Creatine Kinase B-Subunit Activity in Serum after Immunoinhibition of M-Subunit Activity

Willie Gerhardt¹ and Johan Waldenström²

Creatine kinase (EC 2.7.3.2) B-subunit activity in serum may be routinely measured as residual activity after specific immunoinhibition of the M-subunit. We assessed the inhibition kinetics, specificity, completeness of inhibition, and inhibitory capacity of three different anti-M preparations, with use of isolated human BB, MM, and MB isoenzymes. The Scandinavian-recommended reaction system was used. We suggest a set of tentative quality requirements for anti-M for use in diagnosing acute myocardial infarction. We need to measure and subtract sample residual adenylate kinase activity was demonstrated. We describe a routine photometric method for determining B-subunit activity in serum. With the Scandinavian CK method the upper reference value for total creatine kinase in serum was found to be 150 U/L for women, 270 U/L for men. By bioluminescence, we found the upper reference value for B-subunit activity to be 6 U/L for both sexes. We discuss three different modes for applying B-subunit determinations to the diagnosis of acute myocardial infarction.

Additional Keyphrases: diagnosis of acute myocardial infarct · heart disease · photometry · enzyme activity · reference values · sex- and age-related effects · discrimination limits · cardiac arrest

Development in the goad of a specific antibody that is capable of inhibiting M-subunit activity of human creatine kinase (EC 2.7.3.2) was reported by Würzburg et al. (1–3). After immunoinhibition by anti-M, CK B-subunit dependent activity may be determined as the residual CK activity. We demonstrate in this report that the method is specific for CK B activity if assay conditions are appropriate. Theoretically, after such inhibition, in a mixture of all three CK dimers, all of CK isoenzyme BB and half of CK MB will be measured. However, the fraction B measured in the MB hybrid may vary among different batches of anti-M.

The method cannot discriminate between CK BB and CK MB. Consequently, results of such measurements in serum are most correctly expressed as S-CK B activity (4–6), and should not be multiplied by the theoretical factor of two, to be expressed as CK MB activity.

We have previously described a routine S-CK B method at 37 °C based on the jointly developed Scandinavian (37 °C) (7, 8) and German (25 °C) (9) recommended CK methods, and clinical evaluations of the method as applied to the diagnosis of acute myocardial infarction (5, 10, 11).

After the conclusion of these studies, information on the effect of such chelators as ethylenediaminetetraacetate on CK reaction rates (12, 13) and on CK reagent stability (14, 15) necessitated a revision of the Scandinavian CK method (8, 16) and a re-investigation of anti-M in the presence of ethylenediaminetetraacetate. The manufacturer kindly put several experimental preparations of anti-M at our disposal for investigation. The following is a detailed study of the properties of anti-M immunoinhibitors in the revised Scandinavian CK reaction system, a survey of different modes of clinical application of the method, and a summary of clinical results obtained during the last three years.

Materials

The CK reaction medium (Table 1) was prepared according to the Scandinavian recommendation (8).

Components

Creatine phosphate, disodium salt (cat. no. 15402); adenosine 5-diphosphoric acid (ADP) (cat. no. 15016); adenosine 5-monophosphate (AMP), disodium salt (cat. no. 15015); nicotinamide-adenine dinucleotide phosphate (NADP), disodium salt (cat. no. 15600 and cat. no. 128040), and disodium ethylenediaminetetraacetate were all obtained from Boehringer Mannheim, F.R.G. Hexokinase, 370 kU/L (25 °C); glucose-6-phosphate dehydrogenase (EC 1.1.1.49) from yeast, 225 kU/L (25 °C), in glycerol; and P1-P5-diadenosine 5-pentaphosphate, trilithium salt, were donated by Boehringer Research Center, Tutzing, F.R.G. Other chemicals included creatine phosphate (Baker, The Netherlands; no. 3109), 350 mmol/L; imidazole (BASF, F.R.G., “99 % pure,” or Sigma, U.S.A., low-fluorescence grade III no. 1-0250); and N-acetylcysteine (Sigma grade, cat. no. A 7250). The absorbance of the complete reagent at 340 nm was less than 0.250. The reagent blank rate was less than 0.001 absorbance units per minute (corresponding to 3.7 U/L). Details on preparation of stable stock solutions, purity requirements for N-acetylcysteine, auxiliary enzymes, and imidazole are given in ref. 7. Lyophilized anti-M from goat was obtained from E. Merck, Darmstadt, F.R.G. The preparations we investigated are identified in the text by their batch numbers.

