If time factors are considered, transketolase levels are related to thiamine content in the presence of a normal liver. In contrast, there is no correlation between enzyme and vitamin levels in the presence of experimental liver necrosis. This is due to the fact hepatic injury accentuates the disparity between rate of reduction of thiamine and transketolase levels during vitamin depletion and impedes or prevents the restoration of tissue thiamine during replacement therapy. Interference with conversion of free thiamine to its metabolically active coenzyme and to synthesis of its apoenzyme is contributory since, in the presence of active hepatic necroses, there is incomplete response to both *in vitro* and *in vivo* addition of thiamine pyrophosphate.

Summary. Transketolase activity in rat liver and red blood cells is decreased at a significantly slower rate than circulating or tissue thiamine in dietary-induced thiamine deficiency; superimposition of carbon tetrachloride-induced liver necrosis on thiamine deficiency increases the disparity between alterations of vitamin and enzyme levels. Replacement therapy in thiamine deficient animals with a normal liver only slowly restores tissue thiamine and transketolase; in the presence of active liver necrosis low tissue vitamin and enzyme levels persist despite administration of thiamine.

1. Wolfe, S. J., Brin, M., Davidson, C. S., *J. Clin. Invest.*, 1958, v37, 1476.

2. Fennelly, J., Baker, H., Frank, O., Leevy, C. M., *Clin. Research*, 1963, v11, 182.

3. Brin, M., *Ann. N. Y. Acad. Sci.*, 1962, v98, 528.

4. Baker, H., Frank, O., Fennelly, J., Leevy, C. M., *Am. J. Clin. Nutr.*, 1964, v14, 197.

5. Steyermark, A., *Quantitative Organic Microanalysis*, Steyermark, A., ed., Academic Press, 1961, 2 ed., p151.

6. Leevy, C. M., George, W., Deysine, M., Gnassi, A. M., *Exp. and Molecular Path.*, 1962, v1, 457.

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Myosin from Mice with Hereditary Muscular Dystrophy.* (29396)

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Myosin, one of the principal contractile proteins in muscle, is considerably reduced in quantity in various forms of muscular dystrophy and atrophy(1-5), but little is known about any qualitative changes of these proteins and their possible relation to the wasting process. This paper deals with the properties of myosin from mice with hereditary muscular dystrophy(6), particularly in terms of its macromolecular structure in solution.

Materials and methods. Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, supplied dystrophic mice and control litter mates of strain 129. They were sacrificed when 2-3 months old. Myosin was extracted repeatedly from their hind legs by the procedure of Hasselbach and Schneider(7), and it was freed of actomyosin and other impurities by at least 2 cycles of fractional precipitation. Sarcoplasmic proteins were obtained by extraction with 0.1 μ K-phosphate buffer, pH 7.5, in the cold for 2 hours. They were further fractionated in an LKB paper electrophoresis instrument(8) in 0.1 μ K-phosphate buffer, pH 6.4, at 1°-2°C for 16 hours at 5 volts/cm. Quantitative determination of muscle protein fractions (sarcoplasm, myosin, actin, and stroma), which were obtained by differential extraction(7), was done by Nesslerization(9) or the Biuret method (10). Actin was prepared from rabbit skeletal muscle by the procedure of Mommaerts (11). ATPase activity of myosin was assayed at 25°C for 3 minutes in media containing 0.25 mg myosin/ml, 30 mM KCl, 20 mM histidine buffer, pH 7.0, and either 10 mM Ca Cl_2, 5 mM ATP or 1 mM Mg SO_4, 1mM ATP. The latter medium was also used to assay activation of myosin ATPase

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TABLE I. Fractionation of Muscle Proteins from Mice.

	mg protein N/g muscle wet wt		
	Control	Dystrophic	P
Sarcoplasm	7.5 ± 1.0	7.5 ± 1.5	
Myosin	9.4 ± 2.0	4.9 ± 1.2	<.001
Actin	7.0 ± 1.0	7.5 ± .9	
Stroma	5.6 ± .9	6.5 ± .9	<.05

by F-actin (0.025 mg/ml). The inorganic phosphate liberated was determined by the method of Rockstein and Herron(12). The ability of myosin to combine with F-actin was tested in a medium containing 1.5 mg myosin/ml, 0.5 mg F-actin/ml, 0.5 M KCl, pH 7.0, at 25°C. It is expressed according to Weber and Portzehl(13) as ATP sensitivity. The physical-chemical studies of myosin were done in 0.4-0.6 M KCl, 0.05 M K-phosphate buffer, pH 6.8. The viscosity number(14) was determined at 0.15°C in a Cannon-Fenske viscometer. Sedimentation velocity experiments were performed in the Spinco Model E analytical ultracentrifuge. Light scattering was measured in a Brice-Phoenix photometer(15) at 436 mμ over an angular range from 26°-135° after the solutions had been centrifuged in the preparative ultracentrifuge at 30,000 rpm for 3-4 hours and then pipetted off from the tubes in the rotor with a special pipetting assembly(16). The weight-average molecular weight and the z-average radius of gyration of the myosin molecules were derived from the Zimm plot of the data(17). A value of 0.208 ml/g for the refractive index increment was taken from the literature for rabbit myosin(18). Statistical significance of the data was evaluated by Student's "t" test(19).

