Serum enzyme levels in the diagnosis of ischaemic heart disease

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In 1954 LaDue, Wróblewski, and Karmen demonstrated the increase that occurs in the level of serum transaminase following acute myocardial infarction. In the last nine years their observations have repeatedly been confirmed and the estimation of transaminase levels is now a routine procedure whenever myocardial infarction is suspected. Dixon and Webb (1958) list 12 transaminases occurring in animal tissue. Of these, it is the group concerned with the transfer of the amino group from glutamate to oxaloacetate with the formation of aspartate with which we are principally concerned in the study of ischaemic heart disease. The related enzyme involved in the conversion of pyruvate to alanine is the other transaminase of which serum levels are most frequently estimated in the routine laboratory in relation to acute hepatocellular and pancreatic damage. Both enzymes are widely distributed through the cells of the body. The rationality of their use in the investigation of specific organs depends on their uneven distribution. Table I, modified from Wróblewski and LaDue (1956), gives comparative values in units per gram wet weight for some of the principal organs of the body.

TABLE I		
COMPARATIVE VALUES FOR ENZYMES FOR CERTAIN ORGANS OF THE BODY		
Tissue	Glutamate Aspartate Transaminase (GOT) (units/g. tissue)	Glutamate Alanine Transaminase (GPT) (units/g. tissue)
Myocardium	155,000	7,000
Skeletal muscle	100,000	5,000
Liver	140,000	45,000
Pancreas	30,000	3,000
Lung	10,000	750
Serum	20	15

Both enzymes require phosphorpyridoxyl as an activator. Transaminase activity will therefore tend to be reduced in conditions in which there is a deficiency of phosphorpyridoxyl. Jenkins, Orlowski, and Sizer (1959) have recently produced evidence to suggest that isoniazid derivatives may inhibit the enzymic activity of the pyridoxyl transaminase com-

plex. The part pyridoxyl plays is not therefore a matter of pure academic interest.

Inspection of Table I suggests that damage to heart muscle is likely to be associated with release of glutamate aspartate transaminase (GOT) into the blood stream. Though there may well be an associated increase in the serum level of glutamate alanine transaminase (GPT) this will not approach the level associated with enzyme release following liver damage. The concentration of both enzymes in pulmonary tissue is much lower than in either the myocardium or the liver and therefore weight for weight serum levels will be less disturbed in uncomplicated pulmonary infarction than in myocardial or hepatic damage.

Serial studies of patients with uncomplicated coronary occlusions indicate that significant changes in transaminase levels do not occur before the sixth to twelfth hour after the initial myocardial insult. Thereafter the serum level rises rapidly, reaching a maximum between 12 and 36 hours later. If no extension of the lesion occurs the level falls again,

FIG. 1. Serial studies of glutamate aspartate transaminase (GOT) levels and α -hydroxybutyric dehydrogenase levels in the first eight days following uncomplicated myocardial infarction. The glutamate units ● ● are shown on the left. The dehydrogenase units ○ ○ are shown on the right.

reaching normal between the third and fifth days. Figure 1 shows a serial study on a man of 55 suffering his first myocardial infarct. The black dots indicate the transaminase levels which have reverted to normal in four days. The open circles represent the levels of α -hydroxybutyric dehydrogenase which are still raised at the end of eight days. The peak values are very roughly proportional to the extent of cardiac damage. If the peak level exceeds 500 units patients rarely survive, and values over 300 units must be regarded as carrying a grave prognosis.

It is rare in routine experience to get such a clean-cut picture. Because of the relatively sharp rise and fall clinicians often fail to obtain a sample at the optimum time in relation to the initial infarction and it might be wise, as a routine procedure, to take three consecutive blood samples from any patient suspected of having suffered acute myocardial damage at 12 hours, 24 hours, and 36 hours after the first incident suggestive of an attack. The serum from these samples should be stored in the deep freeze, the second being analysed immediately and the first and third kept for reference.

Spuriously low levels may be the result of delays in collecting samples and their analysis. If the specimen is not to be analysed at once the serum should be separated and stored in the deep freeze without delay. The possible inhibitory effects of certain drugs have been mentioned already. Perhaps even more important than spuriously low levels are spuriously high results. By this is meant levels arising from lesions other than myocardial infarctions.

