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Review

Electrophoresis of serum isoenzymes and proteins following acute myocardial infarction

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ABSTRACT

The clinical significance of the serum enzymes creatine kinase (CK, EC 2.7.3.2), lactate dehydrogenase (LD, EC 1.1.1.27) and aspartate aminotransferase (EC 2.6.1.1), and the isoenzymes CK 1–3 and LD 1–5, in acute myocardial infarction (AMI) is reviewed. Particular attention is given to electrophoretic analysis of the isoenzymes (and the CK isoforms/subforms) following AMI and thrombolytic therapy. Other protein markers for the monitoring of AMI, including myoglobin and muscle contractile proteins, are also discussed and the potential for the detection of new marker proteins using high-resolution two-dimensional electrophoretic methods is demonstrated. Whilst emphasis is placed upon electrophoretic methods the value of complementary immunoassays is acknowledged in order to maintain a balanced perspective.

CONTENTS

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AMI	Acute myocardial infarction
AMPD	2-Amino-2-methyl-1,3-propanediol
AST	Aspartate aminotransferase, L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1
ATP	Adenosine triphosphate
CCU	Coronary care unit
CK	Creatine kinase, ATP:creatine N-phosphotransferase, EC 2.7.3.2
GPD	Glucose-6-phosphate dehydrogenase, EC 1.1.1.49
GSH	Reduced glutathione
GSSG	Oxidised glutathione
HK	Hexokinase, ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1
IEF	Isoelectric focusing
LD	Lactate dehydrogenase, L-lactate:NAD oxidoreductase, EC 1.1.1.27
MOPSO	3-(N-Morpholino)-2-hydroxypropanesulphonic acid
MTT	Methylthiazoyltetrazolium
NAD	Nicotinamide-adenine dinucleotide
NADH	Dihydronicotinamide-adenine dinucleotide
NADP	Nicotinamide-adenine dinucleotide phosphate
NADPH	Dihydronicotinamide-adenine dinucleotide phosphate
NBT	Nitro blue tetrazolium
NEPHGE	Non-equilibrium pH gradient electrophoresis
PCMB	*p*-Chloromercuribenzoate
p*I*	Isoelectric point
PMS	Phenazine methosulphate

1. INTRODUCTION

Acute myocardial infarction (AMI) is a major cause of hospitalisation (and death) in the western world. Chest pain is symptomatic of acute ischaemic heart

disease and its onset is routinely followed by admission of the patient to a coronary care unit (CCU) for surveillance and diagnosis by electrocardiography and serum enzyme profile [1–6]. Thrombolytic therapy [7,8] is subsequently used to improve the prognosis. Unfortunately, atypical symptoms ("silent" AMI) result in upto 5% of AMI's remaining undiagnosed [9] whilst > 50% of CCU patients (occupying intensive care facilities) prove to be AMI-negative. Such discrepancies, combined with the new demands of the "thrombolytic era", have highlighted the need for more sensitive and specific markers for early diagnosis and subsequent management of AMI. To date, refinements in the assay and manipulation of established enzyme markers have proven most successful but the development of new protein markers of potential significance continues.

We will attempt an historical review of the use of electrophoresis for "cardiac" enzyme and protein analysis whilst emphasizing recent developments. Although electrophoresis has traditionally been the method of choice for such studies the increasing importance of immunoassays must be acknowledged to maintain a balanced perspective.

2. CREATINE KINASE

Creatine kinase (CK; ATP:creatine N-phosphotransferase; EC 2.7.3.2) reversibly transfers phosphate between ATP and creatine [10]:

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

Cytoplasmic CK is a dimer (molecular mass 86 000–89 000) of two subunits M (43 000) and/or B (44 500), each encoded by a separate gene [11,12]. They combine to give three isoenzymes denoted CK-MM, CK-MB and CK-BB (CK3, CK2, CK1, respectively) [13–15] (Fig. 1).

2.1. Electrophoresis of serum CK isoenzymes following AMI

Electrophoresis is widely used for separation of serum CK isoenzymes in the routine clinical laboratory [16,17]. Early studies of the transient appearance of CK-MB following AMI used agar [18], agarose [19], cellulose acetate [20] or polyacrylamide [21] as a support medium (Table 1). More recently, commercial systems have been widely adopted (*e.g.* Helena Labs., Beaumont, TX, USA; Corning Medical, Palo Alto, CA, USA; Beckmann Instruments, Clinical Instruments Division, Brea, CA, USA; Gelman Sciences, Ann Arbor, MI, USA) with agarose gels [22–24] or cellulose acetate strips [25,26] as the method of choice.

An alternative electrophoretic approach is isoelectric focusing (IEF) which separates the isoenzymes according to differences in isoelectric point (p*I*) rather than net charge (*c.f.* zonal electrophoresis). Wevers *et al.* [27] used preparative IEF (in an LKB sucrose density gradient electrofocusing column) to separate the

