ALTERED RESPIRATION AND PROTON PERMEABILITY IN LIVER MITOCHONDRIA FROM GENETICALLY DYSTROPHIC MICE

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SUMMARY: *Liver mitochondria from dystrophic mice respire at lower rates than do those from normal littermates. Defective respiration is associated with relative exclusion of anions from the mitochondrial interior and with greatly diminished permeability to protons. Similar membrane alterations could, in principle, account for the pathology observed in muscle from these animals.*

Strains of mice with congenital muscular dystrophy resembling Duchenne and Erb's dystrophy of humans provide an attractive model for the human disease (1). Examination of muscle from dystrophic animals has revealed a number of alterations associated with the membrane systems of myofibrils including diminished calcium translocation in vesicles derived from dystrophic sarcoplasmic reticulum (2) and increased Na^+-K^+ stimulated ATPase at the sarcolemma (3). The characteristic degeneration of muscle cellular structure renders judgments about membrane function in genetic dystrophy somewhat ambiguous. Therefore, we have commenced to examine membrane systems from other tissues where alterations, if present, may relate to a primary genetic defect, rather than massive degeneration. We have centered our attention on liver mitochondria where relatively large yields of an intact, extensively characterized, membrane system may be obtained.

METHODS: Genetically dystrophic mice, strain 129/ReJ-dy, were obtained from the Jackson Laboratory, Bar Harbor, Maine, and

were maintained with normal littermates under identical conditions. Littermate pairs were killed by decapitation and liver mitochondria were isolated in 0.25M sucrose (4). Oxygen consumption was measured at 25°C. and pH7.4 using a Clark electrode and employing a reaction medium containing 2 mM $MgCl_2$, 250 mM sucrose, 5 mM Tris-phosphate, 50 mM sodium succinate, where present, 400 μM adenosine diphosphate (ADP), and from 2 to 4 mg of mitochondrial protein. Membrane potentials were estimated by measuring the equilibration of ^{14}C-labeled malonate across the mitochondrial membrane and employing the Nernst equation essentially as described by Harris and Pressman (5) for other anions. The reaction mixture contained 35 mM Tris, 140 mM sucrose, 4.3 mM malonate and 7 mg of mitochondrial protein. Mitochondria were separated after a 90-second incubation from the medium by rapid centrifugation through a silicone layer exactly as described by Harris and van Dam (6). The intramitochondrial volume was estimated by measuring the incorporation of ^{3}H-labeled water and was corrected for the sucrose-permeable space (6). Values for membrane potentials obtained in this fashion represent maximum (positive) values as they do not take into account the differing degree of ionization of the weak acid, malonate, on either side of the membrane owing to any pH gradient across it. Addition of uncouplers such as 2,4-dinitrophenol and carbonyl cyanide, p-trifluoromethoxy phenylhydrazone (FCCP) collapsed the measured potential to zero. Finally, similar potential values were obtained by employing other labeled anions, including acetate and succinate.

Proton movement was followed by using a conventional glass electrode and a Radiometer Model 12 pH meter. A suspension of mitochondria in 2 mM Tris-succinate, pH 7.4 and 250 mM sucrose

was rendered anaerobic by sweeping with argon for 20 minutes. Proton movement was initiated by addition of oxygen dissolved in 250 mM sucrose.

RESULTS AND DISCUSSION: Mitochondria isolated from the livers of dystrophic mice respire at a substantially lower rate than that observed with comparable concentrations of mitochondria from normal littermates (Table I). Spectroscopic examination of the two classes of mitochondria has failed to reveal differences in concentration or oxidation state of the respiratory carriers with, for example, 0.31 and 0.22 nmoles per mg of protein for cytochromes ($\underline{c}$ + $\underline{c}_1$) and for the $\underline{b}$ cytochromes, respectively, in both normal and dystrophic mitochondria. Thus, the basis for different rates appears to reside elsewhere. For instance, mitochondrial respiration can be limited in rate by the translocation of oxidizable substrates across the mitochondrial inner membrane (7) or by being opposed by a hydrogen ion gradient produced by respiration-linked proton efflux (8, 9). In the latter instance, respiration appears limited by the rate at which protons flow back into the intramitochondrial space.

The diminished respiratory rate in the dystrophic case is associated both with a barrier to anion entry and with low permeability to hydrogen ion. When the distribution of the ^{14}C-malonate anion between the intra- and extramitochondrial space is measured, it is seen (Table I) that the anion is excluded to a greater degree from the interior of mitochondria from dystrophic animals. Since, in these experiments, the anions are allowed to reach a steady-state distribution, it is clear that the exclusion is not kinetic and reflects an altered charge distribution about the membrane corresponding to a more negative transmembrane potential in the dystrophic case. The values

Table I. Respiration, membrane potentials and proton relaxation times in mitochondria from normal and dystrophic mice. Potentials are expressed with a polarity of inside positive. Corresponding experiment numbers in the normal and dystrophic categories refer to experiments with a littermate pair. Proton relaxation is expressed as the half-time for proton return in experiments identical to those shown in Figure 1.

