Enzyme tests in the evaluation of thrombolysis in acute myocardial infarction

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SUMMARY   The activity of α-hydroxybutyrate dehydrogenase, creatine kinase, creatine kinase MB and aspartate aminotransferase was measured on serial plasma samples from patients with acute myocardial infarction. The study was part of a multicentre randomised trial of the effect of thrombolytic treatment in the acute phase of acute myocardial infarction. The applicability and comparability of enzyme tests for the estimation of myocardial injury were studied in 76 control patients and 74 patients treated with streptokinase. Treatment with streptokinase caused a considerable acceleration of enzyme release after acute myocardial infarction, both in patients with persistent coronary occlusion and in those with successful reperfusion. But this changed pattern of enzyme release did not affect the rate of enzyme elimination from plasma or the released proportions of different enzymes.

Thus the assessment of infarct size by measurement of these enzyme activities can also be applied to patients treated with streptokinase. Moreover, the enzymes measured in the present study are all equally valid markers of myocardial injury.

The beneficial effects of early thrombolytic treatment in patients with acute myocardial infarction have been demonstrated in several large clinical trials.[1-6] Such treatment leads to patency of the infarct related coronary artery, limitation of infarct size, improved left ventricular function, and decreased mortality. These features are not equally reliable indicators of the efficacy of new thrombolytic agents, however. For example recanalisation of the coronary artery is not always associated with limitation of infarct size, improvement of left ventricular function, or decreased mortality.[5] Large clinical trials are needed to demonstrate improvement of left ventricular function or decreased mortality after thrombolytic treatment.[23] Smaller trials produced false negative results[7] as did earlier trials of low-dose intravenous streptokinase.[89] Negative results in some of these studies were the result of long delays before treatment and exclusion of patients with large areas of ischaemia,

who have most to gain from early thrombolytic treatment.[56]

The most sensitive indicator in the evaluation of thrombolytic treatment is infarct size as estimated by the cumulative release of enzymes from the myocardium.[1 5 6 10] The usefulness of cumulative enzyme release as a marker of myocardial damage and the correlation of such estimates with necropsy data were shown in several studies.[11-14] But streptokinase profoundly influences the time course of enzyme release after acute myocardial infarction.[15 16] Such changes may reflect altered rates of enzyme elimination from plasma and this would invalidate comparison between streptokinase and other treatment. Accelerated washout of enzymes from the infarcted area could also influence the exposure of enzymes to denaturing conditions and could thus change the release of enzyme activity in plasma.[17-19] Such effects would also affect the proportions in which labile and stable enzymes are released.

We studied the validity of enzyme tests after thrombolytic treatment. Multienzyme analysis of plasma samples was included in the protocol of a large trial on the effects of streptokinase after acute

myocardial infarction and time-activity curves of several enzymes were determined. These data were analysed by a method that simultaneously estimates the elimination rates from plasma of various enzymes and by calculation of cumulative release of these enzymes in the first few days after acute myocardial infarction.

Patients and methods

The study, carried out under the auspicies of the Netherlands Interuniversity Cardiology Institute, was part of a randomised controlled trial to compare early thrombolytic treatment with conventional treatment in patients with acute myocardial infarction. Full details on patient selection, treatment protocol, and clinical outcome in 269 treated patients and 264 controls have been published.[12] Briefly, the procedures were as follows.

Patients with acute myocardial infarction aged ≤ 70 years were included in the trial if they were admitted to the coronary care unit within four hours of the onset of symptoms. The diagnosis of acute myocardial infarction was based on chest pain lasting > 20 minutes and electrocardiographic signs typical of myocardial infarction (that is ST segment elevation of at least 0·1 mV in a limb lead or 0·2 mV ST segment deviation in one of the precordial leads). Exclusion criteria included previous bypass surgery or treatment with streptokinase, recent bleeding or trauma, and inability to give informed consent. Eligible patients were registered by a telephone answering service which then allocated treatment. Only patients allocated to treatment with streptokinase were asked for informed consent and had angiography during the acute stages of infarction. When coronary anteriography showed that the infarct related artery was occluded or severely narrowed in the group allocated to thrombolysis, intracoronary streptokinase was infused at a rate of 4000 U/min to a maximum of 250 000 units. Patients randomised to treatment after January 1984 were given an intravenous bolus dose of 500 000 units of streptokinase before the intracoronary dose. Patients who had cardioversion were excluded because this procedure caused a considerable release of creatine kinase from injured skeletal muscle.