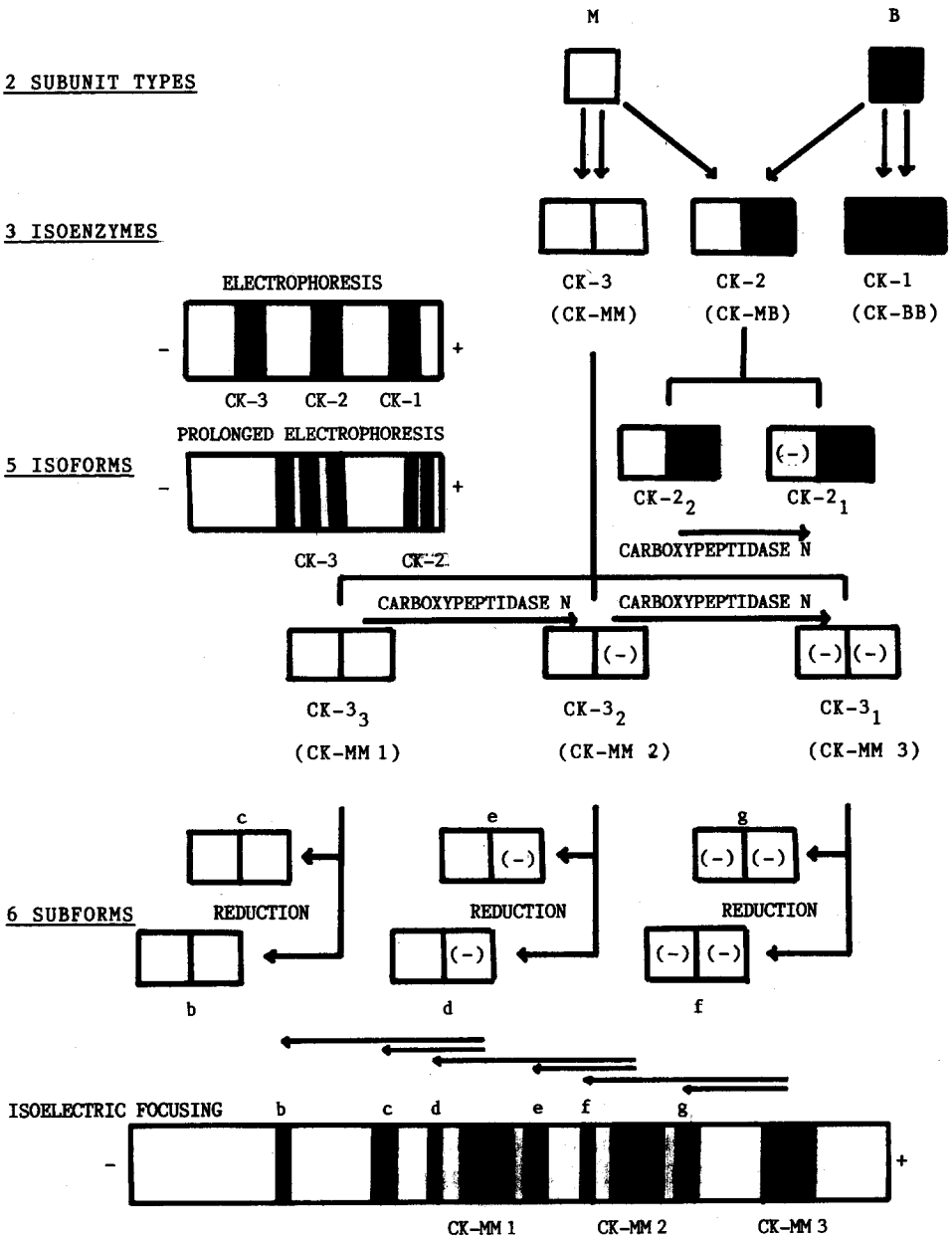


Fig. 1. Serum CK isoenzymes and heterogeneity of CK-MM (CK-3). CK is a dimer of two subunits, M and B, which combine to give three isoenzymes denoted CK-MM, CK-MB and CK-BB (CK-3, CK-2 and CK-1, respectively). The three isoforms of CK-MM (denoted CK-MM 1–3 or CK-3_{1-3}) and two isoforms of CK-MB (denoted CK-MB 1,2 or CK-$2_{1,2}$) result from post-translational modification (by carboxypeptidase N) of the M subunit: (−) indicates loss of carboxy-terminal lysine. The cathodal and intermediate subforms are enhanced by 2-mercaptoethanol reduction of the corresponding isoforms.

TABLE 1

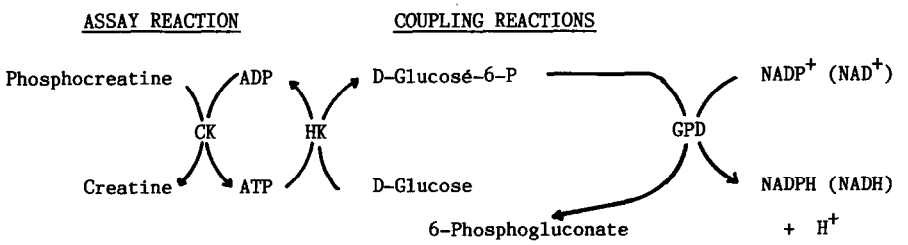
ELECTROPHORESIS OF SERUM CK FOLLOWING AMI

Electrophoretic method	Isoenzymes		Isoforms		Ref.
	Type	Assay time (h)	Type	Assay time (h)	
Agar gel electrophoresis					
Veronal buffer (pH 8.6), 220 V, 2 h	MM, MB	3	–		18
Agarose gel electrophoresis	MM, MB	3.5	–		19
Barbital buffer (pH 8–8.6), 85 V	MM, MB	1	MM 1–MM 3, MB1, MB2	3	22
20 min (isoenzymes)	MM, MB	1	MM 1–MM3	2	23
120 min (isoforms)	MB	0.25	–		24
Cellulose acetate electrophoresis	MM, MB	2	–		20
Barbital buffer (pH 8.4–8.8)	MM, MB	< 1	MM I–MM III	2	25
300 V, 10 min (isoenzymes)	MM, MB	0.5	MM 1–MM 3, MB 1, MB2	1.5	26
300 V, 60 min (isoforms)			$CK3_1$–$CK3_3$	< 0.5	38
800 V, 10 min (isoforms)					
Polyacrylamide gel electrophoresis					
Tris–EDTA borate (pH 8.4)	MM, MB		3MM, 2MB,		21
Tris–barbital (pH 7.4)			MM 1–MM 3, MB1, MB2	4	37
Preparative IEF					
Sucrose density gradients (pH 4–8)	MM, MB	65	MM 1–MM 3, X, MB	65	27
Analytical IEF in polyacrylamide			MM–MM 4, MB1, MB2	3–5	37
Using thin-layer/flat bed systems			MM 1–MM IV	3	25
pH 5–8 or 3.5–10, upto 1500 V for 1–2.5 h			MM 1–MM 3 a–k	3	30,39,40
Analytical IEF in agarose			X, Y, MM 1–MM3	1	23
	MM, MB	1.5	MM1–MM 11, MiMiI, II	1.5	28
	MM, MB	4	MM_0–MM_5		29
NEPHGE	MM, MB		MM1–MM 3 c–j MB1, MB2	1.5	30

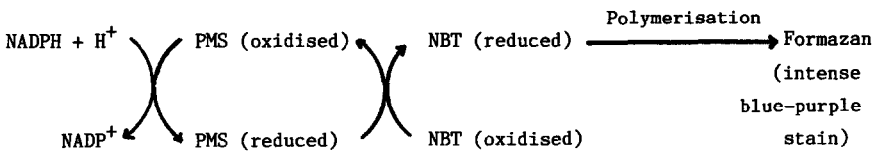
CK isoenzymes prior to hybridisation studies whilst others [28,29] have used analytical IEF (in agarose gels) to monitor serum CK isoenzymes following AMI. IEF may well improve resolution [28,29] but is more time-consuming and technically demanding than electrophoresis, *i.e.* a research tool rather than a routine method. A probable compromise is non-equilibrium pH gradient electrophoresis (NEPHGE) in which electrophoresis of the CK isoenzymes through a pH gradient is terminated before they reach their isoelectric point [30].

