Biochemical Procedures as Aids in Diagnosis of Different Forms of Cancer

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ABSTRACT

The application of biochemical analyses as aids in the diagnosis of cancer is discussed with emphasis on the fact that biochemical testing is more useful in following the regression and progression of disease than in early initial diagnosis. The uses of biochemical analyses of metabolic degradation products, lipids, hormones and their receptors, enzymes, including isoenzymes, and trace metals are included.

Introduction

Diagnostic procedures in cancer must be designed to achieve several purposes. They must define the disease, describe its extent and be useful in following its progression or regression. For more than 75 years, investigators have searched for biochemical defects in cancer cells that could be exploited in diagnosis. Despite these long and continuing studies, few biochemical procedures have been developed for early diagnosis of specific forms of cancer. The most useful application of biochemical assays has been in following the regression and progression of disease and in establishing the presence of metastases.

From the standpoint of general use, the most important tests would be those useful in cancers with the highest population incidence. In the United States these are cancer of the colon and rectum, female breast, lung, bronchus and uterus. However, most well defined biochemical procedures are those related to rarer forms of cancer. As indicated in table I these include neuroblastoma, pheochromocytoma, carcinoid, hepatocellular carcinoma, multiple myeloma, osteogenic sarcoma and other osteoblastic bone tumors. In these diseases, the assay of the listed components is essential for confirmation of the diagnosis and in following the response to therapy.

Serum Enzymes

Historically, the biochemical procedure used for the longest time as a diagnostic aid in cancer is acid phosphatase. This serum enzyme has been used for 35 years in the evaluation of carcinoma of the prostate. The enzyme activity is elevated in only 24 percent of patients with non-metastatic prostate carcinoma and in 81 percent of patients with skeletal metastases. Efforts have been made to increase the sensitivity of the acid phosphatase assay in prostatic disease by utilizing specific tartrate inhibition of prostatic acid phosphatase and assay of the “prostatic” frac-
TABLE I
Biochemical Assays Useful in Initial Diagnosis and Follow-Up of Rare Cancers

<table>
<thead>
<tr>
<th>Disease</th>
<th>Biochemical Constituent</th>
</tr>
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<tbody>
<tr>
<td>Neuroblastoma</td>
<td>Catecholamines, vanillyl-mandelic acid, metanephrine, cystathionine, dopamine β-hydrolase, dopamine, homovanillic acid</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>Catecholamines, metanephrine, vanillylmandelic acid</td>
</tr>
<tr>
<td>Carcinoid</td>
<td>5-Hydroxyindoleacetic acid</td>
</tr>
<tr>
<td>Trophoblastic neoplasms</td>
<td>Chorionic gonadotrophin</td>
</tr>
<tr>
<td>(choriocarcinoma, testicular tumors)</td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>Bence-Jones protein, immunoglobulin</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>α-Fetoprotein</td>
</tr>
<tr>
<td>Osteogenic sarcoma</td>
<td>Alkaline phosphatase</td>
</tr>
</tbody>
</table>

In one study, elevations of tartrate-sensitive acid phosphatase were found in 14 of 34 patients with prostatic carcinoma, 2 of 90 patients with benign prostatic hypertrophy, 15 of 76 patients with other forms of cancer, and 23 patients without cancer. It has been our experience that the prostatic acid phosphatase as determined by tartrate inhibition is not useful as a clinical laboratory tool.8

Disc electrophoresis on polyacrylamide gel has indicated there are three to five acid phosphatase isoenzymes in the serum of healthy adults.31 Electrophoresis of the serum from a patient with prostatic carcinoma (total serum acid phosphatase of 290 units) or of a homogenate of prostatic tissue demonstrated additional isoenzymes in the post albumin area. This technique requires further evaluation as a diagnostic aid in cancer of the prostate.31 Assays of bone marrow serum acid phosphatase have been proposed as a technique for detecting early metastasis to bone in adenocarcinoma of the prostate and in staging of the disease.30