MEASUREMENT OF ENZYME ACTIVITIES

At all centres the activity of α-hydroxybutyrate dehydrogenase was measured in seven serum samples obtained in the first four days after acute myocardial infarction. In three of the five participating centres analysis of enzyme activity was more elaborate and samples were taken at the following times after admission to the coronary care unit: *University Hospital, Leiden:*—0, 4, 8, 12, 16, 20, 32, 44, 56, 68, 92, and 116 hours; *University Hospital, Maastricht*—0, 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84, and 96 hours; *Free University Hospital, Amsterdam*—0, 6, 12, 24, 36, 48, 72, and 96 hours.

We measured enzyme activity in serum samples kept at 4°C and assayed within 16 hours (Amsterdam) or plasma obtained by immediate centrifugation of blood samples for 15 minutes at 2500 rpm and stored at − 20°C (Leiden and Maastricht).

The activity of α-hydroxybutyrate dehydrogenase was measured at 25°C with α-ketobutyrate as substrate[20] using a commercial test kit (Boehringer, HBDH-Optimiert) in Leiden and Maastricht. In Amsterdam, the activity of lactate dehydrogenase was assayed at 25°C according to the recommendations of the Dutch Association for Clinical Chemistry[21] and converted to α-hydroxybutyrate dehydrogenase activity as described in.[10]

Aspartate aminotransferase was assayed at 25°C with aspartate and α-ketoglutamate as substrates[22] with a test kit (Boehringer GOT-Optimiert) in Leiden and Maastricht and by another assay method in Amsterdam.[21]

Creatine kinase was measured at 25° with creatine phosphate as substrate[23] with test kits (that is Boehringer CPK-Optimiert in Leiden and Merck CPK-NAC in Maastricht) and at 30°C by another assay method in Amsterdam.[21]

Creatine kinase isoenzyme MB was determined by immunochemistry[24] using test kits (Boehringer CPK-MB Monotest) at 30°C in Amsterdam and 25°C in Leiden and Maastricht.

Ezyme activities were expressed in units per litre (U/l). One unit of activity converts one μmol of substrate per minute at the indicated temperature. Systematic differences in enzyme activities introduced by the use of different assay conditions at different centres were eliminated by comparison against standards. The activities of α-hydroxbutyrate dehydrogenase measured in Leiden and Maastricht, for instance, were multiplied by a factor of 1·34. Final mean (SD) activities (U/l) in normal plasma were: α-hydroxybutyrate dehydrogenase 126 (21); creatine kinase 39 (19); aspartate aminotransferase 10 (3); and creatine kinase MB 4·4 (2·3). The enzyme content of cytoplasm from human myocardial biopsy specimens, expressed in units per gram wet weight of tissue (U/g), determined by the same assay conditions was as follows (mean (SD))[25 26]: α-hydroxybutyrate 165 (15); creatine kinase 865 (87); aspartate aminotransferase 54 (6); creatine kinase MB 132 (36). These latter values were used to express cumulative release of enzymes in gram equivalents of myocardium (see below).

CALCULATION OF CUMULATIVE RELEASE OF ENZYMES

Time-activity curves were analysed according to the two-compartment model (fig 1), that has been validated in many studies on the turnover of radiolabelled plasma proteins and circulating tissue enzymes.[27] The plasma activity of enzyme C at time t is determined by input of enzyme from the heart and elimination of enzyme determined by a fractional catabolic rate constant (FCR). In addition, there is extravasation of enzyme (measured by a fractional transcapillary escape rate constant (TER)) and return to plasma from the extravascular pool E(t) (measured by a fractional extravascular return rate constant (ERR)). Cumulative release of enzyme per litre of plasma from zero time up to time t is given by:

