"Glycerate Dehydrogenase" Activity in Acute Myocardial Infarction and Myocardial Ischemia

M. J. McQueen, I. W. C. Garland, and H. G. Morgan

Hydroxy pyruvate has been shown to be an alternative substrate for lactate dehydrogenase. The clinical value of this "glycerate dehydrogenase" has been investigated in 60 patients admitted to the hospital with a provisional diagnosis of myocardial infarction. For comparison, lactate dehydrogenase, α-hydroxybutyric dehydrogenase, creatine kinase, aspartate transaminase, and alanine transaminase were also assayed at 37°C. Normal ranges and methods of assay are given for each enzyme. Glycerate dehydrogenase has greater activity than any other enzyme and parallels lactate dehydrogenase and α-hydroxybutyric dehydrogenase in its time of peak activity and duration of increased activity. Clinical and biochemical evidence is provided to show that α-hydroxybutyric dehydrogenase is not specific for the cardiac isoenzymes. Of the enzymes studied, creatine kinase activity increases most quickly after myocardial infarction, and an exclusive role for its use in coronary care units is also suggested.

Additional Keyphrases lactate dehydrogenase • α-hydroxybutyric dehydrogenase • creatine kinase • aspartate transaminase • glutamic-oaloacetate transaminase • alanine transaminase • infarction • normal values • ischemia • diagnostic aid • isoenzyme specificity

Earlier workers (1) have shown that labeled hydroxy pyruvate is incorporated into glucose by rat-liver slices and into liver glycogen by the intact rat. Hydroxy pyruvate is also as effective a substrate as pyruvate for beef heart (8) and skeletal muscle (5) crystalline lactate dehydrogenase, and for the purified enzyme from rat liver (4).

Catalysis of reduction of hydroxy pyruvate by enzymes of human origin has recently been described (6, 7). The evidence for the reversibility of the reaction has been presented (6), and, by analogy with the enzyme involved in the reduction of pyruvate to lactate (LDH)(1) and of 2-oxobutyrate to hydroxy pyruvate (α-HBD), the enzyme catalyzing the reduction of hydroxy pyruvate to glyc erate may be called "glycerate dehydrogenase." We do not claim that this enzyme is the glycerate dehydrogenase that has the code number EC 1.1.1.29. We stress that the enzyme involved appears to be the same one that, when it is involved in the reduction of pyruvate to lactate, is called lactate dehydrogenase. Hydroxy pyruvate is therefore an alternative substrate for LDH and has been shown to give greater activity than pyruvate and 2-oxobutyrate in a normal population (6, 7).

Preliminary work indicated that this assay might have clinical applications. Therefore we have studied 60 patients admitted to the Royal Infirmary with a provisional diagnosis of acute myocardial infarction.

Methods and Materials

Date and time of the acute incident were obtained from the clinical history. Each patient had electrocardiographic evidence available as an aid to diagnosis.

The timing of the first sample varied with the different time intervals at which each patient was admitted to the hospital after onset of chest pain. Assays on further samples almost completely depended on sera being supplied by those looking after the patients in the wards. Hence, every patient did not have all the enzyme assays carried out at each time interval.

All enzyme assays were performed at the physiological temperature, 37°C. LDH, α-HBD, and glycerate dehydrogenase activity were measured under optimal conditions (6, 7).

Alanine transaminase and aspartate transaminase were assayed with ultraviolet test kits

From the Cardiac Surgery Unit and the Department of Biochemistry, Royal Infirmary, Glasgow, U.K.
Received Nov. 17, 1971; accepted Dec. 14, 1971.

1 Nomenclature: LDH is lactate dehydrogenase (l-lactate:NAD oxidoreductase, EC 1.1.1.27); α-HBD is α-hydroxybutyric dehydrogenase; alanine transaminase is l-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2; aspartate transaminase is l-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1; and CK is creatine kinase (ATP: creatine phosphotransferase, EC 2.7.3.2).
(Boehringer Corp., London). All of these enzymes were assayed with a Model 8600 "Reaction Rate Analyzer" (LKB Instruments, Inc., Rockville, Md. 20852).

CK activity was measured by using an "activated" monotest kit (Boehringer Corp.,) with a Unicam SP 800 recording spectrophotometer, temperature control being provided by a Tecam water bath.

