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REVIEW

CREATINE KINASE ISOENZYMES

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CONTENTS

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AK	Adenylate kinase, ATP:AMP phosphotransferase, EC 2.7.4.3
AMI	Acute myocardial infarction
AST	Aspartate aminotransferase, L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1
ATP	Adenosine triphosphate
CK	Creatine kinase, ATP:creatine N-phosphotransferase, EC 2.7.3.2
CNS	Central nervous system
DMD	Duchenne-type muscular dystrophy
GK	Glycerol kinase, ATP:glycerol 3-phosphotransferase, EC 2.7.1.30
GPO	L-α-Glycerophosphate oxidase, glycerol 3-phosphate:O_2 oxidoreductase, EC nil
G-6-PDH	Glucose-6-phosphate dehydrogenase
GSH	Glutathione
HK	Hexokinase, ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1
IgG	Immunoglobulin G
LDH	Lactate dehydrogenase, L-lactate:NAD oxidoreductase, EC 1.1.1.27
NAC	N-Acetyl-L-cysteine
NADP	Nicotinamide-adenine dinucleotide phosphate
NADPH	Dihydronicotinamide-adenine dinucleotide phosphate
NBT	Nitroblue tetrazolium
PMS	Phenazine methosulphate
POD	Peroxidase, hydrogen peroxide oxidoreductase, EC 1.11.1.7

1. INTRODUCTION

ATP:creatine N-phosphotransferase (EC 2.7.3.2, CK) is an enzyme that catalyses the reversible transfer of a phosphate residue during high-energy bonding between ATP and creatine [1]:

$$\text{creatine} + \text{ATP} \overset{\text{CK}}{\rightleftharpoons} \text{phosphocreatine} + \text{ADP}$$

The CK enzyme plays an important role in energy metabolism, especially in the contraction of skeletal muscle. CK is a dimer consisting of two subunits, M and

B. Three isoenzymes, MM, MB and BB, each with a relative molecular mass of 82 000, are found in the cellular cytoplasm [2].

CK is distributed in skeletal muscle, cardiac muscle, brain tissue and other organ tissues. Its activities and isoenzyme patterns are organ-specific [3, 4]. Thus, identification and quantification of the CK isoenzymes released in the sera are extremely useful for confirming damage to the specific organs or increases in membrane permeability, enabling further diagnosis, clinical follow-up and prognosis [5]. The CK isoenzyme assay has been used by clinical laboratories in the development of improved analytical methods. This paper describes routine analytical methods for CK isoenzymes applied in clinical laboratories and the subsequent clinical evaluations.

2. ANALYTICAL METHODS

Assay methods for CK isoenzymes are generally divided into the following five categories: (1) electrophoresis [6–8]; (2) ion-exchange chromatography [9, 10]; (3) immunoinhibition [11–13]; (4) immunoprecipitation [13, 14]; (5) radioimmunoassay [15, 16]. Of these, electrophoresis with agarose gel [7] or cellulose acetate membranes [8] used as supporting material and immunoinhibition with anti-human CK-M subunit goat antibodies [12] are the most commonly used methods. In this section, these two methods, as well as ion-exchange chromatography with mini-columns packed with DEAE Sepharose CL-6B, are described.

2.1. Electrophoresis

2.1.1. Principle

The principle reactions in CK activity staining are as follows:

$$\text{phosphocreatine} + \text{ADP} \xrightarrow{\text{CK}} \text{creatine} + \text{ATP} \quad (1)$$

$$\text{ATP} + \text{glucose} \rightarrow \text{glucose-6-phosphate} + \text{ADP} \quad (2)$$

$$\text{glucose-6-phosphate} + \text{NADP} \rightarrow \text{6-phosphogluconate} + \text{NADPH} \quad (3)$$

$$\text{NADPH} + \text{NBT} + \text{PMS} \rightarrow \text{reduced type of NBT} \quad (4)$$

There are two assay methods for CK activity. The first detects the NADPH fluorescence produced by reactions 1–3. The other is a colorimetry method, which detects the reduced NBT produced by all four reactions. Electrophoresis using Cellogel membranes as a supporting material [17] followed by fluorescent staining [18, 19] is described.

2.1.2. Reagents

A barbital–hydrochloric acid buffer (pH 8.4, $I = 0.041$) is used for electrophoresis [17]. 5,5-Diethylbarbituric acid (8.5 g) and 1 *M* hydrochloric acid (11.5 ml) are dissolved in 500 ml of distilled water and diluted to 1000 ml with additional distilled water. The monotest CK NAC (Boehringer-Mannheim, Mannheim, F.R.G.) is used as a CK reaction mixture. The preparation of the monotest CK

NAC is based on the method of the German Society for Clinical Chemistry (GSCC) [20]. Sucrose is added to the reagent solution to a final concentration of 20% (w/v) in order to decrease the diffusion of the fluorescent bands. A 4-ml volume of the mixture is required per Cellogel (5×18 cm, Chemetron, Milan, Italy). The Cellogel is cut into halves (5×9 cm) and used as the supporting medium.

2.1.3. Procedure

Sample application points are placed 3 cm from the cathodal side of the Cellogel, as shown in Fig. 1A. The membrane is soaked with the barbital–hydrochloric acid buffer for at least 10 min. The distance between the poles in the electrophoresis chamber is set at 7 cm. The excess buffer is wiped from the Cellogel and the membrane is immediately set on the bridges in the chamber. The surface penetrable by the samples is the duller one, recognizable by the cut-off corner of the membrane. A 3-μl volume of serum is applied uniformly to the membrane with a microsyringe. Four samples can be applied on one Cellogel membrane. The sample volume is adjusted according to the total CK activity and electrophoresed at 200 V for 30 min with the barbital–hydrochloric acid buffer. During this time, the chamber must be cooled with an ice-pack to avoid inactivation of the heat-sensitive CK isoenzymes by the amount of Joule heat produced.

