Fundamentals of clinical cardiology

Enzymatic profile of myocardial infarct

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"And thick and fast they came at last
And more, and more, and more . . ."

Alice Through the Looking Glass
Lewis Carroll

The causal relationship between the rise in activity of certain enzymes in the serum and the occurrence of damage to the myocardium has been established without question. Experiments and histochemical investigations have shown that infarcted heart muscle is rapidly depleted of various enzymes, and that these enzymes appear in an active form in the blood that drains from the infarcted myocardium.

Rapid clinical utilization of these observations followed the report by LaDue and associates[1] in 1954, that serum glutamic oxalacetic transaminase (SGOT) is elevated after an acute myocardial infarct. As indicated in the preamble to this report, many other enzymes have been tested for their applicability in the confirmation of the diagnosis of myocardial infarct. The myocardium is a rich source of enzymes, and, in addition to SGOT, elevations of the malate and lactate dehydrogenases, aldolase, phosphoglucose isomerase, and creatine phosphokinase are usually present after infarction of cardiac muscle.

To the present time, however, only five enzymatic assays have withstood critical clinical and laboratory investigation. These are the measurement of (1) serum glutamic oxalacetic transaminase (SGOT); (2) serum lactic dehydrogenase (SLD); (3) isoenzymes of serum lactic dehydrogenase; (4) serum α-hydroxybutyric dehydrogenase (SHBD); and (5) serum creatine phosphokinase (SCPK).

Transaminases. Human tissues and serum contain two transaminases of clinical importance: SGOT and glutamate-pyruvate transaminase (GPT). They catalyze the reversible transfer of an amino (NH_2) group from an amino acid to a receptor keto acid, yielding a new amino acid and a new keto acid:

$$\text{Aspartate} + \text{ketoglutarate} \overset{\text{GOT}}{\rightleftharpoons} \text{Glutamate} + \text{oxalacetate}$$

$$\text{Alanine} + \text{ketoglutarate} \overset{\text{GPT}}{\rightleftharpoons} \text{Glutarate} + \text{pyruvate}$$

The heart and liver are especially plentiful in GOT, but the liver contains much more GPT than does the heart. Thus, the SGOT may be expected to be increased when either of these organs is damaged, but an elevation of SGPT is typical of hepatocellular damage only.

Lactic dehydrogenase. This enzyme is widely distributed throughout most body tissues and catalyzes the reaction:

$$\text{Lactate} + \text{nicotinamide} - \text{adenine dinucleotide (NAD)} \overset{\text{LDH}}{\rightleftharpoons} \text{pyruvate} + \text{reduced nicotinamide} - \text{adenine diculeotide } (NADH_2)$$

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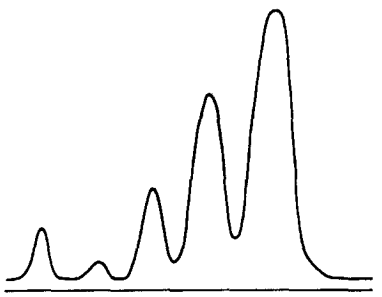

Fig. *1.* Densitometric tracing of a lactic dehydrogenase electropherogram obtained from a patient manifesting an acute myocardial infarct. Note (1) the elevated fast (anodic) isoenzymes and (2) the clear discrimination between these "myocardial" fractions and the cathodic-hepatic isoenzymes.

Table I. *Tetramic molecular association of the two principal polypeptide subunits of lactic dehydrogenase*

Monomers

A B

Tetramers

AA AA	AB AA	AB AB	BB AB	BB BB
LDH_1	LDH_2	LDH_3	LDH_4	LDH_5

Lactic dehydrogenase isoenzymes. Lactic dehydrogenase, in consort with other enzymes, is not a uniform enzyme, but rather occurs in a number of molecular species. These differ in their primary structure, i.e., amino acid sequence, yet possess the same action and substrate specificity (L-lactate). The enzymatically active fractions (isoenzymes or isozymes) can be separated by a variety of means. Under the conditions of electrophoresis (starch gel, agar gel, or cellulose acetate), normal human serum is separable into five distinct isoenzymes.[2] Tissues with a high aerobic metabolism, such as cardiac muscle, have a high content of fast-migrating (anodic) isoenzymes. Conversely, slow-migrating (cathodic) fractions predominate in the liver.[3] The heart and liver fractions occupy extreme poles of the isogram and may be readily distinguished (Fig. 1).

The five isoenzymes are produced by a random tetramic association of two different polypeptide subunits which are considered to be under separate genetic control.[4] The first and fifth are homogeneous tetramers, whereas the three intermediate bands are heterogeneous or "hybrid forms." This suggests that, in fact, only two principal or parent forms of LDH exist. All possible combinations of these two monomers are produced (Table I).

