# LACTATE DEHYDROGENASE ISOENZYMES IN HUMAN HEARTS HAVING DECREASED OXYGEN SUPPLY

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In most animal tissues, lactate dehydrogenase (LDH E.C. 1.1.1.27) contains two polypeptide chains, designated H and M subunits, differing in molecular weight, antigenicity, electrophoretic mobility and kinetic behavior toward substrates and cofactors (Kaplan et al, 1960; Kaplan et al, 1961; Cahn et al, 1962). The intact enzyme is a tetramer of these two subunits. The tissue proportion of the five possible tetramers (isoenzymes $H_4$, $H_3M$, $H_2M_2$, $HM_3$, $M_4$) is determined by the relative percentage of H and M chains available for combination. Lactate dehydrogenase from human hearts having morphologic evidence of diminished tissue perfusion and increased muscle mass showed a higher proportion of M chains than LDH from normal hearts or those with either characteristic alone. Heart tissue of newborn infants revealed a similar pattern. It is suggested that this is an adaptive mechanism for enhanced utilization of anaerobic glycolytic pathways.

At relatively high pyruvate concentrations, LDH exhibits substrate inhibition. This is a property of the H chain (predominant in heart muscle), and the amount of substrate inhibition is directly proportional to the percentage of H chains in the enzyme (Dawson et al, 1964). It has been documented that this inhibition is related to the formation of an abortive terniary complex between $NAD^+$, pyruvate, and LDH (Gutfreund et al, 1968). Kaplan and associates have advanced the hypothesis that inhibition of LDH by high tissue concentrations of pyruvate may assure a constant flow of substrate into pathways

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of oxidative phosphorylation, having observed that tissues, such as the heart, which rely on oxidative phosphorylation for their major source of energy, have predominantly $H_4$ (pyruvate inhibited) isoenzyme, while tissues such as skeletal muscle, relying partially on anaerobic glycolysis for energy production, have a predominance of $M_4$ (non-pyruvate inhibited) isoenzyme. Some investigators have questioned the validity of this hypothesis (Vesell and Pool, 1966). Our findings support the Kaplan hypothesis.

## METHODS

Sections of hearts obtained at autopsy were taken from the left ventricular free wall and frozen. Homogenates were prepared in 100 mM phosphate buffer at pH 7.4. Electrophoretograms were performed on cellulose acetate in 30 mM phosphate buffer, pH 7.0, at 3°C. LDH activity was assayed by measuring the rate of disappearance of NADH at 340 nM (pH 7.5, 100 mM phosphate buffer at 37°C) at low ($3.3 \times 10^{-4}$M) and high ($3.3 \times 10^{-3}$M) concentration of sodium pyruvate.

## RESULTS

As estimated from the electrophoretograms (Figure 1), an increased percentage of M chains was present in cardiac LDH from those patients having a combination of diminished tissue perfusion and cardiac enlargement, as evidenced by coronary artery narrowing, focal cardiac fibrosis, or recent or old infarction, and increased total heart weight (greater than 325 gm for females, 375 gm for males). Other relevant morphologic data are presented in Table I.

Those hearts with a combination of poorly perfused tissue and cardiac hypertrophy (Group I) had relatively greater LDH activity at high pyruvate concentrations than hearts having only one (Group II) or neither (Group III) of these characteristics. Total activity of LDH (in units/gm tissue) was not significantly different among groups. Hearts from 950 to 2600 gm newborns (Group IV) also contained increased M subunits, as evidenced by electrophoretic patterns and high/low pyruvate kinetics.

