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Fatty acid oxidation by liver and muscle preparations of exhaustively exercised rats

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The influence of exhaustive exercise on the capacity of liver and muscle of rats to oxidize fatty acids was investigated *in vitro*. The rate of oxidation of fatty acids by liver preparations was significantly elevated as a result of exhaustion. Concurrently, the concentrations of β-hydroxybutyrate were elevated in the plasma of the exhausted rats, suggesting that oxidation of fatty acids was also elevated *in vivo*. These findings are analogous to the findings of increased oxidation of fatty acids that results from training. In muscle, oxidation of palmitate, palmitoylcarnitine and β-hydroxybutyrate by homogenates and isolated mitochondria was depressed with exercise. Despite the decrease in the oxidative capacity of the muscle preparations, the activities of several enzymes of β-oxidation were either increased or unchanged as a result of exercise, suggesting that the depression in fatty acid oxidation may not be related to alterations in the process of β-oxidation. Further studies showed that oxidation of [2-^{14}C]pyruvate by muscle was depressed, whereas oxidation of [1-^{14}C]pyruvate was not changed as a result of exercise. These results suggest that the decrease in fatty acid oxidation may be related to aberrations in the oxidation of acetyl-CoA. The changes in fatty acid oxidation that were observed, which are at variance with what is reported to occur with training, may have resulted from increased fragility of muscle mitochondria as a result of exercise. This increased fragility may render the mitochondria more susceptible to experimental manipulations *in vitro* and a subsequent loss of normal function.

During prolonged periods of exercise, a large part of the energy required for muscle is derived from the oxidation of fatty acids (Havel *et al.*, 1963; Paul & Issekutz, 1967). There are three sources from which fatty acids are derived. The first is the fat stores in adipose tissue, which are mobilized in response to exercise-induced elevation of adrenaline concentrations (Fell *et al.*, 1980). A second source is the circulating triacylglycerol-rich lipoproteins, which release fatty acids on hydrolysis by lipoprotein lipase (Robinson, 1970). The activity of lipoprotein lipase has been shown to increase in muscle with prolonged exercise (Borensztajn *et al.*, 1975; Nikkila *et al.*, 1978; Lithell *et al.*, 1981). The third source of fatty acids is the fat stored within the tissue itself, which has been shown to be quantitatively greater in physically trained subjects than in untrained subjects (Morgan *et al.*, 1969; Froberg, 1971; Reitman *et al.*, 1973; Stankiewicz-Choroszocha & Gorski, 1978).

Previously we examined the influence of an acute bout of exhaustive exercise on changes in plasma lipid concentration and the activity of hormone-sensitive lipase in adipose tissue (Barakat *et al.*, 1981). We reported that exhaustive exercise resulted in an increase in plasma non-esterified fatty acids and a decrease in plasma triacylglycerols. Furthermore, we reported that the activity *in vitro* of adipose-tissue hormone-sensitive lipase was increased in the exhausted animals. The decrease in plasma triacylglycerols may have resulted from an increase in the uptake of triacylglycerol by the tissues of the exercising animals. Thus the changes in fatty acid concentrations that we observed with exhaustion are similar to those seen during training.

In the present investigation, one aspect of fatty acid metabolism in exhaustive exercise, namely oxidation of fatty acids, was examined. As a first step in a series of on-going studies, we elected to determine the capacity of liver and muscle to oxidize fatty acids and other substrates *in vitro*. This was done to compare the influence of a single ex-

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haustive bout of exercise with the reported influence of exercise-training on the capacity of tissues to oxidize fatty acids. The results reported here show a decrease in fatty acid oxidation by muscles of animals that are exercised to exhaustion, which is in contrast with results in trained animals.

Experimental

Animals

Male Holtzman rats (Holtzman Co., Madison, WI, U.S.A.) weighing 250–300 g were housed in individual cages in a room maintained at 20–23 °C, and kept on a 12h-dark/12h-light cycle (06:00–18:00h light). The animals were given water and food (Wayne Blox, Allied Mills, Chicago, IL, U.S.A.) *ad libitum* until the start of the experimental protocol. The rats were divided randomly into two groups: controls (unexercised) and exercised. The exercised animals were run on a rodent treadmill between 06:00h and 11:00h and made to run at 28 m/min (0% grade) until they could no longer run (220 ± 19 min, mean $\pm$ S.E.M.). Food was removed from the cages of the animals in the control group during the time that the exercised rats were on the treadmill. Both the exercised and control animals were killed at the same time by decapitation. Blood was collected from the severed neck in heparinized test tubes and plasma was prepared. The liver and both gastrocnemius muscles were excised and placed in 0.15 M-KCl over ice.

