Review of Pyridoxal Phosphate and the Transaminases in Liver Disease

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ABSTRACT

In vitro supplementation with the active form of vitamin B₆, pyridoxal-phosphate (PLP), increases measurements of both serum aminotransferase enzymes, L-aspartate: 2-oxoglutarate amino transferase, EC 2.6.1.1 (AST) and L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2 (ALT). The plasma PLP level in normal individuals clearly relates inversely to the degree of stimulation of serum AST and ALT. PLP added in vitro increases the reference values but does not decrease the biological variability of AST measurements in healthy individuals.

Since B₆ deficiency is observed in alcoholics, in some significant percentage of hospitalized patients and in apparently healthy people over age 64, these individuals will show PLP stimulation of their serum aminotransferase enzymes.

Patients with liver disease show lesser activation with PLP of AST activity but not ALT activity than patients with heart disease (myocardial infarction). AST isoenzyme measurements in the form of a mitochondrial AST/total AST ratio may discriminate alcoholic hepatitis from all other hepatic diseases. In renal dialysis patients including transplant patients, it may be desirable to measure the aminotransferases with added PLP in order to reflect better the cytolytic state of the liver. While unconfirmed studies suggest the combination of PLP activation and AST isoenzyme measurements may aid in the diagnosis of hepatoma, PLP activation per se does not provide clear cut improved diagnostic value of AST and ALT in liver diseases. However, in view of PLP incorporation into the IFCC reference methods for AST and ALT, and the National Reference System for the Clinical Laboratory, it is recommended that PLP be included in all AST and ALT measurements.

Introduction

The enzymes AST and ALT carry out the interconversion of amino acids and alpha-keto acids by the transfer of amino groups and thus function as a link between amino acid metabolism and carbohydrate metabolism. Both aminotransferases require vitamin B₆, pyridoxine, at their active sites in the physiologically active form of pyridoxal-5'-phosphate (PLP). Hamfelt, in 1964 was the first to demonstrate and we confirmed in 1973 the increased AST activ-
ity of normal and pathological sera after *in vitro* supplementation with PLP. Subsequently, we demonstrated the rate of association of PLP and the apoenzyme was retarded by phosphate buffer in comparison with tris (hydroxymethyl) aminomethane (Tris) or six other organic buffers. Numerous investigations of the effect of measurement conditions on the catalytic activity of both aminotransferases have been made over the past ten years, which have been reviewed recently by Rej. Clearly the serum aminotransferases are not fully saturated with the coenzyme.

While the activation findings for both normal and pathological sera are highly variable, the analytical reasons for these differences have been only partly clarified and the variation may also reflect nutritional status. Thus, Westerhuis and Halkenscheid found a very significant inverse linear relationship between the plasma PLP concentration and the amount of stimulation of AST or ALT in healthy individuals. Nevertheless, the International Federation of Clinical Chemistry reference methods for AST and ALT as well as the American Association for Clinical Chemistry recommended methods include supplementation with PLP. In addition, several laboratories, reporting in the CAP Enzymology Survey, utilize commercially available kit methods incorporating PLP while many others do not. However, now that Bowers and McComb have introduced the International Clinical Enzyme Scale (ICES) concept, comparison of results by different methods may be less of a problem. They propose that all AST methods can be systematically converted to the ICES Scale and that all AST results will be numerically compatible. Demonstration of its applicability has been made by converting the AST results reported in the 1983 College of American Pathologists' Comprehensive Chemistry Survey and a New York State AST Survey. In each instance, conversion brings about convergence of multiple method mean data bars to a single coherent mean data bar.

**Relationship of Vitamin B₆ Deficiency**

Vitamin B₆ is widely distributed in most common foodstuffs, but losses from food of up to 70 percent can occur during cooking, processing, and refining. The body's need for Vitamin B₆ appears to be directly related to protein intake. Also certain clinical situations such as exposure to radiation, drug therapies such as isoniazid, penicillamine, anovulatory agents, etc. and cardiac failure as well as pregnancy and lactation increase the requirement. The coenzyme forms of Vitamin B₆ are known to participate in more than 50 enzymatic reactions involved in the metabolism of amino acids. A recent review has addressed these and the other functions of PLP in considerable detail.

