Creatine Kinase and the Diagnosis of Myocardial Infarction

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It is well established that serum creatine kinase (CK) is a sensitive index of myocardial damage. The test is very widely used in the routine diagnosis of myocardial infarction, although it is recognized to lack specificity for the myocardium. The MB isoenzyme of CK (CK-MB) is more heart-specific, but is more difficult to measure accurately. Furthermore, many of the methods are, to a greater or lesser extent, unsatisfactory and may cause misleading results.

The role of creatine kinase in the diagnosis of myocardial infarction has been reviewed previously [1-4]. The present review will not attempt to cover in detail the same ground, but will emphasize those aspects that are of current interest and those areas where the literature is confusing; for example, the CK-MB content of various body tissues or the relative merits of different methods of CK-MB measurement. In these areas, where reports in the literature cannot be reconciled with one another, I shall tend to present a personal view based on the experience in this laboratory.

Enzymes other than CK will only be mentioned briefly, to bring their contribution to the diagnosis of myocardial infarction into perspective. Similarly, methodology will be discussed only to the extent that it is necessary to know about the methods and their drawbacks, if results of serum enzyme measurements are to be interpreted correctly.

The overall emphasis of this review will be on practical clinical aspects of the use of CK and its isoenzymes in the diagnosis and management of patients who have had a myocardial infarction, or in whom this diagnosis requires either to be confirmed or excluded.

CREATINE KINASE ISOENZYMES

It is necessary, firstly, to consider the isoenzymes and multiple forms of CK. These are of great importance in diagnosis, often give rise to methodological problems, and have been the subject of considerable interest recently.
Tissues mainly contain CK in the cytoplasm, in various organelles, but a small percentage is present in mitochondria. In serum, the cytoplasmic isoenzymes always predominate. Mitochondrial CK and a number of additional forms, such as macro-CK, may also be present in serum occasionally [5]. Their main importance lies in the fact that they may cause difficulties in interpretation.

**Cytoplasmic isoenzymes**

There are 3 of these—CK-MM, CK-MB, and CK-BB. They are also known, in order of increasing electrophoretic mobility, as CK3, CK2, and CK1 [6]. These isoenzymes are dimers, comprising two polypeptide chains, designated either M or B, each of relative molecular mass (Mr) 40,000.

Somewhat surprisingly, in view of the amount of work that has been performed in this field, the exact distribution of CK isoenzymes within different body tissues remains in doubt, since many investigators do not agree on some of the findings. A simplified summary which attempts to give a consensus view is shown in Table 1; more detailed information is available elsewhere [4]. A number of points are immediately apparent:

1. Skeletal muscle carries the highest activity of CK and this is mainly CK-MM.
2. Cardiac muscle also has a fairly high activity of CK and seems to be the major tissue containing CK-MB.
3. Other tissues contain less CK than skeletal and cardiac muscle, and the CK they contain is mainly, if not all, CK-BB.

There is disagreement as to whether skeletal muscle does or does not contain CK-MB. This is a matter of considerable importance, since the interpretation of serum CK-MB results is based on the premise that CK-MB is specific for the myocardium.

The results of measurements of CK-MB in skeletal muscle seem to depend on two factors: the method of measuring CK-MB and the choice of muscle to be sampled. Thus, the CK-MB content of skeletal muscle has been reported to lie between 0% [7] and 30% [8]. There are a number of reasons for considering that


<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration (activity) (U/g wet weight)</th>
<th>CK-BB (%)</th>
<th>CK-MB (%)</th>
<th>CK-MM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle</td>
<td>2,500</td>
<td>&lt;1</td>
<td>&lt;2*</td>
<td>98</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>500</td>
<td>&lt;2</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Brain</td>
<td>500</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bladder (urinary)</td>
<td>150</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Prostate</td>
<td>100</td>
<td>100</td>
<td>—</td>
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<td>Intestine</td>
<td>100</td>
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<td>Kidney</td>
<td>20</td>
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<tr>
<td>Liver</td>
<td>&lt;1</td>
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*See text for discussion.*
the lower estimates, of below 2%, are more likely to reflect the true position [9], although there may be some muscles with a higher CK-MB content [10]. There is also evidence, however, that diseased muscle (e.g., from patients with muscular dystrophy or polymyositis) has a relatively larger proportion of CK-MB [11].

There is also some doubt about the CK-MB content of the myocardium. It may vary with age, with disease, or with site sampled. Results between 5% and 60% have been reported, but a consensus value would seem to be between 20% and 30%.

**Mitochondrial CK**

This isoenzyme is present in tissues as a dimer ($M_r$ about 80,000). The individual polypeptide chains are different from those of the cytoplasmic isoenzymes and do not react with antisera to them. In serum, the mitochondrial isoenzyme may be present in an oligomeric form (macro-CK2) [12].

**Sub-isoenzymes of CK**

In serum, but not in tissue extracts, CK-MM can be shown to exist as 3 sub-bands (CK-MM1, CK-MM2, and CK-MM3). It now seems likely these are due to the conversion of the serum CK-M subunit to a form with greater anodal electrophoretic mobility by the action of a carboxypeptidase in serum; this enzyme splits off the positively charged C-terminal lysine to give a more negatively charged molecule [13, 14]. In a similar fashion, CK-MB has two sub-bands.

