Lactate Dehydrogenase Isoenzymes

A Comparison of Pyruvate-to-Lactate and Lactate-to-Pyruvate Assays

Arthur F. Krieg, Lawrence J. Rosenblum, and John B. Henry

Five lactate dehydrogenase isoenzymes are normally observed in human serum: H4 (LDH1), H3M (LDH2), H2M2 (LDH3), HM3 (LDH4), and M4 (LDH5). This study presents evidence that these isoenzymes vary in the ratio of LDH activity (pyruvate → lactate) to LDH activity (lactate → pyruvate).

Human L-lactate:NAD oxidoreductase (No. 1.1.1.27, trivial name lactate dehydrogenase) (1) catalyzes reversible conversion of lactate (L) to pyruvate (P) in the presence of nicotinamide adenine dinucleotide (NAD).* Enzyme activity may be measured from the pyruvate side of the reaction (P → L) or from the lactate side of the reaction (L → P):

\[
\text{LDH} \quad \text{Pyruvate} + \text{NADH}_2 \overset{\text{Lactate} + \text{NAD}}{\longrightarrow}
\]

Both assays are accepted reference methods for measurement of lactate dehydrogenase (LDH) activity (2–4). At pH 7.4, pyruvate → lactate (P → L) activity is about three times lactate → pyruvate (L → P) activity, because equilibrium favors formation of lactate. Normal range for the P → L assay is 130–265 spectrophotometric units at 25°C (3); normal range for the L → P assay is 40–98 units at 25°C (5). The ratio obtained by dividing P → L activity by L → P activity varies for different serum specimens (4, 6). We have termed this the “P:L ratio”:

\[
P:\text{L ratio} = \frac{P \rightarrow L \text{ LDH activity}}{L \rightarrow P \text{ LDH activity}}
\]

*Also known as diphosphopyridine nucleotide (DPN).
LDH consists of at least 5 isoenzymes, which differ in electrophoretic mobility, $K_m$ pyruvate, $K_m$ lactate, and other properties (7). Each isoenzyme appears to be a tetramer composed of "H" and "M" polypeptide chains. Five combinations are possible: HHHH (H$_4$ or LDH$_1$), HHMM (H$_3$M or LDH$_2$), HHMM (H$_2$M$_2$ or LDH$_3$), HMMM (HM$_3$ or LDH$_4$), and MMMM (M$_4$ or LDH$_5$). With electrophoretic separation, LDH$_1$ (H$_4$) travels between albumin and alpha$_1$ globulin; LDH$_2$ (H$_3$M) travels with alpha$_2$ globulin; LDH$_3$ (H$_2$M$_2$) travels with beta globulin; LDH$_4$ (HM$_3$) travels with "fast gamma"; LDH$_5$ (M$_4$) travels with "slow gamma." In the older literature, LDH isoenzymes are assigned numbers according to lack of migration on electrophoresis, thus M$_4$ is referred to as "LDH$_1$" (8). A sixth LDH isoenzyme has been found in human sperm and testicular tissue (9). With borate-buffered starch gel, 8 LDH isoenzymes may be identified (10). These additional isoenzymes may represent variants of H and M subunits (11); at present they have no clinical importance.

A second ratio is based on the fact that high concentrations of pyruvate are optimal for isoenzymes rich in M subunits, but cause inhibition of isoenzymes rich in H subunits (8, 12, 13). Inhibition is directly proportional to H subunit content.

The pyruvate ratio (PR) is obtained when $P \rightarrow L$ activity at low pyruvate concentration is divided by $P \rightarrow L$ activity at high pyruvate concentration:

$$PR = \frac{P \rightarrow L \text{ activity (low pyruvate)}}{P \rightarrow L \text{ activity (high pyruvate)}}$$

Isoenzymes rich in H subunits are inhibited by high concentration of pyruvate and have high PR's. Isoenzymes with few H subunits have optimal activity at high pyruvate concentrations and have low PR's. Percentages of H subunits in isoenzymes may be calculated from their PR's.

Isoenzymes containing large proportions of H subunits tend to predominate in tissues with aerobic metabolism (e.g., heart). Isoenzymes containing chiefly M subunits are found in tissues with considerable anaerobic metabolism (e.g., skeletal muscle and liver). At $37^\circ$ optimal pyruvate concentrations may be similar for H$_4$ and M$_4$ (14); hence, physiologic significance of differences found at $25^\circ$ is not established.