A filter paper (5×9 cm) is soaked with 2 ml of the CK reaction mixture, and the Cellogel is placed face down on the filter paper. This is then incubated at 37°C for 30 min. During this procedure, care must be taken to prevent the for-

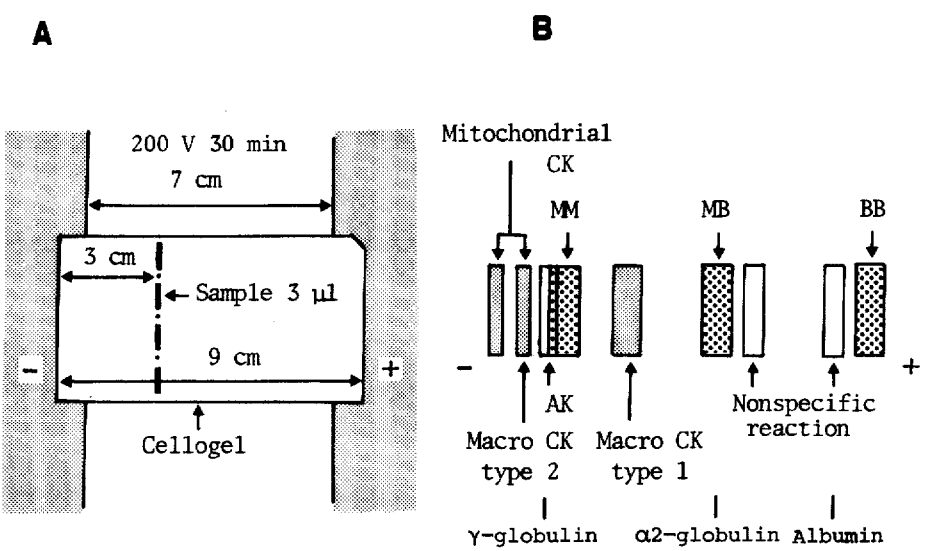

Fig. 1. (A) Electrophoresis of CK isoenzymes on a Cellogel membrane. (B) A diagrammatic representation of CK isoenzymes on a Cellogel membrane. Mitochondrial CK shows four electrophoretic mobilities (cf. Fig. 5B). Here, two dominant bands at the cathodal side of CK-MM are represented. Adenylate kinase runs at the cathodal side of CK-MM on a Cellogel membrane; however, the enzyme runs at the anodal side of CK-MM on agarose gel.

mation of air bubbles between the Cellogel and the filter paper. The fluorescence on the Cellogel is determined by densitometry at 360 nm.

2.1.4. Enzymogram

CK-MM migrates to the fast γ-globulin fraction, CK-MB to the α_2-globulin fraction and CK-BB to the pre-albumin fraction (Fig. 1B). However, only CK-MM can be detected in the sera of healthy adults (Fig. 2A).

2.1.5. Comments

The lower detection limit of the fluorescence method is ca. 10 I.U./l, and a range of 300–1000 I.U./l is determined to be the most suitable for quantitation. When severe haemolysis occurs, fluorescence as a result of the reaction of adenylate kinase (AK) released from the erythrocytes appears at the cathodal side of the CK-MM (Fig. 2F). However, in most cases, this would not be a problem as long as the monotest CK NAC is used. When atypical fluorescent bands appear, a blank reaction should be carried out with reagents excluding phosphocreatine. A CK kit named the autopack, CK NAC (BMY), in which the substrate is excluded, is convenient for the blank reaction. The CK activities on the Cel-

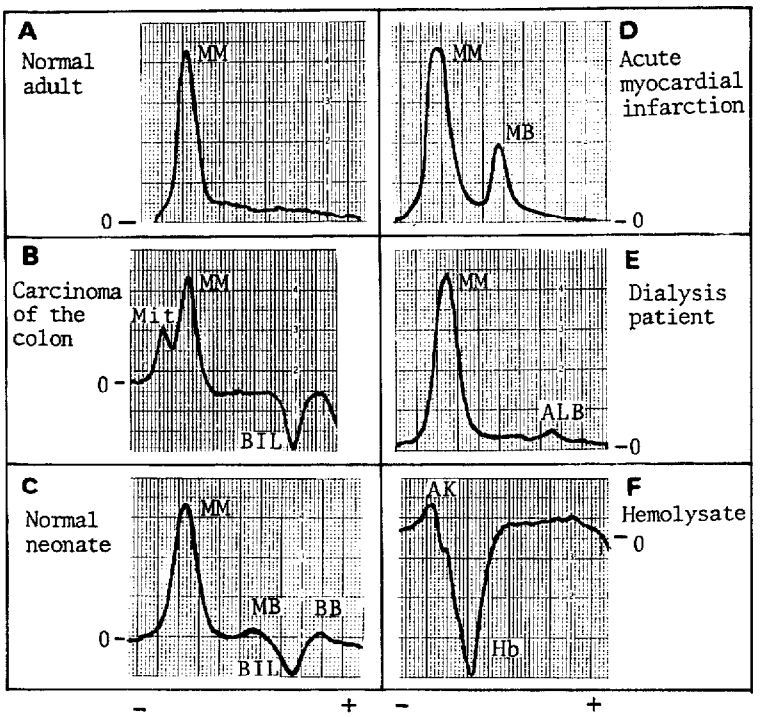

Fig. 2. Densitograms of serum CK isoenzymes separated by Cellogel electrophoresis. Fluorescence does not occur from bilirubin and haemoglobin bands, instead the bands show negative patterns from a baseline (B, C and F). Dialysis patients show fluorescence in the albumin band (E). Mit = mitochondrial CK; BIL = bilirubin; ALB = albumin; AK = adenylate kinase; Hb = haemoglobin; 0 = baseline.

