Basal Energy Utilization in Mammalian Skeletal Muscle* (32916)

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During the course of experiments designed to examine the chemical energetics of normal and dystrophic mouse skeletal muscle, it became evident that a "basal" value for the rate of chemical energy utilization was required. Such a value has been reported for cat papillary muscle (1), but does not appear to be available for mammalian skeletal muscle. To secure a suitable value the disappearance of energy-rich phosphate stores was determined in skeletal muscles maintained under anaerobic conditions and treated with iodoacetic acid (IAA).

Methods. White mice of a Swiss-Webster strain were used. Each was sacrificed by a quick blow to the head followed by decapitation to obtain muscles free of anesthetic agent and almost all blood. Both anterior tibial muscles were removed, were mounted on the electrode assembly of a quick-freeze apparatus (2), and were stretched to their approximate resting length in situ by the addition of a 1 gm effective load to each isotonic lever. Next, the muscles were immersed in mammalian Ringer solution at 20°C containing: 145 mM of Na^+, 5.6 mM of K^+, 2.5 mM of Ca^{++}, 130.6 mM of Cl^-, 25 mM of HCO_3^- and 5.6 mM of glucose, which was and continued to be gassed with 95% O_2–5% CO_2. After 5 min, a single supramaximal shock was delivered and the resulting contractions were recorded. Muscle pairs which exhibited weak or unequal contractions of the members were discarded at this time. In acceptable pairs the Ringer solution was exchanged for one containing 5×10^{-4} M of IAA. This incubation solution, at 20°C, was continuously gassed with 95% O_2–5% CO_2 for the next 30 min. Subsequent to this aerobic incubation in IAA–Ringer solution the muscles were either frozen immediately or immersed in fresh IAA–Ringer solution, which was and continued to be gassed with scrubbed 95% N_2–5% CO_2, for anaerobic incubation of 3, 10, or 13 min duration prior to freezing. Freezing was effected by rapid immersion into isopentane at —160°C, a technique developed by Mommaerts and Shilling (3).

Frozen muscles were freed from the electrodes, stored briefly under liquid nitrogen, trimmed and weighed in the frozen state. Next they were pulverized and extracted with 0.3N $HClO_4$ at 0°C, neutralized and analyzed. ATP, ADP, and AMP were determined by a modification of the technique of Strehler and Totter (4), where in step-wise manner ADP and AMP were converted to ATP by addition of phosphoenolpyruvate, pyruvate kinase and myokinase to muscle extract samples. Repeated checks failed to detect any endogenous myokinase activity in the firefly extract or in the muscles during this procedure. A modification of the Ennor and Rosenberg (5) method was employed for the assay of phosphocreatine (PC); inorganic phosphate (P_i) was determined by the method of Wahler and Wollenberger (6). Concentrations were expressed as μM/gm, of frozen muscle. Data were analyzed by the small sample t test and the null hypothesis rejected at the 5% level.

Results. The mean values and standard errors of the mean values of analytically determined concentrations are presented in Table I. The concentration of ATP, ADP, and AMP at 3, 10, and 13 min is not significantly different from those values at 0 time, i.e., before anaerobic conditions were imposed on the IAA-poisoned muscles. The concentration of PC is statistically significantly different at 3, 10, and 13 min, while the P_i was significantly different at 3 and 13 min. Despite the lack of statistically significant changes in the concentration of ADP,

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TABLE I. Concentrations of ATP, ADP, AMP, PC, and P_i in μM/gm of IAA-Treated Mouse Skeletal Muscles after Varied Periods of Anaerobic Incubation at 20°C.

Min in N_2[a]	ATP	ADP	AMP	PC	P_i	Muscle wt. (mg)
0	3.173	.553	.270	8.065	7.930	38.79
(24)	±.212	±.084	±.042	±.151	±.528	±1.25
3	3.153	.616	.311	7.179[b]	9.429[b]	44.03
(14)	±.165	±.049	±.041	±.437	±.498	±1.71
10	2.793	.624	.279	6.224[c]	8.772	40.59
(18)	±.217	±.051	±.047	±.401	±.786	±2.02
13	2.720	.655	.258	4.490[c]	9.802[b]	45.68
(18)	±.170	±.041	±.037	±.443	±.481	±1.72

[a] Numbers of muscles analyzed are given in ( ).
[b] Indicates $p < .05$.
[c] Indicates $p < .001$.

