Biochimica et Biophysica Acta, 357 (1974) 167–176

BBA 46776

AEROBIC GLYCOLYSIS IN VASCULAR SMOOTH MUSCLE: RELATION TO ISOMETRIC TENSION

JOHN W. PETERSON* and RICHARD J. PAUL**

Biophysical Laboratory and Department of Physiology, Harvard Medical School, Boston, Mass. 02115 (U.S.A.)

(Received February 11th, 1974)

SUMMARY

Steady-state lactic acid production and O_2 consumption rates have been measured simultaneously in a preparation of bovine mesenteric vein, under controlled mechanical conditions. When these metabolic fluxes are expressed in terms of computed ATP synthesis rates, the contribution of aerobic glycolysis to total metabolic energy production is found to be 30 %. Each flux is shown to depend linearly on the developed isometric tension under conditions of varying degree of pharmacological stimulation. Furthermore, the two metabolic fluxes decrease in parallel to near basal levels at the minimum contracted length, where no isometric tension is developed upon maximal stimulation. The relative contribution of aerobic glycolysis is found to be independent of the mechanical state of the muscle.

INTRODUCTION

It has been previously reported [1] that a linear relation is found between the rate of O_2 consumption and the graded isometric tension developed at fixed length in bovine mesenteric vein. This relation was shown to be independent of the specific pharmacological stimulant used [2]. This observation was taken to represent a linear dependence of the steady-state rate of metabolic energy utilization on the maintained, graded isometric force. The validity of using O_2 consumption rate alone to measure total metabolic energy flux depends on the relative contribution of other energy yielding reactions, principally the production of lactic acid. Studies on arterial smooth muscles [3–6] indicate that, in the presence of O_2, the contribution to metabolic energy production by aerobic glycolysis is rather variable, typically between 10 and 40 %. However, the studies are difficult to interpret due to the lack of a well-defined mechanical state.

Preliminary measurements for bovine mesenteric vein indicated a rather sub-

* Present address: Massachusetts Institute of Technology, Research Laboratory of Electronics (26-322), Cambridge, Mass. 02139. Addres for reprint requests.

** Present address: II Physiologisches Institut, Universität Heidelberg, Heidelberg, Germany.

stantial rate of lactic acid production [2]. The more detailed experiments below were undertaken therefore, to determine accurately the contribution of aerobic glycolysis to total steady-state metabolic energy production; and to determine the dependence of this contribution, if any, on the mechanical state of the muscle.

METHODS

Unbranched segments of the superior mesenteric vein were excised from calves generally 6–8 weeks old immediately following death by exsanguination. The veins were stripped of adventitia and immersed in iced physiological saline solution within 0.5 h of death. The saline used is a bicarbonate buffer (pH 7.4) at 37 °C when gassed with air–CO_2 (95 : 5, v/v), and contained: 118 mM NaCl, 5.32 mM KCl, 1.54 mM NaH_2PO_4, 1.19 mM $MgSO_4$, 24.9 mM $NaHCO_3$, 2.53 mM $CaCl_2$, 0.01 mM EDTA, 100 mg/l penicillin-G, and 300 mg/l streptomycin sulfate. Glucose was maintained at 10 mM in all experiments.

Vein segments (approx. 200 mg wet wt) were cut open axially (1 cm × 4 cm, 0.50 mm thick) and sewn into longitudinal loops, since histological studies indicated that the predominant orientation of smooth muscle cells was longitudinal. After overnight cold storage at 4 °C in glucose-free saline [7] the tissues were allowed to equilibrate for several hours at room temperature before mounting in the all glass and stainless steel muscle chamber at 37 °C, which was well stirred by a glass-encapsulated magnetic stirring rod. The loop was placed between a fixed post (lower) and a moveable post (upper), by which length was controlled and force measured. The upper post exited the chamber through a 5 cm long hole in the stainless steel top (clearance 0.1 mm), and was connected to a force transducer mounted on a moveable platform. A second hole gave access to the bathing solution, permitting the introduction of glucose (10 mM) and the addition or dilution of pharmacological stimulants. The chamber was flushed periodically with fresh aerated saline, such that the O_2 tension was maintained between air and 60 % of air. The long diffusion path through these small bore holes effectively prevented the leakage of O_2 into or out of the chamber. O_2 consumption rate was determined polarographically with a Clark-type electrode. This essentially closed system allowed therefore, the simultaneous determination of O_2 consumption rate (J_{O_2}) and active isometric tension (ΔP_0), the difference between total stimulated tension and passive tension at any length. The details of the muscle chamber and other apparatus are reported elsewhere [2]. The tissue was then allowed to further equilibrate for 2 h at 37 °C, during which time basal O_2 consumption rate and passive tension came to stable values. No spontaneous or myogenic tone was noted. The length at which passive tension was stable at 1 g wt (980 dynes) was designated the rest length, L_0.

