ALTERATIONS OF ASPARTATE- AND ALANINE- TRANSAMINASE IN MICE WITH HEREDITARY MUSCULAR DYSTROPHY

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MICHELSON et al¹⁾ have originally described on the spontaneous occurrence of an autosomal recessive mutation resulting in muscular dystrophy in a highly inbred strain of mice (129/Re), concomitant with the pathological and biochemical alterations which have proven to bear a remarkable similarity to those found in human muscular dystrophy.²⁾

Previous studies indicate that many fundamental biochemical processes are altered in mice with hereditary muscular dystrophy and that the principal lesion of the myopathy under discussion seems to be an altered protein metabolism³⁾ possibly leading to a derangement of enzymatic systems. A considerable attention has recently been given to an increase in activity of some serum enzymes associated with tissue destruction in man and other animals, because it is believed that the concentration of serum enzymes reflects either a physiological breakdown of cells or an unavoidable escape of enzymes through a partially permeable cell membrane.

In mice with myopathy some enzymes such as aldolase,⁴⁾ creatine kinase,⁵⁾ acid phosphatase,⁶⁾ aspartate- and alanine-transaminase⁷⁾ are elevated in the serum.

The present report is concerned with the determination of tissue which is most effective in elevating aspartate- and alanine-transaminase in serum. It has been already described that a striking elevation in those enzymatic activities is found in human patients with progressive muscular dystrophy and in mice.⁸⁾ Quantitative changes of those enzymes in affected tissues of dystrophic mice are also discussed compared with those of normal mice.

MATERIALS AND METHODS

Material. Dystrophic mice and their normal litter mates of 129/Re strain were originally obtained from the Jackson Laboratory, Bar Harbor, Maine. This strain develops a disorder of muscle transmitted as an autosomal recessive trait which closely

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resembles a human progressive muscular dystrophy. Mice varying in age from 80 to 100 days were used in the experiments.

Preparation of tissue homogenates. 1) The skeletal muscle was dissected from the hind legs, 2) a piece of the liver lobe was cut off and put on filter paper to remove the blood, 3) the whole heart had its chambers opened and had the blood removed on filter paper, and 4) a left half of the cerebral lobe was cut to obtain a representative sample for analysis. Each material was weighed and homogenized in a Potter-Elvehjem glass homogenizer with a suitable amount of cold distilled water. Immediately after homogenization, the total volume was adjusted by the addition of cold water so as to make 1.0 ml of the homogenate contained 25 mg of tissue. After 30 minutes' elution in an ice box, the homogenate was filtered with filter paper. These eluents were diluted five fold in volume with cold water for a transaminase assay. The serum was diluted in five fold with cold water. All the procedures were carried out at 0°C-5°C.

Transaminase assay. Aspartate-transaminase (GO-T) and alanine-transaminase (GP-T) activities were determined by a modified method of Reitman and Frankel.⁹⁾ A reaction mixture consisted of 1.0 ml of substrate and 0.2 ml homogenate or diluted serum solution was incubated for 30 minutes (GP-T) or for 60 minutes (GO-T) at 40°C, pH 7.4.

RESULTS

1) *Enzyme activities in serum.* The results of individual aspartate- and alanine-transaminase determinations in serum from both normal and dystrophic mice are summarized in TABLE I. The data indicate that a pronounced elevation of both aspartate- and alanine-transaminase activities occurs in dystrophic mice. The differences of both enzymatic activities between normal and dystrophic mice are highly significant ($P < 0.01$); activities of alanine-transaminase in individual dystrophic mice are widely varied. It seems likely that an alteration in these enzyme activities in the serum of dystrophic mice may represent changes in cell permeability or necrosis of affected tissues.

TABLE 1.
Serum transaminase activities in normal and dystrophic mice*

	Aspartate-transaminase (μ moles/hr./ml)	Alanine-transaminase (μ moles/hr./ml)
Normal	11.2 $\pm$ 4.1	14.1 $\pm$ 4.2
Dystrophic	20.8 $\pm$ 4.3	21.0 $\pm$ 7.4
Significance	$P < 0.01$	$P < 0.01$

* Activities were measured as described in the text.