Analyses and assays

Plasma β-hydroxybutyrate was measured spectrophotometrically with β-hydroxybutyrate dehydrogenase by the procedure described by McGarry *et al.* (1970). Because β-hydroxybutyrate is a major ketone body (Williamson & Hems, 1970), it was selected as an indicator of changes in total ketone-body concentration during exercise (Winder *et al.*, 1974). Lipids were extracted from plasma, liver and skeletal muscle by the procedure of Folch *et al.* (1957). Total lipid concentration in liver and muscle was determined gravimetrically, and the concentration of non-esterified fatty acids in the lipid extract from these tissues and plasma was determined by the method of Duncombe (1963).

Liver was homogenized in 0.25 M-sucrose (1:1, w/v) in a glass/Teflon Potter–Elvehjem homogenizer. The homogenate was diluted to 10 vol. with 0.25 M-sucrose and centrifuged at 600 ***g*** for 10 min. A portion (1 ml) of the supernatant was kept for measurement of fatty acid oxidation. The remainder of the 600 ***g*** supernatant was centrifuged at 15000 ***g*** for 10 min. The resultant pellet was suspended in 2 vol. of 0.25 M-sucrose and centrifuged at 15000 ***g*** for 10 min. This procedure was repeated and the resultant mitochondrial pellet was finally resuspended in a volume of 0.25 M-sucrose equivalent to the weight of the liver initially used.

Muscle was pressed through a tissue press, then homogenized in Chappell–Perry buffer (Chappell & Perry, 1954), pH 7.4 (1:10, w/v), in a glass/glass homogenizer. The homogenate was filtered through four layers of cheesecloth and a portion (1.5 ml) was retained for measurement of fatty acid oxidation by the filtered homogenate. Mitochondria were isolated from the homogenate by the method described by Ernster & Nordenbrand (1967). The resultant mitochondrial pellet was suspended in a volume of homogenization buffer equivalent to the weight of the muscle that was homogenized.

The capacity of liver and muscle homogenates and isolated mitochondria to oxidize [1-^{14}C]-palmitate was determined in duplicate by collecting and counting the radioactivity of $^{14}CO_2$ produced during the incubation period in ScintiVerse E (Fisher Scientific Co., Pittsburgh, PA, U.S.A.). A 2 ml incubation volume was used, containing the following cofactors (pH 7.3): 2.0 mM-ATP, 0.05 mM-CoA, 1.0 mM-dithiothreitol, 0.1 mM-malate, 1.0 mM-$MgCl_2$, 0.072 mM-bovine serum albumin (fatty acid-free), 0.1 mM-NAD^+, 100 mM-sucrose, 100 mM-K_2HPO_4, 80 mM-KCl, 0.1 mM-EDTA, 1.0 mM-DL-carnitine and 0.2 mM-[1-^{14}C]palmitate (1 μCi). The reaction was initiated by the addition of sample, and the contents were gassed with O_2/CO_2 (19:1) and stoppered with a rubber septum stopper containing a polypropylene centre cup. The flasks were incubated at 37 °C for 30 min with gentle shaking. Immediately before the termination of the incubation, 0.2 ml of ethanolamine/methylCellosolve (1:2, v/v) was injected into the hanging centre well. The reaction was stopped by injecting 0.2 ml of 2 M-H_2SO_4 into the contents of the flask. Flasks were shaken for 60 min to collect the evolved $^{14}CO_2$. A portion (0.05 ml) of the ethanolamine/methylCellosolve was then taken and counted for radioactivity.

Oxidation of palmitate by teased muscle fibres was determined in the same manner as oxidation of palmitate by homogenates. Muscle fibres (approx. 20 mg each) were teased from the red portion of the gastrocnemius in iso-osmotic KCl. Approx. 50 mg (wet wt.) of these fibres were incubated, in quadruplicate, in the same incubation mixture as that used for homogenates. After a 30 min incubation time, the reaction was terminated, and the fibres from each flask were removed, dried and weighed. The rate of palmitate oxidation by fibres is reported as nmol of palmitate oxidized/min per g dry wt.

