Problems and Usefulness of Serum Enzyme Measurement in the Diagnosis of Diseases

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THE USEFULNESS OF serum enzyme measurement as an aid in diagnosis is well established. In our laboratory during the past five years (1968-1972) the number of requests for some of the clinically important enzymes is shown in the figure.

As from 1968 and earlier, requests for enzyme assays sent to our laboratory showed a steady increase except in 1970 when there was a slight decline (Curva A). If we consider Curve B which represents those enzymes which we measure and which are also measured in major hospitals in Malaysia (e.g. the transaminases and phosphatases), there was a decline in the number of requests as from 1970. This was because more and more hospital laboratories throughout Malaysia are expanding their services to include enzyme assays in addition to the other important laboratory tests. A better reflection of the increasing requests for enzyme assay is shown by Curve C which represents those enzymes which we assay but which are not assayed at State hospital laboratories (e.g. lactate dehydrogenase and creatine kinase). From 1970 there was an increase of about 20% per annum for such enzyme tests and this increase reflects more or less the workload which most hospitals are currently experiencing.

We come now to the important question: namely which enzymes should a hospital laboratory assay? To answer this one must first take into account such factors as the availability of qualified staff, fine chemicals and good instrumentation. The enzymes to be offered under routine should be practical and useful. Sometimes the selection will depend to some extent on local interests. An attempt is now made to outline the usefulness of some clinical enzymes both from the methodological and diagnostic aspect in the light of current knowledge.

Organ-specific Enzymes and Isoenzymes

The ultimate goal in diagnostic enzymology is the discovery of a specific enzyme not only for an organ but also for a particular disease of that organ. Unfortunately this has not yet been achieved satisfactorily. Interest in organ-specific isoenzymes was initiated by Hodson and co-workers (1962) who were able to distinguish the principal bone, liver and intestinal fractions of alkaline phosphatase using starch-gel electrophoresis. This technique was particularly helpful in the differential diagnosis of bone and liver diseases under conditions of raised total alkaline phosphatase activity.

When the heterogeneity of lactate dehydrogenase (LD) was worked out, the diagnostic application of the isoenzyme pattern after electrophoresis showed promise. In myocardial infarction the serum is found to contain an excess of the faster moving isoenzyme fractions (LD-1 and LD-2), while in most cases of infective hepatitis the serum is especially rich in the slowest moving (LD-5) fraction.

Isoenzyme electrophoresis technique is not altogether a convenient tool for the hospital laboratory. Chemical assays are generally preferred whenever possible. Such measurements as the hydroxybutyrate dehydrogenase activity, the heat stability tests for lactate dehydrogenase and alkaline phosphatase and the urea-inhibition LD test were developed and favoured with the enthusiastic advocacy of their originators. Some of these tests

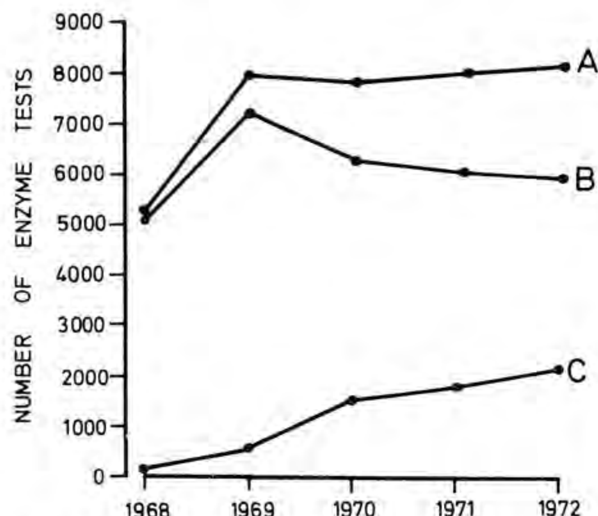

Fig. in "Problems and usefulness of serum enzyme measurement in the diagnosis of diseases.

Enzyme tests done over 5 years. A: Total enzymes. B: Enzymes which are also measured in other hospital laboratories.
C: Enzymes not measured in other hospital laboratories.

are of proven value only in a limited number of circumstances and will be mentioned again later under the appropriate sections.