2) *Enzyme activities in various tissues.* Dystrophic mice exhibited a marked increase in both enzymes in the skeletal muscle (TABLE 2). The differences of these enzymatic activities between normal and dystrophic skeletal muscles was highly significant. A mean activity of each enzyme in the dystrophic

TABLE 2.
Transaminase activities in various tissues of normal
and dystrophic mice*

	Aspartate-transaminase (μ moles/hr./mg wet wt.)		Alanine transaminase (μ moles/hr./mg wet wt.)	
	Dystrophy	Normal	Dystrophy	Normal
Skeletal muscle	0.88 $\pm$ 0.18	0.51 $\pm$ 0.12 P<0.01	0.46 $\pm$ 0.10	0.24 $\pm$ 0.04 P<0.01
Heart muscle	1.12 $\pm$ 0.17	1.20 $\pm$ 0.13 N.S.**	0.47 $\pm$ 0.08	0.54 $\pm$ 0.10 P<0.01
Liver	1.15 $\pm$ 0.40	1.13 $\pm$ 0.22 N.S.	1.22 $\pm$ 0.16	1.21 $\pm$ 0.13 N.S.
Brain	0.98 $\pm$ 0.05	0.92 $\pm$ 0.03 N.S.	0.09 $\pm$ 0.02	0.08 $\pm$ 0.02 N.S.

* Activities were measured as described in the text.

** No significance.

skeletal muscle was elevated approximately 72.5% in aspartate-transaminase, and 91.6% in alanine-transaminase over normal controls.

Aspartate- and alanine-transaminases showed a slight increase in the dystrophic heart muscle. Such increase was not significant in the former enzyme but was significant in the latter enzyme (P<0.01). The liver and brain tissues of dystrophic mice showed a slight increase in both enzymes, which, however, was not significant as compared with that of normal mice.

TABLE 3.
Transaminase activities in normal and dystrophic
muscles in reference as nitrogen base

	Aspartate-transaminase (μ moles/hr./mg wet wt.)		Alanine-transaminase (μ moles/hr./mg wet wt.)	
	Dystrophic	Normal	Dystrophic	Normal
Skeletal muscle	0.155 $\pm$ 0.033	0.061 $\pm$ 0.014 P<0.01	0.081 $\pm$ 0.02	0.029 $\pm$ 0.006 P<0.01
Heart muscle	0.197 $\pm$ 0.032	0.162 $\pm$ 0.017 P<0.01	0.079 $\pm$ 0.017	0.072 $\pm$ 0.014 N.S.

TABLE 3 shows the values of transaminase activities determined in nitrogen base for normal and dystrophic muscles. The skeletal muscle of dystrophic mice shows a high significance in both enzymatic activities (P<0.01). In the heart muscle of dystrophic mice, however, aspartate-transaminase is also significantly increased but an elevation of alanine-transaminase is non-significant,

TABLE 4.
Transaminase activities in subcellular fractions of
dystrophic muscle*

	Skeletal muscle		Heart muscle	
	Mitochondrial	Supernatant	Mitochondrial	Supernatant
Aspartate-transaminase	106**	193	86	94
Alanine-transaminase	108	158	102	85

* Differential centrifuging of homogenates in 0.25 M sucrose was performed as described by Hogeboom et al.¹²⁾

** Number shows a percent changes of enzyme activity compared with those of normal muscles.

3) *Alterations of the enzyme properties in dystrophic muscle.* Aspartate- and alanine-transaminase have been reported to exist in two isozymes, one of which was derived from the soluble supernatant fraction and the other from the mitochondrial fraction.^{10,11)} A proportional distribution of these two enzymes in the skeletal and heart muscles is changed in dystrophic mice as compared with that of normal mice (TABLE 4). A marked increase of supernatant 'free' isozymes was shown in both enzymes in the skeletal muscle of dystrophic mice. In the heart muscle, only a slight difference was observed in both enzymes.