Changes in proportions of LDH isoenzymes may aid clinical diagnosis. In cardiac disease, LDH$_1$ and LDH$_2$ are increased; in hepatic disease, LDH$_4$ is increased (15). The present work was designed to study: (1) the PR's of different purified human LDH isoenzymes, as a first step toward possible clinical use of this ratio; (2) the P:L ratios
of different purified human LDH isoenzymes, as a first step toward possible clinical use of this ratio.

After preparation of pure isoenzymes, pyruvate optimums for H₄ and M₄ were established. The P:L of each isoenzyme was measured repeatedly. P:L ratios for each isoenzyme were measured both at low and high concentrations of pyruvate. P:L ratios for serum samples from patients also were determined, and on some of these samples, LDH zymograms were prepared.

Materials and Methods

Human heart and liver obtained at autopsy 6 hr. after death were separately homogenized and centrifuged at 4°C. Samples (2 ml.) of each supernatant were applied to horizontal starch blocks (30 × 15 × 1.3 cm.) prepared with barbital buffer (pH 8.6, ionic strength 0.03) (16). Electrophoresis was performed in a cold room at 4°C with constant voltage (400 v) for 18 hr. Isoenzyme bands were localized by fluorescence and LDH activity confirmed by the nitroblue tetrazolium reaction.

Isoenzymes were eluted from each band, and LDH activity adjusted to 100 spectrophotometric units (L → P assay at 25°C).

Spectrophotometric assays of P → L LDH activity were performed at 25°C, pH 7.4 (0.1 M potassium phosphate buffer), final concentration of NADH₂ 1.5 × 10⁻⁴ M (3). "High pyruvate" refers to 18 × 10⁻⁴ M pyruvate (final concentration). "Low pyruvate" refers to 2.5 × 10⁻⁴ M pyruvate (final concentration). Assays on serum samples were determined with 6 × 10⁻⁴ M pyruvate (final concentration) (3).

Spectrophotometric assays of L → P LDH activity were performed at 25°C, pH 8.8 (0.05 M sodium pyrophosphate buffer), final concentration of NAD 5.25 × 10⁻³ M, final concentration of lactate 7.75 × 10⁻² M (5).

In all assays, absorbance at 340 nanometers (nm)* was measured at 1-min. intervals for 6 min. in a Beckman DU spectrophotometer. Temperature of 25.0 ± 0.1°C was maintained by dual thermospacers connected to a Haake Type F thermostatically controlled circulating water bath.

Isoenzyme fractionations on serums from patients were performed by agar-gel electrophoresis.

Results

The 5 isoenzyme bands measured 2–4 cm. in width. Midpoint of M₄ was 6.5 cm. on the cathode side of origin; M₄H was 0.7 cm. on the anode

*Also known as millimicrons or mm.
side of origin; $M_4H_2$ was 7.5 cm., $MH_3$ was 14.3 cm., and $H_4$ was 22.8 cm. from the origin. A sixth fluorescent band occasionally observed close to the cathode proved negative for LDH activity by the nitroblue tetrazolium reaction.

The relationships between pyruvate concentration and LDH activity for $H_4$ and $M_4$ isoenzymes are shown in Fig. 1. Figure 2 shows the

![Figure 1. Effect of pyruvate concentration on LDH activity of $H_4$ and $M_4$ isoenzymes. $H_4$ has relatively narrow pyruvate optimum at $2.5 \times 10^{-4}$ M. $M_4$ has relatively broad pyruvate optimum extending from $8.0 \times 10^{-4}$ M to $20.0 \times 10^{-4}$ M.](image)

pyruvate ratios for the 5 LDH isoenzymes. The P:L ratios are given in Fig. 3 and 4, measured at low pyruvate and high pyruvate, respectively. LDH assays on serum specimens from patients are given in Fig. 5, showing relationship between P $\rightarrow$ L and L $\rightarrow$ P. The P $\rightarrow$ L assays were run at $6 \times 10^{-4}$ M pyruvate, an intermediate concentration recommended for clinical use (3). The serial LDH isoenzyme patterns on a single patient recovering from hepatitis are recorded in Fig. 6, together with the P:L ratio for each pattern.

**Discussion**

Our pyruvate optimums for $H_4$ and $M_4$ isoenzymes are similar to those previously reported (12). Serums from patients have pyruvate optimums which vary from $4 \times 10^{-4}$ M to $8 \times 10^{-4}$ M (17), comparable to our pyruvate optimums of about $3 \times 10^{-4}$ M ($H_4$) and about $10 \times 10^{-4}$ M ($M_4$). It appears that serums from patients may show variations in pyruvate optimums comparable to variations observed for purified isoenzymes.