Methods of Enzyme assay

Almost all clinically important enzymes are assayed using the fixed-time or two-point assay principles (e.g. alkaline phosphatase) in hospital laboratories throughout Malaysia. From the practical aspect this method of assay is convenient and suitable for large batch analyses. Unfortunately two-point assays are not altogether ideal especially for some enzymes (e.g. aspartate transaminase), where factors such as the accumulation of inhibitory products, high enzyme concentration, etc., may contribute to errors in measurement. Nevertheless two-point assays are universally accepted and will continue to be used until such time when the availability of suitable recording spectrophotometers will favour their replacement by the more accurate kinetic procedures.

Problems in Enzyme assay

Many factors affect the measurement of enzyme activity and these include purity of the reagents, presence of inhibitors, proper control of pH and concentration of buffer and substrate, strict temperature adherence and instability of the enzyme and reagents can cause varying results. The quality control in enzyme measurement is a perplexing problem that has no easy solution. Commercially available control sera used as standards and for quality control in enzyme assay are not to be advocated (Moss, 1970) unless the activity of the enzyme in question has been checked by a reliable primary method which is often a kinetic method. In practice this is seldom done and laboratories accept the manufacturer's values and assume (or hope) that the control serum enzymes have not deteriorated.

Enzyme activity

The expression of enzyme activity has been rather confusing especially for the physicians. Laboratories sometimes report enzyme activities in International Units following the recommendations of the International Union of Biochemistry. As such enzyme activity should be expressed in International Units/liter (IU/1) and along with the result the normal range by the same method should be included. This is important, for depending on the method of assay for a particular enzyme the normal range can vary. For instance, if serum alkaline phosphatase was assayed by the modified method of King & Armstrong (described in King & Wootton, 1956) as is done here and mostly in Europe, the normal range expressed in International Units is 25 – 92 IU/1. By the Bessey, Lowry & Brock method (1946) the normal range is 13 – 38 IU/1, while the Bodansky method (1933) gives the normal range as 8 – 22 IU/1. Anyone familiar with the normal range of one of these methods could be initially misled when results by another assay method are presented without the inclusion of the normal range. As most of our methods are fixed-time assays, the enzyme activities are usually defined by the originators of the methods e.g. King & Armstrong or Reitman & Frankel, and normal values expressed in such units fortunately do not vary much from one laboratory to another.

Heart

The clinically important enzymes that are found in the heart tissue in high concentrations are lactate dehydrogenase, aspartate transaminase (formerly GOT) and creatine kinase (CK). (The enzyme abbreviations follow the recommendations of Baron *et al.* (1971).) These enzymes are not necessarily equally important in myocardial infarction, as they are released at different rates from the damaged tissue. The activity of creatine kinase in blood can be increased as early as 6 h after an episode of myocardial infarction and the peak value is reached after 12 – 48 h. Aspartate transminase (AST) reaches a peak after 24 h and can remain elevated up to 5 days, while the elevation of total lactate dehydrogenase activity which is less pronounced remains raised for about 10 days or more.

The elevation of LD in myocardial infarction is principally in the fast moving isoenzyme fraction (LD-1) which is found in high concentration in heart tissue. LD-1 has been shown to preferentially reduce 2-oxobutyrate (Elliot & Wilkinson, 1961), and is referred to as hydroxybutyrate dehydrogenase (HBD). While some laboratories measur HBD activity, its advantage over total LD was questioned by McQueen *et al.* (1972) who showed that the activities of HBD and LD parallel each other in patients with a definite diagnosis of myocardial infarction. When they expressed the enzyme activities in arbitrary units, taking unity as the upper limit of normal, CK, AST and LD showed the greatest elevations respectively. Considering that CK activity declines more rapidly and that the measurement of CK is technically more demanding, AST and LD measurement is more usually done, of this, AST is preferred as (a) it is more specific as a "heart" enzyme and (b) the result is not invalidated for specimens moderately haemolysed. Elevation of alanine transminase (formerly GPT) in myocardial infraction is slight and this test is unnecessary when AST is measured.

