Vitamin B$_6$ deficiency in chronic liver disease—evidence for increased degradation of pyridoxal-5'-phosphate

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SUMMARY   Plasma levels of pyridoxal-5'-phosphate (PLP), the active coenzyme form of vitamin B$_6$, were found to be significantly lower than normal in 22 out of 31 patients with decompensated cirrhosis or subacute hepatic necrosis. There was no significant difference in plasma PLP levels between those with liver disease due to alcohol and those with other varieties. When intravenous supplements with pyridoxine hydrochloride were given only 33% responded with an increase in plasma PLP. In contrast, all patients given PLP responded, although peak plasma levels were variable, the response being significantly less than that found in normal control subjects. After supplementation with pyridoxine hydrochloride, and with PLP, the urinary excretion of 4-pyridoxic acid, which is derived from the degradation of PLP, was higher in patients who showed the least increase in plasma PLP levels. Although impaired phosphorylation of pyridoxine hydrochloride may be one factor, the most likely explanation for these findings is an increased rate of PLP degradation which may be important in the pathogenesis of vitamin B$_6$ deficiency in patients with severe liver disease.

Low circulating plasma vitamin B$_6$ levels have been reported in 60% of a patient population with alcoholic cirrhosis, although only a small proportion had clinical symptoms of deficiency (Leevy *et al.*, 1970). Such states of hypovitaminaemia have also been documented in a randomly selected hospital population (Leevy *et al.*, 1965), and in alcoholics without liver disease (Lumeng and Li, 1974). However, similar studies in non-alcoholic liver disease have not been performed.

Plasma levels of pyridoxal-5'-phosphate (PLP), the biologically active form of vitamin B$_6$, have been shown to be a reliable index of vitamin B$_6$ nutrition (Sauberlich *et al.*, 1972), and to correlate with the PLP content of erythrocytes and with such functional indices of vitamin B$_6$ status as the tryptophan loading test and PLP saturability of red blood cell transaminases (Hamfelt, 1967; 1967a). In the present paper we report measurements of plasma levels of PLP and of its main metabolite in the urine, 4-pyridoxic acid, in alcoholic and non-alcoholic patients with decompensated cirrhosis or hepatitis. The response of these patients to supplementation with the vitamin has also been investigated.

Methods

The thirty-one patients investigated all had histologically proven chronic liver disease with two or more of the following clinical or biochemical features: encephalopathy, jaundice, hypoalbuminaemia, prolonged prothrombin time, or ascites. The aetiological groups were as follows: active chronic hepatitis with cirrhosis in four patients, primary biliary cirrhosis (two), secondary biliary cirrhosis (one), cryptogenic cirrhosis (one), haemochromatosis (one), and schistosomiasis (one). Four patients had subacute hepatic necrosis and had been ill for three to six months, and 17 had either severe portal tract fibrosis or cirrhosis attributable to alcohol. Nine of the latter group admitted to a daily alcohol consumption of at least half a bottle of spirits, or the equivalent, within two weeks of study (classified as recent heavy drinking), while four had been drinking

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one third to half a bottle (recent moderate drinking). The remaining four claimed complete abstinence from alcohol for periods ranging from six months to two years before the time of admission. Dietary intake had been erratic in the nine heavy drinkers, and had also been poor for at least one month in the four patients with subacute hepatic necrosis due to anorexia and general malaise. An additional four patients (three alcoholics, one active chronic hepatitis) had been maintained on protein-restricted diets (30 g or less daily) to control hepatic encephalopathy. No patient had received vitamin supplements within four weeks of study.

Blood samples were taken at the beginning of the study, and three and seven days later. Plasma was stored at $-20°C$ for determination of pyridoxal-5'-phosphate by an enzymatic method based on the PLP dependent decarboxylation of 1-^{14}C tyrosine (Chabner and Livingstone, 1970). Recovery experiments with PLP added to plasma from cirrhotic patients excluded the possibility of assay inhibition by any abnormal plasma factor present in liver disease.

Twenty-four hour urine collections were started each time blood was taken, and a filtered urine aliquot was stored at $-20°C$ for subsequent determination of 4-pyridoxic acid (Reddy *et al.*, 1958).

A normal range for plasma PLP and urinary 4-pyridoxic acid was obtained from 17 healthy laboratory personnel.

Nine patients received intravenous vitamin supplements for one week comprising pyridoxine hydrochloride (McCarthy's, 50 mg bd), thiamine hydrochloride (Roche, 100 mg bd), and ascorbic acid (Roche, 500 mg bd). Thiamine and ascorbic acid were given, as they are included, together with pyridoxine hydrochloride, in most parenteral vitamin preparations. In another 11 patients, pyridoxal-5'-phosphate (Roche, 50 mg bd) was substituted for pyridoxine hydrochloride. In all these patients, plasma concentrations of PLP and urinary 4-pyridoxic acid excretion was estimated before administration of vitamins and on the third and seventh day of the course. In some patients, additional samples could be obtained up to seven days after the supplements had been discontinued. Plasma PLP levels were also estimated in four healthy volunteers before and 12 hours after a three day course of supplementation with pyridoxine hydrochloride.