The clinical, laboratory, and diagnostic importance of this molecular heterogeneity lies in the fact that a differential evaluation of an elevated total SLD may be possible when there is concomitant injury to several tissues.

"Alpha-hydroxybutyric dehydrogenase" (HBD). It has been shown that lactic dehydrogenase does not possess an absolute specificity for its usual substrate, but can also convert the oxo-group of ketobutyric acid in the alpha position to the hydroxy group.[5] The fast-migrating SLD isoenzymes exhibit much greater ability than do the slow fractions to reduce α-ketobutyrate relative to their activities with pyruvate as substrate. In deference to the reaction with α-ketobutyrate, the "catalyst" has been designated "α-hydroxybutyric dehydrogenase" (HBD).[5] At present, it is not known what kind of enzyme one is measuring when the assay for SHBD activity is performed, but the measurement has been equated with a chemical differentiation of the fast-migrating SLD isoenzymes.[6]

Creatine phosphokinase (CPK). CPK catalyzes the reversible transfer of a high-energy phosphate group from adenosine triphosphate (ATP) to creatine:

$$\text{ATP} + \text{creatine} \overset{\text{CPK}}{\rightleftharpoons} \text{adenosine diphosphate (ADP)} + \text{creatine phosphate.}$$

The enzyme is found principally in skeletal and heart muscle, and also, in lesser amounts, in brain tissue. Kidney, liver, lung, pancreas, and the erythrocytes have virtually undetectable amounts of CPK activity.[7] The distribution in human tissue has led to the consideration of serum CPK activity as the "most specific and sensitive serum enzyme test currently available for the confirmation of disease or injury to

skeletal muscle and myocardium."[7] In diseases of skeletal muscle, with particular reference to the dystrophies, the estimation of CPK is the measurement of choice, and studies in patients with muscular disorders underscore the value of estimations of CPK in the differential diagnosis between progressive muscular dystrophy and atrophies of neurogenic origin.[8]

Clinical application

Any evaluation of the clinical applicability of an enzyme assay must perforce be a consideration in three fundamental and interrelated areas of consideration: (1) ease of assimilation into the diagnostic laboratory, (2) specificity and sensitivity of the enzyme being measured, and (3) duration of the enzyme's activity in sera after tissue injury.

Laboratory performance. The estimation of the activity of SGOT, SLD, and SHBD may be determined satisfactorily by either spectrophotometric or colorimetric assays. The former has inherent "chemistry" advantages, but equal *clinical* information is derived from both types of assay. As of the present, colorimetric assays of CPK cannot be recommended and are distinctly inferior to ultraviolet spectrophotometric methods.[9]

The electrophoretic separation of SLD isoenzymes is diagnostically superior to techniques relying on heat stability of the fractions. Cellulose acetate fractionation is within the technical ability of any routine laboratory and is readily suited for either a quantitative or qualitative evaluation after separation of the isoenzymes.[3]

Enzyme specificity. There is no enzyme activity measurable in serum which is specific for heart muscle alone. Specificity then becomes a subject of relative evaluation. In decreasing order of relative specificity, the enzymes may be listed as follows: CPK, isoenzyme of LDH, SHBD, SGOT, and SLD.

The nonspecificity of SLD is well known. Table II, listing the tissue activity levels of CPK and GOT, may be considered to represent the two extremes of the relative specificity scale. Table III emphasizes the absence of CPK activity within the erythrocyte, a very important feature when one is considering the effect of hemolysis on serum activity levels.

Early reports attested to a high degree of specificity for heart muscle manifested by α-hydroxybutyric dehydrogenase.[10,11] As clinical and diagnostic experience with the assay was gained, this enhanced specificity was found to be only relative, albeit superior to the measurements of SGOT and SLD.[6] Fig. 2 indicates that elevations above normal may occur in a variety of disorders other than myocardial infarct. Elliott and Wilkinson[5] have resorted to the use of an SHBD/SLD ratio in the differentiation of myocardial disease from other disorders in which the SHBD is elevated. Investigation in our laboratories,[6] and that of Rosalki[12] have not substantiated this discriminatory capacity of the ratio.

Fractionation of the isoenzymes of lactic dehydrogenase has markedly increased the sensitivity and specificity of that enzyme in clinical diagnosis. The various fractions have been designated numerically according to their primary sources as follows[13]:

Table II

GOT activities of human tissues[15]*		*CPK activities of human tissues*[7]†	
Heart	7,800	Skeletal muscle	18,400
Liver	7,100	Heart	5,550
Skeletal muscle	5,000	Brain	3,400
Kidney	4,500	Adrenal gland	136
Pancreas	1,400	Lung	0.5
Spleen	700	Liver	0
Lung	500	Prostate	0
Red blood cell	15	Red blood cell	0
Serum	1	Serum	0

*Relative activity referred to serum as unit.

