

Clinical Evaluation of Models and Markers of Myocardial Ischemia

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Developments of analytical and experimental techniques within the 70's have resulted in much new knowledge of the pathophysiology of myocardial ischemia. This has initiated clinical research on the nature and treatment of myocardial infarction. But the application in daily clinic is still limited. The aim of this paper is to evaluate the clinical usefulness of important new concepts that are available experimentally.

It is now recognized (22) that ischemic heart disease comprises a spectrum of temporally changing disturbances of cardiac functions. The sequence of metabolic changes within the first hours of the infarct process is illustrated in fig 1.

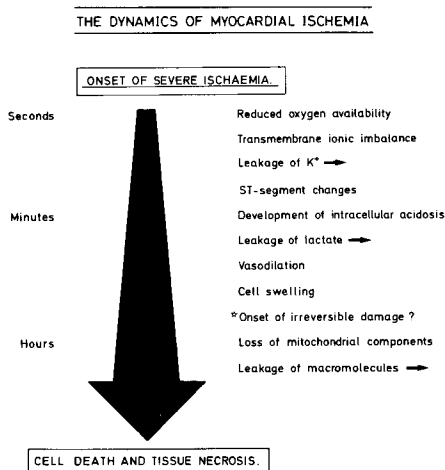


Fig. 1. Changes of cellular properties in myocardial ischemia leading from normal state, reversible and irreversible damage to tissue necrosis.

The changing properties of the myocardial cell membrane are now available for studies by e.g. ECG (23) and measurements of released cellular constituents that serve as markers (10). By these studies it has become clear that myocardial ischemia in man is more variable than known from experimental infarcts in animals. In man two or more types of developments of infarcts must be considered, fig. 2 (28). The typical, rapid developing infarct (type I) reaches final size within 4-8 hours; in type II and III the development is slow and continuous or discontinuous. Type I will be found in about 30-50 % of human infarcts whereas slow developing infarcts constitute the rest. However, the sequence of metabolic changes summarized in fig. 1, will probably be identical independent of the type of infarct. This has important clinical implications because the success of therapeutic interventions will depend on how far the ischemic process has developed.

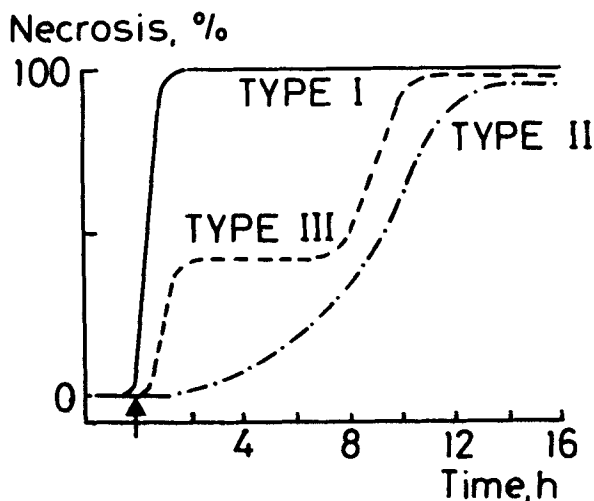


Fig. 2. Schematic presentation of the progression of myocardial damage. The clinical presentations show wide variations, with all kind of modifications between type I and III.

We have therefore considered four aspects of enzymatic models and markers of myocardial ischemia (a) determination of extent of myocardial damage, i.e. the accuracy; (b) the possibility of prediction of extent of damage before full development, i.e. the precision of infarct size at various times after onset (c) the information about ischemic damage in subcellular compartments of the myocardial cell, i.e. the specificity

regarding location; and (d) the detection of minimal changes that may be reversible, i.e. the detectability of the markers and models.

THE CONCEPT OF INFARCT SIZE AND ITS ACCURACY

Direct estimation of infarct size is possible only by histopathological techniques and in those few patients who die from their infarcts.

