

Lactic Dehydrogenase Isoenzymes in Tissues and Serum of Normal And Dystrophic Lambs.* (31124)

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Lactic dehydrogenase (LDH) is known to occur as isoenzymes which represent the 5 possible tetramers of the so-called "H" and "M" subunits(1) under the control of separate genetic loci(2-4). Electrophoretic separations of LDH preparations from various organs of the body show characteristic isoenzyme patterns, and changes in the LDH pattern in human serum have proven useful for detection of necrosis in specific tissues(5). It is assumed that the LDH pattern of necrotic tissue is added to the LDH pattern characteristic of normal serum. In certain types of hereditary muscular dystrophy the LDH isoenzyme distribution becomes altered in the affected muscles with distinctive serum isoenzyme patterns resulting(6,7). Nutritional muscular dystrophy (NMD) in the lamb is characterized by marked increases in serum glutamic oxaloacetic transaminase (SGOT) and in serum LDH activity(8,9).

Boyd(10) has fractionated the LDH isoenzymes in the tissues and serum of normal lambs and from lambs with nutritional dystrophy. Dystrophic serum showed increases in all 5 forms of LDH with the most marked increase in LDH V. The proportions of the isoenzymes in the tissues were very similar for normal and dystrophic animals. The present study describes changes in all 5 isoenzymes both in the serum and in the muscle from lambs made dystrophic on a diet deficient in vit. E and selenium.

Methods. Two-day-old lambs were fed artificial milk of the following percentage composition: torula yeast,† 12.00; cerelose, 4.14; stripped lard, 3.00; methionine, 0.10; minerals,‡ 0.70; choline, 0.02; vitamins,§ 0.04; and water, 80.00. Each of 16 animals in Ex-

periment I was depleted until its SGOT activity increased to 1000 units/ml (average age, 18 days), when it was placed in one of 4 lots for the remainder of an 8-week period. The treatments imposed were Na₂SeO₄ equivalent to 2 parts of selenium per million (dry matter), 250 ppm of dl α -tocopherol, both selenium and tocopherol, or no supplement. Four positive control animals received the artificial milk supplemented with both tocopherol and selenium from birth. Appropriate amounts of an aqueous solution of Na₂SeO₄ or of vit. E in olive oil were given orally once per day. In Experiment II, six lambs were unsupplemented and 4 received selenium and tocopherol supplements throughout the experiment. Three unsupplemented animals and 2 supplemented control animals were sacrificed when the serum LDH activity of the unsupplemented animals first exceeded 2000 units/ml. The other 3 unsupplemented and 2 supplemented control animals were sacrificed when the serum LDH activity of the unsupplemented animals had remained elevated for 18 days.

Blood samples, collected at weekly intervals by jugular venipuncture, were allowed to clot, and the serum was stored at -10°C until analyzed. Tissues were removed immediately after the animals were sacrificed and stored at -10°C. Tissue extracts were prepared for electrophoresis by homogenizing in 0.1 M phosphate buffer (pH 7.4) in a conical all glass tissue grinder and centrifuging at 10,000 × g for 5 minutes in a Servall-automatic refrigerated model RC 2 centrifuge with

† Composition of the mineral premix was: CaCO₃, 71.00%; NaCl, 28.47%; KIO₃, 0.05%; CoCl₂ • 6H₂O, 0.12% and Na₂MoO₄ • 2H₂O, 0.36%.

§ Composition of the vitamin premix was: Inositol, 10.00%; calcium pantothenate, 0.60%; menadione, 0.40%; B₁₂ (1% titration), 1.00%; and sucrose, 88.00%. Lambs were also given 1 ml of olive oil containing 5,000 IU of vit. A and 500 IU of vit. D₃ daily.

* Published with approval of the Director of Wisconsin Agri. Exp. Station. Supported in part by Wisconsin Alumni Research Foundation and Selenium-Tellurium Development Committee.

† Torula yeast (USP), Lake States Yeast Corp., Rhinelander, Wisc.

