Phosphoglucomutase Activity in Hereditary Muscular Dystrophy in Mice.* (25375)

WILLIAM R. HAZZARD† AND SAMUEL L. LEONARD
Dept. of Zoology, Cornell University, Ithaca, N. Y.

Activity of phosphoglucomutase, determined in biopsy samples of skeletal muscles of man with progressive muscular dystrophy, is markedly lower than normal when measured without addition of the coenzyme (glucose 1, 6 diphosphate) to the system(1). Bodansky (2) refined the assay method of Najjar(3), adding coenzyme in varying amounts to eliminate distortions in measured phosphoglucomutase activity due to residual coenzyme in the tissue. The present study concerns determination of mutase activity, both with and without added coenzyme, in leg muscles of mice with hereditary muscular dystrophy, whose myopathy is suggestive of progressive muscular dystrophy in man(4).

Methods and materials. Pairs of dystrophic and control mice of same age and sex were obtained from Roscoe B. Jackson Memorial Laboratory. Three pairs were 51-56 days old while 6 other pairs were 86-106 days old when sacrificed. Leg muscles were excised from nembutal-anesthetized animals, trimmed of extraneous tissue, frozen with dry ice, and stored at -20° until ready for use. Phosphoglucomutase activity was determined by a modification of the method of Najjar(3), as refined by Bodansky(2). The final reaction volume of 1 ml. included 0.3 ml of 1% aqueous homogenate of muscle and freshly prepared cysteine. Glucose-1-phosphate was obtained from Schwarz Labs., lot #2102. The ice cold homogenate and the substrate-buffer mixture were warmed separately at 37° for 3 minutes prior to combining. After 4 minutes, the reaction was stopped by adding 1 ml of 5N H_2SO_4. The mixture was diluted to 5 ml, heated for 7 minutes at 100°, filtered, and 1 ml aliquot taken for P determination(5). The results are reported as mg glucose-6-phosphate P formed/100 mg fresh tissue/hour. Since purified coenzyme was not available, maximum mutase activity was determined(2) by adding to the reaction mixture various amounts of boiled normal muscle homogenate (equivalent to 4, 8, and 12 mg of tissue) prepared according to Cardini *et al.*(6). The relative activities in presence of different amounts of boiled extract containing coenzyme thus permitted extrapolation to activity at infinite coenzyme concentration. The linearity of 1/concentration of coenzyme *vs.* 1/velocity of the reaction, proposed by Sutherland and coworkers(7) and tested by Bodansky(2) for purified coenzyme, was confirmed for addition of boiled homogenate to both crude and crystalline phosphoglucomutase preparations. The non-collagenous protein N (NCPN) content of the homogenates was determined by the method of Lilienthal, *et al.* (8).

Results. Table I indicates that phosphoglucomutase activity is significantly decreased in the dystrophic leg muscles, regardless of the basis upon which the activity is measured. The importance of adding coenzyme to the reaction mixture is emphasized by the difference in values for the 13-week-old mice with and without added coenzyme (39% and 68% decreases, respectively). The further but less striking importance of basing results on NCPN rather than on wet weight(8,9) is seen in the difference between the ratios of activity at infinite added coenzyme concentration in 13-week-old mice: 52.0% on wet weight, 60.7% on NCPN. The NCPN content of dystrophic muscles in older mice was 26.2 $\pm$ 1.1 mg/g wet weight as opposed to 28.5 $\pm$ 1.3 mg/g wet weight in control mice, representing a decrease of 8% in NCPN content of diseased muscles.

Discussion. Values in Table I for phosphoglucomutase activity, based on wet weight of tissue and without added coenzyme, are similar to those obtained in previous experiments on 6 pairs of mice 14-18 weeks of age.

* Aided by grants from Muscular Dystrophy Assn. and by Sage and Sackett Research Funds of Dept. of Zoology, Cornell University.

† Medical Student Fellow of Nat. Fn.

TABLE I. Phosphoglucomutase Activity in Dystrophic Mouse Muscle.