Oxidation of palmitoylcarnitine by mitochondria isolated from liver and muscle was measured polarographically. The incubation mixture was essentially the same as that used for palmitate

oxidation, except 0.2mM-palmitoylcarnitine was used instead of palmitate.

The rate of oxidation of D-β-hydroxy[3-^{14}C]-butyrate by fresh whole homogenates of gastrocnemius muscle was assessed as described by Winder *et al.* (1973) with slight modification. Muscle was homogenized (1:10, w/v) in 175mM-KCl containing 2mM-EDTA and 2mM-dithiothreitol, pH 7.4. The rate of oxidation of D-β-hydroxy[3-^{14}C]butyrate was assessed by measuring the rate of $^{14}CO_2$ production in a reaction mixture which contained, in a final volume of 2ml: 5mM-$MgCl_2$, 107.5mM-KCl, 20mM-potassium phosphate buffer, 2mM-EDTA, 2mM-ADP, 10mM-Tris/HCl, 1mM-dithiothreitol and 1mM-DL-β-hydroxybutyrate, pH 7.4. In addition each flask contained 0.05μCi of D-β-hydroxy[3-^{14}C]-butyrate. Reaction mixtures were placed in 25ml flasks equipped with serum caps and hanging centre wells, as described above. The reaction was started by the addition of β-hydroxybutyrate and ADP. After 20min of incubation at 30°C, the reaction was stopped by injecting 0.2ml of 2M-H_2SO_4 into the reaction vessel. The $^{14}CO_2$ was trapped in ethanolamine/methylCellosolve as described above, and counted for radioactivity.

For the determination of the oxidation rates of pyruvate by muscle homogenates 8mM-[1-^{14}C]-pyruvate (1.1μCi/assay) or 8mM-[2-^{14}C]pyruvate (1.8μCi/assay) was incubated in the same incubation mixture that was used for the determination of palmitate oxidation. The rest of the procedure was the same as that described above.

For the measurement of the activities of the enzymes of β-oxidation, liver and muscle were homogenized in 10mM-potassium phosphate buffer, pH 7.4, containing 1mM-EDTA and 1mM-dithiothreitol. L-3-Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) was assayed spectrophotometrically by following the rate of NADH oxidation that accompanies the conversion of acetoacetyl-CoA into 3-hydroxyacyl-CoA (Bradshaw & Noyes, 1975). Enoyl-CoA hydratase (crotonase, EC 4.2.1.17) was assayed spectrophotometrically by determining the decrease in A_{280} owing to the hydration of $\alpha\beta$-unsaturated acyl-CoA as described by Steinman & Hill (1975). 3-Oxoacyl-CoA thiolase (EC 2.3.1.16) activity was determined by following the breakdown of 3-oxoacyl-CoA at 303nm as described by Middleton (1975).

All assays were performed under conditions of substrate saturation, with the amount of enzyme as the rate-limiting component of the reaction *in vitro*. Protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Comparisons between exercised and rested groups were made by using an analysis of variance. The significance level in these comparisons was set at $P < 0.05$.

Results

As a result of exhaustive exercise, plasma β-hydroxybutyrate concentrations were elevated more than 3-fold, and non-esterified fatty acid concentration was almost doubled (Table 1). Concurrently, the total lipid content in liver was significantly higher and the concentration of non-esterified fatty acids showed the same trend (Table 1). In muscle, however, no significant changes were observed in either the total lipid or fatty acid content in the exhausted rats (Table 1).

The effect of exhaustive exercise on the oxidative capacity of tissue homogenates and isolated mitochondria was assessed in two ways: (i) determination of $^{14}CO_2$ produced from the oxidation of [1-^{14}C]-palmitate and (ii) determination of the amount of O_2 consumed during the oxidation of palmitoylcarnitine. Oxidation of [1-^{14}C]palmitate by liver homogenates and isolated mitochondria was significantly elevated in the exhaustively exercised rats (Table 2). Although not statistically significant, palmitoylcarnitine oxidation by isolated mitochondria tended to be higher in the exhausted rats than their rested counterparts (Table 2).

Oxidation of [1-^{14}C]palmitate and D-β-hydroxy-[3-^{14}C]butyrate by muscle homogenates was signi-

Table 1. *Effect of exhaustive exercise on plasma and tissue lipid contents*

For details see the text. Results are means ± S.E.M. for the numbers of animals shown in parentheses. * indicates a statistically significant difference ($P < 0.05$) between control and exercised animals.

