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Glutathione Peroxidase Activity and Glutathione Concentration in Genetically Dystrophic Mice[1] (39431)

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Dystrophia muscularis is a primary hereditary myopathy exhibited by the inbred strain of mice 129/ReJ-dy. This disease, which is characterized by progressive muscular weakness and atrophy of the skeletal muscles (1, 2), resembles muscular dystrophy in human beings in several aspects (3). Although the disease has been the subject of much investigation, its metabolic basis remains unknown.

It is well documented that dietary selenium deficiency can produce nutritional muscular dystrophy in a variety of species (4) and that the symptoms are similar to those seen in inherited muscular dystrophy. Recently it has been demonstrated that selenium functions as a component of glutathione peroxidase (glutathione: hydrogen peroxide oxidoreductase, EC 1.11.1.9) (5, 6) and that the activity of the enzyme decreases markedly in response to dietary selenium deficiency (7). Glutathione peroxidase evidently serves to protect the cell from lipid free radical damage by reducing fatty acid hydroperoxides to their correspondng hydroxy acids. It also functions in the removal of hydrogen peroxide generated during cellular metabolism (8).

In view of the important role of selenium in the prevention of nutritional muscular dystrophy and selenium's function as a part of glutathione peroxidase, the present study was conducted to determine whether inherited muscular dystrophy of mice is associated with any change in specific activity, substrate availability, or apparent K_m value of glutathione peroxidase in the muscle.

Materials and methods. Female dystrophic mice of the 129/ReJ-dy strain and their age-matched normal controls, 129/ReJ, were obtained from Jackson Laboratories[4] and maintained with stock rodent chow (Wayne Lab Blox). Animals were sacrificed in pairs over a 50-day period after signs of muscular dystrophy had become apparent. The mice were anesthetized with ether and a blood sample was obtained by heart puncture. The whole animal was perfused with saline by a saline drip into the heart in order to remove blood from the tissues. Heart, liver, lung, and skeletal muscle were removed and homogenized in 2 ml or 2 vol of buffer (0.01 *M* phosphate buffer containing 0.135 *M* KCl, pH 7.0). One sample of skeletal muscle was obtained from the hind legs and a second sample from the fore legs. The tissues were homogenized for 5 sec on a Polytron PT 10 homogenizer. Homogenates were centrifuged at 48,000*g* for 1 hr and the supernates were analyzed for glutathione peroxidase activity. Red blood cells were washed with saline and hemolyzed in deionized water before enzyme assay. Glutathione peroxidase activity was determined by the method of Little *et al.* (9). Oxidation of reduced NADP was measured spectrophotometrically at 340 nm in an assay medium containing 0.25 m*M* reduced glutathione (GSH), 0.12 m*M* NADPH, 0.1 *M* Tris-HCl buffer (pH 7.0), 3 m*M* EDTA, 0.2 m*M* cumene hydroperoxide, and excess glutathione reductase. All assays were carried out at 25°. These conditions resulted in minimum nonenzymatic activity. The protein content of the supernate was measured by the method of Lowry *et al.* (10). Glutathione peroxidase activity is expressed as nanomoles of reduced NADP oxidized per minute per milligram of protein.

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For glutathione analysis, the tissues were homogenized by the same procedure, and the concentration of GSH was measured by the method of Mills as modified by Hafeman *et al.* (7).

Results and Discussion. The dystrophic mice weighed significantly less ($P < 0.001$) than their age-matched controls at 40 days of age but showed few signs of dystrophy. During the ensuing 50 days, all dystrophic mice developed the symptoms of dystrophy previously described for this strain (1, 3) including kyphosis, paralysis of hind legs, periodic backward neck extension, denuded eyelids, and periocular inflammation.

Glutathione peroxidase was measured over a period of 7 weeks to determine whether enzyme activity changed as the skeletal muscles exhibited increasing degrees of paralysis (Figs. 1 and 2). Enzyme activity was higher in skeletal muscle of dystrophic mice than in that of normal mice (Table I). This increase was statistically significant ($P < 0.05$) for the muscle from the fore leg. Omaye and Tappel (11) recently reported that glutathione peroxidase activity is increased in skeletal muscle of the hind quarters of dystrophic mice of the 129/ReJ-dy strain. The hind legs exhibit signs of muscular weakness and paralysis earlier than the fore legs in this strain. It is of interest that in the present study the increase in enzyme activity was not confined to skeletal muscle showing severe symptoms of dystrophy but was at least as evident in the less affected muscles of the fore leg. No differences in enzyme activity were found in heart muscle or in the tissues of the liver, lung, or red blood cells (Table I).

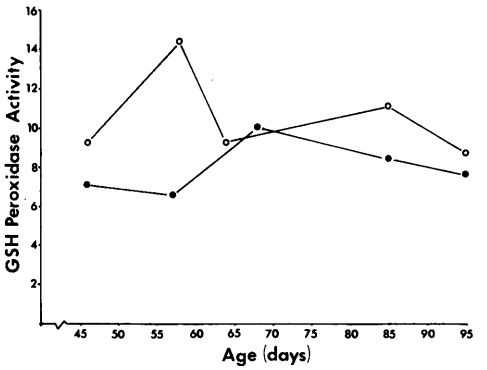

FIG. 1. Glutathione peroxidase activity in muscle from the hind legs of normal (●——●) and dystrophic (○——○) mice. Glutathione peroxidase activity is expressed as nanomoles of NADPH oxidized per minute per milligram of protein.

