Enzyme assays in diseases of the heart and skeletal muscle

SIDNEY B. ROSALKI

From St Mary's Hospital, London

I Disease of the heart

Heart tissue injury may release cardiac enzymes into the circulation and elevate serum enzyme levels (LaDue, Wróblewski, and Karmen, 1954). Many enzymes become raised, but three enzymes— aspartate aminotransferase (EC 2.6.1.1), creatine kinase (EC 2.7.3.2), and lactate dehydrogenase (EC 1.1.1.27)—have proved of particular diagnostic value. In addition, determination of lactate dehydrogenase isoenzymes by electrophoresis or other methods may be helpful, as may be the combined estimation of alanine aminotransferase (EC 2.6.1.2) and aspartate aminotransferase.

Enzyme Determinations in Suspected Myocardial Infarction

Determination of cardiac enzymes is most frequently required for confirmation of suspected myocardial infarction. Diagnosis requires knowledge of enzyme changes following infarction, of enzyme changes produced by other cardiac diseases and pulmonary causes of chest pain, and also of the non-cardiopulmonary conditions which may also result in enzyme elevation.

Aspartate Aminotransferase

Elevation of this enzyme in serum commences some six to eight hours after infarction, reaches a peak after 24 hours, and returns to normal on average by the fifth day (LaDue and Wróblewski, 1955). If serial determinations are made within four days of infarction, elevated values are found in over 95% of patients (Agress, 1959). But, if initial sampling is delayed to the fourth day the test becomes unreliable.

Creatine Kinase

This enzyme begins to increase in serum within three to six hours of myocardial infarction, reaches a peak at 24 hours, and returns to normal on average by the third day (Dreyfus, Schapira, Resnais, and Scebat, 1960). The early elevation is advantageous in permitting early diagnosis and the selection of cases for admission to intensive care units (Smith, 1967). In most published series raised levels were found in approximately 90% of cases of myocardial infarction. This is lower than that found with aspartate aminotransferase because blood sampling is frequently not possible within the short period of increased creatine kinase activity. However, both enzymes are found to be raised with equal frequency when samples obtained within the first 48 hours are compared (Smith, 1967).

Lactate Dehydrogenase

Elevation of serum lactate dehydrogenase commences some 12 hours after infarction, reaches a peak after 48 hours, and returns to normal on average by the 11th day (Wacker, Ulmer, and Vallee, 1956; King and Waind, 1960). This delayed return is valuable if a blood sample cannot be obtained soon after the infarct. A raised level is found in more than 95% of cases and, when initial sampling is carried out after the third day, is more frequent than elevation of aspartate aminotransferase.

Enzyme Levels After Infarction

The degree of enzyme elevation following infarction varies with each enzyme. The most sensitive is creatine kinase, for which raised values average some seven to 10 times the upper limit of normal; next comes aspartate aminotransferase followed by lactate dehydrogenase. With all these enzymes, the smaller the infarct the lower is the peak enzyme level. With low peak values the serum enzyme levels return to normal sooner than the average figures quoted above (LaDue and Wróblewski, 1955; Rosalki, 1963), but in the case of lactate dehydrogenase a return to normal within five days of infarction is very uncommon.
**PROGNOSTIC VALUE OF ENZYME DETERMINATIONS**

The degree of enzyme elevation has some prognostic significance (Chinsky and Sherry, 1957), and higher mortality rates in the early (within six weeks) and late (two and five year follow-up) post-infarctiver period occur with high enzyme levels (Kibe and Nilsson, 1967). For example, patients with aspartate aminotransferase values greater than 150 IU or lactate dehydrogenase (pyruvate → lactate methods) above 1,000 IU per litre at 25°C show mortality figures more than twice those of patients with peak serum enzyme levels below these figures (Rosalki, 1963).

**SUGGESTED SCHEDULE FOR DIAGNOSIS OF INFARCTION BY ENZYME ASSAY**

For the diagnosis of myocardial infarction it is recommended that a blood sample should be taken as early as possible after infarction followed by two additional samples at intervals of 24 hours. This routine has the advantage that transient enzyme changes will not be missed and that peak enzyme levels can be assessed for use as a guide to prognosis. Where initial sampling is delayed, or elevation above the normal range is slight, a rise or fall in enzyme levels greater than the normal variation per day may provide diagnostic confirmation. With careful methodology such variation in normal subjects does not exceed 7 IU per litre at 25°C for aspartate aminotransferase (Goble and O’Brien, 1958), or 40 IU per litre for lactate dehydrogenase (pyruvate→lactate methods) (Nutter, Trujillo, and Evans, 1966). Similarly, variation of creatine kinase (creatine phosphate → creatine methods) does not normally exceed 10 IU per litre at 25°C on ambulant sedentary subjects; levels of this enzyme normally fall with bed rest (Griffiths, 1966) but a rise exceeding this value may be significant.

