EFFECT OF AGE ON THE ISOZYMES OF LACTIC DEHYDROGENASE OF THE HEART AND THE BRAIN OF RAT

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Lactic dehydrogenase (LDH) has five isozymes, H_4, H_3M_1, H_2M_2, H_1M_3 and M_4, which differ from each other in their kinetic properties (Dawson et al., 1964). These workers have shown that the turn over number of the chicken M_4-LDH for the forward reaction, pyruvate ⟶ lactate, is higher than that of H_4-LDH and the isozyme composition of the LDH of the skeletal muscle of the rabbit changes during its development. Brody (1965) has shown that the LDH pattern of the skeletal muscle of adult rat changes after denervation of the muscle. This shows that the isozymes of LDH are subject to alterations with the change in the environment of the tissue. We show here that the isozyme patterns of LDH of the brain and the heart of rat change in a manner that may decrease the capacity of these tissues to tolerate anaerobic condition with increasing age.

MATERIALS AND METHODS. Cerebral hemispheres of 1 day and 4, 12, 30 and 74 week old rats and also their hearts were taken out and washed in 0.1 M phosphate buffer (pH, 7.4). Homogenate of each tissue (10%, w/v) was prepared at 0°C. in the above buffer using a Potter-Elvehjem homogenizer fitted with a teflon pestle. The homogenate was centrifuged at 8,000 g in an International Refri-

gerated Centrifuge. Suitable dilutions of each supernatant were made in the buffer before assaying for the LDH (Kornberg, 1955). Pyruvate (3.0×10^{-4}M) was added last and the rate of decrease in extinction at 340 mμ was recorded for 210 min. in a Double Beam Beckman Spectrophotometer for the determination of units of enzyme/g. wet wt. Also the ratios of the decrease in extinction at pyruvate concentrations of 3.0×10^{-4}M and 1.0×10^{-2}M ($NADH_L/NADH_H$) were calculated and the data of Fine et al. (1963) on the rat isozymes were used for the determination of the proportions of H_4- and M_4-LDH in each tissue. NADH instead of its hypoxanthine analog was used as according to Dawson et al. (1964) the analog only exaggerates the effects at both the concentrations of pyruvate, and therefore has no effect on the ratio.

The Michaelis constants (K_m) of the enzyme in the supernatants of both the tissues were determined by the usual Lineweaver-Burk method by recording the extinction at pyruvate concentrations of 0.033, 0.066, 0.1, 0.30 and 0.66×10^{-3}M.

RESULTS AND DISCUSSION. Fig. 1 shows the changes in the total LDH and the proportions of H_4 and M_4-LDH in the brain with increasing age of the rat. The total LDH increased sharply during the development till 30 weeks of age which is the young adult stage. Thereafter there was a decrease in the activity of the enzyme till 74 weeks which is the post-reproductive phase. The proportion of H_4 increased sharply and that of M_4 less sharply till 12 weeks of age when the rat matures. Then the proportion of H_4 continued to increase slowly and that of M_4 decreased till 74 weeks resulting in an increase in $NADH_L/NADH_H$ ratio. This is supported by the finding that the K_m of LDH decreased after adulthood indicating increasing proportions of H_4 (Fig. 3). This is based on the assumption that the rat H_4-LDH