logel membranes can be stained with tetrazolium. In this case, 0.04 ml of 10 mg/ml NBT and 0.02 ml of 0.1 mg/ml PMS are mixed with 2 ml of monotest CK NAC [21]. This mixture can also be used for CK staining following agarose or agar gel electrophoresis [21].

2.2. Ion-exchange chromatography

2.2.1. Principle

Ion-exchange chromatography utilizes the different affinities of the CK isoenzymes for weak anion-exchange resins. The affinity differs according to the ionic charge on the individual isoenzymes, reflecting their amino acid composition. Worthington's modified Mercer's method with mini-columns (Worthington Biochemical, Freehold, NJ, U.S.A.) is described.

2.2.2. Reagents

Each mini-column (25 mm × 8 mm I.D.) is packed with DEAE Sepharose CL-6B, and 50 m*M* Tris–HCl buffer (pH 7.5) containing 30, 200 or 300 m*M* sodium chloride is used for the elution of CK-MM, CK-MB and CK-BB, respectively.

2.2.3. Procedure

The caps are removed from the columns, allowing the buffer to flow freely, and 6 ml of CK-MM elution buffer are used to rinse the column. Then 0.25 ml of serum is applied to the column and allowed to soak into the ion exchanger. The CK-MM is eluted with 3.0 ml of the buffer and the eluate is collected. The CK-MB and the CK-BB are then eluted with 4.0 and 2.0 ml of their respective elution buffers and their eluates are collected. The CK activity of each fraction is determined and multiplied by a dilution factor (CK-MM, 13; CK-MB, 16; CK-BB, 8).

2.2.4. Reference values

The reference values of CK-MB are as listed in the manual of the reagent kit, namely 0–2.7 I.U./l for men and 0–3.4 I.U./l for women at 30°C.

2.2.5. Comments

The quantitative lower limit for a column is reported to be in the range of 1–5 I.U./l [22], and the application limit of a sample to the column is 2000 I.U./l of the total CK activity. While preparing this manuscript, the authors were informed that the Worthington's column kit will become commercially unavailable by the end of 1987. However, there are suitable substitute columns, and the elution buffers can be prepared in the laboratory. Ion-exchange chromatography using a larger column and gradient elution buffer (Fig. 3B) is useful for the purification and preparation of the CK isoenzymes.

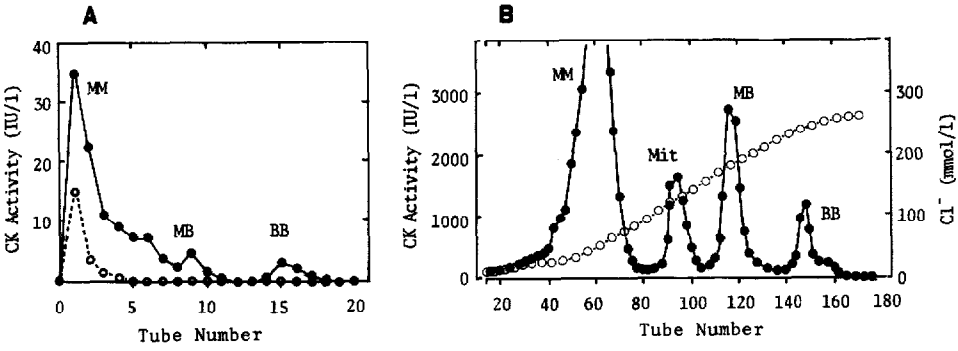

Fig. 3. (A) Elution patterns of CK isoenzymes from a mini-column of Worthington: (○) normal adult; (●) marker. (B) An elution pattern of CK isoenzymes from a DEAE Sephacel ion-exchange column (50 cm×2.5 cm I.D.) with a linear gradient of 0–300 m*M* sodium chloride in 50 m*M* Tris-HCl buffer (pH 7.4) flowing at 35 ml/h. (●) CK activity; (○) chloride concentration; Mit = mitochondrial CK.

2.3. Immunoinhibition

2.3.1. Principle

CK-M subunit activities in the CK-MM and CK-MB isoenzymes are inhibited by anti-human CK-M subunit antibodies, and the remaining CK-B subunit activities in CK-MB and CK-BB are determined (Fig. 4A). CK-BB is seldom present in serum, so CK-MB activity is determined by doubling the CK-B sununit activity. Immunoinhibition using the CK-MB Merckotest (Merck, Darmstadt, F.R.G.) is described.

