# Chemical Energetics of Slow- and Fast-Twitch Muscles of the Mouse

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ABSTRACT The energy utilization associated with contraction was measured in isolated slow- and fast-twitch muscles of the mouse at 20°C. The extent of this utilization was estimated from either the extent of high-energy phosphate splitting occurring during contraction (the initial chemical change, $\Delta \sim P_{init}$) or from the extent of recovery resynthesis calculated from the observed oxygen consumption and lactate production occurring during the recovery period (recovery chemical resynthesis, $\Delta \sim P_{rec}$). For short tetani, the cost to maintain isometric tension in the fast-twitch extensor digitorum longus (EDL) was approximately threefold greater than that in the slow-twitch soleus. With prolonged stimulation, however, the energy cost in the EDL diminished so that after 12 s of stimulation, the energy cost in the EDL was only 50% greater than that of the soleus. For both the slow-twitch soleus and the fast-twitch EDL and for all tetanus durations (up to 15 s), the extent of the initial chemical change was identical with the amount of recovery chemical resynthesis, showing that a biochemical energy balance existed in these muscles.

## INTRODUCTION

A logical necessity to any detailed quantitative study of the energetics of muscle contraction is the knowledge of the nature and extent of the chemical reactions underlying this event. Assurance that the proper chemical species have been measured can only be inferred by comparing the measured extents of reaction with some other independent measurement of the total energy cost. For example, when the total energy liberated during contraction was compared with that predicted from the observed chemical change, a substantial quantitative discrepancy was apparent in isolated frog muscle; total energy is liberated in excess of that accountable by the measured chemical change (Curtin and Woledge, 1978; Homsher and Kean, 1978). On the basis of this observed discrepancy, a "missing reaction" has been inferred, which has not yet been identified by direct measurement.

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If high-energy phosphate (~P) use associated with contraction is confined solely to the period of contraction and if the steady-state content of intermediary metabolites is fully restored at the end of recovery, the amount of energy use during the initial period ($\Delta\sim P_{init}$) must equal the amount of high-energy phosphate resynthesized during the recovery period ($\Delta\sim P_{rec}$). The $\Delta\sim P_{rec}$ can be estimated from the extent of aerobic substrate oxidation and glycolytic ATP production provided that (*a*) the extents of oxidation are coupled to the rephosphorylation of ADP and (*b*) the stoichiometric coupling coefficients relating ~P resynthesis to the extent of these oxidations are known. A comparison then of the initial chemical change ($\Delta\sim P_{init}$) and the recovery resynthesis ($\Delta\sim P_{rec}$) provides an alternative approach to studying the chemical energy balance in contracting muscle. When such an analysis was applied to contracting frog muscle, the estimated recovery chemical resynthesis ($\Delta\sim P_{rec}$) always exceeded the $\Delta\sim P_{init}$ (Kushmerick and Paul, 1976*b*). This result led to the conclusion similar to that obtained with myothermal methods in that the amount of initial chemical change was insufficient to account for the total energy consumption and points independently to the hypothesis of a "missing reaction" occurring during contraction.

It is of interest to ascertain whether the energy imbalance described for frog muscle is peculiar to this animal or representative of other muscles, as has been suggested by others (Gower and Kretzschmar, 1976; Homsher and Kean, 1978; Curtin and Woledge, 1978). We report here the results of a study comparing the initial chemical breakdown during contraction with the recovery chemical resynthesis in isolated hindlimb muscles of the mouse, the slow-twitch soleus and fast-twitch extensor digitorum longus. Some of these data have been presented in preliminary form (Kushmerick and Crow, 1982; Crow and Kushmerick, 1981).

## METHODS

### *Muscle Preparation*

Male CD-1 mice (Charles River Breeding Laboratories, Wilmington, Mass.) between the ages of 21 and 28 days were either anaesthetized with an intraperitoneal injection of sodium pentobarbital (100 mg/kg body weight; Nembutal, Abott Laboratories, North Chicago, Ill.) or killed by cervical dislocation. The soleus and extensor digitorum longus (EDL) muscles were dissected with tendons intact on both ends. During all subsequent manipulations, the muscles were constrained at a length close to their in vivo length.

Unless otherwise indicated, all experiments were performed at 20°C in a bicarbonate Ringer's solution of the following composition: 116 mM NaCl; 4.6 mM KCl; 1.16 mM $KH_2PO_4$; 2.5 mM $CaCl_2$; 1.16 mM $MgSO_4$; 25.3 mM $NaHCO_3$; 10 μg/ml gentamicin sulphate. The solution was gassed with a mixture of 95% $O_2$/5% $CO_2$ (vol/vol) to obtain a pH of 7.4.

Fiber type characterization was determined by histochemical staining of frozen serial cross-sections (10 μm) of the muscles. Fibers were classified according to the system of Barnard et al. (1971); the alkaline myosin ATPase (Padykula and Herman, 1955; Guth and Samaha, 1969) and NADH diaphorase stain (Naclas et al., 1958*a* and *b*) were the basis for delineation. Sections of rat muscle were run in parallel to

provide a reference for the degree of staining. The fiber type composition of each muscle is expressed as the percentage of the total cross-sectional area occupied by each fiber type (Table I).

Fiber lengths and orientation with respect to the long axis defined by the tendons were determined by attaching the muscle at its in vivo length to a wooden stick and fixing overnight in 10% formaldehyde in normal saline. The muscles were then placed in a 30% (wt/vol) $HNO_3$ solution to macerate the connective tissue, and stained with toluidine blue.

The respiratory quotient for resting and partially activated muscle was determined manometrically in a Warburg constant volume respirometer (Gilson Medical Electronics, Inc., Middleton, Wis.) at 20°C in phosphate-buffered oxygenated Ringer's solution (10 mM phosphate, pH 7.4 instead of 25.3 mM $HCO_3^-$). Muscles were restrained at their in vivo length by a stainless steel bracket fabricated at the time of dissection. Rates of oxygen consumption were determined by trapping the evolved $CO_2$ with either 20% KOH or an alkaline tissue solubilizer (Protosol, New England Nuclear, Boston, Mass.) absorbed on filter paper placed in the center well. The resting rates of oxygen consumption determined both polarographically and manometrically were identical.

The steady-state rates of $CO_2$ evolution in resting or partially activated muscles were determined in the following manner. The combined rate of $CO_2$ evolution and oxygen consumption was determined by following the rate of pressure change in the Warburg flask. KOH was then added to the center well and the rate of oxygen consumption alone was determined. The rate of $CO_2$ evolution was then calculated by subtraction of the two rates. Caffeine (10 mM) was added to respiring resting muscles to activate metabolism to a rate similar to that observed during stimulation. $CO_2$ evolution and $O_2$ consumption were then determined on these partially activated muscles.

### *Recovery Oxygen Consumption*

Recovery oxygen consumption was measured polarographically. The construction, operation, and precautions involved in this method have been described (Kushmerick and Paul, 1976*a*). Because of the small size of these muscles (ca. 5 mg), certain modifications of the system were necessary to reduce the basal oxygen consumption of the chamber. Oxidation at the surface of the stainless steel components was avoided by constructing the chamber entirely of glass and ceramic (MaCor; Dow Corning Corp., Midland, Mich.). Oxygen consumption by the electrode itself was reduced by using one with a smaller platinum cathode (25 μm, Radiometer-Copenhagen, No. E5046). Under these conditions, the rate of loss of $O_2$ from the chamber was constant and was ~90 pmol/min or ~12% of the resting basal consumption of a 5-mg mouse muscle (750 pmol/min).

The response time of the system (electrode, chamber, and recorder) was estimated by injecting small volumes of temperature equilibrated solutions saturated with either 100% $O_2$ or 100% $N_2$. The response was exponential with a time constant of ~15 s.

