Alterations in Lactate Dehydrogenase of the Brain, Heart, Skeletal Muscle, and Liver of Rats of Various Ages*

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SUMMARY

The activity of lactate dehydrogenase of the supernatant fraction of brain, heart, skeletal muscle, and liver of 1-day and 4-, 12-, 30-, 74-, and 96-week old rats was determined to study the changes in this enzyme with increasing age. Units of enzyme per g, wet weight, of all the tissues increased during the growth phase, reaching a maximum at 30 weeks, and then decreased. NADH\textsubscript{2}:NADH\textsubscript{4} ratios, \(K_m\) values, and percentage of inhibition of lactate dehydrogenase by oxalate showed that the ratio of H-lactate dehydrogenase to M-lactate dehydrogenase increased after adulthood. This was due to a decrease of M-lactate dehydrogenase and not due to any increase in H-lactate dehydrogenase. Lower levels of lactate dehydrogenase and of M-lactate dehydrogenase in old age may make the tissues more oxygen dependent and decrease the period of tolerance for anaerobic conditions.

One of the well known changes that occur in the tissues of aging animals is the alteration in the activities of several enzymes (1-4). Although a decrease or an increase in the activity of a particular enzyme is known to occur with increasing age, the cause of such a change is not known. Therefore, in an attempt to understand some of the basic mechanisms involved in the aging process, a detailed study was made of the changes of one particular enzyme, lactate dehydrogenase (L-lactate:NAD reductase, EC 1.1.1.27), of the brain, heart, skeletal muscle, and liver of rats of various ages. Lactate dehydrogenase was chosen for this study because a good deal of information is available about its enzymic characteristics and genetic control. Furthermore, lactate dehydrogenase is essential for anaerobic glycolysis, and hence is necessary for muscular work in the absence of oxygen. One of the changes that occur in old age is a decrease in the capacity for muscular work, and alterations in lactate dehydrogenase may be one of the factors contributing to this change.

Lactate dehydrogenase has five isozymes, \(H_5\), \(H_3M_1\), \(H_2M_2\), \(H_1M_3\), and \(M_4\) (5). These are composed of two types of subunits, H and M, which are under the control of two separate genes (6). The isozyme pattern is characteristic for each tissue (7). For example, \(H_5\) is found predominantly in tissues which are essentially aerobic, such as the heart and the adrenal cortex, and \(M_4\) is rich in tissues that are essentially anaerobic, such as the skeletal muscle and the adrenal medulla. The turnover number and \(K_m\) value of \(H_4\) for pyruvate are lower than those of \(M_4\) (8). Also, \(H_5\) is inhibited by 0.33 mM oxalate (9) and high pyruvate (10 mM), whereas \(M_4\) is not (8). These characteristics were used to study the changes in the isozymes of lactate dehydrogenase of the brain, heart, skeletal muscle, and liver of the rat at various ages. The cells of the brain, heart, and skeletal muscle are postmitotic, that is, they do not divide after a certain period of growth of the animal and might be expected to show the maximum effect of age on the activity of the enzyme. The liver was chosen to represent a premitotic tissue. The results reported here show that M-lactate dehydrogenase of these tissues decreases in old age of the rat.

MATERIALS AND METHODS

Animals—The albino rats used were of Wistar strain and were kept at 24 ± 2°C. They were given a commercial rat diet (Anidiet A, Chelsea Laboratories, Poona, India). The rats were also given gram (Cicer arietinum) on alternate days, and water ad libitum. Only female rats were used, since their post-reproductive period is well defined (16 months). The ages of the rats used were 1 day and 4, 12, 30, 74, and 96 weeks. The results for each group were obtained from five or six rats which were killed at a fixed time on successive days.

Preparation of Tissues—The rats were killed by dislocation of the neck, and the brain (cerebral hemispheres), heart, skeletal muscle (gastrocnemius), and liver were removed immediately, washed in cold 0.1 M potassium phosphate buffer (pH 7.4), and weighed. A 10% homogenate (w/v) of each tissue was prepared in ice-cold 0.1 M phosphate buffer with the use of a Potter-Elvehjem homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 12,000 × g in an International refrigerated
The method of assay is given in the text. Data were collected from 5 animals of each age. Standard deviations and the levels of significance are given.

