Enzyme assays in malignant disease

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Although cancer diagnosis remains almost exclusively morphological, with histological examination the lynch pin, there are situations in which other techniques, such as enzyme assays, may provide information of clinical value. It is, however, disappointing that so much of the increase in knowledge of the biochemical properties of tumours has proved to be nonspecific, and therefore of limited diagnostic value. Nevertheless, tumours have occasionally produced an unusual isoenzyme, an observation of considerable biological and diagnostic interest. Assays of normally occurring enzymes in serum may also yield information of clinical value in circumstances where the lack of specificity is not important; thus, whilst not indicating the nature of a tumour, they may nevertheless provide an indication of its activity, of its response to treatment, or of the site of metastases.

Elevation of serum enzymes by tumours may be due to leakage from the tumour or from adjacent normal tissue damaged by the neoplasm. Serum enzyme levels may also be influenced less directly, for example when there is associated haemolysis. Usually more than one of these factors is operative, as emphasized in the following discussion.

Serum Alkaline Phosphatase (EC 3.1.3.1.)

Assay of this enzyme continues to be of great value in the detection of lesions in liver and bone. Different isoenzymes have been demonstrated by various techniques in liver, bone, intestine, and placenta. Some degree of discrimination is possible on the basis of urea inhibition, heat stability and electrophoresis, the relative merits of the latter on various media being discussed elsewhere in this symposium by Wieme and Demeulenaere (p. 51); however, many of these methods are still somewhat specialized. It is often important clinically to distinguish liver and bone isoenzymes in serum, and for most laboratories the assay of a second enzyme specific for liver appears to be the most practical way of achieving this (p. 82). This field has been reviewed by Posen, (1967), Newton (1967), and Fishman and Ghosh (1967).

Some evidence has been produced that certain

alkaline phosphatase isoenzymes may be tumour specific. Stolbach, Krant, and Fishman (1962) discovered such an enzyme in the serum and tumour of a patient with carcinoma of the lung; this is referred to as the Regan isoenzyme after the name of the patient. It closely resembles placental alkaline phosphatase in being heat stable and inhibited by phenylalanine, and is inactivated by the same antiserum. The Regan enzyme was found to be present in serum from about 5% of a series of patients with a wide variety of tumours, but not in normal sera; it has also been found in the tumours of such patients, including those arising from lung, ovary, colon, breast, uterus, lymphoma tissue, and even myeloma. Serum usually requires concentration before the enzyme can be demonstrated by electrophoresis. Stolbach *et al* (1969) suggest that this enzyme is an example of ectopic synthesis by tumour cells of an enzyme protein not normally produced in the tissue of origin, due to repression of specific genomes in the malignant cell.

Other alkaline phosphatase isoenzymes have also been isolated from tumours. Timperley (1968) demonstrated a heat-labile, L-phenylalanine insensitive alkaline phosphatase in the tumour of a patient with squamous carcinoma of the bronchus and raised serum alkaline phosphatase. Another abnormal alkaline phosphatase has been demonstrated in the serum of some non-icteric patients with hepatic metastases (Newton, 1967; Jennings, Brocklehurst, Hirst, and Zircos, 1969). This, however, appears not to be unequivocal evidence of a tumour, as it may be present in patients with obstructive jaundice not associated with neoplasia (Latner, 1965).

5'-Nucleotidase (EC 3.1.3.5.)

This phosphatase, which catalyses the hydrolysis of 5'-nucleotides, is present in bone and liver in high and low concentration respectively (Reis, 1952). Paradoxically, its activity in serum is frequently raised in liver disease and seldom raised in bone disease (Dixon and Purdom, 1954). It has some value, therefore, in distinguishing between hepatic and skeletal causes of a raised serum alkaline phosphatase, but there is a tendency to replace it

by assay of more specific liver enzymes (p. 82). Its value in the diagnosis of liver metastases has been proclaimed by some workers (Schwarz and Bodansky, 1965) but not confirmed by others (Davidge and Philpot, 1966; Belfield and Goldberg, 1969). The substrate generally used in its measurement, adenosine-5'-monophosphate, is also hydrolyzed by alkaline phosphatase, so that the apparent nucleotidase activity may be erroneously high if alkaline phosphatase activity on the substrate is not determined separately or eliminated (Belfield and Goldberg, 1969).

Acid Phosphatase (EC 3.1.3.2.)

Estimation of serum acid phosphatase has long been the most useful enzymatic tool in cancer diagnosis. The acid phosphatase in normal serum is known to be a mixture of isoenzymes from different tissues, including red cells and platelets. The phosphatase in red cells and most of that in platelets is inactivated by formaldehyde.

The separate identity of the formaldehyde-stable tartrate-labile prostatic isoenzyme is supported by immunological studies (Moncure and Prout, 1970) and its molecular heterogeneity is now thought to be due to attachment of different numbers of sialic acid residues to a single enzyme protein (Smith and Whitby, 1968). Small, transient increases of this isoenzyme may occur after pressure on the non-malignant prostate by rectal examination, impacted faeces, or a distended bladder.