$$Q(t) = C(t) + E(t) + \int_0^t FCR.C(\tau)d\tau \qquad (1)$$

where C(t) and E(t) are the activities still present in intravascular and extravascular spaces and the integral term encompasses eliminated activity. The extravascular pool E(t) is determined by the time-dependent plasma activity and TER and ERR[28]:

$$E(t) = TER.\exp(-ERR.t).\int_0^t \exp(ERR.\tau)C(\tau)d\tau \quad (2)$$

Values of C(t) in equations (1) and (2) were obtained by subtraction of the normal activities in plasma from the actual activities measured at time t. Individual values of these normal activities were estimated from the first sample of each patient. Fixed values of TER = 0·014 per hour and ERR = 0·018 per hour were used for all enzymes.[27][28]

ESTIMATION OF FRACTIONAL CATABOLIC RATE CONSTANTS (FCR)

Values of FCR for creatine kinase, creatine kinase-MB, and aspartate aminotransferase were estimated as described elsewhere.[28] The procedure is based on the comparison of the time-activity curve of one enzyme with a simultaneously measured curve of a slowly eliminated reference enzyme. Comparisons were performed over a period of 48 hours after first symptoms (t = 0), using α-hydroxybutyrate dehydrogenase as the reference enzyme. This technique was validated by intravenous infusion of enzymes in the dog[28] and yields the value of FCR as well as the ratio of released quantities of reference enzyme and the test enzyme (ρ_{CK} = released α-hydroxybutyrate dehydrogenase (HBDH) activity/released creatine kinase activity). It should be noted that the values of ρ thus obtained are mainly determined by the initial increases of both time-activity curves. These values may deviate to some extent from the calculated ratios of enzyme release. For instance ρ_{CK}, obtained from the estimation procedure, may differ from Q_{HBDH}/Q_{CK} calculated from equation (1) and (2) for longer intervals.

Results

Table 1 shows the distribution of patients with complete time-activity curves up to at least 48 hours after acute myocardial infarction. Data for some patients were incomplete because of death, early surgery or percutaneous transluminal coronary angioplasty, or refusal of informed consent after allocation to thrombolytic treatment.

Figure 2 shows the plasma curves obtained from the three participating centres. The small number of patients from Leiden explains an increased variability in the right hand panel. Figure 2 shows that results from the three centres were similar and that it was acceptable to pool data. Early release of all enzymes was accelerated in the patients treated with streptokinase. This was further investigated by calculating the time t_h at which cumulative release of enzyme equalled half of the total quantity released ($Q(t_h) = Q(48)/2$). Accelerated release implies an earlier t_h and fig 3 shows that this was indeed found in patients treated with streptokinase. In all three groups treated with streptokinase t_h was significantly different from controls (p < 0·001, Mann-Whitney). It is interesting to find that early release was also increased in patients with unsuccessful thrombolysis. Apparently, the effect is not dependent on early coronary reperfusion.

Estimation of the fractional catabolic rate constants for creatine kinase, creatine kinase-MB, and aspartate aminotransferase is based on a fixed value of 0·015 per hour for α-hydroxybutyrate dehydrogen-
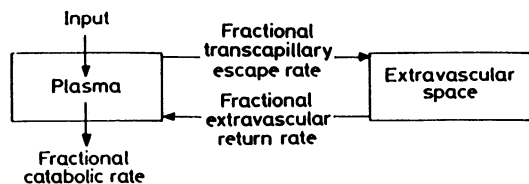