Many other serum enzymes have been suggested as useful parameters in the di-
agnosis of cancer. Serum amylase is elevated in a small percentage of patients with cancer of the pancreas, and alkaline phosphatase is elevated in primary tumors of bone. Alkaline phosphatase is more useful in evaluating metastatic disease to bone and liver. The enzyme is elevated predominantly in osteoblastic bone lesions. In breast carcinoma, most cases of bony metastases are osteolytic, and alkaline phosphatase levels are usually in the normal range or slightly elevated. In prostatic carcinoma, most of the metastatic lesions are osteoblastic, and the serum alkaline phosphatase is elevated predominantly in osteoblastic bone lesions. In breast carcinoma, most cases of bony metastases are osteolytic, and alkaline phosphatase levels are usually in the normal range or slightly elevated. In prostatic carcinoma, most of the metastatic lesions are osteoblastic, and the serum alkaline phosphatase is elevated predominantly in osteoblastic bone lesions. In breast carcinoma, most cases of bony metastases are osteolytic, and alkaline phosphatase levels are usually in the normal range or slightly elevated. In prostatic carcinoma, most of the metastatic lesions are osteoblastic, and the serum alkaline phosphatase is elevated predominantly in osteoblastic bone lesions. In breast carcinoma, most cases of bony metastases are osteolytic, and alkaline phosphatase levels are usually in the normal range or slightly elevated. In prostatic carcinoma, most of the metastatic lesions are osteoblastic, and the serum alkaline phosphatase is elevated predominantly in osteoblastic bone lesions. In breast carcinoma, most cases of bony metastases are osteolytic, and alkaline phosphatase levels are usually in the normal range or slightly elevated. In prostatic carcinoma, most of the metastatic lesions are osteoblastic, and the serum alkaline phosphatase is elevated predominantly in osteoblastic bone lesions. In breast carcinoma, most cases of bony metastases are osteolytic, and alkaline phosphatase levels are usually in the normal range or slightly elevated. In prostatic carcinoma, most of the metastatic lesions are osteoblastic, and the serum alkaline phosphatase is elevated predominantly in osteoblastic bone lesions. In breast carcinoma, most cases of bony metastases are osteolytic, and alkaline phosphatase levels are usually in the normal range or slightly elevated. 

During successful treatment, there may be an elevation in the serum alkaline phosphatase presumably related to the repair of bone. These elevations have been referred to as the "paradoxical rise" in serum alkaline phosphatase. In liver disease, elevations of alkaline phosphatase may be observed in patients with extrahepatic obstruction of the biliary tract and in patients with intrahepatic metastases. In infiltrative disease of the liver such as leukemia, reticulum cell sarcoma and Hodgkin's disease, alkaline phosphatase activity is markedly elevated, the activity being directly related to the extent of liver involvement.

Recently, there has been great interest in the isoenzymes of serum alkaline phosphatase and the so-called "Regan isoenzyme" has been used in studies of patients with cancer. In 1968, Fishman and his associates identified an alkaline phosphatase in the serum and tissue of a patient with carcinoma of the lung. This enzyme was named the Regan isoenzyme after the patient in whom it was found. Investigations have indicated that the isoenzyme is identical to the heat stable, L-phenylalanine sensitive alkaline phosphatase of the human placenta, that it demonstrates identical electrophoretic mobility to that from placenta before and after neuramidase treatment and reacts with antisera to placental alkaline phosphatase. In two studies of 913 patients with a variety of cancers, the Regan isoenzyme was observed in the serum in 66 instances or 7.2 percent. The highest incidence was in patients with cancer of the ovary and lung. With a sensitive immunochemical assay, Regan isoenzyme has been detected in serum of 89 of 91 normal adults and 106 of 112 sera from patients with malignant disease. In the cancer patients, only 11 showed elevated activities to an extent of 3 to 300 times the average normal value. An alkaline phosphatase variant different from the Regan isoenzyme has been observed in serum of patients with hepatoma. In one study, 6 of 21 patients with hepatoma exhibited the alkaline phosphatase variant. All of these patients had a-fetoprotein in their serum. It was concluded that the alkaline phosphatase variant was not useful in hepatoma.

An important demand made of the clinical biochemistry laboratory is to aid the clinician in establishing whether or not an elevated serum alkaline phosphatase is due to liver or bone metastases or to a combination of both. Numerous attempts have been made to use electrophoresis to separate liver from bone alkaline phosphatase. However, in our experience, there is too much overlap in the electrophoretic migration of these isoenzymes to permit their routine clinical use. We have successfully used serum 5'-nucleotidase for this purpose. 5'-Nucleotidase is a substrate specific phosphatase present in liver tissue but not in bone. When it is elevated in the serum, it is indicative of liver involvement.