Standard method for the study and estimation of infarct size by histopathological techniques has only recently become available (9). It has been confirmed that a close relation exists between the results of histopathological and enzymatic determinations of infarct size in man (8). Enzymatic determinations of infarct size are possible in all patients surviving myocardial infarction, and it is therefore of interest to discuss how bias (error) may occur in the various methods for enzymatic estimation of infarct size.

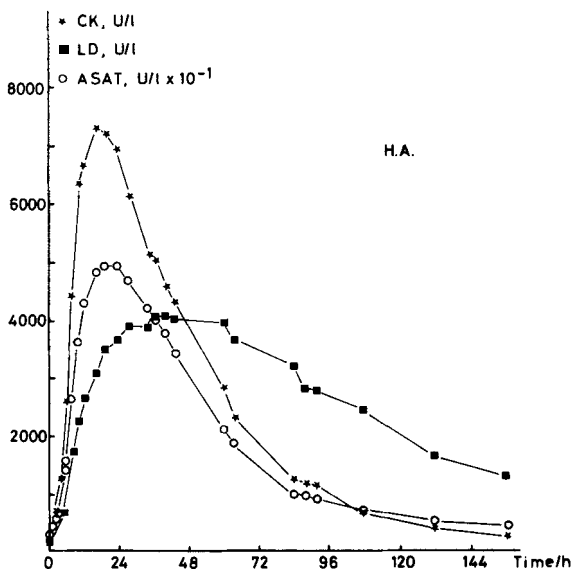


Fig. 3. Simple estimate of infarct size: From peak values of changing enzyme concentrations in the blood with time following acute myocardial infarction.

Table I. Average time intervals (h) from onset of chest pain to peak values in the blood of markers of ischemic, myocardial damage.

Creatine kinase					
Total	23	25	23	22	-
Isoenzyme MB	-	21	21	19	-
Aspartate aminotransferase					
Total	30	28	26	-	-
Isoenzyme M	-	52	-	-	-
Lactate dehydrogenase					
Total	41		41	-	-
Isoenzyme 1					39
Myoglobin	-		10	8	-

References	(7)	(25)	(20)	(27)	(28)
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The most simple method is the maximum value of the plasma curve of enzyme concentrations versus time. In fig. 3 it is demonstrated that the accurate values may be difficult to obtain for those enzymes that appear and disappear very quickly, creatine kinase (CK) and aspartate aminotransferase (ASAT) whereas lactate dehydrogenase (LD) stay at a maximum value plateau for a longer period of time.

However, the plasma curve may also serve as parameter in relatively simple, mathematical functions (e.g. log normal) from which the cumulated amount of enzyme appearing in the plasma compartment may be calculated (12). The only additional informations needed is the disappearance constant of enzyme, k_D ; this can be obtained from the half lifes of the enzymes in plasma. An infarct size is finally obtained through

transformation of the cumulated amount of enzyme in plasma to the amount of enzyme depleted from the infarct (fig. 4). The parameters needed for doing the transformation comprise: E_M^N , concentrations of enzymes in myocardium; E_M^D , the fraction hereof that is released after an infarct; and k_r , the fraction hereof that reaches the blood. On figure 4 are shown acceptable values for these constants for three enzymes.

This kind of quantification rests on two sets of assumptions: firstly that the plasma-enzyme curve empirically, through fitting to an algorithm, may be converted to an amount of enzyme, and secondly that the release, distribution and catabolism of enzyme molecules may be explained by a simple, one-compartment model with few constants.