**Macro-CK**

Two forms of high $M_r$ CK ($M_r$>200,000) may be present in serum, but not in tissue extracts, and are known as macro-CK1 and macro-CK2. Both are relatively uncommon findings [15, 16].

*Macro-CK1* is a complex between CK-BB and an immunoglobulin, usually IgG. It is present in 1 or 2% of patients admitted to the hospital [17], and in an even smaller proportion of healthy individuals [18]. Its significance is unknown, but it is presumably similar in nature to complexes of other enzymes (e.g., alkaline phosphatase or lactate dehydrogenase [19, 20]) with immunoglobulins, which are also of unknown significance. The complex has a relatively long plasma half-life compared with that of uncomplexed CK-BB which is very rarely found in serum, and even then in only very small amounts.

*Macro-CK2* is an oligomeric form of mitochondrial CK found in less than 5% of seriously ill patients [12]. Apart from the fact that it denotes illness of a serious nature, the significance of macro-CK2 is unknown. Its importance, like that of macro-CK1, lies in the fact that it may interfere with some methods of CK-MB measurement.

The electrophoretic mobilities of the various forms of CK that may be present in serum are shown in Fig. 1. However, it is important to point out that, in normal serum, most electrophoretic methods can only demonstrate CK-MM. Some methods
can also demonstrate weak CK-MB bands, but CK-BB is not demonstrable in normal serum.

**MEASUREMENT OF CK AND ITS ISOENZYMES**

**Total CK**

Most laboratories now measure CK by optimised techniques using a reaction sequence in which the reaction catalysed by CK is coupled with the reduction of NADP (e.g., [21]) and the resultant increase in absorbance at 340 nm is monitored:

\[
\text{creatine phosphate} + \text{ADP} \rightarrow \text{creatine} + \text{ATP} \\
\text{ATP} + \text{glucose} \rightarrow \text{glucose-6-phosphate} + \text{ADP} \\
\text{glucose-6-phosphate} + \text{NADP} \rightarrow \text{6-phosphogluconate} + \text{NADPH} \\
\*\text{hexokinase}; \**\text{glucose-6-phosphate dehydrogenase}
\]

The method has evolved and been improved over the years so that values obtained now do not correspond with values obtained in the past (i.e., before about 1980). The main differences between laboratories now arise from differences in assay temperature, since 25°C, 30°C, and 37°C are still all in widespread use.

The reaction sequence used in the assay would also measure adenylate kinase. To eliminate this activity as much as possible, AMP and adenosine pentaphosphate, which both inhibit adenylate kinase, are included in the reaction mixture. However, caution in interpreting results is required if the activity of adenylate kinase in the sample (whether of serum or tissue extract) is markedly greater than that...
of CK, since the inhibitors of adenylate kinase do not inhibit it completely at the concentrations used.

**CK-MB**

In contrast to total CK methodology, which now seems fairly stable, methods to detect and to quantitate the CK-MB isoenzyme continue to give rise to difficulty. These difficulties arise, not so much in terms of the technical performance of the tests, which is usually very simple, but in terms of the sensitivity and specificity of the assay. The problems arise mainly because the methods are being used to measure small amounts of CK-MB in the presence of large amounts of CK-MM.

A large number of methods and variants are in current use. Almost all are available commercially, and very few laboratories use CK isoenzyme methods that they have developed themselves. Three main principles of analysis are used:

1. Electrophoresis;
2. ion-exchange chromatography;
3. immunological.

*Electrophoretic methods* for measuring CK-MB have used various support media, but agarose is now probably the most popular. The main bands of CK activity (Fig. 1) are nowadays usually made visible by generating NADPH at sites of CK activity through the same reaction sequence as is used to measure total CK activity. Fluorescent bands of NADPH, due to CK, are either assessed qualitatively by visual inspection or scanned in a suitable densitometer.

Electrophoresis has a number of advantages. The analyst has confidence in the presence of isoenzyme bands that he can see, so there are few problems with specificity. Secondly, abnormal variants such as macro-CK can usually be recognized immediately, and thus cause no problem in interpretation. The method is also simple and fairly rapid.

Electrophoresis also has a number of disadvantages. Most methods are insensitive and are unable to measure, or even to detect, the CK-MB that is present in normal serum. The precision is less good than is obtainable by other methods, and the densitometer is expensive. Artefacts may occur due to non-specific fluorescent bands, or to adenylate kinase; but these can readily be detected by using a blank test staining procedure, in which creatine phosphate is omitted from the substrate. These disadvantages detract little from the overall clinical value of the test.

*Ion-exchange chromatographic methods* are available as a number of variants of this technique, all based on simple minicolumns [22]. Separations depend on charge differences between the isoenzymes; and it is possible, by eluting with a series of buffers, to collect fractions containing CK-MM, CK-MB, and CK-BB. However, this tends to be tedious and often only two fractions are collected, one containing CK-MM and the other containing CK-MB and CK-BB.