Purified CK Isoenzymes

Lyophilized human CK MM (from psoas muscle), CK MB (from heart muscle), and BB (from brain) were provided by the Boehringer Research Center. The purity of isoenzyme preparations MM and BB was controlled by qualitative fluo-

rescence isoenzyme electrophoresis on agarose in the system previously described (6). For CK MM, 10 μL of a CK MM preparation, 250 kU/L, was applied. With a detection sensitivity of 15 U/L, expressed as CK B, as little as 0.01% contamination of the CK MM preparation by CK MB or BB would be detected. By such criteria the CK MM and CK BB

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³ Nonstandard abbreviations used: anti-M, M-subunit immunoinhibitor; CK, creatine kinase; S-CK B, creatine kinase B-subunit activity in serum (S-).

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Table 1. Reaction Conditions According to Scandinavian CK Reaction System

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>37.0 °C</td>
</tr>
<tr>
<td>pH (reaction mixture, 37 °C)</td>
<td>6.5</td>
</tr>
<tr>
<td>Imidazole acetate</td>
<td>100 mmol/L</td>
</tr>
<tr>
<td>Creatine phosphate</td>
<td>30 mmol/L</td>
</tr>
<tr>
<td>Adenosine-5’-diphosphate</td>
<td>2 mmol/L</td>
</tr>
<tr>
<td>d-Glucose</td>
<td>20 mmol/L</td>
</tr>
<tr>
<td>Nicotinamide adenine dinucleotide phosphate</td>
<td>2 mmol/L</td>
</tr>
<tr>
<td>Hexokinase (EC 2.7.1.1)</td>
<td>3500 U/L</td>
</tr>
<tr>
<td>d-Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)</td>
<td>2000 U/L</td>
</tr>
</tbody>
</table>

preparations were both homogeneous. The MB preparation contained traces of MM and BB.

Methods

Chromatographic Isolation of CK MB from Sera

The B subunit activity in our lyophilized MB preparation constituted only about 40% of the MB activity. Consequently, CK MB was isolated from sera of cases of acute myocardial infarction by chromatography on Boehringer CK MB minicolumns (cat. no. 198219; Boehringer Mannheim). We applied 500 µL of serum to a 2-mL column containing 20 g of DEAE-Sephadex A50 per liter of imidazole acetate buffer (100 mmol/L, pH 6.7 at 25 °C). The elution procedure was slightly modified. After elution of CK MM by washing twice with 5-mL portions of the imidazole acetate buffer, the MB isoenzyme was eluted in five 1-mL fractions with imidazole acetate buffer containing 50 mmol of magnesium chloride per liter. Fractions 2 and 3, containing about 75% of the MB, were pooled and used. Electrophoretic control showed this MB preparation to be pure. However, because CK MB dissociates into MM and BB fractions within 24 h on storage at 4 °C or by freezing and thawing in buffer, we used only freshly isolated MB fractions in titration experiments with anti-M.