Fig 1 *Two-compartment model for circulating proteins.*

Table 1 *Distribution of patients*

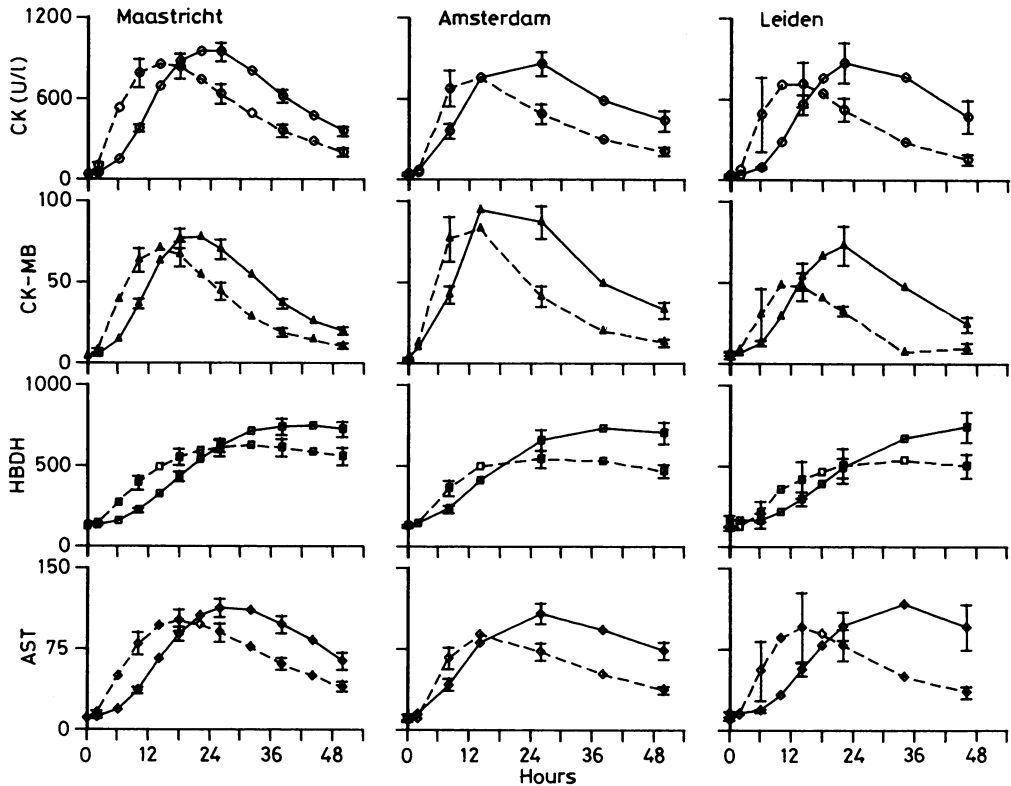| | Maastricht | | Amsterdam | | Leiden | |
	SK	C	SK	C	SK	C
Entered in the trial	61	62	47	46	9	10
Complete enzyme data	47	47	31	37	4	7
Rejected because of cardioversion	3	4	4	10	1	1
Entered in the present study	44	43	27	27	3	6

SK, streptokinase; C, controls.

Fig 2 Mean (SEM) time-activity curves obtained at the three participating centres. Broken lines indicate patients treated with streptokinase and continuous lines indicate the controls. K, creatine kinase; HBDH, α-hydroxybutyrate dehydrogenase; AST, aspartate aminotransferase.

ase as estimated in patients with acute myocardial infarction.[29] In order to check whether this value can
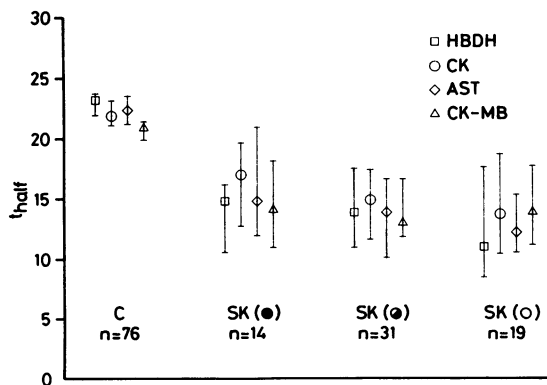

Fig 3 Median values and 95% confidence intervals for the time needed to reach half of the total release in 48 hours. C, controls; SK, streptokinase group. (●) Persisting total occlusion; (◑) reperfusion with more than 90% stenosis remaining; and (○) reperfusion with <90% stenosis remaining. See legend to fig 2 for abbreviations.