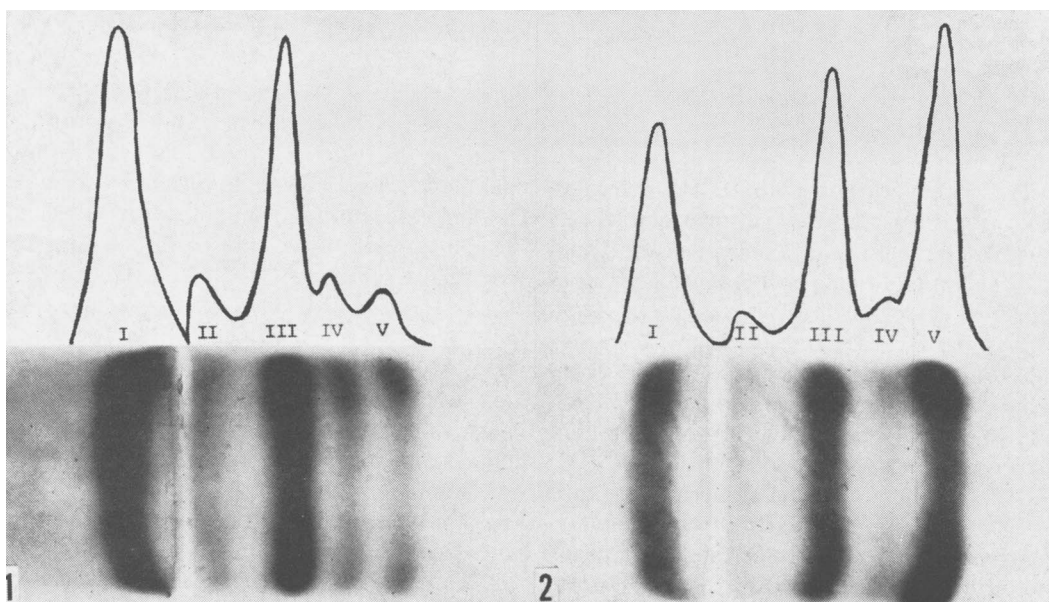


FIG. 1. Zymogram and densitometer tracing for normal lamb serum (3 LDH units per slide).

FIG. 2. Zymogram and densitometer tracing for serum from dystrophic lamb (3 LDH units per slide).

the SS-34 rotor. Total LDH activity in serum and tissue extracts was determined by the method of Amador *et al*(11), and SGOT determinations were as outlined by Sigma Chemical Co.(12). LDH isoenzymes were separated by agar gel electrophoresis for 75 minutes with a voltage gradient of 29.3 volts/cm. The barbital buffer used, electrophoresis apparatus and the details for preparing agar slides have been described(13). Zymograms were stained for LDH activity by the method of Hicks and Nalevac(14) and then scanned with a Densicord Recording Electrophoresis densitometer, model 542. The area under each peak was measured with a planimeter and the percentage of the total represented by each form calculated. The units of each isoenzyme present were then obtained by multiplying the total LDH activity for that sample by the percentage accounted for by each isoenzyme. The results were analyzed statistically by Duncan's multiple range test(15).

Results. Lambs fed the nonsupplemented artificial milk developed clinical and pathological symptoms of NMD as previously described(16). All nonsupplemented animals in Exp. I died (average age, 28 days), while all animals treated therapeutically with both vit.

E and selenium or given both nutrients from birth survived and appeared completely normal when the experiment was terminated. Two out of 4 deficient animals treated with selenium alone and 3 out of 4 which received vit. E alone survived for the remainder of the 8-week period but were visibly inferior to animals which received both nutrients. The average daily gains were 169 ± 31.6 g (average \pm standard error) on vit. E and selenium, 150 ± 37.4 g on selenium alone, and 105 ± 18.6 g on vit. E alone, as compared to 218 ± 10.4 g for controls which received both nutrients from birth. In Exp. II the average daily gains for the supplemented and unsupplemented animals were 171 g and 141 g, respectively.

Serum GOT and LDH activity increased progressively with the development of dystrophy. The largest percentage increase occurred in LDH V, followed by isoenzymes IV, III, II and I in that sequence (Table I). However, the largest increase in unitage occurred in LDH III followed by enzyme forms V, I, IV and II. Fig. 1 and 2 show typical serum LDH patterns for normal and dystrophic lambs. Vitamin E with or without selenium restored SGOT and LDH totals to

TABLE I. Total Enzyme and Isoenzyme in Serum of Normal and Deficient Lambs.