Following electrophoresis, the CK isoenzymes are detected by activity staining [31] which incorporates hexokinase (HK, EC 2.7.1.1) and glucose-6-phosphate

dehydrogenase (GPD, EC 1.1.1.49). This involves overlaying the support medium with a commercial CK assay mixture (*e.g.* CKNac, Boehringer Mannheim, Mannheim, Germany), incubating at 37°C (~ 30 min), and then visualising (under UV) the fluorescent NADPH (NADH) generated by the coupling reactions:



Alternatively, the NADPH (NADH) can be further coupled to reduction of nitro blue tetrazolium (NBT), via reduction of phenazine methosulfate (PMS) [32,33].



Pre-treatment (following fluorescence) of agarose and polyacrylamide gels with the sulfhydryl blocking reagent *p*-chloromercuribenzoate (PCMB) counteracts the adverse effects of sulfhydryl compounds (in commercial CK assay mixtures) and thereby reduces background upon NBT staining [34].

2.2. Electrophoresis of serum CK isoforms following AMI

Early studies suggested multiple forms of CK isoenzymes [35,36]. At least three isoforms of CK-MM (denoted CK-MM 1–3 or $CK3_{1-3}$) and two isoforms of CK-MB (denoted CK-MB 1,2 or $CK2_{1,2}$) are detected in AMI serum when the standard electrophoresis conditions (for CK isoenzyme analysis) are prolonged [21–23,25,26,37] or the voltage increased [38] or, alternatively, IEF [23,25,28–30,34,37,39,40] or NEPHGE [30] are used as the separation method (Table 1). These isoforms are not true isoenzymes. They arise following post-translational modification of the CK-M subunit by a heat-labile serum "CK conversion factor" [41–45] with carboxypeptidase activity [46–49], *i.e.* hydrolytic cleavage of the carboxyterminal lysine results in an anodal shift in electrophoretic mobility compatible with the loss of a single positive charge [50] and this generates two additional CK-MM isoforms and a single additional CK-MB isoform (Fig. 1). For eloquent review see Panteghini [51].

2.3. Electrophoresis of serum CK-MM subforms following AMI

Electrophoresis detects only three CK-MM isoforms [21–23,25,26,37] but IEF reveals more complex patterns [23,25,28–30,37,40] which can be explained by the existence of two cathodic subforms for each of the three isoforms [30,40] (Fig. 1). *In vitro* incubation of AMI serum with 2-mercaptoethanol (2–15 mmol/l) prior to IEF enhances the cathodic subforms (converting CK-MM 1 to b,c, CK-MM 2 to d,e and CK-MM 3 to f,g, Fig. 2) [30,40] whilst glutathione results in a limited but reversible interconversion [52], *i.e.* the major subform of each isoform is enhanced by reduced glutathione (GSH, 1.25 mmol/l) and diminished by oxidised glutathione (GSSG, 2.5 mmol/l) and sequential incubation (GSH followed by GSSG, or GSSG followed by GSH) reverses the earlier effect [52]. This "secondary redox conversion" has not been confirmed *in vivo* but when superimposed upon the "carboxypeptidase conversion" it generates nine isoforms/subforms of CK-MM, perhaps explaining the unexpected heterogeneity noted in earlier studies [23,25,28,37,53].

2.4. Nomenclature of serum CK isoenzymes and CK-MM isoforms/subforms

The electrophoresis of serum CK following AMI has been complicated greatly by contradictions in nomenclature [54]. Table 2 highlights this problem and offers a tentative comparison based upon reported pI values [23,25,27,29,30,37,39,40,55–57]. Strict adherence to the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (multiple forms of en-

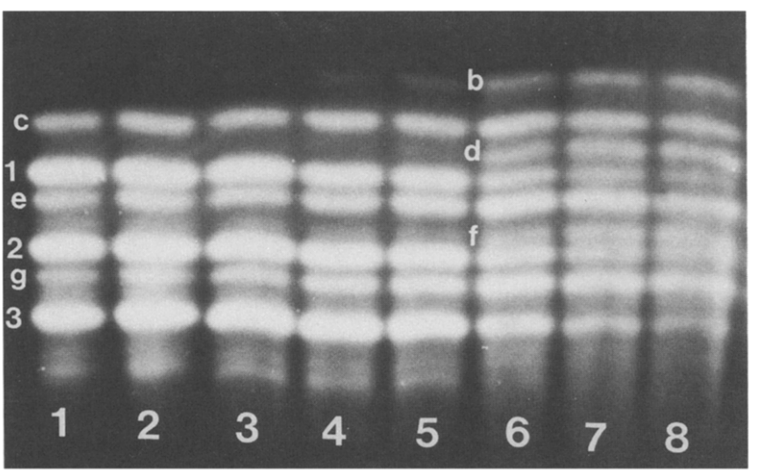


Fig. 2. *In vitro* incubation of AMI serum with 2-mercaptoethanol prior to IEF: effect upon CK-MM. The serum (total CK > 3000 U/l; CK-MB > 20%) was incubated at room temperature with an equal volume of 0.1 mol/l Tris–HCl buffer pH 7.4 (track 1) or buffer containing 0.05, 0.075, 0.38, 0.75, 3.8, 7.5 or 15 mmol/l 2-mercaptoethanol (tracks 2–8, respectively). The major bands (track 1) denoted 1, 2 and 3 correspond to the isoforms CK-MM 1–3 ($CK\text{-}3_3$, $CK\text{-}3_2$ and $CK\text{-}3_1$, respectively); b and c, d and e, and f and g correspond to the cathodal subforms of isoforms CK-MM 1, CK-MM 2 and CK-MM 3, respectively.

TABLE 2

NOMENCLATURE AND p*I* VALUES (IN PARENTHESES) OF SERUM CK-MM ISOFORMS/SUBFORMS

Isoform/ subform[a]		Designation of zone													
		Ref. 27		Ref. 37		Ref. 25		Ref. 23		Ref. 57		Ref. 56		Ref. 29	
a	(7.55)[b]														
b	(7.35)											MM_{-1}	(7.29)	MM5	(7.40)
c	(7.25)			MM	(7.10)		(7.13)					MM_0	(7.10)	MM4	(7.10)
d	(7.05)														
MM1	(6.91)	MM3	(6.86)	MM1	(6.88)	MMI	(6.90)	MM3	(6.90)	MM1	(6.90)	MM_1	(6.88)	MM3	(6.90)
e	(6.85)														
f	(6.72)									MMB1	(6.76)				
MM2	(6.65)	MM2	(6.49)	MM2	(6.70)	MMII	(6.62)	MM2	(6.67)	MM2	(6.62)	MM_2	(6.70)	MM2	(6.70)
g	(6.50)									MM2B	(6.49)				
h	(6.40)														
MM3	(6.35)	MM1	(6.24)	MM3	(6.45)	MMIII	(6.36)	MM1	(6.45)	MM3	(6.36)	MM_3	(6.45)	MM1	(6.20)
i	(6.28)														
j	(6.20)	X	(6.07)	MM4	(6.25)	MMIV	(6.20)	X	(6.23)	MM4	(6.20)	MM_4	(6.25)	MM0	(6.00)
k	(6.15)							Y	(6.05)						

[a] Ref. 39.