A formaldehyde-stable tartrate-stable enzyme is responsible for the raised levels found in 50% of patients with advanced breast carcinoma (Jegatheesan and Joplin, 1962). Serum levels rise significantly only when the architecture of the gland is so disrupted as to prevent the normal exocrine flow, and is particularly marked when there are metastases. Some degree of inverse correlation between the serum enzyme level and the clinical response to treatment was found by Reynolds, Lemon, and Byrnes (1956) and by Joplin and Jegatheesan (1962).

Glycolytic Enzymes in Serum

Serum levels of several glycolytic enzymes, namely, glucose phosphate isomerase (EC 5.3.1.9), phosphoglucomutase (EC 2.7.5.1), ketose 1-phosphate aldolase¹ (EC 4.1.2.7), and lactate dehydrogenase (EC 1.1.1.27) are often raised in disseminated cancer and other diseases. In advanced breast cancer, for example, there is a close correlation of

changes in glucose phosphate isomerase levels with progression of the disease (Bodansky, 1954 and 1957). However, these changes occur too late and are too nonspecific to assist early diagnosis, but may help to elucidate the cause of effusions and CSF changes whose aetiology is obscure. These changes also have been used with some success to evaluate palliative treatment. Serum glucose phosphate isomerase levels were found to be increased in 85% of patients with advanced breast cancer by Joplin and Jegatheesan (1962), and to fall, usually to within the normal range, during clinical remission after pituitary ablation; in patients who showed no clinical improvement there was either no significant change or an increase in the serum level.

The glycolytic enzymes may also give some indication of the site of metastases, particular when more than one is assayed. In breast cancer, Jegatheesan and Joplin (1962) concluded that very high levels of glucose phosphate isomerase, phosphoglucomutase, and ketose 1-phosphate aldolase are found only when liver metastases are present, though with such metastases high levels are not invariable. However, there are now better ways of determining this.

Total serum lactate dehydrogenase (LD) activity is often raised late in the course of malignant disease, and this appears to be independent of the nature of the tumour and the site of metastases (Wróblewski, 1959); hence it is of little diagnostic value. LD activity tends to be higher in malignant tissue than in corresponding normal tissue (Meister, 1950) but there is considerable overlap (Latner, 1964). Attempts have been made to associate LD isoenzyme patterns with malignant tumours. According to Latner (1964), malignant tumours contain predominantly the slower migrating isoenzymes; in the case of carcinoma of the breast, this is supported by Richterich and Burger (1963), and Barnett and Gibson (1964). Serum isoenzyme patterns however do not show any clear cut tumour specificity.

Posen (1970) has emphasised that interpretation of serum LD isoenzyme patterns must be made in the light of knowledge of the clearance rates from the blood stream of the various isoenzymes. (A particular pattern may arise from selective removal of one isoenzyme.) Injection experiments with sheep (Boyd, 1967) have shown that the isoenzyme LD 5 has a much faster clearance rate from plasma than LD 1. There is good reason to believe that this also pertains in man, and to interpret electrophoretic serum LD patterns as indicating particular tissues of origin ignores these different removal rates (Posen, 1970). Differential degradation within the cell of origin also appears to be an important factor in deciding the pattern

¹Also known as aldolase.

'characteristic' of different tissues. For these reasons such patterns cannot yet be relied on to provide consistent tumour-specific information.

Lactate dehydrogenase activity is higher in some malignant effusions than in serum, but this also occurs in nonmalignant effusions containing blood or pus. Anti-tumour therapy of malignant effusions is said sometimes to cause a return to normal values.

Cerebrospinal fluid activity is independent of serum level. Increases do occur in association with malignant meningeal and cerebral lesions, but similar changes accompany inflammatory lesions and cerebrovascular accidents.

Leucine Aminopeptidase (EC 3.4.1.1)

This enzyme was once thought to have some specificity for pancreatic cancer, but it is now accepted that raised levels are found in this disease only when secondary hepatobiliary changes are present, and as patients with jaundice from any cause may show raised serum levels, the assay does not appear to have much diagnostic value (Mericas, Anagnostou, Hadziyannis, and Kakari, 1964). Furthermore, in one method of assay (Goldberg, Pineda, and Rutenburg, 1959) the substrate, leucyl- β -naphthylamide, is carcinogenic.