The basal oxygen consumption of the muscle was measured by multiplying the observed drop in partial pressure of oxygen ($P_{O_2}$) with time by the solubility of oxygen in Ringer's solution and the chamber volume. Recovery oxygen consumption after a tetanus was measured in a similar manner except that the partial pressure drop taken was that defined by the parallel separation of the pre- and post-tetanic basal rates (Kushmerick and Paul, 1976*a*). The solubility of $O_2$ at 20°C was taken as 0.245 μmol/ml·atm (Seidel and Linke, 1965). The chamber volume was ~2 ml and was deter-

mined during each experiment with an extracellular space marker (20 μl [0.1 μCi] of a 1-mM solution of $^{14}$C-inulin or $^{14}$C-sorbitol [New England Nuclear]).

One end of the muscle was attached to either a glass or stainless steel support that functioned as mechanical ground. The other end was connected to a capacitance-type force transducer (Harvard Apparatus Co., Inc., South Natick, Mass.) with a gold chain. A 4- × 0.1-cm (length × diameter) diffusion path between the muscle and the transducer was used as an effective gas seal. All solutions were passed through a 0.2-μm nitrocellulose filter before introduction into the chamber.

Muscles were stimulated with either transverse platinum electrodes or with platinum foil plates placed at opposite ends. Before each experiment, the length of the muscle was adjusted to give the maximum twitch or tetanic tension. To avoid electrolysis of the water at the surface of the platinum, the muscles were stimulated with alternating condenser discharges (RC ca. 3 ms) at a frequency of 66 Hz at 15 V. Whereas this stimulation frequency was sufficient to cause a fused tetanus in both the soleus and EDL, higher frequencies of stimulation resulted in slightly more tension. However, the muscles fatigued rapidly when stimulated at frequencies higher than 66 Hz.

The ability of these muscles to receive oxygen from stirred solutions by diffusion alone was tested in the following manner. The critical $P_{O_2}$, where the diffusion of oxygen would become rate-limiting to muscle respiration, was calculated by solution of the equation given by Krogh (1918), with the assumption that the geometry of the muscles could be approximated by a cylinder and that a 20-fold increase in oxygen consumption occurred upon stimulation. The critical $P_{O_2}$ was also determined experimentally by measuring the total recovery oxygen consumption for a short tetanus at various levels of $P_{O_2}$. The critical $P_{O_2}$ under these conditions was defined as that $P_{O_2}$ where a reduction in oxygen consumption and a compensatory increase in lactate production in response to stimulation is observed. The values of the critical $P_{O_2}$ obtained by both methods were varied between 350 and 450 torr. Consequently, all aerobic experiments were conducted in Ringer's solution equilibrated with an atmosphere significantly greater than the critical $P_{O_2}$, typically between 650 and 700 torr.

The contribution of net aerobic glycolysis to the total recovery metabolism was determined by measuring the accumulation of lactate, pyruvate, and alanine in the oxygen consumption chamber. The amount of lactate released into the chamber after contraction is equal to the net recovery lactate production because the elevated concentration in the medium after stimulation remained steady at its elevated value for at least 1 h and because the muscle lactate content was restored to control values by the end of the recovery period. Accordingly, during the oxygen consumption experiments, an extracellular space marker was injected into the chamber 20 min after a tetanus and allowed to mix for 10 min. An aliquot of the chamber fluid was then removed and the total amount of lactate, pyruvate, and alanine in the chamber was determined by enzymatic analysis (Lowry and Passoneau, 1972).

### *Chemical Measurements*

Muscles were rapidly frozen in pairs at the end of mechanical relaxation in a device similar to that described by Kretzschmar and Wilkie (1969) and either were extracted directly in methanol-EDTA at −30°C (Kushmerick and Paul, 1976*a*) or were pulverized at liquid nitrogen temperature before perchloric acid extraction (Lowry and Passoneau, 1972) in a device similar in design to that described by Seraydarian et al. (1961). One muscle of each pair remained unstimulated, whereas the other muscle was stimulated for various durations. The reported chemical changes refer to

the differences in chemical content between the unstimulated control and the experimental member of each muscle pair.

The reactions of interest were:

$$ATP \rightarrow ADP + Pi \quad (1)$$

$$PCr + ADP \leftrightarrow Cr + ATP \quad (2)$$

$$2ADP \leftrightarrow ATP + AMP \quad (3)$$

$$AMP \rightarrow IMP + NH_3 \quad (4)$$

$$0.5\ (glucose)_n + 1.5\ P_i + 1.5\ ADP \rightarrow lactate + 1.5\ ATP + 0.5\ glucose)_{n-1}. \quad (5)$$

These reactions form a network of reactants and products so that the extent ($\xi$) of any reaction is not necessarily obtained from the measured change of a single compound. The extents of these reactions were calculated from the observed chemical change in the following compounds:

$$\xi_1 = -ATP - PCr + AMP + NH_3$$

$$\xi_2 = -PCr$$

$$\xi_3 = +AMP + NH_3$$

$$\xi_4 = +NH_3$$

$$\xi_5 = +lactate$$

The content of free and total creatine (Cr and $C_t$) in the extracts was measured by the $\alpha$-naphthol procedure (Ennor, 1957). All other metabolites were measured fluorometrically by enzymatically coupling the metabolites to either the reduction or oxidation of NAD(H) and NADP(H) (Lowry and Passonneau, 1972). Protein was determined in the perchloric acid-precipitate by the method of Lowry et al. (1951) with bovine serum albumin as standard.

### *Calculation of the Initial Chemical Change and Recovery Chemical Input*

The initial chemical change was calculated from the extent of high-energy phosphate depletion after accounting for recovery metabolism occurring during the contraction. Aerobic resynthesis was blocked by incubating and stimulating the muscles in a Ringer's solution equilibrated with 95% $N_2$ and 5% $CO_2$ (vol/vol). The extent of glycolytic recovery metabolism was calculated by multiplying the extent of reaction 5 by the stoichiometric coefficient of 1.5 ($\xi_5 \times 1.5$) (Scopes, 1973). A 9-s tetanus was used for a detailed chemical analysis. The adenylate kinase (reaction 3) and adenylate deaminase (reaction 4) reactions did not occur to any significant extent upon stimulation. For a 9-s isometric tetanus in the soleus, $\xi_3 = 0.21 \pm 0.22$ $\mu$mol/g SEM ($n = 5$) and $\xi_4 = 0.09 \pm 0.10$ $\mu$mol/g SEM ($n = 5$). Similarly, for a 9-s isometric tetanus in the EDL, $\xi_3 = 0.27 \pm 0.19$ $\mu$mol/g SEM ($n = 7$) and $\xi_4 = 0.21 \pm 0.18$ $\mu$mol/g SEM ($n = 8$).

The initial chemical change ($\Delta\sim P_{init}$) was then determined from the extent of ATP splitting ($\xi_1$) and from glycolytic recovery ($\xi_5$):

$$\Delta\sim P_{init} = \xi_1 + 1.5\ \xi_5.$$

Because neither the hexosemonophosphates nor hexosediphosphates accumulated to any extent during the 9-s tetanus (0.05 $\pm$ 0.06 $\mu$mol/g [SEM; $n = 10$] and 0.13 $\pm$

0.08 μmol/g [SEM; $n = 9$] for the soleus and EDL, respectively), the extent of reaction 1 could also be calculated from the change in inorganic phosphate content:

$$\xi_{1a} = +P_i.$$

In some instances, both measurements were combined to provide a "best estimate" for reaction 1 using the statistical methods described in Curtin and Woledge (1979). The degrees of freedom involved in this "best estimate" were obtained from the formulae in the Documenta Geigy (p. 172, 1962).