It is advisable to carry out at least two enzymatic procedures to guard against the possibility of methodological error. In the first 48 hours following infarction the combination of aspartate aminotransferase and creatine kinase would be the logical choice. After this, a combination of the former with lactate dehydrogenase or its isoenzymes is preferable. With this regime, elevated serum enzyme activity can be detected in over 95% of patients with recent myocardial infarction. This figure can be compared with the much lower figures generally quoted for definitive diagnosis by clinical means alone (80%), by electrocardiographic examination alone (80%), and by combined clinical and ECG examination (90%) (Kibe and Nilsson, 1967).

**DIFFERENTIAL DIAGNOSTIC ROLE OF LACTATE DEHYDROGENASE ISOENZYMES AND ALANINE AMINOTRANSFERASE DETERMINATION**

Whilst enzyme changes provide convenient confirmation of suspected infarction, and the diagnosis of infarction must be suspect in their absence, it must not be forgotten that other cardiac, chest, and non-cardiopulmonary disorders may also result in serum enzyme elevation.

It is in the differential diagnosis of myocardial infarction from other causes of enzyme elevation that lactate dehydrogenase (LD) isoenzyme determination and the assay of both the aspartate and alanine aminotransferases prove helpful. Separation of the isoenzymes of creatine kinase and aspartate aminotransferase is not of diagnostic value.

**LACTATE DEHYDROGENASE ISOENZYMES**

The lactate dehydrogenase of human somatic tissues can be separated by electrophoresis into five isoenzymes (Wieland and Pfleiderer, 1957). The band moving fastest towards the anode is designated fraction 1, and the remaining fractions are numbered consecutively—fraction 5 being the slowest moving. The relative proportions of the isoenzymes vary with the tissue of origin. Heart, kidney, and red cells contain mainly the fastest moving isoenzymes, LD1 and LD2. Lung contains isoenzymes of intermediate mobility, LD2, LD3, and LD4. Liver contains mainly the slowest moving isoenzyme, LD5, and this isoenzyme is generally the most prominent in skeletal muscle, although other fractions are present (Wieme, 1959; Wróblewski, Ross, and Gregory, 1960).

In normal serum LD1, LD2, and LD3 are readily detected, LD2 being the most prominent fraction. When serum total LD activity is raised, the isoenzyme fractions corresponding to those most prominent in the involved tissues are also increased. Therefore after myocardial infarction it is the fast moving isoenzymes, particularly LD3, that are found to be raised (Vesell and Bearn, 1957; Wróblewski et al, 1960), the characteristic alteration being an LD1 level that is equal to or exceeds that of LD4 (Cohen, Djordjevich, and Ormiste, 1964; Moller and Raabo, 1964). When this LD isoenzyme pattern is found in serum, it suggests that LD elevation is of cardiac origin and this may assist differentiation from other disorders which result in elevation of total LD activity but with a different isoenzyme pattern (Table I). It should be remembered, however, that megaloblastic anaemia, acute haemolysis, renal infarction, and sometimes the Duchenne type of muscular dystrophy (Englhardt-Gökel, Löbel, Seitz, and Waller, 1958; Wróblewski and Gregory, 1961; Richterich et al, 1961; Cohen et al, 1964) may produce a similar alteration in LD isoenzyme patterns.

The ability of all these procedures to discriminate between LD elevation due to fast or slow moving LD isoenzymes in serum is very similar, with measurement of urea-stable LD activity perhaps showing some marginal advantage. The choice of determination must thus be a matter for individual laboratory preference. The author prefers HBD determination, and many years' experience with the method has shown it to be a reliable diagnostic procedure. However, since batches of substrate and co-enzyme (reduced NAD) may contain enzyme inhibitors which affect the fast moving isoenzymes more than the slow and (in the case of the co-enzyme) HBD more than LD (Rosalki; unpublished data), the use of inhibitor-free reagents in all LD procedures is essential.

The advantages of LD isoenzyme procedures are threefold. First, they increase the diagnostic specificity and sensitivity of LD determination by their preferential measurement of the fast moving LD isoenzymes; second, they permit earlier diagnosis than is possible with total LD determination alone because the LD3 isoenzyme fraction may be elevated whilst total LD activity still remains within the normal range; and third they may permit diagnosis of infarction after total LD activity has returned to normal, for LD3 activity may remain elevated several days longer, elevation sometimes extending into the third or fourth week following infarction.

Whilst these procedures usefully extend the value of total LD determination, it is the author's opinion that they are all less informative than full isoenzyme determination and quantitation by electrophoresis. This alone is helpful in determining when LD isoenzymes of intermediate mobility are responsible for total LD elevation and is also the only procedure which can conveniently and sensitively demonstrate combined elevation of anodic and cathodic fractions.