Two other enzymes, y-glutamyl transpeptidase and leucine aminopeptidase have also been used as specific indicators of liver disease. In a comparison of these enzymes in a series of 96 patients in whom the clinician suspected liver involvement, the three enzyme activities were observed within normal limits in 26 individuals and all elevated in 39 others. In seven patients,
TABLE II
ELEVATIONS OF "UBIQUITOUS ENZYMES" IN SERUM OF PATIENTS WITH CARCINOMA METASTATIC TO LIVER

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>No. of Patients</th>
<th>No. with Elevations</th>
<th>Percent Elevated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphohexose isomerase</td>
<td>72</td>
<td>60</td>
<td>84</td>
</tr>
<tr>
<td>Aldolase</td>
<td>100</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>156</td>
<td>108</td>
<td>69</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>70</td>
<td>43</td>
<td>62</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>65</td>
<td>34</td>
<td>53</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>188</td>
<td>94</td>
<td>50</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>68</td>
<td>32</td>
<td>47</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>179</td>
<td>60</td>
<td>33</td>
</tr>
</tbody>
</table>

γ-glutamyl transpeptidase was the only elevation; in two patients, this enzyme was normal when the others were elevated. In each of two other cases, either 5′-nucleotidase or leucine aminopeptidase was elevated when the other activities were within normal limits. In 11 cases, the leucine aminopeptidase was normal when the other two were elevated; in seven cases, the 5′-nucleotidase was normal when leucine aminopeptidase and γ-glutamyl transpeptidase were at supranormal activities. In the extreme sensitivity of γ-glutamyl transpeptidase and its elevation in a large variety of situations, particularly alcoholism, may mitigate against its use in establishing the presence of liver metastases in cancer. The main points suggested by these data are the need for multiple enzyme assays and the fact that no single determination is universally applicable to the evaluation of liver diseases.

The ubiquitous enzymes are a large group of metabolically involved enzymes present in all tissues. They include lactic dehydrogenase, phosphohexose isomerase, aldolase, transaminases, isocitric dehydrogenase, etc. They are not useful in primary diagnosis but are helpful in following the course of disease in patients with liver metastases. Of all these enzymes, serum phosphohexose isomerase appears to be elevated in the greatest number of patients (table II).

Body Fluid Enzymes

Attempts have been made to use the ubiquitous enzymes in fluids other than blood in cancer diagnosis and follow-up. Urinary lactic dehydrogenase has been extensively studied as a diagnostic aid in cancers of the bladder and kidney. It has been concluded from our study of 263 patients with various diseases of the genito-urinary tract that these assays are not useful as a routine diagnostic procedure in the evaluation of patients with genito-urinary symptoms. Elevations of urinary LDH seem to reflect more closely pyuria, hematuria or bacteriuria than the presence of a specific proved tumor.

The activities in vaginal washings of 6-phosphogluconate dehydrogenase, phosphohexose isomerase and the lysosomal enzyme β-glucuronidase have been used in the evaluation of cervical cancer. Elevations of these enzymes were observed in
washings from a majority of women with uterine cancers and also from many women with benign disease. Although these assays flag patients with gynecological disorders, the high false positive rate led to a conclusion that it is unlikely that vaginal fluid enzymology can provide a screening test for cervical carcinoma. These findings must be reevaluated and extended to studies of these and other enzymes in tissue washings or brushings from the lung, stomach and colon as well as the cervix.

Tissue Enzymes

The metabolically involved enzymes have also been evaluated in tissue biopsy material as aids to the pathologist in establishing the presence of cancer and the tissue of origin of a metastatic lesion. A recurrence of interest in tissue LDH isoenzymes has focused particularly on the ratio of LDH$_5$ and LDH$_1$. A shift to LDH$_5$ (the muscle variant) has been observed in human cancerous breast, uterus, brain, lung, stomach and kidney. The most impressive study is that of 619 surgical prostate specimens. In these cases the LDH$_5$/LDH$_1$ ratio was greater than one in 90 of 116 (77.6 percent) specimens with histologically verified carcinoma of the prostate and in only 70 of 503 (13.9 percent) of tissue specimens from patients with benign disease.$^{23}$