In another seven patients with liver disease, and nine normal controls, plasma PLP concentrations were estimated before intravenous supplementation with PLP (50 mg bd) for three days, and subsequent determinations were made on the fourth, seventh, ninth, and 11th days.

Results

Plasma levels of pyridoxal-5'-phosphate (PLP) were below the lower limit of the range found in the normal control subjects (mean $\pm$ 2 SD) in 22 of the 31 patients with liver disease, mean values for the liver disease and control groups of $3\cdot0 \pm 1$ SEM $0\cdot4$ and $11\cdot7 \pm 1\cdot0$ ng/ml respectively (Fig. 1). There was no significant difference, however, between plasma PLP levels in the alcoholic patients and the remaining patients ($3\cdot5 \pm 0\cdot6$ ng/ml and $2\cdot5 \pm 0\cdot5$ respectively), and levels did not correlate with the clinical state, aetiology or duration of illness, or dietary history. In the 11 patients in whom plasma PLP levels were estimated over a period of one week, values remained remarkably constant, the mean for the group being $3\cdot0 \pm 0\cdot5$ ng/ml on day 0 and $2\cdot9 \pm 0\cdot5$ on day 7.

The response to supplementation with pyridoxine hydrochloride in the nine patients studied was variable (Fig. 2) with only three showing an increase in plasma PLP. Even in these, values were significantly lower than those attained after similar supplementation in normal controls with values of $29\cdot0 \pm 2\cdot5$ ng/ml and $115\cdot3 \pm 11\cdot0$ respectively after three days ($P < 0\cdot001$). The change in plasma levels of PLP after pyridoxine hydrochloride administration could not be correlated with any clinical or biochemical parameters nor with recent alcohol intake. Thus, although all three patients who showed an increase in plasma levels of PLP had alcoholic liver disease, a further three alcoholics showed no significant changes, and in both groups two patients had a
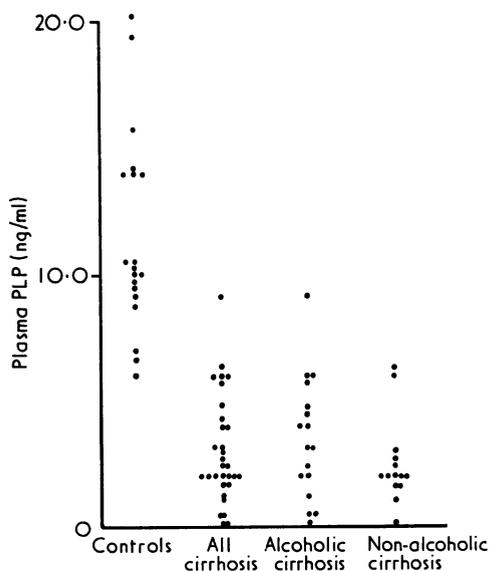
Fig. 1 *Plasma pyridoxal-5'-phosphate levels in normal controls and patients with decompensated liver disease.*
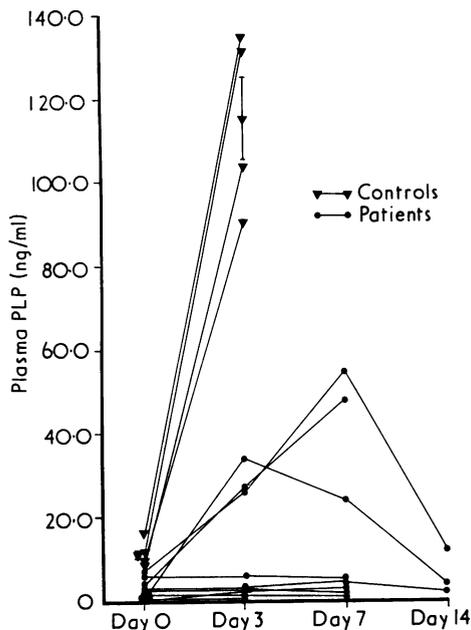
Fig. 2   *Plasma pyridoxal-5'-phosphate levels in normal controls and patients with liver disease before and after the administration of pyridoxine hydrochloride.*

Fig. 3   *The effect of intravenous pyridoxal-5'-phosphate on plasma PLP levels in patients with liver disease.*

history of heavy alcohol intake and one of the patients who showed an increase in plasma levels of PLP was drinking moderately heavily in the two weeks preceding the study.

