Clin. Biochem. 2, 171–176 (1969)

LDH ISOENZYMES BY UREA INHIBITION AND SUBSTRATE (LACTATE) MODIFICATION: A CLINICAL EVALUATION*

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(Received December 3, 1968)

SUMMARY

1. Differentiation of the anodic (LDH_1 and LDH_2) and cathodic (LDH_5) isoenzymes of lactate dehydrogenase in human sera can be accomplished by combining variable substrate (lactate or pyruvate) concentrations with specific inhibitors such as urea.

2. One technic useful in the clinical laboratory is based upon a colorimetric assay for total LDH activity in which the oxidation of lactate is coupled to the reduction of a tetrazolium salt. The *ratios* of activity in 2M lactate to the activity in 0.02 lactate—2M urea yields an index of the predominant LDH isoenzymes in sera.

3. Clear-cut distinction between myocardial and hepatic isoenzyme predominance was achieved in the majority of 98 patients studied, but the isoenzyme index can be misleading and non-confirmatory in patients who have liberation of LDH isoenzymes from several organ sources.

4. The LDH isoenzyme index is considered to be an additional and informative test only and cannot serve as a *diagnostic* estimation.

THE FRACTIONATION OF LACTATE DEHYDROGENASE (LDH) into its isoenzymes has significantly increased that enzyme's clinical specificity and usefulness. At present, the two most widely used clinical laboratory techniques for determining the predominating LDH isoenzymes in sera are electrophoresis and relative thermal stability. Recently, however, the investigation of selective *inhibitors* of serum LDH indicates that a third and *biochemical* estimation of the isoenzymes of LDH is practical for the clinical laboratory. (*1*, *2*)

Sulfite was found by Wieland et al. (*3*) to preferentially inhibit the electrophoretically fast (LDH_1 and LDH_2) isoenzymes. Subsequently oxalate was determined to have a similar action. (*4*) Several investigators also demonstrated

*This study supported by grants from the Michigan and Virginia Heart Associations. Presented in part at the Annual Meeting of The American Society of Clinical Pathologists and The College of American Pathologists, October 11–19, 1968, Miami Beach, Florida.

that LDH_1 and LDH_5 were inhibited when incubated with urea; the latter more so than the former.

The foregoing observations and additional studies (*5*, *6*, *7*, *8*) of the various kinetic properties of LDH have recently been exploited by several workers to yield a combination of *in-vitro* conditions capable of selectively measuring the fast (anodic-"cardiac") and slow (cathodic-"hepatic") isoenzymes of LDH.

A convenient procedure has been described by Babson, (*1*) in which the hepatic isoenzyme is assayed in 2M lactate solution and the cardiac isoenzyme in 0.02M lactate and 2M urea solution. The ratio of the respective absorbances yields an index which is used to determine the predominance of LDH isoenzymes in sera. A clinical evaluation of this form of LDH isoenzyme differentiation is presented in this report.

Materials and Methods

Principle of LDH isoenzyme assay system: The biochemical differentiation of the cardiac and hepatic subunits (isoenzymes) of LDH is achieved by the incorporation of urea *and* modifications of the concentration of *lactate* substrate used in an estimation of total serum LDH activity.* The latter is a colorimetric assay in which the oxidation of lactate is coupled to the reduction of a tetrazolium salt (*9*).

The ratio of the enzymatic activity in 2M l (+) lactate (heart-inhibiting substrate) to the activity in 0.02M L (+) lactate—2M urea (liver-inhibiting substrate) expressed as a ratio of the absorbance is an indication of the predominant isoenzymes:

$$\text{LDH index} = \frac{\text{O.D. 2M L (+) lactate}}{\text{O.D. 0.02M L (+) lactate—2M urea}}$$

Analytical Studies: After the establishment of normal range (N = 50; 2 S.D. range of 0.8–1.2) for the LDH index, the LDH isoenzyme assay system was tested in a variety of clinical disorders from the patient population of The University of Michigan Medical Center and The Johnston-Willis Hospital, Richmond, Virginia. The latter consisted of the following categories of disease:

A. Uncomplicated myocardial infarcts (41 patients). "Uncomplicated" indicates that: (1) no other major disease was present and (2) the patient was free from episodes of shock or congestive heart failure.

B. Complicated myocardial infarcts (5 patients). In this group of patients, temporary alterations of the expected "myocardial" configuration of the LDH index were induced by a co-existent pathological state.