The following procedures were used for chemical sampling from the chamber. A 26-gauge hypodermic needle was connected by a Teflon line to a Hamilton 3-way valve fitted with a 2-ml tuberculin syringe, and remained inserted in the muscle chamber throughout these experiments. This allowed the periodic withdrawal of samples (1.5 ml) of the total bathing solution (19.7 ml). These samples were immediately frozen (−30 °C) until assayed for lactic acid by the enzymatic method described by Hohorst [8], as modified by Lundholm et al. [9]. The volume withdrawn in sampling was simultaneously replaced by flooding the top of the chamber with saline

and allowing the solution to enter the chamber passively as the sample was withdrawn. This procedure results in a dilution effect (about 8 %) with each sample taken, and was accounted for in all calculations. The volume of the sampling system was determined to be 75 μl, and all assays were corrected for this dead volume, i.e. mixing between the previous sample retained in the line and the sample currently being taken. The adequacy of these two correction procedures was checked by introducing a calibrated solution of lactic acid into the chamber (control without tissue) and sampling 3 times successively. Agreement between the assay values when corrected as above, and the values calculated from the standard solution was better than ± 2 %, which is at the level of resolution of the biochemical assay procedure. The frozen lactate solutions were found to be stable indefinitely. Calibrations of the biochemical assay for lactic acid were linear and reproducible to ± 3 %, which can be taken as the minimum resolvability of samples.

The contribution of bacterial contamination to both J_{O_2} and lactic acid production rate (J_{LA}) were minimized using bacteriostats and Millipore sterilizing filters (0.22-μm pore) on all solutions entering the chamber. Control measurements indicated that background J_{O_2} and J_{LA} from all sources was less than 2 % of tissue rates.

To compute the total metabolic energy production (assuming J_{O_2} and J_{LA} account for the principal sources of ATP production), both fluxes are converted to equivalent ATP synthesis rates (J_{ATP}) using the commonly accepted biochemical description of glycolysis and oxidative phosphorylation [10]. Measurements of the respiratory quotient in vascular smooth muscle indicate that the predominant substrate is carbohydrate [4, 11]. The literature suggests that, in the presence of glucose, comparable quantities of lactic acid may come from both endogenous glycogen and external glucose [5]. While extensive glycogenolytic capacity has been demonstrated in bovine carotid artery [12], substrate depletion experiments indicate a dependence on external glucose in bovine mesenteric vein [2]. The numerical conversion of J_{O_2} and J_{LA} to J_{ATP} accounts for this possible dependence on carbohydrate source by assuming a 1 : 1 ratio of glycogen and glucose utilization, as shown in Table I. The uncertainties shown express the possible variation in the conversion factors due to

TABLE I

CONVERSION FACTORS FOR COMPUTING J_{ATP} FROM OBSERVED J_{O_2} AND J_{LA}

The quantity of ATP produced by the complete oxidation of glucose depends on the P/O ratio (discussed in text) and the mechanism for the re-oxidation of extramitochondrial NADH produced by glyceraldehyde-3-phosphate dehydrogenase. For an FAD-linked mechanism, oxidation of extramitochondrial NADH yields only 2 ATP. For the purposes of setting an upper bound, an NAD-linked mechanism has been assumed, yielding 3 ATP per cytoplasmic NADH; hence, 38 moles ATP per mole glucose oxidized. The $\pm$ notation expresses the maximum range of the conversion factors. J_{LA}, lactic acid production rate.