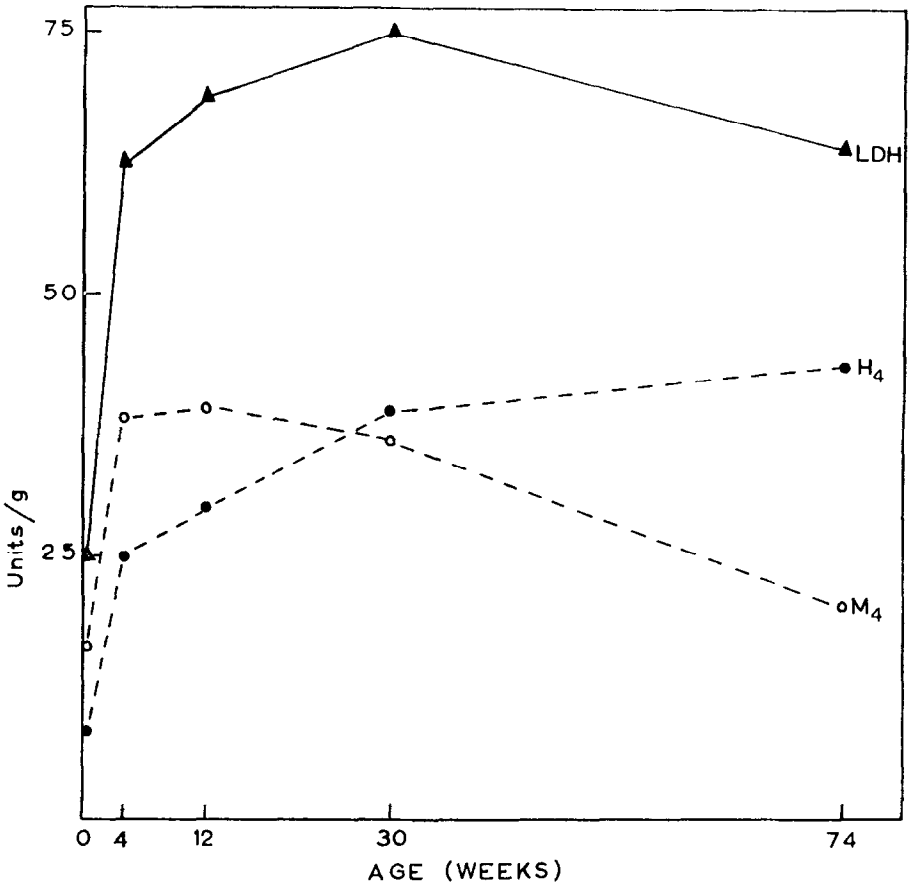

Fig. 1. Activity of lactic dehydrogenase (units/g. wet wt. of total LDH and of H_4 and M_4 isozymes) in the brain of rats of different ages. The assay methods are described in the text. Each point represents the average of the data from five animals. ▲, total LDH; ●, H_4; o, M_4.

has a lower K_m value than that of M_4 as in the case of chicken isozymes (Dawson et al., 1964).

In the heart tissue (Fig. 2) the total LDH increased sharply till 30 weeks. Then the activity declined, the total LDH at 74 weeks being about one-half of that at 30 weeks. The H_4 and M_4 also increased in activity up to 30 weeks followed by a decrease in both the isozymes till 74 weeks. However, the rate of decrease of M_4 was