The main form of the vitamin in the plasma is PLP which upon hydrolysis yields pyridoxal (PL). Human body stores are minimal and can be depleted quickly. While either urinary 4-pyridoxic acid in 24-hour specimens or the 4-pyridoxic acid per mg of creatinine excretion in random urine can be used to measure vitamin B₆ status in humans, the plasma or red cell level of PLP as well as the tryptophane load test are good indices of the nutritional status of B₆.21 Thus, Hamfelt and Ranke et al showed marked decreases in PLP level with age which diet supplement corrected in some but not all individuals. Furthermore, Hamfelt found that persons over 64 years of age had 30 percent lower levels of serum AST than groups aged 41–63 or 18–40, and showed a higher percentage stimulation by AST (19 percent vs. 6.5 percent vs. 5 percent); enzyme activity was not restored to the levels shown by the younger groups.
Although the more recent work of Deledda\textsuperscript{12} tends to support Hamfelt's findings and they appear to hold for ALT as well, more definitive studies are needed.

While an increased intake of pyridoxine for a few days had only a slight effect on the concentration of circulating AST apoenzymotransferase\textsuperscript{34} in healthy individuals, Vitamin B\textsubscript{6} deficiency may contribute significantly to interindividual differences in the PLP stimulation of serum ALT and AST in hospitalized patients.\textsuperscript{22} In another study\textsuperscript{39} Leevy et al observed that 27 percent of randomly selected municipal hospital patients had low serum levels of vitamin B\textsubscript{6}.

Very recently, Westerhuis and Hafkenscheid\textsuperscript{77} reported a very significant inverse relationship between the plasma PLP concentration in normal, healthy individuals and the stimulation of serum ALT and AST by \textit{in vitro} PLP, i.e., the degree of stimulation of the apoenzyme of the two serum aminotransferases clearly depends inversely on the plasma PLP concentration \textit{in vivo}. Plasma from men showed a 30 percent higher mean value than plasma from women but an age factor was not included. The coefficient of variation for the ALT determinations is about twice as large as for the AST determinations on the same healthy individuals, which may reflect a difference in biological variation. However, their most significant observation was the fact that the degree of \textit{in vitro} stimulation of both serum AST and ALT in healthy normal individuals is inversely proportional to the \textit{in vivo} plasma level of PLP. While the authors speculated about the underlying cause in terms of sex, aging of cells, etc., no valid explanation was arrived at.

Does the addition of PLP decrease the biological variability of the enzyme measurements? Rej and Vanderlinde\textsuperscript{38} postulated that the dispersion of reference values of AST might decrease if all of the catalytically inactive apo-AST in serum were activated by saturating it with pyridoxal phosphate. However, Hörder and Bowers\textsuperscript{24} found the biological variability (CV's) of serum AST measurements of nine healthy individuals over a two week period with and without \textit{in vitro} PLP stimulation to be the same. They presumed that their results regarding the biological variabilities of AST measured with and without PLP for their selected subset of persons would hold true also for a universe of the healthy population. While they observed an average 39 percent increase in serum AST of normal healthy individuals upon the addition of PLP, a 49 percent increase was observed for 48 sera from hospitalized subjects all of whom had AST values within normal range. Obviously the incorporation of PLP alters the reference ranges upward for ALT and AST as shown by Plebani et al\textsuperscript{51} who established reference values for optimized ALT and AST measurements supplemented with PLP.

**General Clinical**

A very important question is whether the addition of PLP leads to greater diagnostic utility of the aminotransferases, i.e., better differentiation between disease states.

In 1966 Hamfelt\textsuperscript{22} measured AST with and without added pyridoxal in old persons, in acute intermittent porphyria, in heart muscle necrosis, and in thyrtoxicosis. In addition, he measured the effect of pyridoxal on both aminotransferases in patients with chronic alcoholism. Significant changes were observed in all groups of patients except for three of six measurements of ALT in chronic alcoholic patients. Later Hamfelt and Tuvemo\textsuperscript{53} studied the effect of exogenous vitamin B\textsubscript{6} on the maternal serum and red cell AST activities at delivery, assayed in the presence and absence of PLP, as well as the cord blood activity.
Many groups during the middle nineteen seventies including Ury and Chassy,73 Cheung and Briggs,10 Rosalki and Bayoumi,62 Moss,47 Ratnaike and Moss,54 Jung and Egger,26 and Jung and Bohm25 studied the effect of in vitro PLP supplementation on aminotransferase assays in various diseases and conditions. These data, based on various assay systems for AST and ALT, were reviewed by Campbell and Sanderson9 in 1979. In general, they suggest no clear cut increased diagnostic utility perhaps owing to the highly variable factors affecting the extent of aminotransferase activation with PLP including: (1) concentration of PLP, (2) duration of preincubation, (3) the buffer system used since phosphate inhibits60 the reassociation of PLP and apoenzyme, (4) concentration of aminotransferase substrates, and (5) patient variability. However, significant stimulation was observed in a number of disease categories including myocardial infarction (MI), liver diseases, conditions requiring extracorporeal blood treatment, nutritional deficiency and pregnancy which demonstrated the need for the better controlled studies which have evolved more recently.

