as much as 5 to 10 times that of the saline treated dogs.

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Evaluation of Glycolytic and Citric Acid Cycles in Homogenates of Dystrophic Mouse Muscle.* (27821)

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The purpose of this study was to compare the two major metabolic pathways operating in the skeletal muscles of littermate normal and dystrophic mice in the hope of elucidating the underlying chemical defect in this hereditary myopathy. The affected extremity muscles in mice show histologic changes similar to those in human muscular dystrophy(1).

In both human and mouse muscular dystrophy there is abnormal distribution of such glycolytic enzymes as serum aldolase(2,3,4, 5), phosphohexoisomerase(3,4), lactic dehydrogenase(3,5), muscle aldolase(2,3,6), and phosphorylase(2). This information suggested a primary defect in glycolysis and prompted evaluation of the complete cycle. Canal(7) injected fructose into the blood stream of patients with muscular dystrophy and found abnormal retention of lactic and pyruvic acids. This observation fortified the possibility of a deficiency either in glycogenolysis in muscle or glycogenesis in the liver. It could also reflect a disturbance at or below the acetyl Co A level, resulting in a failure to oxidize pyruvic acid to carbon dioxide and water in the citric acid cycle. A failure in the cycle below the acetyl Co A level could conceivably drive the reaction in the direction of excess fat synthesis. Excessive fat is deposited in the muscles of both human beings and animals afflicted with muscular dystrophy.

Our experiments indicate that both the glycolytic cycle and the citric acid cycle, as com-

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plete units, operate efficiently in dystrophic muscle.

Materials and methods. Dystrophic mice, strain #129, obtained from Jackson Memorial Laboratory, and their normal littermates 2-4 months old, were killed by interperitoneal injection of decamethonium bromide (syncurine).† The muscles of all extremities were immediately removed in the cold room (4°C). The tissue was finely ground with isotonic KCl in a motor-driven conical glass tube. Cellular debris and lipids were removed by centrifugation at 23,500 *g* at 4°C for one hour.

The over-all reaction for glycolysis is: glycogen + 3 ADP + 3 $PO_4^{\equiv}$ ⟶ 2 lactate + 3 ATP. The reaction mixture was essentially the same as that described by Stoesz and Lepage(8). 0.025 ml of a 10% homogenate was incubated in a 1:10 dilution of Lepage's reaction mixture for 30 minutes at 37°C. Lactic acid was determined by the method of Barker and Summerson(9).

Citric acid cycle studies were conducted on homogenates of normal and dystrophic mouse muscle prepared in the cold room (4°C) as follows: A 10% homogenate, obtained from 1-2 g of wet tissue prepared for glycolysis, was first centrifuged at 600 *g* for 15 minutes. The supernatant was then centrifuged at 23,500 *g* for one hour, yielding a mitochondrial pellet with microsomes. This pellet was resuspended in approximately 3 ml of 0.25 M sucrose per gram of wet tissue. One ml of mitochondrial suspension was incubated in a medium described by Azzone(10) and respiration was recorded on a Warburg Apparatus.

Results. The data in Table I indicate that the 2 tissues differ very little in their ability to carry on glycolysis. The citric acid cycle was studied by measurements of oxygen consumption recorded at 20-30-minute intervals for 3-4 hours. After appropriate corrections the quantity of oxygen in micromoles, plotted against time, indicated an induction period varying from 20-60 minutes. We attribute this induction period to the time required for the multiple reactions to reach a steady state.

† Syncurine was obtained from Burroughs-Wellcome. It was chosen because it paralyzed skeletal muscle without significantly affecting muscle metabolism.

TABLE I. Comparison of Lactic Acid Produced after Glycolysis.

	mg lactic acid/g wet tissue					
Exp	1	2	3	4	5*	Mean & S.D.
Normal	27.6	36.4	27.9	28.8	33.7	
	29.6	36.4	27.3	28.8	33.0	30.9 ± 3.13
					30.7	
					30.7	
Dystrophic	30.2	39.0	35.7	31.3	31.2	
	35.3	36.6	31.9	28.5	35.0	33.6 ± 3.13
	33.4					

* These determinations were not performed simultaneously.

Table II depicts the micromoles of O_2 consumed per hour while the reaction was proceeding at a steady state. Inasmuch as the residual concentration of O_2 was essentially unchanged at the conclusion of each experiment, it seemed futile to determine reaction rates. Unfortunately, the low oxygen consumption of mitochondrial suspensions precludes final evaluation of the complete cycle.

Discussion. Glycolysis was carried out with fructose-diphosphate for several reasons: 1) Glucose fails as a good substrate because it inhibits the glycolytic cycle(11). 2) One measure of a functioning glycolytic cycle is the ability to generate DPNH. Glycolysis, as measured by lactic acid production, seemed unimpaired in the homogenates of dystrophic mouse muscle. Dreyfus(3) found that enzymatic ability to convert glycogen to lactic acid is reduced in human dystrophic muscle. However, we cannot compare his results with ours, inasmuch as we used diphosphate sugar

TABLE II. Comparative Respiration.

μmoles of O_2 consumed/g wet tissue/hr			
Normal*		Dystrophic*	
1.10	1.08	1.28	1.26
1.27	.97	.88	1.80
1.54	1.58	2.10	2.17
1.42	.82	2.55	2.18
1.92	2.13	2.00	1.68
2.01	1.98	1.20	1.90
1.06	1.28	2.48	2.22
1.08	1.24	1.51	
2.46	1.92		
1.49 ± .44†		1.82 ± .54†	

* Each value is derived from a unique run.
† Mean and S.D.

instead of glycogen for reasons given above.

Preliminary studies on the efficiency of the glycolytic cycle in the livers of normal and dystrophic mice fail to suggest a defect.

The activity of a number of enzymes of normal and dystrophic human muscle, including some operative in the glycolytic and citric acid cycles, has been appraised by histochemical technics. No significant differences were reported(12).

Suspensions of normal and dystrophic muscle mitochondria appear to respire at about the same rate. The intactness of the cycle has also been verified by Baker(13), who found the mean value of carbon dioxide production per gram of body weight equal in normal and dystrophic mice. This suggests that both oxygen consumption and carbon dioxide output are proceeding in an orderly fashion in all cycles tested. The respiratory rate is slow in both normal and abnormal muscle homogenates.

Preliminary experiments with liver homogenates of normal and dystrophic mice also fail to suggest significant differences in the respiratory rates.

Summary. The intactness of the glycolytic cycle was evaluated in homogenates of dystrophic mouse muscle, and contrasted with their normal littermates. The cycle, as measured by lactic acid production, appeared to operate efficiently. Similarly, the citric acid cycle was tested by measuring O_2 consumption of mitochondrial and microsomal suspensions derived from normal and abnormal mouse muscle. This cycle also seemed intact. Preliminary experiments with liver homogenates suggested that both cycles were unimpaired in dystrophic mice.

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Inductive Factors in Gliosis.* (27822)

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Recent work utilizing morphologic histochemical methods has indicated that a marked and sustained increase in activity of a number of oxidative enzymes occurs in reactive glia(1,2,3). There is a resemblance between this phenomena and that of adaptive enzyme synthesis in bacteria(4), as well as inductive enzyme synthesis in mammalian liver(5,6). Accordingly, a search for a possible inducing factor or factors responsible for this increase in enzyme activity as well as the reactive gliosis itself would appear warranted. Two questions which immediately arise are: (1) does

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