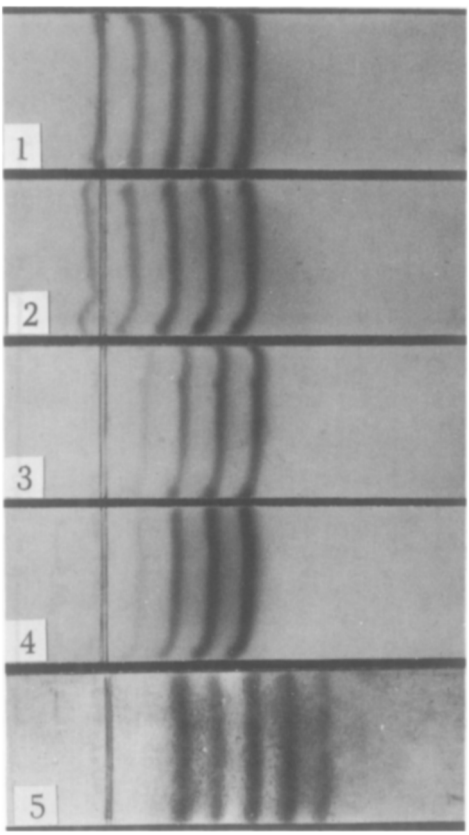


Figure 1. Cellulose acetate LDH electrophoretograms of heart homogenates. From left to right the bands represent isoenzymes $M_4$, $M_3H$, $M_2H_2$, $MH_3$, and $H_4$. The heavy double line is the application point; the anode (-) is to the right. (1) and (2) Coronary artery disease in association with cardiac enlargement (Group I), showing increased concentrations of $M_4$ and $M_3H$ isoenzymes; (3) Coronary artery disease alone, showing normal pattern (Group II); (4) Neither cardiac enlargement nor coronary artery disease (Group III), showing normal pattern; (5) 1700 gm newborn (Group IV), showing pronounced M-chain shift in LDH isoenzyme distribution.

## DISCUSSION

Estradiol stimulation of the uterus, neoplasia, and embryonic development all exhibit enhanced rates of protein synthesis, and alterations in LDH

Table I

CLINICAL, MORPHOLOGIC, AND BIOCHEMICAL CHARACTERIZATION OF PATIENT GROUPS

| | Patient Group* | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Number of Cases | 8 | 13 | 4 | 5 |
| Male/Female | 5/3 | 6/7 | 3/1 | 4/1 |
| Age (years) | 60 ± 6 | 70 ± 4 | 55 ± 9 | --- |
| Heart Weight (gm) | 496 ± 46 | 399 ± 30 | 330 ± 23 | --- |
| Left Ventricular Wall Thickness (cm) | 2.0 ± .10 | 1.50 ± .10 | 1.40 ± .05 | --- |
| Right Ventricular Wall Thickness (cm) | .20 ± .04 | .18 ± .02 | .15 ± .03 | --- |
| Percent M Chains** | 35 ± 10 | 15 ± 5 | 15 ± 5 | 45 ± 10 |
| Activity high/low (%)*** | 40.1 ± 3.7 | 29.9 ± 2.0 | 30 ± 1.1 | 42.8 ± 4.3 |
| | | p = .015 | | |
| | | p = .040 | | p = .025 |

* Group I. Coronary artery disease with cardiac enlargement (hypertrophy); II. Either coronary artery disease or cardiac enlargement; III. Neither coronary artery disease nor cardiac enlargement; IV. Newborns.
** Percent M-type isoenzyme present estimated from electrophoretograms.
*** Activity high/low is the relative activity (in %) of LDH at high concentrations of pyruvate ($3.3 \times 10^{-3}$M) as compared to that at low concentrations ($3.3 \times 10^{-4}$M).
Values are population means ± standard error of the mean and p values are derived from corrected small sample statistics.

isoenzyme composition have been found in each state (Goodfriend et al, 1964; Goldman, et al, 1964; Fine et al, 1963). In experiments with rat hamstring muscles, Goldberg found that compensatory work-induced hypertrophy involved increases in both soluble and contractile proteins (Goldberg, 1968). Adult rats exposed to chronic low oxygen tensions showed a shift in cardiac LDH isoenzyme distribution toward a higher proportion of M chains, while newborn rats reared under similar conditions showed delayed achievement of the adult H-predominant pattern (Mager et al, 1968), findings consistent with our observations in the human heart. It is of interest, however, that cardiac enlargement was not a necessary co-existing factor in the adult rat.

Our findings may be interpreted as demonstrating an adaptive change in heart LDH isoenzyme composition associated with a combination of decreased tissue perfusion and cardiac enlargement. This adaptation may underlie the enhanced contribution of anaerobic glycolysis to total heart energy requirements, evidenced by the increased capacity of the chronically under-perfused heart tissue for lactate production. The relative resistance of fetal heart tissue to low oxygen tension may reflect a similar mechanism.

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