grouped mice averaged 1556. It is concluded that behavior may affect resistance and it is suggested that this phenomenon may be an important factor in epidemics and in development of host-parasite relations.

1. Christian, J. J., *Ecol.*, 1956, v37, 258.
2. ———, 1959, in press.
3. Christian, J. J., Davis, D. E., *J. Mamm.*, 1956, v37, 475.
4. Coker, C. M., *J. Inf. Dis.*, 1956, v98, 187.
5. ———, *J. Parasitol.*, 1956, v42, 479.
6. Davis, D. E., Christian, J. J., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 728.
7. Gould, S. E., *Trichinosis*, Chas. C. Thomas, Springfield, 1945, p356.
8. Larsh, J. E., Jr., Kent, D. E., *J. Parasitol.*, 1949, v35, 45.
9. Larsh, J. E., Jr., Race, G. J., *J. Inf. Dis.*, 1954, v94, 262.
10. Markell, Edward K., *ibid.*, 1958, v102, 158.
11. Read, C. P., *Rice Institute Pamphl.*, 1958, v45, 36.
12. Stoner, R. D., Godwin, J. T., *Am. J. Path.*, 1954, v30, 913.
13. Weinstein, P. P., *Am. J. Trop. Med. Hyg.*, 1955, v4, 61.

Received July 25, 1958. P.S.E.B.M., 1958, v99.

Enzyme Studies in Muscular Dystrophy. III. In Hereditary Muscular Dystrophy in Mice.*† (24320)

I. M. WEINSTOCK,‡ SANDRA EPSTEIN AND A. T. MILHORAT
Depart. of Medicine, Cornell University Medical College, Russell Sage Institute of Pathology, and N. Y. Hospital, N. Y. City

Michelson, Russell and Harman(1) reported on spontaneous occurrence of a genetic mutation resulting in muscular dystrophy in a highly inbred strain of mice. The muscular areas involved, the nature of the lesions in these muscles, and lack of involvement of the nervous system, are similar to those seen in muscular dystrophy in man. By these criteria, this form of muscular dystrophy in the mouse would appear to be related more closely to muscular dystrophy in patients than are the experimentally induced types of muscle wasting. To characterize further this myopathy in mice, and if possible, to find some relationship to other forms of muscle wasting, we have investigated a number of enzyme systems in the muscles. Our results will be discussed in relation to those of comparable studies in muscular wasting of Vit. E-deficiency and of neurotomy, and that occurring in clinical forms of muscular dystrophy in man.

Methods and materials. Mice of strain 129 (dystrophic animals and normal litter mates) obtained from the Roscoe B. Jackson Memorial Laboratory were given a commercial mouse pellet diet. (Purina Laboratory Chow). The animals were allowed free access to food and water. According to the original investigators(1), symptoms of muscular dystrophy are evident by the third week of life, and dystrophic mice survive from one to 6 months, or occasionally longer. In these investigations, age of the dystrophic mice averaged 68 days (46-110), and weight averaged 14.1 g (9.7-19.0), when they were sacrificed. The control group consisting of heterozygous litter mates of the dystrophic mice, averaged 63 days (47-116) of age, and 21.4 g (16.4-25.4) in weight when they were studied. Dystrophic animals were smaller in size and lighter in weight than controls, and exhibited various degrees of ataxia, atrophy, and paralysis. However, there was no close correlation between age or weight of the animals, within the ranges observed, and gross symptoms of muscular dystrophy.

* Aided by grants from Muscular Dystrophy Associations.

† A preliminary report was presented before Fed. Am. Soc. Exp. Biol., March, 1957.

‡ Present address: Dept. of Physical Medicine and Rehabilitation, N. Y. Medical College, and Bird S. Coler Hospital, N. Y. City.

TABLE I. Effect of Muscular Dystrophy on Composition of Mouse Muscle.

Types and No. of animals	Content/g wet wt*				$\frac{CN}{TN}$§ × 100,
	Total N_2	Protein† N_2	Collagen‡ N_2	Dry wt	
	mg				%
Controls (14)	20.3 ± 2.3‖	18.9 ± 2.0	1.5 ± .5	165 ± 21	7.4
Dystrophic (12)	17.3 ± 1.9	16.3 ± 1.8	1.9 ± .2	156 ± 16	11.0
% change	−15	−14	+27	−5.5	
P¶	<.01	<.01	<.02	<.3	

* Tissues were kept saturated with water at 0°C, while visible fat and connective tissue were removed. Small pieces and strips of muscle tissue were blotted before weighing, but appreciable water still adhered to tissue surfaces. Dry wt determinations indicate reproducibility of technic.

† Includes collagen nitrogen.

‡ Collagen was determined directly by analysis of alkali insoluble residue.