The aldolase isoenzymes differ in some human cancers and tend to revert to a fetal form. Aldolase exists as three families of isoenzymes; aldolase A in muscle, aldolase B in liver and aldolase C (the most anodic form on electrophoresis) in brain and nerve tissue. Primary tumors of the liver have been observed to contain aldolase A and not the B form found in normal liver.$^{24}$ Serum from patients with cancer contains a greater proportion of muscle variant aldolase than serum from normal persons.$^{44}$ Other than patients with cancer, this has been found only in serum of persons suffering from muscular dystrophy. Gliomas contain aldolase C as does normal brain tissue; in meningiomas, aldolase C is not detected and only aldolase A is present.$^{25}$ Aldolase A is found in fetal brain and liver and in tumors. Cervical carcinoma and carcinoma of the uterine corpus exhibit an electrophoretically distinct hexokinase isoenzyme not found in normal tissue.$^{14}$

Tissue enzymes other than the ubiquitous ones may also be useful in evaluation of cancer. A glutaminase with a low affinity for phosphate appears to be present in cancerous lung with activity inversely proportional to the mitotic rates and degrees of differentiation of the tumors.$^{19}$ Two sulfate activating enzymes, sulfate adenylyltransferase and adenylsulfate kinase are stated to be absent in hormone non-responsive breast tissue. The assay of the sulfurylation enzymes has been proposed as a useful tool in conjunction with estrogen receptor protein in evaluation of patients prior to ablative endocrine surgery or hormonal therapy.$^7$

A discussion of enzymes in cancer would be incomplete if mention were not made of muramidase and its role in leukemia.$^{26}$ This enzyme has been found only in the urine of patients with granulocytic leukemia who do not have a Philadelphia chromosome.$^{27}$ The elevations in these cases are presumably due to release of the enzyme from monocytes and from more mature members of the granulocyte series. Serum muramidase has been found elevated in patients with acute myelogenous and chronic granulocytic leukemia but not in acute lymphatic leukemia.$^{29}$ The elevations in patients with chronic granulocytic leukemia are up to 10 times the upper limit of normal.

Steroid Discriminants

The use of individual steroid analyses in primary tumor diagnosis or predicting re-
sponsiveness to adrenalectomy, hypophysectomy or chemotherapeutic hormonal therapy has not been very successful. This lack of success is undoubtedly due in part to the effect of age, weight, the emotional stress of hospitalization and the extent of the illness on hormone excretion, as well as the relatively large analytical error in steroid analysis.35

During the past decade, numerous workers have used a grouping of steroid analyses to predict hormone responsiveness in breast, prostate and lung cancer. Bulbrook and his associates in England found that urinary etiocholanolone levels tended to be high in breast patients who subsequently had positive clinical responses to adrenalectomy or hypophysectomy and that the 17-hydroxycorticosteroids (17-OHCS) tended to be low in successful cases.3'17 By means of a discriminant function of these parameters, it was possible to predict the success or failure of adrenalectomy or hypophysectomy in breast cancer. The more positive the discriminant, the greater the chance of palliative success following ablative surgery. Other workers have designed other discriminants using other steroid analyses (estriol, 11-oxysteroids, 17-ketosteroids) and other factors, including age and the free period (time between primary diagnosis and recurrence). Their findings have been similar to those of the English group.7

**Estrogen Binding**

The binding of estrogen to macromolecular components (receptor protein) has received wide attention as a possible indicator of response to ablative hormone surgery. In vivo and in vitro studies have shown that human breast cancer often accumulates more estrogen than normal breast tissue. In vivo studies are limited because of possible radiation hazards, the expense of the large amount of needed radioactive hormone and the many uncontrolled factors that can affect the metabolism and excretion of hormones.

Jensen and his colleagues found that the estrogen receptor was in an 8S sucrose density gradient peak.18 Using *in vitro* techniques, these workers observed that 19 patients without binding ability failed to respond to adrenalectomy and 5 of 11 patients with binding protein had remissions. Wittliff et al also found the estrogen binding protein in the 8S to 9S fraction.46 Of 75 tumor specimens, 29 had positive binding (43.0 ± 5.3 fmoles of receptor per mg protein), 36 were negative and 10 were borderline. One of 21 normal and noncancerous breast tissue specimens demonstrated specific estrogen binding. The binding in tumor tissue was not related to the presence of metastases or the number of carcinoma cells in the studied specimen. Wittliff reviewed the literature and reported that of 329 primary breast tumors examined, 158 or 48 percent contained specific estrogen receptors. Long term studies are required to correlate studies of hormone binding proteins and the clinical response of patients to therapy.

