SUMMARY Lactic dehydrogenase (LD) isoenzymes were determined by a rapid, simple technique and their utility in the diagnosis of acute myocardial infarction (AMI) was evaluated. LD isoenzymes were separated by ion-exchange column chromatography using DEAE-Sephadex. The cardiac fractions (LD-1 and LD-2) were measured separately on an Abbott ABA-100 analyzer and ratio of LD isoenzyme 1 to LD isoenzyme 2 (LD1:2) calculated. Daily serum samples were obtained from 100 patients selected only for a history of chest pain of abrupt onset. In 47 patients whose diagnosis was acute myocardial infarction (AMI), confirmed by typical clinical presentation and typical rise in cardiac-specific creatine kinase isoenzyme fractions of the effluent from the 150 mMol/liter sodium chloride buffer were collected in the second vial (Fraction 2). Column elution was completed with 2–4 ml fractions of the 200 mMol/liter sodium chloride buffer in the third vial (Fraction 3). LD isoenzyme separation by column was evaluated by electrophoresis on polyacrylamide gel as shown in figure 2. LD isoenzymes 3, 4, and 5 were eluted in Fraction 1, LD isoenzyme 2 in Fraction 2 and LD isoenzyme 1 in Fraction 3.

Figure 3 depicts the ion-exchange chromatographic behavior of LD isoenzymes. LD activity of column fractions was measured by the Wacker method at a 5-min interval with the Abbott ABA-100. LD activity in column fractions as low as 10 U/L was detected. Results of column fractionated LD were expressed as the ratio of LD isoenzyme 1 to LD isoenzyme 2 (LD1:2) (Fraction 3/Fraction 2).

Results

Method reliability for the detection of LD isoenzymes 1 and 2 by column chromatographic procedure was followed by a daily analysis of a lyophilized serum control (Moni-Trol II-X). The mean (N = 39), standard deviation, and coefficient of variation for the determination of LD isoenzyme 1 and 2 in this serum pool were 89 U/L for LD-1 and 250 U/L for LD-2, 12.5 U/L for LD-1 and 34.7 U/L for LD-2, and 11.5% for LD-1 and 7.6% for LD-2, respectively. The 100 patients were divided into groups on the basis of their clinical diagnosis and maximum values for LD1:2 (i.e., the peak value of LD1:2 in a series of determinations for each patient). Group I included 47 patients in whom the diagnosis of acute myocardial infarction was confirmed by typical clinical history and MB (table 1). The peak LD1:2 in this group ranged from 0.77–2.26. Seven of these patients had peak values above 0.76 but below 1.00.

Group II included 44 patients in whom the diagnosis of acute myocardial infarction was excluded (table 1). No elevation of LD1:2 was noted in these patients. The maximum LD1:2 ranged from 0.25–0.76. Therefore, the patients in groups I and II (91 out of 100) could be accurately separated by an LD1:2 of 0.76.

There were two patients (group III) who had the abrupt onset of prolonged chest pain, 41 and 91 hours before hospital admission, respectively (table 2). The diagnosis of

ISOENZYME ANALYSIS has been increasingly used in the diagnosis of acute myocardial infarction. The value of separation, recognition, and quantification of the isoenzymes of creatine kinase (CK)\(^1\)–\(^2\) and to a lesser extent, lactic dehydrogenase (LD)\(^3\)–\(^4\) has been demonstrated in several studies. In a previous report\(^1\) we demonstrated the clinical usefulness of a rapid method for the separation and quantification of the cardiac specific isoenzyme (CK-MB). Routine utilization of LD isoenzyme analysis in the diagnosis of acute myocardial infarction has been hindered by the lack of a similar rapid and simple method.

In the present study, LD isoenzymes were separated and quantified by a column technique similar to that used for CK isoenzymes. The clinical usefulness of this procedure in the diagnosis of acute myocardial infarction was evaluated in a series of unselected patients admitted to the Coronary Care Unit (CCU).

Methods

Lactic dehydrogenase isoenzyme analysis was performed on the sera of 100 patients admitted to the Coronary Care Unit (CCU) of Montefiore Hospital in November and December of 1976. Patients in whom an abrupt onset of chest pain could not be documented were excluded from the study. The diagnosis of acute myocardial infarction was made on the basis of typical prolonged retrosternal and/or left arm pain plus typical rise and fall of MB. In the absence of the latter, acute infarction was confirmed by the appearance of new diagnostic Q waves. Serum samples for LD isoenzyme analysis were obtained on admission and daily thereafter. LD isoenzyme results were not reported back to the CCU.

Figure 1 is a diagrammatic representation of the chromatographic process. Sample (0.25 ml) was applied to the column. Sample effluent and 2–4 ml fractions of the effluent from the 100 mMol/liter sodium chloride buffer were collected in the first vial (Fraction 1). Another 2–4 ml
acute myocardial infarction was confirmed by ECG changes alone. The percent MB was normal throughout. The LD1:2 however, was definitely elevated on admission. The subsequent LD1:2 values correlated well with the interval between symptoms and hospitalization, rising further in the latter infarction and falling in the earlier case.

Group IV was composed of three patients with elevated LD1:2 who did not have an acute myocardial infarction (table 3). One patient was an 81-year-old woman with Gaucher's disease and active hemolysis. The second patient was a 48-year-old man with diabetes, gout, hyperkalemia, and hypertriglyceridemia. He had no evidence for hemolysis or renal necrosis. The third patient had no medical illness. Therefore, only one of these false positive results could be explained.