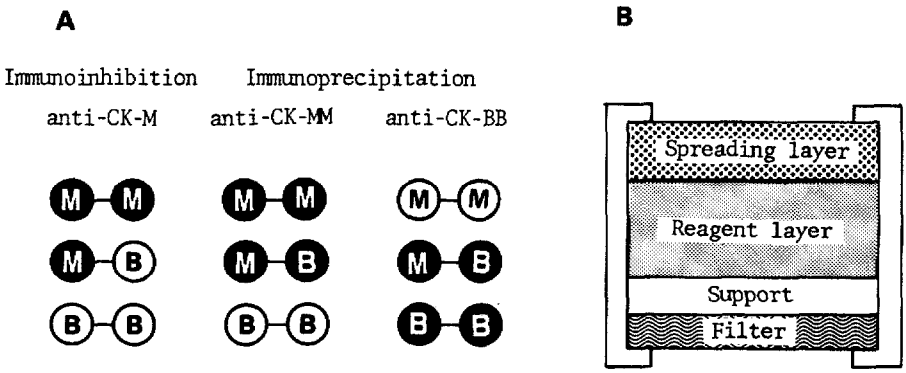

Fig. 4. (A) Principle of immunoinhibition with anti-CK-M subunit antibodies and immunoprecipitation with anti-CK-MM and anti-CK-BB antibodies. Enzyme activities represented with open circles are determined in each method. (B) Structure of an Ektachem Clinical Chemistry Slide for CK-MB. The spreading layer contains surfactants, NAC, ethylene-bis(oxyethylenenitrile)tetraacetic acid and anti-CK-M subunit antibodies. The reagent layer contains phosphocreatine, ADP, glycerol, magnesium acetate, diadenosine pentaphosphate, GK, leuco dye, POD, GPO and imidazole buffer (pH 7.0).

2.3.2. Reagents

Antibodies (goat) to the human CK-M subunit, which inhibit 1000 I.U./l CK-M, are contained [23] in the CK assay reagents based on the method of GSCC [20].

2.3.3. Procedure [12]

A mixture of 0.1 ml of serum and 2.5 ml of the reagent containing anti-CK-M antibodies is incubated at 37°C for 10 min. A 0.1-ml volume of substrate solution is added to the mixture, and after 2 min the CK-B activity is determined at 340 nm.

2.3.4. Reference values

The CK-B subunit activity in sera obtained from 208 healthy adults ranged from 0 to 5 I.U./l at 30°C.

2.3.5. Comments

The quantitative lower limit of this method is 3 I.U./l CK-B activity, and the maximum limit of inhibition is 1000 I.U./l CK-M activity. Since the CK-B activity in serum is very low, a sample blank value based on non-specific reactions must be subtracted from the CK-B activity value. CK-MB can be rapidly determined by the "Kodak Ektachem Clinical Chemistry Slide Assay" (Kodak, Rochester, NY, U.S.A., Fig. 4B), which uses immunoinhibition [24]. The principle enzyme reactions are as follows:

$$\text{CK isoenzymes} + \text{anti-CK-M subunit antibodies} \rightarrow \text{CK-B} \quad (5)$$

$$\text{phosphocreatine} + \text{ADP} \xrightarrow{\text{CK-B}} \text{creatine} + \text{ATP} \quad (6)$$

$$\text{ATP} + \text{glycerol} \rightarrow \text{L-}\alpha\text{-glycerophosphate} + \text{ADP} \quad (7)$$

$$\text{L-}\alpha\text{-glycerophosphate} + O_2 \rightarrow \text{dihydroxyacetone phosphate} + H_2O_2 \quad (8)$$

$$H_2O_2 + \text{dye precursor} \rightarrow \text{dye} + 2H_2O_2 \quad (9)$$

There is a high degree of correlation between the CK activities determined by the Ektachem method and the immunoinhibition method [24]. Although the Ektachem method is expensive, it is convenient for emergency use.

3. EVALUATION OF ANALYTICAL METHODS

The electrophoretic method is reliable for identification of CK isoenzymes (Table 1), but it is time-consuming and semi-quantitative. A blank reaction procedure must be performed in order to distinguish the CK bands from the non-specific reactions. The sensitivity of the colorimetric method is generally low, so the fluorescence method is recommended for more accurate results. It is noted that the linear range in this latter method is relatively narrow. Electrophoretic methods have been developed by Helena Labs. (Beaumont, TX, U.S.A.) and

TABLE 1

COMPARISON OF THREE ANALYTICAL METHODS FOR CK ISOENZYMES

	Electrophoresis	Ion-exchange column chromatography	Immunoinhibition
Time	30–65 min	30–90 min	Less than 20 min
Stat capability	No	No	Yes
Sensitivity	10 I.U./l	1–5 I.U./l	3 I.U./l
Specificity	Yes (but AK)	Yes (but carry over)	Yes (but macro CKs)
Quantity	Semi	Yes	Yes
Visibility	Yes	No	No
Training	Needed	Needed	Not needed
Special equipment	Needed	Not needed	Not needed

Corning Medical (Palo Alto, CA, U.S.A.). The supporting media used are cellulose acetate and agarose, respectively, and the enzyme activity is detected by fluorescence staining. Diffusion of the fluorescent bands on the cellulose acetate is less than on agarose; however, the resolution of agarose is better than that of cellulose acetate. Both methods are useful and each allows the easy separation and semi-quantitation of CK isoenzymes.

Ion-exchange chromatography is time-consuming and thus is not recommended for emergency use. However, it is useful for the determination of CK-MB catalytic concentrations [25]. The disadvantages of this method are the overlaps of the isoenzyme with the neighbouring fractions and the inevitable dilution of the CK activity with the elution buffer. However, these problems can be solved by lowering the pH of the first elution buffer [10], selecting a suitable ion exchanger [26] and suitably diluting the serum with the first elution buffer [27]. DEAE Sepharose CL-6B is superior to CK fractionation, while better resolution is obtained with DEAE cellulose than DEAE Biogel A or DEAE Sephadex A-50 [28].