Bergmeyer, Scheibe and Wahlefeld4 in 1978 developed optimized methods for both serum AST and ALT and as part of their study described the effects of PLP on pooled sera from either patients with diagnosed heart (myocardial infarction) or liver disease. Their careful studies included individual sample blanks. Both serum AST and ALT from liver disease patients showed about 15 percent stimulation by PLP. On the other hand, serum AST from heart disease patients showed a 49 percent stimulation whereas serum ALT showed only 7.3 percent. Thus, sera for AST from patients with heart disease show greater activation with PLP than sera for AST or ALT activation from patients with liver diseases. These results confirm the earlier findings of Raitke and Moss54 and Rosalki and Bayoumi.62

As part of the IFCC recommended procedure for serum AST,5 as modified for use with a centrifugal analyzer, Bruns, Savory et al8 in 1981 evaluated the effect of PLP on serum AST from four types of patients: liver, heart, pediatric and renal. Sera from patients with heart disease (myocardial infarction) showed more stimulation by PLP than those from any of the other three groups. In 1983 Dols and van Zanten15 published their findings comparing the German Society for Clinical Chemistry (DGKC) recommended AST method, the first “optimized” procedure which is carried out in phosphate buffer and does not utilize PLP, with the IFCC method5 which utilizes PLP and tris buffer. The indices of intra- vs. interindividual variability showed that reference ranges by both methods are insensitive to intra-individual variations in serum AST activities, in agreement with results published by Hördér and Bowers.24 Thirty of 745 results for patients’ samples were identified as abnormal by the IFCC-based method but normal by the DGKC method; 13 were classified as normal by the IFCC-based method but abnormal by the DGKC method.

The patients’ samples were subdivided into the categories of kidney, liver, heart and unclassified. The correlation between AST values by each method for samples obtained from heart patients was poorer than for the other patient categories. This phenomenon apparently is produced by the large spread in PLP activation for samples from patients with heart disease as has been previously observed by others not using IFCC-based methodology.54,62 Thus PLP stimulation of samples from patients with heart disease is more variable as well as larger than observed for patients with kidney, liver or unclassified diseases. However, Dols and van Zanten’s stud-
ies demonstrated that AST/ALT ratios obtained by the IFCC methods with PLP added, carried out on sera from 101 patients suffering from biopsy-proven liver disease, were still utilizable. In a comparative study of improved DuPont ACA methodology with added PLP in 1981, Garber, Feldbruegge, and Hoes sel found highly variable percentages of stimulation by PLP in a small number of patients with MI, on dialysis with carcinoma of the colon with hepatic involvement, with hepatoma, or metastatic melanoma with liver involvement. The patients with various types of malignant liver pathology showed a greater increase in observed AST than reported earlier by Rosalki and Bayoumi for chronic liver disease. Thus studies involving correlation of various generalized categories of pathology with PLP stimulation of the aminotransferases have shown higher results but no clear cut improved diagnostic utility.

Liver

A number of investigations involving PLP activation of the aminotransferases have been directed to the study of patients with specific liver diseases. In table I are summarized the effect of PLP on aminotransferase assays in various liver diseases and other conditions to be described.

In evaluating the effects of in vitro PLP supplementation on serum AST and ALT in patients with liver disease, several observations should be kept in mind. According to Mitchell et al, base line plasma PLP concentrations are significantly lower in cirrhotic patients than in normal persons owing to the abnormal regulation of plasma PLP by cirrhotics as measured by a test dose of PLP. Roussov et al who observed that approximately 90 percent of patients with severe cirrhosis are vitamin B deficient, found that treatment with B increased the mean PLP stimulated AST level from 118 ± 17 to 146 ± 20 U/l which is not statistically significant. Secondly, plasma PLP tends to be reduced in patients with extrahepatic obstruction. This may be due to the hydrolysis of plasma PLP by alkaline phosphatase in conditions with raised alkaline phosphatase. Thirdly, the plasma clearance of 31 ml/min for PLP in healthy individuals is increased in several types of liver disease: namely, alcoholic cirrhosis—63 ml/min (2× normal); acute viral hepatitis—106 ml/min (3× normal) and in extrahepatic obstruction—132 ml/min (4× normal). These increases in the plasma clearance of PLP are presumably due to the increased removal of plasma PLP by the “sick” liver, possibly by an alkaline phosphatase located in the hepatocyte plasma membrane. On the other hand, Roussow et al found that in fulminant hepatic failure the plasma level of PLP changed markedly, increasing within one week of the onset of symptoms from a mean of 11.3 to 79.8 ng/ml. In parallel with the rise in PLP, there was an increase in serum AST holoenzyme activity.