	Carbohydrate source		Conversion factor for equal utilization
	Glucose	Glycogen	
$J_{O_2} : J_{ATP}$	38 ATP/6 O_2	39 ATP/6 O_2	6.42 ± 0.08 ATP/O_2
$J_{LA} : J_{ATP}$	2 ATP/2 lactic acid	3 ATP/2 lactic acid	1.25 ± 0.25 ATP/lactic acid

changes in carbohydrate source, and introduce an uncertainty of less than ± 0.04 in the absolute value of the relative contribution of J_{LA} to J_{ATP}.

Because in vivo P/O ratios have not been measured in vascular smooth muscle, the ideal ratio of 3.0 has been used. This probably represents an overestimate. The in vitro measurement of P/O = 2.3 in isolated smooth muscle mitochondria [13] suggests that the absolute value of the fraction of J_{ATP} due to lactic acid production, using the above conversion factors, may be underestimated by as much as 0.05. Comparisons of the relative contribution of aerobic glycolysis between different tissues or between different mechanical states of the same tissue remain valid to within ± 0.06; as long as any variations in the P/O ratio do not exceed the above range (2.3–3.0).

RESULTS

Lundholm and Mohme-Lundholm [5, 14] found that there appeared to be a threshold limit for the internal lactate concentration in bovine mesenteric artery. Beyond this limit, all further lactate production appears in the external bathing solution, while the internal lactate concentration remains constant; that is, a chemical steady state is attained. If such a threshold value exists in bovine mesenteric vein, it is reached rather quickly. The constancy of the rate of appearance of lactic acid in the muscle chamber with time is shown in Fig. 1 for two typical unstimulated vein

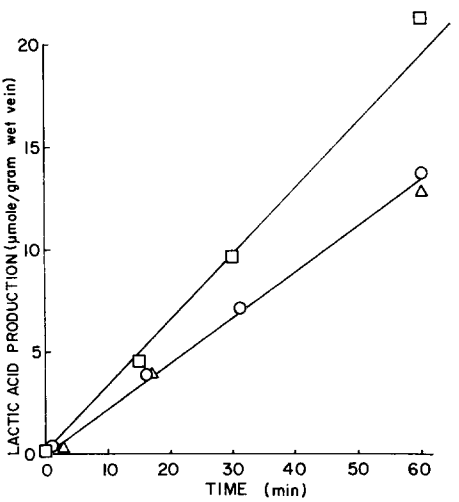

Fig. 1. Zero time on the plot indicates times during the experiment at which the chamber was thoroughly flushed with 5–6 vol. of fresh saline, such that the concentration of lactic acid in the bathing solution was essentially zero. The constancy of the rate of production of lactic acid ($\pm 5\%$) over a subsequent 1-h measurement period is illustrated by the squares, which represent the total lactate appearing in the muscle chamber determined by 4 sequential samples. Triangles represent the production rate in another vein, measured during the hour immediately following the initial 2-h equilibration period. Following a stimulation (45 min), the tissue was returned to the basal condition by a thorough flushing out of the stimulant. Basal lactic acid production rate during the subsequent hour is shown by the circles, and was not affected by the intervention of a period of increased lactic acid production (not shown) or residual effects of pharmacological stimulation.

TABLE II

BASAL J_{O_2}, J_{LA} AND J_{ATP} IN BOVINE MESENTERIC VEIN

J_{LA}/J_{O_2} and % J_{ATP} from J_{LA} are the means of the values of these quantities determined in each vein preparation, ±S.E. These numbers are of greater significance statistically than the quotients of mean values. J_{LA}, lactic acid production rate.

	μmole/min per g wet vein	Number of determinations
J_{O_2}	0.069±0.005	10
J_{LA}	0.166±0.013	12
J_{ATP}	0.637±0.033	10
Molar ratio:		
J_{LA}/J_{O_2}	2.27±0.34	11
% J_{ATP} from J_{LA}	30.8±2.6 %	11

segments. Following an induced change in the lactic acid production rate, the rate of appearance of lactic acid externally is constant in less than 10 min.

The production of lactic acid in the unstimulated (basal) tissue was determined simultaneously with O_2 consumption rates. The appearance of lactic acid in the bathing solution was linear with time and, on a molar ratio basis, averaged 2.3 times the observed value of basal J_{O_2}. Measurements were made on samples from seven veins. The results, including computed J_{ATP}, are summarized in Table II.