The advantages of ion-exchange chromatography lie in its simplicity and the fact that it requires only simple instrumentation. It is also fairly precise. The method's disadvantages mainly relate to problems due to the incomplete separation of
CK-MM and CK-MB fractions, if insufficient care is taken with the assay. The method is also rather insensitive, while unusual isoenzymes such as macro-CK, if present, usually cause spurious results.

*Immunological methods* all use antibodies directed against CK-M or CK-B, or both, and several techniques may be included under this general heading.

Anti-CK-M may be used to precipitate [23], or more usually to inactivate [24], all the CK-MM in the sample and a variable fraction (usually 50% with commercial antisera) of the CK-MB. Anti-CK-B antibody may be used, in conjunction with anti-CK-M antibody, in 'sandwich' assays, which are based on the following principle:

\[
\text{Solid-phase} \\
\text{anti-CK-M} \\
\downarrow \\
\text{+CK-MB (in sample)} \\
\rightarrow \\
\text{Solid-phase} \\
\text{---CK-MB} \\
\text{anti-CK-M} \\
\downarrow \\
\text{+anti-CK-B}\ast \\
\rightarrow \\
\text{Solid-phase} \\
\text{---CK-MB---anti-CK-B}\ast \\
\text{anti-CK-M}
\]

The anti-CK-B\ast antibody is labeled with a radioactive isotope [25], fluorescent molecule or an enzyme [26], which allow the amount of solid phase complex, and hence CK-MB, to be measured. By this use of two antibodies with different specificities, problems with interference by both CK-BB and CK-MM are minimized. Other immunological techniques to increase specificity have also been described [27].

The straightforward immuno-inhibition methods using anti-CK-M have several advantages that make them ideal for use in routine laboratories. They are simple and rapid to perform, and are precise. However, they are prone to error due to certain disadvantages, as follows:

1. Incomplete inhibition of CK-MM, which must be inhibited by over 99%.
2. Variable blank activity due to adenylate kinase.
3. Interference by CK-BB, macro-CK, and mitochondrial CK, none of which are inhibited by anti-CK-M.

It is possible to select antisera which have excellent inhibition properties. It is possible also, with slight inconvenience, to perform a 'blank' in the absence of creatine phosphate, to correct for adenylate kinase activity. However, it is important always to be aware of the possibility that the method will yield spurious 'CK-MB' results, if abnormal CK forms are present in the serum.

More complex immunological assays, such as the 'sandwich' assays just described, largely eliminate these problems and seem likely to gain popularity over the simple methods that have mostly been used so far.

There are many methods currently in use. As yet they do not always give the same results; this makes interpretation (discussed in the section on reference values)
difficult. In general, electrophoresis or the more complex immunological methods are preferred in U.S.A., whereas in Europe immuno-inhibition methods are very widely used.

REFERENCE VALUES

Total CK

Most laboratories use ‘optimized’ methods of enzyme activity measurement. These give similar results, provided the assay temperature is the same or an appropriate for temperature has been applied, but this is not an advisable procedure in most instances. Until fairly recently, methods used by various laboratories were not comparable and the values given for CK reference limits have in the past varied considerably, mainly due to differences in analytical technique.

Some difficulties remain, even if modern ‘optimized’ methods are used. Firstly, in most populations, serum CK activity has a distribution that is skewed to the right and approximates to log-Gaussian. This tends to render the upper reference limits somewhat indeterminate with a wide span of ‘borderline’ values, i.e., values that may or may not be abnormally high. Secondly, there are ethnic differences in CK values [28] which may cause differences between laboratories depending on the population served. Thirdly, there are differences due to sex, activity, and possibly age [28, 29]. For example, serum CK activity is 50% to 100% higher in males than in females, and is approximately twice as high in out-patients as in in-patients.

In healthy ambulant adults, one survey using optimized methods reports an upper reference limit for CK of 270 U/liter for males and of 150 U/liter for females [30]. These values were obtained using an assay temperature of 37°C; to convert to 30°C and 25°C these figures should be multiplied by 0.65 and 0.40, respectively.

It is advisable, because of the above factors, for laboratories to determine their own reference limits. Even if they do so, however, they must define the nature of the reference population very carefully because it may not be relevant to the diseased population in which CK measurements are to be used as a diagnostic test, i.e., mainly patients admitted with acute chest pain. In spite of these necessary caveats, diagnostic problems are only rarely caused by difficulties in defining the upper reference value, because the rise in serum CK after myocardial infarction is usually fairly large, to between 5 and 10 times the upper reference limit.

CK-MB

The problems in defining reference values for CK-MB activity in serum are much greater. In the first instance, the units or method of reporting may vary; examples include activity expressed in units/liter, or as a percentage of total CK activity, or as mass in ng/liter. Mass units are required for immunological methods of measurement which do not involve inhibition, e.g., ‘sandwich’ methods or immunoassays. It should also be noted that activity and mass results may not

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correlate with one another since not all CK-MB that is immunologically detectable necessarily has CK activity.

A second problem with CK-MB measurements, in normal healthy individuals, lies in the insensitivity of most of the methods used. For example, most electrophoretic methods can only measure with confidence activities of CK-MB greater than 10 U/liter. Immunological methods may have sample blank values as high as 15 U/liter; if the sample blank is subtracted, the detection limit is about 5 U/liter.