**Table 1**  Non-cardiopulmonary diseases causing serum enzyme elevation

<table>
<thead>
<tr>
<th>Disease</th>
<th>Aspartate Amino-</th>
<th>Alanine Amino-</th>
<th>Lactate Dehydro-</th>
<th>Isoenzymes</th>
<th>Creatine Kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver disease</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>LD3,</td>
<td>-</td>
</tr>
<tr>
<td>Prolonged hypotension</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>LD1,</td>
<td>-</td>
</tr>
<tr>
<td>Acute pancreatitis</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>LD1,</td>
<td>-</td>
</tr>
<tr>
<td>Renal infarction</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>LD1,</td>
<td>±</td>
</tr>
<tr>
<td>Acute haemolysis</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>LD1,</td>
<td>±</td>
</tr>
<tr>
<td>Megaloblastic anaemia</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>LD1,</td>
<td>±</td>
</tr>
<tr>
<td>Disseminated malignancy</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>LD4,5,6,7</td>
<td>-</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>LD or 5,</td>
<td>+</td>
</tr>
<tr>
<td>Muscle disease or trauma</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>LD or 5,</td>
<td>+</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Acute psychoses</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>LD,</td>
<td>±</td>
</tr>
<tr>
<td>Drug toxicity</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>LD,</td>
<td>±</td>
</tr>
</tbody>
</table>

+ = frequent enzyme elevation  ± = occasionally elevated  - = elevation absent or rare

Provided these conditions are remembered, they are not likely to be a source of diagnostic confusion with cardiac disease.

LD isoenzyme examination is normally carried out by electrophoresis of serum, isoenzymes being demonstrated by staining, and a quantitative assessment of the stained bands being made densitometrically. However, several non-electrophoretic techniques are available. Amongst the most popular are the heat treatment of serum, determination of serum alpha-hydroxybutyrate dehydrogenase\(^1\) activity, and the measurement of urea-stable LD activity.

**HEAT-STABLE LACTATE DEHYDROGENASE**

The slow moving LD isoenzymes are relatively heat-labile so that measurement of heat-stable LD activity in serum provides a measure of the fast moving isoenzymes.

**ALPHA-HYDROXYBUTYRATE DEHYDROGENASE (HBD)**

If 2-oxobutyrate is substituted for pyruvate as substrate for LD it is preferentially reduced by the faster moving LD isoenzymes (Rosalki and Wilkinson, 1960), and estimation of this activity in serum is another way of measuring the proportion of the faster LD isoenzymes.

**UREA-STABLE LACTATE DEHYDROGENASE**


The ability of all these procedures to discriminate between LD elevation due to fast or slow moving LD isoenzymes in serum is very similar, with measurement of urea-stable LD activity perhaps showing some marginal advantage. The choice of determination must thus be a matter for individual laboratory preference. The author prefers HBD determination, and many years' experience with the method has shown it to be a reliable diagnostic procedure. However, since batches of substrate and co-enzyme (reduced NAD) may contain enzyme inhibitors which affect the fast moving isoenzymes more than the slow and (in the case of the co-enzyme) HBD more than LD (Rosalki; unpublished data), the use of inhibitor-free reagents in all LD procedures is essential.

The advantages of LD isoenzyme procedures are threefold. First, they increase the diagnostic specificity and sensitivity of LD determination by their preferential measurement of the fast moving LD isoenzymes; second, they permit earlier diagnosis than is possible with total LD determination alone because the LD3 isoenzyme fraction may be elevated whilst total LD activity still remains within the normal range; and third they may permit diagnosis of infarction after total LD activity has returned to normal, for LD3 activity may remain elevated several days longer, elevation sometimes extending into the third or fourth week following infarction.

Whilst these procedures usefully extend the value of total LD determination, it is the author's opinion that they are all less informative than full isoenzyme determination and quantitation by electrophoresis. This alone is helpful in determining when LD isoenzymes of intermediate mobility are responsible for total LD elevation and is also the only procedure which can conveniently and sensitively demonstrate combined elevation of anodic and cathodic fractions.

**ALANINE AMINOTRANSFERASE**

The combination of determination of this enzyme with that of aspartate aminotransferase is helpful in differentiating a high serum level of the latter of cardiac origin from one of hepatic origin (Wróblewski...
Enzyme assays in diseases of the heart and skeletal muscle

Paroxysmal arrhythmias
In the absence of infarction, these show elevation of serum aspartate aminotransferase in more than 20% of patients who develop heart rates exceeding 140 per minute and lasting for more than 30 minutes (Runde and Dale, 1966). Elevation is generally the result of hepatic congestion so that elevation of alanine as well as aspartate aminotransferase is usually present, levels of the latter generally not exceeding 40 IU per litre at 25°C. Both creatine kinase and lactate dehydrogenase are usually normal but, should the latter be elevated, it is generally the LD₅ fraction which is found to be increased. Occasionally, pulmonary congestion causes elevation of intermediate LD isoenzymes, or impairment of coronary perfusion may result in release of cardiac isoenzymes.