[b] Detected in only 4% of AMI patients and suspected to be of mitochondrial origin.

zymes) dictates that the most anodal isoenzyme be given the lowest arabic numeral (*i.e.* CK-BB = CK-1; CK-MB = CK-2; and CK-MM = CK-3) and the most anodal isoforms the lowest lower case letter (*i.e.* CK-3_{a-c}) [15,38,58,59]. In practice, letter subscripts have been replaced by numbers; "tissue" CK-MM = $CK3_3$ and the anodal conversion is represented by CK-3_3 → CK-3_2 → CK-3_1 [38,51,58–61].

However, problems arise when this nomenclature is extended to the subforms of the CK-MM isoforms, *i.e.* the most cathodal subform of CK-3_1 is more cath-

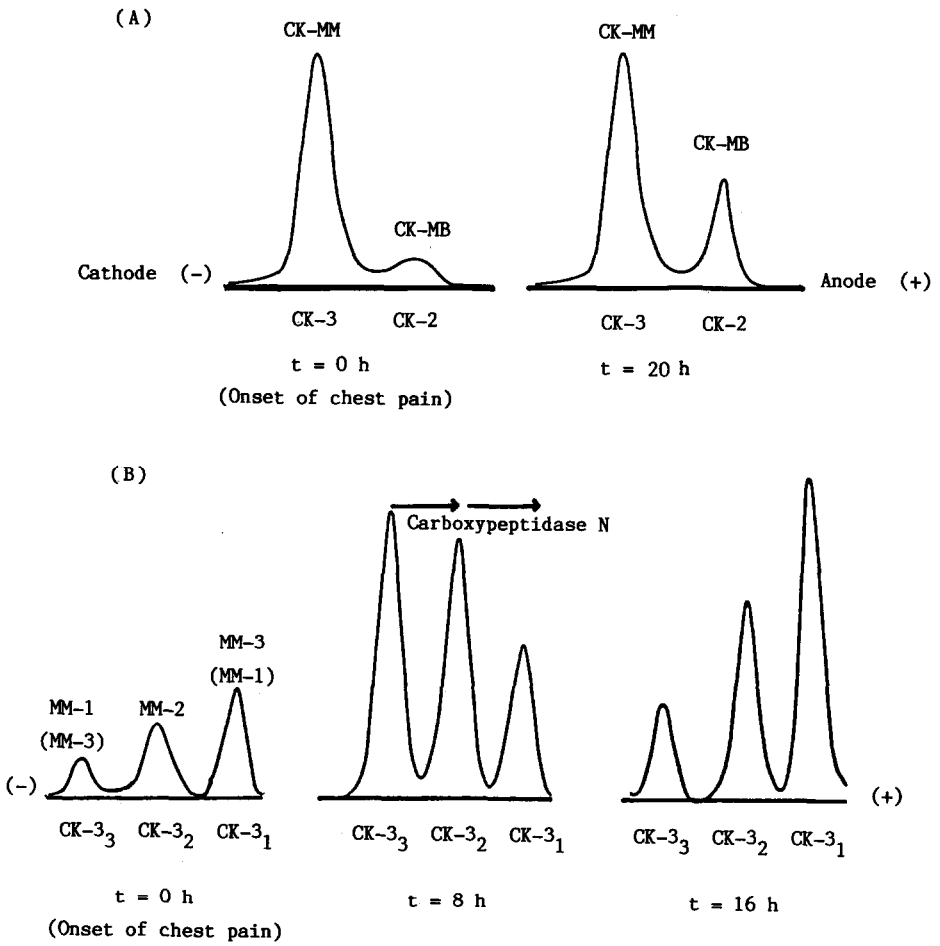


Fig. 3. Kinetics of serum CK isoenzymes/isoforms following AMI. (A) Isoenzymes: the CK-2 (CK-MB) isoenzyme is a "gold standard" for diagnosis of AMI; it increases within 6 h of infarction, peaks within 20 h and normalises within three days. (B) Isoforms: early release of "tissue" CK-3_3 is followed by its carboxypeptidase N-catalysed anodal conversion to CK-3_2 and CK-3_1. CK-3_3 provides an early indicator of AMI and successful reperfusion; its kinetics of conversion can be used to predict the time of onset of infarction. *Note*: by convention the anode of an electrophoresis gel is normally shown to the left, however, with CK isoenzymes and CK-3 isoforms this convention is commonly reversed.

odal than CK-3_2 and likewise, the most cathodal subform of CK-3_2 is more cathodal than CK-3_3 (Fig. 2). In common with others [25,37,55–57] we have denoted CK-3_3 ("tissue" CK-MM) as CK-MM 1 and CK-3_2 and CK-3_1 as CK-MM 2 and CK-MM 3, respectively, in order of sequential appearance on anodal conversion; the respective subforms are denoted b, c; d, e; and f, g [39,40,52] (Figs. 1 and 2 and Table 2). This is not unreasonable since the traditional CK-MM/CK-MB nomenclature is still widely used and it does simplify interpretation of our complex isoform/subform patterns. Perhaps the greatest source of confusion is the nomenclature that adopts paradoxically the IUPAC recommendations for the isoforms but not the true isoenzymes, *i.e.* "tissue" CK-MM = CK-MM 3 and anodal conversion is represented by CK-MM 3 → CK-MM 2 → CK-MM 1 [23,27,29,62,63].

2.5. Clinical significance of serum CK isoenzymes following AMI

The tissue distribution of CK isoenzymes and their clinical significance in diseases other than AMI is outside the scope of this contribution. For excellent reviews see Lott and Abbott [64], Kanemitsu and Okigaki [65], and Lotţ and Stang [66].

