dot{V}O_2$ was not limited by O_2 delivery (7, 8, 21, 22), and neither hypoxic nor anoxic loci were found; *3*) at all work rates including those near $\dot{V}O_{2max}$, lactate efflux was not a simple function of intracellular P_{O_2}; *4*) Lactate efflux was not linearly correlated with $[LA]_T$; and *5*) $[LA]_T$ was related to $\dot{V}O_2$ but not $P_{t_{O_2}}$. We interpret these results to mean that an accelerated increase in blood lactate above a critical work rate need not reflect O_2-limited metabolism. Thus the concept of an anaerobic threshold work rate (1, 38) is erroneous when applied to red muscle. Moreover, in the absence of anoxia neither blood lactate nor $[LA]_T$ can be used to impute muscle O_2 availability or glycolytic rate. Finally, a small population of anoxic cells is not sufficient to produce a detectable increase in lactate efflux above that from fully aerobic muscles at the same $\dot{V}O_2$.

Use of Lactate to Detect Anoxia

Glycolytic flux is strongly regulated by [ATP] and [ADP]. In anoxia the fall in [ATP] and rise in [ADP] greatly increase glycolytic flux above that seen in aerobic work (7, 8). The effect of regional anoxia can be demonstrated in working dog gracilis by clamping the small artery which supplies the distal third of the muscle. Under these abnormal conditions $[LA]_T$ in the distal portion of the gracilis rises above levels in the aerobic proximal portion and lactate efflux from the whole muscle increases (manuscript in preparation). Thus $[LA]_T$ and lactate efflux can furnish evidence of regional ischemia and anoxia. The practical problem in the use of lactate measurements as an O_2 probe is that aerobic $[LA]_T$ and aerobic lactate efflux for the observed condition must be known for reference.

Aerobic lactate accumulation increases with increasing $\dot{V}O_2$. Moreover, the standard deviation for $[LA]_T$ and lactate efflux increase at high-stimulation frequency. This increased variability may reflect different ratios of $\dot{V}O_2/\dot{V}O_{2max}$ in individual muscles (7). Variability under aerobic conditions is, in effect, background noise tending to obscure an anoxic lactate signal. The maximum $[LA]_T$ in uniformly anoxic gracilis muscles is only about double that observed at 8 Hz in the virtual absence of anoxia (7, 8). Since the potential anoxic signal does not greatly exceed the background noise, a large fraction of the cell population must be O_2-limited to produce statistically significant increases in $[LA]_T$ and/or lactate efflux above aerobic levels. The foregoing severely limits the sensitivity of lactate measurements as an O_2 probe in experimental and clinical studies.

Interpretation of $[LA]_a$ in Whole Animal

The existence of a threshold work rate for accelerated increase in $[LA]_a$ has been confirmed in several species. We are by no means the first to challenge the inference that this threshold reflects recruitment of glycolysis caused by muscle anoxia (4, 12, 19, 21, 23, 33, 37). The anaerobic threshold has been defined by an increase in the ratio of ventilation to $\dot{V}O_2$ as well as by increased $[LA]_a$. Recent evidence indicates that a change in the ventilatory ratio is not quantitatively related to lactate efflux from muscle (3), and that the anaerobic threshold determined from $[LA]_a$ is not necessarily the same as the threshold defined by change in ventilation (17, 18, 30, 35). $[LA]_{v\text{-}a}$ depends on rate of washout and hence flow. The necessity for measuring both components of lactate efflux is clearly demonstrated by the data in this report. Nevertheless, the quantity generally measured in studies on humans is $[LA]_a$. Further difficulty arises because $[LA]_a$ is strongly influenced by circulating catecholamines and by lactate clearance by liver and heart (5, 13, 37). Indeed, trained human skeletal muscles are capable of taking up lactate from blood during submaximal exercise (34).

Despite the foregoing, the relation between $[LA]_a$ and work rate is highly reproducible. Experiments to clarify the underlying mechanisms could significantly increase the usefulness of $[LA]_a$ measurements as descriptors of performance in natural exercise. It is possible that the rate of lactate efflux increases in natural exercise above a defined (threshold) work rate because of a change in the pattern of fiber recruitment (1, 23, 32). In this view work below the threshold would be performed by slow- and fast-oxidative fibers, whereas fast-glycolytic fibers would be recruited above the threshold. This possibility is unlikely to be the sole explanation because the threshold in natural exercise and the threshold work rate for accelerated lactate efflux from stimulated dog gracilis occur at comparable fractions of $\dot{V}O_{2max}$, even though the gracilis contains no white glycolytic fibers (27) and all fibers were recruited in each twitch. An alternative explanation for the relation between $[LA]_a$ and work rate in natural exercise is that a regulated lactate transporter exists in the sarcolemma, which responds to the neurohumoral changes associated with the stress of relatively heavy exercise. Evidence for active transport of lactate in skeletal muscle is considered in the following section.