Enzyme form	Normal,* units/ml	Deficient,† units/ml	Increase over normal	
			%	Units/ml
LDH I H ₁	151 ± 7‡	595 ± 67	294	444
LDH II H ₂ M	21 ± 3	115 ± 23	447	94
LDH III H ₂ M ₂	96 ± 5	812 ± 193	705	716
LDH IV HM ₃	9 ± 2	117 ± 36	1200	108
LDH V M ₄	5 ± 1	455 ± 140	9000	450
Total LDH	281 ± 10	2094 ± 355	645	1813
SGOT	63 ± 4	1916 ± 260	2941	1853

* All observations from all positive control animals were utilized for a total of 36 values.

† This includes 16 observations from animals depleted of vit. E and selenium at the time of initial supplementation.

‡ Average ± standard error.

TABLE II. Total Lactic Dehydrogenase Activity and Isoenzyme Distribution in Tissues of Normal and Dystrophic Lambs.*

Tissue	% of total activity					Total LDH activity, units/mg dry matter
	I	II	III	IV	V	
Normal						
Adrenal	63 ± 12	18 ± 6	18 ± 6	1 ± 1	—	48 ± 7
Pancreas	40 ± 6	32 ± 2	28 ± 4	—	—	70 ± 7
Lung	41 ± 4	18 ± 2	39 ± 1	2 ± 1	—	66 ± 8
Spleen	41 ± 4	20 ± 2	38 ± 2	1 ± 1	—	123 ± 4
Brain	42 ± 6	20 ± 2	35 ± 6	2 ± 1	1 ± 1	186 ± 24
Liver	85 ± 4	8 ± 1	7 ± 4	—	—	224 ± 36
Kidney	67 ± 7	22 ± 4	11 ± 3	—	—	216 ± 6
Heart	82 ± 5	12 ± 3	6 ± 1	—	—	355 ± 21
Diaphragm	11 ± 5	5 ± 1	31 ± 4	13 ± 3	40 ± 5	824 ± 74
Tongue	—	1 ± 1	41 ± 8	20 ± 1	38 ± 6	1155 ± 69
Gluteus medius	—	2 ± 1	10 ± 2	8 ± 1	80 ± 2	1352 ± 274
Longissimus dorsi	—	1 ± 0	4 ± 1	7 ± 1	88 ± 2	1294 ± 123
Early dystrophy						
Gluteus medius	—	1 ± 1	19 ± 2	21 ± 2	59 ± 3	848 ± 34
Longissimus dorsi	—	2 ± 1	11 ± 2	18 ± 5	69 ± 1	881 ± 81
Advanced dystrophy						
Gluteus medius	2 ± 1	5 ± 2	24 ± 1	16 ± 2	54 ± 2	920 ± 101
Longissimus dorsi	2 ± 1	3 ± 1	17 ± 6	23 ± 3	56 ± 5	841 ± 410

* Average ± standard error.

normal and also restored the normal distribution of LDH isoenzymes. Supplementation with 2 ppm of Se resulted in only a temporary decline in blood enzyme activity with a return to an abnormal isoenzyme distribution within 1 week after initiation of treatment.

Ten lambs which had developed spontaneous NMD under field conditions showed the same highly elevated serum enzyme activity and abnormal serum LDH isoenzyme distribution as in those fed artificial milk; normal total activity and isoenzyme patterns resulted when selenium and vit. E were administered.

Table II shows total LDH activities in various tissues and percentages of the total activity represented by the various isoenzymes.

Tissues in which LDH I predominated tended to have low total activity, and tissues in which LDH V predominated were high in total activity. In most of the tissues total enzyme activity and isoenzyme distribution were very similar in normal and in dystrophic animals (data not shown). However, in skeletal muscle from dystrophic lambs there was a highly significant ($P < 0.01$) decrease in the percentage of LDH V, and a highly significant ($P < 0.01$) increase in the percentages of LDH III and LDH IV both in early and advanced dystrophy. The total LDH activity in dystrophic skeletal muscle tended to decrease, and the percentage of LDH I and II tended to be higher than normal. Skeletal muscle from 4

lambs in which NMD had developed under field conditions showed similar changes in LDH distribution.