It should also be noted that some laboratories, notably those in Scandinavia, report CK-MB as “CK-B subunit activity,” i.e., the activity remaining in a sample after immuno-inhibition by anti-CK-M antibody. However, since these antisera inhibit CK-MB by 50%, the residual activity after immuno-inhibition by anti-CK-M represents only 50% of the CK-MB present in the sample. Therefore, it is standard practice in most countries other than in Scandinavia to multiply the residual activity by two and report this as serum “CK-MB” activity.

Healthy individuals probably have serum CK-MB activity values of less than 5 U/liter. As the values for each CK-MB method are likely to be specific for that method, laboratories should determine their own reference limits, as otherwise they have to rely on the guidance given by the manufacturers of the test kit, which may be misleading [31].

There are additional problems with reference values when attempting to differentiate myocardial damage from skeletal muscle damage. If the results of serum CK activity are only expressed in terms of absolute activity, e.g., U/liter, then rises in serum CK-MB activity may be found whenever large rises in CK-MM are present, due either to the lack of specificity of the CK-MB method or to the release of CK-MB from skeletal muscle. Therefore, many laboratories advise that interpretation of raised activities of CK-MB should also be based on the total serum CK activity. In other words, if a raised CK-MB activity is found, it should only be interpreted as being of myocardial origin if the CK-MB:CK ratio, expressed as a percentage, exceeds a specified value. This may be 6%, 5%, or 4%, depending on the method used.

It follows from the above that every laboratory that reports CK-MB results must also issue clear guidelines for interpreting these results. Reliance on the literature, or even the guidance given by the manufacturers of the analytical kit method being used, may give rise to incorrect interpretation of results.

**Effect of exercise on serum CK and CK-MB activity**

Prolonged strenuous exercise, e.g., a 20-mile run, causes a rise, that is often very large, in serum CK activity [29]. The rises are less in trained athletes than in those individuals who are sedentary [32]. It follows that serum CK is of little value in making a diagnosis of myocardial infarction in patients who have recently been engaged in very strenuous activity, since the rises in serum CK activity tend to peak several hours after the exercise and thereafter decay relatively slowly over

the next day or so [33]. As discussed later, this pattern of changes is similar to that occurring after acute myocardial infarction [34]. Although there is little doubt that these rises in serum CK activity originate from skeletal muscle, in trained athletes serum CK-MB activity also rises after exercise [34], due to the presence of an increased CK-MB:CK ratio in skeletal muscle in these individuals [35].

SERUM CK AFTER ACUTE MYOCARDIAL INFARCTION

In a patient who has had an acute myocardial infarct the changes in serum enzyme activity usually follow a predictable pattern, unless there is co-incident disease or complications occur. Modifications to this pattern may occur, but these are best considered in relation to the changes seen in what I shall call the "typical case" of uncomplicated acute myocardial infarction.

*The "typical case"*

Each enzyme, including total CK and CK-MB, shows its own pattern of change (Fig. 2). The following phases can be recognized:

1. A lag phase which follows the onset of symptoms but during which there is no rise in serum enzyme activity.
2. A phase of rapidly increasing enzyme activity.
3. A peak of activity, around which the rate of change of activity may be relatively low, followed by
4. A phase of exponential fall in serum enzyme activity until normal values are reached.

There is little doubt that CK-MB is detectable in serum sooner than CK-MM.

![Fig. 2. Time-course of changes in serum CK activity (---) and CK-MB activity (-----) after myocardial infarction.](image)
As might be expected, this is method-dependent, more sensitive methods being capable of detecting the changes in activity of serum CK-MB earlier than less sensitive methods. The time at which rises are first seen is important, since most patients are admitted to a Coronary Care Unit between 2 and 8 h after the onset of symptoms. In our experience, using an electrophoretic method of only average sensitivity, about 90% of patients with myocardial infarction have raised CK-MB at 6 h, whereas the corresponding figure for total CK is about 70% [36]. Other workers, using radioimmunoassay methods, have reported that CK-MB was raised at 4 h in all patients with myocardial infarction whom they studied [37].

Peak activity of total CK usually occurs between 16 and 24 h after the onset of pain. The peak of CK-MB activity occurs slightly earlier, usually between 15 and 20 h post-onset. This earlier peak for CK-MB is to be expected since the MB isoenzyme has a shorter plasma half-life (about 12 h) than that of CK-MM (about 18 h).

The duration of rise in enzyme activity depends on the height of the peak serum enzyme activity, the duration of enzyme release from damaged myocardium, and the kinetics of removal of the enzyme. While the height of the enzyme peak is usually known and it seems likely that significant enzyme release ceases to occur after 24 h, the rate of removal of enzyme activity from serum varies from patient to patient. In general, patients with small rises of CK-MB may show normal serum activity again after as little as 36 h, whereas patients with larger infarcts may have raised CK-MB activities for 4 or 5 days after infarction. The duration of total CK rise is longer than the corresponding CK-MB rise.