An additional complexity to keep in mind is that while ALT is a single soluble cytoplasmic enzyme, AST activity consists of a soluble or cytoplasmic enzyme (c-AST) and in addition a mitochondrial isoenzyme (m-AST), each of which can be stimulated differently by PLP and is best measured with different substrate concentrations of alpha-ketoglutarate. As an example, the AACC recommended method for total AST (t-AST) utilizes a higher alpha-ketoglutarate concentration than does the IFCC recommended method for total AST and would therefore be expected to measure more optimally m-AST when both isoenzymes are present in the serum.

Recently, Rej has shown that catalytical measurements and immunological measurements give about equivalent values for the two isoenzymes of AST
<table>
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<tr>
<th>Reference</th>
<th>Diseases or Conditions Studied</th>
<th>Significant Findings</th>
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<tr>
<td>Horder and Bowers (1977)(^{22})</td>
<td>Healthy persons</td>
<td>Biological variability of AST measurements is the same with or without in vitro PLP supplementation.</td>
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<tr>
<td>Westerhuis and Hafkenscheid (1983)(^{6})</td>
<td>Healthy individuals</td>
<td>Plasma PLP levels are inversely proportional to AST/ALT stimulation.</td>
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<tr>
<td>Bergmeyer, Scheife, and Wahlefeld (1978)(^{32})</td>
<td>Myocardial infarction (MI) Liver diseases</td>
<td>Sera for AST from patients with heart disease (MI) showed 3X greater activation with PLP than AST and ALT activation from patients with liver disease.</td>
</tr>
<tr>
<td>Bruns, Savory et al (1981)(^{33})</td>
<td>Liver disease</td>
<td>Sera from patients with heart disease showed more stimulation of AST by PLP than patients in other three groups.</td>
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<tr>
<td>Dols and van Zanten (1983)(^{34})</td>
<td>Heart disease</td>
<td>Sera from patients with heart disease showed markedly different activation with PLP as compared with sera from other patients categories.</td>
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<tr>
<td>Dols and van Zanten (1983)(^{34})</td>
<td>Heart disease Liver disease</td>
<td>Ratio for AST/ALT, as used in the differentiation of liver disease, can be used either with or without PLP activation.</td>
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<tr>
<td>Roussouw, Labadarios et al (1978)(^{37})</td>
<td>Cirrhotic patients</td>
<td>In vivo treatment with PLP increased the mean in vitro PLP stimulated AST level from 118 to 146 U/l (not significant).</td>
</tr>
<tr>
<td>Jung, Ohlrich et al (1978)(^{42})</td>
<td>Healthy persons Chronic liver disease (7 subtypes) Acute viral hepatitis</td>
<td>Serum AST is activated by 37% and ALT by 15% for healthy persons. Apo AST activity in patients with chronic liver diseases is not changed as compared with that of healthy persons. However, the relative stimulation rate is significantly smaller. Apo ALT activity and corresponding relative stimulation is significantly greater as compared with healthy persons. In acute viral hepatitis, a decrease of AST and ALT activity is followed by a decrease in the PLP stimulation (less apoenzyme) in the course of the disease. In the authors' experience, the diagnostic value of determination of the aminotransferases could not be improved by the addition of PLP.</td>
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TABLE I (continued)

Effect of Pyridoxal Phosphate on Aminotransferase Assays in Various Liver Diseases and Other Conditions