also be used in patients treated with streptokinase the apparent fractional disappearance constant (k_d) of α-hydroxybutyrate dehydrogenase from plasma was estimated in both groups between 72 and 96 hours after acute myocardial infarction. Mean (SD) values of $k_d = 0.0097$ (0.0001) per hour and $k_d = 0.0094$ (0.0002) per hour respectively were found in 60 controls and in 54 patients treated with streptokinase. This difference is not statistically significant. This value of k_d is lower than the fractional catabolic rate for α-hydroxybutyrate dehydrogenase because enzyme release tails off and there is extravascular return of the enzymes during the elimination phase.[27] Table 2 shows the values of the fractional catabolic rate and ρ. Patients with a cumulative release equivalent to $\leqslant 300$ U/l of α-hydroxybutyrate dehydrogenase activity in the first 48 hours after acute myocardial infarction (n = 21) were omitted because such small infarctions produce ill-defined curves with unreliable values. Six patients with a creatine kinase activity of $\geqslant 100$ U/l in the first sample were also omitted because infarctions in these patients may have occurred more than four hours

Table 2 *Mean (SEM) results of the estimation procedure with HBDH as the reference enzyme*

	Controls (n = 67)	Thrombolysis (n = 58)
FCR_{CK}	0·19 (0·02)	0·19 (0·02)
FCR_{AST}	0·082 (0·006)	0·090 (0·007)
FCR_{CK-MB}	0·37 (0·07)	0·38 (0·07)
ρ_{CK}	0·19 (0·01)	0·18 (0·01)
ρ_{AST}	2·95 (0·11)	2·76 (0·12)
ρ_{CK-MB}	1·27 (0·17)	1·21 (0·14)

FCR_{HBDH} equals 0·015 per hour.[29] Values of ρ in human myocardium are ρ_{CK} = HBDH/CK = 0·19; ρ_{AST} = HBDH/AST = 3·05 and ρ_{CK-MB} = HBDH/CK-MB = 1·25.

before the first sample was obtained. It follows from the values presented in table 2 that despite the considerably altered time course of enzyme release after streptokinase (see fig 2) both groups had identical values for fractional catabolic rate and ρ. Values of ρ in table 2 are approximately equal to the values calculated from the cytoplasmic enzyme content of myocardium. This implies that estimates of injury from different enzymes will be similar if the released quantities are expressed in gram-equivalents of myocardium. Figure 4 shows the result of this calculation in both groups of patients. The overall reduction of enzyme release after treatment with streptokinase that has been reported for α-hydroxybutyrate dehydrogenase was also apparent for the other enzymes. Although creatine kinase-MB somewhat underestimated and aspartate aminotransferase somewhat overestimated the infarct size calculated from α-hydroxybutyrate dehydrogenase and

creatine kinase, this figure shows that there is a global release of cytoplasmic enzymes that reflects their proportions in the myocardium. More importantly, it also shows that streptokinase does not influence the proportions in which the various enzymes are released.

Release of α-hydroxybutyrate dehydrogenase and creatine kinase-MB was faster in the treated group than in the controls and the reduction of enzyme release by streptokinase only became apparent after 36 hours (fig 5).