Immunoinhibition is specific, quantitative and rapid. The anti-human CK-M subunit goat antibodies inhibit more than 99.9% of the CK-M subunit activity without affecting the CK-B subunit activity [12, 23]. The immunologic reaction between the CK-M subunit and the antibodies increases to a plateau phase within 10 min [12, 23]. This method can be applied to various types of automatic analyser [29], and is superior to both routine and emergency clinical CK-MB analyses. The CK-MB activity determined by NAC activation is between 5 I.U./l (fresh sera) and 50 I.U./l (frozen sera) higher than that determined by glutathione (GSH) activation [30]. Inactivated CK, especially in aged sera, is not completely activated by GSH. Therefore, NAC is recommended for the CK activation. The antibodies do not inhibit mitochondrial CK and CK–immunoglobulin complexes, thus when the CK-MB activity is greater than 25% of the total CK activity, the serum must be electrophoresed for further identification of the remaining CK [31].

In addition to immunoinhibition, another immunological method is immunoprecipitation using precipitating antibodies against CK-MM or CK-BB [23]. CK-MM and CK-MB are precipitated with anti-CK-MM antibodies, and CK-MB and CK-BB are precipitated with anti-CK-BB antibodies (Fig. 4A). Although this method is specific and sensitive, it requires 5 h to precipitate immune complexes. Thus, this method is not recommended for routine analyses.

Radioimmunoassay is another specific and sensitive assay method. However, unlike the other four methods, it measures the isoenzyme mass concentration [15, 16]. An inactivated form of CK-BB was reported using radioimmunoassay [16].

4. CLINICAL SIGNIFICANCE

4.1. Creatine kinase MM

The half-life of CK-MM in human serum is 18 h [32]. The isoenzyme is distributed in the organs as shown in Table 2 [3, 4, 13, 33]. CK-MM is further

TABLE 2

CONCENTRATION AND ISOENZYME PATTERNS OF CK IN HUMAN TISSUES [13]

Tissue	Total CK activity (I.U./g frozen tissue)	Distribution of CK isoenzymes (%)		
		MM	MB	BB
Skeletal muscle	860–1310	96–100	0–3	0–1
Tongue	225–292	90–99	1–5	0–5
Diaphragm	140	96	4	2
Heart (adult)	100–280	71–96	4–27	0–2
Heart (infant)	78–250	96–100	0–4	0
Aorta (arcus aortae)	3–7	81–88	8–14	3–5
Kidney	0–1	70–100	0	0–30
Spleen	0–1	65–75	0	25–35
Thyroid	13–34	64–79	6	15–30
Adrenal	0–1	35–60	0–10	25–40
Lung	2–9	27–72	0–4	18–69
Carotis	2	56	2	42
Artery (aorta ascendens)	1	39	7	54
Liver*	0–1	50	0	50
Prostate	7–10	34–39	2–6	59–60
Uterus	3–9	5–16	2–20	64–93
Pancreas	0–1	21–29	5–9	66–73
Intestine (mesentery)	5–6	11–13	7–9	78–80
Bladder	14–35	2–7	3–5	89–93
Stomach	15–23	3	2–6	91–95
Hypophysis	11	0	0	100
Spinal cord	23–27	0	0	100
Cerebellum	50–87	0	0	100
Cerebrum	55–90	0	0	100

*From six specimens assayed, one showed CK activity.

TABLE 3

SERUM CK AND CK-B SUBUNIT ACTIVITIES OF PATIENTS IN VARIOUS CLINICAL CONDITIONS

Clinical conditions	Number of cases	Total CK activity (I.U./l)*: mean range (positive %)	CK-B subunit activity (I.U./l)**: mean (% against total activity) range (positive %)	CK-BB***
Normal adults	M 108	84 37–130	2.5 0.0–5.0	
	F 100	61 21–100	2.5 0.0–5.0	
Muscular dystrophy	11	8877 215–35 190 (100)	109.3 (1.2) 4.5–428.0 (90)	(+)
Dermatomyositis	5	2038 36–8970 (60)	24.1 (1.2) 1.0–101.4 (40)	(+)
Polymyositis	7	945 199–2025 (86)	12.0 (1.3) 4.3–29.0 (83)	(+)
Myasthenia gravis	2	143 88–197 (50)	3.9 (2.7) 3.0–4.8 (0)	(+)
Multiple sclerosis	13	57 17–286 (8)	3.1 (5.5) 0.8–5.5 (15)	
Amyotrophic lateral sclerosis	2	81 33–128 (50)	2.0 (2.4) 1.5–2.4 (0)	
Acute myocardial infarction	167	2225 85–26 506 (100)	77.2 (3.5) 3.1–262.5 (97)	(+)
Myocarditis	12	381 11–1595 (42)	9.4 (2.5) 0.5–36.3 (58)	
Endocarditis	11	291 14–1039 (55)	7.3 (2.5) 0.3–20.2 (36)	
Old myocardial infarction	38	174 33–1141 (50)	4.1 (2.3) 1.1–17.0 (19)	
Ventricular fibrillation	44	106 7–698 (20)	6.0 (5.7) 0.3–37.0 (17)	
Cardic angina	75	91 22–402 (23)	3.2 (3.3) 0.2–17.1 (12)	
Coronary insufficiency	28	80 10–321 (21)	2.6 (3.3) 0.0–5.1 (4)	
Pericarditis	4	32 10–48 (0)	2.8 (8.7) 0.4–5.2 (25)	
Open heart surgery	69	1393 123–4084 (100)	44.6 (3.2) 4.7–226.7 (99)	(+)
Aortocoronary bypass surgery	7	548 52–1182 (86)	21.1 (3.9) 2.6–41.2 (71)	(+)
Subarachnoidal haemorrhage	16	187 7–2393 (19)	4.5 (2.4) 0.0–21.1 (19)	(+)
Cerebral haemorrhage	9	55 10–113 (0)	3.4 (6.2) 0.8–10.4 (13)	(+)
Cerebral embolism	38	70 10–387 (11)	2.9 (4.1) 0.2–8.7 (14)	(+)
Meningitis	11	93 13–431 (18)	4.2 (4.5) 0.2–18.1 (13)	(+)