In Fig. 2, isoenzymes rich in H subunits show high PR’s and isoenzymes rich in M subunits have low PR’s. This may be explained by the fact that isoenzymes rich in H units are inhibited by high pyruvate, while isoenzymes rich in M units show optimal activity with high pyruvate. Our finding that intermediate isoenzymes have intermediate ra-
Fig. 2. Pyruvate ratios for 5 pure LDH isoenzymes. On successive days, pyruvate ratios for $H_2$ were 1.5, 1.4, and 1.3; corresponding ratios for $M_4$ were 0.9, 0.7, and 0.8. Intermediate isoenzymes had intermediate pyruvate ratios directly proportional to their contents of $H$ units.

Fig. 3. P:L ratios (low pyruvate) for 5 pure LDH isoenzymes. On successive days, ratios for $H_2$ were 4.8, 4.3, and 4.1; corresponding ratios for $M_4$ were 0.6, 0.9, and 1.2. Intermediate isoenzymes had intermediate ratios approximately proportional to their contents of $H$ units.

Fig. 4. P:L ratios (high pyruvate) for 5 pure LDH isoenzymes. Ratios for $H_2$ and $M_4$ were 3.2 and 2.1 respectively. Intermediate isoenzymes had intermediate ratios approximately proportional to their contents of $H$ units.
tios proportional to H unit content agrees with Kaplan (11). Although Plagemann et al. (8) and Wróblewski and Gregory (12) found a semi-logarithmic relationship between "% H units" and PR, they used different pH's for LDH assays at high and low pyruvate concentrations.

Although the PR provides an estimate of the percentage of H units, several problems are apparent. First, small variations in assayed LDH activity cause relatively large variations in the PR; it is difficult to achieve precision without multiple assays. Second, there is considerable day-to-day variation in position and slope of the standard curve used to calculate the percentage of H units from PR (cf. Fig. 2). Hence, daily standardization with several isoenzyme fractions is necessary.

Several workers have noted lack of a constant numerical relationship between LDH activity as measured by the forward and reverse assays. The P:L ratio is not constant for different serum samples (4, 6).

Weinberg and Adler (6) compared the $L \rightarrow P$ spectrophotometric assay with a $P \rightarrow L$ colorimetric method. P:L ratios varied from under 3.9 to over 13.2. In a similar study by Amador et al. (4), P:L ratios varied from 2.1 to 6.1. In this study, P:L ratios of purified isoenzymes (low pyruvate) varied from 0.6 ($M_4$) to 4.8 ($H_4$). Ratios for serums ranged from 2.1 to 4.8.

We believe that 2 factors may influence the P:L curve in Fig. 3. First, isoenzymes rich in M units have low numerators owing to sub-optimal pyruvate concentration in the $P \rightarrow L$ assay. Second, isoenzymes

---

**Fig. 5.** $P \rightarrow L$ and $L \rightarrow P$ LDH activity in serum samples from patients. $P \rightarrow L$ assays were run at $6 \times 10^{-4}$ M pyruvate.
rich in H units have low denominators owing to inhibition by high lactate concentration in the L → P assay; there is evidence that H, may be inhibited by the concentration of lactate normally used in this assay (14).

If concentration of pyruvate in the P → L assay were raised and P:L ratios redetermined, one would expect lower ratios for isoenzymes rich in H units, due to pyruvate inhibition. Also, one would expect isoenzymes rich in M units to give higher ratios due to optimal pyruvate concentration in the P → L assay. This prediction is confirmed by Fig. 4.

Figure 5 illustrates the relationship between P → L and L → P LDH activity in serum specimens from patients. At low levels of LDH activity, there is relatively good correlation between the 2 assays, while at high levels of LDH activity there is no constant numerical relationship. In each assay, a graphical plot provided evidence of zero order kinetics. High LDH activity may be due to either marked increase in H4 (LDH1) and H3M (LDH2) in cardiac disease, or to marked increase in M4 (LDH5) in hepatic disease, while normal individuals show less variation in isoenzyme patterns. We believe that variations in isoenzyme pattern may have contributed to the variations in P:L ratio in serums with high LDH activity. Figures 3, 4, and 6 provide
evidence that variations in isoenzyme pattern may cause variations in P:L ratio.

If lyophilized preparations of pure isoenzyme fractions were readily available, the PR or P:L ratio might provide clinically useful estimates of the percentage of H units in serum samples with increased LDH activity. Owing to marked day-to-day variation in calibration curves, (cf. Fig. 2 and 3), clinical use of these ratios would require daily standardization.

Since optimal conditions (at 25°C) differ for individual isoenzymes, and since pathologic serums contain varying amounts of individual isoenzymes, it may be difficult to achieve an LDH assay ideal in all cases.

References