<table>
<thead>
<tr>
<th>Age</th>
<th>Brain</th>
<th>Heart</th>
<th>Skeletal muscle</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>24.80 ± 2.05</td>
<td>156.90 ± 25.70</td>
<td>49.60 ± 8.54</td>
<td>195.56 ± 24.78</td>
</tr>
<tr>
<td>4 weeks</td>
<td>62.64 ± 2.74</td>
<td>383.56 ± 62.05</td>
<td>263.20 ± 33.66</td>
<td>301.08 ± 27.87</td>
</tr>
<tr>
<td>12 weeks</td>
<td>69.27 ± 2.52</td>
<td>581.81 ± 86.81</td>
<td>425.04 ± 51.93</td>
<td>286.06 ± 23.93</td>
</tr>
<tr>
<td>30 weeks</td>
<td>74.54 ± 5.07</td>
<td>712.00 ± 73.80</td>
<td>644.22 ± 53.68</td>
<td>251.44 ± 14.99</td>
</tr>
<tr>
<td>74 weeks</td>
<td>63.67 ± 5.30</td>
<td>387.00 ± 10.11</td>
<td>480.95 ± 44.57</td>
<td>266.65 ± 40.93</td>
</tr>
<tr>
<td>96 weeks</td>
<td>45.13 ± 3.89</td>
<td>271.86 ± 31.94</td>
<td>403.64 ± 58.84</td>
<td>201.72 ± 11.55</td>
</tr>
</tbody>
</table>

| CENTRIFUGE for 30 min to remove the nuclei, cell membranes, and mitochondria. The supernatant fraction was collected and suitably diluted with 0.1 M phosphate buffer, pH 7.4, for the assay of lactate dehydrogenase.

**Spectrophotometric Assay of Lactate Dehydrogenase—**NADH was obtained from CalBion, and sodium pyruvate, KH2PO4, and K2HPO4 were obtained from E. Merck, West Germany. The method of assay of lactate dehydrogenase was that of Kornberg (10). The assay mixture consisted of 1.84 ml of water, 1.0 ml of phosphate buffer (pH 7.4), NADH to give an absorbance value of 0.5, 0.05 ml of supernatant, and 0.1 ml of pyruvate (0.33 mM final concentration). The total volume was 3.0 ml; the reaction was started by adding pyruvate last and noting the decrease in A405 at intervals of 30 sec in a Beckman DB model spectrophotometer. The activity of lactate dehydrogenase of each tissue was expressed as units per g of tissue, wet weight.

**Determination of Proportions of Subunits—**Measurements of the activity of lactate dehydrogenase at “low” (0.33 mM) and “high” (10.0 mM) pyruvate concentrations were made to determine the NADH4:NADH3 ratios of each tissue, according to the method of Goodfriend, Sokol, and Kaplan (11), since this ratio is directly proportional to the concentration ratio of H subunits to M subunits. The change in the proportions of H and M subunits were also studied by determining the apparent Km value of lactate dehydrogenase for pyruvate and by determining the extent of inhibition of the activity of the enzyme by 0.33 mM oxalate in the presence of 0.33 mM pyruvate (9).

The concentration of protein in the supernatant fraction was determined (12), and the standard deviation of the mean of the results obtained from 5 or 6 animals and the levels of significance between two sets of data were calculated.

**RESULTS**

**Units of Enzyme—**Table I shows that the activity of lactate dehydrogenase in the brain, heart, and skeletal muscle increased significantly until 30 weeks and then declined. The activity at 30 weeks was 3-, 5-, and 10-fold higher than that at 1 day, and 2-, 2.5-, and 1.8-fold higher than that at 96 weeks in the brain, heart, and skeletal muscle, respectively. In the liver, the activity increased until 4 weeks and then decreased until 12 weeks, after which there was no significant change.

**Proportions of Subunits—**Fig. 1 shows that in all four tissues the NADH4:NADH3 ratio increased significantly after 30

![Fig. 1. NADH4:NADH3 ratios of the brain (O), heart (C), skeletal muscle (A) and liver (V) of rats of various ages. Lactate dehydrogenase was assayed at 0.33 mM (NADH4) and 10.0 mM (NADH3) pyruvate. Each point represents the mean for five or six animals. Vertical bars represent standard deviations.](https://via.placeholder.com/150)

![Fig. 2. Effect of age on apparent Km for pyruvate of lactate dehydrogenase of the brain (O), heart (C), skeletal muscle (A), and liver (V) of rat. Data were obtained from five or six animals. The enzyme was assayed in 0.033, 0.066, 0.11, 0.33, 0.66, and 1.3 mM pyruvate.](https://via.placeholder.com/150)
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FIG. 3. Percentage of inhibition of the activity of lactate dehydrogenase of the brain (●), heart (○), skeletal muscle (△), and liver (□) of rats of various ages. The enzyme was assayed at 0.33 mM pyruvate in the presence 0.33 mM oxalate.