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<tr>
<th>Reference</th>
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<th>Significant Findings</th>
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<tr>
<td>Matloff, Selinger, and Kaplan (1980)</td>
<td>Healthy individuals, Alcoholic liver disease, Primary biliary cirrhosis</td>
<td>The AST/ALT ratio in serum and liver tissue was increased only in individuals with alcoholic liver disease but did not reach statistical significance. The addition of saturating amounts of PLP to the Karmen assays did not increase ALT or AST activity of liver tissue of patients with alcoholic liver disease and chronic active hepatitis.</td>
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<tr>
<td>Diehl, Potter et al (1984)</td>
<td>Alcoholic hepatitis</td>
<td>Addition of PLP to liver homogenates increased liver ALT but not AST, in all patients with initially low plasma PLP. Significantly higher ratios of apo (PLP stimulation) to holo AST activity for both the serum mito and cytoplasmic AST isoenzymes were observed in patients with hepatoma than in patients with acute hepatitis (6 pts.) and in patients with miscellaneous liver diseases (5 pts.)</td>
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<tr>
<td>Kamei, Ohkubo, and Yamanaka (1979)</td>
<td>Healthy individuals, Hepatoma, Acute hepatitis, Miscellaneous liver disease</td>
<td>PLP produced a stimulation of 29-136% in serum AST from 7 to 9 dialysis patients and of 64-258% in serum ALT with all 9 dialysis patients showing increases. PLP supplementation resulted in 56% higher AST and 71% higher ALT in patients with kidney transplants in the absence of chronic renal failure. PLP must be added to the reaction mixture if one is to determine the aminotransferases, especially ALT, correctly in patients with kidney transplants and thus to diagnose liver diseases in such patients. The corresponding relative stimulation rates of serum AST and ALT by PLP were not changed in the hemodialysis patients but in renal transplant recipients the relative stimulation of AST was significantly smaller and that of serum ALT was greater. Therefore, PLP addition to the reaction mixture for determination of ALT activities in patients with renal allografts should be used for liver assessment in these patients. While serum AST stimulation was the same for patients with renal allografts as for healthy individuals, the stimulation of ALT was greatly increased except for patients who received cyclosporine A instead of azathioprine.</td>
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<tr>
<td>Campbell and Sanderson (1979)</td>
<td>Dialysis patients</td>
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<td>Jung, Mildner et al (1981)</td>
<td>Chronic hemodialysis patients, Kidney transplant patients</td>
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<tr>
<td>Hafkenscheid, Rosier and van Dijk (1984)</td>
<td>Patients with renal allografts</td>
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present in liver tissue. However, he demonstrated that the catalytic methods which we currently use to measure the AST isoenzymes in plasma grossly underestimate the mitochondrial proportion of the total AST activity and the amount of plasma enzyme present by catalytic measurements to be far less than found by immunological assay. On the other hand, Leung et al\textsuperscript{41} found that the proportion of catalytically active c-AST found in serum samples to be about one-half that of the available isoenzyme, whereas nearly all of the m-AST was catalytically active. Thus, in either event, current methodologies for serum AST may fail to measure a major portion of the t-AST enzyme actually present, presumably because it is not enzymatically active. Immunologic mass unit (ng/ml) measurement of the AST isoenzymes as well as total AST and total ALT may one day provide us with data offering many new insights into the pathophysiology of liver disease.

Let us now consider currently available published investigations on the role of PLP activation of AST and ALT in liver diseases. Jung et al\textsuperscript{29} investigated the amounts of c-AST, m-AST, apo and total AST and apo and total ALT in the serum of healthy individuals as well as patients having any of seven types of chronic liver disease. They found the apo AST activity in patients with various chronic liver diseases to approximate those found in healthy persons even though the latter had increased amounts of total AST activity. Thus, patients with chronic liver disease showed significantly smaller PLP stimulation rates for AST than healthy persons. On the other hand, apo ALT activity and its corresponding relative stimulation was significantly greater in patients with chronic liver disease than in healthy persons. While there were some partly significant differences between the various groups with liver diseases, the small numbers of patients in the groups limit the conclusions which are available. For healthy persons there was significant correlation between the value of either apo AST or relative stimulation and m-AST, but this correlation was lost in liver disease confirming the earlier findings of Rosalki and Bayoumi\textsuperscript{62} and Ratnake and Moss.\textsuperscript{54} In the case of acute viral hepatitis, a decrease of AST and ALT activity is followed by a decrease of ALT apoenzyme activity in the course of the disease. However, in their overall experience the diagnostic value of determinations of AST and ALT aminotransferase activities in liver disease could not be improved by addition of PLP.

