Recognition and quantification of myocardial injury by means of plasma enzyme and isoenzyme activities after cardiac surgery

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SUMMARY Serial plasma enzyme determinations were carried out in 32 patients who underwent cardiac surgery with the aid of extracorporeal circulation. Plasma creatine kinase (CK), the cardiospecific isoenzyme of CK (CKMB), and alpha-hydroxybutyrate dehydrogenase (HBDH) were determined from the onset of surgery up to 100 to 120 hours after operation. From the plasma enzyme activities, the total amount of enzyme released by the injured heart into the circulation could be calculated using mathematical equations solved numerically by means of a computer. The calculated amount of CK, CKMB, and HBDH released by the heart correlated well with (1) postoperative mortality, and (2) peak activities of the respective enzymes. The calculated amount of any of the 3 enzymes released showed poor or no correlation with (1) electrocardiographic criteria of myocardial infarction, (2) duration of cardiopulmonary bypass, and (3) duration of total aortic cross-clamping.

This study shows that the extent of myocardial injury after surgery can be assessed quantitatively using the calculated amounts of enzyme released, as well as using peak plasma activities of CKMB and HBDH.

Cardiac surgery using extracorporeal circulation may be complicated by the per- and postoperative development of myocardial infarction. The diagnosis of myocardial infarction is generally based on electrocardiographic criteria of which the most specific, though not very sensitive, is the appearance of Q waves (Brewer *et al.*, 1973; Espinoza *et al.*, 1974; Rose *et al.*, 1974). However, in a number of cases the postoperative occurrence of ventricular conduction disturbances and arrhythmias such as bundle-branch block, complete heart block, or ventricular tachycardia hampers the detection of new Q waves.

Rises in the plasma enzymes aspartate aminotransferase (AST, EC 2.6.1.1), creatine kinase (CK, EC 2.7.3.2), and lactate dehydrogenase (LDH, EC 1.1.1.27) are commonly used for the diagnosis of acute myocardial infarction. This method is sensitive but non-specific, as enzymes can leak into the circulation from sources other than

the heart. Examples include SGOT (AST) from the liver in the presence of congestive heart failure, LDH from erythrocytes when there is haemolysis, and CK from skeletal muscle after muscle trauma.

The introduction of isoenzyme determinations has improved the diagnostic value of enzyme tests. The cardiospecific isoenzyme of CK (CKMB) has been used successfully for the detection of acute myocardial infarction in patients in coronary care units (Konttinen and Somer, 1972; Roe *et al.* 1972; Roberts *et al.*, 1975a) and in patients undergoing aortocoronary bypass surgery (Dixon *et al.*, 1973; Oldham *et al.*, 1973; Pyle *et al.*, 1976; Warren *et al.*, 1977). Likewise the 'heart' fraction of LDH (LDH-1) has been proved to be valuable in the detection of acute myocardial infarction post-operatively (Codd *et al.*, 1975; Mohiuddin *et al.*, 1976; Codd *et al.*, 1977).

In the present study CK, CKMB, and alpha-hydroxybutyrate dehydrogenase (HBDH, an accepted method of assessing the sum of LDH-1 and LDH-2 activities) were determined in serial plasma

Table 1 Clinical data of 3 groups of patients: (a) coronary artery bypass surgery, (b) aortic valve replacement, and (c) mitral valve replacement