Figure 6 shows the relations between estimates of myocardial damage based on different enzymes. The strongest correlations were found between α-hydroxybutyrate dehydrogenase and aspartate aminotransferase (r = 0·95 and r = 0·89 in streptokinase and control groups respectively) but creatine kinase and creatine kinase-MB also correlate well with α-hydroxybutyrate dehydrogenase (r = 0·73–0·89). This implies that estimates of myocardial injury can in principle be based on any of the enzymes studied. The choice of specific marker enzymes may be decided by considerations such as those of convenience, specificity, and accuracy.[26 30-32] Again this situation was not changed by treatment with streptokinase, despite the somewhat steeper slope of the regression line for the treated group in the creatine kinase panel of fig 6. The ratio of α-hydroxybutyrate dehydrogenase to creatine kinase release is identical in both groups (fig 4) and the changed slope in fig 6 is an effect of the non-uniform distribution of data.
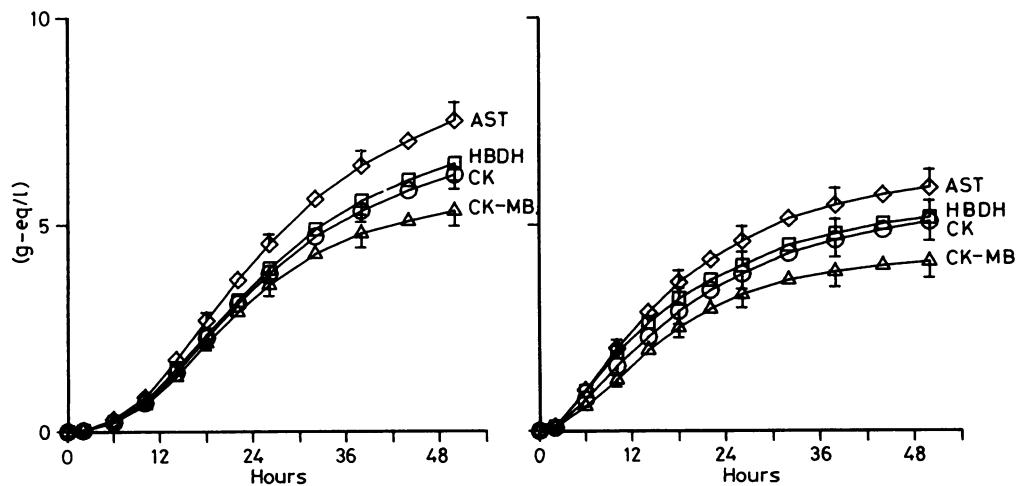

Fig 4 *Mean infarct size assessed by enzyme activity (expressed as gram-equivalents of myocardium per litre of plasma) based on cumulative release of HBDH (□), CK (○), AST (◇), and CK-MB (△) after treatment with streptokinase (right panel) and in controls (left panel). See legend to fig 2 for abbreviations.*
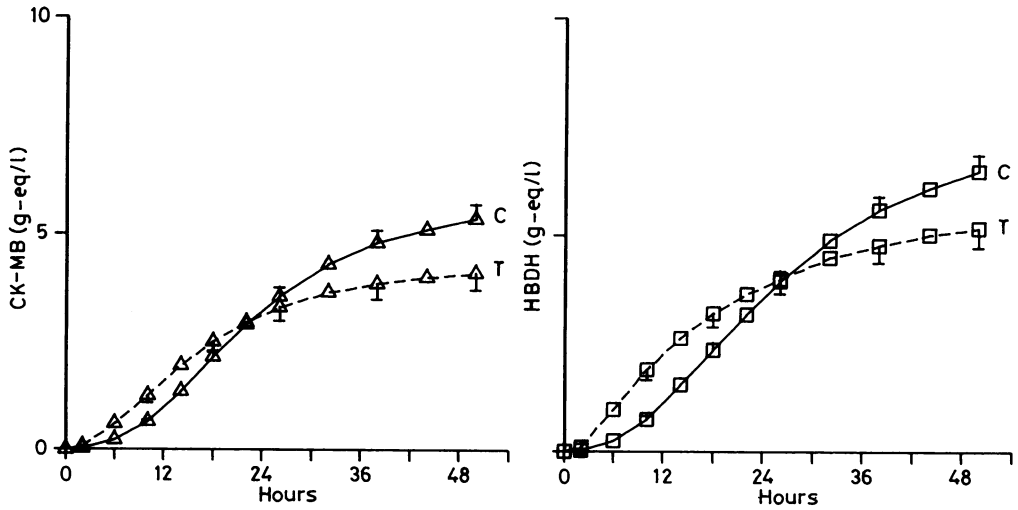
Fig 5 *Mean infarct size assessed by enzyme activity (expressed in gram-equivalents of myocardium per litre of plasma) and based on cumulative release of HBDH and CK-MB. Broken lines indicate patients treated with streptokinase and continuous lines indicate controls. See legend to fig 2 for abbreviations.*
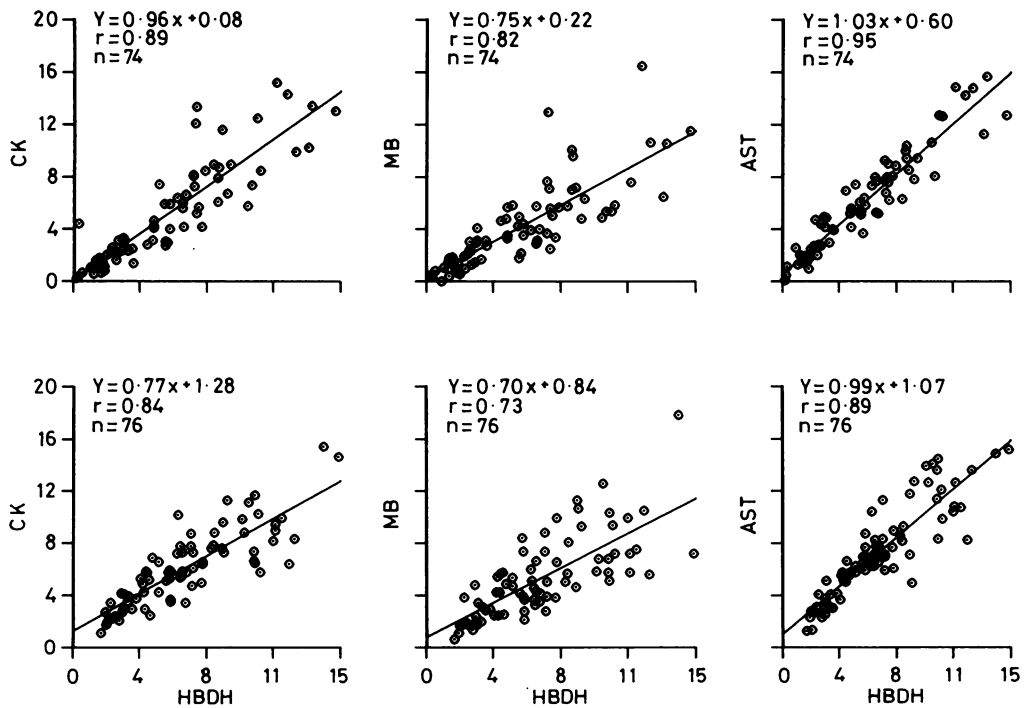

