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Method Variation in Lactate Dehydrogenase Isoenzyme Determination

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Differences between methods for determining lactate dehydrogenase isoenzymes are illustrated, which can account for discrepant results. They should be taken into consideration in the interpretation of test results for the diagnosis of myocardial infarction.

Myocardial infarction (MI) is a leading cause of morbidity and mortality in the population. Its diagnosis is of critical importance, particularly in the coronary-care and intensive-care units. For these reasons it is of great importance for the laboratory to provide accurate diagnostic information. Determination of serum lactate dehydrogenase (LD; EC 1.1.1.27) has been a sensitive, albeit nonspecific, indicator of MI. Determination of LD isoenzymes has enhanced diagnostic specificity and the estimation of the ratio of the cardiac-associated LD isoenzyme-1 to isoenzyme-2 (LD1/LD2) has been established as a reliable biochemical index in the diagnosis of MI (1–3). The degree of accuracy and reliability of the information provided by the LD isoenzyme test is in some dispute among various laboratories (1–4). This is due in part to methodological differences. The purpose of this report is to illustrate that methodological differences can account for variable results.

Methods

An agar gel electrophoretic procedure originally developed for the quantitative determination of LD isoenzymes (5) has recently been improved and applied in clinical investigations (4). The main features of the technique are: (a) Electrophoretic separation of LD isoenzymes on microscope slides covered with agarose gel. (b) Colorimetric development of the isoenzymic activity by a tetrazolium reduction procedure. (c) Quantitation of the isoenzyme zones by densitometric scanning. The original procedure was improved by: (a) Use of the same method to reveal LD isoenzyme patterns in normal tissues, cells, and fluids, as well as in serum samples from patients with defined pathological conditions (6). These reference patterns form a data base for meaningful comparisons and correlation of test results. (b) Analysis of measured volumes of serum containing standard enzymic activity, which enables accurate quantitation.

A similar electrophoretic technique was selected for comparison, one used routinely in clinical laboratories. The Corning agarose-gel colorimetric electrophoretic procedure was performed according to the manufacturer's instructions. (Corning Medical, Medfield, MA 02052).

Erythrocyte lysates were prepared from normal blood samples collected into heparinized test tubes. The blood samples were centrifuged (180 × g, 10 min) to separate the cells. An 1-mL aliquot of erythrocytes was removed and washed twice with 2-mL portions of phosphate-buffered-isoionic saline. Two milliliters of a hypotonic solution (water/phosphate-buffered saline, 2/1) was placed in the test tube containing the cells, and they were lysed by one cycle of freezing and thawing at −20 °C and 50 °C, respectively. The lysed cells were centrifuged at 10 000 × g for 5 min. From the supernatant hemolysates, aliquots were removed, diluted 15-fold with phosphate-buffered saline, centrifuged (10 000 × g, 5 min), and used for electrophoretic determinations of isoenzymes.

Results and Discussion

Figure 1 shows typical LD isoenzyme electrophoretic patterns and densitometric scans of a hemolysate obtained by the two methods. While the same methodological principles are used in the two methods, the results are entirely different. The electrophoretic pattern of and percentage values for the hemolysate analyzed by my technique demonstrate an increased density of LD1 and LD2, with the LD1 approaching but not exceeding LD2. In contrast, by the commercial method, a different picture and values have been found; the LD1 exceeded LD2, producing a reversal of the LD1/LD2 ratio.

While the difference in results between the two methods is accounted for by methodological details such as the concentration of the agarose gel, the conditions of electrophoresis, and the composition of reagents, all of which influence the quantitative distribution of the isoenzymes, the implications of such differences are of great importance in the diagnosis of MI. In this diagnosis, one criterion is the reversal of the
usual LD1/LD2 ratio (the so called "flip"). A false-positive result is possible: in the coronary care unit by hemolysis owing to handling of the blood and in the intensive-care unit in surgical patients owing to operative procedures and blood transfusions. For these reasons, previous investigators (1) have called attention to false-positive results produced by hemolysis in the detection of MI. These limitations of the LD isoenzyme test for the detection of MI have been eliminated by improving my procedure, as demonstrated by its evaluation in patients undergoing heart operations (4). No reversal of the LD1/LD2 ratio was found, in patients after cardiac operations with an uncomplicated clinical course and recovery, by hemolysis due to operative procedures and transfusions, but there was a clear-cut reversal of the ratio, which persisted for several days, in patients who suffered MI.

Table 1 further validates the LD isoenzyme test. Control values for serum and hemolysates were determined in blood samples from normal volunteer blood donors and normal values for myocardial extracts from biopsy and autopsy specimens. Table 1 shows that the LD1/LD2 ratio is less than 1 in the serum and hemolysate controls; consequently, hemolysis alone cannot cause a reversal of the LD1/LD2 ratio by my technique. The preponderance of LD1 in the myocardium after heart surgery can produce a sharp reversal of this ratio.

Another example pertinent to methodological difference, not related to the diagnosis of MI, is also described. In a recent report (7) the appearance of a sixth LD isoenzyme was demonstrated in serum of critically ill patients.

A serum sample from such a patient showing the sixth isoenzyme has been provided to us by the authors. The sample was analyzed by the two electrophoretic tests, and the results are shown in Figure 2. A sixth LD isoenzyme is demonstrable by the commercial procedure, but not with my technique. It is interesting to note that by the cellulose acetate electrophoretic technique used by the original authors (7), the sixth LD isoenzyme was completely separated from the other five; by the commercial agarose procedure the LD6 was close to the LD5 and by my method no LD6 was evident. Whether the sixth LD isoenzyme is not separated by my technique or is an artifact produced by the commercial procedures owing to the higher density of the supporting medium remains to be elucidated. The point here is that different methods can produce different quantitative and qualitative results, and for this reason special attention should be paid by the laboratorian in the interpretation of test results produced by the respective laboratory methods.

Table 1. Relative Percentage Distribution of Lactate Dehydrogenase Isonzyme Fractions *

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal serum</td>
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<td>29.9</td>
<td>40.4</td>
<td>20.3</td>
<td>5.7</td>
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<td></td>
<td></td>
<td>(0.4)</td>
<td>(0.34)</td>
<td>(0.4)</td>
<td>(0.2)</td>
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<td>Erythrocyte hemo-</td>
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<td>37.9</td>
<td>40.1</td>
<td>19.3</td>
<td>1.5</td>
</tr>
<tr>
<td>lysates</td>
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<td>(1.2)</td>
<td>(0.9)</td>
<td>(2.0)</td>
<td>(0.5)</td>
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<tr>
<td>Myocardial extracts</td>
<td>4</td>
<td>56</td>
<td>35</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

* Results expressed as mean (and SD).

References