Normal Values

The normal ranges used in this study were all established at 37°C on the same group of 280 apparently healthy volunteers who were not hospital patients.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>U/liter</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerate dehydrogenase</td>
<td>300-850</td>
<td>(6)</td>
</tr>
<tr>
<td>LDH</td>
<td>240-525</td>
<td>(7)</td>
</tr>
<tr>
<td>α-HBD</td>
<td>80-440</td>
<td>(7)</td>
</tr>
<tr>
<td>Alanine transaminase</td>
<td>10-37</td>
<td>(9)</td>
</tr>
<tr>
<td>Aspartate transaminase</td>
<td>6-39</td>
<td>(9)</td>
</tr>
<tr>
<td>CK</td>
<td>up to 130</td>
<td>(unpublished)</td>
</tr>
</tbody>
</table>

The distribution of each of these enzymes was lognormal and positively skewed, so the ranges given are the 98% confidence limits (8).

Results

Although all the patients had the common presenting symptom of chest pain, sufficient to arouse the suspicion of myocardial infarction and to warrant hospital admission, they are divided into two groups.

Group 1 comprised 38 patients with unequivocal electrocardiographic evidence of acute transmural myocardial infarction.

Group 2 comprised 22 patients in whom the available electrocardiographic evidence was not conclusive as to whether necrosis of some part of the heart muscle had occurred.

Results for Group 1. Distribution of activity of each enzyme at the various sampling times after the onset of chest pain is illustrated in Figures 1 to 3. The greatest absolute activity is attained by glycerate dehydrogenase. This is more clearly illustrated when the mean activity of each enzyme at each time interval is plotted (Figure 4). LDH and α-HBD are next and have a similar pattern of activity against time to that of glycerate dehydrogenase. Creatine kinase and aspartate transaminase also show significant increases above their normal ranges.

Results for Group 2. The mean activities of each of the enzymes at the different time intervals remains within normal limits (Figure 5). These findings lend further support to the doubts raised by the electrocardiographic reports in this group of patients. However, three of these patients had enzyme activities at some time that were above normal limits. Each time it was the CK that was supernormal. The first of these patients had a CK of 138 U/liter during the 24-48 h period. All the other enzyme assays done during this and the 0-12 and 48-72 h periods were consistently at the lower end of each normal range. This patient had electrocardiographic evidence of posterior myocardial ischemia. The second patient had CK activities of 167 U/liter and 150 U/liter at the 24-48 and 48-72 h periods, respectively. All the other assays at these times together with the CK at the 0-12 h period remained well within normal limits. This patient had a myocardial infarction one month before his current admission. The third patient had a CK of 300 U/liter at the 24-48 h period. All other assays at this and the 0-12 and 48-72 h periods were within normal limits. The electrocardiogram also suggested myocardial ischemia.

Discussion

Although the mean activities of the six enzymes assayed on sera from the patients in group 1 are shown in Figure 4, this takes no account of the increase of activity of each enzyme relative to its normal range. In Figure 6 the upper limit of nor-
mal for each enzyme is taken as unity and the mean increase of activity of each enzyme at each time interval is related to this upper limit.

The first important point that emerges from this study is the time of greatest elevation and the duration of elevation of each enzyme when measured at 37°C. CK activity is greatest at 12–24 h and has virtually returned to normal by 73–96 h. This is similar to aspartate transaminase, although the graph (Figure 6) suggests that this enzyme may reach its peak activity slightly later than does CK. The dehydrogenases parallel each other and reach their peak activities later than the other enzymes, at 25–48 h, and still show a mean activity at 73–96 h almost twice their upper limit of normal values.

There have been many other reports relating to the times of peak elevation of various enzymes (10–17, 19, 20). This list of references is not comprehensive, but in these and other studies we could not find comparative results for the number of enzymes we have investigated, or the effects of
optimal assay conditions at 37°C. Our findings as to the timing of peak elevations under these conditions generally agree with the earlier reports.