	Coronary artery bypass surgery	Aortic valve replacement	Mitral valve replacement
No. of patients	15	10	7
Mean age (range) (y)	50.5 (35-62)	46.5 (22-69)	58.0 (46-68)
Sex	12 M, 3 F	8 M, 2 F	2 M, 5 F
Mean no. of grafts (range)	1.9 (1-3)		
Preoperative functional class (NYHA)			
II	1	4	0
III	13	6	5
IV	1	0	2
Cardiopulmonary bypass time (mean $\pm$ SD) (min)	118.0 $\pm$ 50.4	156.4 $\pm$ 66.8	119.6 $\pm$ 37.6
Aortic cross-clamp time (mean $\pm$ SD) (min)	34.3 $\pm$ 15.8	23.2 $\pm$ 13.1	26.1 $\pm$ 17.8
Electrocardiographic evidence of acute myocardial infarction	4	2	0
Mortality (within a week postoperatively)	2	1	0

samples for a period of 5 days in patients treated surgically for coronary artery bypass grafts or valvular replacement. From the activity curves of the plasma enzymes, the total amount per litre plasma (A) of CK, CKMB, and HBDH released by the heart into the circulation postoperatively could be calculated using a method published earlier (Witteveen *et al.*, 1975), which was slightly modified and in this study also applied to CKMB. The present study investigates the relation of the calculated A values with type of operation, postoperative mortality, electrocardiographic criteria of myocardial infarction, peak activity of plasma CK, CKMB, and HBDH, and duration of cardiopulmonary bypass and aortic cross-clamping.

Subjects and methods

PATIENTS

Thirty-two patients are included in this study. Table 1 shows the clinical data. Three groups are distinguished according to the kind of operation: aortocoronary bypass grafting (15 patients), aortic valve replacement (10 patients), and mitral valve replacement (7 patients).

METHODS

All operations were performed with standard cardiopulmonary bypass techniques. Selective coronary perfusion was used in the valvular surgery groups. Mild systemic hypothermia of 28-30°C was used in all cases, together with topical myocardial cooling with iced saline. The pulmonary bypass system was primed with lactated Ringer solution with dextrose (5%) and albumin, lowering the haematocrit from an average of 41.9 (range 50 to 33) to an average of 27.2 (range 34 to 21).

Defibrillation was effected by DC countershock of 50 J when necessary during rewarming. Duration of cardiopulmonary bypass and aortic cross-clamping is shown in Table 1.

A standard 12 lead electrocardiogram was recorded before operation and repeated at least once daily after operation. The only electrocardiographic criterion to assess myocardial infarction was the appearance of Q waves of 0.04 s duration or more (Brewer *et al.*, 1973; Espinoza *et al.*, 1974).

Ventricular conduction disturbances, such as bundle-branch block, total heart block, or severe ST-T segment changes, were considered as symptoms of acute ischaemic injury only (Codd *et al.*, 1975; Hultgren *et al.*, 1977).

ENZYME ASSAYS

Venous blood samples were taken before the first incision, immediately after termination of cardiopulmonary bypass, and subsequently every 6 hours for 100 to 120 hours. After the addition of 0.2 ml 20 per cent trisodium citrate each 10 ml sample was centrifuged at 1174 *g* for 10 minutes to remove blood cells. Thereafter the samples were stored at -20°C until they were assayed for CK, CKMB, and HBDH. CK was assayed with creatine phosphate as a substrate (Oliver, 1955) using a testkit (Baker). The isoenzymes of CK were separated by ion-exchange chromatography on DEAE-Sephadex into the 3 isoenzyme fractions: CKMM, CKMB, and CKBB (Pharmacia) (Mercer and Varat, 1975). HBDH was assayed with alpha-ketobutyrate as a substrate (Rosalki and Wilkinson, 1960) using a testkit (Boehringer). Enzyme activities were expressed in units (U) per litre, in which 1 U of enzyme catalyses the conversion of 1 μ mol substrate per minute at 25°C.

MATHEMATICAL ANALYSIS OF ENZYME ACTIVITY DATA

By following the plasma enzyme levels sequentially one can calculate the amount of enzyme released from the infarcted or damaged area into the circulation. The quantity of enzyme released per litre of plasma during the first 24 hours after operation

is denoted as A. The restriction to 24 hours for the calculated quantity of enzyme released is discussed below. Previously this quantity was indicated by Q (Witteveen *et al.*, 1975), but in order to avoid confusion with the electrocardiographic Q the former Q is replaced by A in the present study. A is no longer expressed in terms of grams of destroyed tissue since the quantity of enzyme released per litre of plasma is a better clinical index (Norris *et al.*, 1975).