(Continued on p. 410)

TABLE 3 (continued)

Clinical conditions	Number of cases	Total CK activity (I.U./l)*: mean range (positive %)	CK-B subunit activity (I.U./l)**: mean (% against total activity) range (positive %)	CK-BB***
Encephalitis	2	20 18–22 (0)	2.7 (13.5) 1.6–3.7 (0)	
Alcoholism	4	122 48–233 (50)	5.8 (4.8) 2.3–10.0 (50)	
Schizophrenia	5	92 12–168 (20)	2.5 (2.7) 0.6–5.1 (20)	
Neurosis	8	62 27–128 (13)	1.8 (2.9) 0.7–3.5 (0)	
Hypothyroidism	38	455 16–3500 (51)	8.2 (1.8) 0.3–56.1 (43)	
Systemic lupus erythematodes	14	58 16–288 (7)	2.4 (4.2) 0.5–5.7 (15)	
Pulmonary infarction	7	37 7–102 (0)	2.2 (5.9) 0.3–7.1 (14)	
Malignant tumours (hepatoma, carcinomas of the stomach, breast, colon and others)	125	81 4–1360 (16)	4.3 (5.2) 0.0–19.4 (29)	(+)
Normal neonates	118	529 180–1090	27.7 3.5–50.0	+
Asphyxia of newborn	38	1169 137–4029 (45)	79.2 (6.8) 3.1–517.4 (53)	+

*Total CK activity was determined with monotest CK NAC (Boehringer-Mannheim) at 30°C on a Hitachi 705.
**CK-B subunit activity was determined with monotest CK-MB (Boehringer-Mannheim) at 30°C on a Hitachi 705.
***+ = CK-BB is detected in the sera of most cases; (+) = CK-BB is detected in the sera of some patients.

separated into five bands, designated as CK-MM and CK-MM1 to CK-MM4 by isoelectric focusing [35]. CK-MM and CK-MM1 appear in sera of patients with acute myocardial infarction (AMI) during the fast stage. Thus, the diagnostic value of the bands for AMI is high [36].

Serum CK-MM activity increases in such disorders as progressive muscular dystrophy [37–42], dermatomyositis [43], polymyositis [44], AMI [45] and hypothyroidism [46] (Table 3). In Duchenne-type muscular dystrophy (DMD), serum CK-MM activity increases from 8 to 90 times the normal upper limit [42]. DMD is a sex-linked recessive genetic symptom appearing only in young males, generally before they reach the age of 3. Serum CK activity also increases in ca. 70% of the genetically proven carriers in families with DMD [40, 41]. Thus, the CK screening test is very significant for confirming DMD patients and detecting carriers [40, 41]. In DMD, CK-MB is also detected in serum when total CK activity increases [42], and skeletal muscle is known to be an origin of CK-MB.

CK-MM activity increased in the sera of almost half the patients with myositis [43]. A small increase in CK-MM activity has been determined in the sera of some patients with myasthenia gravis [44] or other diseases of the nervous sys-

tem, such as neuromyopathy [47]. Serum CK-MM activities also increase after physical exercise [48], intramuscular injection [49] or surgical operation [50].

4.2. Creatine kinase MB

The half-life of CK-MB is 12 h [51]. CK-MB can be hybridized in vitro by the reaction of CK-MM and CK-BB with guanidine hydrochloride (Fig. 5A) [52]. CK-MB1 is formed by M1 and B subunits, and CK-MB2 by M2 and B subunits

