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## Serum Levels of the Mitochondrial Isoenzyme of Aspartate Aminotransferase in Myocardial Infarction and Muscular Dystrophy

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*Summary.* The cationic (mitochondrial) isoenzyme of aspartate aminotransferase is a normal constituent of plasma. After myocardial infarction the serum level usually rises, reaching a peak 24 to 48 h later, and returns to normal more slowly than does the total aspartate aminotransferase activity. The serum level was found to be raised in all 6 cases of muscular dystrophy tested.

### *Introduction*

Aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1, glutamic-oxalacetic transaminase, GOT) comprises two isoenzymes. One is anionic and is associated principally with the soluble fraction of the cytoplasm, the other is cationic and is associated with the mitochondria [4]. A sensitive semiquantitative assay has been developed for cationic aspartate aminotransferase in serum or plasma and it has been demonstrated that this isoenzyme is a normal constituent of plasma [5]. This paper is concerned with the changes in plasma level of the cationic isoenzyme occurring after myocardial infarction and in muscular dystrophy.

### *Material and Methods*

The patients included in the first part of this study (cases 13-27, [3]) are those admitted to the Royal Victoria Infirmary, Newcastle upon Tyne, between 24th June and 12th July, 1966, with a diagnosis on admission of acute myocardial infarction. One frail old lady died shortly after admission and no specimens were obtained. In 3 cases there was no abnormality of the ECG, plasma enzymes, WBC, ESR or temperature at any time. These patients were all discharged within 7 days of admission and are not further considered. The medical firms of the hospital rotate their emergency admission liability day by day.

One firm has a strong predilection towards cardiology and most of the cases admitted otherwise than as emergencies came into this unit. No form of selection was practised.

Because of the existing evidence [8, 9, 16], that the appearance of cationic isoenzyme in the blood following infarction was likely to be a transient early phenomenon, it was planned to take 3 specimens during the course of the first 24 h after admission and thereafter daily specimens for one week and then twice weekly specimens until discharge. Only 8 out of 15 cases were admitted within 24 h of the onset of the disease, and for various other reasons it was not always possible to carry through this programme in its entirety.

Several cases require comment because of unusual clinical features. Case 1 had right hemiplegia and aphasia following an unsuccessful carotid thrombarterectomy 5 years previously; he had had attacks of myocardial infarction 3 months and 10 months before the present admission and he died of bronchopneumonia 2 months after discharge. Case 2 had hypercholesterolaemia under treatment with "Atromid": he was free of pain and subjectively recovered before his plasma enzyme levels began to rise. Case 3 was treated with streptokinase intravenously for 4 days: on the 8th day he suffered an embolism of the left vertebral artery, but recovered completely. Case 4 called in his practitioner because of severe back pain, he was given 30 mg of morphine and was admitted to hospital because he had been unconscious for 24 hours after this. On arrival he "woke up" after the administration of 5 mg of nalorphine, vomited a large quantity of altered blood and complained of chest pain. There was a leucocytosis, hypotension, bradycardia and marked but transient E.C.G. changes. He had oliguria, the blood urea reached a peak of 248 mg/100 ml on the 5th day, declining thereafter as his urine output increased. The lactate dehydrogenase isoenzyme pattern suggested that the liver rather than the heart was the source of the abnormal plasma activity (courtesy of Dr. *A. W. Skillen*). It may be thought justifiable to ignore this case in evaluating the plasma cationic isoenzyme response to myocardial infarction, but the assay results are given in figure 1 for completeness sake.

Case 12 had had anorexia and dysphagia for 3 months: for 36 h before admission she had had a nagging retrosternal pain and twice vomited blood during this period; faecal occult blood tests were positive. The ECG tracings clearly indicated myocardial infarction. On the 7th day she was noted to be in congestive cardiac failure and

digoxin was given by injection, but she died on the 8th day. Autopsy was not carried out. Case 14 had a pericardial friction rub throughout his admission: he died suddenly 10 days after discharge.