The amount of high-energy phosphate resynthesized during the recovery period (recovery chemical input, $\Delta\sim P_{rec}$) was calculated from the extent of the recovery oxygen consumption and lactate production by the following equation:

$$\Delta\sim P_{rec} = \kappa\xi O_2 + \lambda\xi_\delta = \kappa\xi O_2 + \lambda\xi_{lac}$$

where $\xi O_2$ is the amount of recovery oxygen consumption, $\xi_{lac}$ is the amount of recovery lactate production (reaction 5), and $\kappa$ and $\lambda$ are stoichiometric coefficients relating the extent of oxidation or accumulation of end-products with the degree of ATP resynthesis. For a muscle-metabolizing glycogen, the value of these constants are (Kushmerick, 1978; Scopes, 1973):

$$\kappa = P/O_2 = 6.3$$

$$\lambda = P/lac = 1.5.$$

*Expression of Results and Data Interconversions*

All chemical changes and recovery oxidations are expressed with respect to the blotted wet weight of the muscle. A potential problem incurred by the use of small muscles (5–7 mg) is the underestimation of metabolite concentrations in muscle cytosol due to the relatively large tendons in these muscles. This problem is especially serious in the soleus muscle because a large portion of the Achilles tendon must accompany the muscle during dissection. This factor alone can introduce a 30–40% error in the estimation of muscle mass and hence, the metabolite levels.

The following procedures were employed in an attempt to avoid this problem. For oxygen consumption and manometric measurements, the blotted wet weight was determined at the end of the experiment after carefully removing all tendon visible with the aid of a dissecting microscope. For the experiments involving assessment of the initial chemical change, this wet weight could not be obtained. Instead, the total creatine in the perchloric acid extract was used to estimate the muscle mass. The equivalent blotted wet weight was obtained from a conversion factor determined in an independent series of experiments relating total creatine content to blotted wet weight. The values of these factors were constant over the range of muscle weights used (3–10 mg) and are described by the following regressions (± SD):

Soleus: g wet wt = 2.9 ± 2.1 + 19.8 ± 1.5 μmol $C_t$/g wet ($n = 25$).

EDL: g wet wt = 1.9 ± 3.2 + 29.5 ± 2.5 μmol $C_t$/g wet ($n = 21$).

The conversion of the data to a gram-wet weight basis was necessary because the two muscles differed greatly in total creatine content. Any difference in intrinsic ATP hydrolysis rates between these muscles would be obscured were the data expressed on a total creatine basis.

Measurements of the extent of recovery oxidation or initial chemical breakdown have been expressed relative to the tension-time integral rather than tetanus duration. The value of this integral (N·m·s/g) was obtained by multiplying the area enclosed by the isometric myogram by the fiber length of the muscle. When expressed on a

per-gram basis, the tension-time integral is the time integral of the force developed per cross-sectional area (assuming a density of 1.05 g/cm$^3$). This quantity is used as the basis for comparison because an adequate comparison of the cost for tension maintenance in the two muscle types studied must involve an accounting of the difference in force development per cross-sectional area (0.19 ± 0.02 [SD] N/m$^2$ [$n$ = 26] for the soleus and 0.21 ± 0.03 [SD] N/m$^2$ [$n$ = 25] for the EDL), rate of tension development and relaxation, and resistance to fatigue. Within a given muscle type, and the tension-time integral was linearly related to the tetanus duration (± SD):

$$\text{Soleus: } L_0 \int \text{Pdt} = 0.02 \pm 0.01 + 0.17 \pm 0.04t \quad (n = 41)$$
$$\text{EDL: } L_0 \int \text{Pdt} = 0.14 \pm 0.09 + 0.15 \pm 0.03t \quad (n = 52)$$

where $L_0 \int$ Pdt is the tension-time integral per gram blotted weight and $t$ is the tetanus duration.

### *Statistical Methods*

Regression analyses were performed using the Gauss-Newton method of analysis (Duggleby, 1981) on a digital computer. Calculation of the rates of energy use in Fig. 4 and Table III were obtained from the regression of both $\Delta \sim P_{init}$ or $\Delta \sim P_{rec}$ and tension-time integral by performing the regression with the data points grouped to form sequential windows of 3 s of stimulation. At least 10 points were used in each regression.

Statistical significance was inferred from the unpaired $t$ test (Armitage, 1971). When the variance of the two means were different, the degrees of freedom were adjusted as described by Beyer (1968, p. 19).

## RESULTS

### *Muscle Characterization*

The slow-twitch soleus contained both slow- (SO) and fast-twitch oxidative type (FOG) fibers. The fast-twitch EDL, on the other hand, was composed almost exclusively of oxidative (FOG) and glycolytic (FG) fast-twitch fiber types. The cross-sectional distribution of these fiber types is summarized in Table I.

The general arrangement of tendons and muscle fibers was similar to that reported by Close (1964) for the rat soleus and EDL. Within any muscle, all fibers were approximately the same length and ran nearly parallel to the longitudinal axis of the muscle. The value of the angle describing the deviation of the longitudinal axis of the fiber from that of the muscle was 6° for the soleus and 4° for the EDL. All measurements of tension development could be corrected for this angle. Because the error introduced by not making such a correction is <0.2%, it has been neglected.

The rate of oxygen consumption in unstimulated muscles at rest length was determined both manometrically and polarographically and found to be identical for the two muscles (147 ± 18 nmol/g·min [SEM, $n$ = 16] and 150 ± 10 nmol/g·min [SEM, $n$ = 47]). Under resting conditions, the rate of oxygen consumption was equal to that for $CO_2$ evolution (147 ± 21 nmol $CO_2$/g·min [SEM, $n$ = 11]), suggesting a respiratory quotient of unity. The

value of the respiratory quotient remained near unity (0.98 ± 0.03 [SD, $n = 5$]) despite a five- to eightfold increase in the rate of oxygen consumption resulting from the addition of 10 mM caffeine. No exogenous substrate was added to the medium, and a respiratory quotient of unity suggests that the substrate supporting recovery oxidations is glycogen.

*Recovery Chemical Input*

The decay of recovery oxygen consumption from its accelerated rate immediately after a tetanus to the post-contractile basal rate followed an apparent exponential time-course in both muscles. The values of the time constants obtained by fitting an exponential function were independent of tetanus duration and substantially different for the EDL and soleus muscles. For the EDL, the value of this constant was 2.3 ± 0.5 min (SD, $n = 43$). Because significant aerobic resynthesis occurred during the period of tetanic stimulation in the soleus, the value of the time constant could only be reliably obtained either from tetani of such short duration (<3 s) that no significant aerobic recovery metabolism occurred, or from the decay of oxygen consump-

TABLE I

FIBER CHARACTERIZATION OF MOUSE SOLEUS AND EDL

| | Soleus | EDL |
|---|---|---|
| Fiber composition* | | |
| % SO | 75.5(±1.8) | 1.0(±0.6) |
| % FOG | 24.5(±1.2) | 63.3(±2.1) |
| % FG | —‡ | 35.7(±2.3) |
| Fiber length§ | 10.0(±0.2) | 9.2(±0.6) |
| Fiber length/muscle length | 0.83 | 0.68 |

* Mean percentage of total cross-sectional area occupied by each fiber type ± SE of mean ($n = 10$).

‡ No detectable fibers of this type.

§ Mean fiber length in millimeters ± SD. Data from 20 muscles and 500 fibers.

tion after the termination of a stimulus sufficient to cause the muscle to obtain an elevated steady-state rate of consumption. The values obtained under both conditions were 0.6 ± 0.2 min (SD, $n = 27$) and 0.58 ± 0.2 min (SD, $n = 4$), respectively, and were not adjusted for the time constant of the oxygen-measuring apparatus (0.25 min). The constancy of the value for the recovery time constant for different stimulus durations is consistent with the observations of Hill (1940) and Mahler (1978).