Quality Controls

Preparations of CK isoenzymes MM, MB, and BB were diluted to suitable activity in a modification of the method described by Rosalki (17). In a mixture consisting of Tris-HCl buffer (50 mmol/L, pH 7.0, 25 °C), β-mercaptoethanol (50 mmol/L), glycerol (5 mol/L), bovine γ-globulin (Sigma, 10 g/L), and ethylenediaminetetraacetic acid, 3 mmol/L, dispensed in 2-mL portions into small vials and stored at −70 °C, the CK MM and BB preparations showed no loss and CK MB only a slight decrease in activity during four months. Additionally, we used a control serum, “Precipath E” (Boehringer). As daily quality controls we used Precipath E and CK BB, MB, and MM, for control of the total CK and CK B determinations. In addition, every tenth sample in all series was a Precipath E.

Data on imprecision are given in Results.

Measuring Techniques

Photometry. For all routine measurements except determination of the reference range for S-CK B we used an LKB model 8600 or 2086 photometric reaction-rate analyzer with the temperature set at 37 °C. We incubated 50 µL of sample in LKB cuvets with 1000 µL of CK reagent (without creatine phosphate), and without and with anti-M, for a minimum of 15 min in the thermostating tunnel of the instrument. The reaction was started by injecting creatine phosphate. For routine measurements, the injection nozzle was moved two positions ahead of the measuring position. Thus, creatine phosphate was injected and the reaction initiated 2 min before the cuvets reached the photocell, to allow monitoring of reaction rates under steady-state conditions 120–180 s after the start. This permitted a through-put of 45 to 60 samples per hour (18). We determined residual adenylate kinase (EC 2.7.4.3) activity in all serum samples with use of the same reagent, except that creatine phosphate was omitted.

Bioluminescence. This method was used only in determining the reference range of S-CK B. Because photometric imprecision was too high for accurate determination of normal values for S-CK B in the range 1 to 7 U/L (increase of absorbance at 340 nm, 0.0002 to 0.0019 A/min in the photometric method), we made these measurements in the LKB 1250 luminometer with the bioluminescence reagent of Lundin et al. (19, 21). With use of a specially purified luciferase (no EC no. assigned), a constant ratio is maintained between the amount of ATP and light emission for long enough to permit continuous monitoring of the reaction rates for several minutes. Reaction conditions were as follows: 50 µL of sample in a total volume of 1200 µL (volume fraction 0.04). Reaction temperature 25 °C, as luciferase is unstable at higher temperatures. Imidazoleacetaete buffer (100 mmol/L, pH 7.5, 25 °C) containing anti-M, luciferase, and, per liter, 350 µmol of luciferin, 30 µmol of ADP, 10 mol of creatine phosphate, 20 µmol of P1, P5-diadenosine 5-pentaphosphate, 20 mmol of ethylenediaminetetraacetic acid, and 10 mmol of Mg2+. The bioluminescence assays were calibrated by the above-described routine photometric method. For each series, CK BB and MM controls and three patients' sera with a CK B activity of about 30 U/L, for use in calibration, were measured in both instruments.

Residual adenylate kinase activity was first monitored for 60 s in the absence of creatine phosphate (22). As reaction conditions in the bioluminescence assay with ADP as low as 30 µmol/L are unfavorable for adenylate kinase, this residual activity was nearly always less than 0.5 U/L. The CK reaction was then started by adding creatine phosphate and monitored for 60 s. The reaction curves were linear. Observed rates were proportional to CK B concentration in the range 1 to 60 U/L. The analytical sensitivity corresponded to about 0.5 U/L. Within-series imprecision (CV) for 1 U/L was 5% (our own observations and ref. 20).