Miyake\textsuperscript{46} studied the release of the AST isoenzymes and ALT in 43 healthy controls and in 280 patients with liver diseases. High m-AST activity was found in the acute stage of acute hepatitis, in subacute hepatitis and in primary biliary cirrhosis in which conditions were generally associated with high total AST activities. The activity ratio of m-AST/t-AST varied depending on the stage and severity of the liver disease. The m-AST/t-AST ratio was decreased in acute, fulminant and subacute hepatitis while no significant reduction in ratio was found in bile duct obstruction, alcoholic liver injury, or metastatic liver cancer. Although relatively high m-AST/t-AST ratios were found in some patients with severe hepatic injury, Miyake found no definite association with a poor prognosis. Also observed was a marked elevation of t-AST over ALT in advanced chronic hepatitis, liver cirrhosis and primary hepatoma perhaps as Miyake suggests owing to a preferential leakage of cytoplasmic AST. Skrha et al\textsuperscript{70} also investigated the isoenzymes of AST in chronic liver diseases which included chronic hepatitis in 27 patients, liver cirrhosis in 23 patients, and secondary neoplastic affection of the liver in 6 patients. All patients with biochemically active
forms of liver disease manifested increased t-AST as well as cytoplasmic AST and 57% manifested simultaneously increased m-AST. Skrha and his associates observed that 13% of the patients with stabilized forms of liver disease manifested an isolated increase of the m-AST which they believe could be of value in evaluating the course of the disease. Also they observed a strikingly high share of m-AST in focal processes of malignant character in the liver. PLP stimulation tests were not carried out as a part of either Miyake's or Skrha et al.'s investigations.

More recently, Jung and Pergande in association with Rej, Schreiber and Schimmelpfennig measured the activities of two mitochondrial enzymes AST and glutamate dehydrogenase (EC 1.4.1.2) (GLDH), in the sera of healthy persons and in 43 patients with chronic liver diseases. The distribution of activities for GLDH, but not m-AST, was sex dependent with men showing higher values. While there was a very weak correlation between the activities of the two mitochondrial enzymes in healthy persons (r = 0.439), patients with chronic liver disease exhibited a greater increase in the activity of GLDH than of m-AST and the correlation between the two mitochondrial enzymes was stronger. The diagnostic sensitivity and specificity of either mitochondrial enzyme are reported to be less than that of total AST, ALT or gamma-glutamyltransferase (EC 2.3.2.2).

Chronic Alcohol Abuse and PLP

In 1974 Lumeng et al suggested a possible relationship between chronic alcohol abuse, PLP deficiency and lowered levels of hepatic ALT activity. Thus, Matloff, Selinger and Kaplan investigated AST and ALT levels with and without added PLP in liver biopsy specimens and in sera obtained the same day from patients with alcohol problems. Hepatic ALT activity was significantly decreased in the liver tissue of patients with alcoholic hepatitis and cirrhosis as compared with the activity in individuals with normal livers and individuals with primary biliary cirrhosis. The decreased hepatic ALT activity was not related to the presence of the cirrhosis in the biopsy specimens and was not increased by the addition of saturating amounts of PLP to the assay mixture. The AST/ALT ratio in serum and liver tissue increased only in individuals with alcoholic liver disease and not in four other types of chronic liver disease. Since Matloff and his coworkers did not measure the serum or hepatic vitamin B6 content of their patients and did not perform liver biopsies in alcoholic patients without liver disease, their data do not shed major light on the role of PLP deficiency in chronic alcohol abuse.

More recently, Kakuma, Leevy, Frank and Baker have shown that PLP protects in vivo and in vitro against the liver cytotoxicity of acetaldehyde, an hepatotoxic metabolite of ethanol. In a subsequent 1984 paper, Diehl et al studied the relationship between PLP deficiency and activities of serum and liver AST and ALT in 12 patients with alcoholic hepatitis. According to their findings, the patients with lower initial plasma PLP levels did not have significantly more severe acute hepatocellular injury histopathologically. Hence, Diehl et al's data do not support the contention that adequate PLP levels protect the liver from alcohol induced cytotoxicity. Neither does PLP deficiency seem to explain the low liver AST levels in patients with alcoholic hepatitis because neither in vivo nor in vitro did PLP supplementation bring about a change in AST levels in the liver. However, PLP in vivo did bring about a decrease in serum AST and an increase in serum ALT. Hence, PLP depletion appears to be par-
tially responsible for the high serum AST/ALT ratio that is typical of patients with alcoholic hepatitis.