FIG. 4. Concentration of protein (milligrams per g, wet weight) in the supernatant fraction of the brain (●), heart (○), skeletal muscle (△), and liver (□) of rats of various ages.

weeks. In the brain, this increase was evident even earlier, that is, from 12 weeks. The $K_m$ for pyruvate of lactate dehydrogenase in the two aerobic tissues, the brain and the heart, decreased during the growth period followed by a rise until 12 weeks and then a gradual decrease until 96 weeks (Fig. 2).

The two anaerobic tissues (skeletal muscle and liver), on the other hand, showed an increase during the growth period followed by a decrease. Particularly, in the skeletal muscle the $K_m$ value increased 2-fold during growth. The percentage of inhibition of the activity of the enzyme by oxalate was also similar for the heart and the brain on one hand and skeletal muscle and liver on the other (Fig. 3).

Protein Content of Supernatant Fraction—Fig. 4 shows that the protein content of the supernatant fraction of the brain did not change significantly until 74 weeks, and decreased thereafter. In the heart, skeletal muscle, and liver, however, there was a significant increase until 30 weeks, which was followed by a decrease.

DISCUSSION

The life span of an animal may be broadly divided into two periods: growth and senescence. In this set of experiments, 1-day and 4-, 12-, and 30-week old rats represent the various stages in the growth period; the 30-week old rat represents the peak of the growth period. The 74- and 96-week old rats represent the senescence phase. Thus, the changes in lactate dehydrogenase studied here cover the entire life span of the animal.

Our studies show that the activity of lactate dehydrogenase (units per g, wet weight) in the brain, heart, and skeletal muscle increases during growth and decreases in old age. In the liver, however, no decrease was observed in old age. The general nature of change in the activity of lactate dehydrogenase of the four tissues on the basis of dry weight may also be considered as similar to that of wet weight after 12 weeks of age, as there was no significant change in the water content of the tissues after this age. During the earlier period of growth (0 to 12 weeks), the water content declines by 5 to 10%. However, the increase in the activity of the enzyme during this period is more than 200% in the brain, heart, and skeletal muscle, and 50% in the liver.

The alterations in the specific activity (units per mg of protein) of lactate dehydrogenase as calculated on the basis of soluble protein in the supernatant fraction were also similar to those of units per g, wet weight, in all the four tissues studied.

Alterations in H- and M-Lactate Dehydrogenase—NADH₄: NADH₄ ratios, or the ratios of H to M subunits of all the four tissues, are higher in old age (96 weeks) than in the adult or younger rats. The studies on $K_m$ values and percentage of inhibition by oxalate indicate that this increase is due to a decrease in the proportion of M subunits, and not due to any change in H subunits. Hinks and Masters (13) have reported an increase in H, during the developmental period beginning from the fetus to the adult stage. Our studies show that in the old age also there is a higher proportion of M subunits. This may be a disadvantage to the tissues in carrying out anaerobic glycolysis when oxygen is absent, since the turnover number of $H_4$ is lower than that of $M_4$. Thus, the capacity of the tissues of the older animal to work in anaerobic conditions may be lower than that of the adult tissues. This is particularly true of the heart, which is aerobic and in which the increase in H:M ratio is the highest. The synthesis of more M subunits by heart cells in culture and their inhibition in the presence of oxygen (11) support the above conclusions.

The skeletal muscle, which is rich in M subunits, is of special interest in this context. During vigorous muscular activity in the adult, when oxygen cannot be supplied as fast as is required, ATP is generated by the breakdown of glycogen anaerobically, with the result that lactate accumulates. A high level of lactic acid is not harmful to the muscle, although it may be harmful...
to the heart (14). The increase in the proportion of M subunits which occurs between 12 and 30 weeks may be of advantage to this tissue for producing energy in the absence of oxygen in adulthood when it is most active.

Furthermore, the possibility of a change in the regulation of lactate dehydrogenase by the intermediates of the citric acid cycle in old age cannot be discounted, since Fritz (15) has shown that M, but not H, is stimulated by these intermediates. Preliminary reports showing a decrease in the subunits of the brain, heart, and skeletal muscle of old rats have been published (16).

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REFERENCES
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