Results

Time Course of Immunoinhibition

We studied the kinetics of immunoinhibition at 37 °C with different preparations of anti-M. Racks of cuvets with 1 mL of CK reagent without creatine phosphate but with antibody included were preheated for 15 min in the thermostating tunnel of the LKB 2086. The tunnel lid was then removed and 50-µL samples were pipetted as rapidly as possible, starting with the last cuvet. Contents of all cuvets were mixed manually with a glass rod and the reaction rates were measured at the time intervals shown on the abscissa of Figure 1. Qualitative differences were noted between two anti-M preparations (numbers 7915327 and 14326) at a concentration of 250 mg/L in the CK reagent A. With preparation no. 14326, equilibrium of inhibition was reached within 10 min, and inhibition of CK MM was more than 99.9% complete, with about 50% inhibition of CK MB and negligible inhibition of CK BB. In contrast, immunoinhibition of the isolated isoenzymes MB and MM by the experimental preparation no. 7915327 took
nearly three times longer to reach equilibrium. Maximal inhibition of CK MM by no. 7915327 was about 99–99.5%, but never 100% (Table 2), with about 60% inhibition of CK MB and about 7% inhibition of CK BB. Consequently, for routine determination with preparation no. 14326, sample racks could be inserted into the LKB 2086 directly after pipetting, whereas preparation no. 7915327 required a minimum of 20 min incubation at room temperature before the racks were placed into the instrument. Extension of incubation time with no. 7915327 beyond 20 min did not increase inhibition of M-subunit activity.

For all antibody preparations it was essential to mix the contents of all cuvets very thoroughly immediately after pipetting (we used an LKB Multimixer for a minimum of 30 s). Omission of thorough mixing resulted in nonlinear reaction curves with decreasing reaction rates after the start with creatine phosphate. S-CK B activity was stable at 37 °C for at least a further 30 min after inhibition equilibrium had been reached.

**Anti-M Concentration in CK Reagent A**

We studied the completeness of inhibition, the specificity, and the residual CK B subunit activity in the CK MB hybrid as functions of anti-M concentrations in the CK reagent (Figure 2). The duration of incubation was as given in the legend. In the range 250 to 1000 mg/L, preparations number 7915327 and 1976 caused a mean inhibition of CK MM of 99.5% (Figure 2A, Table 2). In contrast, anti-M no. 14326 in such concentrations inhibited CK MM by more than 99.9%. The mass concentrations (mg/L) required for inhibition of at least 2000 U of CK MM activity per liter depended on the particular preparation of anti-M.

Within the given concentration interval, the preparations showed different specificity. No significant inhibition of CK BB was observed with preparation no. 14326, in contrast to...

**Table 2. Day-to-day Imprecision of CK MM Daily Control**

<table>
<thead>
<tr>
<th></th>
<th>Total CK, U/L</th>
<th>Fraction Inhibited</th>
<th>Residual MM, U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>1066</td>
<td>0.994</td>
<td>6.3</td>
</tr>
<tr>
<td>SD</td>
<td>64</td>
<td>0.003</td>
<td>3.6</td>
</tr>
<tr>
<td>CV, %</td>
<td>0.06</td>
<td>0.003</td>
<td>0.56</td>
</tr>
</tbody>
</table>

*Scandinavian CK reagent ± anti-M no. 7915327 (250 mg/L)*

**Fig. 1. Time course of immunoinhibition**

Residual activities of CK isoenzymes as a function of duration of incubation at 37 °C in CK reagent with anti-M. CK BB and MM, human, purified lyophilized preparations; and CK MB, freshly isolated by chromatography of infant patient's serum immediately before analysis. ●—●, anti-M 14326 (Merckotest CK MB); △—△, anti-M 7915326 250 mg/L of CK reagent

**Fig. 2. Residual activities of human CK isoenzymes BB, MB, and MM as a function of concentration of three different anti-M preparations in CK reagent**


3–6% inhibition with no. 1976. Increasing concentrations of preparation no. 7915327 caused increasing BB inhibition, as shown in Figure 2. The residual CK B activity determined in a freshly isolated CK MB hybrid is shown in Figure 2B.

