Alterations in Tissue and Serum Aldolase in Rabbits Due to Vitamin E Deficiency (35365)

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(Introduced by X. J. Musacchia)

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Patients suffering from progressive muscular dystrophy usually exhibit an increased level of aldolase in their sera during the early stages of the disease (1–3). Dreyfus *et al.* (4), in addition, found a reduction in muscle aldolase in dystrophic individuals whether the units were expressed in terms of wet weight of the tissue or noncollagen nitrogen (NCN). Decreased levels of the muscle enzyme based on various parameters have likewise been described in another form of dystrophy, hereditary muscular dystrophy of mice (5). Hyperaldolasemia was also demonstrated to exist in these animals (6) as well as in the inherited disease which affects chickens (7).

This investigation was undertaken to establish whether similar alterations occurred in serum and tissue aldolase in a third dystrophic state, namely, the nutritional disease induced by vitamin E deficiency. Enzymatic activity in rabbit muscle and liver were expressed on the parameters of dry fat-free weight (DFF wt) and NCN in order to localize the organ or organs affected and to determine whether a change in serum aldolase accompanied cellular destruction or resulted from another cause. Glutamic-oxaloacetic transaminase (GOT) was also assayed in the liver so as to establish whether the alteration in aldolase concentration seen there was a unique phenomenon or an example of a generalized effect.

Materials and Methods. Eight white New Zealand rabbits, weighing 1–1.65 kg, were housed individually in steel metabolism cages and placed on the diet of Young and Dinning (8) with 10% of the sucrose replaced by cellulose (Alphacel).

Four of the animals were orally supplemented with 8 mg of α-tocopherol acetate/kg of body weight (2.5 mg/0.5 ml of olive oil) twice weekly and served as controls, while the experimental animals received olive oil alone. Blood samples were periodically withdrawn by cardiac puncture and the resulting serum was stored at $-10°$ for enzymatic assays. Creatine and creatinine were determined intermittently on 24-hr urine specimens by the method of Bonsnes and Taussky (9). Symptoms of muscular dystrophy appeared in 5–8 weeks and the experimental period terminated when "state III," as described by Mackenzie and McCollum (10) was reached.

The animals were then sacrified by decapitation and were exsanguinated. Both gastrocnemius muscles and the liver were immediately excised, weighed, and a 2% homogenate (w/v) was prepared in ice-cold distilled water in a Potter-Elvehjem homogenizer. Duplicate 10-ml aliquots of each tissue homogenate were pipetted into weighing bottles and dried for 24 hr at 108° for estimation of dry weight. Portions of the dried homogenates were then subjected to the extraction procedure outlined by Neuman and Logan (11) for DFF wt and collagen.

Tissue collagen was determined from its hydroxyproline content by multiplication of the amount of the amino acid contained in a aliquot of hydrolysate by the factor 7.42 after correction for hydroxyproline destruction by acid during hydrolysis. The protein was then converted to its nitrogen equivalent by use of an average value of 17.5% nitrogen for collagens derived from various sources (12). Hydroxyproline was determined by the

method originally proposed by Neuman and Logan (13) but with those modifications suggested by Martin and Axelrod (14) and Miyada and Tappel (15).

Total nitrogen was determined on dry defatted material derived from tissues by the Kjeldahl digestion procedure of Niederl and Niederl (16). Distillation was performed using the apparatus of Parnas and Wagner (17) and $(NH_4)_2SO_4$ as a standard. The amount of NCN in tissue represented the difference in values between total nitrogen and collagen nitrogen analyzed.

Studies on tissue enzymes were performed on 1-ml aliquots of the original tissue homogenates. Aldolase was assayed for its activity in tissue and serum by the Dounce *et al.* (18) and White and Hess (2) modifications of the method of Sibley and Lehninger (19). Additional changes were made in methodology and reagents to produce a more reliable and reproducible enzymatic test. These were: (i) The temperature of reaction was elevated from 25 to 38° as originally proposed by Sibley and Lehninger (19); (ii) A 0.5-ml aliquot of 0.2 *M* collidine buffer was employed instead of 1.0 ml of a 0.1 *M* solution; and (iii) Methyl cellosolve was mixed with 0.75 *N* NaOH in the proportion of 2:1 and 7.0 ml of the final solution used for color development as described by Lowry *et al.* (20). Enzymatic activity was expressed as micrograms of triose phosphate phosphorus formed per minute. If serum was determined, the units would be expressed per milliliter of serum; if the tissue level was analyzed, the units would be denoted per gram of DFF wt or NCN. Inorganic phosphate liberated in the aldolase reaction was determined by the micromethod described by Chen and coworkers (21).