The calculation and the mathematical model used for the calculation are described in detail elsewhere (Witteveen *et al.*, 1975). Briefly, the model is composed of two compartments, an intravascular space (volume V_i), and an extravascular space (volume V_e). Enzymes diffuse from one compartment to another with a permeability constant P and are eliminated from the plasma with a certain disappearance constant k. Using these indices, the total amount of enzyme (A) released by the infarcted area into 1 litre of plasma (U/l) can be calculated.

The period of observation was too short to obtain reliable values for the apparent disappearance rate of HBDH in individual cases, so a fixed mean value of 0.012/h was used (Witteveen *et al.*, 1975). In order to preserve comparability of results obtained for different enzymes, the same procedure was used for CK and CKMB, using the mean values of 0.046/h and 0.096/h, respectively, as determined for the total group of patients.

STATISTICAL METHODS

Statistical significance of differences between means was calculated using Student's t test for small samples. Linear regression analysis was employed to detect whether a linear correlation existed between pairs of values of two variables. Where necessary

equations were used which allow for errors in both variables.

Results

Postoperative occurrence of myocardial infarction diagnosed on the basis of the development of Q waves was observed in 4 of 15 patients who received aortocoronary bypass grafts (27%) and in 2 of 17 patients with valvular replacement (12%).

Mortality during surgery and in the postoperative period of 1 week was 2 of 15 patients in the coronary bypass group (13%) and 1 of 17 patients in the valvular replacement group (6%) (Table 1). At necropsy these hearts showed definite evidence of recent myocardial infarction, confirming the electrocardiographic diagnosis made during life.

The Figure shows the A_{CK} , A_{CKMB} , and A_{HBDH} in the 3 groups of patients. The mean A values and the standard errors of the mean are listed in Table 2. The mitral valve replacement group shows significantly lower release of CK ($P < 0.05$) than the aortic valve replacement group.

Linear regression analysis was performed on the values of A_{CKMB} which is known to be cardiospecific,

Table 2 Calculated total enzyme release per litre plasma (A, in U/l) of CK, CKMB, and HBDH in 3 groups of patients

	Coronary artery bypass surgery (n = 15)	Aortic valve replacement (n = 10)	Mitral valve replacement (n = 7)
A_{CK}	2069 $\pm$ 500	3898 $\pm$ 1280	924 $\pm$ 130*
A_{CKMB}	92 $\pm$ 27	214 $\pm$ 93	74 $\pm$ 13
A_{HBDH}	448 $\pm$ 124	921 $\pm$ 302	299 $\pm$ 60

Indicated are mean values $\pm$ SEM.

* $P < 0.05$ compared with aortic valve replacement.

Fig. Calculated total release (A) of CK, CKMB, and HBDH for 3 groups of patients undergoing open-heart surgery: aortocoronary bypass grafting (CB, n = 15), aortic valve replacement (AVR, n = 10), and mitral valve replacement (MVR, n = 7). Indications are given of patients who did not survive the postoperative period of 1 week (o) and of patients who developed electrocardiographic signs of acute myocardial infarction postoperatively (Q waves) (x). Bars represent mean values $\pm$ SEM.