**Correction for Incomplete M-Subunit Inhibition and for Serum Adenylate Kinase Activity**

With proper corrections, routine use of an anti-M that inhibits less than 99.9% of M-subunit activity is possible. For...
two experimental immuno-inhibitors, no. 1976 and 7915327, a residual M-subunit activity of about 0.5-1% was always present (Figures 1, 2; Table 2). Added to the normal S-CK B activity, which may be as much as 6 U/L (see below), a falsely positive result may be obtained. Routine subtraction of an arbitrary 1% of the total S-CK activity in the sample from the apparent S-CK B activity will prevent this and consequently will increase the analytical specificity. The necessity for this correction when working with such anti-M preparations as no. 1976 or no. 7915327 was demonstrated in several patients being treated with intramuscular injections of procainamide every 4 h (10, 11). An example is shown in Figure 3. S-CK B activity, uncorrected for 1% of total S-CK, showed a prolonged increase, thus indicating a continued enzyme release from the myocardium. In contrast, S-CK B, when corrected for 1% of total S-CK, rapidly returned to and remained below the discrimination limit for acute myocardial infarction (Figure 3). The method can thus discriminate between S-CK B and total S-CK, even when the proportion of S-CK B fraction is as low as 0.5%.

Interference by Serum Adenylate Kinase

In the Scandinavian CK reagent, the adenylate kinase inhibitor combination AMP, 5 mmol/L, and 3,5-diadenosine-5'-pentaphosphate, 10 μmol/L, efficiently inhibits S-adenylate kinase from erythrocytes, thrombocytes, muscle, and liver, but not completely (7, 25, 26). Figure 4 shows the relative frequency of residual S-adenylate kinase activities in 1015 samples. About 85% of these were < 8 U/L. Discrimination limits of S-CK B activity in the diagnosis of acute myocardial infarction of 15 U/L (5, 10, 11) and 12 U/L (23) have been used. Added to the upper normal value for S-CK B activity of 6 U/L, residual S-adenylate kinase activity may be expected to cause falsely positive results (5, 10, 11). Consequently it is necessary to measure and subtract individual sample S-adenylate kinase from the measured apparent S-CK B activity.

Imprecision of the Routine Photometry Method

Because of limitations inherent in spectrometry, imprecision increases sharply with decrease of reaction rates below 15 U/L, as illustrated in Figure 5. This situation cannot be remedied by simply increasing the sample volume, because there is no proportionality between sample volume fraction and the corresponding reaction rates (7, 8, 16). The within-day imprecision (CV) of a determination of S-adenylate kinase having an activity of 5 U/L is about 15%. Consequently, subtraction of these low residual S-adenylate kinase activities increases the imprecision of apparent CK B results, as shown in Figure 5. (For details see ref. 4.) The within-day imprecision of corrected S-CK B values at the discriminatory level of 12 U/L was about 8%, corresponding to a day-to-day imprecision of 10%. The subtraction of increased residual S-adenylate kinase activities has previously been shown to prevent falsely high S-CK B results (10, 11). Day-to-day imprecision of total S-CK and S-CK B in daily quality controls is given in Table 3.

Reference Ranges of S-CK and S-CK B

Sera from patients judged to be healthy by several clinical and laboratory criteria were put at our disposal by a current reference-range project (the Kristianstad survey). Figure 6 shows the frequency distribution of total S-CK for 109 men and 127 women, as measured by the routine spectrophotometric method. A sex-related difference is observed. The upper reference values, given as either the 97th or 95th percentiles, were found to be the same values, 150 and 270 U/L, for women and men, respectively. Persons performing usual amounts of bodily work are included in the material, but persons with known excessive muscular activities are excluded.

S-CK B reference values were measured by bioluminescence, calibrated against the routine photometric method. The

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**Table 3. Day-to-day Imprecision, Merckotest CK MB (14326), 23 Daily Controls; Photometric Routine Method**

<table>
<thead>
<tr>
<th>Precipath 526, U/L</th>
<th>CK BB, U/L</th>
<th>CK B in BB</th>
<th>% B in BB</th>
<th>CK MB, U/L</th>
<th>CK B in MB, % B in MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \bar{X} )</td>
<td>496.7</td>
<td>747</td>
<td>737</td>
<td>98.7</td>
<td>552</td>
</tr>
<tr>
<td>SD</td>
<td>12.54</td>
<td>28.2</td>
<td>31.2</td>
<td>2.99</td>
<td>24.6</td>
</tr>
<tr>
<td>CV, %</td>
<td>2.5</td>
<td>3.8</td>
<td>4.2</td>
<td>3.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

\* Residual (B-subunit) activity in control preparations BB and MB measured in the presence of anti-M.