Aerobic glycolysis was found to account for some 30 % of the total observed metabolic energy production in the basal state. Several Pasteur effect experiments were performed to investigate the adequacy of applying idealized biochemical models to this preparation. In two vein samples, J_{LA} was measured under both aerobic and anaerobic conditions. The increase in J_{LA} under anaerobic conditions appears sufficient to replace the metabolic energy production of aerobic metabolism. The observed ratio of anaerobic and aerobic lactic acid production was approx. 2.8, which agrees with similar measurements in arterial smooth muscle [15].

A series of experiments were performed measuring J_{LA} and J_{O_2} under the same mechanical conditions that were used in the previous O_2 consumption studies. Following a determination of basal J_{O_2} and J_{LA}, the vein segment was stimulated to varying sub-maximal levels of isometric tension with epinephrine (5 μg/ml for a maximal response of typically 50 g wt). Upon changes in epinephrine dosage by addition or dilution, isometric tension adjusts rapidly to new stable values, generally attained within 2–3 min. Fig. 2 shows a sequence of lactate samples (expressed as total content of the muscle chamber versus time) with sequential increasing doses of epinephrine, each giving stable graded isometric tensions at varying degrees of activation. Analogous to the rate of oxygen consumption, J_{LA} was found to increase linearly with active isometric tension.

Fig. 3 shows a series of lactate samples for a passive, then maximally stimulated tissue. At 100 min, the tissue was allowed to freely contract against a small load to its minimum contracted length. Analogous to J_{O_2}, J_{LA} clearly decreases toward the basal lactic acid production rate. Fig. 4A illustrates the results of similar experiments on another vein. The simultaneous measurements of J_{O_2} and J_{LA} (expressed in μmoles/min/g wet vein) are plotted against active isometric tension for varying degrees of

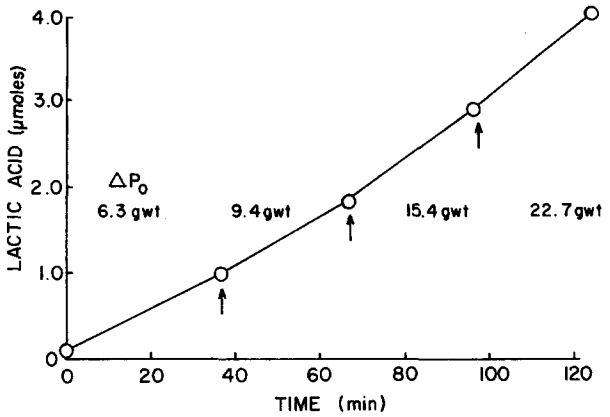

Fig. 2. Total lactic acid in the bathing solution is shown as a function of time for a series of graded isometric tensions. Arrows indicate additions of epinephrine and corresponding increases in isometric tension. Lines connecting the samples represent therefore, the lactate production rates during the four intervals of stimulation, which is seen to increase in parallel with isometric tension.

stimulation at rest length (open symbols), and for the minimum contracted length with maximal stimulation (closed symbols). The two curves are summed in terms of the computed ATP production rates in Fig. 4B. Aside from a simple multiplicative factor in converting to J_{ATP}, the major features of the plot of J_{O_2} or J_{LA} alone are retained identically. The experimental range typically observed in the contribution of aerobic glycolysis to total ATP production rate is shown for a series of such mechanical states in a single tissue in Table III.