Mechanisms of Lactate Efflux from Dog Gracilis

Release of lactate from myocytes could be driven by passive diffusion and/or carrier-mediated transport. The dependence of efflux on the concentration gradient can be used to test both possible mechanisms of lactate release. Since venous lactate concentrations change by a factor of ~1.5 from rest to maximal work, whereas $[LA]_T$ changes 20-fold a plot of efflux vs. $[LA]_T$, $[LA]_v$ is essentially the same as Fig. 4.

Passive diffusion. Diffusive flux depends on the surface area available for exchange and the concentration gradient. The limiting surface area for lactate efflux from tissue is at the capillary. The number of perfused capillaries in dog gracilis increases almost to a maximum at 1 Hz (21). This should promote lactate efflux. However, the diffusing species appears to be unionized lactic acid (31); so the concentration gradient depends on the pH gradient as well as $[LA]_T$. Work at a small fraction of aerobic capacity increases intracellular pH by a few tenths of a pH unit, thereby decreasing the gradient for outward diffusion of lactic acid (24, 26). Opposing effects of stimulation on the concentration gradient and ex-

change area could account for the small increases in lactate efflux observed at low-twitch frequencies.

Between 2 and 6 Hz capillary density remains constant (21), intracellular pH probably falls (28, 29), and both $[LA]_T$ and lactate efflux increase. These changes are consistent with the hypothesis that passive diffusion contributes to the principal change in lactate efflux. Between 6 and 8 Hz the number of perfused capillaries in dog gracilis decreases by about a factor of 2 (21). This decrease in exchange area could account for the relatively small increase in lactate efflux between 6 and 8 Hz despite a twofold rise in $[LA]_T$. Though passive diffusion is qualitatively consistent with our data its quantitative contribution cannot be evaluated.

Carrier-mediated transport. Studies on erythrocytes (12), tumor cells (36), kidney tubule preparations (2), and perfused heart (11) confirm the existence of a saturable carrier-mediated lactate transport system in cell membranes. Values for K_m in various preparations range from 4 to 8 mM. The $[LA]_T$ at half-maximal efflux is ~8 mM for the data in Fig. 4. The carrier is sensitive to pH gradients, suggesting H^+ cotransport or transport of the unionized form (11, 25, 36). Low efflux at 1–2 Hz could be explained if an increase in intracellular pH at low work rates modifies dependence of flux on $[LA]_T$. The plateau in Fig. 4 between 6 and 8 Hz is consistent with saturation of a carrier. Thus our data are both qualitatively and quantitatively consistent with the carrier hypothesis. If lactate efflux is indeed dominated by a carrier and its biochemical controls, blood lactate would not be expected to furnish reliable information about $[LA]_T$ and glycolytic rate.

Functional Significance of Glycolysis and Blood-Tissue Lactate Gradient

Does glycolysis serve as a significant energy source during steady work? Our experiments can be used to evaluate this question. If the lactate efflux reflects the steady-state rate of glycolysis then the contribution to the overall energy supply can be estimated from the equation: 1.5 (LA efflux)/(6/$\dot{V}O_2$). This assumes all lactate is produced from muscle glycogen and a P/O ratio of 3. Applying this calculation to the data in Fig. 2*A*, the contribution of glycolysis to the energy supply at all $\dot{V}O_2$ $> 2\ \mu mol \cdot g^{-1} \cdot min^{-1}$ is 1–2%. If glucose uptake is contributing to the glycolytic substrate the value will be lower. If glycolysis also supplies all of the substrate for oxidative metabolism then the contribution is <10% at all stimulation frequencies between 1 and 8 Hz. The contribution of glycolysis to the energy supply is low. Moreover the fraction of the energy supplied by glycolysis is essentially constant at all steady-state work rates studied. Therefore the recruitment of glycolysis does not reflect a threshold phenomenon resulting from the ATP demand exceeding the capacity of mitochondria to produce ATP. Thus it is probable that glycolysis plays a role independent of its function as an ATP source.

We have previously proposed that $[LA]_T$ functions as a redox buffer, providing readily accessible reducing equivalents and carbon for mitochondria in support of oxidative phosphorylation (7–9). This function requires $[LA]_T$ be a function of $\dot{V}O_2$, as observed. Small lactate efflux in spite of a large blood-tissue lactate gradient permits buffering with low flux through the glycolytic pathway. This in turn conserves muscle glycogen that is known to be required for sustained work (1). Thus the data in this report are consistent with the concept that glycolytic rate, under aerobic conditions, is regulated to maintain $[LA]_T$ by compensating for losses of lactate to oxidation and washout.