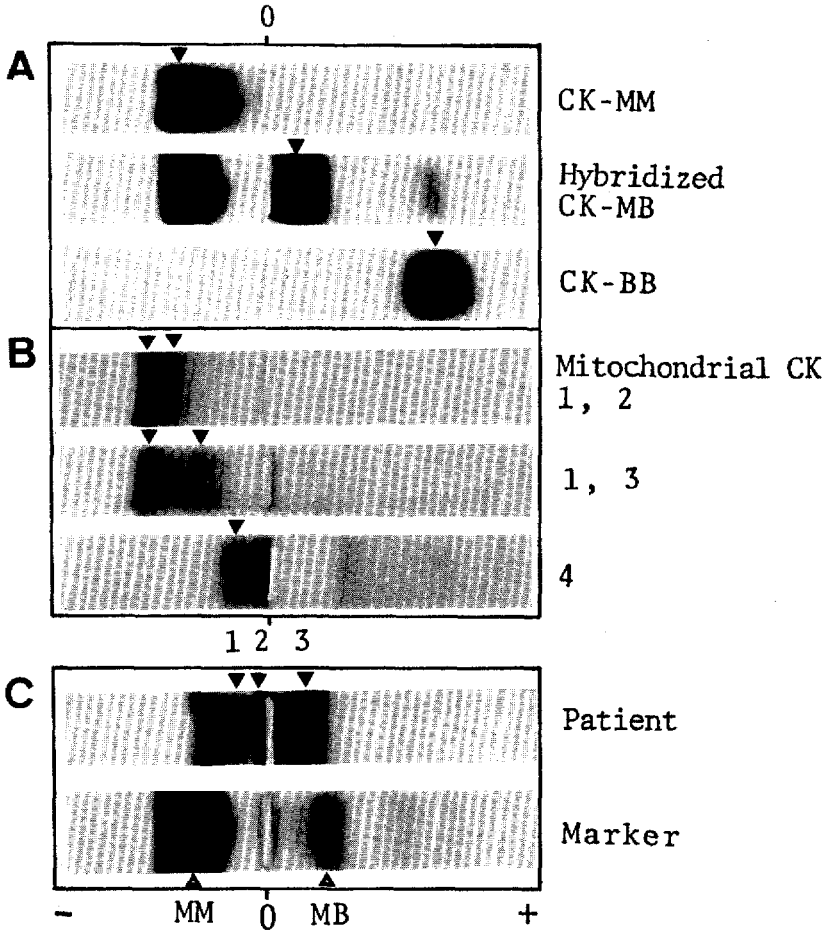

Fig. 5. (A) Hybridization of CK-MM from human skeletal muscle and CK-BB from human stomach tissue. CK-MM (2 I.U.) and CK-BB (6 I.U.) were incubated with 50 mg of guanidine hydrochloride in 1 ml of 100 m*M* Tris-HCl buffer (pH 8.0) containing 0.01 ml of 2-mercaptoethanol. 0 = Sample application point. (B) Mitochondrial CK separated by agarose-agar gel electrophoresis. Mitochondrial CK shows four electrophoretic mobilities around CK-MM, the position of band 3. Bands 1 and 3 are monomeric forms and bands 2 and 4 are oligomeric ones. Electrophoresis was performed at 100 V for 60 min and CK activity was stained with tetrazorium. (C) Three atypical CKs found in serum of a patient with carcinoma of the colon. Band 1 is CK-IgG complex, band 2 is membrane-bound CK and band 3 is CK-IgA/β-lipoprotein complexes. 0 = sample application point.

[53]. CK-MB mainly exists in cardiac muscle; thus, the diagnostic value of CK-MB in AMI is very high, and 6% CK-MB (or 3% CK-B subunit) activity of total CK activity is recommended as a discrimination limit of AMI from skeletal muscle damage [54]. In our study, blood samples from 167 patients with AMI were taken initially at 2-h intervals and later at 4-h intervals, and CK-MB assays were performed. The positive rate of CK-MB in the sera was 63% for the first 8 h after attack and 97% for the following 16 h. However, in five patients, serum CK-MB activities were within the normal range. The maximum activities of CK-MB ranged between 6 and 525 I.U./l, as determined by immunoinhibition at 30°C. After CK-MB activities reached their maximum values, they began to decrease and returned to normal after 72 h. The recovery time was thus shorter than the four days required by total CK, the seven days by aspartate aminotransferase (AST) and the fourteen days by lactate dehydrogenase (LDH). For the diagnosis of AMI, CK-MB is sensitive to the next to total CK activity and is specific to the next to electrocardiography [54]. Shell et al. [55] have reported a formula with which the infarction size of myocardium can be calculated.

4.3. Creatine kinase BB

The half-life of CK-BB is very short, less than 2 h [56], owing to the high clearance rate and rapid inhibition of the isoenzyme [34]. Albumin dimers [57] and IgG [58] are considered to be inhibitors of CK-BB. CK-BB activity in the central nervous system (CNS) is relatively high, ca. 100 I.U./g in fresh tissue [32]. However, CK-BB is negligible in the sera of patients with diseases of the CNS, except those in an acute phase. This is because as long as the blood–brain barrier is intact, CK-BB cannot be released in the peripheral blood [59]. Furthermore, because of its very short half-life, CK-BB cannot be detected in serum.

CK-BB is also present in smooth muscle, but it is not detectable in the sera of patients with benign diseases in this tissue. CK-BB frequently appears in the sera of patients with malignant tumours of the stomach [60], lung [61] and prostate [62, 63]. Wold et al. [64] histochemically detected CK-BB in the tissues of carcinomas of the breast, stomach, urinary bladder, pancreas and lung, and confirmed that this isoenzyme was formed in tumour cells [64]. Thus, CK-BB is now reported to be a tumour-associated marker [63].

CK-BB activity is relatively high in the sera of normal neonates, although CK-MM activity is dominant [65]. It was reported that CK-BB activity increased when neonates received brain damage during labour [66]. According to our data, CK-BB activities in the sera of 53% of neonates with asphyxia increased within 72 h of birth.

4.4. Mitochondrial creatine kinase

The relative molecular mass of mitochondrial CK is 84 000. However, the enzyme often forms oligomers in serum [67], known as macro CK type 2 [31]. Mitochondrial CK shows four electrophoretic mobilities around CK-MM (Fig. 5B) [67, 68]. The activation energy of mitochondrial CK is more than 100 kJ/

TABLE 4

FREQUENCY OF MITOCHONDRIAL CK IN SERA OF PATIENTS WITH MALIGNANT TUMOURS

This table is modified from Tables 1A, II and III of ref. 70.