The basal rates of lactate production by these muscles were 5.4 ± 0.3 (SD, $n = 5$) and 9.2 ± 0.5 (SD, $n = 8$) nmol lactate/g·min for the soleus and EDL, respectively. During contraction, the extent of release of lactate as well as other possible termination products of the glycolytic sequence (pyruvate, alanine, glutamine, and ammonia) was similar in amount to that reported for rat muscles (Goodman and Lowenstein, 1977). Of these end-products, however, only lactate accumulated to an extent that was energetically significant. The contribution of these other metabolites to the total recovery input was usually <1% of the recovery oxygen consumption.

In Fig. 1, the contributions of aerobic and glycolytic recovery processes to the total recovery chemical input are shown for both the soleus (circles) and EDL (squares). The open symbols represent the recovery chemical input derived from the consumption of oxygen alone ($\xi O_2$), whereas the closed

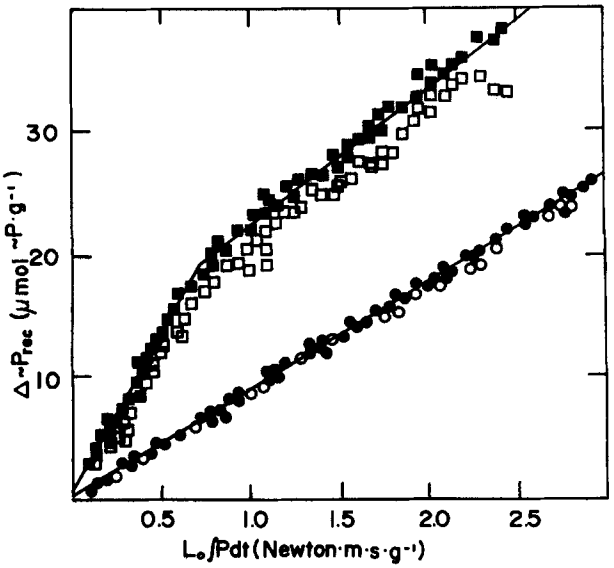


FIGURE 1. The relationship between total recovery chemical input and the tension-time integral. The recovery chemical input ($\Delta\sim P_{rec}$) is expressed in μmol ~P/g and obtained from the following equation: $\Delta\sim P_{rec} = \kappa\xi O_2 + \lambda\xi lac$, where $\kappa$ and $\lambda$ are stoichiometric factor equal to 6.3 and 1.5, respectively. Open symbols represent the contribution to the total recovery chemical input from oxygen consumption alone ($\kappa\xi O_2$) in the soleus (○) and EDL (□). Closed symbols represent the total recovery chemical input after the contribution from glycolytic ATP production ($\lambda\xi lac$) was added to the aerobic contribution in soleus (●) and EDL (■). In a majority of the data points for the soleus, the contribution from lactate production was negligible, and for the sake of visual clarity only the points corresponding to the total recovery input were graphed. The lines are linear functions fitted to the data with the following parameters:

Soleus: $\Delta\sim P_{rec} = 0.34 \pm 0.71 + 8.73 \pm 0.51\ L_0 \int Pdt$

EDL: $\Delta\sim P_{rec} = 0.68 \pm 1.3 + 23.4 \pm 3.55\ L_0 \int Pdt\ (0 < L_0 \int Pdt < 0.75)$

$\Delta\sim P_{rec} = 11.3 \pm 1.5 + 11.1 \pm 1.2\ L_0 \int Pdt\ (1.0 < L_0 \int Pdt < 2.5)$.

Data from 13 soleus and EDL muscles each. Multiple determinations were performed on each muscle. Muscles were stimulated and allowed to recover for 30 min before stimulating again.

symbols represent the total recovery chemical input after addition of the contribution arising from glycolytic recovery ($\xi O_2 + \xi lac$). For the soleus, >95% of the total recovery chemical input arose from aerobic oxidations; lactate accumulated only to a minor energetically significant extent (<5%) for

tetani of all durations. For the EDL, the contribution of recovery glycolysis to the total recovery chemical input was larger than in soleus and it increased with the duration of the tetanus.

The relationship describing the total recovery chemical input and tension-time integral for the soleus was linear:

$$\Delta\sim P_{rec} = 0.34 \pm 0.71 + 8.73 \pm 0.51\ L_0 \int Pdt \qquad (n = 34)$$

where $\Delta\sim P_{rec}$ is the total recovery chemical input in μmol ~P/g.

In contrast, the relationship between the recovery chemical input and tension-time integral was nonlinear in the EDL. This relationship was conveniently fitted by two linear functions. For short tetani (<6 s), this relationship was described by the following function:

$$\Delta\sim P_{rec} = 0.68 \pm 1.31 \pm 23.4 \pm 3.55\ L_0 \int Pdt \qquad (n = 21).$$

For longer tetani (≥9 s), the relationship was described by the function:

$$\Delta\sim P_{rec} = 11.3 \pm 1.45 + 11.1 \pm 1.21\ L_0 \int Pdt \qquad (n = 25).$$

*Initial Chemical Change*

SOLEUS The observed chemical change in the compounds of interest are shown in Table IIA for tetani of various duration occurring under both aerobic and anaerobic conditions. The initial chemical change ($\Delta\sim P_{init}$) is also listed and was calculated from the observed changes by the procedures described in Methods. In Fig. 2, the initial chemical change has been plotted as a function of tension-time integral. The open symbols represent the chemical change occurring under aerobic conditions, whereas the closed symbols represent the initial chemical change assessed under anaerobic conditions. Different types of symbols were used to represent the data obtained at different tetanus durations. For tetani >3 s, the initial chemical change in aerobic

TABLE IIA

INITIAL CHEMICAL CHANGE IN THE MOUSE SOLEUS

| Tetanus duration | Tension-time integral | PCr ($\xi_2$) | ATP ($\xi_1$) | Pi ($\xi_{1A}$) | 1.5 × ξ Lac | $\Delta\sim P_{init}$ | $n$ |
|---|---|---|---|---|---|---|---|
| $n$ | *N-m-s/g* | | *μmol/g* | | | | |
| Aerobic | | | | | | | |
| 1 | 0.20(±0.11) | −1.35(±0.23) | −0.02(±0.02) | 1.35(±0.22) | 0.10(±0.21) | 1.35(±0.38)* | 5 |
| 3 | 0.55(±0.14) | −3.11(±0.17) | −0.09(±0.04) | 3.18(±0.17) | 0.21(±0.32) | 3.18(±0.40)* | 9 |
| 6 | 1.05(±0.18) | −4.35(±0.37) | −0.72(±0.36) | 4.79(±0.25) | 0.06(±0.10) | 5.15(±0.53)* | 8 |
| 9 | 1.52(±0.13) | −5.32(±0.55) | −0.52(±0.29) | 5.76(±0.31) | 0.15(±0.31) | 5.78(±0.56)* | 9 |
| 12 | 1.89(±0.18) | −5.23(±0.26) | −0.61(±0.31) | 5.00(±0.32) | 0.21(±0.31) | 5.90(±0.69) | 8 |
| 15 | 2.36(±0.21) | −5.35(±0.21) | −0.54(±0.28) | 5.61(±0.39) | 0.18(±24) | 5.93(±0.66) | 6 |
| Anaerobic | | | | | | | |
| 1 | 0.23(±0.13) | −1.92(±0.09) | −0.06(±0.08) | 1.97(±0.11) | 0.31(±0.14) | 1.97(±0.22) | 6 |
| 3 | 0.66(±0.16) | −3.52(±0.39) | −0.75(±0.08) | 4.31(±0.40) | 1.45(±0.23) | 5.76(±0.36) | 6 |
| 6 | 1.16(±0.16) | −7.52(±0.29) | −1.32(±0.24) | 8.97(±0.22) | 2.10(±0.17) | 10.6(±0.43) | 8 |
| 9 | 1.51(±0.14) | −10.8(±0.12) | −2.47(±0.26) | 13.4(±0.16) | 1.03(±0.21) | 13.8(±0.36) | 8 |
| 12 | 1.93(±0.19) | −12.4(±0.19) | −2.61(±0.29) | 14.9(±0.32) | 2.15(±0.28) | 16.8(±0.45) | 9 |
| 15 | 2.30(±0.17) | −14.5(±0.26) | −2.83(±0.37) | 17.4(±0.229) | 2.31(±0.31) | 19.6(±0.53) | 7 |