C. *Patients without myocardial infarcts*:

1. Hepatic parenchymal disease (12 patients)
2. Pulmonary thromboembolism (5 patients)
3. Angina pectoris or congestive heart failure (18 patients)
4. Renal infarct (2 patients)
5. Disorders manifesting a major hemolytic component (15 patients)

Confirmation of the diagnosis of acute myocardial infarct in the 46 patients

*Reagents obtained as "Profile LDH", General Diagnostics Division Warner Chilcott Laboratories, Morris Plains, New Jersey.

with that disease was achieved by a combination of clinical history, specific electrocardiographic changes and serial estimation of serum creatine phosphokinase (CPK) and serum glutamic oxalacetic transaminase (GOT).

RESULTS

A. *Uncomplicated myocardial infarcts*: All patients with acute, uncomplicated myocardial infarcts manifested LDH isoenzyme ratios less than 0.75 at some time during the first 48 hours after the infarct. Ten of the 41 patients exhibited this within the first 24 hours. In four patients, the abnormal ratio was manifest *before* total serum LDH activity had risen to significant abnormal levels.

A normal ratio was usually achieved by the eleventh post-infarct day (Table 1) but four of the 20 subjects who were followed with serial activity determinations

TABLE 1

LDH ISOENZYME RATIOS IN ACUTE MYOCARDIAL INFARCT

Day	LDH ratio
1	0.94
2	0.56
3	0.58
4	0.65
5	0.67
6	0.68
7	0.70
8	0.71
9	0.75
10	0.79
11	0.80
12	0.90
13	0.98

Serial LDH isoenzyme ratio in a prototypic patient with an "uncomplicated" myocardial infarct. Note the normal ratio during the first day of *clinical* infarct and the persistence of abnormal ratios through the eleventh day.

continued to have abnormal ratios for as long as 14 days after the initial change from normal.

The LDH electropherogram (10) correlated in *all* instances with the abnormal isoenzyme index.

B. *Complicated myocardial infarcts*: Five patients comprising this category manifested "complications" that altered the "myocardial" predominance of their LDH indices.

Three of the five patients did not initially demonstrate low ratios in their sera. In each instance their heart isoenzyme dominance became evident during the second day after infarct when their isoenzyme ratios fell below 0.75.

In a fourth patient, a similar "neutralization" or equilibration of the isoenzyme occurred during the third through the fifth post-infarct day, when the patient's ratio was altered abruptly from 0.65 to 1.32 by a bout of severe congestive heart failure.

The fifth patient of this group represents another potential tissue source for obscuring "myocardial isoenzymes". In this instance, LDH_5 in the serum from a traumatic skeletal muscle injury was great enough to obscure the altered ratio brought about by the myocardial infarct.

C. *Patients without myocardial infarcts*:

1. *Hepatic parenchymal disease*: A clear-cut distinction between the myocardial and hepatic isoenzymes was achieved by the use of the ratio assay in this group of disorders. Twelve patients with active hepatic parenchymal disease in the form of acute viral hepatitis (*6*), chronic active hepatitis (*3*), ruptured liver (*2*), and decompensated cirrhosis (*1*), all manifested isoenzyme ratios greater than 1.3(3.5–1.4) (Table 2).

TABLE 2

LDH Isoenzyme Ratios in Hepatic Parenchymal Disease

	Disease	LDH ratio
1	Acute viral hepatitis	3.5
2	Acute viral hepatitis	2.0
3	Acute viral hepatitis	2.2
4	Acute viral hepatitis	2.7
5	Acute viral hepatitis	3.0
6	Acute viral hepatitis	2.0
7	Chronic active hepatitis	1.4
8	Chronic active hepatitis	1.9
9	Chronic active hepatitis	2.0
10	Ruptured liver	2.2
11	Ruptured liver	2.5
12	Decompensated cirrhosis	2.5

Active parenchymal disease of the liver uniformly produced abnormal LDH isoenzyme ratios (greater than 1.3) and was a sensitive indicator of hepatic disease.

2. *Pulmonary thromboembolism*: The ratios in the five patients studied in this category were variable but did not fall below 0.8. The respective ratios were 3.1, 1.1, 1.2, 1.2, and 0.8. In the first instance, the high ratio was attributed to a dominance of LDH_5 from hepatic congestion.

3. *Angina pectoris, and congestive heart failure*: Ten patients with angina pectoris were studied. None of this group manifested isoenzyme ratios less than 0.9. The LDH electropherogram was within normal limits.

The isoenzyme ratio of the eight patients with clinical congestive heart failure ranged from 1.70 to 0.9.