Exp. (No. of mice)	Without added coenzyme			At infinite coenzyme conc.		
	Fresh tissue*	NCPN†	Ratio‡ ×100	Fresh tissue*	NCPN†	Ratio‡ ×100
8 wk old						
Dystrophic (3)	7.4 ± .3	2.9 ± .3	53.4 ± 5.8	23.5 ± 1.8	8.8 ± .1	74.5 ± 4.8
Control (3)	14.4 ± 1.9	5.5 ± .2		30.5 ± 2.1	11.9 ± .6	
13 wk old						
Dystrophic (6)	5.7 ± 1.7	2.3 ± .7	32.0 ± 9.5	17.9 ± 2.1	7.4 ± .9	60.7 ± 4.4
Control (6)	18.8 ± 1.3	6.6 ± .4		34.2 ± 2.3	12.2 ±1.6	
P (significance)	<.001	<.001		<.001	<.01	

* Activity = mg P/100 mg tissue/hr ± stand. error.
† Activity = mg P/mg non-collagenous protein nitrogen/hr ± stand. error.
‡ Ratios of dystrophic/control, based on NCPN results.

This group decreased 65% in mutase activity in leg muscles, and 70% in abdominal muscles, while heart muscle exhibited no significant change. The importance of basing muscle analyses on NCPN has been previously demonstrated(8,9). In the present study, however, there was little difference between enzyme activity ratios of dystrophic to normal muscle based on NCPN and those ratios calculated on wet weight basis. On the other hand, it is very important to add sufficient coenzyme to permit calculation of maximum activity, independent of the coenzyme content of the tissue.

When so calculated, not only is mutase activity increased in both dystrophic and normal muscle as compared to activities recorded in the absence of added coenzyme, but also the activity ratio of dystrophic to normal is raised. This fact suggests that the coenzyme content of the dystrophic muscle is also decreased, only enough coenzyme remaining to permit a much reduced conversion of glucose-1-phosphate. Furthermore, it might be inferred that this loss of coenzyme is relatively greater than the loss of the enzyme: if the opposite were true, the ratio of dystrophic/normal activities would decrease upon addition of coenzyme, and if both enzyme and coenzyme were decreased to the same degree (thus remaining in constant proportions), the ratio in the absence of added coenzyme would be identical to that at infinite coenzyme concentration. Direct determination of amounts of coenzyme in small muscle samples would substantiate this inference(6,10). The role of glucose-1-phosphate kinase in producing the coenzyme(10) should also be investigated.

Although observations on 8-week-old mice are few, the results suggest a progressive decline in mutase activity as the dystrophic mice mature and the myopathy becomes more severe. This corroborates previous reports that changes in some enzyme activity in dystrophic mouse muscle are correlated with age of mice(11).

The decreases in mutase activity in human muscular dystrophy, determined in the absence of added coenzyme and based on wet weight and NCPN, are of the same order of magnitude as those found for dystrophic mice (1). No results calculated at infinite added coenzyme concentration are available for comparison. A slightly smaller decrease in the glycogenolytic enzyme, phosphorylase, has previously been reported for dystrophic mice (12). The decrease in human dystrophy is somewhat more marked(1).

Information of the biochemical constituents of dystrophic and normal mouse muscle is being accumulated with the object of comparing the findings with those observed in clinical dystrophies. Some resemblances and some differences between the two have been found; as yet, however, no definite pattern has emerged to permit broad generalizations(11, 13,14).

Summary. A modified method of assaying maximum phosphoglucomutase activity in crude muscle homogenates is presented. Mutase activity is significantly decreased in leg muscles of mice with muscular dystrophy whether determined as total enzyme activity (at infinite coenzyme concentration) or as

residual enzyme-coenzyme activity (in the absence of added coenzyme). Evidence is presented for a decrease in coenzyme content of dystrophic muscle and for a correlation of these enzyme changes with age of mice and severity of myopathy.

The authors thank Dr. Oscar Bodansky for his counsel and generous contribution of crystalline phosphoglucomutase.