Results. Table I shows distribution of protein nitrogen among various muscle fractions. The decrease of myosin to about half the normal value was statistically very significant ($P<0.001$). The amounts of sarcoplasmic proteins and actin were alike in 2 groups. Protein in the insoluble residue after exhaustive extraction (largely collagen) increased somewhat ($P<0.05$) in the dystrophic group. Further fractionation of the sarcoplasmic proteins by paper electrophoresis yielded 5 bands. The relative concentrations for corresponding fractions were similar in the 2 groups, except for the fastest component, presumably myoalbumin, which, in the dystrophic group, was significantly increased from 3.2 ± 1.6% to 6.9 ± 2.8% ($P<0.01$).

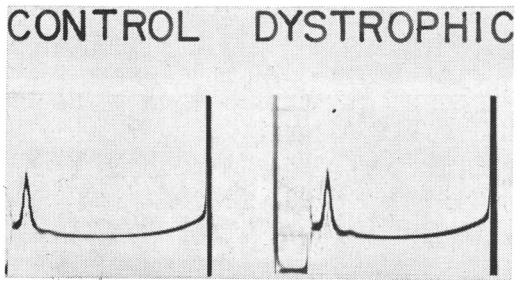
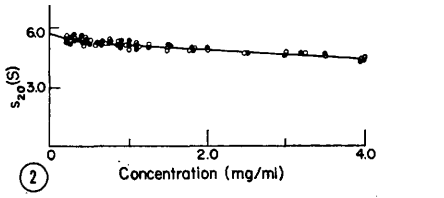


FIG. 1. Ultracentrifugal patterns of myosin from control and dystrophic mice.



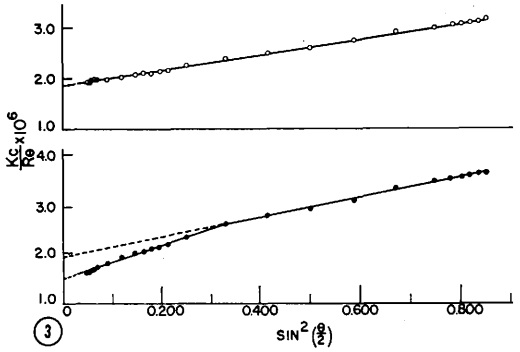


FIG. 2. Concentration dependence of sedimentation coefficients ($s_{20}(S)$) of myosin from control (○) and dystrophic (●) mice.

FIG. 3. Zimm plot of light scattering intensities of myosin from control (○, 0.57 mg/ml) and dystrophic (●, 0.53 mg/ml) mice.

The ultracentrifuge patterns of myosin from both groups of mice were very much alike (Fig. 1). The peak appeared homogeneous, but there was always a small amount (less than 5% of the total) of another, faster sedimenting, component. Fig. 2 shows the variation of sedimentation coefficient with concentration; there was no difference between the two myosins. The extrapolation to zero concentration yielded a sedimentation constant of 5.8 S.

TABLE II. Light Scattering of Mouse Myosin.

	Molecular wt $\times 10^{-3}$	Radius of gyration, Å	Length of rod, Å	Diameter, Å
Control	510 ±40	448 ±23	1550 ± 80	27.6 ±1.4
Dystrophic	567 ±90, 770 ±146	448 ±45, 705 ±125	1550 ±160, 2440 ±440	29.0 ±2.2, 27.0 ±2.1

Fig. 3 illustrates a Zimm plot of the light scattering intensities of myosin of about the same concentration in the 2 groups. The curves for the controls usually were close to linear over a wide angular range and thus could readily be extrapolated to zero angle. Those for the dystrophic mice, however, consisted of 2 segments of different slopes both of which were extrapolated to zero angle for calculation of the molecular parameters. On the assumption of a rod-like shape, as with rabbit myosin(18), the length and diameter of the myosin molecules were calculated from the molecular weight and radius of gyration. Table II presents mean values with their standard deviations of the light scattering results for 11 experiments in each group. Both the molecular weight and radius of gyration were significantly increased ($P<0.001$) when the extrapolation was done from the lower part of the angular range (70°-26°). Their standard deviations indicate a much wider range of values than for the controls. The extrapolation from the upper part of the angular range (135°-70°), however, yielded values similar to those for the controls. Thus the myosin from dystrophic mice appeared to be more polydisperse. It seemed to consist largely of a component of the same weight and size as myosin from controls, but also of heavier and larger particles. Both myosins had a viscosity number of 0.17 ml/mg; thus the overall shape of the molecules was not changed in the dystrophic process.

Table III shows that the characteristic properties of myosin, *i.e.*, its ATPase activity and its ability to combine with actin (ATP sensitivity), were not affected by the disease.