Tissue GOT was assayed by a modification (22) of the method proposed by Tonhazy *et al.* (23). Enzymatic activity was expressed in terms of the "Q_T^{10} unit" as proposed by Ames and Elvehjem (24) and either based on the parameters milligrams of DFF wt or milligrams of NCN.

Results. Table I demonstrates the effect of normal and vitamin E-deficient diets on the serum aldolase activity in rabbits. The enzyme is increased from 5 to 15 times in the deficiency state and precedes and parallels the onset of creatinuria by 1 to 2.5 weeks (10). The change in serum aldolase in dystrophic rabbits is initiated at a time when body weight is either stationary or increased slightly in these animals. Normal animals show no overall alteration in enzymatic activity except a slight rise and fall at about 20

TABLE I. Effect of Duration of Dietary Regimen on Rabbit Serum Aldolase Activity.

	(U/ml of serum)[a]							
(days):	0–1	2–3	4–5	6–10	20–24	25–29	30–35	50–58
Normal[b c] animals								
2	0.4	—	—	—	1.1, 1.5	—	—	0.2, 0.2
3	0.2	0.2	—	0.1	0.3	0.3, 0.6	0.3	—
4	0.1	—	0.1	—	0.6, 0.7	0.3, 0.4	—	0.2, 0.3
Dystrophic[c] animals								
1	1.1	1.2	0.6	2.6, 2.9	—	5.8, 6.3	—	—
2	0.8	0.4	0.1	0.2, 0.3	0.7, 1.1	1.1, 1.3	—	2.4, 3.2
3	0.2	0.2	—	1.1, 1.3	1.9, 2.2	2.2, 2.4	3.2, 3.1	—
4	0.4	—	0.1, 0.3	—	1.7, 2.2	—	—	2.8, 3.1

[a] Meyerhof units = μg of phosphorus of triose phosphate produced/min at 38°.

[b] Animal 1 became sick during experimental period and was not included for statistical reasons.

[c] Normal animals 2–4 were on E-supplemented diet a total of 57, 50, and 51 days, respectively; dystrophic animals 1–4 were on E-deficient diet 45, 54, 35, and 51 days, respectively.

TABLE II. Fat Content, Dry Fat-Free Weight Content and Percentage Distribution of Nitrogen in Tissue (mean ± SEM; sample no. in parentheses).

(%)	Rabbit gastrocnemius muscle		
	Normal	Dystrophic	p[d]
Fat[ab]	0.85 ± 0.16 (6)	2.06 ± 0.25 (7)	<0.005
DFF wt[b]	23.82 ± 0.24 (6)	20.76 ± 1.03 (7)	<0.05
Coll N[c]	0.1 ± 0.0 (6)	0.57 ± 0.09 (7)	<0.001
NCN[c]	2.88 ± 0.05 (6)	1.61 ± 0.13 (7)	<0.001

[a] Abbrev.: DFF wt = dry fat-free weight; Coll N = collagen nitrogen; NCN = noncollagen nitrogen.

[b] Percentage dry weight equals percentage fat plus percentage DFF wt in each group.

[c] Percentage total nitrogen equals percentage Coll N plus percentage NCN in each group.

[d] Comparison of normal and dystrophic muscle contents.

days. Both groups of animals tend to show an initial dip in aldolase levels due to adjustments to their respective diets.

Turning to skeletal muscle, Table II shows that the average fat content of the vitamin E-deficient tissue based on wet weight is elevated approximately three times over that of the normal. A great increase in the collagen nitrogen content is observed in these dystrophic organs which is accompanied by a 45 and 25% reduction, respectively, in the levels of NCN and total nitrogen.

Liver, on the other hand, displays somewhat different results. Table III reveals a 40% increase both in NCN and total nitrogen with essentially no change in collagen content. However, hepatic DFF wt is depressed by 25% in the dystrophic state.

The activity of aldolase in diseased muscle is reduced to one seventh of normal when expressed on a DFF wt basis and to one fifth of normal in terms of NCN (Table IV). However in liver, this activity is elevated to three times normal on the former base line, although the increase is not significant ($.10 < p < .05$) when NCN is considered.