Chronic congestive cardiac failure
About 15% of patients with chronic congestive cardiac failure are found to show elevated aspartate aminotransferase and lactate dehydrogenase levels. This results from liver damage (Agress, 1959) so that it is the LD₅ fraction that is increased and elevation of the alanine aminotransferase is common, though all three generally remain below twice the upper limit of normal. Creatine kinase activity remains normal.

ENZYME CHANGES IN CARDIAC DISORDERS OTHER THAN MYOCARDIAL INFARCTION

Angina of effort
In this condition no alteration of serum enzyme levels takes place (LaDue and Wróblewski, 1955).

Acute coronary insufficiency
In patients with prolonged cardiac pain but without other clinical or ECG evidence of infarction (which is presumed not to occur), increased enzyme activity in serum is observed in more than 10% of cases (Goble and O'Brien, 1958; Agress, 1959; Resnik, 1962). The levels generally remain less than twice the upper limit of normal and when LD is elevated it is the LD₅ fraction that is increased. Occasionally the elevation is delayed until the fourth to 10th day following the attack. Whether the relatively small rise in serum enzymes indicates some myocardial necrosis is unknown although this is generally assumed.

Pericarditis
Serum enzyme levels are normal in more than 90% of patients (Agress, 1959). Where raised values are found, minor myocardial damage is thought to be the reason.

Myocarditis
This may be accompanied by raised serum enzyme levels if the condition is active (Nydick, Tang, Stollerman, Wróblewski, and LaDue, 1955; Chesler, 1958). LD₅ activity is prominent when total LD activity is elevated (Cohen et al, 1964).

Pneumonia
In pneumonia, aspartate aminotransferase and creatine kinase generally remain normal. Reports of elevation of the latter in pulmonary disease have frequently been traced to release of the enzyme from muscle damaged by intramuscular injections. Lung tissue itself contains minimal amounts of this enzyme. Up to one third of patients with pneumonia may show elevated total LD activity but such elevation is slight; the LD₅ fraction may be increased (Mager, Blatt, and Abelmann, 1966) presumably as a result of intrapulmonary red cell breakdown.

SOME ENZYME CHANGES IN NON-CARDIAC CAUSES OF CHEST PAIN
Three conditions deserve special consideration because of the possibility of confusion with myocardial infarction. These are pulmonary infarction, pneumonia, and dissecting aortic aneurysm.

Pulmonary infarction
As a result of pulmonary infarction aspartate aminotransferase is elevated in some 25% of patients (Agress, 1959). The increase is usually small, the levels rarely exceeding 40 IU per litre at 25°C, and occurs only in severe cases, frequently being delayed until the fourth day. In myocardial infarction of comparable severity more pronounced changes are usual. It is not easy to assess the incidence of LD elevation following pulmonary infarction, because of widely diverging reports, possibly due to varying methodology. It appears that minor degrees of elevation are a frequent but inconstant accompaniment. Elevation, of the LD₅ fraction as a result of accompanying haemolysis of red cells in the infarcted lung tissues, of LD₅ as a result of liver damage from hepatic congestion, or of LD₅, LD₅, and LD₅ released from the damaged lung tissue itself, may be observed (Cohen et al, 1964). Creatine kinase activity remains normal.
Acute dissecting aortic aneurysm
Acute dissection of the aorta is generally unaccompanied by an alteration in serum cardiac enzyme levels unless the aortic arch is involved. When this occurs, more than a third of patients show increased enzyme activity which may reach very high levels (Wilkie, 1969). This is believed to result from heart muscle damage secondary to occlusion of the coronary orifices.

Non-cardiopulmonary diseases causing serum enzyme elevation
Non-cardiopulmonary conditions which may result in serum enzyme elevation are listed in Table I. Provided that they are remembered, they rarely cause confusion in the diagnosis of myocardial infarction.

Comparative value of assays of serum aspartate aminotransferase, lactate dehydrogenase, and creatine kinase in differential diagnosis
It seems appropriate at this point to sum up briefly the value of assays of these enzymes in serum in differentiating myocardial infarction from other disorders.

Aspartate aminotransferase activity is raised in a variety of conditions in many of which liver damage is a feature. Concomitant elevation of alanine aminotransferase helps to identify the latter.

Elevation of lactate dehydrogenase occurs in a wide variety of conditions as the aspartate aminotransferase. However, elevation of the LD1 isoenzyme, which is found mainly in the heart, red cells, and kidney, limits the number of non-cardiac disorders which need be considered.

Creatine kinase activity possesses the highest degree of specificity. It is not elevated in liver disease, blood diseases, or malignant diseases nor is elevation a feature of pulmonary disease. It must, however, be remembered that spurious elevation may result from intramuscular injections (Hess, MacDonald, Frederick Jones, Neely, and Gross, 1964) and that raised levels may follow recent, prolonged and severe exercise (Griffiths, 1966).