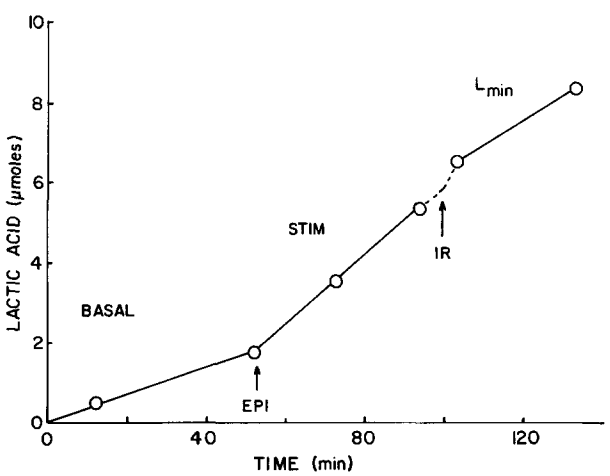

Fig. 3. Immediately following a determination of the basal lactate production rate, this vein segment was stimulated to maximum isometric tension (about 50 g wt) with epinephrine (EPI). The two samples following the stimulation illustrate that J_{LA} increases abruptly to a new and constant rate. IR ("release from isometric") indicates the initiation of a lightly-loaded contraction, which attains the minimum contracted length (L_{min}) in about 5 min. The broken line represents the extrapolated lactic acid production between the samples taken before and after the active contraction.

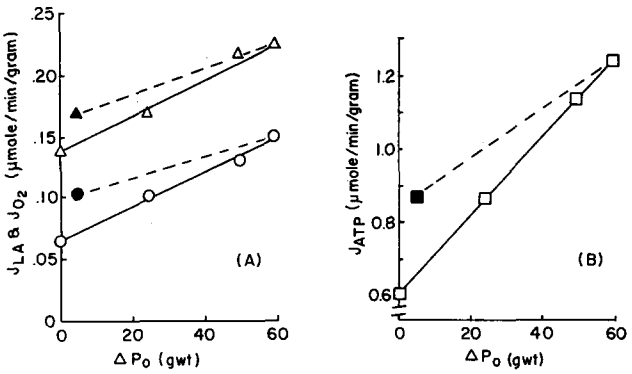

Fig. 4. (A) A plot of the data obtained from the procedures illustrated in Figs 2 and 3 for a single vein segment. J_{LA} (△) and J_{O_2} (○) depend linearly on the graded active isometric tension maintained at rest length. At the minimum contracted length (closed symbols), where the developed isometric tension with maximal stimulation is small, both J_{LA} and J_{O_2} are found to be about 20% greater than their respective basal values. (B) J_{LA} and J_{O_2} are each converted to J_{ATP} and summed. A linear dependence on isometric force and an elevation of J_{ATP} at L_{min} are evident. Note that the plot has been expanded in that the ordinate does not begin at zero.

TABLE III

PERCENT J_{ATP} FROM J_{LA} FOR DIFFERENT MECHANICAL STATES IN A SINGLE TISSUE

It should be noted that, due to the small ATP production associated with aerobic glycolysis, the fractional contribution of J_{LA} (lactic acid production rate) to metabolic energy production is rather insensitive to small variations in the molar ratio J_{LA}/J_{O_2}.

	ΔP_o (g wt)	$(1.25 \times J_{LA})/J_{ATP}$ (%)	J_{LA}/J_{O_2}
Basal	0	28.9	2.09
Stimulated	59	22.8	1.52
	49	24.6	1.67
	24	24.7	1.61
L_{min}	5	24.4	1.66
Mean±S.E.:		25.1±2.3	1.71±0.22

For the three primary mechanical states studied, the fractional contribution of aerobic glycolysis to total energy production is constant. The average results for all such experiments with these mechanical conditions are presented in Table IV.

For bovine mesenteric vein, O_2 consumption rates reflect identically the total steady state energy production rates. This is shown statistically in Table V, which compares the primary observations based on all preceding J_{O_2} experiments with the results obtained in terms of total energy production rates determined in the currently described set of experiments.

With only two determinations, a slight difference in the fractional contribution of J_{LA} to total J_{ATP} was seen with histamine as stimulant. Histamine averaged 0.05 lower aerobic glycolysis relative to J_{ATP} than stimulation with epinephrine at the same isometric force. Many more experiments would be required to determine with statistical certainty, this slight reduction in J_{LA}/J_{ATP} under histamine stimulation.

TABLE IV

CONTRIBUTION OF J_{LA} TO COMPUTED J_{ATP} FOR DIFFERENT MECHANICAL STATES

For the 3 mechanical states, the mean percent contributions of J_{LA} (lactic acid production rate) to J_{ATP} are not different at the 80 % confidence level. Paired comparisons are the mean values of differences observed between the given mechanical states in the same vein; that is, the mean of differences rather than the difference of means. With this more restrictive statistical test, the contributions of J_{LA} to J_{ATP} are not different at the 30 % confidence level.