1. Dreyfus, J. C., Schapira, G., *Compt. rend. Soc. biol.*, 1954, v147, 1145.
2. Bodansky, O., *J. Biol. Chem.*, 1958, v232, 859.
3. Najjar, V. A., *ibid.*, 1948, v175, 281.
4. Michelson, A. M., Russell, E. S., Pickney, J. H., *Proc. Nat. Acad. Sci.*, 1955, v41, 1079.
5. Fiske, C. H., Subbarow, Y., *J. Biol. Chem.*, 1925, v66, 375.
6. Cardini, C. E., Paladini, A. C., Caputto, R., Leloir, L. F., Trucco, R. E., *Arch. Biochem.*, 1949, v22, 87.
7. Sutherland, E. W., Cohn, M., Posternak, F., Cori, C. F., *J. Biol. Chem.*, 1949, v180, 1285.
8. Lilienthal, J. L., Jr., Zierler, K. L., Folk, B. P., Buka, R., Riley, M. J., *ibid.*, 1950, v182, 501.
9. Barnes, B. A., Gordon, E. B., Cope, O., *J. Clin. Invest.*, 1957, v36, 1239.
10. Paladini, A. C., Caputto, R., Leloir, L. F., Trucco, R. E., Cardini, C. E., *Arch. Biochem.*, 1949, v23, 55.
11. Tassoni, J. P., Curtis, R. L., Hollinshead, M. B., *Anat. Rec.*, 1959, v133, 342.
12. Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 720.
13. Weinstock, I. M., Epstein, S., Milhorat, A. T., *ibid.*, 1958, v99, 272.
14. Dreyfus, J. D., Schapira, G., Schapira, F., *J. Clin. Invest.*, 1954, v33, 794.

Received September 28, 1959. P.S.E.B.M., 1959, v102.

Effect of Lactose on the Utilization of Phosphorus from Colloidal Phosphate by Chicks.* (25376)

J. N. BARUAH, R. E. DAVIES, B. L. REID† AND J. R. COUCH
Departments of Biochemistry and Nutrition and Poultry Science, Texas A & M College System, College Station

The effect of lactose on absorption of calcium and phosphorus has been of considerable interest, and a great deal of work has been done on the over-all effects of lactose feeding. Lactose, when included at a level of 25% in the basal diet, increased the amount of calcium and phosphorus absorbed due to formation of lactic acid by fermentation in the intestine(1). Simultaneous feeding of lactose and bone meal to dairy calves resulted in increased calcium retention from bone meal (2). These authors found that feeding a diet containing 25% lactose caused marked lowering of pH of contents of the digestive tract below the duodenum. Inclusion of lactose at a level of 40% in the diets of young chicks of a rachitogenic ration produced a favorable effect on calcium absorption, as well as having a positive influence on maintaining a more acid condition in the entire intestinal tract (3). Colloidal phosphate (soft phosphate with colloidal clay), obtained as by-product in the mining of rock phosphate, has been offered as a phosphorus supplement, to be used in animal rations. Recent workers(4,5, 6) reported that this supplement, when used as sole source of phosphorus in the diet, gave lower growth rate and (percent) bone ash in chicks than was produced by tricalcium phos-

* These studies were supported in part by grants-in-aid from Soft Phosphate Research Inst., and Butler Chem. Co. The following products were supplied through courtesies as indicated: choline chloride by Hoffman-Taff, penicillin and other B-vit. by Merck and Co., D-alpha tocopheryl acetate by Distillation Products Industries, condensed fish solubles by Philip R. Park, aureomycin by Am. Cyanamid Co., stabilized Vit. A by Stabilized Vitamins, Vit. D_3 by Charles Bowman and Co., menadione sodium bisulfite by Heterochemical Corp., butylated hydroxy-toluene by Koppers Co., colloidal phosphate by Soft Phosphate Research Inst., and defluorinated rock phosphate by Butler Chemical Co.

† Present address: The Pillsbury Co., Clinton, Iowa.