**Polyamines**

Several biochemical procedures have been described which require further clinical evaluation to establish their true role in cancer diagnosis. Polyamine synthesis is accelerated in rapidly growing normal or malignant tissue, presumably owing to increases in ornithine decarboxylase activity in stimulated growing tissues. These effects appear to be hormone dependent. Russell and her associates demonstrated elevated urinary excretion of polyamines in cancer patients.32 In 50 normal persons excretions were: spermine 3.4 ± 0.67 mg per 24 hours; spermidine, 3.1 ± 0.56 mg per 24 hours and putrescine, 2.7 ± 0.53 mg per 24 hours. In cancer patients, excretions were increased 5 to 10 fold, and surgical
removal of a tumor mass always led to a decrease in urinary polyamine concentrations. In 24 patients with acute myelocytic leukemia in relapse, the values were as follows: Putrescine, $5.5 \pm 1.1$ mg per 24 hours; spermidine $26.4 \pm 3.4$ mg per 24 hours; and spermine $38.1 \pm 4.2$ mg per 24 hours. In 5 patients with ovarian carcinoma, the 24 hours values were putrescine, $29.9 \pm 5.7$ mg, spermidine $43.5 \pm 6.7$ mg and spermine $38.7 \pm 6.1$ mg.

In a study of blood polyamines, putrescine and spermine were not detected and spermidine was found at a concentration of $0.32 \pm 0.07$ nM per ml in 10 normal persons. In cancer patients, putrescine was not detected and spermine was found in 3 of 18 patients at levels between 0.14 and 0.28 nmol per ml. However, spermidine was observed in the serum of all 18 cancer patients at levels between 0.44 and 3.30 nmol per ml. The highest value was in a case of mediastinal choriocarcinoma. Sixteen of the 18 patients had values greater than two standard deviations above the mean value in the control group.

**Trace Elements**

Trace elements may play an important role in cancer. Their use as diagnostic aids has not been outlined. Serum copper concentrations have been reported to be abnormally high in untreated cases of Hodgkin's disease. In one study, the high levels returned toward normal when the disease was successfully treated and became abnormal during relapse, usually before clinical indications were noted.

**Lipids**

Lipids also play an important role in the molecular biochemistry of the cancer cell. Serum lipoproteins are reported present in decreased amounts in patients with advanced breast cancer and gynecological tumors. In a study of 122 patients with malignant tumors, lipoproteins, phospholipids and cholesterol were markedly decreased and the lipoprotein decreases were not related to extent of disease or type of treatment. Another interesting lipid observation is elevated urinary excretion of non-esterified cholesterol in women with carcinoma of the steroid producing glands and in men with testicular and prostate cancers. Abnormal excretions (above 1.20 mg per 24 hours) were observed in 29 of 32 patients with adenocarcinoma of the prostate and in each of eight patients with choriocarcinoma, teratocarcinoma and embryonal cell carcinoma of the testis. Excretions were within normal limits in 19 patients with testicular seminomas. The abnormal values seemed to correlate with the clinical course of the disease.

**Tryptophane Metabolites**

Tryptophane metabolites appear to be excreted in abnormal amounts in patients with breast cancer. Exploiting this observation a tolerance test has been described for use in cancer diagnosis and follow-up in patients with Hodgkin's disease, breast and bladder cancer. In this test, urinary tryptophane metabolites are measured following an oral dose of tryptophane. In a study of 36 patients with bladder cancer, increased urinary kynurenine was found in 12 subjects, 3-hydroxy-kynurenine in 25, kynurenic acid in 9, xanthurenic acid in 4 and acetylkynurenine in 10. The supranormal excretions reverted toward normal levels when there was a favorable response to therapy. The rationale for the test is the finding of increased activity of tryptophane pyrrolase and decreased kynureninase in the liver of patients with cancer.

**Conclusions**

It is not possible to review all of the biochemical tests that have been proposed as diagnostic aids in cancer. Most of these have not had the specificity necessary to make them useful in cancer screening and
cancer control. It is to be hoped that as new and more sensitive biochemical techniques are developed, a diagnostic procedure of a general nature will be found. In this review there purposefully has not been a discussion of tumor associated antigens. However, it should be kept in mind that these procedures are properly designated biochemical assays.

References