There were four patients (group V) with documented myocardial infarction in whom the LD1:2 ratio did not rise above normal values (table 4). Three of these patients had relatively slight rises in MB. One patient with continuous chest pain had only borderline elevations of MB in the samples that could be obtained before he died. In none of these patients did the LD1:2 reach the derived cut-off point of 0.76. However, there was an elevation to at least 0.70 in all of them.

**Discussion**

The enzymatic diagnosis of acute myocardial infarction has been well established over the past 20 years. More recently, isolation and quantification of CK-MB isoenzyme have greatly improved diagnostic accuracy$^1$. Although CK-MB isoenzyme determination is probably sufficient in the great majority of cases, it is of no value in infarctions occurring several days prior to admission. In these situations, LD isoenzyme analysis may provide the necessary enzymatic confirmation.

Lactic dehydrogenase has a wide distribution in the body.$^7$ There are five different isoenzymes with different organ distributions. LD-4 and LD-5 are found in skeletal muscle and the liver. The myocardium has a preponderance of LD-1 with lesser amounts of LD-2. Necrosis of the myocardium results in the release of relatively more LD-1 than LD-2 into the blood, reversing the normal ratios. A similar situation occurs with hemolysis and renal injury. Extensive use of LD isoenzymes in the routine diagnosis of acute myocardial infarction has been hindered up to now because of the limitations of the electrophoretic method which is tedious, time consuming, and is only semi-quantitative. The column chromatographic method used in this study is quantitative and can be easily performed on a daily basis as a routine laboratory test.

Cohen et al.$^4$ demonstrated in 1973 that a rise in the

<p>| Table 1. Peak LD 1:2 and Clinical Diagnosis (Groups I and II) |
|-----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Diagnosis</th>
<th>Peak LD 1:2 (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>Myocardial infarction</td>
<td>0.77-2.26</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>No infarction</td>
<td>0.25-0.76</td>
</tr>
</tbody>
</table>
LD1:2 above 1.0 was seen in all their patients with myocardial infarction. LD1:2 did not change in patients with angina pectoris who did not have evidence of myocardial infarction. They also pointed out that since LD-1 and LD-2 are also normally found in the kidneys and red blood cells, renal infarction and hemolysis can produce similar elevation of LD1:2.

Roe et al. studied the use of LD isoenzymes in conjunction with CPK isoenzymes in the diagnosis of acute infarction. They demonstrated that a rise in the LD1:2 to more than 1.0 confirmed the diagnosis. In their study, the LD isoenzyme rise occurred about 12 to 24 hours after an acute infarction.

Wagner et al. noted 90% sensitivity and 95% specificity when they utilized the LD1:2 in conjunction with CPK isoenzymes. They concluded that although the LD1:2 was relatively specific and sensitive, it was less so than the CPK isoenzyme. They showed that the LD1:2 usually increased about 24 to 48 hours after the onset of symptoms. In their study, the LD1:2 was especially useful in patients whose infarction occurred several days prior to admission.

The present study confirms the results of previous workers on the value of determining LD1:2. In this study, we have demonstrated a rapid method that allows the use of the LD1:2 on a day-to-day basis. This can be accomplished without losing the specificity and sensitivity noted by Wagner and co-workers.

In our study of 100 patients, a peak LD1:2 above 0.76 appeared to be the cut-off point for the diagnosis of infarction. Of 44 patients with no myocardial infarction, there was none in whom the peak ratio exceeded 0.76. In 47 of the patients with documented infarction, the value of the peak LD1:2 always exceeded 0.76. Our diagnostic cut-off point of 0.76 is less than the reported ratio of 1.0 noted in previous studies. It is likely that the upper limit of normal for LD1:2 by this method is between 0.70 and 0.80. This may be due to differences in the methods.

Perhaps the greatest usefulness of routine LD1:2 determinations is in patients with acute myocardial infarctions occurring more than 48 hours prior to admission. In these patients the value of MB determination is limited, since the levels may have returned to normal by the time of admission. Because LD1:2 rises later during an infarction and remains elevated longer than does MB, its determination may be critical in evaluating this clinical subgroup.

There were three patients in Group IV who constituted false-positives for the LD1:2. In one patient, the elevation of LD1:2 could be satisfactorily attributed to the occurrence of hemolysis. However, there was no explanation for the abnormal results in the two other patients. These may represent unavoidable false-positive results. There is a more satisfactory explanation for the normal LD1:2 in the four patients with myocardial infarction in Group V. One patient died before the LD1:2 could have become positive. The other three patients had a small myocardial infarction as evidenced by a peak MB of less than 10 International Units. It is likely that LD isoenzymes are not as sensitive as CK isoenzymes in detecting myocardial necrosis.

This report demonstrates that the LD isoenzyme determination, when carried out by a rapid and sensitive method, is a useful addition to the enzyme diagnosis of acute myocardial infarction. In most cases, the LD1:2 will serve to confirm the CK-MB results. However, the LD1:2 values become especially critical when the infarction has occurred 48–72 hours prior to admission, after the CK-MB has returned to normal. The determination of both CK-MB and LD1:2 by the column technique results in extremely high levels of diagnostic accuracy.

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