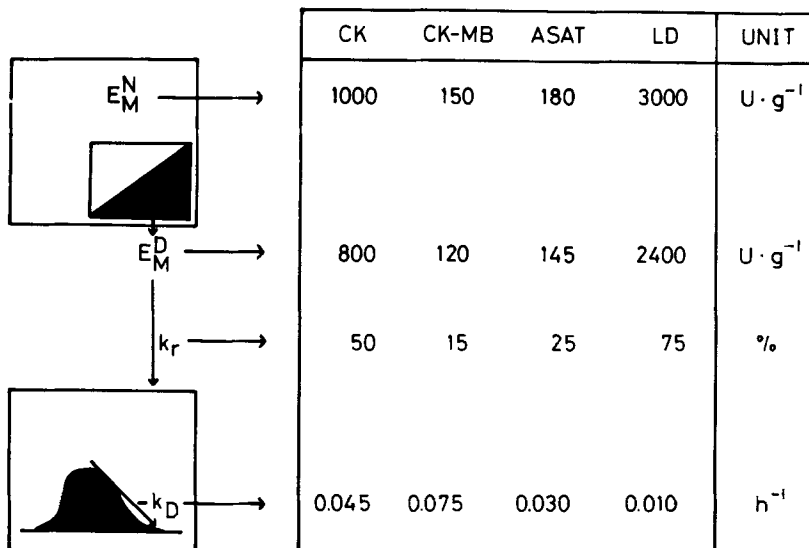


Fig. 4. Estimate of infarct size from a one-compartment model of enzyme turnover and myocardial depletion of enzyme: Parameters of enzyme turnover in acute myocardial infarction. E_M^N is the tissue concentration of enzyme in normal myocardium. E_M^D is the concentration of enzyme that is released from necrotic tissue. k_r is the fraction of released enzyme that reaches the peripheral blood. k_D is the fractional disappearance constant of enzyme from the blood. The values are average values for animal and human tissues.

It soon became clear, that some of these assumptions about enzyme turnover are too simple. Fitting of the plasma curve to a log-normal model is not always possible. Other algorithms have consequently been tried, e.g. gamma - exponential by Danner (5) and modifications of the model of the Shell model (20) by e.g. Tommassini (25). These calculations are not based on assumptions about physiological processes. They are therefore not subject to testing by comparing to observations about physiological phenomenon.

Doubts have also been raised about the validity of the second set of assumptions: a one-compartment distribution space of enzyme and the constancy in release and distribution parameters of enzymes.

Thus Vatner (26) showed that coronary perfusion influences the shape of the plasma curve. Reperfusion leads to a rapid rise in the plasma curve compared to a slow rise at low perfusion of the myocardium. Cairns (1) has shown that the space of distribution of enzymes depend on therapy by e.g. propranolol. The k_D values also change, not due to change in catabolism but to the variation in space of distributions. Clark (4) has shown how the fractional disappearance rates of creatine kinase from blood, plasma and lymph, which is a major way of transport of enzymes from the heart.

An alternative approach to estimation is a conversion of the plasma curve, based on assumptions on turn-over of macromolecules in general (14), figure 5. Enzymes released from the myocardium will be distributed in at least two compartments, extravascular and plasma. The shape of the plasma curve depends on the appearance of enzyme in plasma, AF. This rate of enzyme appearance depends on the values of flux parameters for proteins of similar molecular weights. By varying the values of the constants the appearance function, AF, will vary, figure 6. Thus it is possible to test the assumptions of the model.