The authors thank James L. Frierson and Willie Reaves for providing expert technical assistance.

This research was supported by National Institutes of Health Grants AM-22124 and HLB-03290.

Received 27 November 1985; accepted in final form 12 February 1986.

REFERENCES

1. ÅSTRAND, P. -O., AND K. RODAHL. *Textbook of Work Physiology.* New York: McGraw-Hill, 1977, p. 293–318.
2. BARAC-NIETO, M., H. MURER, AND R. KINNE. Asymmetry in the transport of lactate by basolateral and brush border membranes in rat kidney cortex. *Pfluegers Arch.* 392: 366–371, 1982.
3. BARBEE, R. W., W. N. STAINSBY, AND S. J. CHIRTEL. Dynamics of O_2, CO_2, lactate, and acid exchange during contractions and recovery. *J. Appl. Physiol.* 54: 1687–1692, 1983.
4. BROOKS, G. A. Anaerobic threshold: review of the concept and directions in future research. *Med. Sci. Sports Exercise* 17: 22–34, 1985.
5. CARLSON, K. I., J. C. MARKER, D. A. ARNALL, M. L. TERRY, H. T. YOUNG, L. G. LINDSAY, M. E. BRACKEN, AND W. W. WINDER. Epinephrine is unessential for stimulation of liver glycogenolysis during exercise. *J. Appl. Physiol.* 58: 544–548, 1985.
6. CLARK, A., AND P. A. A. CLARK. Capture of spatially homogenous chemical reactions in tissue by freezing. *Biophys. J.* 42: 25–30, 1983.
7. CONNETT, R. J., T. E. J. GAYESKI, AND C. R. HONIG. Lactate production in a pure red muscle in absence of anoxia: mechanisms and significance. *Adv. Exp. Med. Biol.* 154: 327–335, 1983.
8. CONNETT, R. J., T. E. J. GAYESKI, AND C. R. HONIG. Lactate accumulation in fully aerobic, working dog gracilis muscle. *Am. J. Physiol.* 246 (*Heart Circ. Physiol.* 15): H120–H128, 1984.
9. CONNETT, R. J., T. E. J. GAYESKI, AND C. R. HONIG. Energy sources in fully aerobic rest-work transitions: a new role for glycolysis. *Am. J. Physiol.* 248 (*Heart Circ. Physiol.* 17): H922–H929, 1985.
10. CONNETT, R. J., T. E. J. GAYESKI, AND C. R. HONIG. An upper bound on the minimum PO_2 for O_2 consumption in red muscle in vivo. *Adv. Exp. Med. Biol.* 191: 291–300, 1985.
11. DENNIS, S. C., M. C. KOHN, G. J. ANDERSON, AND D. GARFINKEL. Kinetic analysis of monocarboxylate uptake into perfused rat hearts. *J. Mol. Cell. Cardiol.* 6: 259–272, 1985.
12. DEUTICKE, B., I. RICKERT, AND E. BEYER. Stereospecific, SH-dependent transfer of lactate in mammalian erythrocytes. *Biochim. Biophys. Acta* 507: 137–155, 1978.
13. DONOVAN, C. M., AND G. BROOKS. Endurance training affects lactate clearance not lactate production. *Am. J. Physiol.* 244 (*Endocrinol. Metab.* 7): E82–E92, 1983.
14. GAYESKI, T. E. J. *Cryogenic Microspectrophotometric Method for Measuring Myoglobin Saturation in Subcrellular Volumes; Application to Resting Dog Gracilis Muscle* (Ph.D. thesis). Rochester, NY: Univ. of Rochester, 1981. (University Microfilms, No. DA8224720, Ann Arbor, MI, 1981).
15. GAYESKI, T. E. J., R. J. CONNETT, AND C. R. HONIG. Oxygen transport in rest-work transition illustrates new functions for myoglobin. *Am. J. Physiol.* 248 (*Heart Circ. Physiol.* 17): H914–H921, 1985.
16. GAYESKI, T. E. J., AND C. R. HONIG. Shallow intracellular O_2 gradients and absence of perimitochondrial O_2 "wells" in heavily working red muscle. *Adv. Exper. Med. Biol.* In press.