§ $\frac{CN}{TN} = \frac{\text{Collagen nitrogen}}{\text{Total nitrogen}}$. ‖ Stand. dev. ¶ P = Level of significance.

The mice were sacrificed by decapitation. Muscle from the entire hind quarters, after removal of visible fat and connective tissue, was minced, then homogenized in water in a glass Potter-Elvehjem apparatus. Aliquots of the homogenate were diluted with water and used for assays of enzyme activity. Samples of the homogenate were dried in an oven at 105-110°C for 18-20 hours for dry weight determinations. Collagen was determined by a slight modification of the method of Lilienthal *et al.*(2), and nitrogen was estimated by direct nesslerization after digestion.

Aldolase was determined in the system of Sibley and Lehninger(3), with some modifications as suggested by Dounce *et al.*(4). Triose phosphate was measured as the difference in amounts of inorganic phosphate, before and after alkaline hydrolysis(5). Inorganic phosphate was determined by the Lowery-Lopez method(6) modified by Potter(7). Two different levels of enzyme concentration, in duplicate, were used in each determination of aldolase activity, and the results were averaged. Succinoxidase and cytochrome oxidase activities were determined by the methods of Schneider and Potter(8,9). Oxygen consumption was linear for at least 30-40 minutes, and average values of the linear portion of the curve were used to calculate oxygen consumption per hour. Cytochrome oxidase activity was determined at 3 levels of enzyme concentration, and the results were extrapolated and corrected for zero enzyme concentration(9). Cathepsin activity was determined by a modified Anson procedure(10) previously described(11). The substrate was hemoglobin at pH 4.0, and proteolysis, in the absence of added activators, was measured by increase in optical density at 280 mμ.

Results. Because of the changes in composition of muscle associated with most wasting conditions, a proper reference base for enzyme activity is critical. Concomitantly with these enzyme studies, we determined dry weight, and total-, protein-, and collagen-nitrogen of the muscle. Results of the enzyme assays are reported on the basis of wet weight, dry weight, protein-nitrogen content and non-collagen protein nitrogen content of the muscle. Probably the most accurate index of functioning muscle mass upon which to base enzyme activity is non-collagen protein nitrogen content(2,12). An apparent decrease in enzyme activity, unless based on content of non-collagen protein, might be nonspecific and only a reflection of over-all breakdown of muscle. The effects of muscular dystrophy on composition of the muscle of these mice are shown in Table I. There was a significant 15% decrease in total nitrogen and protein nitrogen content of the muscle when estimated on a wet weight basis, although dry weight of the muscle was unchanged. Collagen nitrogen increased as much as 27%, but accounted for only 11% of the total nitrogen because of the relatively small amounts present initially.

Data on aldolase activity are shown in Table II. In dystrophic muscle, aldolase activity is decreased when estimated on the basis of either wet or dry weight of muscle. How-

TABLE II. Effect of Muscular Dystrophy on Aldolase Activity of Mouse Muscle.

Types and No. of animals	μmoles fructose-diphosphate split/hr			
	Per g wet wt	Per mg dry wt	Per mg PN*	Per mg NCPN†
Controls (12)	1390 ± 350	8.4 ± 1.6	72 ± 14	79 ± 16
Dystrophic (11)	1090 ± 330	7.0 ± 1.9	68 ± 23	76 ± 25
% change	−22	−17	−6	−4
P	<.05	<.05	<.6	<.7

* PN = Protein nitrogen. † NCPN = Non-collagen protein nitrogen.

ever, when based on content of either protein nitrogen or non-collagen protein nitrogen in the muscle, aldolase activity in dystrophic muscle is not significantly different from that in the normal controls. Succinoxidase and cytochrome oxidase activities of the muscles are shown in Table III. There was no significant difference between succinoxidase activities of control and dystrophic groups, regardless of which reference base was used for estimating enzyme activity. On the other hand, cytochrome oxidase activity was considerably increased. When referred to either wet or dry weight of the muscle, it was increased about 35%, and when this activity was based on non-collagen protein nitrogen content of the muscle, the increase was about 50%, since non-collagen protein nitrogen content of the muscle decreased during the course of dystrophy. Cathepsin activity of muscle of dystrophic mice was increased over the levels of the litter mate controls (Table IV). This increase in cathepsin activity was significant when based on any of the employed standards of muscle mass, and, as in the case of cytochrome oxidase activity, the increase was greatest when based on non-collagen protein nitrogen content of the muscle.