NORMAL FLUCTUATIONS OF GOT LEVELS IN SERUM

Infants, though ischaemic heart disease is hardly likely to concern them, tend to have higher levels than their mothers.

The mean level for GOT in adult serum lies between 20 and 25 units though the normal range given is up to 40 units. It is evident, therefore, that while little attention should be paid to an isolated value of 40 units two consecutive daily analyses showing a rise from 25 to 40 units should make the pathologist ponder. Gross cardiac arrhythmias with transient anoxias may produce such modest elevations, as will gross anoxia from other causes, such as violent intestinal haemorrhage, though in such instances the diagnosis is rarely in doubt.

DAMAGE TO ORGANS OTHER THAN THE HEART PRODUCING RAISED GOT LEVELS IN SERUM

The three worth considering are the liver, the pancreas, and the lungs.

THE LIVER Infective hepatitis in its early stages is often accompanied by a rise in the serum GOT level but at the same time there will be an associated and even greater rise in the serum GPT level. The elevated levels tend to persist and neither the rise nor the fall is as rapid as seen in uncomplicated myocardial damage.

Liver damage may be the consequence of hepatic anoxia from cardiac decompensation but here again, though at a more modest level, the rise and fall of enzyme levels is slower than in uncomplicated myocardial damage.

Iatrogenic liver damage is increasingly common and is frequently associated with fluctuations in transaminase levels. The so-called 'tranquillizers' are most commonly at fault but occasionally the dicoumarol derivatives used as anticoagulants may have a toxic effect on the liver. Though the incidence is less than 1 in 30,000 the confusion arising from neglecting this possibility is so important that it must be mentioned.

THE PANCREAS Acute attacks of pancreatitis may be confused in the early stages with acute myocardial insults. In both the level of serum GOT may be significantly raised but in acute pancreatitis the serum GPT and the blood amylase levels will be raised.

LUNG EMBOLI In seven patients having pulmonary emboli the highest recorded transaminase level was 50 units even though large areas of pulmonary infarction were demonstrable.

It is evident that pulmonary infarction *per se* is not associated with gross elevations of serum transaminase levels. One of the most valuable applications of transaminase assay is to identify a second myocardial incident following within a few weeks of the first when E.C.G. changes are of little help, and it is at this time that it may be important to decide whether or not a demonstrable rise in the serum GOT level was the consequence of associated pulmonary infarction or of a fresh myocardial infarction.

THE LACTIC DEHYDROGENASES

The lactic dehydrogenases are a group of enzymes distributed widely throughout the animal tissue. They convert lactic acid to pyruvic acid in the presence of N.A.D. (nicotinamide-adenine-dinucleotide) which acts as a hydrogen acceptor. These enzymes contain zinc.

CARDIAC ISCHAEMIA In cardiac ischaemia there is a transient rise in the level of serum lactic dehydrogenase which persists for seven to 12 days. Thus it may be that a week after the primary attack the

lactic dehydrogenase levels may still be raised when the serum GOT level has returned to normal. There is a very rough correlation between the level of serum lactic dehydrogenase with the severity of the initial attack as there is with the level of serum GOT. Thus levels of over 3,000 units must be regarded as indicative of a poor prognosis. There are, however, so many complicating factors that it is only rarely in practice, and that in extreme cases, that the correlation is of practical value.

The wide distribution of lactic dehydrogenase in the tissues of the body, its raised levels in a wide variety of clinical conditions, and the fact that it is known to be a member of a large family of enzymes whose business is in catalyzing oxidation reduction reactions, has created some caution in the minds of thoughtful pathologists on the abiding value of the measurements of lactic dehydrogenase levels in the diagnosis of myocardial damage.

THE ISOENZYMES The demonstration in starch and agar electrophoresis that there were at least three dehydrogenases of recognizable different mobility (Vesell and Bearn, 1958) caused no surprise, for it was already known that there were at least two serum pseudocholinesterases circulating in the serum and separable by electrophoresis (Kekwick 1955). It was, however, gratifying when it was demonstrated (Plagemann, Gregory, and Wróblewski, 1960) that individ-

FIG. 2. The lactic dehydrogenase isoenzymes isolated by agar electrophoresis from serum, myocardium, and liver tissue.