The pattern of enzyme changes just described is fairly constant from patient to patient, in the absence of complicating factors. Furthermore, the relative magnitude of the changes in various enzymes tends towards a constant value; i.e., patients showing large rises in CK will also show large rises in CK-MB, aspartate aminotransferase, and 'heart-specific' lactate dehydrogenase. This follows from the fact that peak enzyme activity, for all these enzymes, is closely related to infarct size. These predictable features mean that, when deviations from the pattern do occur, the possibility of alternative diagnoses needs to be considered.

The CK-MB:CK ratio varies during the course of the changes described above. In general, a relatively high proportion of CK-MB is present in serum early after infarction, often between 10% and 20%; whereas after 2 days the percentage of CK-MB is usually less than 5% due to its more rapid elimination. The proportion of CK-MB at any one time is more variable than might have been expected if myocardium contains a constant proportion of CK-MB; but correlations between site and size of infarct, age and sex of patient, and the CK-MB proportion in serum have been absent or very weak [36]. Nevertheless, at the time of peak CK-MB values, or before, the CK-MB:CK ratio is nearly always greater than 5%. In patients in whom the value is less than 5%, the CK-MB is more likely to have arisen from skeletal muscle.
Variations from the “typical pattern”

Variations caused by release of enzyme from other tissues, or from myocardium for causes other than myocardial infarction, will be considered later.

A slow and delayed rise in serum enzyme activity may be seen in the occasional patient, and is assumed to be due to a slowly evolving infarct. This is very uncommon.

Sometimes a plateau of enzyme activity may be present, possibly due to extensions of the infarct within a relatively short period. In these cases, CK-MB may be useful in detecting extension since its peak is so short-lived.

It is possible that impaired circulation such as may be found in heart failure or shock, may alter the characteristics of “washout” of enzymes from damaged myocardium and delay enzyme release.

A number of therapeutic procedures may be associated with alterations in the pattern of release of enzymes, or may cause problems in diagnosis if infarction has not occurred.

Intramuscular injections may cause serum CK activity to rise [38], but there is no rise in CK-MB activity [39]. Increases in CK are usually small, to twice the upper reference limit or less, unless irritant or hypertonic solutions are injected.

Resuscitative measures such as external cardiac massage and direct current countershock may both cause release of CK into serum. The enzyme is released from skeletal muscle in the anterior chest wall, so CK in serum rises but CK-MB nearly always remains normal. The rises in serum CK are usually very small unless the external cardiac massage has caused significant trauma to the anterior chest or the DC countershock has been repeated several times [40].

Thrombolytic therapy is being used increasingly in an attempt at early dissolution of the thrombus blocking the affected coronary artery. Initially, intracoronary streptokinase was used, but it has been replaced by less invasive methods using intravenous streptokinase analogues or tissue plasminogen activators. Where early recanalisation occurs, there seems to be a “washout” of enzyme from the ischaemic area causing the following changes [41]:

1. Peak values for CK and CK-MB to be reached early, at 10 h to 12 h after onset of symptoms.
2. The height of the CK and CK-MB peaks to be larger than they would otherwise have been.

The rate of decay of enzyme activity remains unchanged.

Policy for blood sampling

The more samples that are obtained, the more accurate the picture of evolving enzyme changes is likely to be. However, in the vast majority of cases, all the information needed can be obtained from a small number of properly timed blood samples. In practice, this normally requires a minimum of two samples, one before significant changes in enzyme activity have occurred (i.e., less than 6 h after the infarction), and one at the time of peak enzyme activity—for CK and CK-MB.
this is usually between 16 and 24 h after the onset of symptoms.

Unfortunately, the time of peak enzyme activity cannot be predicted with accuracy, or it may occur at an inconvenient time, or sample collection may be forgotten by staff concerned with other more urgent matters. For these reasons, it is often most practicable to collect blood from patients on admission to the hospital, for an early (baseline) sample, and on 2 or 3 successive occasions in the next 24–36 h at fixed times of the day, say 0600 and 1800 h. This allows a regular routine to be established for the Coronary Care Unit and the laboratory, and the measurements are unlikely to miss the peak of enzyme activity by more than a few hours.

In many hospitals, specimens for measurement of serum CK and CK-MB continue to be collected from patients more than 48 h after the onset of symptoms. In most patients this is totally unnecessary, since any enzyme changes will have occurred by this time and no further information will be obtained from such late specimens. Naturally, it may be necessary to collect specimens after 48 h if there has been a delay in referring the patient to the hospital, but CK measurements are not appropriate under these circumstances and ‘heart-specific’ lactate dehydrogenase should be measured instead.

Interpretation of results

The diagnosis of myocardial infarction depends on a combination of clinical factors, electrocardiographic and enzyme results. It is generally accepted that two of these must indicate infarction before the diagnosis can be made. The enzyme activities required to support the clinical diagnosis may depend on practical considerations, i.e., whether the therapeutic ‘penalty’ of missing a case by setting diagnostic criteria too stringently is acceptable. In a Coronary Care Unit, for example, one of the major decisions to be made will be whether to transfer the patient elsewhere in the hospital, or even back home; so there will be a tendency to sacrifice specificity for sensitivity (see below) to ensure that all patients with infarcts are recognised.