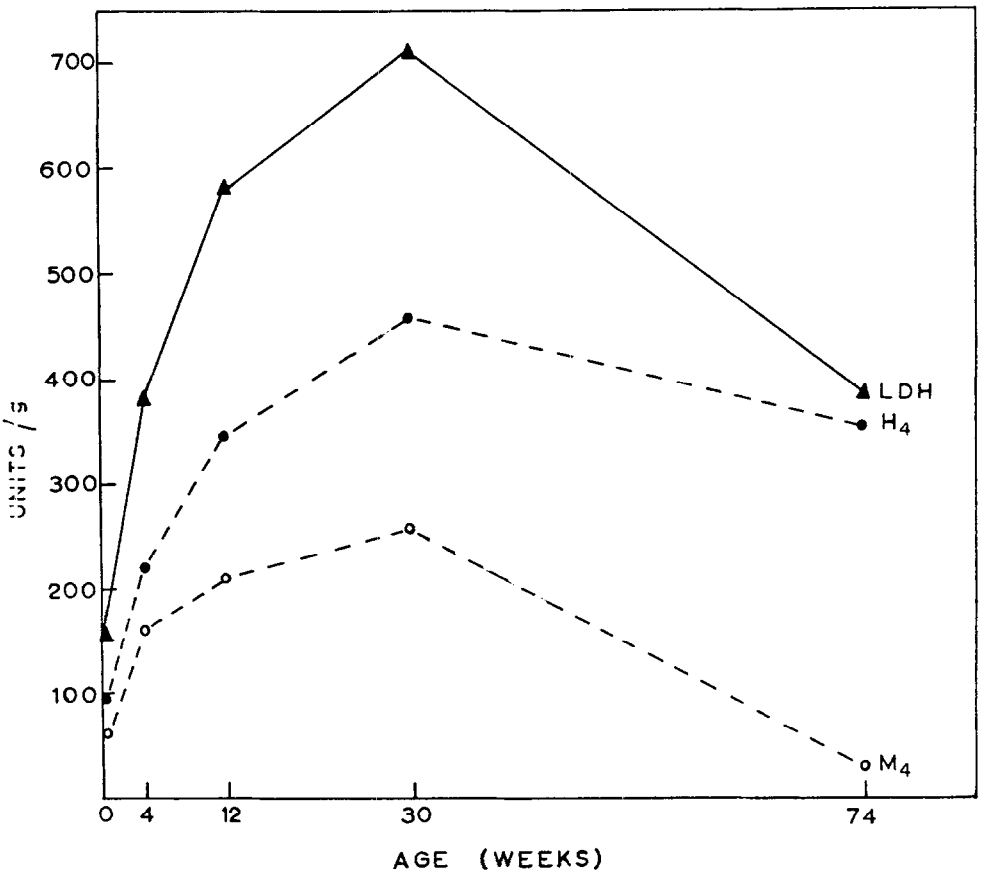

Fig. 2. Activity of lactic dehydrogenase (units/g. wet wt. of total LDH and of H_4 and M_4 isozymes) in the heart muscle of rats of various ages. The assay methods are described in the text. Each point represents the average of the data from five animals. ▲, total LDH; ●, H_4; o, M_4.

greater than that of H_4. The K_m of the enzyme also decreased with age (Fig. 3) indicating an increase in the proportion of H_4 after adulthood. So here too the $NADH_L/NADH_H$ ratio increased showing increase of H_4 in the old age.

The cells of both the brain and the heart do not divide and are believed to show the maximum effect of age. They are highly oxygen dependent and cannot withstand anaerobic condition for long periods.

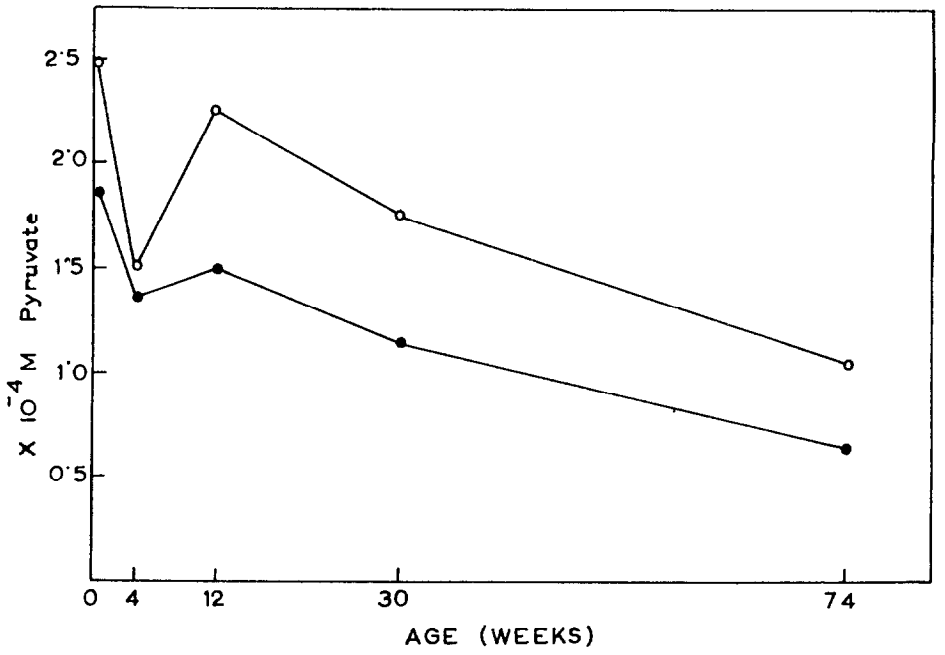

Fig. 3. Effect of age on the K_m of lactic dehydrogenase of the brain and the heart muscle. The assay methods are described in the text. Each point represents the average of the data from five animals. o, brain; •, heart.

Our results show that in both the tissues total LDH increases till adulthood. So the efficiency of glycolysis and the capacity of the tissues to withstand anaerobic condition may increase with development till adulthood. This may account for the capacity for sustained work which is seen in the adult. After this age total LDH decreases in both the tissues, particularly in the heart. This may not only decrease the efficiency of glycolysis but also may decrease the capacity of the tissues to withstand anaerobic condition which is of definite disadvantage to the organs. A possible reason for the decrease in total LDH may be the decrease in the cell number in these tissues which is known to occur with increasing age.

The increase in H_4-LDH may further enhance the above disadvan-

tage, since this isozyme has a lower turn over number than M_4-LDH. It is possible that this alteration in the isozyme composition may be due to the greater activity of the gene responsible for the synthesis of H subunit in the old age in comparison to that of the gene for the M subunit.

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