Fig 6 *Correlations between infarct size assessed by enzyme activity (expressed in gram-equivalents of myocardium released per litre of plasma during the first 48 hours) calculated for different enzymes. Upper figures are for patients treated with streptokinase, lower figures are controls. See fig 2 for abbreviations.*

Discussion

Rapid release of aspartate transaminase, shown by early peak activities in plasma, was noted the first time that patients with acute myocardial infarction were treated with streptokinase.[15] Since then the effect has been reported for creatine kinase[16] and lactate dehydrogenase[33] and after the administration, of urokinase.[34] Early investigators ascribed this phenomenon to accelerated wash-out of enzymes from the infarcted area caused by reperfusion. Later on the phenomenon of "reperfusion damage" became apparent: reperfusion of the myocardium after temporary ischaemia may result in a paradoxical exacerbation of cellular damage, associated with an immediate release of intracellular enzymes.[35 36] Accelerated wash-out probably contributes only marginally to this effect because it was also seen after reoxygenation of hypoxic monolayer heart cell cultures.[37] In these preparations typical wash-out effects can be excluded. So reperfusion seems to result in more rapid necrosis, probably of myocardial tissue that has already been irreversibly damaged.

Reperfusion obviously occurs after spontaneous coronary reopening or successful thrombolysis—that is in 85% of the patients treated with streptokinase in the original trial[2]—and this could explain the early release of enzymes in the treatment group (fig 2). In fact, early peaking of creatine kinase activities has even been regarded as proof of successful reperfusion.[38-40] Figure 3, however, shows that such early release also occurs in patients with persistence of coronary occlusion after treatment with streptokinase. One explanation for this observation is that reperfusion occurred in most of these patients in the first few hours after the end of coronary catheterisation. In five of these patients, however, persisting coronary occlusion was demonstrated before discharge from hospital and the median values of t_h for α-hydroxybutyrate dehydrogenase, creatine kinase, creatine kinase-MB, and aspartate aminotransferase were 12·9 h, 17·0, 14·3 h, and 13·3 h respectively—not different from the values in fig 3. These findings suggest that streptokinase may have other effects as well as a thrombolytic action, which are responsible for accelerated enzyme release. A similar conclusion follows from some experimental studies in which streptokinase had a protective effect on the myocardium during non-thrombogenic hypoxia and ischaemia.[41 42] One of these studies showed that streptokinase had a vasodilatory effect on the non-ischaemic isolated rat heart.