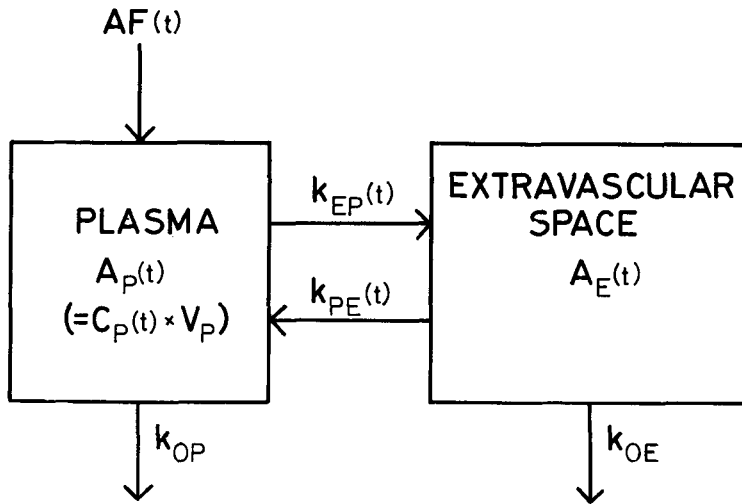


Fig. 5. A two-compartment model for enzyme turnover after myocardial infarction: $A_P(t)$ and $A_E(t)$ are the plasma and extravascular compartments, respectively. AF is the appearance function (rate of appearance of new enzyme). $k_{EP}(t)$, $k_{PE}(t)$, k_{OE} are rate constants for enzyme transfer (14).

The interest in information of the extent of acute myocardial infarct rests on the expectation that infarct size may be a major prognostic determinant. This has been difficult to critically investigate due to lack of simple and reliable methods of estimating infarct size. As already mentioned the accuracy of enzymatic estimates of the infarct size seem to be comparable to that obtained with histopathological methods. Furthermore there is now enough experimental evidence to conclude that peak values of the plasma enzyme curve provides reliable estimates of infarct size when compared to the more complicated models described above (12,19). It should be noted, however, that this may be true only for those cases of myocardial infarcts that follow the type I infarct development as described in fig 2. In type II or III it may be necessary to follow the development of the plasma curve for longer periods than the 36-48 hours that suffice in type I to reach the peak. And more frequent sampling intervals than three per day should be used.

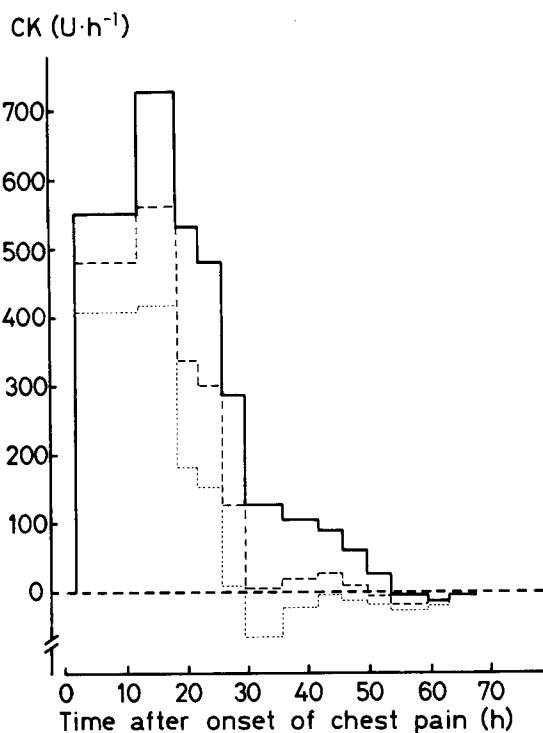


Fig. 6. Estimate of infarct size from a two-compartment model of enzyme turnover:

Appearance functions (AF) of an enzyme, CK, according to the two-compartment model described in fig. 5. The three AF curves are the results of computations using three different sets of values for the rate constants of enzyme catabolism, k_{OP} and k_{OE} . The estimate of the infarct size is the (integrated) area of the AF curve.

The choice of enzymatic marker should take into account the avoidance of bias, i.e. over- or underestimation of infarct size due to contributions of enzyme from non-cardiac sources and loss of cardiac enzymes during turnover of enzyme. The probability of contribution of enzyme from noncardiac sources increases if total enzymes rather than (heart specific) isoenzymes are used; it also increases if the sampling period is extended and in particular in patients with insufficiency of cardiac function and stasis in the lungs and the liver.

On the other hand loss of enzyme during transport of released enzyme may be more pronounced for specific isoenzymes in low concentrations than for enzymes that occur in tissues other than heart.