In general, to indicate myocardial infarction, the enzymes should show the following changes:

1. A rise in CK—preferably to values above any intermediate area arising out of difficulties in interpreting the reference range.
2. A rise in CK-MB. The exact value has to be determined, depending on the method.
3. A CK-MB:CK ratio, at less than 24 h after the onset of symptoms, of more than 5% (with some methods, 6%).
4. Associated changes in other selected enzymes, e.g., abnormal LD1/LD2 ratio [42].

Using these, or similar, criteria the sensitivity and specificity of various diagnostic methods including the various enzyme tests have been assessed by many authors. A few of the results will be summarized here, but it must be emphasized
that both sensitivity and specificity of tests are critically dependent on the nature of the population being sampled and upon the decision criteria. Most studies have used patients admitted to a Coronary Care Unit in whom the incidence of myocardial infarction is usually about 50%.

**Sensitivity and specificity.** In the present context: Sensitivity is the number of patients with myocardial infarction with a positive test, expressed as a percentage of the total number of patients with myocardial infarction. Specificity is the number of non-myocardial infarction patients with a negative test, expressed as a percentage of the total number of non-myocardial infarction patients tested.

Many assessments of sensitivity and specificity have been published, but they cannot readily be compared with one another since the results are critically dependent on the value selected to separate ‘infarct’ from ‘non-infarct.’ Selection of more stringent criteria for diagnosis, e.g., a higher value for serum CK-MB activity required to support the diagnosis of myocardial infarct, tends to decrease the sensitivity of the test and to increase its specificity. Sensitivity and specificity will also vary with the time of blood sampling [43].

The sensitivity indicated by most studies (see [3]) of total CK measurements is about 98%, with very few reports suggesting a sensitivity of less than 95%. The sensitivity of CK-MB measurements is method-dependent; and with electrophoresis and ion-exchange chromatography, values of about 98% or 99% are usually found.

Immuno-inhibition methods tend to have a lower sensitivity, but in the past this was mainly due to difficulties in methodology. A recent large study has indicated a sensitivity of 97%, using a method measuring CK-B subunit activity [44].

The specificity of total CK measurements is reported to lie between 65% and 85% [3], though much lower values may be found in populations other than those admitted to a Coronary Care Unit. Serum CK-MB is more specific than total CK, most studies indicating a value between 95% and 98%. The immuno-inhibition methods are probably less specific than other CK-MB methods [45], because patients occasionally have raised CK-B activity in serum due to causes other than CK-MB [46].

For comparison, the sensitivity of the ECG is usually found to be about 70%, and its specificity is 100% if only stringent criteria are accepted.

These figures suggest that, for most purposes, CK and CK-MB measurements are adequately sensitive in detecting myocardial infarction and compare favourably with other techniques such as scintigraphic or ECG methods [47]. The specificity of total CK is poor, and in cases where there is any doubt, measurement of CK-MB is desirable in addition or some other relatively specific test, e.g., the LD1/LD2 ratio, may be measured.

On the basis of these or similar figures for sensitivity and specificity, it is possible to define a rational policy for investigating patients with myocardial infarction such as the one described above. Very few patients should be mis-diag-
nosed provided the lack of specificity of total CK (and of aspartate aminotransferase and lactate dehydrogenase) is recognized.

Myocardial infarction and other forms of CK

Macro-CK may cause diagnostic difficulty, mainly due to interference with immuno-inhibition and ion-exchange chromatography methods of CK-MB analysis, by giving rise to falsely elevated values. Total CK is rarely falsely increased outside reference limits due to this cause. It is not difficult to recognize the spurious nature of the CK-MB rise, since the activity remains constant and does not show the characteristic changes with time that are seen after myocardial infarction. However, immediate recognition of the problem requires the sample to be examined by electrophoresis.

Mitochondrial CK is not found in serum of most patients after myocardial infarction if current methods of CK isoenzyme analysis are used.

Sub-isoenzymes of CK. Since the interconversion of the sub-isoenzymes is time-dependent, it has been suggested that the proportion of the sub-forms might allow a rapid assessment of the age of the infarct [48]. The practical value of this has not been assessed to date.

SERUM CK AND INFARCT SIZE

It is known, from both animal experiments and results in man, that the amount of enzyme released from damaged myocardium is related to infarct size. Whereas peak enzyme activity was used as an index of enzyme release at first, later on (in the 1970's) more sophisticated analysis of multiple, serial serum enzyme results was used to determine enzyme release, and hence, infarct size. Details of the methods are given elsewhere [49].

In order to quantitate cardiac damage in this way a number of assumptions and additional calculations have to be made. For example, it must be assumed (1) that the enzyme is not destroyed before reaching the circulation, (2) that a constant, known, proportion of contained enzyme is liberated from the infarcted area, and (3) that enzyme activity is evenly distributed both between different areas in the heart and from one patient to another. The volume of distribution of the enzyme and its rate of disappearance from the circulation must also be known. In spite of these problems, “enzymic infarct size” thus obtained correlates fairly well with other more reliable ways of estimating infarct size [50].