Abbreviations used: determinations represent mean ± SEM. * $\Delta\sim P_{init}$ determined from "best estimate" procedure described in Methods.

muscles was far less than that in anaerobic muscles. In anaerobic muscles, $\Delta\sim P_{init}$ was a linear function of tension-time integral:

$$\Delta\sim P_{init} = 0.03 \pm 0.34 + 8.75 \pm 0.98\ L_0 \int Pdt \qquad (n = 22).$$

In aerobic muscles (open symbols), on the other hand, the amount of ~P utilization was nonlinearly related to the tension-time integral; it eventually

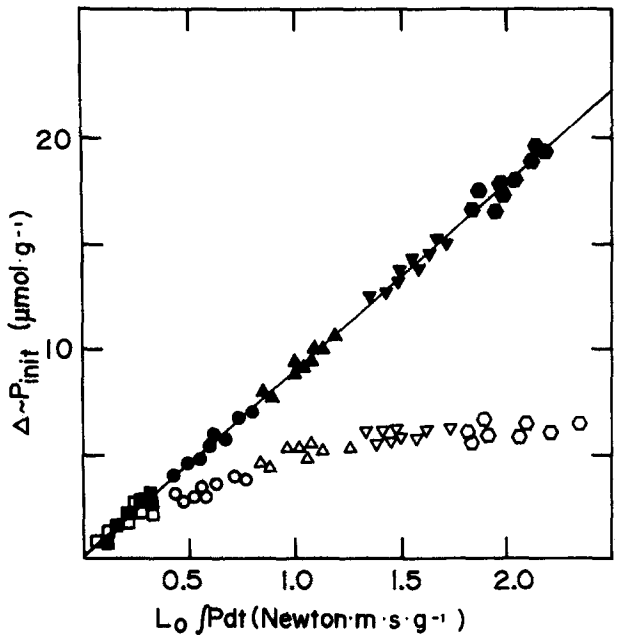


FIGURE 2. The relationship between initial chemical breakdown and tension-time integral in the soleus. Units are the same as in Fig. 1. Each data point represents the initial chemical breakdown ($\Delta\sim P_{init}$) in a single muscle compared with its control from observed changes in ATP, PCr, and lactate in the manner described in Methods. The open symbols represent the initial chemical change assessed under aerobic conditions, whereas the closed circles represent the initial chemical change assessed under anaerobic conditions. The solid line is the linear regression function fitting the anaerobic data:

$$\Delta\sim P_{init} = 0.03 \pm 0.34 + 8.75 \pm 0.98\ L_0 \int Pdt.$$

The different symbols represent muscles stimulated for different tetanus durations. The symbols used are (□, ■), 1 s; (○, ●), 3 s; (△, ▲), 6 s; (▽, ▼), 9 s, (◌, ◉), 12 and 15 s.

reached a plateau and became independent of further stimulation, all of which suggested the achievement of a steady state in which aerobic resynthesis matched ~P hydrolysis.

EDL The observed chemical change in the compounds of interest are shown in Table IIB for tetani of various duration occurring under both aerobic

and anaerobic conditions in the EDL. The initial chemical change was calculated as described in Methods. In Fig. 3, the initial chemical change ($\Delta\sim P_{init}$) has been plotted as a function of the tension-time integral. The open symbols represent the data for the chemical change occurring under aerobic conditions, whereas the closed symbols represent those points determined under anaerobic conditions. This plot displayed the same lack of linearity that was evident in the plot of recovery chemical input (Fig. 1). The anaerobic data in Fig. 3 were also conveniently fitted by two linear functions. For tetani of ≤6 s duration, the data were fitted by the function:

$$\Delta\sim P_{init} = 0.10 \pm 0.4 + 22.9 \pm 2.3\ L_0 \int Pdt \qquad (n = 16).$$

For tetani of ≥9 s duration, the data were fitted by the line:

$$\Delta\sim P_{init} = 11.8 \pm 1.2 + 11.4 \pm 1.2\ L_0 \int Pdt \qquad (n = 15).$$

TABLE IIB

INITIAL CHEMICAL CHANGE IN THE MOUSE EDL

| Tetanus duration | Tension-time integral | PCr ($\xi_2$) | ATP ($\xi_1$) | Pi ($\xi_{1A}$) | 1.5 × $\xi$ Lac | $\Delta\sim P_{init}$ | $n$ |
|---|---|---|---|---|---|---|---|
| *s* | *N-m-s/g* | | *μmol/g* | | | | |
| Aerobic | | | | | | | |
| 1 | 0.24(±0.14) | −4.36(±0.19) | 0.09(±0.19) | 4.41(±0.35) | 0.15(±0.22) | 4.36(±0.35)* | 5 |
| 3 | 1.56(±0.13) | −8.78(±0.44) | −1.21(±0.15) | 10.1(±0.52) | 0.93(±0.24) | 10.6(±0.53)* | 5 |
| 6 | 1.05(±0.15) | −14.4(±0.74) | −2.07(±0.44) | 15.8(±0.82) | 2.47(±0.45) | 18.4(±0.97)* | 8 |
| 9 | 1.56(±0.17) | −15.9(±0.48) | −2.81(±0.62) | 19.6(±0.35) | 4.61(±0.42) | 23.6(±0.89)* | 9 |
| 12 | 1.89(±0.21) | −17.9(±0.83) | −3.35(±0.81) | 20.3(±0.72) | 7.01(±0.19) | 28.3(±1.17)* | 8 |
| 15 | 2.11(±0.26) | −18.5(±0.41) | −4.00(±0.83) | 20.8(±0.29) | 8.68(±0.26) | 29.6(±1.16)* | 6 |
| Anaerobic | | | | | | | |
| 1 | 0.27(±0.13) | −4.26(±0.14) | −0.04(±0.13) | 4.26(±0.13) | 0.15(±0.22) | 4.26(±0.28)* | 5 |
| 3 | 0.56(±0.13) | −8.4 (±0.45) | −0.96(±0.17) | 9.12(±0.48) | 0.99(±0.12) | 10.2(±0.62)* | 8 |
| 6 | 1.04(±0.17) | −14.9(±0.65) | −2.35(±0.60) | 16.8(±0.88) | 3.18(±0.52) | 20.4(±1.03)* | 9 |
| 9 | 1.51(±0.11) | −19.8(±0.38) | −2.61(±0.29) | 22.1(±0.54) | 5.31(±0.41) | 26.8(±0.63) | 13 |
| 12 | 1.83(±0.21) | −19.2(±0.83) | −4.8(±0.35) | 24.1(±0.41) | 7.01(±0.39) | 31.1(±0.98)* | 12 |
| 15 | 2.01(±0.29) | −20.1(±0.41) | −4.91(±0.23) | 24.8(±0.31) | 9.21(±0.31) | 34.2(±0.82)* | 5 |

Abbreviations used: determinations represent the mean ± SEM. * $\Delta\sim P_{init}$ determined from "best estimate" procedure described in Methods.

*Comparison of Initial Chemical Change and Recovery Chemical Input*

In Fig. 4 the initial chemical change measured directly in muscles rapidly frozen at the end of contraction is compared with the recovery chemical input obtained from the extent of recovery oxidations. These bar graphs compare the normalized and averaged rates of energy use in μmol/(N·m·s) occurring over the 3-s interval marked; the actual rates along with the results of the statistical test of their significance are given in Table III. Three features are worthy of note.

(*a*) In both muscles, an apparent balance between use and resynthesis occurs for all the conditions illustrated (compare the open and cross-hatched bars in each set). This is evident both from Fig. 4 and Table III.