Panteghini et al.\(^4^9,5^0\) sought to establish a relationship between the AST isoenzyme levels in serum and the degree of hepatic damage in a group of 69 patients with various hepatic diseases. During hepatic damage, c-AST was found in greater quantities than m-AST but the latter increased to a greater extent in alcoholic hepatitis. Thus, the most significant new data were the much greater increase in plasma m-AST and the ability of the m-AST/total AST ratio to discriminate between alcoholic hepatitis and all other hepatic diseases. PLP was not added to any of the assays in their study which emphasized the role of the AST isoenzymes. The role of m-AST in estimating liver cell structural damage has been previously emphasized by Skrha et al.\(^7^0\) and the condition has been described as a “necrotic type” by Schmidt and Schmidt.\(^6^6\)

Recently, Evans et al.\(^1^6\) have reported that serum AST and GGT measurements in alcoholics admitted to a detoxification unit are not valid indices of alcohol intake since the correlations were rather low and the sensitivity was only about 50 percent.

Hepatoma

The most striking study reported to date is by Kamei et al.\(^3^3\) who combined immunological separation of the AST isoenzymes with their stimulation by PLP in patients with a variety of hepatic diseases. Significantly higher ratios of apo to holo AST activity for both the mito and soluble isoenzymes were observed in the patients with hepatoma in contrast with cirrhosis, acute hepatitis, chronic hepatitis and other hepatic disorders. Thus patients with hepatoma differed significantly in the amount of PLP stimulation observed from patients with all other liver diseases. There was negligible overlap of the hepatoma group with all other groups. To date there has been no followup of these intriguing findings reported in 1979; perhaps owing to the technical availability and complexity of the assays combined with the greater rarity of hepatoma in the American population. However, an immunoprecipitation assay, using antiporcine antibody conjugated to sheep erythrocytes, has been described\(^7^4\) and is the basis for a diagnostic “kit” method from Eiken Chemical Co. Ltd. in Japan, and now available in North America.\(^5^6\)

Reye’s Syndrome

Similarities between Reye’s syndrome and fulminant hepatic failure suggest that these illnesses may share many biochemical features. Thus Faraj et al.\(^1^7\) showed the concentrations of PLP in plasma are significantly higher at the onset of disease than after treatment. Further, the concentrations of PLP in plasma at the time the patients entered the hospital correlated at the 0.73 level with their ALT activities and suggests that the two may be released together from the liver. The low plasma concentrations of PLP observed after treatment presumably reflect the reversible process of hepatic injury and also may represent decreased hepatic stores of PLP in Reye’s patients.

Blood Banking

Although not involving PLP stimulation, three major prospective studies have established a correlation of elevated levels of ALT in donor blood with increased risk of non-A, non-B hepatitis (NANB) in blood recipients.\(^1^,2,3^1\) Thus directors of donor programs such as the Greater New York Blood Program have instituted ALT testing of all donor bloods, while other experts question
whether such enzymatic screening of donor bloods should be done.\textsuperscript{52} While answers are not available at this time on this important issue, Kolins\textsuperscript{35} has shown recently that although the predicative value of a negative ALT test is 95 percent, the predicative value of a positive ALT test is only 30 percent. This means only 30 percent of the samples with elevated ALT levels will cause non-A, non-B post transfusion hepatitis, the remaining 70 percent being false positives. Kolins feels this low predicative value has prevented acceptance of the test. On the other hand, Silverstein et al\textsuperscript{69} through a cost-effectiveness analysis found that sensitivity analyses indicate that screening would be cost-saving for a wide range of cost estimates and number of units per transfusion. They concluded that ALT screening is warranted until more sensitive and specific screening tests for transmissibility of non-A, non-B hepatitis become available.