D. *Renal infarct and cortical necrosis*: Only two patients in this category were studied but their low ratios of 0.60 and 0.56 illustrated the dominance of the anodic or "myocardial-like" isoenzymes in the renal cortex.

E. *Disorders with a major hemolytic component*: The effect of hemolysis on

serum enzyme activity, and particularly on assays of LDH activity is presented in Table 3. The rich component of LDH_1 and LDH_2 in the erythrocyte is responsible for the depressed or low isoenzyme ratio.

TABLE 3

LDH Isoenzyme Ratios in Disorders Accompanied by Hemolysis

Disease		LDH ratio
Homozygous sickle cell anemia	(3)	0.5–0.6
Untreated pernicious anemia	(2)	0.4, 0.4
Hemolysis associated with Starr valve prosthesis	(4)	0.6–0.5
Acquired hemolytic anemia	(2)	0.6, 0.7
Hemolysis and leukemia	(4)	0.7–0.45

The low ratios accompanying hemolysis is an expected finding because of the dominance of LDH_1 and LDH_2 in the erythrocyte.

Discussion

Lactate dehydrogenase (LDH) catalyzes the reversible conversion of lactate to pyruvate in the presence of nicotinamide adenine dinucleotide (NAD). Activity may be estimated from either the pyruvate or lactate side of the reaction:

$$\text{pyruvate} + \text{NADH}_2 \xrightleftharpoons{\text{LDH}} \text{lactate} + \text{NAD}$$

It has also been demonstrated that an alteration of the concentration of pyruvate *or* lactate is capable of differentiating various isoenzymes of LDH in tissue or serum. Enhancement of this differentiation has been achieved by combining the effects of substrate concentration with specific chemical inhibitors of LDH. Urea has better than other substances been proven to fulfill the demands of a selective inhibitor of LDH isoenzymes (*11*).

The effect of varying concentrations of urea and pyruvate on LDH isoenzyme activity has been studied by Konttinen and Lindy (*12*) who found high concentrations of pyruvate increased the activity of the heart (LDH_1 and LDH_2) isoenzymes in the presence of urea while the liver (LDH_5) isoenzyme was virtually destroyed. They reported good separation of cardiac from non-cardiac isoenzymes with a 2.0M urea-LDH test using a 10^{-3} M pyruvate concentration.

Recently it has been noted that the activity of LDH_1 may be increased in the presence of urea irrespective of whether the substrate is pyruvate or lactate (*11*). Babson (*1*), using a rapid colorimetric LDH assay in which the oxidation of lactate is coupled to the reduction of a tetrazolium salt, found that the *ratios* of activity in 2M lactate to the activity in 0.02M lactate—2 M urea yielded good heart and liver isoenzyme differentiation.

The isoenzyme ratio or LDH index as determined by these biochemical means adds a distinct measure of specificity to total LDH activity estimations, but expectations for its *clinical utility* must be conservative. The ratio depends on the relative proportions of cardiac (LDH_1 and LDH_2) or hepatic (LDH_5) isoenzymes

in the test sera. In *uncomplicated* acute disorders of the myocardium the biochemical index has a high degree of discriminating ability and has correlated well with the electrophoretic separation of LDH. Fine "diagnostic" discrimination, however, is not possible by the use of the isoenzyme ratio system. Changes occurring in the intermediate isoenzymes ($LDH_{2, 3}$, and $_4$), the so-called isomorphic pattern of the LDH electropherogram, are not only not detected, they also are capable of spuriously affecting the ratio. More serious, in the clinical context, is the "normalization" of the ratio by concomitant increases in both cardiac and liver fractions. Finally, regardless of the technic used to determine the dominance of one or more of the isoenzymes of LDH in patients' sera, no organ specificity can be attached to the results because of cross-reactions between tissues. There is a predominance of LDH_1 and $_2$ in the erythrocyte and renal cortex and hemolysis or infarct of the kidney yield an isoenzyme pattern often indistinguishable from that observed after a myocardial infarct. The isoenzyme ratios in patients with leukemia or lymphoma are unpredictable since they depend on the combination of (1) tumor metabolism, (2) hemolytic component and (3) hepatic involvement.

Lubran and Jensen (*6*) have most appropriately defined the place of the LDH isoenzyme ratio in clinical medicine, "an additional laboratory test in the diagnosis of myocardial (and other) disease, not as a diagnostic or even screening test". In this respect the ratio is not unlike the LDH electropherogram, i.e. informative and possibly confirmatory rather than diagnostic, and must be related to the entire clinical-patient mosaic.

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