Table 3 Correlation coefficients obtained from linear regression analysis on data of calculated total enzyme release per litre plasma (A) of CK, CKMB, and HBDH in patients after cardiac surgery

	All patients (n=32)	Coronary artery bypass surgery (n=15)	Aortic valve replacement (n=10)	Mitral valve replacement (n=7)
ACKMB vs. ACK	0.846	0.911	0.818	0.736
ACKMB vs. A _{HBDH}	0.840	0.832	0.827	0.867
A _{HBDH} vs. ACK	0.959	0.872	0.989	0.775

together with those of ACK and A_{HBDH}, to investigate whether CK and HBDH released postoperatively also originate from the myocardium. Table 3 shows the correlation coefficients. In the mitral valve replacement group a relatively low correlation coefficient (0.736) is found, partly because of the low A values in this group of patients (see Figure) and the small number of patients (n=7). Nevertheless, the data in Table 3 seem to indicate a close overall correlation between the A values of the 3 enzymes, showing that the greater part of CK and HBDH released into the circulation in the postoperative period originated from the heart muscle tissue. The whole range of A values has been divided arbitrarily into 3 parts in order to interpret A values in terms of myocardial damage (Table 4). The lowest range of A values (ACK<1500 U/l; ACKMB<75 U/l; and A_{HBDH}<400 U/l) represents slight myocardial damage, present in 60 to 73 per cent of the coronary bypass patients, in 30 to 40 per cent of the patients with aortic valve replacement, and in 71 to 100 per cent of the patients with mitral valve replacement, these percentages varying with the enzyme used. Patients in this low range of A values all survived operation and the postoperative

period of 1 week. However, a few patients in the coronary bypass group with low A values had new Q waves postoperatively. Since new Q waves are specific indicators of acute myocardial infarction, infarctions may have occurred, which were small in size as concluded from the low values of enzyme release, and had no harmful effect on the postoperative recovery of the patients.

The intermediate range of A values (ACK: 1500-3000 U/l; ACKMB: 75 to 150 U/l; and A_{HBDH}: 400 to 800 U/l) represents moderate myocardial damage, present in 7 to 20 per cent of the coronary bypass patients, in 20 to 30 per cent of the patients with aortic valve replacement, and in 0 to 29 per cent of the patients with mitral valve replacement, these percentages varying with the enzyme used. Patients in this intermediate range of A values all survived. Only 1 patient in the coronary bypass group with an intermediate ACKMB value showed new Q waves on the electrocardiogram, probably indicating the postoperative development of non-fatal myocardial infarction.

The highest range of A values (ACK>3000 U/l; ACKMB>150 U/l; and A_{HBDH}>800 U/l) represents extensive myocardial damage, present in 13 to 20 per cent of the coronary bypass patients, the percentage varying with the enzyme used, and in 40 per cent of the patients with aortic valve replacement. None of the patients in the mitral valve replacement group had A values in the highest range.

Postoperative death was associated with the high A values: in the coronary bypass group 67 to 100 per cent of the patients with high A values died, the percentage varying with the enzyme used, and in the aortic valve replacement group 25 per cent of the patients with high A values died. The hearts of these patients all showed extensive recent myocardial infarctions at necropsy. Application of the

Table 4 Comparison of 3 ranges of values of ACK, ACKMB, and A_{HBDH} with number of patients, postoperative development of new Q waves in electrocardiogram, and postoperative mortality in 3 surgery groups

	Coronary bypass surgery (n=15)			Aortic valve replacement (n=10)			Mitral valve replacement (n=7)			Extent of myocardial damage based on the value of A
	No. of cases	Q waves	Postop. death	No. of cases	Q waves	Postop. death	No. of cases	Q waves	Postop. death	
ACK (U/l)										
< 1500	9	2	0	4	0	0	7	0	0	Slight
1500-3000	3	0	0	2	0	0	0	0	0	Moderate
> 3000	3	2	2	4	2	1	0	0	0	Extensive
ACKMB (U/l)										
< 75	11	1	0	4	0	0	5	0	0	Slight
75-150	1	1	0	2	0	0	2	0	0	Moderate
> 150	3	2	2	4	2	1	0	0	0	Extensive
A _{HBDH} (U/l)										
< 400	11	2	0	3	0	0	6	0	0	Slight
400-800	2	0	0	3	0	0	1	0	0	Moderate
> 800	2	2	2	4	2	1	0	0	0	Extensive