Liver

The liver tissue is principally rich in LD, AST and alanine transaminase (ALT). Alkaline phosphatase (ALP) is also found in the liver, although not as abundant as the above enzymes, its diagnostic importance in liver disease is well established. Raised serum ALP especially above 35 King-Armstrong Units per 100 ml is suggestive of extrahepatic or intrahepatic cholestasis, while in acute hepatitis the serum level though raised, is usually about 30 KA/100 ml or less. The reason for the higher serum elevation of ALP during cholestasis is generally believed to be the result of failure of the liver to excrete the enzyme into the bile (retention theory). Recent findings by Kaplan & Righetti (1969) seem to suggest that the elvation in serum ALP during biliary obstruction is due to the increased pressure in the bile canaliculi which exert some effect on the cell membrane to induce synthesis of the enzyme.

Alkaline phosphatase exists in multiple molecular forms which can be distinguished by electrophoresis as bone, liver, intestine and placental isoenzymes. The placental ALP is heat stable and many workers have demonstrated a rise of this fraction with advancing pregnancy, while in placental dysfunction it is associated with a fall of its activity in serum. Fishman *et al.* (1972) have recently suggested an optimal method for the measurement of placental ALP for normal pregnancies. The normal values which they reported and those of Bean & Stott (1972) show such a wide range that it becomes difficult to confirm the diagnosis of placental dysfunction in individual cases on the basis of the heat stable ALP values.

The measurement of total ALP to investigate liver disease in adolescents or in patients with concurrent bone disease is sometimes difficult. Both liver and bone disorders show raised serum ALP, thus making the differential diagnosis difficult or uncertain. Isoenzyme separation to distinguish liver and bone ALP is technically difficult and not practical for a busy routine laboratory. Some laboratories measure 5'-nucleotidase which is increased in hepatobiliary disorder as is also ALP, but its activity is unaltered in bone disease. Besides these enzymes, leucine aminopeptidase and gamma-glutamyltranspeptidase have also been shown to rise in hepatobiliary obstruction. We are ourselves still undecided which one of these three enzymes to choose from as the back-up for elevated ALP in a clinically uncertain situation. Alkaline phosphatase is perhaps the only important enzyme for investigating bone disease and together with its usefulness in liver disease, the measurement of this enzyme is a must.

Damage of the parenchymal cells of the liver can be investigated by measuring the serum transaminase activity. The activity starts to rise during the prodromal illness and peak values coincide with the onset of clinical icterus. Unless the illness resolves itself, there is a persistent raised level which suggests an unfavourable prognosis. Alanine transaminase has been associated as principally a liver enzyme. In acute hepatitis for instance, the serum alanine transaminase is highly elevated together with aspartate transaminase. In some cases alanine transaminase levels exceed that of aspartate transaminase, more so when both enzymes are measured by the kinetic method. When both these enzymes are assayed by the colorimetric method (e.g. Reitman & Frankel method) as is done in all hospitals in Malaysia, their usefulness when expressed as their activity ratio is questionable because of the lack of sensitivity and precision of the method (Goldberg, 1971). At best the colorimetric method measures the elevations in transaminase levels and as such there is little advantage in measuring both the transaminases. As most laboratories would have set up the aspartate transaminase assay for cardiological investigation, this enzyme becomes the obvious and practical choice for the assessment of liver cell damage as well.

The serum LD activity is often markedly raised in the early stages of viral hepatitis, moderately raised in toxic jaundice, while in obstructive jaundice and in cirrhosis variable LD activities are found (Wilkinson, 1962). Hepatocellular damage releases the liver-rich LD isoenzyme fraction, that is the LD-5, which can be seen from the serum LD isoenzyme pattern. This isoenzyme separation techni-

que, though not difficult to perform (Baron & Buttery 1972) is seldom done routinely. The chemical measurement of this isoenzyme as the heat-labile or urea-sensitive fraction has been advocated but has gained little acceptance generally. Perhaps due to the rather broad normal ranges and the difficulties encountered in methodology to ensure proper optimal and exacting conditions of assay, results have not been discriminating enough to distinguish normal values from pathological values.