\* B-subunit activity in control expressed as a fraction of total CK. Nominal value for the MB control: 40% active B-subunits.
upper reference value, given as the 97th percentile, was found to be 6 U/L for both women and men (Figure 7). These values agree well with other published reference values (21).

S-CK and S-CK B Activities in a Typical Case of Acute Myocardial Infarction

S-CK and S-CK B activities as determined by the routine photometry method are shown as a function of time in a typical acute myocardial infarction case in Figure 8. Beside the activities in IU/L per liter, S-CK B has been calculated as the fraction of total S-CK. S-CK B activity increased rapidly to nearly fourfold the discriminatory limit of 12 U/L within 6 h of onset of acute symptoms.

Discussion

Reaction Conditions

Our routine photometric method is based on the Scandinavian CK reagent (8) and a standard reaction temperature of 37 °C. Under these conditions the CK reaction follows apparent zero-order kinetics at 2000 U/L for at least 180 s (7). At this rate NADPH concentration will reach 250 μmol/L in 180 s, corresponding to a NADPH/NADP⁺ ratio of 0.1, above which NADPH becomes significantly inhibitory (9). The anti-M concentration in the reagent was adjusted to inhibit more than 2000 U of CK-MM activity per liter. CK B subunit activity was stable at 37 °C in the CK-reagent for at least 30 min after equilibration of inhibition had been reached. Because photometric imprecision increases steeply at low reaction rates, it is an advantage to use the highest reaction temperature compatible with CK B stability.

CK-MB Control

For establishing inhibition properties of anti-M it is essential to use a CK-MB isoenzyme freshly isolated from serum from an acute myocardial infarction patient. On storage in buffer at various temperatures ranging from 25 to −20 °C, the isolated MB hybrid rapidly dissociates and forms MM and BB. In imidazole buffer, pH 6.7 at 25 °C, B subunits stored at 4 or −20 °C are less stable than MB subunits (10), leading to a relative loss of B subunit activity. Apparently, a similar loss occurs on lyophilization, as observed in our lyophilized CK-MB control, which had only about 40% CK B activity.

Theoretically, unspecific inhibition of B-subunit activity in CK-BB and CK-MB should correlate, so that a 5% inhibition of B-subunit activity should result in a 10% inhibition of CK-BB and a 5% inhibition of B in MB. Expressed as fraction residual activity of the uninhibited activity, this would correspond to 90% BB and 45% MB activity. Such a correlation was not found with anti-M preparation 7915327. At an anti-M mass concentration of 250 mg/L, the residual BB and MB activities were about 96 and 35%, respectively. These results indicate that binding of this particular anti-M to the M-subunit of the MB hybrid induced a conformational change, causing partial inhibition of the catalytic activity of the B-subunit. With other anti-M preparations this phenomenon was not observed. Any MB preparation with an established fraction active B-subunits may be used for routine quality control.

Immunoinhibition of M subunit activity may be incomplete because of competition between inhibiting and non-inhibiting antibodies in a particular anti-M preparation. The results obtained with experimental anti-M preparations 1976 and 7915327 can be so explained. In such cases, CK-M activity can not be completely inhibited by increasing the concentration of anti-M. Conversely, anti-M 14326 completely inhibited CK MM activity.

Quality Requirements for Anti-M for Diagnostic Use

Quality requirements for an anti-M preparation may be formulated as follows:

1. Immunoinhibition shall reach equilibrium within 10 min.
2. Inhibition of CK-M subunit activity should exceed 99.9%.
3. At an anti-M concentration that inhibits CK-MM by more than 99.9% the measurable fraction of CK-B subunit activity in a freshly isolated CK-MM hybrid should be at least 45 to 50% of the total CK-MM activity.
4. At the same anti-M concentration the inhibition of CK-BB should be less than 5%.