Although the activities of both cytochrome oxidase and cathepsin in dystrophic muscle were elevated, there was no obvious correlation between these changes, and age or weight of the dystrophic mice, within the ranges used in this investigation. However, there did seem to be some general relation between increases in enzyme activity and gross symptoms of muscular dystrophy. Cytochrome oxidase and cathepsin activities in the muscles of 2 homozygous normal control mice were of the same order as those in the heterozygous normal control group. Concurrently, with the studies reported here, we similarly assayed and found no change in the activities of these enzymes in liver tissue.

Discussion. The changes in protein content of muscles of the dystrophic mouse observed (Table I) were similar to those reported in 3 other types of muscle wasting, *i.e.*, wasting that occurs after Vit. E-deficiency (13,14), atrophy that results from neurotomy (12), and degeneration of muscles that represents the main pathologic alteration in muscular dystrophy in patients(14,15). Although in these forms of muscle wasting, increase in collagen content of the muscle is quantitatively greater, the general result of all forms of wasting is a reduction in non-collagen protein nitrogen content of the muscle.

Since aldolase activity of dystrophic muscle appears decreased only when based on wet or

TABLE III. Effect of Muscular Dystrophy on Succinoxidase and Cytochrome Oxidase Activities of Mouse Muscle.

Types and No. of animals	Succinoxidase activity, mm^3 O_2/hr				Cytochrome oxidase activity, mm^3 O_2/hr			
	Per g wet wt	Per mg dry wt	Per mg PN	Per mg NCPN	Per g wet wt	Per mg dry wt	Per mg PN	Per mg NCPN
Controls (9)	7400 ± 1200	45 ± 9	380 ± 79	424 ± 84	18,300 ± 4200	116 ± 35	990 ± 260	1080 ± 290
Dystrophic (7)	7100 ± 1100	46 ± 9	415 ± 81	470 ± 92	24,900 ± 3700	159 ± 20	1460 ± 250	1660 ± 280
% change	−4	+2	+5	+11	+36	+37	+47	+54
P	<.6	<.8	<.3	<.2	<.01	<.01	<.01	<.01

TABLE IV. Effect of Muscular Dystrophy on Cathepsin Activity of Mouse Muscle.

Types and No. of animals	Cathepsin activity*			
	Per g wet wt	Per mg dry wt	Per mg PN	Per mg NCPN
Controls (8)	10.7 ± 2.7	.066 ± .017	.58 ± .13	.62 ± .14
Dystrophic (6)	17.7 ± 4.6	.110 ± .037	1.02 ± .26	1.16 ± .31
% change	+65	+67	+76	+87
P	<.01	<.01	<.01	<.01

* Cathepsin activity = Change in optical density at 280 mμ/ml of incubation mixture/hr.

dry weight of muscle, and is of normal value when estimated on the protein- or non-collagen protein nitrogen, it is probable that any changes noted are only a reflection of over-all reduction in functioning muscle mass. Serum aldolase of dystrophic mice has been found to be increased(16). A similar relationship, between blood serum and muscle enzyme activities, has been reported for transaminase in patients with muscular dystrophy; *i.e.*, serum transaminase activity was increased, while muscle transaminase levels, when referred to non-collagen protein nitrogen, were unchanged (17). Increase in activity of an enzyme in blood serum does not necessarily indicate that the metabolic lesion specifically involves that particular enzyme, since the increase may be only a reflection of tissue destruction. Phosphorylase activity of muscle of dystrophic mice has been investigated by Leonard(18), who found approximately a 30% decrease in activity of dystrophic thigh muscle, when based on wet weight of tissue. Data on non-collagen protein nitrogen content of the muscles were not reported. Reduction in activity of both aldolase and phosphorylase in the muscle of the dystrophic mouse is much less than has been observed in patients with muscular dystrophy. The muscles of these patients may show reductions in aldolase and phosphorylase activities to one-third their normal values, when based on non-collagen protein nitrogen content of the muscle(17).

Although succinoxidase activity of dystrophic mouse muscle tends to show a slight increase on the basis of non-collagen nitrogen content, this difference is not significant, and one must conclude that succinoxidase activity is essentially unchanged. On the other hand, cytochrome oxidase activity is significantly increased in the muscle of the dystrophic mouse. However, none of the respiratory or glycolytic enzymes investigated in this laboratory (succinoxidase, cytochrome oxidase, and aldolase), or other representative respiratory or glycolytic enzymes observed by others, show a pattern of change that is similar in the 4 types of muscle wasting mentioned here. Alterations in activities of respiratory and glycolytic enzyme systems of muscle, associated with various muscle wasting conditions, have been reported(12-15,17,19,20). A possible, though tentative conclusion, that may be drawn from available evidence, is: in clinical muscular dystrophy and in muscle atrophy due to neurotomy, glycolytic enzyme systems are generally decreased, and respiratory enzymes are not primarily affected; in muscular dystrophy resulting from Vit. E-deficiency, and in the hereditary muscle dystrophy in mice, respiratory activity tends to be increased, and glycolytic enzymes are relatively unchanged. This conclusion is based on experimental observations that often are not definitive, and, occasionally are conflicting. It is perhaps equally likely that the changes observed in enzyme activity are a reflection of specifically different causative mechanisms of muscle wasting, or are merely secondary effects of the atrophy.