Discussion. Lambs with NMD may show visible pathological changes not only in the skeletal muscle, but also in the heart, liver or kidney(17,18). The predominant isoenzymes in these tissues are LDH V in skeletal muscle, and LDH I in heart, liver and kidney tissue. Thus the observed increases in serum LDH I and V in the serum of our dystrophic animals is not surprising. On the other hand, the extremely large increase in serum LDH III would be difficult to explain on the basis of a simple release of normal isoenzymes from tissues known to be damaged in NMD. However, a significant increase in LDH III was observed in the muscle as dystrophy developed, and hence it is likely that this is a source of the LDH III in the serum of dystrophic lambs. But blood levels of isoenzymes do not necessarily parallel those in damaged muscle as shown by the fact that in most cases the increase in LDH IV in skeletal muscle exceeded the increase in LDH III, yet the rise in serum LDH IV was much smaller than that of LDH III. Presumably variations in blood enzymes also reflect phenomena such as the release of enzymes from tissues other than muscle, unequal rates of release of different enzymes from damaged tissue, and unequal rates of inactivation or removal from the blood.

Other workers have observed pronounced changes in the isoenzyme distribution of skeletal muscle from humans(6) and chickens(7) with hereditary muscular dystrophy. Kaplan and Cahn(7) have postulated that the hereditary disease in chickens causes a block in the synthesis of the M subunit. More recently Brody(19) observed a reversion to an embryonic isoenzyme pattern in experimentally denervated guinea pig muscle. The dystrophic muscle in the present nutritional study likewise showed an isoenzyme pattern which tended to revert toward that in embryonic ovine tissue in which the LDH I predominates(20).

Summary. Serum from lambs with nutritional muscular dystrophy showed increases in all 5 forms of lactic dehydrogenase (LDH).

The largest percentage increase occurred in LDH V, followed by isoenzymes IV, III, II and I. The largest absolute increase was in LDH III. Therapy with vit. E alone or with vit. E plus selenium restored normal levels of all isoenzymes. Treatment with selenium alone diminished serum LDH only temporarily. Data are presented for the LDH isoenzymes in lamb tissues. Skeletal muscle from dystrophic lambs showed a decrease in the percentage of LDH V and increases in the percentages of the other LDH forms. The other tissues from dystrophic animals showed an essentially normal LDH isoenzyme distribution.

1. Cahn, R. D., Kaplan, N. O., Levine, L., Zwilling, E., *Science*, 1962, v136, 962.
2. Appella, E., Markert, C. L., *Biochem. Biophys. Res. Commun.*, 1961, v6, 171.
3. Costello, L. A., Kaplan, N. O., *Biochim. Biophys. Acta*, 1963, v73, 658.
4. Boyer, S. H., Fainer, D. C., *Science*, 1963, v141, 642.
5. Wroblewski, F., Gregory, K. F., *Ann. N. Y. Acad. Sci.*, 1961, v94, 912.
6. Weime, R. J., Herpol, J. E., *Nature*, 1962, v194, 287.
7. Kaplan, N. O., Cahn, R. D., *Proc. Nat. Acad. Sci.*, 1962, v48, 2133.
8. Blincoe, C., Dye, W. B., *J. Animal Sci.*, 1958, v17, 224.
9. Clincoe, C., Marble, D. W., *Am. J. Vet. Res.*, 1960, v21, 866.
10. Boyd, J. W., *Biochem. J.*, 1964, v92, 17P.
11. Amador, E., Dorfman, L. E., Wacker, W. E. C., *Clin. Chem.*, 1963, v9, 391.
12. Sigma Chemical Co., *Tech. Bull.* 410, 1958.
13. Yakulis, V. J., Heller, P., Josephson, A. M., Singer, L., *Am. J. Clin. Path.*, 1960, v34, 28.
14. Hicks, G. P., Nalevac, G. N., *Anal. Biochem.*, 1965, v13, 199.
15. Steel, G. D., Torrie, J. H., *Principles and Procedures of Statistics*, McGraw-Hill Co. Inc., New York, 1960, p107.
16. Hopkins, L. L., Pope, A. L., Baumann, C. A., *J. Animal Sci.*, 1964, v23, 674.
17. Young, M. S., Keeler, R. F., *Am. J. Vet. Res.*, 1962, v23, 966.
18. Hartley, W. J., Grant, A. B., *Fed. Proc.*, 1961, v20, 679.
19. Brody, I. A., *Nature*, 1965, v205, 196.
20. Hinks, M., Masters, C. J., *Biochemistry*, 1964, v3, 1789.

Received February 24, 1966. P.S.E.B.M., 1966, v122.