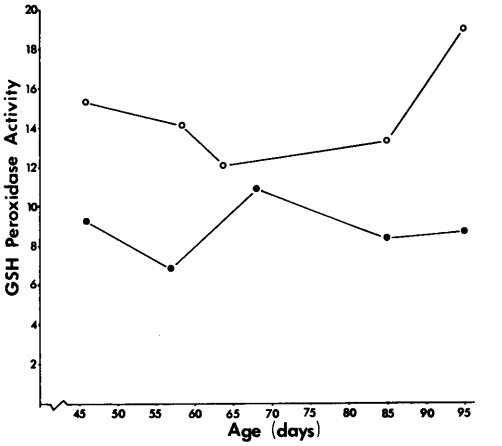

FIG. 2. Glutathione peroxidase activity in muscle from the fore legs of normal (●——●) and dystrophic (○——○) mice. Glutathione peroxidase activity is expressed as nanomoles of NADPH oxidized per minute per milligram of protein.

TABLE I. GLUTATHIONE PEROXIDASE ACTIVITY

Tissue	Nanomoles of NADPH oxidized/min/mg protein	
	Normal	Dystrophic
Muscle (hind)	8.00 ± 1.04[a]	10.56 ± 0.61
Muscle (fore)	9.03 ± 0.83	14.77 ± 1.15[b]
Heart	18.48 ± 2.42	17.04 ± 1.13
Lung	100.63 ± 11.18	99.27 ± 8.36
Liver	394.50 ± 70.0	385.38 ± 66.70
Red blood cell	34.24 ± 5.14	27.77 ± 3.15

[a] Mean of five samples ± standard error.
[b] Significantly different from normals ($P < 0.05$).

Caution must be exercised in interpreting changes in enzyme specific activity based on protein concentration in dystrophic tissues. It has been demonstrated that differences between normal and dystrophic muscle in the concentration of enzymes and other constituents may reflect a change in the protein concentration of dystrophic muscle rather than a change in enzyme activity in the cells. The protein content of dystrophic mouse muscle may be only 25% of that found in normal muscle and all protein constituents are not reduced to the same extent. Many reported differences in enzyme activities between dystrophic and normal muscle can be attributed to secondary changes in muscle

biochemistry which can be mimicked by denervation of normal muscle rather than to primary changes characterizing dystrophic muscle (12). Whether this is also true for glutathione peroxidase activity cannot be determined from the present or previous (11) studies. These studies do demonstrate, however, that muscular dystrophy in the 129/ReJ-dy mouse is not due to lack of glutathione peroxidase actvity in skeletal muscle or other tissues.

Since glutathione peroxidase is highly specific for GSH (13) and enzyme activity increases with increasing GSH concentration within the physiological range, levels of GSH were compared in various tissues from normal and dystrophic mice (Table II). GSH concentration was greater in skeletal muscle of dystrophic mice; this increase was statistically significant for the muscles of the hind legs. These data agree with previously reported values for GSH in skeletal muscle of normal and dystrophic mice (14). There were no differences between dystrophic and normal mice in GSH concentration in the heart, lungs, or liver.

Previous studies on glutathione peroxidase have shown that the kinetic behavior of the enzyme is not readily investigated due to the extremely low apparent K_m for H_2O_2 and the high velocity of the enzymatic reaction itself (9, 13). In addition, the apparent K_m for the hydroperoxide increases with increasing GSH concentrations. Utilizing the assay conditions previously outlined (with 0.25 m*M* GSH) apparent K_m values of 3.1 and 5.6 μM cumene hydroperoxide were obtained for normal and dystrophic muscle, respectively. Apparent K_m values for liver enzyme for normal and dystrophic mice were 16 and 19 μM cumene hydroperoxide. These values are valid only for the assay conditions described, but they serve to indicate that there were no detectable differences in the properties of glutathione peroxidase from normal and dystrophic muscle. The K_m values for the muscle enzyme are similar to those reported for glutathione peroxidase isolated from pig blood (9).

TABLE II. TISSUE GLUTATHIONE CONCENTRATION

Tissue	Milligrams of GSH per 100 g wet weight	
	Normal	Dystrophic
Muscle (Hind)	22.8 ± 0.9[a] (4)[c]	27.2 ± 1.6[b] (3)
Muscle (Fore)	23.5 ± 2.3 (4)	29.5 ± 1.7 (3)
Heart	36.8 ± 1.6 (4)	36.9 ± 4.2 (3)
Lung	32.0 ± 3.4 (4)	31.2 ± 1.1 (3)
Liver	190.2 ± 7.6 (8)	198.2 ± 8.6 (7)

[a] Mean ± standard error.
[b] Significantly different from normals ($P < 0.05$).
[c] Entries in parentheses represent number of samples analyzed.

Summary. The present studies were conducted to determine whether inherited muscular dystrophy in the 129/ReJ-dy mouse was associated with differences in specific activity, substrate availability, or apparent K_m of glutathione peroxidase. The results indicate that glutathione peroxidase is elevated in skeletal muscle of mice with genetic muscular dystrophy when the activity is expressed on a protein basis. This elevation precedes the development of severe paralysis since muscles from the fore legs showed increased enzyme activity as early as the more severely affected hind legs. There was no difference in glutathione peroxidase activity in tissues other than skeletal muscle. GSH concentration was elevated in muscle and normal in other tissues of dystrophic mice, showing that adequate substrate was available to the enzyme. The apparent K_m for cumene hydroperoxide was also similar for muscle of normal and dystrophic mice. This report provides further evidence that mice with *dystrophia muscularis* have a functional glutathione peroxidase system in all tissues including skeletal muscle, and that a defect in this *in vivo* protective system is apparently not a contributing factor in the pathology of the disease.

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