Theoretically, early peaking of plasma enzyme activities, as shown in fig 2, could also be the result of an increase in the fractional catabolic rate after treatment with streptokinase. This would be a

serious complication because the reported reduction of enzyme release in the patients treated with streptokinase[2 10] could be an artefact caused by underestimation of the fractional catabolic rate. This is why we analysed the effect of streptokinase on fractional catabolic rate. Estimation of fractional catabolic rate, however, is based on the assumption that myocardial enzymes are released simultaneously. A theoretical analysis showed that a delay of two hours between the release of α-hydroxybutyrate dehydrogenase and creatine kinase would produce a 50% error in values of fractional catabolic rate and ρ.[27] But a delay was not found in studies of experimental hypoxia or ischaemia in heart cell culture, isolated hearts, and hearts in situ: creatine kinase, aspartate aminotransferase, and α-hydroxybutyrate dehydrogenase were released in parallel.[27] It seems that the onset of permeability of the sarcolemma to cytosolic proteins is an all-or-none phenomenon. The validity of the estimation procedure was confirmed when ρ values accorded with myocardial enzyme content (table 2). Increases in enzyme activities in the plasma during the first few hours after acute myocardial infarction, when the role of extravasation and catabolism of enzymes is still minor, were also proportional to myocardial enzyme content.[29] Finally, any delay between the release of, say, creatine kinase and α-hydroxybutyrate dehydrogenase would probably be altered by the more rapid release after treatment with streptokinase and this would produce different values in the streptokinase and control groups; as the results in table 2 show, this did not happen.

In several clinical studies necropsy has shown highly significant correlations between infarct size and the release of creatine kinase (and creatine kinase-MB) in plasma.[11-13] A similar result was obtained for the release of lactate dehydrogenase.[14] Moreover, the reduction in α-hydroxybutyrate dehydrogenase activity in the heart was equivalent to the increase in plasma activity[26] and the results shown in fig 4 confirm this relation for other enzymes as well. Figures 4 and 6 show that after acute myocardial infarction various enzymes are released in fixed proportions that are independent of thrombolytic treatment. This implies that several validation studies[11-14] are relevant to the enzymes we studied. It is important to note that the release in plasma in some of these studies was seriously underestimated because the apparent disappearance constant k_d was used in the calculations instead of the fractional catabolic rate constant.[26]

The two main conclusions of the present study—that (a) infarct size is independent of the enzyme chosen for estimation and (b) thrombolytic treatment does not affect the values of fractional catabolic rate and ρ—imply that any of the enzymes studied is

an equally valid marker of myocardial injury and each can be used for the evaluation of thrombolytic treatment. α-hydroxybutyrate dehydrogenase was chosen as the reference enzyme in the original trial[2 10] because of its slow elimination from plasma—that is, low fractional catabolic rate—which permitted cumulative release to be calculated accurately from a few samples.[31] A potential hazard is the occurrence of bleeding. Infarct size would be considerably overestimated if α-hydroxybutyrate dehydrogenase activity from extravasated erythrocytes entered the plasma. Estimates based on α-hydroxybutyrate dehydrogenase were similar to those based on creatine kinase and aspartate aminotransferase, however, and neither of these enzymes are found in erythrocytes. The strong correlation between α-hydroxybutyrate dehydrogenase and aspartate aminotransferase ($r = 0.95$) in the streptokinase group (fig 6) in which bleeding was frequent, precludes any important release of α-hydroxybutyrate dehydrogenase from erythrocytes.

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