	Control	Exercised
Plasma		
β-Hydroxybutyrate (μmol/ml)	0.34 ± 0.03 (11)	1.18 ± 0.07* (11)
Non-esterified fatty acids (μg/ml)	125 ± 13 (11)	203 ± 14* (10)
Liver		
Total lipids (mg/g)	48.8 ± 0.91 (11)	59.4 ± 2.44* (13)
Non-esterified fatty acids (mg/g)	1.08 ± 0.18 (11)	1.27 ± 0.11 (13)
Muscle		
Total lipids (mg/g)	17.9 ± 0.41 (11)	16.9 ± 0.24 (13)
Non-esterified fatty acids (mg/g)	0.31 ± 0.03 (11)	0.33 ± 0.04 (13)

Table 2. *Effect of exhaustive exercise on fatty acid oxidation by liver and muscle homogenates and isolated mitochondria*
Values represent means ± S.E.M. for the numbers of observations shown in parentheses. * indicates a statistically significant difference ($P < 0.05$) between control and exercised animals. For details see the text.

	Liver		Muscle	
	Control	Exercised	Control	Exercised
Homogenates				
[1-^{14}C]Palmitate oxidation				
(nmol/min per mg of protein)	0.084 ± 0.011 (12)	0.150 ± 0.011* (13)	0.096 ± 0.014 (8)	0.041 ± 0.008* (12)
(nmol/min per g of tissue)	17.9 ± 2.3 (12)	38.7 ± 3.0* (13)	9.89 ± 0.06 (8)	3.12 ± 0.63* (12)
D-β-Hydroxy[3-^{14}C]butyrate (nmol/min per g of tissue)			3.59 ± 0.26 (11)	1.11 ± 0.14* (11)
Mitochondria				
[1-^{14}C]Palmitate oxidation (nmol/min per mg of protein)	0.19 ± 0.02 (11)	0.33 ± 0.02* (12)	0.19 ± 0.02 (12)	0.11 ± 0.02* (12)
Palmitoylcarnitine oxidation (ng-atoms of O/min per mg of protein)	34.6 ± 1.9 (11)	40.6 ± 2.5 (12)	66.0 ± 6.0 (11)	38.8 ± 4.0* (11)
Mitochondrial yield				
(g of protein/liver per kg body wt.)	2.14 ± 0.03 (11)	1.84 ± 0.05* (12)		
(mg of protein/2 muscles per kg body wt.)			44.3 ± 2.2 (11)	35.2 ± 3.8* (11)

Table 3. *Effect of exhaustive exercise on the activity of some enzymes of β-oxidation in liver and muscle homogenates*
Values represent means ± S.E.M. with numbers of observations shown in parentheses. * indicates a statistically significant difference ($P < 0.05$) between control and exercised animals. For details see the text.

	Liver		Muscle	
	Control	Exercised	Control	Exercised
3-Hydroxyacyl-CoA dehydrogenase				
(nmol/min per mg of protein)	28.0 ± 1.7 (6)	36.7 ± 3.0* (6)	16.4 ± 1.7 (6)	21.6 ± 2.7 (6)
(μmol/min per g of tissue)	6.4 ± 0.32 (6)	9.2 ± 0.63* (7)	1.2 ± 0.1 (6)	1.6 ± 0.2 (6)
Enoyl-CoA hydratase (crotonase)				
(μmol/min per mg of protein)	3.5 ± 0.17 (12)	3.5 ± 0.19 (12)	0.38 ± 0.02 (11)	0.49 ± 0.03* (13)
(μmol/min per g of tissue)	797 ± 35 (11)	937 ± 41 (13)	26.8 ± 2.0 (12)	34.0 ± 2.1* (12)
3-Oxoacyl-CoA thiolase				
(nmol/min per mg of protein)	70.2 ± 3.6 (11)	67.9 ± 6.6 (14)	42.0 ± 7 (12)	34.0 ± 3.0 (12)
(μmol/min per g of tissue)	17.2 ± 1.13 (12)	17.6 ± 1.23 (14)	2.46 ± 0.42 (11)	2.48 ± 0.29 (12)

Table 4. *Effect of exhaustive exercise on oxidation of [1-^{14}C]pyruvate and [2-^{14}C]pyruvate by muscle homogenates*
Values represent means ± S.E.M. for the numbers of observations shown in parentheses. * indicates a statistically significant difference ($P < 0.05$) between control and exercised animals. For details see the text.

