

# Hydrolysis of pyridoxal-5'-phosphate in plasma in conditions with raised alkaline phosphatase

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**SUMMARY** Hydrolysis of pyridoxal phosphate in plasma was demonstrated in patients with liver disease and other conditions with raised alkaline phosphatase, and this usually closely paralleled the alkaline phosphatase level, whether of liver or bone origin. The endogenous plasma pyridoxal phosphate was inversely related to the alkaline phosphatase, and plasma hydrolysis of pyridoxal phosphate may at least in part be responsible. Very large doses of vitamin B<sub>6</sub> may be necessary to compensate for this hydrolysis.

Pyridoxal-5'-phosphate is the active coenzyme form of vitamin B<sub>6</sub>. It is also the main form in plasma circulating as an albumin bound complex.<sup>1-3</sup> The plasma level of pyridoxal phosphate is commonly low in liver disease.<sup>4-6</sup> The reason for this is uncertain but there is evidence that it may be due to an increased degradation of pyridoxal phosphate.<sup>5,6</sup>

Pyridoxal phosphate is known to be hydrolysed to pyridoxal by alkaline phosphatase.<sup>7</sup> This also occurs when pyridoxal phosphate is bound to albumin, but at a slower rate.<sup>2</sup> Dialysis studies have suggested that there is some free pyridoxal phosphate in equilibrium with the albumin-pyridoxal phosphate complex and this could explain why hydrolysis by alkaline phosphatase occurs in spite of binding to albumin.<sup>2</sup>

We have investigated whether there is a significant hydrolysis of pyridoxal phosphate in the plasma of patients with a raised alkaline phosphatase.

## Methods

Twenty-five subjects were studied. Ten were control subjects and were healthy members of the staff. Fifteen were patients with conditions associated with a raised alkaline phosphatase. Eleven of these had hepatobiliary disorders—namely, common bile duct obstruction (two), primary biliary cirrhosis (one), chronic active hepatitis (one), haemochromatosis (two), secondary carcinoma (one), hydatid cyst (one), drug-induced hepatitis (one), cirrhosis (one),

and peliosis hepatis (one). The other four had conditions associated with a raised bone alkaline phosphatase—namely, Paget's disease (two) and secondary carcinoma (two) (the primaries being carcinoma of prostate and breast).

Serum alkaline phosphatase was measured by the method of Kind and King as adapted to a Vicker's M300 (normal range 25-200 IU/l). Bone alkaline phosphatase was distinguished from liver alkaline phosphatase by electrophoresis and heat stability.

In order to measure plasma hydrolysis of pyridoxal phosphate, plasma from heparinised blood was incubated at 37°C for one hour with and without the addition of 148 ng/ml pyridoxal phosphate (equivalent to 100 ng pyridoxal). The endogenous level of pyridoxal phosphate and pyridoxal in plasma, and the amount of pyridoxal formed from hydrolysis of pyridoxal phosphate incubated with plasma, was measured by *Lactobacillus casei* microbiological assay as we have described previously.<sup>8,9</sup> This assay is specific for pyridoxal. After acid hydrolysis pyridoxal phosphate is dephosphorylated to pyridoxal and can thus be assayed in this form. The endogenous pyridoxal phosphate level is the difference between the assays of untreated and acid hydrolysed plasma. The dephosphorylation of exogenous pyridoxal phosphate by plasma is measured as the increase in pyridoxal after *in vitro* incubation as described above.

## Results

There was a rapid hydrolysis of pyridoxal phosphate to pyridoxal in plasma from patients with high alkaline phosphatase. A close correlation between

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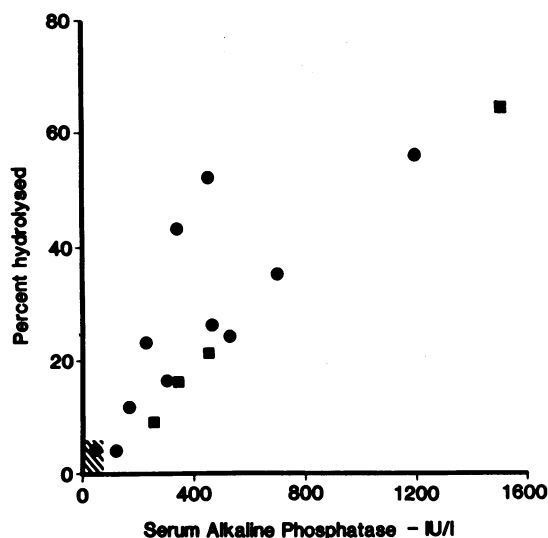


Fig. 1 The relationship between plasma hydrolysis of 148 ng pyridoxal phosphate per ml (equivalent to 100 ng pyridoxal) to pyridoxal, and serum alkaline phosphatase level in 10 control subjects (hatched), 11 patients with a liver disorder (●), and four patients with a condition associated with increased bone alkaline phosphatase (■).