	Basal	L_{min}	Full and partial stimulation
Mean	30.8±2.6 ($n = 11$)	30.2±2.4 ($n = 7$)	30.7±1.1 ($n = 32$)
	Paired comparisons		
% basal − % L_{min}	2.0±2.1 % ($n = 7$)		
% basal − % stimulated	2.6±2.5 % ($n = 11$)		

TABLE V

COMPARISON OF J_{O_2} AND J_{ATP} EXPERIMENTS

In each case, the probability that the observed differences arose through random sampling error are greater than 30 %.

	J_{O_2}	J_{ATP}	Difference
Increase on stimulation (multiple of basal)	1.76±0.04 ($n = 47$)	1.86±0.06 ($n = 10$)	0.10±0.07
Elevation at L_{min} above basal (% of basal)	27.8±4.4 ($n = 12$)	26.7±2.0 ($n = 7$)	1.1±5.3

DISCUSSION

The computation of steady state J_{ATP} from the observed values of J_{O_2} and J_{LA}, using the standard (but idealized) biochemical pathways for glycolytic and oxidative metabolism, is justified primarily by the identification of a large number of glycolytic and tricarboxylic acid cycle enzymes in smooth muscle [16], and the occurrence, though few in number, of otherwise normal mitochondria [13]. Further confirmation of this approach is found in the ability of bovine mesenteric vein to support mechanical activity on a variety of substrates (glucose, pyruvate, lactate, and succinate were investigated).

Measurements of the high-energy phosphate compounds in bovine mesenteric vein indicate that both ATP and creatine are present at less than 1 μmole/g wet wt, in agreement with other vascular smooth muscles [5, 12]. The computed steady state J_{ATP} (1.2 μmoles/min/g when fully stimulated) suggests therefore that metabolic stationary states must be rapidly attained in order to provide a continuous supply of energy to support contractile activity. Our observation that stable, elevated values of J_{O_2} and J_{LA} are quickly reached upon stimulation of the intact tissue is consistent with the observation in poisoned arterial tissue that steady state utilization of ATP+ creatine phosphate prevails in less than 1 min [17].

The basal levels of J_{O_2} (0.07 μmole/min/g) and J_{LA} (0.17 μmole/min/g) reported here for bovine mesenteric vein lie well within the values found in the literature. For determinations in arterial tissues, J_{O_2} ranges from 0.04 to 0.16 [3, 4] and J_{LA} from 0.04 to 0.25 μmole/min/g [4, 6, 14, 15]. The observed contribution of aerobic glycolysis to total metabolic energy production (30 %) is lower than that found in human aorta [4], but higher than that reported for bovine aorta [6]. This suggests that O_2-diffusion limitations, which should be more important in the thicker aortae [3], cannot be the sole determinant of this fraction. On the contrary, we have observed that aerobic glycolysis proceeds in constant proportion to the rate of energy utilization, even when the oxidative capacity of the tissue is not saturated (as in submaximal stimulations). This strongly suggests that the high level of aerobic glycolysis observed is not imposed by external constraints or O_2 availability, but rather reflects an inherent metabolic regulation.

We have shown that our previous observations based on O_2 consumption rates alone are applicable to considerations of total metabolic energy fluxes in bovine mesenteric vein. The argument for linearity between the rate of energy utilization and active isometric tension, which was established in depth on the basis of J_{O_2} alone, can now be extended to total aerobic energy metabolism. Furthermore, measurements of J_{O_2} and J_{LA} at the minimum contracted length provide a convincing argument that approx. 20 % of the increase in energy expenditure during maximum isometric contraction is associated solely with activation energetics, and not the generation of force [2].

To convert from J_{O_2} to J_{ATP} however, requires that an additional factor be included to account for aerobic glycolysis:

$$J_{ATP} = (6.42 \pm 0.08) \times (1.43 \pm 0.10) \times J_{O_2},$$

where the first factor is the assumed biochemical ratio (discussed above) and the second takes lactate metabolism into account.