It is therefore not amazing that publications disagree on the choice of the marker enzyme. Total creatine kinase and its isoenzyme specific for myocardium, MB, gave identical results provided the infarct size is small or medium, whereas total CK seems to overestimate the size of larger infarcts (17). In another publication (2) the value of total LD at the time of peak of total CK was a better predictor of overall risk of the patient than peak value of either CK or CK-MB.

PRECISION OF ENZYMATIC MODELS IN PREDICTING MYOCARDIAL INFARCT SIZE

The effect of treatments in the acute phase of myocardial infarct aiming at reducing infarct size is now the most important issue in lowering the risk of death and complications. Therefore experiments have aimed at assessing the size of the infarct at a very early stage, thus being able to predict its final extent before intervention is started. More clinical studies have now been published on the precision of size estimates at various times before the enzyme curve is fully described. The reliability of the peak value for predicting final infarct size has been discussed already, and has repeatedly been confirmed (12, 19, 7). However, as shown in table I the times of the peak value of the isoenzymes and also of myoglobin (3) do not fulfil the need of reliable prediction within 4-6 hours after onset of the infarct (24). Whether other metabolic markers of ischemia may be useful is not clear. At present the only way of assessing the effects of intervention therefore is the controlled clinical trial in which patients are allocated to two comparable groups of which one serve as the control - non treated - group.

MARKER SPECIFICITY FOR SUBCELLULAR ISCHEMIC DAMAGE

Most of the knowledge described above, has been gained from enzymes that are released from the cytoplasm of the myocardial cells. It has become clear also, that release of cytoplasmic components may occur even if the cellular damage is reversible (11). Occurrence of mitochondrial markers outside the myocardial cell would probably indicate irreversible damage due to the role of the mitochondrion for energy production (27). One isoenzyme of aspartate aminotransferase, m-ASAT, may be used for this purpose and has become available by analytical techniques within the latest five years (15). We have recently studied the occurrence of m-ASAT in blood during acute myocardial infarction (16). A typical case is shown in fig. 7.

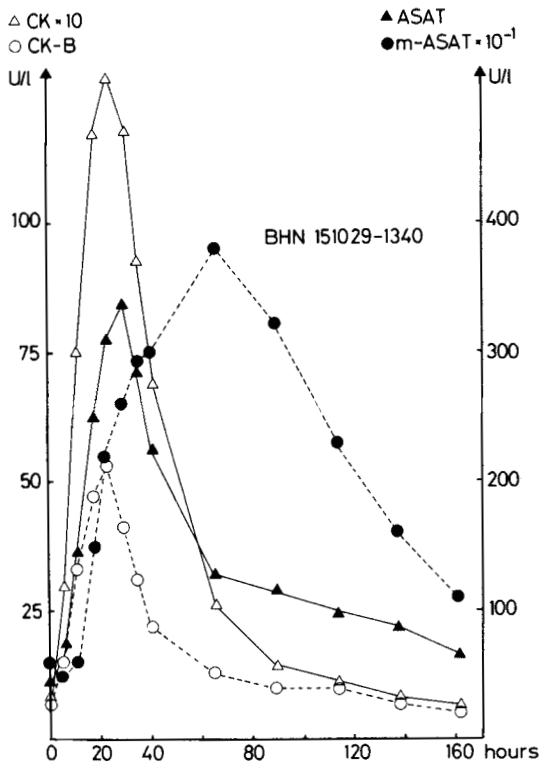


Fig. 7. Time course of changing enzyme concentrations in the blood for three cytoplasmic markers, CK, CK-B and ASAT (the cytoplasmic form) and for one mitochondrial marker enzyme, m-ASAT.

It is apparent that the mitochondrial marker enzyme occurs later and in much less concentration than the three cytoplasmic marker enzymes: CK, CK-B and ASAT. It also disappears at a slower rate than the cytoplasmic markers. We found mitochondrial marker in all 17 patients studied.