Discussion. The increase of collagen nitrogen and fat content observed herein in vitamin E-deficient rabbit muscle, with a decrease in total nitrogen, has been previously described by other investigators (25, 26, 29). In addition a chemical alteration common to the hereditary dystrophies (5, 27), namely, a reduction in NCN was also seen in our studies.

Liver, on the other hand, shows in the E-dystrophic state an approximate rise of 40% in NCN. The reference base NCN has often been employed in conditions which involve muscle wasting (4) and enzymatic activities in both tissues studied exhibit less of a change in concentration when expressed on the nitrogen parameter than on DFF wt.

The decrease in muscle aldolase and increase in serum enzyme observed in dystrophic rabbits is similar in magnitude to that found in progressive muscular dystrophy patients (1, 4). Serum aldolase is also elevated

TABLE III. Fat Content, Dry Fat-Free Weight Content and Percentage Distribution of Nitrogen in Tissue (mean ± SEM; sample no. in parentheses).

(%)	Rabbit liver		
	Normal	Dystrophic	p[b]
Fat	2.63 ± 0.27 (3)	3.28 ± 0.75 (4)	NS
DFF wt	27.20 ± 0.71 (3)	21.22 ± 1.09 (4)	<0.005
NCN[a]	1.73 ± 0.24 (3)	2.45 ± 0.12 (4)	<0.05

[a] Percentage total nitrogen equals percent NCN since Coll N is <0.05% in liver.

[b] Comparison of normal and dystrophic liver contents.

TABLE IV. Aldolase Activity in Rabbit Tissues (mean ± SEM; sample no. in parentheses).

	Animals		
Units[a]/g	Normal	Dystrophic	p[c]
DFF wt[b] (muscle)	86.30 ± 8.11 (6)	12.03 ± 3.25 (7)	<0.001
NCN (muscle)	710 ± 63 (6)	144 ± 28 (7)	<0.001
DFF wt (liver)	34.23 ± 4.58 (3)	99.72 ± 4.82 (4)	<0.001
NCN (liver)	584 ±145 (3)	887 ± 70 (4)	NS

[a] Meyerhof units = μg of phosphorus triose phosphate produced/min at 38°.

[b] Abbrev.: DFF wt = dry fat-free weight; NCN = noncollagen nitrogen.

[c] Comparison of enzymatic activity in normal and dystrophic tissue based on parameter specified.

in inherited muscular dystrophy of mice (6) and chickens (7), though the reports on tissue levels of the enzyme in the above animals are either incomplete or conflicting (5, 27).

Previous investigations of this enzyme in vitamin E-deficient rats by Beckmann and Buddecke (28) revealed an elevation in muscle and serum aldolase activities but no alteration in the liver concentration. Although their evidence appears to conflict with our observations on dystrophic rabbits, these discrepancies may be obviated if their values for enzymatic activity were expressed in terms of NCN and consideration was given to the more rapid rate of muscle regeneration in rats compared to herbivora (29).

The increased level of aldolase observed in the liver in this investigation is possibly the result of a clearing effect by this organ of enzymes from the blood stream. A similar explanation was previously used by Sherlock and Walshe (30) to describe increased levels of hepatic alkaline phosphatase in generalized bone disease.

An elevated level of NCN and total nitrogen of the liver in our studies also favors such a hypothesis. Although a second enzyme, GOT, exhibited an increase in concentration in this organ during dystrophy, the results exhibited too much variation to be significant.

Leakage of aldolase from vitamin E-deficient muscle appears to be an early chemical event in the course of the nutritional disease for it preceded and paralleled the usual onset of creatinuria by 1 to 2.5 weeks. Although the physiologic role of the vitamin remains unknown in this process, it may function to maintain the integrity of the lipoprotein complex which composes the muscle membrane so that the membrane acts as a diffusion barrier for the enzyme.

Summary. Dystrophic rabbit muscle showed a decrease in total nitrogen, noncollagen nitrogen (NCN), dry weight and dry fat-free weight (DFF wt). Collagen nitrogen and the fat content of the tissue were found to be increased over that seen in the controls. Livers of E-deficient animals displayed a 40% rise in total nitrogen and NCN versus normals. Aldolase activity was markedly decreased in dystrophic muscle, but liver revealed an elevation in its enzyme concentration compared to controls. The elevation of serum aldolase in E-deficient rabbits preceded and paralleled the creatinuria associated with the development of dystrophy by a period of 1 to 2.5 weeks. These conclusions were compared to those observed in other forms of muscle wasting.

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