Diagnosis of recurrence of myocardial infarction
Should myocardial infarction recur in the early post-infarction period, renewed elevation of serum enzymes takes place (LaDue and Wróblewski, 1955). The ECG diagnosis of re-infarction may be impossible because of persistence of the ECG changes of the initial infarction. Renewed elevation of serum creatine kinase is the most easily detected serum enzyme change, since this is the first serum enzyme to return to normal following the initial episode.

Diagnosis of myocardial infarction in the early postoperative period
Myocardial infarction occurring during the early postoperative period is an important condition and carries a high mortality (Dack, 1963). It is most frequent within three days of operation, the incidence being highest in major operations on older subjects with a previous history of cardiac disorder. Enzyme elevation during the postoperative period may result from operative trauma alone, and the readiness with which creatine kinase increases after muscle trauma renders this enzyme valueless for the detection of myocardial infarction in the early postoperative period. The fast moving LD isoenzyme shows the least elevation as a result of operative trauma (Killen, 1968) whereas this fraction is, typically, consistently elevated following infarction. Determination of LD1 would therefore appear to be the procedure of choice in this diagnostic situation, but some reports suggest that postoperative infarction may occur with only minimal changes in this fraction (Hunter, Endrey-Walder, Bauer, and Stephens, 1968).

D-glutamyltransferase (EC 2.3.2.1) activity after myocardial infarction
The enzyme D-glutamyltransferase has been found to show protracted elevation following myocardial infarction (Agostoni, Ideo, and Stabilini, 1965; Hedworth-Whitty, Whitfield, and Richardson, 1967), raised values occurring after the fourth day and persisting for up to a month. Whilst such changes are frequent following infarction, and are unusual following cardiac pain without infarction (Rosalki, Rau, Lehmann, and Prentice, 1970), they must be interpreted with caution because raised values are also found in liver disease and chronic ischaemic heart disease.

The source of the elevation following myocardial infarction is uncertain but in most cases is thought to be the liver, hence the test cannot be regarded as a reliable indication of either infarction or ischaemic heart disease. D-glutamyltransferase activity is negligible in normal heart tissue and though an increase in cardiac content following infarction has been reported in animal studies (Ravens, Gudbjarnason, Cowan, and Bing, 1969) the author has examined several infarcted human hearts without observing any significant enzyme activity.

Other applications of enzyme determinations in cardiac disease
Diagnosis of cardiac transplant rejection
Measurement of serum LD activity has been claimed

*Also known as γ-glutamyltranspeptidase.
to be of value in the diagnosis of cardiac transplant rejection, particularly in the first month when ECG changes are unreliable. Rejection may be signified by LD₂ activity exceeding that of LD₁, LD₂ activity greater than 35% of total LD activity, and LD₁ activity more than twice the upper limit of normal for this fraction (Nora, Cooley, Johnson, Watson, and Milam, 1969). However, LD alteration resulting from operation, from the use of extracorporeal circulation or from immunosuppressant drugs may obscure the interpretation of post-transplant LD changes.

**Assessment of Cardiotoxic Effect of Drugs**

Serum enzyme determinations have been used to assess the cardiotoxic effects of drugs. Patients treated with emetine derivatives have sometimes been monitored in this way (Dempsey and Salem, 1966).

**Detection of Intravascular Haemolysis Resulting from Leaking Heart-Valve Prostheses**

Leakage of ball-valve prostheses used to replace heart valves is accompanied by intravascular haemolysis. Such haemolysis liberates red cell LD and results in elevated serum activity, which can be used to assess the degree of haemolysis and to detect valve leakage (Myhre and Rasmussen, 1970).

### II Diseases of Skeletal Muscle

Disorders of skeletal muscle include those which are secondary to an abnormality of motor innervation, and the myopathies, which are primary disorders of skeletal muscle. As a group the myopathies are uncommon and include conditions of exceptional rarity. The most important myopathies clinically are the dystrophies, polymyositis, and myopathies associated with metabolic or endocrine disorders.

In the myopathies, enzymes may be released from muscle and increased serum enzyme activity may result. When this occurs raised levels of creatine kinase (Ebashi, Toyokura, Monoi, and Sugita, 1959), ketose 1-phosphate aldolase (EC 4.1.2.7) (Sibley and Lehninger, 1949), lactate dehydrogenase (Schapira and Dreyfus, 1957), and aspartate and alanineaminotransferases (Dreyfus and Schapira, 1955) may all be found. In every variety of myopathy, however, creatine kinase is the serum enzyme most frequently raised and shows the greatest degree of elevation, so that assay of this enzyme is the assay of choice for the investigation of muscle disease. The changes that occur in some important myopathies and in other muscle disorders from which they must be differentiated are summarized in Table II.