Stability of enzymes to heat was tested in the homogenates of several tissues (FIG. 1). No difference in the thermostability of the enzymes between normal and dystrophic mice was observed in the homogenates of the liver and heart or in diluted sera. On the other hand, both enzymes in the homogenates of the skeletal muscle of dystrophic mice were less thermostable than

TABLE 5.
Changes of transaminase activities in skeletal muscle
when supplemented a heat denaturated serum*

	Alanine-transaminase			Aspartate-transaminase		
	Initial	Serum added	Percent increase	Initial	Serum added	Percent increase
Normal	0.68**	0.98	44.1	1.10	1.12	1.8
Dystrophic	1.02	1.05	2.9	1.44	1.46	1.3

* Normal mice serum was diluted with five fold of distilled water and heated in boiling water for five minutes. Heat denaturated serum was mixed with the same volume of tissue homogenates. After five minutes preincubation, the mixture was used as enzyme source.

** Optical density.

FIG. 1. Activity loss of transaminases in liver (a), heart (b), skeletal muscle (c), and serum (d) during 30 minutes heating (60°C). broken line, GO-T. unbroken line, GP-T. open circles, normal mice. solid circles, dystrophic mice.

those of normal mice.

An elevated ratio of enzymatic activity in the skeletal muscle homogenates of normal and dystrophic mice by addition of a heat denatured serum which included enzyme activating factor¹⁸⁾ was examined. No activation was observed in either enzyme of the dystrophic skeletal muscle, while a marked elevation was observed in the alanine-transaminase activity in the normal skeletal muscle (TABLE 5).

DISCUSSION

Alterations of transaminase activities (GO-T, GP-T) in muscular dystrophy developed in several animals have been described by many investigators. In a human progressive muscular dystrophy, WHITE and HESS¹⁴⁾ have been observed a distinct elevation of serum transaminase only in children but not in adults. CORNELIUS et al¹⁵⁾ also described an elevation of plasma aspartate-transaminase activity in domestic chicken with onset of inherited muscular dystrophy.

Levels of transaminases (GO-T, GP-T) were determined in the tissues from rabbits deficient in vitamin E and mice with hereditary muscular dystrophy by RAFERTE et al.⁷⁾ These authors classified dystrophic animals in three groups on severity, mild, moderate, and severe, and concluded that each animal had a striking elevation of transaminases in tissue in a mild stage but normal or rather a decrease in a severe stage. They discussed from the results of experimentally induced dystrophy in rabbits that an elevation of serum alanine-transaminase was originated from muscle cell destruction. They also stated that a marked elevation of alanine-transaminase activity in the liver tissue indicated a main route from glutamate to pyruvate for energy supply.

In the present experiment, a marked elevation of aspartate- and alanine-transaminase activities in both serum and skeletal muscle of dystrophic mice were also observed. The elevation of these two enzymes in the serum of dystrophic mice is considered to be originated mainly from a liberation of skeletal muscle enzymes which are drastically increased in the course of disease. It is assumed that serum alanine-transaminase which is widely distributed in individuals, may be caused by a liberation of the enzyme not only from the skeletal muscle but also from the heart muscle or other tissues resulting in a secondary lesion as severity advances. This assumption will be supported by the present data which revealed that the alanine-transaminase activity in the heart muscle of dystrophic mice decreased significantly in reference to wet weight base and that a relative hypertrophy of heart volume usually occurred in the final stage of the disease.

Quantitative changes in the isozyme compositions of transaminases, which are caused mainly by an increase of enzyme activity in the supernatant fraction, were also observed in the dystrophic skeletal muscle. However, what should not be excluded is a possibility of liberation of enzymes from mitochondria which show an enlargement, swelling, and decrease of density of the matrix in the dystrophic muscle¹⁶⁾ through a homogenizing process.