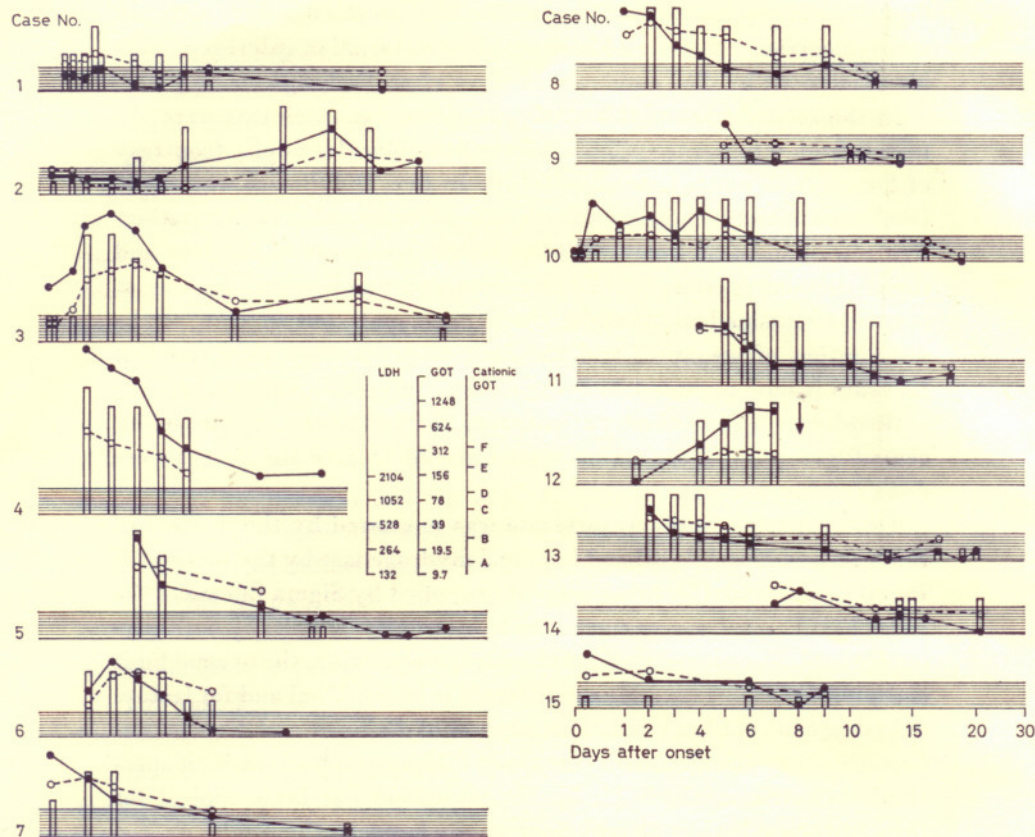
In the second part of this study, single serum specimens were obtained from 6 patients suffering from muscular dystrophy (courtesy of Dr. J. N. Walton. Cases 31-36 [3]). In five, the disease was of the Duchenne variety, these were boys of 5-14 years old. The last patient was a woman of 24 years old with severe weakness of the legs and slight pseudohypertrophy of the calves and quadriceps. She was at one time classified as "female, Duchenne-type", but in view of the distribution of weakness and the lack of progression over the last 12 years is now regarded as being of the limb-girdle variety.

Blood drawn from these patients was allowed to clot, separated by centrifugation, and the serum stored at  $-20^{\circ}\text{C}$  until the assays were done (within 4 days).

Total aspartate aminotransferase was measured by the method of *Reitman and Frankel* [18] and lactate dehydrogenase by the method of *Berger and Broida* [1], using reagents supplied by Sigma Chemical Co. In each case, results were converted to international units by multiplying by 0.48. For purposes of graphical representation, the normal limit accepted for aspartate aminotransferase is 19.5 mU/ml and for lactate dehydrogenase 264 mU/ml. The method of *Reitman and Frankel* underestimates cationic isoenzyme three fold by comparison with spectrophotometric methods [2, 3]. No correction has been applied for this, and there is thus no real discrepancy between the results quoted for total and cationic aspartate aminotransferase activities in cases 18 and 21. Cationic aspartate aminotransferase was estimated by the method of *Boyde* [5], and is reported as grades A to F, equivalent approximately to 3, 5, 7, 11, 19 and 35 mU/ml respectively [5]. Grade A is normal. For purposes of graphical representation, the normal limit is set between grades A and B. Creatine phosphokinase was estimated by the method of *Hughes* [11], and the results expressed in terms of international units by multiplying by the factor 16.7. The normal range is then 17-50 mU/ml.

### Results

The results of the study on myocardial infarction are set out in figure 1. Of the 15 patients studied, in only 2 was there no rise of cationic isoenzyme, and one of these was not admitted until 5 days after the onset. Of the remaining 13 cases, specimens were taken during the first



*Fig. 1.* Serum levels of lactate dehydrogenase and total and cationic aspartate aminotransferase in patients admitted with acute myocardial infarction. The enzyme activity scales are logarithmic and are so adjusted that for each the conventional normal limit falls at the same point on the graph (see Key). Symbols: —●—●— total aspartate aminotransferase; —○—○— lactate dehydrogenase; □ cationic aspartate aminotransferase (this symbol was chosen to make clear the semiquantitative nature of the assay, and the discontinuous mode of expressing the results). In measuring cationic isoenzyme activities it was occasionally felt to be impossible to choose whether to allot a sample to a particular grade or to the next higher grade. In these instances an intermediate grading was adopted (e.g. in case 3, samples 1, 2, 3 and 8 were graded A-B).

24 h in 5 instances; in none of these was the peak level of cationic isoenzyme reached until at least 24 h after onset. The cationic isoenzyme level was slower to return to normal than the total aspartate aminotransferase level. In 8 cases there was at least one occasion when the cat-

ionic isoenzyme level was raised in the presence of a normal total aspartate aminotransferase, and in 6 of these 8 cases, but no others, the cationic isoenzyme level was raised in the presence of a normal lactate dehydrogenase level on at least one occasion. The mean interval between first and last recorded abnormal specimens was 6.7 days. If cases 2, 3, 4, 7, 12 and 14 are eliminated as being in various ways non-representative, or not adequately studied, the mean interval becomes 6.2 days, and this can only be an underestimate of the duration of the elevation of the plasma level.