**Table II Creatine Kinase in Muscle Disease**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Change²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myopathy</td>
<td></td>
</tr>
<tr>
<td>Dystrophy</td>
<td>+ to +++</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>− to + ++</td>
</tr>
<tr>
<td>Metabolic myopathies</td>
<td>±</td>
</tr>
<tr>
<td>McArdle etc</td>
<td>+</td>
</tr>
<tr>
<td>Alcoholic</td>
<td>−</td>
</tr>
<tr>
<td>Thyrotoxic</td>
<td>−</td>
</tr>
<tr>
<td>Steroid</td>
<td>−</td>
</tr>
<tr>
<td>Disorders of motor innervation</td>
<td></td>
</tr>
<tr>
<td>Werding-Hoffman</td>
<td>− to ±</td>
</tr>
<tr>
<td>Benign congenital hypotonia</td>
<td>−</td>
</tr>
<tr>
<td>Kugelberg-Welander syndrome</td>
<td>− to +</td>
</tr>
<tr>
<td>Motor neurone disease</td>
<td>− to ±</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>−</td>
</tr>
<tr>
<td>Polynucleosis</td>
<td>−</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>−</td>
</tr>
<tr>
<td>Trauma</td>
<td></td>
</tr>
<tr>
<td>including intramuscular injections and surgery</td>
<td>+ to +++</td>
</tr>
<tr>
<td>Convulsive disorders</td>
<td></td>
</tr>
<tr>
<td>including epilepsy and tetanus</td>
<td>+</td>
</tr>
</tbody>
</table>

¹Elevation of muscular origin is also found in hypothyroidism, hyperthermia, cerebrovascular disease, and psychotic disorders. Physiological elevation occurs after exercise, in early childhood and in early postpartum period. An increase of cardiac origin is found in heart disease.

²+ = elevation, + + + = marked elevation, ± = mild, inconstant elevation, − = normal.

**Serum Creatine Kinase Activity in Myopathies**

**Muscular Dystrophy**

The muscular dystrophies are genetically determined primary degenerative disorders of muscle. The most important varieties are the Duchenne, the limb-girdle, and the facioscapulohumeral dystrophies, of which the Duchenne dystrophy is the commonest and the most severe. Each is characterized by progressive weakness and wasting of proximal skeletal muscles. The clinical features of these dystrophies are summarized in Table III.

---

¹Commonly known as aldolase or fructose 1-phosphate aldolase.
Duchenne dystrophy

In Duchenne dystrophy elevated serum creatine kinase activity is a constant feature and is a prerequisite for diagnosis. The highest values occur in early childhood (Okinaka, Kumagai, Ebashi, Sugita, Momoi, Toyokura, and Fujiie, 1961), elevation averaging some 70 times normal. Enzyme levels may be of prognostic importance (Dreyfus, Schapira, and Schapira, 1958), for it is suggested that the higher the value relative to the age of the child, the more rapid the progression of the disease. With progression, the serum activity falls as a result of loss of muscle mass from which enzymes can leak into the serum, and also because of reduced patient activity. Even in chair-bound or preterminal patients, however, some elevation persists.

Determination of this enzyme in serum permits diagnosis of Duchenne dystrophy years before it becomes apparent clinically. Very high enzyme levels have been noted in symptomless children with a family history of the disorder, who have subsequently developed the disease (Aebi, Richterich, Stillhart, Colombo, and Rossi, 1961). Conversely, the finding of persistently normal serum activity in early childhood may be used to reassure parents that the disorder will not develop. Reliable diagnostic information has been obtained as early as the age of 3 months.

Duchenne dystrophy is transmitted as a sex-linked recessive disease by female carriers who are clinically normal or near normal. Approximately 1 in 5,000 females are carriers of the disorder, and half the sons of such carriers will develop the disease, and half the daughters will be carriers. Serum creatine kinase assay is the procedure of choice for the detection of the carrier state and thereby assists genetic counselling. Over 80% of symptomless female carriers of Duchenne dystrophy show raised values, the highest incidence and levels occurring in younger subjects (Thompson, Murphy, and McAlpine, 1967). Thus the carrier state may be confirmed by elevated serum activity of this enzyme, whereas with persistently normal values the odds against it are at least 4 to 1. In the detection of carriers by creatine kinase estimation, great care must be taken to minimize the possibility of false positive or negative results (Thompson, 1969). Necessary precautions are indicated in Table IV Should a carrier become pregnant, amniocentesis may be used to determine the sex of the foetus, but enzyme determinations on amniotic fluid are of no value in determining whether or not the child is affected.