This report demonstrates qualitative and quantitative differences among different anti-M preparations, even from the same species of animal. Consequently, new anti-M preparations for diagnostic use should be tested and their immunoinhibition properties, as described above, should be stated. Our quality criteria were fulfilled by only one anti-M preparation, no. 14326, which now is commercially available in Europe as CK MB 14326 reagent kit (E. Merck, F.R.G.).

Analytical Specificity

Two factors primarily determine the analytical specificity for CK B: (a) residual M-subunit activity left by incomplete immunoinhibition, and (b) residual sample adenylate kinase activity. It is possible routinely to correct for both to prevent false-positive results.

Two of the three anti-M preparations tested left a residual M-subunit activity of 0.5–1%. In a serum sample with g, e.g., 2000 U of CK MM activity per liter such an immunoinhibitor will give a residual M-subunit activity of 10–20 U/L, depending on the anti-M preparation and the photometric imprecision at this level of activity (Figure 5). This activity will exceed the discrimination limit for S-SK B (5, 10, 11, 20, 24) in acute myocardial infarction in some cases. We have previously documented that subtracting an arbitrary 1% of total S-C k from the apparent S-C k B activity (after correction for residual S-adenylate kinase activity) reduced the frequency of false-positive results from 4% to 1% in 303 patients without myocardial infarction (5, 11).

Residual sample adenylate kinase activity depends on the efficiency of the adenylate kinase inhibitors used in the reagent. The efficiency of the combined AMP and diadenosine-5'-pentaphosphate used in the jointly recommended Scandinavian and German CK reagents has been extensively investigated (7, 9, 25, 26). No inhibitor combination known to us, including AMP and fluoride (27, 28), inhibits adenylate kinase completely without a concurrent unacceptable inhibition of CK. From Figure 4 it can be estimated that the probability for a sample residual adenylate kinase activity of at least 9 U/L is about 0.1. The probability for a normal S-C k B of at least 3 U/L is 0.5. Consequently, it may be estimated that the probability for the combination of a residual adenylate kinase activity of at least 9 U/L plus a S-C k B of at least 3 U/L will lead to a falsely positive apparent S-C k B in at least 5% of the samples. In a previous study with the relatively high discrimination limit of 15 U/L, subtracting sample adenylate kinase prevented 8% false-positive results in 1117 samples from 303 patients without myocardial infarction (11). With an average sampling frequency of four samples per patient this procedure prevented 20% of these patients from having a false diagnosis of myocardial infarction. The diagnostic utility of anti-M no. 1976, used with both of the above-mentioned corrections, has been extensively reported in ref. 11.

Serum CK B in Acute Myocardial Infarction

For use in the diagnosis of acute myocardial infarction S-C k B determination may be utilized in at least three modes:
1. using a relative discrimination limit, expressing S-C k B as a fraction of total S-C k
2. relating to a S-C k B discriminating threshold expressed in U/L
3. requiring minimal changes of S-C k B during a certain interval

The use of a relative discrimination limit of 3% S-C k B—i.e., 6% expressed as S-C k MB—was initiated in Germany (2, 29) in order to avoid false-positive results owing to extracardial S-C k MB activity.

In our opinion, this procedure suffers from the following disadvantages:
1. As with any ratio, it comprises the analytical imprecision of two separate assays.
2. False negatives can occur with samples from cases of acute myocardial infarction that have high total S-C k activity, owing to extracardial CK MM—e.g., as a consequence of intramuscular injections (11, 30).
3. The S-C k B/S-C k ratio does not convey the information required for the clinician to monitor the course of the infarction (Figure 8) or calculate the amount of CK B released (30, 31). Furthermore, the fractions S-C k B calculated in Figure 8 are similar at peak S-C k B and for the 42-h sample. As is evident from the reference values, the fraction S-C k B of total S-C k actually exceeds 3% in many healthy persons with low total CK activity.