Two other "liver-specific" enzymes need mention, namely sorbitol dehydrogenase (SD) and ornithine carbamoyltransferase (OCT). Serum SD is markedly raised in acute liver cell necrosis and OCT raised in viral hepatitis. Sorbitol dehydrogenase is a very labile enzyme and the preferred method of assay is the kinetic method. Both these factors do not favour SD as an enzyme to be measured under routine condition. Ornithine carbamoyltransferase measurement is technically laborious and care must be taken to choose a method that is not in error (Buttery & Baron, 1972). Despite the claims of these two enzymes to be "liver-specific", few laboratories measure their activities as AST can often supply the diagnostic information required.

Other clinically useful enzymes

Some of the other useful enzymes which we assay are acid phosphatase, amylase, trypsin, creatine kinase, cholinesterase and caeruloplasmin (copper oxidase).

Of the many enzymes assayed for the investigation of malignancy, acid phosphatase is one of the most specific and raised serum levels are found in carcinoma of the prostate. This enzyme, unfurtunately is also found in erythrocytes, liver and bone. Raised prostatic acid phosphatase should be confirmed by the tartrate-labile acid phosphatase test where this enzyme is inhibited by L (+) tartrate.

Raised serum amylase activity is found during acute pancreatitis and any form of parotitis. Serum lipase is sometimes done as it is more specific and remains elevated longer than amylase. Lipase assay however, is more difficult and takes longer to do than amylase and in practice is not popular with clinical laboratories. In assessing pancreatic function serum enzymes measurement alone is not reliable. Stimulation test of the pancrease with either secretin or pancreozymin and duodenal aspirate measurement of pancreatic enzymes (e.g. trypsin, amylase) yield better information.

Creatine kinase has superseded aldolase as the enzyme of choice in the investigation of diseases of muscle as it is more sensitive and specific. Marked increases in the serum level are observed in most cases of muscular dystrophy especially in the Duchenne-type muscular dystrophy. Moderate increases are noted in a number of clinical situations but generally they have little importance in diagnosis.

Requests for the measurement of serum cholinesterase entertained in this laboratory have usually been for people who are engaged in using organophosphorus compounds. Excessive exposure to these compounds would depress serum enzyme level. The normal range of the enzyme is rather large so that slight intoxication is ofter difficult to determine on the basis of a single estimation, more so if the basal value prior to intoxication is not known. Some authorities use the serum enzyme activity to determine the extent of liver cell damage as this enzyme is formed principally in the liver. The more usual request is to ask for total proteins or albumin as these tests are much easier to perform.

Sometime we measure the inhibition of serum cholinesterase with dibucaine in individuals who show prolonged apnoea after succinylcholine administration. This prolonged muscle paralysis may be due to cholinesterase deficiency as in liver disease or due to an atypical enzyme variant resulting from an inborn error of metabolism. Normal cholinesterase is 80% inhibited by dibucaine (Dibucaine Number 80) while the homozygote for the atypical enzyme has Dibucaine Number 20.

Serum caeruloplasmin (copper oxidase) level is reduced in patients with Wilson's disease. This disease is characterised by gross deposition of copper in tissues, increased urinary excretion of copper resulting in low serum copper and low caeruloplasmin concentration. In nephrotic syndrome where there is increased urinary excretion of caeruloplasmin, its level in serum is also low. The measurement of this enzyme for the diagnosis of other diseases is seldom done.

Conclusions

The importance of serum enzymes in diagnosis is well established. For a laboratory with adequate staff, chemicals and instruments it is possible to offer a fairly comprehensive range of clinically useful enzymes. For the small laboratory there is still a place for enzyme measurement. Such enzymes as amylase, acid and alkaline phosphatase and aspartate transminase are not only clinically useful but are relatively easy to do. It is much better for a laboratory with limited facilities to do a small range accurately.

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