Oxidation	Control	Exercised
[1-^{14}C]Pyruvate		
(nmol/min per mg of protein)	4.4 ± 0.06 (10)	4.7 ± 0.074 (11)
(nmol/min per g of tissue)	408 ± 54 (10)	425 ± 72 (11)
[2-^{14}C]Pyruvate		
(nmol/min per mg of protein)	0.72 ± 0.08 (10)	0.47 ± 0.05* (11)
(nmol/min per g of tissue)	68 ± 9.0 (10)	42 ± 3.8* (11)

ficantly depressed as a result of exhaustive exercise (Table 2). Similarly, the rates of oxidation of [1-^{14}C]-palmitate and palmitoylcarnitine were depressed in the mitochondria isolated from the muscles of exhausted rats (Table 2). The decrease in the rate of oxidation of these substrates appears to be not solely related to the observed decrease in mitochondrial yield, since the magnitude of the depression in the oxidation rate exceeds the magnitude of the depression in the mitochondrial yield (Table 2).

In efforts to determine the possible causes of the observed changes in fatty acid oxidation, the activi-

ties of three enzymes that are involved in β-oxidation were measured in homogenates prepared from liver and muscle. The activity of liver 3-hydroxyacyl-CoA dehydrogenase was elevated, but the activities of enoyl-CoA hydratase (crotonase) and 3-oxoacyl-CoA thiolase were not affected by exercise (Table 3). In muscle, the activities of 3-hydroxyacyl-CoA dehydrogenase and 3-oxoacyl-CoA thiolase were unchanged, whereas the activity of enoyl-CoA hydratase was significantly elevated (Table 3). These findings suggest that the observed decrease in the oxidative capacity of muscle preparations may be due not to changes in the activity of the enzymes of β-oxidation, but rather to possible changes in oxidation of acetyl-CoA. To test this hypothesis, the capacity of muscle homogenates to oxidize pyruvate was determined. Oxidation of [1-^{14}C]-pyruvate was unchanged, whereas oxidation of [2-^{14}C]pyruvate was significantly depressed (Table 4). These observations suggest that the decrease in oxidation of pyruvate is not due to aberrations in the activity of pyruvate dehydrogenase, since no changes in [1-^{14}C]pyruvate oxidation were observed. The decrease in oxidation results after the pyruvate dehydrogenase-catalysed step, which is oxidation of acetyl-CoA.

Discussion

The increase in non-esterified fatty acids in the plasma of exhausted rats (Table 1), which may have resulted from enhanced mobilization of adipose-tissue stores, would lead to increased uptake of fatty acids by the tissues of the exhausted animals. The internalized fatty acids are either esterified or oxidized, depending on the physiological needs of the animals. It appears that both processes take place simultaneously in the liver during exhaustive exercise. Evidence of enhanced esterification is reflected in the increase in the concentration of total lipids in the livers of exhausted rats (Table 1).

The increase in the capacity of liver to oxidize fatty acids occurred despite a decrease in mitochondrial yield (Table 2), suggesting that there may have been an overall activation of the process of fatty acid oxidation during the exercise bout. Similar findings of an increase in the oxidative capacity of liver by exercise were reported by Tate *et al.* (1981). Such an activation process is known to occur in starvation and other stressful situations, which are analogous to exercise. It has been reported that liver switches from carbohydrate utilization and fatty acid synthesis to fatty acid oxidation and ketone-body production during starvation or in uncontrolled diabetes (McGarry & Foster, 1980, and references therein). This switch is reportedly hormone-induced and involves changes in which insulin and glucagon concentrations play a major role. Since changes in the concentrations of these hormones with exercise (Fell *et al.*, 1980) resemble those seen with starvation, it is tempting to speculate that the results of increased fatty acid oxidation may be related to hormonal changes. Regardless of the mechanisms involved, however, it appears that the capacity of liver to oxidize fatty acids *in vivo* is enhanced with exercise, as evidenced by the elevation of β-hydroxybutyrate concentration in plasma that we observed (Table 1).