(*b*) The height of the bars provides an inverse measure of the "economy" for tension maintenance. For short tetani, the EDL uses approximately three

times as much ~P as the soleus in the maintenance of an equivalent tension per cross-sectional area. Stated simply, for short tetani the EDL was three times less economical in the maintenance of tension than the soleus.

(*c*) In the soleus, the "economy" of tension maintenance, and hence the height of the bars, is independent of the interval chosen for analysis. In contrast, the economy for the EDL is dependent on previous contractile

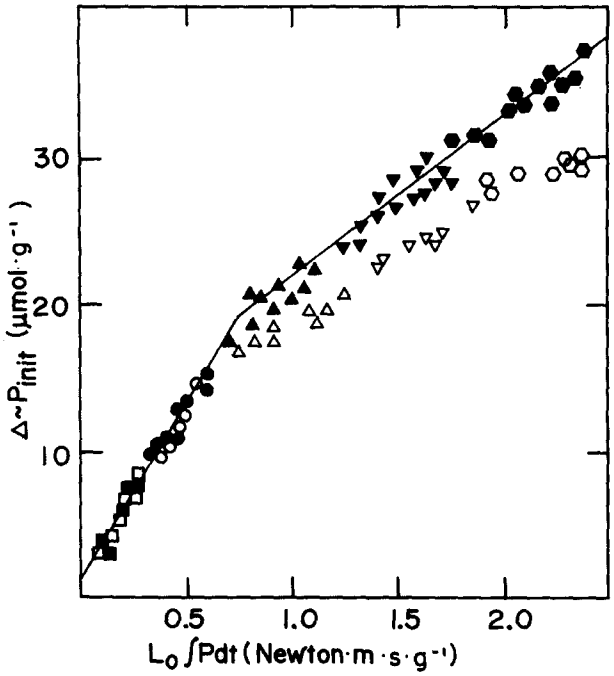


FIGURE 3. The relationship between initial chemical change and tension-time integral in the EDL. The data was obtained as described in Fig. 2. The open symbols represent the initial chemical change assessed under aerobic conditions, whereas the closed symbols represent the initial chemical change assessed under anaerobic conditions. The solid lines are regression functions fitting the anaerobic data.

(*a*) $\Delta\sim P_{init} = 0.10 \pm 0.4 + 22.9 \pm 2.3\ L_0 \int Pdt\ (0 < L_0 \int Pdt < 0.75)$
(*b*) $\Delta\sim P_{init} = 11.8 \pm 1.2 + 11.4 \pm 1.2\ L_0 \int Pdt\ (1.0 < L_0 \int Pdt < 2.5)$.

The different symbols represent muscles stimulated for different tetanus durations. The symbols used for the tetanus durations are defined in Fig. 2.

activity. With increasing tetanic durations, the energy cost for tension maintenance in the EDL decreased.

*Comparison of Initial Chemical Change and Recovery Input under Steady-State Conditions*

From the data represented in Fig. 2, it was evident that after 6 s of stimulation under aerobic conditions, the concentration of metabolites in the soleus

remained at a constant level, indicating the existence of a steady state between ~P resynthesis and use. Appropriately, for tetani of long duration, the oxygen consumption rate of the soleus was steady at a rate above basal. This rate represented the rate of aerobic resynthesis required to maintain the metabolites at a steady level. This steady-state $O_2$ consumption under these conditions

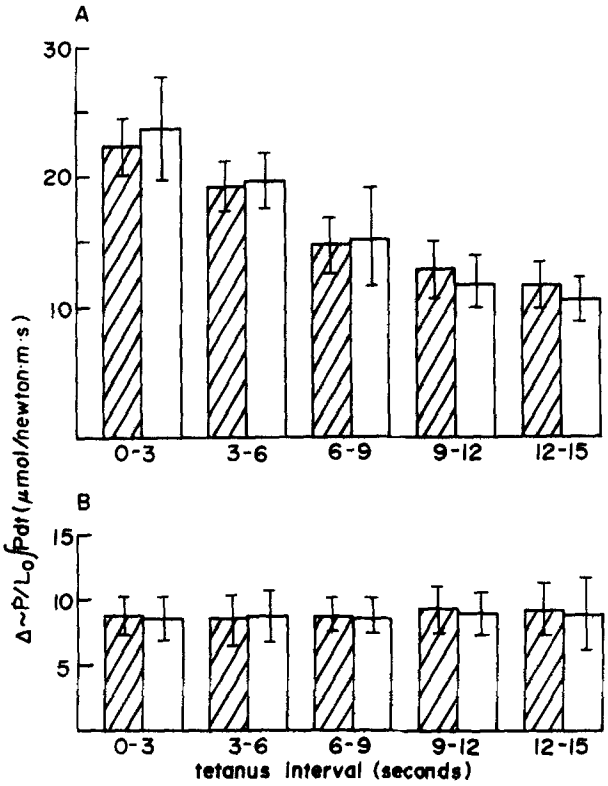


FIGURE 4. Comparison of the rate of recovery chemical resynthesis ($\Delta\sim P_{rec}$) and of initial chemical change ($\Delta\sim P_{init}$) associated with the maintenance of tension in mouse soleus and EDL at 20°C. The cross-hatched bars represent the rate of recovery chemical resynthesis ($\Delta\sim P_{rec}$), whereas the open bars represent the rate of initial chemical change ($\Delta\sim P_{init}$) averaged over a 3-s interval and normalized to the tension-time integral. The data was obtained from regression analysis of data in Figs. 1–3 by the techniques described in Methods. The different sets of bars correspond to data averaged over the following intervals of tetanus: 0–3 s; 3–6 s; 6–9 s; 9–12 s; 12–15 s. The upper set of graphs represent the data for the EDL, whereas the lower set is that for the soleus. The vertical lines on each bar graph corresponds to ± 1 SD.

was satisfactorily determined in four muscles and found to be 0.23 ± 0.02 μmol $O_2$/g/s. With a coupling coefficient of 6.3 ($P/O_2$), this steady rate of oxygen consumption would represent a resynthesis rate of 1.45 ± 0.13 μmol ~P/g/s. From the initial chemical data determined under anaerobic conditions (Fig. 2 and Table IIA), the rate of ~P hydrolysis was 1.41 ± 0.09 μmol

~P/g/s. These numbers were not statistically different ($P > 0.10$) and indicated the existence of an energy balance in the soleus under conditions of steady-state tetanic stimulation as well.

## DISCUSSION

### *Chemical Energy Balance*

A chemical energy balance in contracting muscle prevails when the amount of high-energy phosphate used during contraction ($\Delta\sim P_{init}$) is equal to that resynthesized during the recovery period ($\Delta\sim P_{rec}$). A valid comparison of these two measures of the chemical energy cost for contraction is based on the following premises: (*a*) the steady-state content of intermediary metabolites in the muscles is fully restored at the end of recovery; (*b*) all of the metabolic reactions occurring during contraction have been identified and accurately measured; (*c*) these reactions are confined to the initial period of contraction

TABLE III

COMPARISON OF INITIAL CHEMICAL CHANGE AND RECOVERY RESYNTHESIS IN MOUSE MUSCLES

| Tetanus interval | Soleus | | EDL | |
|---|---|---|---|---|
| | $\Delta\sim P_{init}$* | $\Delta\sim P_{rec}$* | $\Delta\sim P_{init}$* | $\Delta\sim P_{rec}$* |
| *s* | *μmol·N/cm·s* | | | |
| 0–3 | 9.1(±1.8) | 8.9(±0.4) | 24.1(±3.6) | 22.1(±1.1) |
| 3–6 | 8.4(±1.4) | 8.6(±0.5) | 19.4(±2.1) | 19.1(±1.3) |
| 6–9 | 8.9(±1.9) | 8.7(±0.3) | 14.8(±3.9) | 14.5(±1.9) |
| 9–12 | 8.6(±1.0) | 8.6(±0.21) | 11.8(±3.3) | 10.5(±1.2) |
| 12–15 | 8.8(±2.5) | 8.8(±0.3) | 10.0(±1.2) | 11.4(±1.4) |

Mean values ± standard deviation obtained from nonlinear regression analysis as described in Methods.