Enzymic infarct size is related to prognosis [51]. However, peak enzyme activity, which is much easier to determine, is almost as good an index of infarct size [52, 53] and prognosis [54]; and in consequence frequent serial enzyme measurements at intervals of 2 h to 4 h, for estimating enzymic infarct size, are now considered unnecessary for routine diagnosis.

Enzymic infarct size, as described above, is determined by estimating the shape of the serum enzyme activity curve from measurements of serum CK (CK-MB
or LD1) at 2-4 hourly intervals over the rise, peak, and decay of serum enzyme activity. If a mathematical formula can be devised to predict accurately the shape of the serum enzyme activity curve (e.g., a log-Gaussian distribution curve), then very frequent, e.g., half-hourly or hourly, measurements of serum enzyme activity over the early rising phase can be used to predict the rest of the curve, and hence enzyme release. In this way, seven or eight hours after the onset of symptoms it is possible to obtain a "predicted enzymic infarct size." Using such models, some authors have found very close agreement between predicted and actual enzymic infarct size. The value of such measurements lies in the fact that therapeutic measures introduced fairly early on in treatment can thereby be assessed for their effect on enzymic infarct size [55]. Thus, if actual enzymic infarct size is less than that predicted, it may be assumed that the treatment has been effective in limiting infarct size.

Although these methods of estimating infarction have been widely used, they are subject to a number of assumptions which may be difficult to validate. For example, they assume (1) that the drug treatment does not change "washout" characteristics of the enzyme from the infarcted area, (2) that the volume of the distribution of the enzyme remains unchanged, and (3) that its rate of removal is unaffected by the drug. Perhaps the greatest problem, however, lies in the imprecision of the estimate of infarct size at such an early time, due to imprecision in timing and serum enzyme measurement and failure of the model to fit the observed serum enzyme results [56].

My conclusion is that serum CK and CK-MB results are valuable indices of infarct size and of short-term prognosis, but that more sophisticated analysis of enzyme results adds little information of diagnostic value.

MYOCARDIAL INFARCTION COMPLICATING SURGERY AND OTHER CONDITIONS

Sometimes acute myocardial infarction may complicate disorders or procedures which themselves may be associated with changes in serum enzyme activity. This may make diagnosis difficult. Some more important examples are considered below.

Surgery

There is no doubt that there is an increased incidence of myocardial infarction in the operative and post-operative period, especially in those with hypertension or other forms of heart disease [57].

Non-cardiac surgery causes changes in serum CK activity which are virtually identical to those after myocardial infarction. In general, the extent of the rise is related to the extent of the surgery [58], although some anaesthetics (in the absence of surgery) are capable of causing a rise in serum CK activity [59]. Serum CK-MB activity remains normal or, if raised, the serum CK-MB:CK ratio is low,
i.e., less than 3% [58]. Occasionally, operations on organs containing CK-BB may be associated with release of this isoenzyme into the circulation; e.g., there may be a rise in CK-BB (and CK-MB) after prostate resection [60].

Cardiac surgery is always associated with release of both CK and CK-MB into the circulation. However, patients with peri-operative myocardial infarcts can be identified in the majority of cases by the following features (Fig. 3):

1. In the uncomplicated case, without infarction, the rises in serum CK and CK-MB tend to be smaller than in patients who have had peri-operative infarction [61, 62]. Thus, it is rare for CK-MB to exceed 50 U/liter in the average uncomplicated case.

2. In patients who have not had infarcts, peak serum CK-MB activity usually occurs between 4 and 8 h post-operatively, whereas peaks at 16 to 24 h are usually seen when infarction has occurred. This difference in timing is much less apparent for total CK than for CK-MB.

The diagnosis of peri-operative infarction is important both in terms of prognosis for the individual patient and in terms of allowing the surgeon to assess his operative procedures. The precise cut-off values for distinguishing infarction from the normal post-operative rise in serum enzymes need to be determined at each center locally. To do this, additional blood sampling is required, for instance at the end of the operation and at 4, 8, 12, and 24 h post-operatively. The evidence provided by serum enzyme measurement, especially CK and CK-MB, is particularly important as the ECG is unreliable in these patients [61].

Other conditions

Trauma is usually associated with skeletal muscle damage and, therefore, with
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a rise in serum CK activity roughly proportional to the severity of the damage. CK-MB activity in serum usually remains normal or only a small proportion of total serum CK, though exceptions occasionally occur [63].

Coronary angiography may be associated with complications which can give rise to the suspicion of myocardial infarction. In general, coronary angiography is not normally associated with a rise in either serum CK or CK-MB activity.

Coronary angioplasty is followed by a rise in serum CK-MB in about 20% of patients. It seems likely that, in most patients showing these changes, a small amount of myocardial necrosis has occurred [64].

Coma is a fairly common cause of raised serum CK activity. The extent of the rise is correlated with the depth of the coma rather than with its cause. Thus, these changes are to be found in patients following drug overdose, cerebrovascular accidents, diabetic ketoacidosis, etc. [65, 66]. The CK arises from skeletal muscle, and CK-MB is not present in serum in excess [36]. However, exceptions have been reported to this generalization, e.g., in a patient poisoned with theophylline [67].