The thermostability of the enzymes in the skeletal muscle of dystrophic animals differs from those of normal animals, while the enzymes in the liver

or heart show no changes. An elevation ratio of alanine-transaminase activity with a denatured serum preparation which contained enzyme activating factor,¹³⁾ was prevented markedly in the dystrophic skeletal muscle. Thus, a possibility is suggested that qualitative abnormalities exist in the transaminase enzymes of the dystrophic skeletal muscle. Further work is in progress on a structural analysis of these enzymes in dystrophic animals.

SUMMARY

1. An increase in transaminase activities in the serum and skeletal muscle from mice with hereditary muscular dystrophy was confirmed. Almost a two fold elevation of aspartate- and alanine-transaminase activities was observed in the serum of dystrophic mice. An increase of these two enzymes in the skeletal muscle was significant on a wet weight or total nitrogen basis.
2. Quantitative alterations in the transaminase enzymes of the dystrophic skeletal muscle were measured with regard to the isozyme compositions. Difference of thermostability in the enzymes of skeletal muscle homogenates between dystrophic and normal mice was also observed. No elevation of alanine-transaminase activity was observed in the dystrophic skeletal muscle following addition of a heat denatured serum, while a marked elevation was observed in the normal skeletal muscle.

REFERENCES

- 1) MICHELSON, A. M., RUSSEL, E. S., AND HARMAN P. J. (1955), *Proc. Natl. Acad. Sci.*, (Washington) **41**: 1079.
- 2) HARMAN, P. J., TASSONI, J. P., CURTIS, R. L., AND HOLLINSHEAD, M. B. (1963), in "Muscular dystrophy in man and animals" Chapter X, 407.
- 3) DINNING, J. S., AND FITCH, C. D. (1958), *Proc. Soc. Exp. Biol. Med.*, **97**: 190.
- 4) SCHAPIRA, F., SCHAPIRA, G., AND DREYFUS, J. C. (1957), *Compt. rend. Acad. Sci.*, **245**: 754.
- 5) HOSENFELD, D. J., WIESMANN, U., AND RICHTERICH, R., (1962/63), *Enzymol. Biol. Clin.*, **2**: 246.
- 6) PENNINGTON, R. J., (1963), *Biochem. J.*, **88**: 64.
- 7) LAFERTE, R. O., ROSENKRANTZ, H., AND BERLINGUET, F., (1963), *Canad. J. Biochem. Physiol.*, **41**: 1423.
- 8) COOPER, A. C., AND MILLER, J. R., (1962), *Rev. Canad. Biol.*, **21**: 337.
- 9) REITMAN, S., AND FRANKEL, S., (1957), *Am. J. Clin. Pathol.*, **28**: 56.
- 10) BOYD, J. W. (1962), *Clin. Chim. Acta*, **7**: 424.
- 11) FLEISHER, G. A., POTTER, C. S., WAKIM, K. G., RANKOW, M., AND OSBORNE, D. (1960), *Proc. Soc. Exp. Biol. Med.*, **103**: 229.
- 12) HOGEBOOM, G. H., SCHNEIDER, W. C., AND PALLADE, G. E. (1948), *J. Biol. Chem.*, **172**: 619.
- 13) KATSUNUMA, N., MATSUZAWA, T., YOSHIDA, T., AND MIKUMO, K. (1962), *Proc.*

- Symp. Chem. Physiol. Pathol.*, **2**, 186.
- 14) WHITE, A. A., AND HESS, W. C., (1957), *Proc. Soc. Exp. Biol. Med.*, **94**: 541.
 - 15) CORNELIUS, C. E., LAW, G. R. J., JULIAN, L. M., AND ASMUNDSON, V. S. (1959), *Proc. Soc. Exp. Biol. Med.*, **101**: 41.
 - 16) ROSS, M. H., PAPPOS, G. D., AND HARMAN, P. J. (1960), *Lab. Invest.*, **9**: 338.