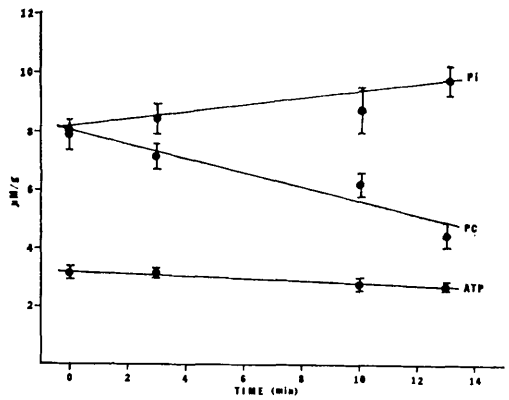

FIG. 1. Graph of mean and standard error of mean change in concentration (μM/gm) of ATP, PC, and P_i in mouse anterior tibial muscles with duration of exposure to IAA–Ringer solution gassed with 95% N_2–5% CO_2. Least mean square line of best fit is applied to each set of data.

the ratio of ADP:ATP increased progressively from 0.17 to 0.24 after 13 min.

Figure 1 depicts the change in concentration with time, as fitted by the method of least means squares. The rate of ATP utilization was 0.038 ± 0.019 μM/gm per min ($p \simeq .05$), while that for PC utilization was 0.245 ± 0.033 μM/gm per min ($p < .001$). The rate of disappearance of the sum of PC and ATP was also highly significant, although there was no significant difference ($p > .50$) between it and the rate for PC alone. Inorganic phosphate, according to the regression analysis, increased at about one half the rate (0.127 ± 0.060 μM/gm per min) of PC decline.

Discussion. Although not tested directly in this study, sufficient evidence has been presented, and cited, by Pool and Sonnenblick (1) to assume that 30-min exposure to 5×10^{-4} M of IAA is adequate to inhibit muscle glycolysis without affecting the activity of creatine phosphoryltransferase:

$$PC + ADP \overset{\text{CP-transferase}}{\rightleftharpoons} ATP + \text{free creatine}$$

This conclusion, at least in regard to the CP-transferase enzyme, is supported by the evidence in Table I of an insignificant depression of ATP and highly significant decline in PC during the periods of anaerobic incubation.

How soon truly anaerobic conditions prevail within the muscle after transfer to 95% N_2–5% CO_2 cannot be assessed directly within the framework of the present experiments. However, estimates of the time at which O_2 has completely disappeared from a muscle suddenly immersed in an O_2-free medium can be obtained by using equations provided by Hill (7). Even if a very low, but steady, Q_{O_2} of unity is assumed in making the calculation of the time, the anterior tibial muscles at 20°C would have depleted the available O_2 in less than 1 min. The changes in [ATP] and [PC] found after 3 min anaerobic incubation would not suggest any appreciable carry-over oxidative metabolism.

The rate of PC utilization was nearly twice that of P_i accumulation. This discrepancy may arise either because (a) glycogen is being converted to glucose-1-phosphate with consequent P_i depletion, or because (b) "extra" PC is being used to satisfy esterification reactions with the formation of fructose diphosphate (8). In alternative (b) the rate of accumulation of P_i, instead of the rate of utilization of PC, would be the more accurate estimate of effective energy utilization. Actually (b) is not the more likely alternative in view of the results obtained by Seraydarian *et al.* (9).

Pool and Sonnenblick (1) found a resting rate of energy utilization of 0.84 μM/gm per min (8.4 mcal/gm per min) in cat papillary muscles subjected to IAA and N_2 under 1 gm resting tension. The ATP disappearance under the circumstances was negligible, and they concluded that the total utilization of high-energy phosphates was represented by that of PC. Our smaller value of approximately 0.25 μM of PC/gm per min (2.5 mcal/gm per min) is in accord with the generally observed lower metabolic rate of skeletal compared to cardiac muscle. Frog sartorius muscles were exposed to IAA and N_2 by Dydynska and Harris (10) during an investigation of the relation between active ion transport and high-energy phosphate consumption. Their data exhibits too great a variability for meaningful regression analysis, but yields average values of about 0.13 μM/gm per min and 0.16 μM/gm per min for rates of PC and PC + ATP utilization, respectively. These rates, as expected, are slower than those observed in mammalian skeletal muscle and would not totally exhaust the available PC stores (15.5 μM/gm) for nearly 2 hours.