Alcohol excess is not uncommon in patients who complain of chest pain and who are admitted to Casualty units. Some of these patients show raised serum CK activity [68] sometimes attributable to trauma or hypothermia, but rarely to the polymyositis which occurs in some alcoholics. In most patients CK-MB is not raised, but this is not always so.

Hypothermia is fairly common in the old in winter, but only rarely associated with classical causes such as hypothyroidism. Whatever the cause, most patients with hypothermia have a raised serum CK [69]. The presence of a normal serum CK-MB should exclude coincident myocardial infarction, but CK-MB has been reported to increase in such patients [70]; so the interpretation of a raised value may not always be easy.

Muscle diseases are very commonly associated with a rise in serum CK. These are seen in polymyositis and the various forms of muscular dystrophy. Serum CK-MB activity may also be raised [71].

Malignant hyperthermia is a related condition in which severe hyperthermia, and often death, follows administration of anaesthetics, such as halothane, to susceptible individuals. The acute episode is characterised by huge rises in serum CK activity [72] and in relatively small rises in CK-MB, but even the asymptomatic susceptible individual may have a raised serum CK activity.

Since the changes in serum CK-MB activity in muscle disorders may be caused either by an increased CK-MB:CK ratio in the diseased muscle [11], or be due to damage to myocardium, it is incorrect to use CK-MB results as evidence of myocardial involvement in diseases which primarily affect muscle.

SERUM CK ACTIVITY IN ANGINA AND OTHER MYOCARDIAL DISORDERS

In general, any inflammatory, toxic, or ischaemic condition which affects the
myocardium may cause an increase in serum CK and CK-MB.

Angina

It is recognized that there are patients with prolonged anginal attacks who fall into a category between angina and overt infarction. This condition may be called unstable angina (previously acute coronary insufficiency); it is essentially a clinical diagnosis. There is little doubt that some of the patients have had small infarcts; certainly some cases are associated with small rises in serum CK and, to an even greater extent, in serum CK-MB. Similar small serum enzyme changes may also occur in some post-infarction patients after a graded exercise test [73], suggesting that CK-MB and, to a lesser extent CK, are released from myocardium which has been reversibly damaged by ischaemia. Although it may be technically inaccurate, it is simplest to take the view that, if there are typical enzyme rises in a patient who has unstable angina, then a diagnosis of myocardial infarction can be made.

Other disorders

Myocarditis is often caused by virus infection, or occasionally by non-specific inflammation. The serum enzyme changes are similar to those of myocardial infarction.

Pericarditis causes relatively minor changes in serum CK and CK-MB, providing evidence of underlying myocardial inflammation.

Cardiac failure does not, per se, affect CK or CK-MB in serum though it may complicate a condition which itself causes such increases.

Arrhythmias do not usually affect either serum CK or CK-MB activity. Occasionally, small increases in both enzymes may follow a prolonged rapid tachycardia.

CK AND THE DIFFERENTIAL DIAGNOSIS OF MYOCARDIAL INFARCTION

A number of conditions which may cause diagnostic confusion have already been discussed, e.g., myositis and angina. Occasionally, other disorders may give rather similar symptoms.

Pulmonary infarction. The enzyme changes of pulmonary infarction are inconstant, usually due either to breakdown of red cells in the infarcted area or to associated liver damage. Serum CK and CK-MB remain normal [74]. Early reports of CK rises were probably due to intramuscular injections. Similarly, pneumonia does not cause a rise in CK or CK-MB in serum.

Acute abdominal conditions, such as peptic ulcer or hiatus hernia, may cause confusing clinical signs but do not usually cause serum CK and CK-MB to rise.

It is very unusual for other disorders which may cause a rise in serum total CK, such as hypothyroidism or muscular dystrophy, to cause any difficulty in differential diagnosis.
CONCLUSIONS

Although I have attempted to simplify the results obtained by various authors, it will be apparent that there are substantial discrepancies between them. These relate to choice of method, to overall diagnostic efficacy of the various methods, and to the changes in CK-MB to be expected in conditions other than myocardial infarction.

A rational policy for making a diagnosis of myocardial infarction, and for managing patients in whom this diagnosis is possible, is fairly simple to achieve. Firstly, the time of blood sampling must be stipulated—this is probably the single most important factor in obtaining interpretable results. Secondly, a rational choice of enzymes to measure must be made. Measurement of total CK and the ‘heart-specific’ isoenzymes of LD in the first instance, to be followed by CK-MB and LD1/LD2 ratio in cases with any diagnostic problem, might be a rational compromise between economy and efficiency. Thirdly, there are likely to be problems related to defining reference ranges: if at all possible a local study using the method of choice should be undertaken. Finally, it should be remembered that it is much more important to distinguish between ‘infarct’ and ‘non-infarct’ than between ‘infarct’ and health.

With the above policy, virtually all cases of myocardial infarction should be capable of being diagnosed providing there are no complicating factors. Complicating factors such as cardiac surgery require the adoption of alternative protocols if optimal diagnostic efficiency is to be achieved.

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