Limbgirdle dystrophy

In this dystrophy raised levels of creatine kinase in

<table>
<thead>
<tr>
<th>Timing of Specimens</th>
<th>Specimen Handling</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>During the evening, since levels are then maximal in carriers, whereas they may be normal in a waking specimen, even in known carriers.</td>
<td>Analyse while fresh up to one week at 4° or after preservation up to one month at −18°</td>
<td>Sensitive established method, with thiol included in substrate. Duplicate determination.</td>
</tr>
<tr>
<td>After normal activity which, in carriers, causes the normal resting values to rise to abnormal levels. It has no effect on values in normal subjects.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not for at least 48 hours after severe, prolonged exercise which raises the level in normal subjects.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three specimens at weekly intervals to avoid the possibility of cyclical variation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table IV Precautions to ensure accuracy in the detection of the carrier state of Duchenne muscular dystrophy
Enzyme assays in diseases of the heart and skeletal muscle

the serum are found in about 75% of patients. The increase is less than in Duchenne dystrophy, levels averaging some 20 times normal. Such assays are of no value for detecting heterozygotes (Richterich, Rosin, Aebi, and Rossi, 1963).

Facioscapulohumeral dystrophy
In this condition elevation of serum creatine kinase occurs in some 80% of patients, levels averaging about five times normal.

Polymyositis
Polymyositis is an inflammatory myopathy which may be secondary to bacterial, viral, or parasitic infection of muscle, or may occur as a primary muscle disorder of unknown aetiology. The latter, so-called idiopathic polymyositis, occurs at any age and in both sexes, though females are more commonly affected. It is sometimes associated with skin disease, collagen disease, or malignancy. The onset may be acute, with fever, weakness, and muscle pain, or it may be insidious with progressive muscle weakness but no constitutional disturbance.

In idiopathic polymyositis, serum creatine kinase is elevated in about two-thirds of patients. Enzyme levels correlate with the activity of the disease. The highest values are found in affected children, and low or normal values occur in chronic, slowly progressive disease in adults. The levels may fluctuate widely, in contrast to the minor fluctuations seen in the muscular dystrophies (Richterich et al, 1963) from which this condition must frequently be distinguished.

Enzyme estimation may be used to assess the response of idiopathic polymyositis to steroid treatment. Raised serum creatine kinase activity may sometimes revert to normal within 48 hours if therapy is successful, and failure of enzyme levels to diminish indicates a lack of response or possibly the need for an increased dose of steroid.

In other varieties of polymyositis creatine kinase elevation is inconstant and relatively minor.

Other myopathies
Myopathy in childhood
A very large number of rare myopathies occur in infancy and childhood. In many of these (eg, central core disease and glycogenosis affecting muscle) moderate elevation of serum creatine kinase is common, so that assays are of no value in their differentiation. However, this mild elevation contrasts with the high levels found in the muscular dystrophies and with the normal values found in disorders of motor innervation, from which these rarer myopathies must be distinguished.

Thyrotoxic and steroid myopathies
The cause of myopathy in adults is commonly metabolic or endocrine, and the condition is found in some patients with thyrotoxicosis or receiving very large doses of steroids. Both these latter varieties are characterized by normal serum creatine kinase activity (Vassella, Richterich, and Rossi, 1965; Ekblom, Hed, Herdenstam, and Nygren, 1966). It is possible that thyroid and steroid hormones interfere with the permeability of the muscle fibre to the enzyme despite the presence of fibre damage.

Alcoholic myopathy and myopathy associated with malignant hyperpyrexia
Two varieties of myopathy in which elevation of serum creatine kinase has been observed recently merit special consideration; these are the syndromes that may accompany chronic alcoholism and the myopathy associated with malignant hyperpyrexia.

1 In chronic alcoholics, both acute and chronic muscle syndromes sometimes occur. The acute syndrome follows recent alcoholic excess and is characterized by painful, tender muscles, muscle cramps, and sometimes myoglobinuria. Over 80% of such patients show increased creatine kinase activity and such elevation is also found in a similar proportion of chronic alcoholics following acute intoxication even in the absence of previous or concomitant symptoms of muscle disease (Nygren, 1966; Perkoff, Dioso, Bleisch, and Klinkerfuss, 1967; Lafair and Myerson, 1968). The enzyme increases some three to five days following a drinking bout and returns to normal within two weeks if drinking is discontinued. Enzyme levels average some four times the upper limit of normal.

The chronic muscular syndrome is unrelated to recent heavy drinking, and is characterized by progressive weakness and wasting of proximal muscles, occasionally with muscle tenderness. In this condition increased creatine kinase activity is found in some 60% of patients, levels averaging twice the upper limit of normal (Perkoff et al, 1967; Lafair and Myerson, 1968).

Chronic alcoholics without evidence of chronic myopathy who have not been drinking recently show normal values.