χ^2 test on the distribution of non-survivors among the 3 ranges of A values showed a highly significant ($P < 0.01$) association of mortality with the highest range of A values of any of the 3 enzymes. Of the patients in the coronary bypass group having high A values, 67 to 100 per cent showed Q waves postoperatively, dependent on the enzyme used. In the mitral valve replacement group this incidence was 50 per cent. Since Q waves can be found through the whole range of A values and mortality was always associated with the highest range of A values, it is believed that A values are more relevant than the presence of Q waves in the electrocardiogram to assess the extent of myocardial damage after open-heart surgery. When the ranges (low, intermediate, and high) in A_{CK} , A_{CKMB} , and A_{HBDH} were compared for each patient, it was found that in 75 per cent of the cases the 3 ranges were identical (for example, A_{CK} : low, A_{CKMB} : low, and A_{HBDH} : low). In 25 per cent of the cases the ranges for 2 enzymes were identical, the range for the third enzyme having shifted to a neighbouring category (for example, A_{CK} : low, A_{CKMB} : low, and A_{HBDH} : intermediate).

Investigations were carried out to see if the maximum plasma level (peak activity) was an equally reliable index of myocardial injury as the calculated A value. Therefore we correlated the peak activities in plasma of CK, CKMB, and HBDH corrected for the respective preoperative activities with A_{CK} , A_{CKMB} and A_{HBDH} , respectively, in the 3 groups of patients. Table 5 shows the correlation coefficients, which show that for diagnostic purposes one may rely on peak plasma enzyme activities corrected for respective preoperative activities in addition to A values. The former are obtained more easily than the latter.

The time lapse between the start of the operation and the moment the enzyme reaches its maximal activity in plasma is referred to as peak arrival time of that enzyme. Table 6 shows the peak arrival

Table 5 Correlation coefficients obtained from linear regression analysis on data of calculated total enzyme release per litre plasma (A) and peak* enzyme activity in plasma corrected for preoperative activity, both expressed in U/l, in 3 groups of patients

	All patients (n = 32)	Coronary bypass surgery (n = 15)	Aortic valve replacement (n = 10)	Mitral valve replacement (n = 7)
CK	0.989	0.986	0.996	0.815
CKMB	0.962	0.974	0.957	0.982
HBDH	0.991	0.993	0.995	0.524

*Highest enzyme activity in plasma in the first 24 hours after onset of surgery.

Table 6 Peak arrival times, expressed in hours, of CK, CKMB, and HBDH in plasma of 3 groups of patients

	Coronary artery bypass surgery (n = 15)	Aortic valve replacement (n = 10)	Mitral valve replacement (n = 7)
CK	30.7 $\pm$ 3.0	29.2 $\pm$ 4.8	20.2 $\pm$ 4.0
CKMB	12.9 $\pm$ 1.4	12.9 $\pm$ 2.0	6.5 $\pm$ 1.1
HBDH	23.2 $\pm$ 3.0	21.1 $\pm$ 2.2	22.8 $\pm$ 6.4

Indicated are mean values $\pm$ SEM.

times of CK, CKMB, and HBDH in the 3 groups of patients. It appears that the peak arrival time of CKMB is significantly shorter in the mitral valve replacement group than in the coronary bypass group ($P < 0.01$) and the aortic valve replacement group ($P < 0.01$). The A values of CK, CKMB, and HBDH did not show any significant correlation with aortic cross-clamp time in either of the 3 groups of patients. The same is true for the cardiopulmonary bypass time, except for A_{CKMB} ($r = 0.94$) and A_{HBDH} ($r = 0.80$) in the mitral valve replacement group.