Serum CK-MB (CK-2) orginates primarily from heart and is the "gold standard" for diagnosis of AMI [67]; it rises within 6 h of infarction, peaks within 10–20 h (~ 10 h earlier than total CK) and normalizes within three days (Fig. 3). Electrophoresis, ion-exchange chromatography and immunoinhibition are the classical methods for assay of CK-2 [64,65] but the recent trend is towards immunometric assay using enzyme-conjugated monoclonal antibodies [63], *e.g.* the *TANDEM* CK-MB enzymometric assay [68,69], the *MAGIC LITE* chemiluminometric assay [70,71], and the *STRATUS (DADE)* immunofluorometric assay [71,72].

2.6. Clinical significance of serum CK isoforms following AMI

This topic has also been extensively reviewed [51,56,62,63,73] but merits special attention since electrophoresis remains the analytical method of choice, *i.e.* chromatofocusing [74] and anion-exchange high-performance liquid chromatography [75] do not achieve the same degree of resolution with respect to "extraneous" subforms, and immunometric assays with specificity for different isoforms [76,77] have yet to be fully developed.

The rationale for serum CK isoform analysis following AMI is early diagnosis (which is essential if myocardial damage is to be minimised by thrombolytic therapy [78]) and non-invasive monitoring of reperfusion [51,62,73]. Myocardial damage/necrosis quickly results in release of "tissue" CK-MM (CK-3_3) and its anodal conversion (CK-3_3 → CK-3_2 → CK-3_1) *in vivo* [41]. Experimental studies (coronary occlusion) in dogs [45,79–81] and sequential sampling in humans following AMI or coronary surgery [23,25,26,28–30,37–40,42,82] have confirmed the value of CK-MM isoforms (usually expressed as a ratio: CK-3_3/CK-3_1) for

TABLE 3

EFFECT OF REPERFUSION UPON SERUM CK ISOENZYMES/ISOFORMS FOLLOWING AMI

Peak values, hours after onset of chest pain			Thrombolytic therapy	Ref.
CK-3_3/CK-3_1	CK-2	Total CK		
10.6 ± 2	25 ± 5	23 ± 4	(−)	23
9.3 (8.6–12.9)[a]	20.5 (9.2–28.2)	25.2 (10.0–33.5)	(−)	82
10.7 ± 3.8	18.5 ± 6.7	20.7 ± 8.6	(−)	85
5.5 ± 1.0	8.6 ± 2.1	12.1 ± 3.1	(+)	
11.8 ± 3.2	20.1 ± 3.2	20.8 ± 3.8	(−)	58
7.5 ± 2.9	10.1 ± 2.3	12.2 ± 4.4	(−)[b]	
4.2 ± 1.8	11.0 ± 2.3	12.0 ± 3.1	(+)	
9.9 (4.0–12.3)	CK-2_2, 17.8 (8.6–27.4) CK-2_1, 26.8 (10.9–29.1)		(−)	61
5.2 (3.0–7.9)	CK-2_2, 9.1 (5.7–13.6) CK-2_1, 11.8 (10.6–18.5)		(+)	
12.2 ± 4.8	20.3 ± 4.3	22.3 ± 5.0	(−)	62
10.8 ± 5.6	14.8 ± 6.4	15.9 ± 5.6	(+)	

[a] Range.

[b] Spontaneous reperfusion.

early detection, *i.e.* peak values of CK-3_3/CK-3_1 precede those of CK-2 and total CK (Table 3). The kinetics of isoform conversion (Fig. 3) can be used to predict the time of onset of infarction [26] and the ratio (CK-3_3/CK-3_1) can apparently distinguish angina and AMI within 4 h of onset (when total CK and CK-2 are normal) [83]. Whilst the CK-3 isoforms show the highest diagnostic efficiency for early diagnosis (< 3–9 h) in AMI [38] simultaneous determination of the CK-2 isoforms would enhance the diagnostic specificity [38,61].

The onset of the "thrombolytic era" for management of AMI, whereby plasminogen activators are intravenously injected to effect fibrinolysis and coronary recanalisation [7,8], has stimulated a demand for non-invasive clinical indicators of successful reperfusion. The use of serum CK for this purpose [58,61,84,85] is based upon the "washout phenomenon" whereby successful reperfusion leads to early release (and elevated levels) of "cardiac enzymes" [84,86–89]. The concept has been experimentally confirmed by coronary occlusion in dogs [81] and validated in humans by comparison of reperfused and non-reperfused patients following AMI [38,58,61,85]. Although total CK and CK-2 are elevated and peak earlier following reperfusion [61,90,91] the peak value of the CK-3_3/CK-3_1 ratio offers the earliest indication of success (Table 3).

The cathodal subforms of the CK-3 isoforms have also been electrophoretically monitored following AMI [30,39,40,52,92] and evaluated for early diagnosis and reperfusion [56]. Chapelle proposed that the major cathodal subform of CK-3_3 is a myocardial CK-3 precursor and an early "direct" marker of functional impairment, predominantly associated with left ventricular failure [92]. This subform showed a higher rate of increase than CK-3_3 following endomyocardial biopsy, AMI or reperfusion [56]. Our studies lack the quantitative data to confirm this but demonstrate, *in vitro*, a "secondary redox conversion" between the CK-3 isoforms and subforms [30,40] and their reversible interconversion by glutathione [52]. Thus the early appearance of cathodal subforms following AMI may indicate a redox type reaction (initiated by myocardial glutathione? [52]) which is rapidly reversed by oxidation [29], *i.e.* a chemical imbalance [30] rather than specific tissue CK subforms [56,92].

3. LACTATE DEHYDROGENASE

Lactate dehydrogenase (LD; L-lactate:NAD oxidoreductase; EC 1.1.1.27) reversibly catalyzes the interconversion of lactate and pyruvate:

$$\text{L-lactate} + \text{NAD}^+ \overset{\text{LD}}{\rightleftharpoons} \text{pyruvate} + \text{NADH} + \text{H}^+$$

LD is a tetramer (molecular mass 134 000) of two subunits M (34 000) and/or H (34 000), each encoded by a separate gene [93]. They combine to give five isoenzymes denoted M_4, HM_3, H_2M_2, H_3M and H_4 (LD 5, LD 4, LD 3, LD 2 and LD 1, respectively) (Fig. 4). For review, see Maekawa [94] and Wolf [95].

3.1. Electrophoresis of serum LD isoenzymes following AMI

Electrophoresis is the method of choice for separation of LD isoenzymes [94,96] and detection of the characteristic LD "flip" (LD 1 > LD 2) associated with AMI (Fig. 4). Early studies of LD in serum following AMI used agar [97], cellulose acetate [98–100] or starch [101] as a support medium. Polyacrylamide gel electrophoresis revealed only four bands unless the stacking gel was omitted [102]. As with CK, routine analysis of LD now commonly involves the use of commercially available systems (*e.g.* Helena Labs. Corning Medical, Beckmann Instruments and Gelman Sciences). They exploit agarose gels or cellulose acetate strips as a support medium and use a variety of assay conditions (Table 4). The methods have recently been compared [96] against the reference method of McKenzie and Henderson [103]; their precision was adequate for routine use but their accuracy was suspect with underestimation of LD-1, LD-2 and LD-3, and overestimation of LD-4 and LD-5 [96]. The reference method [103] is included in Table 4.

