Glutamic Acid Dehydrogenase Activity in Muscles of Dystrophic Mice

Numerous biochemical investigations on a hereditary muscular dystrophy of mice have dealt with metabolic processes involved in glycolysis[1] or in proteolysis[2] or with oxidative steps[3]. An increase of some dehydrogenases in the dystrophic muscle has previously been described with a histochemical method[4]. Levels of some glutamic acid transaminase (GO-T, GP-T) were also elevated in the dystrophic muscle[5,6]. In view of such enzyme elevation, it seemed pertinent to confirm biochemically the activity level of glutamic acid dehydrogenase, which is coupled with glutamic acid transaminase activity. A total nitrogen concentration in the muscle was also determined in normal and dystrophic mice.

Method. Determinations of glutamic acid dehydrogenase activity and a total nitrogen concentration were made in skeletal muscles of strain C57BL/6J dystrophic mice and their normal littermates originally obtained from the Jackson Laboratory. The mice were fed with a complete diet of Oriental Farm NMF pellets and water ad libitum. The muscle was dissected from the hind legs and was homogenized in cold water. After $^1/_2$ h elution in an ice box, the homogenate was filtered through cotton gauze, and used for determination of a total nitrogen concentration and for enzyme assay. Glutamic acid dehydrogenase activity was determined in an anaerobic thunberg tube with NAD^+ in terms of a rate of decolorization of methylene blue[7].

Results and discussion. A marked increase of glutamic acid dehydrogenase activity was shown in the skeletal muscle of mice with hereditary muscular dystrophy when the methylene blue method was used (Table). These data coincided well with the results of other investigators obtained with the use of a TPTZ technique[8].

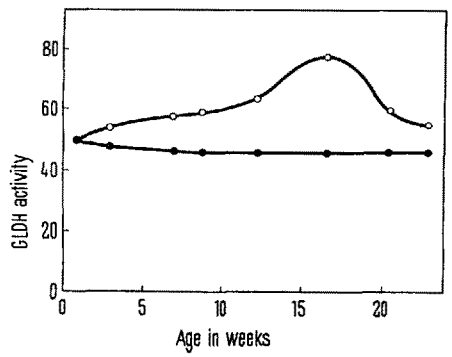
It is well known that the composition of the muscle is markedly altered in dystrophic mice. Thus it was attempted to measure a total nitrogen concentration in the muscle so that a proper reference base might be established in comparing enzyme activities between normal and dystrophic muscles. As a result, a considerable difference was found in enzyme activity when calculated on the basis of a total nitrogen.

Changes in glutamic acid dehydrogenase activity of the muscle were also examined in dystrophic mice and their controls at various ages (Figure). 20 days after birth, when paralysis had progressed considerably in the hind legs, enzyme activity of dystrophic muscle became higher than that of normal mice. Although the enzyme activity was nearly constant in the normal muscle, an increase of the activity was shown in the dystrophic muscle as severity of disease increased. At a severe stage of the disease (about 4 months of age), the greatest activity of the enzyme occurs, followed by a marked decrease of the activity in the terminal stage of the disease.

Glutamic acid dehydrogenase activity and total nitrogen concentration in muscles of dystrophic mice

	Enzyme activity		Total nitrogen
	(γ NADH/mg wet weight/h)	(γ NADH/mg nitrogen/h)	(mg nitrogen/g wet weight)
Controls	53.24 ± 14.33	2475.12 ± 666.20	21.51 ± 0.53
Dystrophic	78.41 ± 4.17	4864.14 ± 258.68	16.12 ± 0.42
Significance	$P < 0.05$	$P < 0.01$	$P < 0.01$

In view of this increase of glutamic acid dehydrogenase in the dystrophic muscle, one would suggest that glutamic acid obtained from the muscle could be transformed into α-ketoglutaric acid which would enter the citric acid cycle in order to keep the cycle properly. A high level of α-ketoglutaric acid dehydrogenase[9] or glutamic acid transaminase (GO-T, GP-T) in a severe stage of the disease, which is the enzyme of coupling reaction with glutamic acid dehydrogenase, found in the dystrophic muscle, is in agreement with the present results. On the other hand, an increase in the level of α-ketoglutaric acid excretion[10] means, in spite of the intactness in a total oxygen uptake[11], that the glutamic acid to the α-ketoglutaric acid system for energy supplement is not indispensable for a primary deletion in the disease.



Variation of the activity of muscle glutamic acid dehydrogenase (GLDH) from normal (●—●) and dystrophic (o—o) mice, as a function of age. Activity is expressed as μg of NADH formed per mg of tissue wet weight/h. Each point represents the average value from 3 different animals.

Résumé. L'activité de la déshydrogénase glutamique a été étudié dans le tissu musculaire de la souris atteinte de dystrophie musculaire héréditaire. La déhydrogénase glutamique est peu active dans le muscle dystrophique de la souris jeune, alors que l'activité de cet enzyme est fortement augmentée dans le muscle de la souris plus âgée (12–18 semaines). D'autre part, l'activité de l'enzyme est diminuée dans le muscle de la souris lorsque la maladie évolue.

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