Discussion

Acute myocardial infarction is a frequent complication of cardiac surgery, especially when the cardiopulmonary bypass procedure is used. Postoperative electrocardiographic evidence of acute myocardial infarction occurs in 6 to 30 per cent of the patients undergoing aorto-coronary bypass grafting (Hultgren *et al.*, 1971; Bolooki *et al.*, 1972; Alderman *et al.*, 1973; Bolooki *et al.*, 1973; Brewer *et al.*, 1973; Oldham *et al.*, 1973; Espinoza *et al.*, 1974; Ghani *et al.*, 1974; Rose *et al.*, 1974; Codd *et al.*, 1975; Mohiuddin *et al.*, 1976; Codd *et al.*, 1977; Hultgren *et al.*, 1977; Klein *et al.*, 1977; Warren *et al.*, 1977). In patients undergoing valvular replacement 0 to 14 per cent developed electrocardiographic abnormalities characteristic of acute myocardial infarction (Ghani *et al.*, 1974; Rose *et al.*, 1974; Hultgren *et al.*, 1977; Klein *et al.*, 1977). Mortality during aortocoronary bypass grafting and in the postoperative period is reported to be 1.1 to 10.7 per cent (Bolooki *et al.*, 1972; Brewer *et al.*, 1973; Dixon *et al.*, 1973; Oldham *et al.*, 1973; Espinoza *et al.*, 1974; Ghani *et al.*, 1974; Rose *et al.*, 1974; Mohiuddin *et al.*, 1976; Codd *et al.*, 1977; Hultgren *et al.*, 1977; Warren *et al.*, 1977). The mortality in a group of patients who were surgically treated for valvular replacement is 3.9 per cent (Ghani *et al.*, 1974).

The development of new Q waves in the electrocardiogram can be considered a specific, but

rather insensitive criterion for acute myocardial infarction (Espinoza *et al.*, 1974; Warren *et al.*, 1977). Other criteria sensitive for myocardial injury, either ischaemia or infarction, that can be used are rises in plasma enzymes (Shirey *et al.*, 1970; Hultgren *et al.*, 1971; Alderman *et al.*, 1973; Bolooki *et al.*, 1973; Ghani *et al.*, 1974; Neutze *et al.*, 1974; Rose *et al.*, 1974; Hultgren *et al.*, 1977). More recently, rises in isoenzymes of CK (CKMB) and LDH (LDH-1) in plasma are used for the diagnosis of myocardial injury (Dixon *et al.*, 1973; Oldham *et al.*, 1973; Codd *et al.*, 1975; Mohiuddin *et al.*, 1976; Pyle *et al.*, 1976; Codd *et al.*, 1977; Warren *et al.*, 1977). Both CKMB and LDH-1 are raised in myocardial infarction, or more generally in myocardial injury. In patients with acute myocardial infarction admitted to the coronary care unit plasma enzymes and isoenzymes have not only provided the clinician with a means to diagnose acute myocardial infarction, but also with a method to assess the size of the infarct (Sobel *et al.*, 1972; Witteveen *et al.*, 1975).

Infarct sizes of patients having acute myocardial infarction in the coronary care unit showed good correlation with prognosis (Sobel *et al.*, 1972; Norris *et al.*, 1975), functional class according to the NYHA criteria (Sobel *et al.*, 1972), the presence of pulmonary venous congestion or pulmonary oedema (Norris *et al.*, 1975), left ventricular haemodynamics (Mathey *et al.*, 1974; Rogers *et al.*, 1977), and incidence of ventricular arrhythmias (Roberts *et al.*, 1975b).

In this study we investigated patients who underwent cardiac surgery with the aid of extracorporeal circulation. The calculated amounts of CK, CKMB, and HBDH released postoperatively by the heart into the circulation showed good correlations with each other (Table 3), with mortality (Table 4), and with peak plasma activities of CK, CKMB, and HBDH, respectively (Table 5).