Under aerobic conditions, Keynes and Maisel (11) obtained a metabolic rate ranging from 11 to 16 mcal/gm per min (130–194 mm^3 O_2/gm per hour) and secretory work rate ranging from 1.0 to 1.4 mcal/gm per min (.057–.084 cal/gm per hour) when Na-ion secretion was stimulated by using 10 mM K^+–Ringer solution. The ion shift amounted to 24–35 mM/kg per hour, and the work accounted for about 10% of the aerobic resting metabolism. The data of Dydynska and Harris (10) show a somewhat smaller mean ion shift of about 15.6 mM/kg per hour. By accepting the conclusion of Keynes and Maisel (11) that frog sartorius muscle Na-ion efflux is not drastically reduced by exposure to 5×10^{-4} M of IAA and 3×10^{-3} M of CN for over 3 hours and by applying the formula they used for secretory work $[W + kC_i\ F(E_{Na} + E_r)]$ with the values they assumed for the sodium equilibrium potential (E_{Na}) and for the membrane potential (E_r) in 10 mM K^+–Ringer solution, one can calculate for the data of Dydynska and Harris (10) a secretory work rate of about 0.6 mcal/gm per min. The total rate of energy expenditure in the latter study ranged from 1.3 mcal/gm per min (PC) to 1.6 mcal/gm per min (PC + ATP), i.e., 38–46% of the "basal" energy expenditure.

It is possible to extend the "bookkeeping" approach to mammalian skeletal muscle by reference to the data of Zierler *et al.* (12). Their study of the K-ion fluxes in rat extensor digitorum longus muscle, under aerobic conditions (95% O_2–5% CO_2), provides values for solving the secretory work equation. The solution, however, requires the assumptions that an energy utilizing, coupled Na^+–K^+ exchange is operating and that active K-ion influx is a measure of active Na-ion efflux, i.e., a one-to-one link between Na-ion and K-ion movement. Recalculation of their data (for groups A and C in Table VI) suggests a minimum secretory work rate between 1.1 and 1.4 mcal/gm per min. This rate would be higher if the membrane potential were greater (say 85 mV instead of the 61 mV reported) and if the coupling ratio were larger than 1:1. Certainly, under aerobic conditions 1 gm of muscle with a Qo_2 of 1 (16.8 mcal/gm per min) could maintain the minimum secretory rate by expending only about 10% of the energy. The rate of energy utilization (2.5 mcal/gm per min) under the "basal" conditions examined by us would support such a minimum aerobic secretory work rate (44–56% of "basal" expenditure) for about 32 min before total exhaustion of PC stores. However, metabolic inhibitors may alter passive ion fluxes by increasing membrane per-

meability (12) and make even greater demands upon the limited energy reserves. When both respiration and glycolysis are inhibited (by a different technique) in mouse soleus muscle (13), the membrane potential falls precipitously within 30 min. The obvious conclusion from our data as applied to that of others is that mammalian skeletal muscle is at a greater disadvantage than amphibian skeletal muscle whenever a diminished metabolism is experienced.

Summary. The "basal" rate of energy utilization, as measured by PC disappearance, in mouse anterior tibial muscles exposed to a N_2–IAA environment is about 0.25 μM/gm per min or 2.5 mcal/gm per min. This rate, as expected, is greater than the "basal" value for amphibian skeletal muscle and less than the "basal" value for mammalian cardiac muscle. By making certain assumptions about active ion transport and by using certain data from other investigators, the conclusion is reached that mammalian skeletal muscle sustains itself with a restricted energy supply much less well than amphibian skeletal muscle.

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Density Gradient Ultracentrifugation of Rubella Virus (32917)

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The development of a hemagglutination test provided a sensitive tool for defining the properties of rubella virus. Some of the biological and biophysical characteristics of rubella virus hemagglutinin (HA) were described in earlier reports from this laboratory (1–3).

Using sucrose, cesium chloride (CsCl) and cesium sulfate (Cs_2SO_4) density gradient techniques, it has been possible to band rubella virus HA and infectivity, and to define the buoyant density of rubella virus. These studies have enabled us to demonstrate the similarity of rubella virus strains grown in several mammalian cell systems and harvested at various passage levels. These experiments indicate that the sedimentation behavior of the HA and infectious virus are closely related.

Materials and Methods. Cells. The BHK 21 (4), BS-C-1 (5), and primary African green monkey kidney (GMK) cell cultures were obtained from the Tissue Culture Section, Division of Biologics Standards, Nation-

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