2 Malignant hyperpyrexia is a rare condition which may follow the administration of a general anaesthetic, particularly when halothane or succinylcholine is used. The incidence is 1 per 15,000 anaesthetics and there is a 66% mortality (Kalow, Britt, Terreau, and Haist, 1970). The condition is commoner in children and young adults and in males and there is frequently a family history of the disease indicating inheritance as an autosomal dominant. Clinically it is characterized by impaired respiration,
muscle spasms, shock, raised body temperature, and general convulsions. Extremely high serum levels of creatine kinase have been noted in the early post-anæsthetic period (Denborough, Forster, Hudson, Carter, and Zapf, 1970b).

Increased serum levels are also found in relatives, some of whom are symptomless whereas others show a myopathy characterized by weakness and wasting, especially of the distal part of the thigh muscle (Isaacs and Barlow, 1970; Denborough, Ebeling, King, and Zapf, 1970a). It is advisable that relatives showing such increased serum levels be treated with caution should they require a general anaesthetic. It may be that persistent elevation of serum creatine kinase from any cause, particularly if associated with a myopathy, constitutes a special anaesthetic risk. It has been suggested that screening of the general public for elevated serum creatine kinase might be a means of preventing this disorder (Denborough et al, 1970a). However, apart from the obvious practical and economic difficulties of this procedure, its usefulness is questionable. Many healthy subjects show marked lability of serum values (Griffiths, 1966) and show an exaggeration of the elevation which normally occurs on exercise. Moreover, some apparently healthy patients with persistently elevated levels are said to have no predisposition to hyperpyrexia following general anaesthesia (Emery and Spikesman, 1970).

Muscle Disease Secondary to Disorder of Motor Innervation

Disorders of motor innervation may result in muscular weakness and wasting resembling the myopathies. In these conditions, the most important of which are shown in Table II, serum creatine kinase activity is generally normal or shows only infrequent or modest elevation (Okinaka et al, 1961). When an increase does occur it is usually confined to the period when the neurological disorder is in an early progressive phase. Levels are generally less than twice the upper limit of normal.

In childhood, differentiation of neurogenic disorder from muscular dystrophy is generally easy because of the high creatine kinase values found in dystrophy, but in adults enzyme determinations may not always distinguish between them.

Other Muscular Conditions Associated with Elevation of Serum Creatine Kinase

MUSCLE TRAUMA

This may be accidental or the result of surgery and may result in pronounced elevation of serum creatine kinase. Values up to five times the upper limit of normal may also result from intramuscular injections (Hess et al, 1964). The possibility of such iatrogenic elevation must therefore always be borne in mind in the interpretation of raised serum levels.

Increased levels may be found following convulsions (Fukuyama and Kawazura, cited by Okinaka, Sugita, Momoi, Toyokura, Watanabe, Ebashi, and Ebashi, 1964) and in tetanus (Mullan and Dubowitz, 1964), the enzyme possibly originating from the convulsing muscle tissue. In tetanus, however, serum levels may rise before the onset of convulsions, probably as a result of changes in the permeability of the muscle fibre membrane caused by tetanus toxin (Brody and Hatcher, 1967).

Other Conditions

In hypothermia (Maclean, Maclean, Griffiths, and Emslie-Smith, 1968), hypothyroidism (Graig and Ross, 1963), cerebrovascular disease (Acheson, James, Hutchinson, and Westhead, 1965; Kalbag, Park, and Pennington, 1966), and acute psychoses (Meltzer, 1968) creatine kinase of muscle origin may appear in the serum. In these, abnormal muscle cell permeability is thought to be the explanation, although why it should occur in psychoses or cerebrovascular disease is obscure.

In hypothyroidism some 80% of patients show raised values, which average eight times the upper limit of normal. There is an inverse relationship between creatine kinase and protein-bound iodine levels in serum (Graig and Smith, 1965) and it has been suggested that thyroid hormone normally influences muscle cell permeability to the enzyme so that, when the hormone level is reduced, permeability is increased.

Finally, it should be remembered that physiological elevation occurs in early childhood (Okinaka et al, 1964) in the early post-partum period (Hughes, 1963), and following prolonged severe exercise (Baumann, Escher, and Richterich, 1962), and that elevation of cardiac origin may result from heart disease (Dreyfus et al, 1960).

References


Enzyme assays in diseases of the heart and skeletal muscle.

S B Rosalki

*J Clin Pathol* 1970 s1-4: 60-70
doi: 10.1136/jcp.s1-4.1.60

Updated information and services can be found at:

[http://jcp.bmj.com/content/s1-4/1/60.citation](http://jcp.bmj.com/content/s1-4/1/60.citation)

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
[http://group.bmj.com/group/rights-licensing/permissions](http://group.bmj.com/group/rights-licensing/permissions)

To order reprints go to:
[http://journals.bmj.com/cgi/reprintform](http://journals.bmj.com/cgi/reprintform)

To subscribe to BMJ go to:
[http://group.bmj.com/subscribe/](http://group.bmj.com/subscribe/)