The activities with the substrates pyruvate, 2-oxobutyrate, and hydroxypyruvate so clearly parallel one another that this provides further supportive evidence that they are all substrates for the same enzyme, LDH. It has been suggested that 2-oxobutyrate provides a measure of the concentration of the fast-moving cardiac isoenzymes (18), and that this leads to greater specificity as a test for myocardial infarction (19). The LDH/α-HBD ratio has been found to be less than 1.23 in cases of myocardial infarction and greater than 1.6 in cases of liver disease (19). Again it has been argued that this shows that α-HBD has greater specificity for the cardiac isoenzymes. The evidence has previously been presented that this specificity is not inherent to the substrate 2-oxobutyrate but has been created by the use of suboptimal substrate concentrations at the nonphysiological temperature of 25°C (5, 7). The original ratios do not hold under optimal concentrations at temperatures above 25°C. In our normal ranges established at 37°C the ratio of LDH/α-HBD is 1.15 to 3.19. In this series of patients with proven myocardial infarction, the ratio of LDH/α-HBD falls within this range and gives further proof that under optimal conditions at 37°C ratios yield no useful information and that 2-oxobutyrate does not possess the specificity claimed for it.

One final point relating to α-HBD that emerges from this study is that LDH and α-HBD both reach peak activities of 3.3 times the upper limit of normal (Figure 6), compared with the findings of Elliot and Wilkinson (20) of a mean peak activity for α-HBD of 4.5 times the upper limit of normal and for LDH of about 3 times the upper normal limit when suboptimal concentrations are used.

The enzymes showing the greatest activity increase are CK, with a peak activity 6.1 times the upper limits of normal, and aspartate transaminase, 4.1 times. As expected, alanine transaminase activity shows no significant rise above its normal range.

The time relationships of peak activity of each enzyme indicate the important part serial estimations of several enzymes play in avoiding false negatives and false positives. A knowledge of such time relationships will aid clinical diagnosis at the varying times at which, after the acute myocardial incident, the physician sees such patients. We believe that the multiplicity and frequency of enzyme assays has helped to contribute to clear enzymatic differentiation between the patients in Group 1 and those in Group 2. This does not mean that an individual patient in Group 2 cannot have increased enzyme activity. The three patients mentioned in the results show that this is not the
case. Here, CK was the enzyme with increased activity in each patient, which shows that one assay carried out randomly can be misleading. The consensus of many factors in these three cases supported a diagnosis of myocardial ischemia, not of myocardial infarction.

Such differentiation can further help the physician in deciding how long any doubtful case of myocardial infarction should occupy a valuable bed in a coronary care unit. CK appears to have a most useful role as a nonemotive arbiter at the 24-h period after onset of chest pain. This enzyme index could be used to decide whether a patient with suggestive history and yet inconclusive electrocardiographic evidence has had a myocardial infarction.

The information regarding α-HBD presented in this paper and elsewhere (5, 7) highlights a discrepancy between biochemical methodology and clinical usefulness. We are not disputing that α-HBD at 25°C under suboptimal conditions has diagnostic value. However, it has not been previously recognized that its value arises from its suboptimal conditions of assay and not from the biological specificity of 2-oxobutyrate for certain isoenzymes. Its real advantage has been that it enabled the diagnostically useful ratio LDH/α-HBD to be established. An increase of the standard assay temperature and the use of optimal concentrations are currently two problems in the field of diagnostic enzymology. The clinical usefulness of α-HBD is lost at 37°C for two reasons. First, under optimal conditions for both assays the ratio LDH/α-HBD has too wide a range to be of value. Second, if optimal and the original concentrations are used for LDH and α-HBD (18, 19), respectively, at 37°C their ratios would be chemically doubtful but would also be difficult to keep sufficiently narrow to be of clinical use. We have shown in this paper that the usefulness of serum enzyme assays in the diagnosis of myocardial infarction is not diminished even when the artificial specificity of 2-oxobutyrate is not present.

Glycerate dehydrogenase emerges as having a useful part to play in the diagnosis of myocardial infarction, as shown by the information it provides when measured with enzymes previously recognized as being of value in clinical diagnosis. It has the greatest activity of any of the enzymes and therefore lends itself to greater speed and accuracy in rate of reaction assays. It also adapts well to the increasing automation required in clinical chemistry. By use of the LKB 8600 Reaction Rate Analyzer, more than 100 assays can be done per hour.

This research program is supported by a grant from the Scottish Hospital Endowments Research Trust. We wish to thank the physicians in this hospital for allowing their patients to be included in this study. The assistance and encouragement of Mr. J. King is also gratefully acknowledged.

References