The fact that palmitoylcarnitine oxidation by muscle preparations was depressed in a manner analogous to that of palmitate (Table 2) suggests that the depression in palmitate oxidation is not due to impairment of the process of translocation of the fatty acid from the cytosol into the mitochondrial matrix. Despite the decrease in the oxidative capacity of muscle preparations, the activities of the enzymes of β-oxidation that we measured were either unchanged or elevated (Table 3). It could be argued that the observed depression in oxidation may have resulted in a depression of the catalytic activity of the FAD-linked acyl-CoA dehydrogenase that we did not actually determine. This was ruled out by the fact that oxidation of ketone bodies, where acyl-CoA dehydrogenase is not involved, was also depressed to the same extent as fatty acid oxidation (Table 2). Thus it appears that the decrease in the oxidative capacity of muscle resulting from exhaustion is due not to alterations in the process of β-oxidation but rather to another process.

Because the depression in fatty acid oxidation by muscle preparations of exhausted rats was at variance with what is known to occur during training and what is expected to occur, the need to ascertain the validity of the system *in vitro* was necessary. Thus, in another experiment, we measured fatty acid oxidation in muscle homogenates, teased muscle fibres and cardiac muscle of exhausted and control rats (see the Experimental section). As in previous experiments, fatty acid oxidation was depressed by 42% in the homogenates from exhausted rats, and oxidation of fatty acids by teased muscle fibres was depressed by 47% (21.0 ± 2.6 nmol/min per g dry wt. for controls and 11.2 ± 1.4 nmol/min per g for exhausted rats). On the other hand, oxidation of fatty acids by heart homogenates of controls and exhausted rats was unchanged (172.8 ± 16.5 nmol/min per g of tissue for controls, 173.6 ± 16.4 nmol/min per g for exhausted rats). These results therefore ascertain the reliability of the system *in vitro*, since the same effects of exhaustion were repetitively and consistently observed by different experimental approaches.

The data presented so far suggest that the depression in fatty acid oxidation that results from exhaustion is not caused by aberrations in the processes of β-oxidation. In a preliminary study, we examined

the influence of exhaustion on oxidation of [1-^{14}C]-pyruvate and [2-^{14}C]pyruvate in order to determine if exhaustion influences the tricarboxylic acid cycle. Whereas [1-^{14}C]pyruvate oxidation by muscle homogenates was not significantly influenced by exhaustion, oxidation of [2-^{14}C]pyruvate was depressed by 37% (Table 4). This suggests that the observed decrease in fatty acid oxidation is not due to formation of acetyl-CoA by β-oxidation, but rather may have been a result of aberrations in the oxidation of acetyl-CoA after it enters the tricarboxylic acid cycle. Evidence in support of this conclusion comes from the studies of Dohm *et al.* (1973), who reported that the activities of several enzymes of the tricarboxylic acid cycle were decreased in muscle homogenates of trained rats that were run to exhaustion. However, no changes in the activities of these enzymes were seen in untrained rats that were run to exhaustion. The discrepancy between our results and those of Dohm *et al.* (1973) may be due to the difference in the exhaustion regimen. Our animals were subjected to a much more rigorous exhaustive regimen than that used in the previous work.

The data that we obtained from the studies with the liver preparations show that exhaustive exercise affects fatty acid oxidation in a manner that is comparable with that seen in training, and that the findings *in vitro* correspond to the expected physiological response. In muscle, on the other hand, exhaustive exercise resulted in changes that are at variance with those reported to occur during training, and which are not expected to occur physiologically. One possible explanation for the discrepancy between our findings *in vitro* and the physiological outcome of exhaustive exercise is that, during exercise, certain changes in the membranes of mitochondria might have occurred that alter their physiological characteristics. This hypothesis is supported by two related observations. When mitochondria from exhausted rats are incubated in phosphate buffer for fixation for electron-microscopic studies, they become swollen and enlarged. Furthermore, Dohm *et al.* (1980) showed that lysosomes of muscle of exhausted rats become more fragile, and that the activities of certain lysosomal enzymes are increased in the muscle of exhausted animals. These enzymes conceivably could damage the mitochondria in such a fashion that may render them more susceptible to damage. Thus, on homogenization or incubation with the phosphate buffer that we used in our studies, it is possible that some cofactors that are necessary for maximal oxidation may have leaked out of the mitochondria, thereby decreasing their oxidative capacity. Obviously, resolution of this hypothesis awaits further investigation.

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