Following electrophoresis, the LD isoenzymes are detected by activity staining

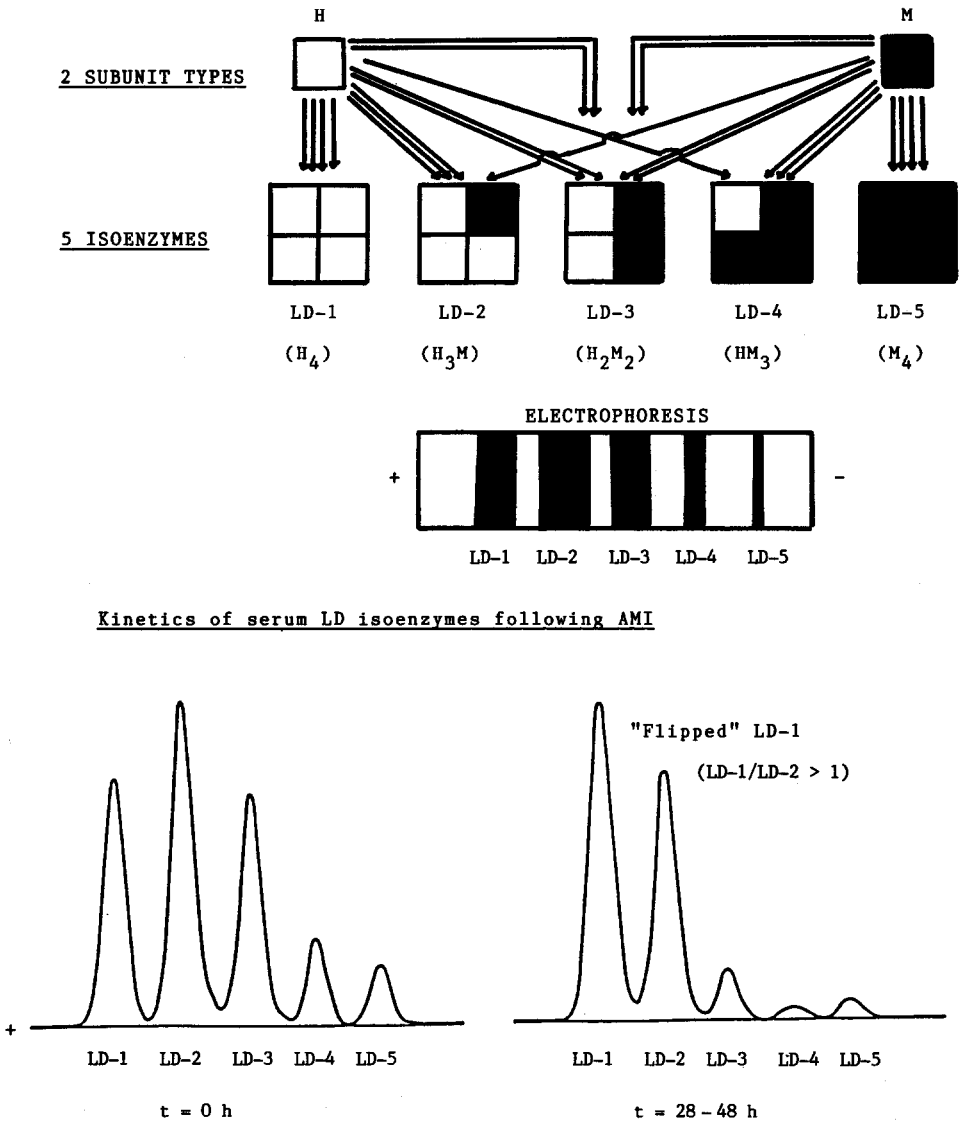


Fig. 4. Serum LD isoenzymes. LD is a tetramer of two subunits, H and M, which combine to give five isoenzymes denoted LD-1 to LD-5 in order of decreasing anodal mobility upon electrophoresis. Following AMI LD-1 increases faster than LD-2 and 80% of patients show a highly specific "flipped" pattern within 48 h.

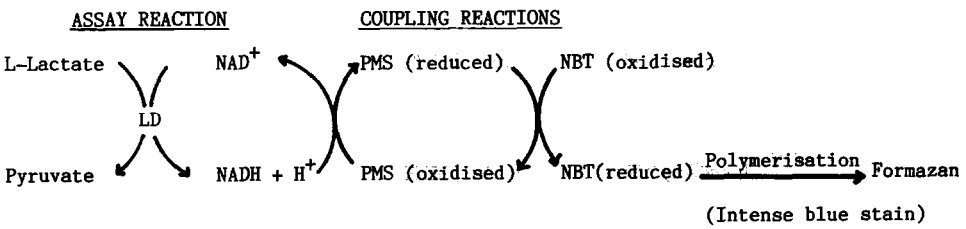
and quantitated by densitometry. The support medium is overlaid with substrate–coenzyme solution [L(+)-lithium lactate–β NAD^+], incubated at 37°C (~ 30 min), and the generated NADH visualised by fluorescence under UV [103].

TABLE 4

ROUTINE ANALYSIS OF SERUM LD WITH COMMERCIAL ELECTROPHORESIS SYSTEMS

Method	Support medium	Buffer, electrophoretic conditions	Detection, incubation conditions
Ref. 103	Agarose gel	Barbital, 200 V, 60 min	Fluorescence, 37°C, 15 min
Beckman Paragon	Agarose gel	Barbital/AMPD, 100 V, 20 min	NBT, 45°C, 30 min
Corning LD Vis-Flur	Agarose gel	Barbital, 200 V, 35 min	Fluorescence, NBT, 37°C, 20 min
Gelman LDH isozyme	Cellulose acetate	Tris–barbital, 210 V, 25 min	MTT, 37°C, 30 min
Helena LD Vis–Flur	Cellulose acetate	Tris–Barbital, 300 V, 10 min	Fluorescence, NBT, 37°C, 20 min
Helena gel LDH	Agarose gel	MOPSO, 100 V, 20 min	NBT, 45°C, 25 min

Alternatively, the formation of NADH is coupled to reduction of NBT, via reduction of PMS or methylthiazolyltetrazolium [94,96].