†Expressed as mean activity of soluble protein (units (× 10^4)/mg.).

Table III. *Normal erythrocyte and serum content of enzyme activity*

	GOT	*LDH*	*HBD*	*CPK*
RBC/serum content	8/1	1,000/1	1,000/1	0

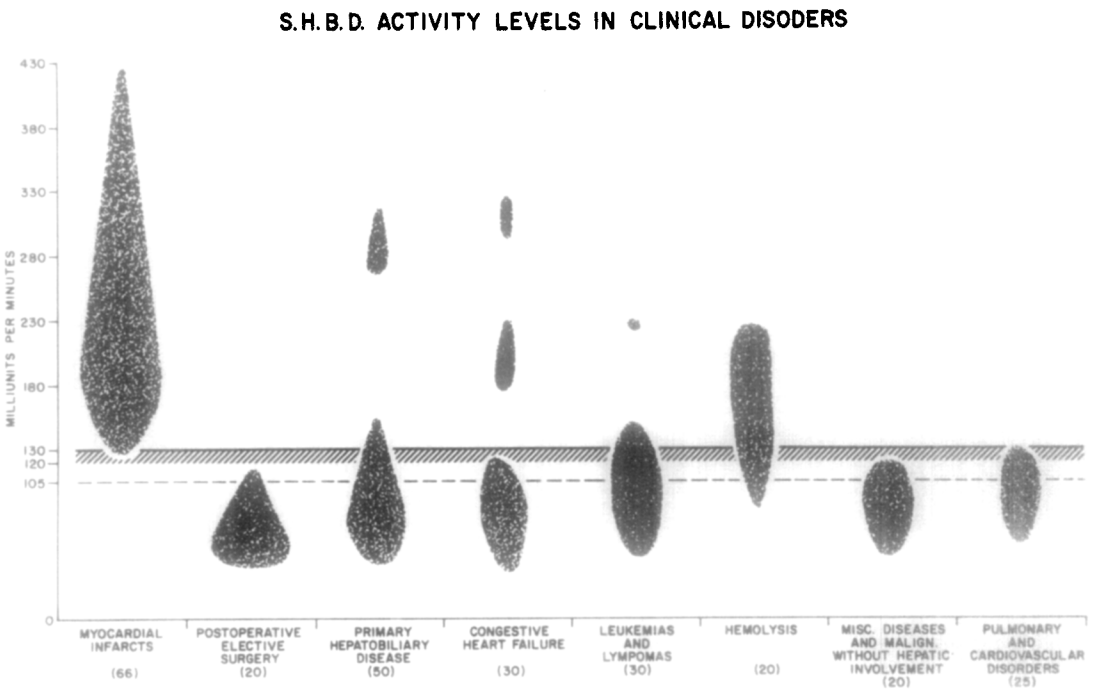

Fig. 2. Although SHBD activity may be considered to be more specific for myocardial lesions than the SGOT or SLD, false positive levels are seen in hepatobiliary disorders, congestive heart failure, and hematopoietic disorders, especially hemolysis. Note, however, the absence of false negatives in the 66 cases of documented myocardial infarct. In the University of Michigan series, all infarcts have produced activity levels above 120 milliunits.

1 from skeletal muscle and liver, *2* and *3* from lungs, *3* and *5* from the pancreas, *3* and *4* from the lungs, *3*, *4*, and *5* from the kidney, *4* and *5* from cardiac muscle.

In actual laboratory and clinical practice, similar and often identical patterns for myocardial infarct are seen in patients manifesting infarcts of the kidney, patients with pernicious anemia or lymphomas with hemolytic anemia, and patients suffering from progressive muscular dystrophy.[3] To emphasize the effect of hemolysis, a common practice is to use hemolyzed serum as a positive control for electrophoretic separations.

Enzyme sensitivity. Under the proper conditions of appropriate selection of test and due consideration of the duration of activity in sera, all five procedures will yield positive results. False negatives are rare and may be attributed to laboratory error in most instances.[14] SLD isoenzyme fractionation appears to have a superior sensitivity. The appearance of a positive increase in the fast fractions may antedate both electrocardiographic changes and a rise in the total enzyme activity in the serum. Ten from our series of over 2,000 isograms have manifested this phenomenon, and the experience of Freeman and Opher[13] corroborates these observations.

Reliance on the estimation of the total activity in sera alone may be misleading in the discrimination between superimposed congestive heart failure and a second infarct or extension of the primary myocardial infarct. In these instances, isoenzyme fractionation and/or estimation of CPK offer considerable assistance: in the former, by the emergence of a hepatic band in the isogram, and in the latter by an additional "peaking" after normalization has occurred. The absence of CPK in the liver makes a secondary rise in activity almost pathognomonic of a superimposed or extended myocardial infarct.