There is statistical evidence indicating that variations in J_{LA} and J_{O_2} occur in an inverse fashion which tends to maintain the value of J_{ATP} constant for a given mechanical state. Such behaviour can be seen in Fig. 4. Further, if ΔJ_{LA}, ΔJ_{ATP} and ΔJ_{O_2} (where Δ means suprabasal) are expressed relative to the isometric tension developed per unit area upon maximal stimulation, the computed value for J_{ATP} is found to be significantly less variable than the other force-normalized quantities.

TABLE VI

ANALYSIS OF VARIANCE IN FORCE-NORMALIZED METABOLIC FLUXES

Snedecor's F distribution gives the probability that the observed reduction in variance occurs by random sampling error; which, in the above, is less than 5 %. The reduction in variance is statistically significant.

	$\frac{\Delta J_{O_2}}{\Delta P_0/\text{area}}$	$\frac{\Delta J_{LA}}{\Delta P_0/\text{area}}$	$\frac{\Delta J_{ATP}}{\Delta P_0/\text{area}}$
Mean	0.137 ($n = 18$)	0.408 ($n = 19$)	1.248 ($n = 18$)
S.E. (%)	±5.6	±11.4	±3.9
F value	2.1	9.0	Relative to J_{ATP}

This is shown in Table VI. This evidence of an inverse variation reinforces the argument for a strictly linear relation between total energy utilization rates and active isometric tension by suggesting that, were total energy utilization measurements consistently made, the data would show even less variability than indicated by J_{O_2} or J_{LA} alone.

While steady-state aerobic glycolysis reduces substantially the efficiency of carbohydrate utilization, extensive glycolytic capacity may be retained in order to meet large transient energy demands. There is some indication that lactate production rate increases by a factor of 2–3 times during rapid contraction (cf. Fig. 3 immediately following the release from isometric). However, as such contraction times are short compared to the temporal resolution of the chemical sampling techniques employed, this hypothesis remains speculative.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Professor S. R. Caplan for his support of this research, to Dr M. J. Kushmerick for the use of his facilities, and to Professor J. G. King for his support during the preparation of this manuscript. This work was supported by National Science Foundation Grant GB24697, and a Massachusetts Heart Foundation Fellowship to R.J.P.

REFERENCES

1 Paul, R. J., Peterson, J. W. and Caplan, S. R. (1973) Biochim. Biophys. Acta 305, 474–480
2 Paul, R. J., Peterson, J. W. and Caplan, S. R. (1974) J. Mechanochem. Cell Motility 2, in the press
3 Lehninger, A. L. (1959) in The Arterial Wall (Lansing, A. I., ed.), pp. 220–246, Williams and Wilkins, Baltimore
4 Kirk, J. E., Effersoe, P. G. and Chiang, S. P. (1954) J. Gerontol. 9, 10–35
5 Lundholm, L. and Mohme-Lundholm, E. (1960) Acta Pharmacol. Toxicol. 16, 374–388
6 Pantesco, V., Kempf, E., Mandel, P. and Fontaine, R. (1962) Pathol. Biol. 10, 1301–1306
7 Sparks, H. V. and Bohr, D. F. (1962) Am. J. Physiol. 202, 835–840
8 Hohorst, H. J. (1963) in Methods of Enzymatic Analysis (Bergmeyer, H. V., ed.), pp. 266–270, Academic Press, New York
9 Lundholm, L., Mohme-Lundholm, E. and Vamos, N. (1963) Acta Physiol. Scand. 58, 243–249
10 Mahler, H. R. and Cordes, E. H. (1966) Biological Chemistry, Chaps 10 and 13, Harper and Row, New York
11 Kosan, R. L. and Burton, A. C. (1966) Circ. Res. 18, 79–88
12 Daemers-Lambert, C. (1964) Angiologica 1, 249–274
13 Stephens, N. L. and Wrogemann, K. (1970) Am. J. Physiol. 219, 1796–1801
14 Lundholm, L. and Mohme-Lundholm, E. (1962) Acta Physiol. Scand. 55, 45–63
15 Südhof, H. (1949) Arch. Ges. Physiol. 252, 551–565
16 Kirk, J. E. (1963) in Atherosclerosis and Its Origin (Sandler, M. and Bourne, G., eds), Chap. 3, Academic Press, New York
17 Daemers-Lambert, C. and Roland, J. (1967) Angiologica 4, 69–87