The peak values for the three cytoplasmic enzymes correlated closely and do probably indicate the size of the infarct. The peak values of the m-ASAT - the mitochondrial marker enzyme - showed much less agreement with the cytoplasmic markers. This may indicate differences in release and turnover of cytoplasmic and mitochondrial markers, but may also indicate that mitochondrial damage may differ in myocardial infarcts of identical size. The implications of this for treatment and prognosis remains to be seen. The importance of this study is the demonstration of the availability of an experimental method for detecting mitochondrial damage in patients that survive their infarcts.

DETECTION OF MINIMAL MYOCARDIAL DAMAGE

The static conceptuel model of myocardial infarction has been replaced by a knowledge of temporal changes as well as a spectrum of degrees of myocardial ischemia (22). This has led to doubt about one of the criteria for distinguishing angina pectoris from infarct, i.e. that no rise in enzymes will occur in angina pectoris. In view of dynamic nature of ischemia this criterion seems equivocal.

We have investigated this problem (13) and found that enzymes are released also during transient myocardial ischemia (fig. 8). This finding was further evaluated in four groups (fig. 9): 14 patients with no evidence of ischaemic heart disease, 17 with stable angina pectoris, 10 with unstable angina pectoris, and 7 in which typical signs of infarction appeared during the first 24 hours after admission. The within-person standard deviation based on 10-20 determinations of creatine kinase in plasma throughout a period of 48 hours, starting within 6 hours after onset of typical chest pain, was estimated. In each group the within-person standard deviations, when cumulated and plotted on a percentage, probit scale vs the logarithmic values of the standard deviation fitted straight lines.

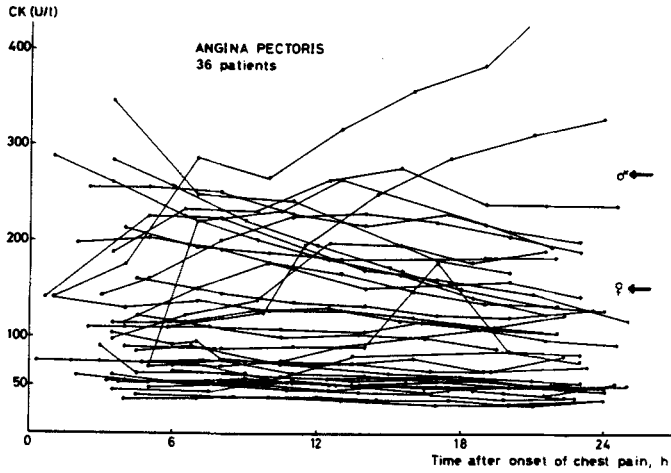


Fig. 8. Variability of enzyme concentrations in patients with clinical symptoms of angina pectoris and minimal myocardial damage. The arrows indicate the upper limits of the reference values interval for healthy females (♀) and males (♂).

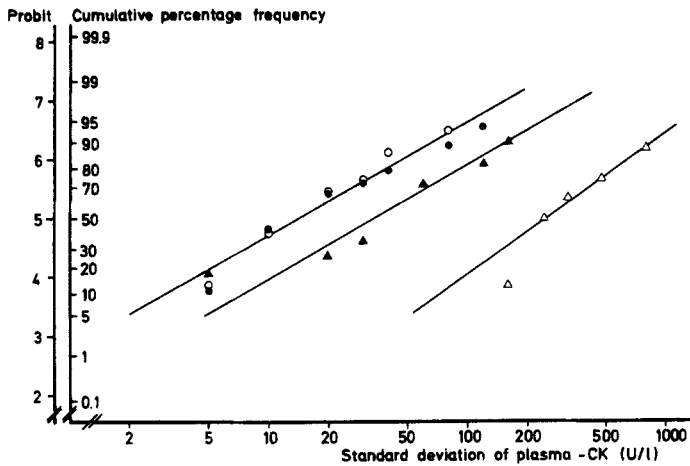


Fig. 9. Cumulative percentage frequency of standard deviations of plasma creatine kinase concentration during the first 48 hours following onset of chest pain. The four groups are: ○ 14 patients without ischemic heart disease; ● 17 patients with stable angina pectoris; △ 10 patients with unstable angina pectoris; ▲ 7 patients with acute myocardial infarction.