Discussion. The pronounced loss of myosin and, at the same time, the relative increase of myoalbumin in dystrophic muscle may perhaps be related to an interesting study by Kasavina *et al*(20). These authors found that in rabbits the amount of myoalbumin was highest in the embryonic state and then gradually decreased as the animals matured. Myosin or actomyosin, however, showed the opposite trend. Thus the dystrophic muscle may still be in an embryonic state or may have returned to it. Similar conclusions have recently been suggested by findings on lactic dehydrogenase isozymes (21) and myoglobin(22) in clinical myopathies.

By the Svedberg equation(23) an approximate molecular weight of 500,000 for myosin was calculated from the observed sedimentation constant and from the literature values (24) of the diffusion constant (1.05×10^{-7} cm^2/sec) and partial specific volume (0.728 ml/g) for rabbit myosin; it was assumed that the latter two are about the same for mouse myosin. This calculated value agreed well with that found by light scattering for the controls. The small amount of the faster

TABLE III. Characteristic Properties of Mouse Myosin.

		ATPase activity, μmole P_i/mg myosin/min			Actin binding ability (ATP sensitivity)
		In presence of			
Exp No.		Ca^{++}	Mg^{++}	Mg^{++}, actin	Mg^{++}
1	Control	1.96	.03	.58	120
	Dystrophic	2.09	.03	.54	125
2	Control	1.35	.02	.39	113
	Dystrophic	1.46	.03	.47	110
3	Control	1.78	.03	.59	131
	Dystrophic	1.69	.02	.42	117

sedimenting component most likely represented aggregated myosin in view of the instability of this protein and its tendency to aggregate.

The light scattering results for myosin from control mice were very similar to those reported for myosin from rabbits(18). This method seemed to indicate polydispersity of myosin from dystrophic mice. Since it is very sensitive to the presence of heavier and larger particles, a small increase of such particles, although not sufficiently resolved in the ultracentrifuge, could account for the observed shape of the light scattering curves. Another possibility would be that myosin from dystrophic mice contained a special kind of aggregate of a somewhat loose structure which would cause them to sediment at the same rate as the bulk of myosin. In contrast to our studies, Horvath(3) noted polydispersity in the ultracentrifugal patterns of myosin from mice, both control and dystrophic, but he prepared myosin by a different procedure.

Summary. The molecular weight and size of myosin from mice with hereditary muscular dystrophy and from their normal littermates as controls were studied by various physical-chemical methods. The ultracentrifugal patterns were similar for the two kinds of myosin, but light scattering seemed to indicate polydispersity of their molecular parameters for dystrophic mice. The ATPase enzymatic activities and the ability to combine with F-actin were not affected by the disease.

1. Bonetti, E., Aloisi, M., Merucci, P., *Experientia,* 1952, v8, 69.

2. Vignos, P. J., Jr., Lefkowitz, M., *J. Clin. Invest.,* 1959, v38, 873.

3. Horvath, B., *Neurology,* 1958, v8, suppl. 1, 52.

4. Oppenheimer, H., Terry, H. R., Forsyth, E., Milhorat, A. T., *Fed. Proc.,* 1959, v18, 116.

5. Fischer, E., Ramsey, V. W., *Am. J. Physiol.,* 1945, v145, 571.

6. Michelson, A. M., Russell, E. S., Harman, P. J., *Proc. Nat. Acad. Sci.,* 1955, v41, 1079.

7. Hasselbach, W., Schneider, G., *Biochem. Z.,* 1951, v321, 462.

8. Valmet, E., Svensson, H., *Science Tools,* 1954, v1, 3.

9. Koch, F. C., McMeekin, T. L., *J. Am. Chem. Soc.,* 1924, v46, 2066.

10. Gornall, A. G., Bardawill, Ch. J., David, M. M., *J. Biol. Chem.,* 1949, v177, 751.

11. Mommaerts, W. F. H. M., *ibid.,* 1952, v198, 445.

12. Rockstein, M., Herron, P. W., *Anal. Chem.,* 1951, v23, 1500.

13. Weber, H. H., Portzehl, H., *Advances in Protein Chemistry,* v7, Anson, M. L., ed., Academic Press, Inc., New York, 1952, 161.

14. Portzehl, H., Schramm, G., Weber, H. H., *Z. Naturforsch.,* 1950, v5b, 61.

15. Brice, B. A., Halwer, M., Speiser, R., *J. Opt. Soc. Am.,* 1950, v40, 768.

16. Schneider, N., *J. Polym. Sci.,* 1956, v32, 255.

17. Zimm, B., *J. Chem. Phys.,* 1948, v16, 1099.

18. Holtzer, A., Lowey, S., *J. Am. Chem. Soc.,* 1959, v81, 1370.

19. Youden, W. J., *Statistical Methods for Chemists,* J. Wiley & Sons, Inc., New York, 1951, 24.

20. Kasavina, B. S., Torchinskii, Y. M., *Biokhimiya,* 1956, v21, 510.

21. Wieme, R. J., Herpol, J. E., *Nature,* 1962, v194 287.

22. Perkoff, G. T., *New Engl. J. Med.,* 1964, v270, 263.

23. Svedberg, T., Pedersen, K. O., *The Ultracentrifuge,* Oxford Univ. Press, 1940.

24. Parrish, R., Mommaerts, W. F. H. M., *J. Biol. Chem.,* 1954, v209, 901.

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