Primary malignancy	Positive/total (%)	Number of cases (%)	
		With metastasis and/or infiltration	With histological diagnosis
Gastric	14/45 (31)	14/14 (100)	Poorly differentiated adenocarcinoma 7 (50) Tubular adenocarcinoma well differentiated type 4 (28) moderately differentiated type 3 (22)
Liver	17/29 (59)	10/17 (59)	Hepatoma 14 (82) Cholangioma 3 (18)
Pulmonary	12/25 (48)	10/12 (83)	Adenocarcinoma 5 (42) Adenosquamous carcinoma 2 (17) Squamous cell carcinoma 1 (8) Undifferentiated carcinoma 4 (33)
Mammary	5/13 (38)	5/5 (100)	Duct carcinoma 5 (100)
Uterine cervix	4/8 (50)	4/4 (100)	Squamous cell carcinoma 4 (100)
Bile duct	3/6 (50)	3/3 (100)	Adenocarcinoma 3 (100)
Gall bladder	2/6 (33)	2/2 (100)	
Colon	3/4 (75)	3/3 (100)	Tubulopapillary adenocarcinoma 3 (100)
Ovarian	1/4 (25)	1/1 (100)	
Prostate	1/4 (25)	1/1 (100)	
Others (peritoneum, skin and ethmoid cell carcinomas, gastric leiomyosarcoma)	4/23	4/4 (100)	
Total	66/167 (40)		

mol and is distinguishable from the other CK isoenzymes, which have activation energies of less than 50 kJ/mol [31]. Mitochondrial CK is often detected in the sera of patients with advanced malignant tumours (Table 4) [68–71] and AMI [72, 73]. Its existence indicates severe damage for the originating tissues and is considered to be a poor prognostic sign [70, 72]. Mitochondrial CK activity remains after immunoinhibition with anti-M subunit antibodies, thus immunoinhibition can be used for the screening of mitochondrial CK.

4.5. Atypical creatine kinase

Atypical CK is generally a complex of CK and immunoglobulin (Fig. 5C), with a relative molecular mass of more than 240 000. The complex is known as macro CK type 1 [31], as opposed to macro CK type 2 of mitochondrial CK. The complex is electrophoresed between CK-MM and CK-MB. CK-BB usually binds with IgG [74, 75] and the isoenzyme stoichiometrically binds onto the F_{ab} site of the IgG molecule at a CK-BB/IgG ratio of 2:1 [76]. The CK-BB–IgG complex seems to provide a route for the intravascular catabolism of CK-BB [58]. Complexes formed between CK-MM and IgA [21, 77] or mitochondrial CK and IgA [17]

TABLE 5

FREQUENCY OF CK-IMMUNOGLOBULIN COMPLEX IN SERA OF PATIENTS WITH MALIGNANT TUMOURS

This table is modified from Table II of ref. 17. N.D. = not determined.

Primary malignancy	Positive/total (%)	Number of cases (%)	
		With recurrence or metastasis in 27 patients	With tumour staging
Colon	8/28 (29)	8/8 (100)	N.D.
Gastric	6/50 (12)	6/6 (100)	II, 1/6 (17) IV, 5/6 (83)
Liver	5/51 (10)	1/5 (20)	N.D.
Pancreatic	4/20 (20)	4/4 (100)	N.D.
Pulmonary	3/26 (12)	2/3 (66)	N.D.
Mammary	1/10 (10)	1/1 (100)	N.D.
Others	0/49 (0)		
Total	27/234 (12)	22/27 (81)	

are also known. The CK–immunoglobulin complex is more stable when heated than CK-MM and CK-MB.

The CK–immunoglobulin complex is often present in the sera of patients with advanced malignant tumours (Table 5) [17, 77] and AMI [78], and seems to be a poor prognostic sign [17, 78]. The complex remains after immunoinhibition with anti-CK-M subunit antisera [31]; thus, care must be taken in assessing the results obtained by immunoinhibition so as not to interpret a false positive for CK-MB.

Other types of atypical CK, such as CK and β-lipoprotein complex [21, 79] or genetic variants (CK-BB) in blood cells that do not normally show CK activity [80], have been reported.

5. CONCLUSIONS

Analytical methods for and the clinical significance of CK isoenzymes have been described. The use of CK isoenzymes in the field of clinical diagnosis is relatively new, however, a decade has passed since the determination of CK isoenzymes was initiated in clinical laboratories. With the development of newer and better assay methods, many interesting findings in accordance with enzymatic, immunological and physicochemical characteristics have been reported, and further clinical significance of CK isoenzymes has been confirmed. The analysis of CK isoenzymes is a very useful tool for diagnosing patients with increasing total CK activities in sera. As the nature and function of CK isoenzymes are further elucidated, isoenzymes will find further applications in the clinical field.

6. SUMMARY

Analytical methods for and the clinical significance of ATP:creatine-N-phosphotransferase (EC 2.7.3.2, CK) isoenzymes have been described. The main assay methods for the isoenzymes are electrophoresis, ion-exchange chromatography and immunoinhibition. Electrophoresis is a time-consuming technique, but it is essential for the identification of CK isoenzymes. Ion-exchange chromatography is reliable for the determination of CK-MB and useful for the isolation and purification of CK isoenzymes. The disadvantages of this method are overlapping of isoenzymes with neighbouring fractions and the inevitable dilution of enzyme activity by the elution buffer. Immunoinhibition is a rapid, sensitive, specific and quantitative technique. However, mitochondrial CK and CK–immunoglobulin complex remain after the inhibition of the M subunit with the antibodies. Thus, when assessing the experimental results, it is necessary to be certain that a false positive for CK-MB has not been obtained. Since each technique has both merits and demerits, the technique most suited to the particular laboratory should be selected.

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