* Differences between $\Delta\sim P_{init}$ and $\Delta\sim P_{rec}$ are not statistically different for any tetanus intervals at the level ($P > 0.10$).

so that no post-contractile suprabasal chemical energy use occurs; (*d*) the stoichiometric coefficients relating ADP rephosphorylation to substrate oxidations and used in the calculation of $\sim P_{rec}$ are known.

In the mouse soleus and EDL muscles contracting at 20°C, a chemical balance was shown to exist between the initial and recovery phases of contraction (Fig. 4 and Table III). In addition, a balance was also shown to exist in stimulated soleus muscles during the steady state that was achieved during prolonged tetanus under aerobic conditions. Under this condition, the rate of recovery resynthesis estimated from the steady-state rate of oxygen consumption was equal to the steady-state rate of ~P use estimated from the rate occurring in the absence of aerobic recovery (anaerobic data in Fig. 2).

The existence of a balance in these muscles suggests that the extent of depletion of accumulation of metabolites listed in Tables IIA and IIB were sufficient to characterize the metabolic events surrounding contraction. Although additional reactions such as hexose-monophosphate accumulation and

the reaction catalyzed by adenylate kinase and deaminase may occur upon prolonged stimulation and under certain conditions (Kushmerick and Davies, 1969; Goodman and Lowenstein, 1977; Meyer and Terjung, 1980), these reactions were shown not to occur at any energetically significant rate (the extent of accumulation of these end-products or of depletion of reactants was <0.2 μmol/g during a 9-s tetanus). It may be argued that in the longest stimulation (15 s) these reactions would become important in the calculation of the initial chemical change. However, since a chemical energy balance prevailed for all tetani using the initial chemical change calculated from the extent of depletion or accumulation of the metabolites listed in Tables IIA and IIB, it would then be necessary to conclude that the muscles were no longer in chemical energy balance at prolonged stimulus durations and that there was an excess of initial chemical change. Such a conclusion would not be expected; in muscles in which a biochemical or myothermal energy imbalance has been shown to exist, a deficit, and not an excess, of initial chemical change has been reported (Curtin and Woledge, 1978; Kushmerick, 1978; Homsher et al., 1979).

This is the first description of a chemical energy balance occurring in contracting muscle. In contrast, a substantial quantitative discrepancy has been reported to exist in contracting frog muscle between the extent of recovery oxidations and the energy used during contraction (Kushmerick and Paul, 1976 *a* and *b*). Significantly more ~P was resynthesized during recovery than was used during contraction. The fact that the chemical discrepancy was present under both aerobic (Kushmerick and Paul, 1976 *b*) and anerobic conditions (DeFuria and Kushmerick, 1977) and that an imbalance had also been reported between the initial heat plus work and chemical change (Curtin and Woledge, 1978; Homsher and Kean, 1978) suggests that this discrepancy arises from an unidentified reaction sequence occurring during contraction and possibly extending into the recovery period (Kushmerick and Crow, 1982).

The observation of a biochemical energy balance in mouse muscles raises the possibility that a myothermal balance may also prevail. Such a quantitative comparison has been reported only for a 10-s tetanus of the rat soleus muscle (Gower and Kretzschmar, 1978). The results of that investigation, however, indicated that only ~70% of the energy input of the muscle could be accounted for by the observed chemical change. A detailed myothermal investigation of the time-course of explained and unexplained enthalpy in mammalian muscles is therefore warranted.

### *Comparative Aspects of the Cost to Maintain Tension*

The pattern of energy use in the fast-twitch EDL differed in two respects from that described for the soleus. For short isometric tetani (<6 s), the EDL consumed and resynthesized approximately three times as much ~P in the maintenance of comparable tension per cross-section area as the soleus. Furthermore, although the rate of chemical energy use during the tetanus was constant for all tetanus durations in the soleus, the rate of chemical energy use

in the EDL decreased with increasing tetanus durations (Fig. 4). The threefold greater chemical energy cost for the fast-twitch EDL could result from either a less efficient coupling of chemical to mechanical energy in this muscle or from a threefold greater rate of actomyosin turnover. The fact that the maximum velocities of shortening (Close, 1964) and the isolated myosin ATPase activities (Barany, 1967) of these muscles also differ by about threefold suggests that this latter hypothesis is correct and that energy conversion occurs at about the same chemomechanical efficiency in these two muscle types. This last hypothesis has not yet been adequately tested in working mammalian muscles.

Other studies have appeared on a comparative analysis of the cost to maintain tension. Using heat production, Gibbs and Gibson (1972) and Wendt and Gibbs (1973) have shown that five to six times as much initial heat is produced by the rat EDL in the maintenance of an equivalent tension as compared with the rat soleus. In comparison, we have demonstrated only a 2.9-fold difference in the chemical energy cost for tension maintenance in the same muscles of the mouse. Heat measurements performed at only one tetanus duration or in the absence of an accompanying set of chemical determinations are not necessarily useful for comparing the chemical energy cost of contraction because both the nature of the underlying chemical events as well as the fraction of the total heat released without a chemical equivalent may depend on both the tetanus duration and the muscle type.

### *Change in the Apparent Cost to Maintain Tension in the EDL*

An additional feature of the data concerns the lack of a constant relationship between the energy cost and tension maintenance in the EDL. Although for short tetani, the EDL consumed ~2.9 times the energy consumed by the soleus for an equivalent tension-time integral, the difference in the rate of energy consumption between the two muscles decreased with prolonged stimulation. By 12 s, the EDL consumed energy at a rate only 1.5 times that of the soleus. Two explanations for this time-dependent decrease in the apparent energy cost for tension maintenance are considered.

FATIGUE OF SPECIFIC FIBER TYPE Approximately 36% of the total cross-sectional area of the EDL is composed of fast-twitch fibers that rely on glycolysis to support recovery metabolism (Table I). These fibers are easily susceptible to fatigue. Since all chemical measurements were expressed relative to the tension-time integral, in order for the observed decrease in the maintenance cost of the whole muscle to account for the selective fatigue of this particular fiber type (FG), it would be necessary to assume that the energy cost for the maintenance of tension in FG-type fibers is significantly greater than that in the FOG-type fibers. There is no evidence either to suggest or disclaim such an assumption.

Furthermore, since the FG type of fibers occupies ~36% of the total cross-sectional area of the EDL muscle, fatigue of these fibers would be associated with a drop in the maintained force by the same percentage. During a tetanus,

force fell to no less than 0.9 $P_0$ at 6 s and to 0.8 $P_0$ at 12 s. With continued stimulation the force did eventually fall to a level consistent with significant fatigue of the FG fiber population (~20 s). However, the lack of correspondence between the rate of fatigue of tension and the time-course of the change in apparent energy cost renders unlikely the suggestion that fiber type-selective fatigue accounts for the observed change in the cost for tension maintenance. Our experiments cannot exclude the possibility that some of the largest FG fibers with possibly the highest rates of energy consumption had either completely fatigued or had entered into a rigor state; if so, these possible changes, including rigor, were completely reversible during recovery.

ACTIVATION COSTS AND ACTOMYOSIN ATPASE The extent of ~P use and resynthesis that is measured reflects the energy costs associated not only with actomyosin turnover but also with parallel reaction sequences involved in activation of the muscle (Curtin and Woledge, 1978; Homsher and Kean, 1978). The reduction in the whole muscle energy cost for contraction with continuous stimulation could arise from a reduction in either class of energy-consuming reactions (actomyosin turnover or activation processes). At present, neither possibility can be excluded, although preliminary experiments indicate that the maximum velocity of shortening in the EDL decreased with prolonged stimulation (Crow and Kushmerick, 1981). This observation suggests that the reduction in energy cost with prolonged stimulation of the EDL is, in fact, due to a reduction in the rate of actomyosin turnover.