Although there was some overlapping of values among the groups, the unstable angina group was clearly discernible from the three other groups. The stable angina pectoris group was indistinguishable from the group without ischaemic heart disease, but separated from the two others.

These results suggest firstly that patients with unstable angina might be identified among other patients admitted with acute chest pain. Secondly that the range of within-person standard deviations in patients with no evidence of ischaemic heart disease and with stable angina pectoris delineates the "noise" level on which enzyme values in patients suspected of having an acute myocardial infarction might be evaluated.

Enzymatic markers are therefore not restricted in their application to myocardial infarct. They are able to reflect - in time and degree - the full spectrum of ischemic damage of myocardial tissue in man.

CONCLUSION

From this review of enzymatic markers of myocardial ischemic damage appears that new important applications are clinically available. Peak values of enzyme plasma curves are reliable prognostic indicators. Amount of released enzyme can be used in randomised clinical trials to assess the effects of therapeutic intervention. Selected cytoplasmic isoenzymes can be used as markers for the integrity of the cell membrane and other for the mitochondrial membrane. Enzymes are used to follow in time and degree the dynamics of myocardial ischemia from minimal, reversible changes to the fully developed myocardial infarct.

REFERENCES

1. Cairns, J.A., Missirlis, E. and Fallen E.L.: Myocardial infarction size from serial CPK: Variability of CPK serum entry ratio with size and model of infarction. *Circulation* 58:1143, 1979.
2. Chapelle, J.P., Albert, A., Smeets, J.P. et al.: Early assessment of risk in patients with acute myocardial infarction. *Eur Heart J* 2:187, 1981.
3. Chapelle, J.P., Albert, A., Smeets, J.P., Boland, J., Heusghem, C. and Kulbertus, H.E.: Serum myoglobin determinations in the assessment of acute myocardial infarction. *Eur Heart J* 3:122, 1982.
4. Clark, G.L., Robinson, A.K., Gnepp, D., Roberts, R. and Sobel, B.E.: Effects of lymphatic transport of enzyme on plasma creatine kinase time activity curves after myocardial infarction in dogs. *Circ Res* 43:162, 1978.
5. Danner, S.A., Smit, E.M., Hart, G.A.M., Oosting, H. and Dunning, A.J.: Prediction on infarct size from serial CK determinations: Evaluation by clinical studies and computer simulation. *Clin Chim Acta* 97:45, 1979.
6. Drexel, H., Dworzak, E., Kirchmair, W., Milz, M.M., Puschendorf, B. and Dienstl, F.: Myoglobinemia in the early phase of acute myocardial infarction. *Am Heart J* 105:642, 1983.
7. Fiolet, J.W.T., Welle, H.F.T., Capelle, F.J.L van. and Lie, K.I.: Infarct size estimation from serial CK MB determinations: Peak activity and predictability. *Br Heart J* 49:373, 1983.
8. Grande, P., Hansen, B.F., Christiansen, C. and Naestoft, J.: Acute myocardial infarct size estimated by serum CK-MB determinations. Clinical accuracy and prognostic relevance utilizing a practical modification of the isoenzyme approach. *Am Heart J* 101:582, 1981.
9. Hansen, B.F.: Heart autopsy in ischemic heart disease, an autopsy protocol. *Acta Pathol Microbiol Scand (Sect A)* 86:241, 1978.