Enzyme release and duration of activity. After an initial elevation following myocardial infarction, enzyme activity in serum declines rather rapidly and the half-

Table IV. *Reaction time or release-activity span in serum after uncomplicated myocardial infarction*

Enzyme activity in serum	*SGOT*	*SLD*	*SHBD*	*SCPK*
Onset of rise	12 hr.	12-24 hr.	12-24 hr.	3-4 hr.
Return to normal	4 days	7 days	10 days	3 days
Peak × normal	4.2	3.6	4.8	11
Time of peak	24 hr.	72 hr.	72 hr.	33 hr.

lives of the enzymes important in the diagnosis of myocardial infarct are short, i.e., the enzymes are rapidly eliminated or inactivated. It may be seen then that, although a normal serum activity level in the course of serial determinations excludes the presence of a "fresh" or superimposed infarct, it does not, depending upon the time of collection, exclude the diagnosis of an initial myocardial infarct.

Table IV summarizes the average time-reaction span and "peaks" of the serum activity of four of the five enzyme assays under consideration. After a myocardial infarction, activity increases in the serum in the following order: (1) CPK, (2) GOT, (3) LD, and (4) HBD. The return to normal levels also follows this sequence. Serum CPK begins to rise before any of the others, often as early as 3 to 4 hours after the infarction. Peak activity is usually reached at 36 hours, followed by a rather precipitous drop to normal levels by the second to the fourth day. The length of time during which SGOT is elevated after the infarction is relatively short—average of 4 to 5 days—and, although SLD is elevated for a longer time, SHBD remains at abnormal levels the longest—10 to 15 days. Large myocardial infarcts may cause persistence of abnormal levels of SHBD for as long as 5 to 10 days after the total enzyme activity of SLD has normalized[13,14] (Fig. 3). If restricted to the choice of one specimen, it would appear to be wisest to delay sampling until the period 24 to 36 hours after infarction. It is this time period which will give the greatest likelihood of obtaining abnormal values for all enzyme activity.

Conclusions

There is no enzyme activity measurable in serum which is specific for myocardial

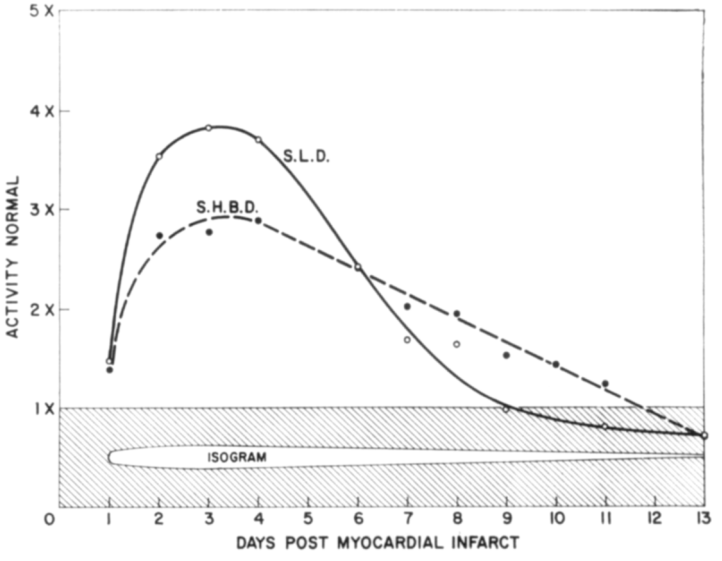

Fig. 3. Graphic depiction of serial isoenzymes and SLD and SHBD activities in the serum from a patient manifesting an uncomplicated myocardial infarct. Note the persistence of elevated levels of SHBD and positive isograms *after* the normalization of SLD.

infarction. Of the five most applicable enzymatic procedures (SGOT, SLD, SLD isoenzymes, SHBD, and CPK) for the confirmation of the diagnosis of myocardial infarct, the estimations of CPK and the fractionation of SLD isoenzymes enjoy the greatest degrees of specificity and sensitivity.

Specificity and sensitivity notwithstanding, the *single* most important variable in the diagnostic application of the tests is the time of collection of the sample in relation to the clinical history. False negatives are the rare exception if the appropriate test is used in the light of activity spans in serum.

Estimations of CPK are most useful in the acute clinical situations, since a rise in its activity may precede that of the other enzymes by 8 hours. All measurements will be positive within 24 to 36 hours after the onset of the infarction. The major significance of estimations of SHBD resides in the fact that its activity persists after other activity levels have returned to normal.

Isoenzyme fractionation and serial estimations of CPK, because of their enhanced discriminatory ability, are superior to all other enzyme tests for purposes of differential diagnosis and prognostic follow-up evaluation.

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