Edwards et al. (1975) have observed a similar phenomena in the mouse soleus at 25°C. They noticed that with prolonged tetanization, the apparent rate of ATP hydrolysis by the whole muscle fell. This effect was only apparent after 15 s of tetanization and was associated with a slowing in the rate of tension relaxation after tetanus. Our data for the soleus show no such change in the apparent energy cost for tension maintenance. The reason for the discrepancy between the data reported here and that of Edwards et al. (1975) is unclear; however, it should be pointed out that the two sets of experiments were performed at different temperatures and that the range of stimulus durations used by Edwards et al. (1975) was far greater than that reported here.

In summary, a chemical energy balance prevailed in both mouse soleus and EDL for all tetanic durations. These results were qualitatively different from the energy balance described for amphibian muscles. As expected from differences in intrinsic speed of shortening, the cost to maintain tension in the fast-twitch EDL was significantly greater than that for the slow-twitch soleus. With prolonged stimulation, however, this difference in the apparent energy cost diminishes.

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REFERENCES

ARMITAGE, P. 1971. *Statistical Methods in Medical Research.* Blackwell Scientific Publ., Oxford, England. 118–126.

BARANY, M. 1967. ATPase activity of myosin correlated with speed of muscle shortening. *J. Gen. Physiol.* **50:**197–216.

BARNARD, R. J., V. R. EDGERTON, T. FURUKAWA, and J. B. PETER. 1971. Histochemical, biochemical and contractile properties of red, white, and intermediate fibers. *Am. J. Physiol.* **220:**410–414.

BEYER, W. H. 1968. Handbook of Tables of Probability and Statistics. Chem. Rubber Co., Cleveland, Ohio. p. 19.

CLOSE, R. I. 1964. Dynamic properties of fast and slow skeletal muscles of the rat during development. *J. Physiol. (Lond.).* **173:**74–95.

CURTIN, N. A., and R. WOLEDGE. 1978. Energy changes and muscle contraction. *Physiol. Rev.* **58:**690–761.

CURTIN, N. A., and R. WOLEDGE. 1979. Chemical change and energy production during contraction of frog muscle: how are their time courses related? *J. Physiol. (Lond.).* **288:**353–366.

CROW, M., and M. J. KUSHMERICK. 1981. Light chain phosphorylation and muscle energetics. *Biophys. J.* **33:**236*a*.

DEFURIA, R., and M. J. KUSHMERICK. 1977. ATP utilization associated with recovery metabolism in anaerobic frog muscle. *Am. J. Physiol.* **1:**C30–C36.

DUGGLEBY, R. G. 1981. A non-linear regression program for small computers. *Anal. Biochem.* **110:**9–18.

EDWARDS, R. H. T., D. K. HILL, and D. A. JONES. 1975. Metabolic changes associated with the slowing of relaxation in fatigued mouse muscles. *J. Physiol. (Lond.).* **251:**387–301.

ENNOR, A. H. 1957. *Methods Enzymol.* **3:**850.

GIBBS, C. L., and W. R. GIBSON. 1972. Energy production of rat soleus muscle. *Am. J. Physiol.* **223:**864–871.

GOODMAN, M. N., and J. M. LOWENSTEIN. 1977. The purine nucleotide cycle: studies of ammonia production by skeletal muscle *in situ* and in perfused preparations. *J. Biol. Chem.* **252:**5054–5060.

GOWER, D., and K. M. KRETZSCHMAR. 1976. Heat production and chemical change during isometric contraction of rat soleus muscle. *J. Physiol. (Lond.).* **258:**659–671.

GUTH, L., and F. J. SAMAHA. 1969. Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. *Exp. Neurol.* **25:**138–152.

HILL, D. K. 1940. The time course of the oxygen consumption of stimulated frog's muscle. *J. Physiol. (Lond.).* **98:**207–227.

HOMSHER, E., and C. J. KEAN. 1978. Skeletal muscle energetics and metabolism. *Annu. Rev. Physiol.* **40:**93–131.

HOMSHER, E., C. J. KEAN, A. WALLNER, and V. GARIBIAN-SARIAN. 1979. The time course of energy balance in an isometric tetanus. *J. Gen. Physiol.* **73:**553–567.

KRETZSCHMAR, K. M., and D. R. WILKIE. 1969. A new approach to freezing tissues rapidly. *J. Physiol. (Lond.).* **202:**66–67p.

KROGH, A. 1918. The rate of diffusion of gases through animal tissues, with some remarks on the coefficient of invasion. *J. Physiol. (Lond.).* **52:**391–408.

KUSHMERICK, M. J. 1978. Energy balance in muscle contraction: a biochemical approach. *Curr. Top. Bioenerg.* **6:**1–37.

KUSHMERICK, M. J., and M. CROW. 1982. Chemical energy balance in amphibian and

mammalian skeletal muscles. *Fed. Proc.* In press.

KUSHMERICK, M. J., and R. E. DAVIES. 1969. The chemical energetics of muscle contraction. II. The chemistry, efficiency and power of maximally working sartorius muscles. *Proc. R. Soc. Lond. B Biol. Sci.* **174:**315–353.

KUSHMERICK, M. J., and R. J. PAUL. 1976 *a*. Aerobic recovery metabolism following a single isometric tetanus in frog sartorius muscle at 0°C. *J. Physiol. (Lond.).* **254:**693–709.

KUSHMERICK, M. J., and R. J. PAUL. 1976 *b*. Relationship between initial chemical reactions and oxidative recovery metabolism for single isometric contractions of frog sartorius at 0°C. *J. Physiol. (Lond.).* **254:**711–727.

LOWRY, O. H., and J. V. PASSONNEAU. 1972. A Flexible System of Enzymatic Analysis. Academic Press, Inc., New York.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265–275.

MAHLER, M. 1978. Kinetics of oxygen consumption after a single isometric tetanus of frog sartorius muscle at 20°C. *J. Gen. Physiol.* **71:**559–580.

MEYER, R. A., and R. L. TERJUNG. 1980. AMP deamination and IMP reamination in working skeletal muscle. *Am. J. Physiol.* **239:**C32–C38.

NACLAS, M. M., D. G. WALKER, and A. N. SELIGMAN. 1958 *a*. A histochemical method for the demonstration of diphosphopyridine nucleotide diaphorase. *J. Biophys. Biochem. Cytol.* **4:**29–38.

NACLAS, M. M., D. G. WALKER, and A. N. SELIGMAN. 1958 *b*. The histochemical localization of triphosphopyridine nucleotide diaphorase. *J. Biophys. Biochem. Cytol.* **4:**467–474.

PADYKULA, H. A., and E. HERMAN. 1955. The specificity of the histochemical method for adenosine triphosphate. *J. Histochem. Cytochem.* **3:**170–195.

SCOPES, R. K. 1973. Studies with a reconstituted muscle glycolytic system: the rate and extent of creatine phosphorylation by anaerobic glycolysis. *Biochem. J.* **134:**197–208.

SEIDEL, A., and W. R. LINKE. 1965. Solubilities of Inorganic and Metal Organic Compounds. Van Nostrand, Princeton, N. J. **2:**1228.

SERAYDARIAN, K., W. F. H. M. MOMMAERTS, A. WALLNER, and R. J. GUILLORY. 1961. An estimation of the true inorganic phosphate in muscle. *J. Biol. Chem.* **236:**2071–2075.

WENDT, I. R., and C. L. GIBBS. 1973. Energy production of rat extensor digitorum longus muscle. *Am. J. Physiol.* **224:**1081–1086.