The alternative methods for separation and assay of LD isoenzymes (*e.g.* ion-exchange chromatography, "2-hydroxybutyrate dehydrogenase" activity) are beyond the scope of this review: for methodological details see Henderson [104]; for evaluation of relative merits see Maekawa [94].

3.2. Electrophoresis of serum LD isoforms following AMI

Additional LD isoenzyme/isoform bands have been detected by electrophoresis but not following AMI. Briefly, they include (i) LD–immunoglobulin (Ig) complexes usually migrating between LD-3 and LD-4 [105–108], and (ii) cathodal LD-6 [109–111] which is associated with arteriosclerotic cardiovascular/liver dis-

ease [112] and indicative of a poor prognosis [111,113]. Immunochemical studies indicate that LD-6 contains the M subunits but is not an IgG–LD macroisoenzyme complex [95]. Upto fourteen LD isoforms were detected following high-resolution polyacrylamide IEF of human tissue extracts, erythrocyte lysates and serum concentrates. LD-1 and LD-3 appeared as doublets whilst intermediate bands were detected between LD-1 and LD-2, LD-2 and LD-3, and LD-3 and LD-4 [114]. The source of this heterogeneity is unknown but perhaps a similar study of serum LD isoenzymes following AMI is justifiable.

3.3. Clinical significance of serum LD isoenzymes following AMI

LD increases 8–12 h after the onset of chest pain, peaks between 28 and 48 h, and normalises by about day 10 [115]. However, LD-1 increases faster than LD-2 and 80% of patients show a highly specific "flipped" pattern (LD-1 > LD-2) within 48 h [1–4]. The disproportionate increase in LD-1 can be expressed as a percentage of total LD or as a ratio of LD-2 [103,116]. Early studies gave excessively high values for the LD-1/LD-2 ratio following AMI [97,117] and a value of $\geqslant 1$ has often been recommended. However, the LD-1/LD-2 ratio does not always "flip" following AMI [116,118] and Henderson and co-workers [119,121] have repeatedly stressed that, for diagnostic purposes, the LD-1/LD-2 ratio need only be increased above the reference limit. Decision thresholds as low as 0.74 have been suggested [116,118,119] but values of 0.92–0.94 may be more realistic [122].

The diagnostic efficacy of the LD-1/LD-2 ratio may be improved by use of the LD-1/LD-4 ratio, *i.e.* the disproportionate increase in LD-1 is magnified by a proportionate decrease in LD-4 [120,121]. However, serum CK (CK-2) is undoubtedly the "gold standard" for diagnosis of AMI and the continued use of LD has been questioned [123–126] except for cases of late presentation, *i.e.* > 48 h after the onset of chest pain (when CK, CK-2 may have normalised) [126,127].

Recent studies indicate an *in vivo* interaction between LD and streptokinase in AMI patients undergoing thrombolytic therapy [128,129]. The phenomenon has been attributed to amino acid homology between the M subunit and the streptokinase binding site on plasminogen [128]. The complex affects serum LD electrophoresis (leaving a precipitate of activity at the sample origin) but can be removed by ultracentrifugation of the serum prior to analysis [129].

4. ASPARTATE AMINOTRANSFERASE

Aspartate aminotransferase (AST; L-aspartate:2-oxoglutarate aminotransferase; EC 2.6.1.1) reversibly catalyses the interconversion of aspartate and oxaloacetate:

$$\text{L-aspartate} + \alpha\text{-oxoglutarate} \overset{\text{AST}}{\rightleftharpoons} \text{oxaloacetate} + \text{L-glutamate}$$

There are two isoenzymes: an anodic cytoplasmic c-AST (molecular mass 120 000) and a cathodic mitochondrial m-AST (100 000) [130]. They differ in amino acid composition and immunochemical properties [131,132]. For review of AST see Rej [133].

4.1. Electrophoresis of serum AST

AST and its isoenzymes have been separated by electrophoresis using paper [130,134], starch [135–137], agar [138], polyacrylamide [139–141] and cellulose acetate [142,143] as a support medium. However, electrophoresis has been criticised for its poor sensitivity and the technical difficulties inherent in activity staining [141,143]. Product inhibition (oxaloacetate) can be prevented by including oxaloacetate decarboxylase in the overlay reagent and quantitating the formation of glutamate with a glutamate dehydrogenase–diaphorase-coupled enzyme system linked to formazan dye formation [141]. However, alternative methods of isoenzyme separation/quantitation are to be preferred. The classical methods are immunoprecipitation [144] or column chromatography [145,146] but radioimmunoassay [147], enzyme-linked immosorbent assay [148,149] and fast protein liquid chromatography [150] have recently been developed for this purpose. The earlier methods have been directly compared [151,152].

4.2. Clinical significance of serum AST following AMI

Immunochemical assay of serum from AMI patients indicates that c-AST increases before m-AST (within 7 and 10 h, respectively) and peaks earlier (within 30 and 50 h, respectively) [146,153,154]. m-AST also normalises more slowly (> six days) [153–155]. The peak activity of c-AST reflects total CK and CK-2 and appears to correlate with infarct size [146,154,155]. In contrast, m-AST may [155] or may not [146,154] but could provide a useful prognostic indicator [156]. Both isoenzymes are further enhanced by the hepatic response to left ventricular failure [155] whilst only c-AST demonstrates a "washout" response to urokinase-induced reperfusion; m-AST is probably retained intracellularly in the damaged tissue [157]. However, it must be emphasized that electrophoresis will only contribute to such studies if detection sensitivity can be greatly enhanced.

5. MYOGLOBIN

Myoglobin (molecular mass 17 000) is an oxygen-binding protein of uncertain function. Its release into serum (myoglobinaemia) and excretion in urine (myoglobinuria) following AMI [158–162] has been monitored by immunoassay [163–167]. Serum myoglobin increases within 4 h of infarction, peaks in < 12 h and normalises by 24 h [163,164,168,169]. Its diagnostic sensitivity for early diagnosis is $\geqslant$ total CK (and CK-2) but its specificity is suspect [168–170]. Electrophoresis is not routinely used to monitor serum or urine myoglobin levels.