17. GLADDEN, L. B., J. W. YATES, R. W. STREMEL, AND B. A. STAMFORD. Gas exchanges and lactate anaerobic thresholds: inter- and intraevaluator agreement. *J. Appl. Physiol.* 58: 2082–2089, 1985.
18. GREEN, H. J., R. L. HUGHSON, G. W. ORR, AND D. A. RANNEY. Anaerobic threshold, blood lactate, and muscle metabolites in progressive exercise. *J. Appl. Physiol.* 54: 1032–1038, 1983.
19. HOLLOSZY, J. O., AND E. COYLE. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.* 56: 831–838, 1984.
20. HONIG, C. R., AND J. L. FRIERSON. Neurons intrinsic to arterioles initiate post contraction vasodilation. *Am. J. Physiol.* 230: 493–507, 1976.
21. HONIG, C. R., C. L. ODOROFF, AND J. L. FRIERSON. Active and passive capillary control in red muscle at rest and in exercise. *Am. J. Physiol.* 243 (*Heart Circ. Physiol.* 12): H196–H206, 1982.
22. HORSTMAN, D. H., M. GLESER, AND J. DELEHUNT. Effects of altering O_2 delivery on $\dot{V}O_2$ of isolated working muscle. *Am. J. Physiol.* 230: 327–334, 1976.
23. IVY, J. L., R. T. WITHERS, P. J. VAN HANDEL, D. H. ELGER, AND D. L. COSTILL. Muscle respiratory capacity and fiber type as determinants of the lactate threshold. *J. Appl. Physiol.* 48: 523–527, 1980.
24. MACDONALD, V. A., AND F. JÖBSIS. Spectrophotometric studies on the pH of frog skeletal muscle. pH change during and after contractile activity. *J. Physiol. Lond.* 268: 179–195, 1976.
25. MAINWOOD, G. W., AND P. WORSLEY-BROWN. The effects of extracellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. *J. Physiol. Lond.* 250: 1–20, 1975.
26. MAISON, G. L., O. S. ORTH, AND K. E. LEMMER. pH changes in rabbit and human striated muscle after contraction. *Am. J. Physiol.* 121: 311–324, 1938.
27. MAXWELL, L. C., J. K. BARKLAY, D. E. MOHRMAN, AND J. A. FAULKNER. Physiological characteristics of skeletal muscles of dogs and cats. *Am. J. Physiol.* 233 (*Cell Physiol.* 2): C14–C18, 1977.
28. MEYER, R. A., T. R. BROWN, AND M. J. KUSHMERICK. Phosphorus NMR of fast- and slow-twitch muscle. *Am. J. Physiol.* 248 (*Cell Physiol.* 17): C279–C289, 1983.
29. MOLÉ, P. A., R. L. COULSON, J. R. CATON, B. G. NICHOLS, AND T. J. BARSTOW. In vivo ^{31}P-NMR in human muscle: transient patterns with exercise. *J. Appl. Physiol.* 59: 101–104, 1985.
30. POOLE, D. C., AND G. A. GAESSER. Response of ventilatory and lactate thresholds to continuous and interval training. *J. Appl. Physiol.* 58: 1115–1121, 1985.
31. ROOS, A. Intracellular pH and distribution of weak acids across cell membranes. A study of D- and L-lactate and of DMO in rat diaphragm. *J. Physiol. Lond.* 249: 1–25, 1975.
32. SALTIN, B. Muscle fibre recruitment and metabolism in prolonged exhaustive dynamic exercise. *Ciba Found. Symp.* 82: 41–52, 1981.
33. SALTIN, B., AND J. KARLSSON. Muscle ATP, CP and lactate during exercise after physical conditioning. In: *Muscle Metabolism During Exercise*, edited by B. Pernow and B. Saltin. New York: Plenum, 1971, p. 395–399.
34. SALTIN, B., K. NAZAR, D. L. COSTILL, E. STEIN, E. JANSSON, B. ESSEN, AND P. D. GOLLNICK. The nature of training response; peripheral and central adaptations to one-legged exercise. *Acta Physiol. Scand.* 96: 289–305, 1976.
35. SIMON, J., J. L. YOUNG, B. GUTIN, D. K. BLOOD, AND R. B. CASE. Lactate accumulation relative to anaerobic and respiratory compensation thresholds. *J. Appl. Physiol.* 54: 13–17, 1982.
36. SPENCER, T. L., AND A. L. LEHNINGER. *L*-lactate transport in Ehrlich ascites-tumor cells. *Biochem. J.* 154: 405–414, 1976.
37. STAINSBY, W. N., C. SUMNERS, AND G. M. ANDREW. Plasma catecholamines and their effect on blood lactate and muscle lactate output. *J. Appl. Physiol.* 57: 321–325, 1985.
38. WASSERMAN, K., B. J. WHIPP, S. N. KOYAL, AND W. L. BEAVER. Anaerobic threshold and respiratory gas exchange during exercise. *J. Appl. Physiol.* 35: 236–243, 1973.