Mitochondria	Experiment Number	Respiration Rate µatoms/min/mg -ADP	+ADP	Membrane Potential mV	Proton Relaxation Half-time (sec.)
Normal	1	0.036	0.109	+18.6	60
	2	0.036	0.114	+21.7	53
	3	0.035	0.125		68
Dystrophic	1	0.030	0.080	+12.8	1680
	2	0.019	0.101	+13.4	1000
	3	0.022	0.110		>1800

given in the table should be compared with those of about +30 mV obtained by Harris and Pressman (5) for normal rat liver mitochondria.

Examination of permeability to protons reveals a dramatic difference betwen the two classes of mitochondria, a difference that can probably account for the altered membrane potential and respiratory rates observed. Figure I shows that mitochondria

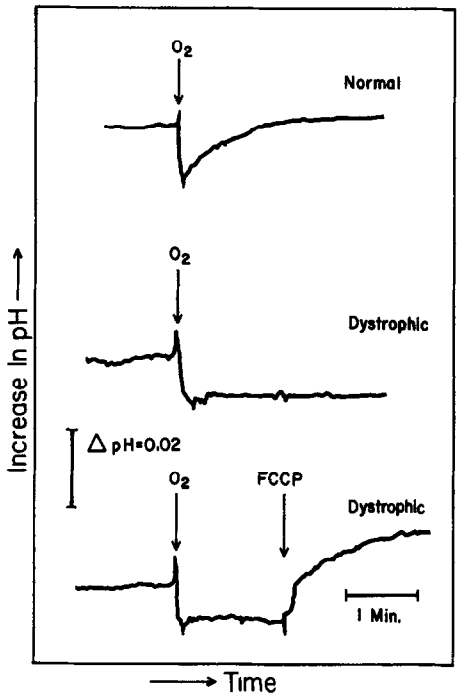

Figure 1. Proton movement in normal and dystrophic mouse liver mitochondria. These are pH tracings obtained in representative experiments when oxygen is added to a suspension of previously anaerobic mitochondria. FCCP denotes addition of 1 μM p-trifluoromethoxy carbonyl cyanide phenylhydrazone. See Table I for examples of half-times for the proton return in such experiments.

from dystrophic animals are virtually impermeable to protons while normal mitochondria exhibit permeability characteristic of mitochondria from a variety of other sources (8, 9). The figure illustrates the respiration-linked extrusion of protons from anaerobic mitochondria which have been presented with a pulse of oxygen, and the subsequent pH relaxation due to the passive permeation of protons back into the mitochondria. The half-time for the relaxation provides an inverse measure of proton permeability and half-times of the order of one minute are characteristic of mitochondrial suspensions from a variety of sources under these conditions (9).

Extremely low proton permeability across membranes of dystrophic mitochondria can account for the observed low rates of respiration since respiration-linked proton efflux is opposed by a larger hydrogen ion gradient. Exclusion of anions from the mitochondrial interior is also a consequence, since a diminished proton leak back into mitochondria renders the inside surface of the membrane less positive. Thus anions traverse a less favorable charge gradient to enter mitochondria isolated from dystrophic animals. In other experiments (not shown) results similar to those illustrated in Figure I have been obtained with a different strain of dystrophic mouse, 129 B6-F_1dy and with a strain of dystrophic hamster, BIO 14.6, obtained from TELACO, Bar Harbor, Maine.

The discovery of a dramatic alteration in liver mitochondrial membranes from animals with muscular dystrophy leads us to suggest that the disease in these animals may reflect a systemic anomaly of cellular membranes. Since liver tissue in these animals appears, by microscopic and various biochemical criteria, to be otherwise normal, it seems that the anomaly does

not in itself render the tissue inviable. A drastic alteration in cation flux across cellular membranes might be expected to be more prejudicial in the case of muscle, owing to the central role of cation movement about the sarcolemma in connection with excitation coupling.

Finally, the availability of mitochondria genetically altered with respect to proton permeability, and membrane potential may prove valuable in exploring the general topic of cellular energy coupling. Thus, the chemiosmotic coupling hypothesis maintains that a combination of the membrane potential and proton gradient provides the driving force for respiration-linked energy conservation including ATP synthesis (8, 9). This hypothesis leads to explicit predictions about energy coupling in mitochondria altered in proton permeability and the consequences of these predictions are presently under study.

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REFERENCES

1. H. Meier and J. L. Southard, Life Sci. 9, 137 (1970).
2. F. A. Streter, N. Ikemoto, and J. Gergeley, Exploratory Concepts in Muscular Dystrophy and Related Disorders, Ed. A. T. Milhorat, Excerpta Medica Foundation, Amsterdam (1967), p. 289.
3. P. V. Sulakhe, M. Fedeldesova, B. McNamara and N. S. Dhalla, Biochem. Biophys. Research Commun., 42, 793 (1971).
4. D. K. Myers and E. C. Slater, Biochem. J. 67, 888 (1957).
5. E. J. Harris and B. C. Pressman, Biochim. Biophys. Acta, 172, 66 (1969).
6. E. J. Harris, K. van Dam, Biochem. J. 106, 759 (1968).
7. K. van Dam, Biochim. Biophys. Acta, 131, 407 (1967)
8. P. Mitchell, Biol. Rev., 41, 445 (1966).
9. P. Mitchell and J. Moyle, Biochem. J., 104, 588 (1967)