10. Hearse, D.J.: Cellular damage during myocardial ischemia: Metabolic changes leading to enzyme leakage. In: Diagnosis and Research (eds. Hearse, D.J., de Leiris, J.) pp. 1. John Wiley and Sons, Chichester, New York, Brisbane, Toronto, 1979.
11. Hearse, D.J.: Release of enzymes from ischemic myocardium. In: Degradative processes in heart and skeletal muscle (ed. Wildenthal W.) pp. 419. Elsevier, Amsterdam, 1980.
12. Hørder, M., Petersen, P.H., Thygesen, K. and Nielsen, B.L.: Plasma enzymes in myocardial infarction. An appraisal of quantitative, clinical and pathophysiological information. Scand J clin Lab Invest 41:41, 1981.
13. Petersen, P.H., Haghfelt, T., Hørder, M., Krøll, L. and Thygesen, K.: Within-person standard deviations of creatine kinase serving as reference values for grouping of patients with ischaemic heart disease. Proceedings of the III International Meeting on Clinical Laboratory Organization and Management. Graz, Austria, September 6, 1981.
14. Petersen, P.H., Hørder, M., Thygesen, K., Nielsen, B.L. and Hansen, L.: Plasma enzymes in myocardial infarction. Application of a two compartment model in assessing myocardial release of enzymes. Scand J clin Lab Invest 41:735, 1981.
15. Rej, R. and Graffunder, B.: Measurement of aspartate aminotransferase isoenzymes. Six procedures compared. Clin Chem 27:535, 1981.
16. Rej, R., Hørder, M., Petersen, P.H., Thygesen, K. and Christensen, L.: Isoenzymes of aspartate aminotransferase and creatine kinase in acute myocardial infarction. Clin Chem 29: 1233, 1983.
17. Roberts, R., Ambos, H.D. and Sobel, B.E.: Estimation of infarct size with MB rather than total CK. Int J Cardiol 2:479, 1983.
18. Rosleff, F., Angelo-Nielsen, K., Klarholt, E., Thygesen, K., Hørder, M. and Petersen, P.H.: Diagnostic value of lactate dehydrogenase isoenzyme-1 in acute myocardial infarction. Dan Med Bull 30:30, 1983.

19. Ryan, W., Karlinder, J.S., Gilpin, A. et al.: The creatine kinase curve area and peak creatine kinase after acute myocardial infarction: Usefulness and limitations. *Am Heart J* 101:162, 1981.
20. Shell, W.E., Kjekshus, J.K. and Sobel, B.E.: Quantitative assessment of the extent of myocardial infarction in the conscious dog by means of analysis of serial changes in serum creatine phosphokinase activity. *J Clin Invest* 50:2614, 1971.
21. Shell, W.E., Lavelle, J.F., Covell, J.W. and Sobel, B.E.: Early estimation of myocardial damage in conscious dogs and patients with involving acute myocardial infarction. *J Clin Invest* 52:2579, 1973.
22. Swan, H.J.C.: Ischemic heart disease is a dynamic phenomenon. In: *Clinical strategies in ischemic heart disease. New concepts and current controversies* (eds. Corday, E. & Swan, H.J.C) pp. 3. Williams and Wilkins, 1979.
23. Thygesen, K. *Assessing acute myocardial infarction by electrocardiography*. Odense: Odense University Press, 1981 (Disp.).
24. Thygesen, K., Hørdler, M., Petersen, P.H. and Nielsen, B.L.: Limitation of enzymatic models for predicting myocardial infarct size. *Br Heart J* 50:70, 1983.
25. Tommasini, G. and Presta, M.: Prediction of infarct size by enzymatic techniques: Modification of a method and clinical application. *Br Heart J* 42:326, 1979.
26. Vatner S.F., Baig, H., Manders. W.T. and Maroko, P.R.: Effects of coronary artery reperfusion on myocardial infarct size calculated from creatine kinase. *J Clin Invest* 61:1048, 1978.
27. Williamson, JR.: Mitochondrial function in the heart. *Ann Rev Physiol* 41:485, 1979.
28. Yusuf, S., Lopez, R., Maddison, A. and Sleight, P.: Variability of electrocardiographic and enzyme evolution of myocardial infarction in man. *Br Heart J* 45:271, 1981.