Migration is from left to right. The enzymes from heart muscle travel with the α_1 globulins. The enzymes from the liver travel with the γ globulins.

ual tissues showed a quite distinct pattern. In normal serum five principal bands of enzyme activity are usually seen, the principal enzyme migrating in the region of the α_2 globulin. In heart muscle extracts, the dominant band is the fastest moving enzyme migrating somewhat ahead of the α_1 globulin, while in hepatic tissue extracts it is the enzymes migrating in the region of the γ globulin that predominate (Fig. 2). The slower moving isoenzymes, those increased in liver damage, are more sensitive to temperature, losing activity at 55° to 60°C., than the faster moving enzymes. It does seem that these differential isoenzyme studies might help materially in diagnostic problems though the techniques are somewhat intricate and are unlikely ever to become a routine procedure.

α - HYDROXYBUTYRIC DEHYDROGENASE Recently Wilkinson and his collaborators (Elliott and Wilkinson, 1961), in a study of the dehydrogenases, have investigated the α -hydroxybutyric acid dehydrogenase, a first cousin to lactic dehydrogenase in somewhat the same way that butyryl cholinesterase is related to acetyl cholinesterase. Initially the Wilkinson group measured the ratio of serum lactic dehydrogenase to serum α -hydroxybutyric dehydrogenase and showed that, whereas in normal persons the ratio never fell below 1.18, in 16 out of 19 patients with myocardial infarction the ratio was below 1.18. In liver damage the ratio of serum lactic to α -hydroxybutyric dehydrogenase is raised and is usually above 2. What this means, in simple terms, is that by comparison with serum lactic dehydrogenase, the level of α -hydroxybutyric dehydrogenase is much more markedly raised in myocardial ischaemia, being relatively little disturbed in hepatic disease excepting in infective hepatitis and hepatic neoplasm, when serum GPT levels are noticeably raised. The level of serum α -hydroxybutyric dehydrogenase is not raised in pulmonary infarction. Increasing experience with the measurement of serum α -hydroxybutyric dehydrogenase levels suggests that its elevation in myocardial damage is so consistent that it may supplant the measurement of lactic dehydrogenase levels when help is needed in the diagnosis five days or more after the initial event. The normal ranges of this enzyme are 150-300 spectrophotometric units and rarely exceed 500 units whereas with cardiac ischaemia values from 300 up to 2,000 units are commonly met. Prolonged digitalization can result in slightly raised levels of serum α -hydroxybutyric dehydrogenase and this possibility must be borne in mind in doubtful cases. Perhaps one of the most interesting of Wilkinson's observations was that in 67 patients with angina of effort the levels in 58 were normal but in nine showed a marginal increase.

STRUCTURE AND RELATIONSHIPS OF THE ISOENZYMES

Implicit in the estimation of serum enzyme levels as an index of tissue damage is that the enhanced activity in the serum is the consequence of release of the enzymes from the damaged tissue in sufficient amounts to be measurable.

Serum levels must reflect the stability of the enzyme released from the cell. Further, it must be assumed that if acting levels are to be meaningful no specific inhibitors are present in the circulation at the time of release. The capacity of the body to excrete the released enzyme is an important factor in determining levels in the serum. Thus, the use of serum alkaline phosphatase assay as an index of biliary obstruction is based on the assumption that the principal channel of excretion of the enzyme is through the bile. We know relatively little of the relative stabilities of the different tissue enzymes released into the blood following tissue damage, though it is known that the lactic dehydrogenase released from degenerate myocardium is more heat stable than that from the liver. Recently Cahn, Kaplan, Levine, and Zwilling (1962) have suggested that the lactic dehydrogenase molecule, which has a molecular weight of about 120,000, in the presence of 12M urea may be split into four subunits of approximately equal size. These subunits differ one from another. It appears that the behaviour of the indi-

vidual lactic dehydrogenase may be dependent on the composition of their subunits, thus the relatively stable lactic dehydrogenase from cardiac muscle is formed from four identical subunits whereas the relatively unstable lactic dehydrogenase from liver tissue is formed from four different subunits. The intermediate isoenzymes are formed from hybrids of subunits of varying composition. This attractive concept has still to be confirmed in detail, though there are analogies in other body proteins, haemoglobin and γ globulin, which make it very likely that the concept of Cahn and his co-authors is correct. The genetic background of these subunits has not yet been worked out.

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