the amount of pyridoxal formed and the level of serum alkaline phosphatase is demonstrated in the 10 control subjects (represented by the hatched area) and the 15 patients detailed above (Fig. 1) ( $r=0.9393$ ,  $P<0.001$ ). Serum alkaline phosphatase levels in healthy control subjects varied from 26–65 IU/l and little or no pyridoxal phosphate was hydrolysed under these experimental conditions. In addition, there was little or no hydrolysis of pyridoxal phosphate in the plasma of two patients with liver disease but with normal serum alkaline phosphatase (haemochromatosis and cryptogenic cirrhosis). In the two patients with the highest phosphatase levels (one of liver origin and the other bone) 56% and 64% pyridoxal phosphate respectively was hydrolysed. The relationship of pyridoxal phosphate hydrolysis to the alkaline phosphatase level appeared to be the same whether the alkaline phosphatase was of liver or bone origin (Fig. 1). However, in the two patients with common bile duct obstruction the hydrolysis appeared relatively greater for the level of alkaline phosphatase.

In view of the binding of pyridoxal phosphate to albumin in plasma, the albumin was measured. The amount of hydrolysis at one hour did not bear any relationship to these levels, which ranged from 26 g/l to 47 g/l.

There is a close inverse relationship between the

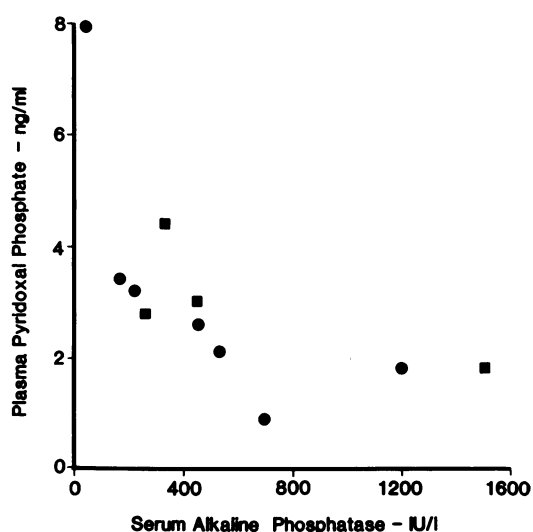


Fig. 2 The relationship between endogenous plasma pyridoxal phosphate (in terms of pyridoxal) and serum alkaline phosphatase level in seven patients with a liver disorder (●) and four patients with a condition associated with increased bone alkaline phosphatase (■).

endogenous plasma pyridoxal phosphate and the alkaline phosphatase level in 11 patients (Fig. 2) ( $r=-0.893$ ,  $P<0.001$ ). The other four patients had been taking mixed vitamins containing pyridoxine and consequently had higher  $B_6$  levels. The patient with the lowest pyridoxal phosphate level (Fig. 2) had neurological and haematological signs of  $B_6$  deficiency (Griffin *et al.*, in preparation).

## Discussion

The evidence that low plasma levels of pyridoxal phosphate might be explained by increased degradation of pyridoxal phosphate in liver disease was based on observations in normal subjects and patients with liver disease after intravenous pyridoxal phosphate. There was a smaller rise in plasma pyridoxal phosphate and a greater urinary excretion of vitamin  $B_6$  derivatives in patients with liver disease.<sup>5,6</sup> Mitchell *et al.*<sup>5</sup> proposed that  $B_6$  degradation took place in the liver because they were unable to demonstrate increased degradation of pyridoxal phosphate *in vitro* in plasma of patients with liver disease. However, they appear to have studied only two patients and one control subject.

We have demonstrated *in vitro* in the plasma of patients with a raised alkaline phosphatase that pyridoxal phosphate is hydrolysed to pyridoxal, and that the amount of pyridoxal formed closely parallels the alkaline phosphatase level (Fig. 1). This might,

in part, explain the low endogenous levels of pyridoxal phosphate reported in liver disease; in fact we have shown that there is a close inverse relationship between the plasma levels and the alkaline phosphatase (Fig. 2). The physiological significance of these findings is, however, uncertain; nor is it known how much hydrolysis of pyridoxal phosphate occurs in the liver of normal subjects or patients with liver disease.

It is possible that some of the pyridoxal formed from plasma hydrolysis of pyridoxal phosphate could be re-utilised, for it might be taken up by the tissues and subsequently rephosphorylated.<sup>2,9,10</sup> It is not certain, therefore, whether hydrolysis of pyridoxal phosphate in plasma would necessarily cause severe depletion in the body. However, one subject with long-standing liver disease and a grossly raised level of alkaline phosphatase presented clinically with epileptiform attacks and sideroblastic anaemia, both of which responded to large doses of pyridoxine (Griffin *et al.*, in preparation). In addition, the observations of Ellis and Presley<sup>11</sup> suggest that a subclinical deficiency of B<sub>6</sub> may not commonly be recognised. It seems, therefore, reasonable that vitamin B<sub>6</sub> should be included in the nutritional management of patients with liver disease as suggested by Labadarios *et al.*<sup>6</sup> In fact, patients with a grossly raised level of alkaline phosphatase might require large doses of vitamin B<sub>6</sub> to compensate for hydrolysis.

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