The relation between postoperative plasma enzyme or isoenzyme activity and duration of cardiopulmonary bypass as well as total aortic cross-clamping time was established earlier in patients receiving aortocoronary bypass grafts and valvular replacement (Oldham *et al.*, 1973; Ghani *et al.*, 1974; Neutze *et al.*, 1974; Codd *et al.*, 1975; Pyle *et al.*, 1976). However, except for the patients undergoing mitral valve surgery, no significant correlation was found between cardiopulmonary bypass time and A values. No correlation was observed between aortic cross-clamping time and A values in either group of patients. The A values for CK in patients of the aortic valve replacement group were significantly higher ($P < 0.05$) than those in patients of the mitral valve replacement group.

Table 7 Peak arrival times, expressed in hours, of CK, CKMB, and HBDH in patients who underwent coronary artery bypass surgery and in patients with acute myocardial infarction admitted to coronary care unit (CCU)

	Surgery	Acute myocardial infarction in CCU	P
CK	30.7 $\pm$ 3.0 (15)	22.9 $\pm$ 1.7 (10) (Witteveen <i>et al.</i> , 1975)	< 0.05
CKMB	12.9 $\pm$ 1.4 (15)	18.0 $\pm$ 0.5 (31) (Fiolet <i>et al.</i> , 1977)	< 0.01
HBDH	23.2 $\pm$ 3.0 (15)	39.1 $\pm$ 2.2 (10) (Witteveen <i>et al.</i> , 1975)	< 0.01

Indicated are mean values $\pm$ SEM; number of patients is indicated in parentheses. P: surgery vs. acute myocardial infarction.

Associated with this difference is a longer cardiopulmonary bypass period in the aortic valve replacement group (156.4 min) compared with that in the mitral valve replacement group (119.6 min), though this difference was not significant. Peak arrival time of CKMB was significantly longer in the patients of the aortic valve replacement group (12.9 hour) than in the patients of the mitral valve replacement group (6.5 hour) ($P < 0.01$) (Table 6). An explanation for these differences between the valvular replacement groups is probably given by the hypertrophied state of the hearts in the aortic valve replacement group. In these hypertrophied hearts, whether concentric or dilated, the selective perfusion of the coronary arteries during the period of cardiopulmonary bypass may be inadequate in perfusing the subendocardial layers of the heart. As cardiopulmonary bypass lasts for an average of 156 minutes, this period may be long enough to promote the development of extensive subendocardial ischaemia, leading to a considerable amount of enzyme released before and directly after disconnection of extracorporeal circulation.

As shown in Table 7, the peak arrival times of CKMB and HBDH are earlier after surgery than after acute myocardial infarction, while for CK the reverse is true. The same phenomenon was apparent from inspection of the release curves, that is the time derivative of the quantity A. While the release of enzyme after acute myocardial infarction usually lasts for at least 48 hours (Witteveen *et al.*, 1975), the release of CKMB and HBDH was completed within 24 hours after operation in the majority of cases, while the release of CK continued for more than 60 hours. This observation strongly suggests a prolonged leakage of CK from damaged skeletal muscle after surgery. The earlier appearance of cardiac enzymes after surgery, compared with acute myocardial infarction, has been described as a washout phenomenon after reflow of ischaemic myocardium (Jarmakani *et al.*, 1976; Maroko and

Vatner, 1977). As a consequence of these findings the calculation of A was restricted to the first 24 hours after operation in order to ensure that myocardial enzyme release was studied. As skeletal muscle contributes late to the CK activity in plasma, the CK release during the first 24 hours will be mainly from the heart; this explains the high correlations of A_{CK} with A_{CKMB} and A_{HBDH} (Table 3).

Of the 3 enzymes used in this study CKMB and HBDH are most appropriate for the calculation of the total amount of enzyme released by the injured heart into the circulation. As the chromatographic separation of CKMB is a time-consuming procedure, especially if many plasma samples have to be assayed, the use of HBDH is preferred to that of CKMB. Only when moderate or severe haemolysis is present in the plasma samples, is CKMB the enzyme of choice for recognition and quantification of myocardial injury after cardiac surgery.

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