Extracardiac S-C k MB has been reported in cases of Duchenne muscular dystrophy (32, 33), and in some other conditions such as shock, polytrauma, acute psychosis, and severe alcohol intoxication (34). In recent reports on extracardiac conditions (29, 34) determinations of CK-MB were made with a reagent containing only AMP as the adenylate kinase inhibitor. It was not stated whether corrections were made for residual S-adenylate kinase activity or correction for incomplete immunoinhibition, or both. Most of these conditions do not pose any differential diagnostic problems with respect to acute myocardial infarction. For surveys see refs. 11, 35, and 36. During three years of routine S-C k B determinations on more than 1000 patients suspected of having had a myocardial infarction we have observed only one case of extracardiac CK MB (23). Additionally, CK isoenzyme electrophoresis on more than 300 patients without myocardial infarction failed to demonstrate increased CK MB activity in more than 1%.

However, we did find CK isoenzyme BB in five cases of cardiac arrest, probably a result of leakage from hypoxic brain tissue. Also, a variant CK BB isoenzyme with abnormal electrophoretic mobility was demonstrated at a frequency of about 1% of all cases (Figure 7, ref 6).

The second mode is to use a fixed discrimination value for S-C k B. Ljungdahl (11) used a discrimination limit of 15 U/L in a retrospective study from a coronary-care unit, based on 6-h sampling intervals and peak enzyme values in each patient. In 227 classifiable patients with a prevalence of infarction of 0.47, he found a diagnostic sensitivity of 0.98 and a diagnostic specificity of 0.98. Also using an immunoinhibition method, Werner and Werbrouck (37) reported technical (diagnostic) sensitivity and specificity of 0.93 and 0.95, respectively, within 24 h of infarction. Hofvendahl et al. (23) lowered the S-C k B discrimination limit to 12 U/L in an evaluation of 143 patients in a coronary-care unit with a prevalence of infarction of 0.46. Eight-hour sampling intervals were used. Diagnostic sensitivity and specificity were 0.99 and 1.00, respectively. Based on the first positive S-C k B value in each patient, the predictive value for a positive result (PV pos) was 1.00. Based on all negative values in the 10- to 20-h interval after onset of acute symptoms the PV neg was 0.99. These results correlate very well with an evaluation of S-C k MB aftercolumn chromatography of samples collected during three consecutive days after onset of symptoms in a prospective study of 201 patients suspected of having had an acute myocardial infarction, with acute myocardial infarction
The prevalence of 0.5, giving diagnostic sensitivity and specificity of 0.98 and 0.97, respectively (38). The third mode is to monitor changes of S-CK B activity in serial samples taken within a relatively short time interval after admission to the hospital. Depending on the time that has elapsed after the onset of the acute symptoms, an increase, peaking, or decrease of S-CK B activity are observable for at least 24 h after infarction. With the bioluminescence method, changes even within the reference range can be observed. Conversely, a series of constant S-CK B values within an 8-h period rule out an acute myocardial infarction with great certainty. Besides a high diagnostic sensitivity, a high diagnostic specificity is obtained because constantly high S-CK B activities owing to extracardial causes are not misinterpreted.

For purely diagnostic purposes it is not necessary to determine S-CK B on all patients. We have previously described the following strategy (5, 23). Total S-CK is monitored on samples taken at 8-h intervals from all patients admitted to our coronary-care unit. If total S-CK exceeds 200 U/L, S-CK B is determined. Only if S-CK B exceeds 12 U/L is sample residual adenylate kinase measured and subtracted. With this procedure, S-CK B determinations constitute about a third of the total number CK and CK B analyses, with no loss of diagnostic information. A clinical evaluation of this strategy has been given in ref. 23.

References