Detection of Liver Infiltration

Serum levels of hepatic enzymes released from damaged tissue have been widely used for the detection of metastatic spread to liver. The value of serum alkaline phosphatase estimation has already been mentioned, its main limitation being the difficulty of distinguishing between liver and bone isoenzymes. Enzymes more specific for liver disease can also be estimated, and assay of aminotransferases together with alkaline phosphatase gives a higher percentage of positive results in metastatic liver disease than either separately. Other sensitive enzyme indicators of liver lesions with adequate specificity are serum isocitrate dehydrogenase (EC 1.1.1.42) (Bell *et al.*, 1962; Okuruma and Spellberg, 1960), ornithine carbamoyltransferase¹ (EC 2.1.3.3) (Reichard, 1961), and D-glutamyl-transferase² (EC 2.3.2.1) (Zein and Discombe, 1970). However, none of these has been found to facilitate the differentiation of liver malignancy from other disorders with which it might be confused, nor do they appear to provide more information than phosphatase and alanine aminotransferase assays. Positive results with the latter enzyme are

seen not uncommonly as a result of anaesthetic, tranquillizers, cytotoxic and other drugs; conversely there is often no increase in serum enzymes despite diffuse hepatic infiltration due to lymphoma or leukaemia.

Lysozyme (EC 3.2.1.17) in Leukaemia

Serum levels of this bacteriolytic enzyme, normally present in saliva, tears, and other secretions, have been found to change in leukaemia (Osserman and Lawlor, 1966; Perillie, Kaplan, Lefkowitz, Rogawski, and Finch, 1968; Pruzanski and Saito, 1969). They appear to reflect marrow leucopoietic activity and are high in most patients with acute or chronic granulocytic leukaemia, very high in monocytic leukaemia, and low or normal in lymphocytic leukaemia. Where the type of leukaemia is doubtful on morphological grounds, however, the level of lysozyme may also be equivocal and its place in diagnosis at present appears limited.

Enzyme Assays in Cancer Screening

Clearly no enzyme has been found to be sufficiently specific for neoplasia to provide the basis for a cancer screening programme. The possibility remains, however, that enzyme changes associated with tumour growth may be of diagnostic value in particular situations, and several workers have studied enzyme changes in vaginal secretions, urine, and other biological fluids in relation to local malignant change.

Bonham and Gibbs (1962) reported encouraging results using phosphogluconate dehydrogenase (EC 1.1.1.43) activity in vaginal secretion as a screening test for uterine cancer, after somewhat doubtful results had been obtained by previous workers studying other enzymes.

Subsequently it was found that, although the test appears reliable in selecting patients with invasive cancer of both body and cervix, it fails to detect carcinoma in situ (Bell and Egerton, 1965; Cameron and Husain, 1965; Boyd, Gibbs, Labrum, and Philips, 1967). Moreover the proportion of false positive results is unacceptably high.

The measurement of lactate dehydrogenase and alkaline phosphatase activity in urine has been recommended by several groups as a screening procedure for urothelial tumours (see Wacker, 1967). Whilst the majority of patients with carcinoma which is apparent clinically do show increased amounts of one or both of these enzymes in urine, the changes are nonspecific, and raised levels of lactate dehydrogenase and other enzymes such as β -glucuronidase (EC 3.2.1.31) are found in asso-

¹Also known as ornithine transcarbamylase.

²Also known as γ -glutamyltranspeptidase.

ciation with many non-malignant renal lesions (Rosalki and Wilkinson, 1959). At most, an increased enzyme level in the urine may be regarded as indicating some abnormality in the urinary tract; a normal level by no means excludes the possibility of cancer or other serious disease of kidney or bladder (Kiser and Riggins, 1966).

The enzyme content of gastric juice has been investigated in patients with gastric carcinoma. Some abnormalities have been noted (Piper, Macoun, Broderick, Fenton, and Builder, 1963) but the technique has not found wide application.

Future Prospects

It remains to be seen whether tumour-specific tests based on enzyme assays will become a reality. Increasing knowledge of differences between tissue isoenzyme patterns may improve the accuracy of tumour localization but greater knowledge of the factors governing the clearance of enzymes from the blood will be needed to interpret such patterns. However, the development of radioisotopic scanning procedures for tumour localization is proceeding at a rapid pace (see McCready *et al*, 1969) and may supersede the use of enzyme assays for this purpose.

A new application of enzyme assays to clinical problems can be expected from recent work on the testing *in vitro* of cancer chemotherapeutic drugs. The response of an individual patient to the cytotoxic agents used in the treatment of malignant disease is unpredictable. The drugs are potentially hazardous, and furthermore may have to be given for six weeks before it is certain that no response will occur. Clearly, any test that would help avoid such delays would be welcome. The use of enzyme assays for predicting the response to treatment has given encouraging results in leukaemia treated with cytosine arabinoside (Kessell, Hall, and Rosenthal, 1969). This synthetic nucleoside must be converted to nucleotide by phosphorylation before it is incorporated into nucleic acid. Comparison of the cytosine arabinoside phosphotransferase activity of leukaemic cells from different patients has shown that a good therapeutic response is associated with high phosphorylation capacity and vice versa. Similar work is proceeding on the cellular enzymes governing the cytotoxic effects of other drugs and it seems probable that, in the leukaemias at least, some form of drug sensitivity test based on enzyme assay may come to occupy a place in patient management, similar to that of antibiotic sensitivity tests in treating bacterial disease.

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