However, in all of the 3 forms of muscle wasting in which it has been investigated, cathepsin activity has been found to be increased. Moreover, of all the enzyme systems investigated, cathepsin alone shows a common pattern of change. In the present studies, cathepsin activity was increased nearly 2-fold in dystrophic mouse muscle. Similar increments have been observed in the muscle of rabbits with muscular dystrophy due to Vit. E-deficiency(11), and in muscle following neurotomy(21,22). Although the role of the catheptic enzymes, *in vivo*, has not yet been clearly established, they may be in-

volved in protein catabolism, and may be the common mechanism in muscle wasting due to a number of different causes. It is of interest that cathepsin activity, *in vitro*, has been reported to correlate with gross changes in protein metabolism and mass in regressing tumors(23), and with certain hormonal influences(24). Investigation of this enzyme system in the muscles of patients with muscular dystrophy is planned.

Summary. Changes in muscle composition, and activity of 4 enzyme systems in mice with *dystrophia muscularis*, have been investigated. Total nitrogen and protein nitrogen contents of dystrophic muscle were reduced as compared to controls, while collagen nitrogen was increased. Percentage dry weight was unchanged. Aldolase activity of dystrophic muscle was decreased on the basis of wet or dry weight, but was unchanged on the basis of protein- or non-collagen protein nitrogen content of muscle. Succinoxidase activity was not significantly affected by muscle dystrophy. Cytochrome oxidase and cathepsin activities were markedly increased in the muscle of dystrophic mice as compared to controls. Liver tissue from these mice showed no change as a result of the dystrophy. These results are compared to those observed in other forms of muscle wasting.

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

2. Lilienthal, J. L., Jr., Zierler, K. L., Falk, B. P., Buka, R., Riley, M. J., *J. Biol. Chem.*, 1950, v182, 501.

3. Sibley, J. A., Lehninger, A. L., *ibid.*, 1949, v177, 859.

4. Dounce, A. L., Barnett, S. R., Beyer, G. T., *ibid.*, 1950, v185, 769.

5. Herbert, C., Gordon, H., Subrahmanyan, V., Green, D. E., *Biochem. J.*, 1940, v34, 1108.

6. Lowery, O. H., Lopez, J. A., *J. Biol. Chem.*, 1946, v162, 421.

7. Potter, V. R., *ibid.*, 1947, v169, 17.

8. Schneider, W. C., Potter, V. R., *ibid.*, 1943, v149, 217.

9. Potter, V. R., in Umbreit, W. W., Burris, R. H., Stauffer, J. F., *Manometric Techniques and Tissue Metabolism*, Burgess Publishing Co., Minneapolis, 1949, p139.

10. Anson, M. L., *J. Gen. Physiol.*, 1938, v22, 79.

11. Weinstock, I. M., Goldrich, A. D., Milhorat, A. T., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 257.

12. Fischer, E., *Am. J. Phys. Med.*, 1955, v34, 212.

13. Mackenzie, C. G., in Herriott, R. M., *Symposium on Nutrition*, The Johns Hopkins Press, Baltimore, 1953, p136.

14. Schapira, G., Dreyfus, J. C., *Exposés annuels biochim. méd.*, 1955, v17, 251.

15. Dreyfus, J. C., Schapira, G., Schapira, F., *J. Clin. Invest.*, 1954, v33, 794.

16. Schapira, F. S., Schapira, G., Dreyfus, J. C., *Compt. rend.*, 1957, v245, 754.

17. Dreyfus, J. C., Schapira, G., Schapira, F., Demos, J., *Clin. Chim. Acta*, 1956, v1, 434.

18. Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 720.

19. Sheves, G. S., Rumina, V. I., *Biokhimiya*, 1956, v21, 389.

20. Aronson, S. M., Valk, B. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 360.

21. Sheves, G. S., Epelbaum, E., Rumina, V. I., *Biokhimiya*, 1956, v21, 71.

22. Chen, K. K., Meek, W., Bradley, H. C., *J. Biol. Chem.*, 1924, v61, 807.

23. Fodor, P. J., Funk, C., Tomashefsky, P., *Arch. Biochem. and Biophys.*, 1955, v56, 281.

24. Mathies, J. C., Palm, L., Gaebler, O. H., *Endocrinology*, 1951, v49, 571.

Received July 25, 1958. P.S.E.B.M., 1958, v99.