**Renal**

In 1972 Wolf and his colleagues\textsuperscript{78} observed, as a clinical laboratory finding, low AST activity in the sera of patients undergoing chronic hemodialysis. Although the effect of \textit{in vitro} PLP supplementation was not examined by them, the authors did attempt to explain their results in terms of the depletion of plasma pyridoxine during long-term dialysis. Subsequently, Dobbelstein et al\textsuperscript{14} first presented evidence for B\textsubscript{6} deficiency in erythrocytes in uremia and Stone and his colleagues demonstrated clearly not only the decreased plasma AST\textsuperscript{75} levels but showed decreased plasma PLP\textsuperscript{72} levels in uremia. The same group\textsuperscript{71} in 1977 found the mean plasma clearance rate of uremic patients to be almost two-fold greater than for control patients. More recently, Lacour et al\textsuperscript{37,38} have documented further the decreased plasma PLP levels in undialyzed and dialyzed uremic patients, and note loss of PLP does not occur in the plasma ultrafiltrates as a result of the hemodialysis. A similar PLP deficiency was observed in kidney transplant patients with normal or near normal renal function, that is, in the absence of chronic renal failure. They believe that a chronic deficiency in tissue PLP concentration induces the decrease in apo-transaminase synthesis, which is observed generally in hemodialysis patients in the absence of cytolytic liver disease.\textsuperscript{72,75,78} Major metabolic effects through \textit{in vivo} repletion with B\textsubscript{6} have been demonstrated in maintenance dialysis patients by Kleiner et al.\textsuperscript{34}

Following up on Wolf’s\textsuperscript{78} and Wannock’s\textsuperscript{75} findings of low AST activity in hemodialysis patients, Campbell and Sanderson\textsuperscript{9} investigated the effect of \textit{in vitro} PLP supplementation on aminotransferase assays of serum from dialysis patients. They obtained an increase stimulation of 29–136 percent in serum AST from 7 of 9 dialysis patients and an even greater stimulation of 64–258 percent in serum ALT with all 9 dialysis patients showing increases. The extent of activation in several of the samples was sufficient to cause the observed enzyme level to increase from a normal or borderline normal value to an elevated or pathological level. While both Jung and his colleagues\textsuperscript{27,28} and Lacour’s group\textsuperscript{37,38} have recommended PLP be added to the reaction mixture if one is to determine correctly the aminotransferases, especially AST, in patients with kidney transplants, and thus to diagnose liver disease in such patients, their findings suggest strongly that serum AST and ALT aminotransferase assays on hemodialysis patients as well should be supplemented with PLP if liver disease is to be detected. More recently, Haffenscheid et al\textsuperscript{19} have demonstrated the type of drug treatment, e.g., cyclosporin or azathioprine, may effect the percentage of AST and ALT stimulation observed in
renal allograft patients in contrast to normal patients.

Miscellaneous

Several patient cases in which serum AST was complexed with immunoglobulin G (IgG) have been reported as well as one patient whose serum contained complexes between AST and both IgA and IgG. The latter patient suffered from lung cancer with metastasis to the liver. Nagamine and Okochi were able to demonstrate that only c-AST binds to IgG, whereas IgA binds to both c-AST and m-AST. While serum AST-IgG complexes have been demonstrated in both healthy and diseased individuals, and have been associated with various liver diseases, their clinical significance is unclear.

Recently, Leklem and Shultz have shown for the first time that exercise in the form of long distance running dramatically alters plasma levels of PLP and total B₆. They speculate that the significant changes in vitamin B₆ metabolites found were related to an increased need for cofactor for gluconeogenesis.

Summary

The literature relative to PLP and the measurement of aminotransferase (AST and ALT) activity has been reviewed in detail recently by Rej. In 1979 Campbell and Sanderson summarized the published work on the diagnostic significance of PLP supplementation on aminotransferase assays, thus this review has focused on findings since their publication.

The plasma PLP level in normal individuals clearly relates inversely to the degree of in vitro stimulation of serum AST and ALT. PLP added in vitro increases the reference values for AST but does not decrease the biological variability of such measurements in healthy individuals.

Since B₆ deficiency is observed in alcoholics, in some significant percentage of hospitalized patients and in apparently healthy people over age 64, these individuals will show PLP stimulation of their serum aminotransferase enzymes.

Patients with liver disease show lesser activation with PLP of AST activity but not ALT activity than patients with heart disease. AST isoenzyme measurements in the form of a mitochondrial AST/total AST ratio may discriminate alcoholic hepatitis from all other hepatic diseases. Unconfirmed studies suggest the combination of PLP activation and AST isoenzyme measurements may aid in the diagnosis of hepatoma. In renal dialysis patients including transplant patients, it may be desirable to measure the aminotransferases with added PLP in order to reflect better the cytolysis of the liver. Table I summarizes the effect of PLP on aminotransferase assays in the various liver diseases and conditions described.

In conclusion, although PLP activation per se does not provide clear cut improved diagnostic value of AST and ALT in liver diseases, it is recommended that AST and ALT be included in all routine AST and ALT measurements in view of its inclusion in the IFCC reference methods for AST and ALT and their adoption into the National Reference System for the Clinical Laboratory.

References