It seems reasonable to conclude that a raised plasma level of cationic aspartate aminotransferase is a more sensitive and persistent indication of myocardial damage than a rise in the total plasma aspartate aminotransferase activity, and that it is probably more sensitive if not more persistent than the lactate dehydrogenase.

The results of the study on muscular dystrophy are set out in Table I. In every case there was a marked elevation of both creatine phosphokinase and cationic aspartate aminotransferase, although the total aspartate aminotransferase, as estimated by the Reitman-Frankel procedure, was normal in two specimens.

TABLE I  
Serum enzyme activities in cases of muscular dystrophy

Case No.	16	17	18	19	20	21
Total aspartate aminotransferase	57	37	11	37	71	5.5
Cationic aspartate aminotransferase	E	E	E	E	E	D
Creatine phosphokinase	3850	3520	4550	2680	5910	209

Case 16-20 were of Duchenne type in young boys. Case 21 was of limb-girdle type (see text).

Total aspartate aminotransferase and creatine phosphokinase activities are expressed in terms of mU/ml. Cationic aspartate aminotransferase activity is expressed as grades A (normal) to F (see text).

### Discussion

*Fleisher, Potter and Wakim* [7] first reported detecting cationic aspartate aminotransferase in the serum following myocardial infarction. *Boyde and Latner* [6], and, more recently, *Zázvorka and Kamarýt* [19], likewise found this isoenzyme in the serum in isolated cases. *Massarat and Lang* [16] describe the results of a more complete study. They used a differential assay based upon immune inhibition of the

isoenzymes by specific antisera (the isoenzymes are immunologically distinct) and applied this to 10 patients. In 4, serial specimens were obtained. In every case the level found during the first 24 h after onset was higher than that found in later specimens. In the remaining 6 cases, a single specimen only was available, taken between 2 and 4 days after the onset of the disease: in none of these was it possible to identify the cationic isoenzyme with certainty. *Fleisher and Wakim* had found, in dogs, that the cationic isoenzyme was cleared from the plasma with extraordinary rapidity [8, 9]. *Massarat and Lang* used these results to explain the findings in their subjects. They suggested that the presence of cationic isoenzyme in plasma is a transient phenomenon characteristic of the "early phase" following myocardial infarction.

Actually, as is plain from the results given above, and from earlier work [5], cationic enzyme does not appear in the plasma only as a transitory phenomenon in disease, but is a normal constituent, and the plasma level is raised for many days after a myocardial infarction. The time-scale of the plasma response resembles that of lactate dehydrogenase rather than that of creatine phosphokinase (as suggested by *Massarat and Lang* [16]) or even that of anionic aspartate aminotransferase.

This conclusion does not imply any unavoidable conflict with *Fleisher and Wakim* [8, 9]. If it is assumed that human cationic isoenzyme, like canine, is rapidly cleared from the circulation, the findings reported here can then be regarded firstly as evidence for a normal rapid turnover of this isoenzyme through the circulation (as envisaged also by *Fleisher and Wakim* [10]) and secondly as evidence for gradual release of the isoenzyme from damaged heart muscle. This seems rather more likely than the alternative – a single massive outpouring shortly after the infarction. A gradual release could result from the slow diffusion of enzyme from an area of ischaemic necrosis, or, perhaps, from tissue damaged by anoxia but still perfused with blood. There does not seem to be any way yet of deciding between these pathological mechanisms. Perhaps each operates in certain cases, and it is even possible that both may operate in the one individual. *Massarat and Lang* [16] suggest that the appearance of cationic isoenzyme in plasma might be an indication that actual necrosis had occurred. This may be correct, but there is no strong experimental foundation for the hypothesis that mitochondrial enzyme can only be released from dead, or even only from severely damaged cells. Besides,

one must consider the possible influence of interference with the clearance mechanism. It seems likely that myocardial infarction could result in damage or overloading of the reticuloendothelial system and thus to raised cationic isoenzyme levels even if the rate of release was normal or only slightly increased [10].

*Kar and Pearson* [12] were unable to detect cationic isoenzyme in the serum of cases of muscular dystrophy. By a similar technique, *Mannucci et al.* [13] found a trace of cationic activity in 1 out of 2 cases of Duchenne-type muscular dystrophy. The finding (above) that the cationic isoenzyme level was very markedly increased in all 6 cases of muscular dystrophy, serves to emphasise the greater sensitivity of the present procedure. Although single random samples were used, it is most unlikely that the levels found were in every case non-representative. It is thus probable that these high serum levels are maintained for long periods during the course of the disease, and this must be attributed to abnormal release from the mitochondria, or abnormal clearance from the plasma, or both. This is another example indicating that high serum levels may occur without acute lethal damage to the cells.

There is now substantial evidence that the "isoenzymes" of aspartate aminotransferase are themselves heterogeneous, but it seems unlikely that this will seriously disturb the differential assay method used for this work, since the subforms appear to be closely comparable in charge and in kinetic properties [14, 15, 17].

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