6. CONTRACTILE PROTEINS

Myosin (molecular mass 480 000) is the major contractile protein. It hydrolyses ATP and displays isoenzymes due to the variable composition of its constituent

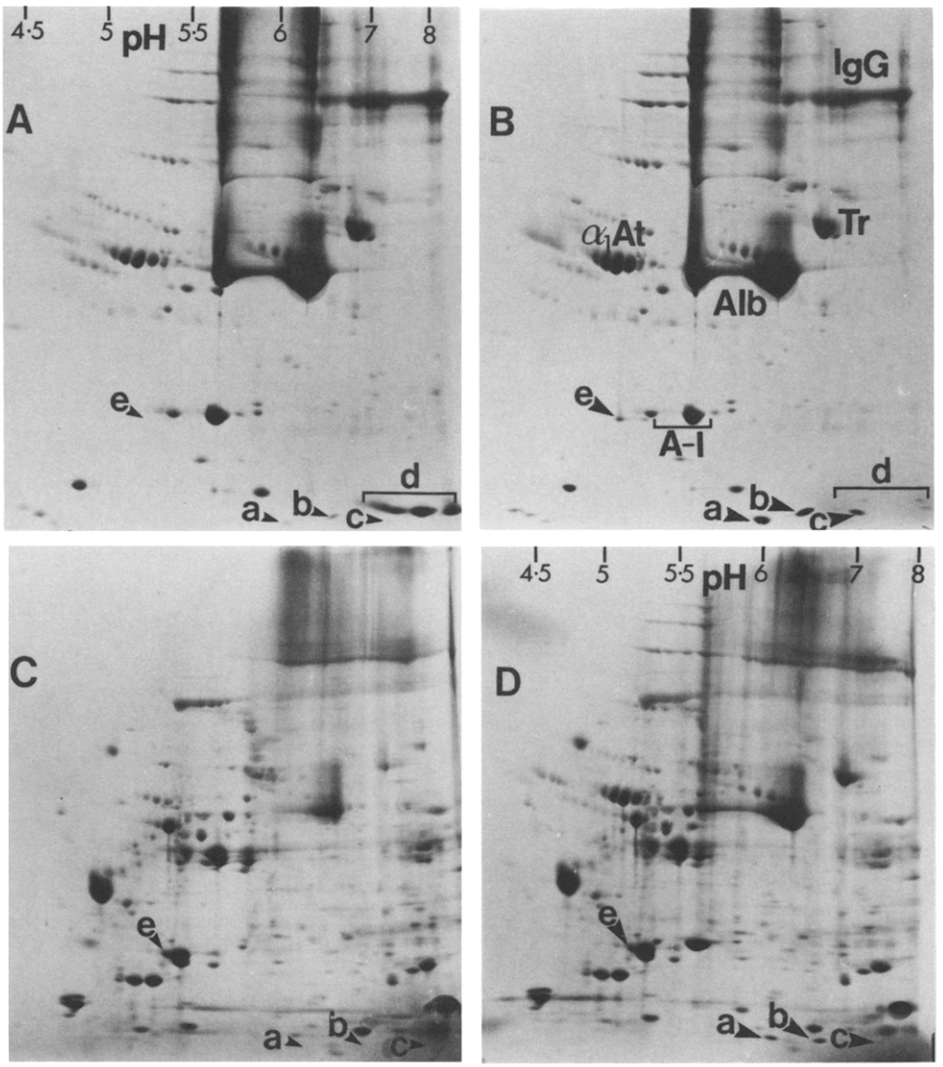


Fig. 5. High-resolution two-dimensional electrophoresis of human serum and myocardial extracts. The AMI serum (3 μl) was sampled 6 and 42 h after admission (A and B, respectively). The abnormal proteins "a"–"c" are probably isoforms of apo serum amyloid A protein whilst "d" and "e" have been tentatively identified as haemoglobin and myosin light chain, respectively. Proteins "a"–"c" were not detected in extracted myocardium (C, 4 mg wet weight) but protein "e" co-electrophoresed with myosin light chain when a mixture of AMI serum and extracted myocardium was electrophoresed (D). The proteins were analyzed under non-reducing conditions and stained with Coomassie Brilliant Blue R-250. Identified serum proteins include albumin (Alb), α_1-antitrypsin (α_1At), transferrin (Tr) and immunoglobulin G (IgG).

heavy (molecular mass 200 000) and light chains ($\sim$ 20 000) [171]. Electrophoresis played a major role in early studies of myosin heterogeneity [172–175] and this tradition has been sustained by recent application of high-resolution two-dimensional electrophoresis [176–178]. However, immunoassay has proven to be the method of choice for monitoring elevation of serum levels of cardiac myosin light chain following AMI [179–183]. Its time course mirrors CK (CK-2) in the early stages of infarction but the specificity of the assay is suspect due to cross-reactivity with skeletal myosin light chain [179,182,183].

Immonoassays have also been developed to monitor the elevated levels of other contractile proteins in serum following AMI [184–187]. Cardiac tropomyosin increases with CK-2 and remains elevated after CK normalisation [184] but monitoring of troponin I [185,186] and troponin T [187] has the additional advantage of cardiospecificity [187]. Recently, serum elevation of cardiac S-100a_0 protein (molecular mass 21 000) has been reported following AMI. Enzyme immunoassay indicates a faster and more prolonged elevation than CK-2 and a potential for differential diagnosis of AMI and angina pectoris [188].

Whilst immunoassays are ideal for quantitating known marker proteins, the detection of new markers demands a technique such as high-resolution two-dimensional electrophoresis [189] which combines two independent electrophoretic parameters (p*I* and molecular mass) for simultaneous analysis of upto 1000 polypeptide constituents. The application of this method to human serum following AMI reveals abnormal low-molecular-mass proteins [190,191] which appear $\sim$ 30 h after infarction, peak within 50 h and normalise after five days [191,192] (Fig. 5A and B). Protein "e" (molecular mass 27 000; p*I* 5.2) was tentatively identified as myosin light chain (Fig. 5C and D) whilst proteins "a"–"c" (molecular mass 13 000; p*I* 6.2, 6.7 and 7.5, respectively) are probably isoforms of apo-serum amyloid A protein [193], one of a number of acute phase reactants elevated following AMI [194–198].

7. CONCLUSIONS

Early zonal electrophoretic methods have been adopted for routine monitoring and clinical diagnosis of AMI. These methods will become automated or be replaced by immunoassays using monoclonal antibodies specific to CK-2, CK-2_2 and CK-3_3. The more sophisticated electrophoretic methods (*e.g.* IEF and high-resolution two-dimensional electrophoresis) are technically demanding and unsuitable for routine use. IEF has enormous research potential for investigating the molecular events, *e.g.* the kinetics of the primary (carboxypeptidase-catalysed) and secondary (redox) conversions of CK-2 and CK-3 following AMI. High-resolution two-dimensional electrophoresis has yet to fulfil its promise but its resolution and sensitivity should lead ultimately to the detection of new and highly specific marker proteins. If so, the innovator (electrophoresis) will again delegate its success to the practitioner (immunoassay).

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