In contrast, all patients supplemented with PLP showed a significant increase in plasma levels of PLP (Fig. 3) with mean levels of 44·0 ± 13·5 ng/ml on day 3 of supplementation, and 44·9 ± 10·3 on day 7, compared with baseline values of 3·7 ± 0·8 ng/ml (P < 0·02 and 0·005 respectively using paired *t* test). However, the extent of the increase in individual patients was again variable and, as with pyridoxine hydrochloride, the rise in blood levels could not be correlated with clinical or biochemical findings or recent alcohol intake. Furthermore, the plasma PLP levels attained in the patients after supplementation with PLP were significantly lower than in normal controls (Fig. 4).

Values for urinary 4-pyridoxic acid excretion were similar in patients with liver disease and normal controls (2·30 ± 0·45 μmol/24 h and 3·30 ± 0·80 respectively), and there was also no significant difference between those with alcoholic (2·70 ± 0·75) and non-alcoholic disease (1·90 ± 0·50). In 11 patients investigated over a period of seven days, urinary 4-pyridoxic acid excretion did not change significantly (2·7 ± 0·7 μmol/24 h on day 1, and 3·7 ± 1·0 on day 7).
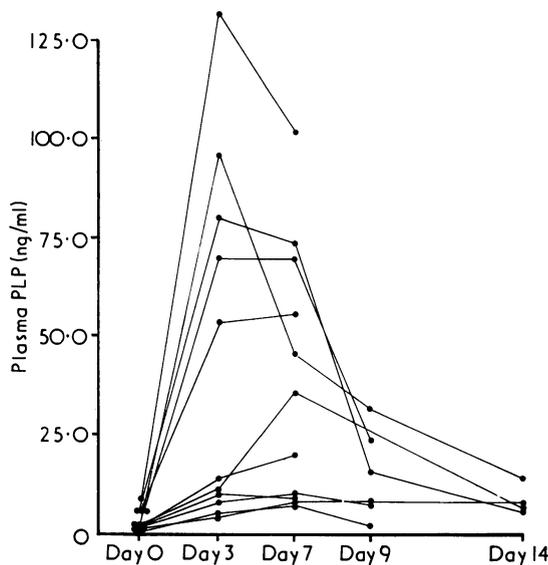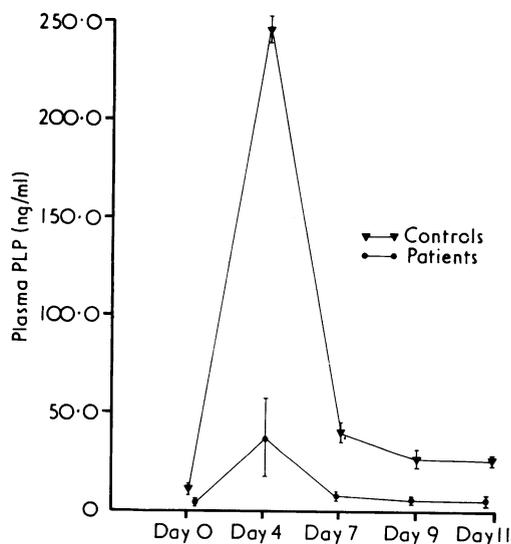

Fig. 4   *The effect of intravenous pyridoxal-5'-phosphate on mean concentrations of pyridoxal-5'-phosphate in normal controls and patients with liver disease.*

Table   *Urinary 4-pyridoxic acid in patients with liver disease after supplementation with pyridoxine hydrochloride or pyridoxal-5′-phosphate*

| | Urinary 4-pyridoxic acid ($\mu mol/24$ hours) | | |
	Pyridoxine hydrochloride (n = 7)		Pyridoxal-5′-phosphate (n = 11)
Pre-supplementation	$1\cdot3 \pm 0\cdot5$	NS	$3\cdot1 \pm 0\cdot8$
Day 3 of supplementation	$107\cdot7 \pm 27\cdot6$	$p<0\cdot05$	$270\cdot3 \pm 79\cdot0$
Day 7 of supplementation	$129\cdot3 \pm 47\cdot3$	$p<0\cdot05$	$255\cdot8 \pm 56\cdot7$

After vitamin supplementation, urinary 4-pyridoxic acid excretion increased in all patients, but significantly higher values were found after PLP than pyridoxine hydrochloride (Table). Although there was no statistical correlation between plasma levels of PLP and urinary 4-pyridoxic acid excretion, the six patients who showed no increase in plasma levels of PLP after pyridoxine hydrochloride excreted considerably more 4-pyridoxic acid than those whose PLP levels were increased ($130\cdot2 \pm 35\cdot2$ $\mu mol/24$ h and $62\cdot6 \pm 37\cdot7$ $\mu mol/24$ h respectively), although these differences just failed to reach statistical significance. Similarly, 4-pyridoxic acid excretion was higher in the four patients who showed the least increase in plasma levels of PLP after PLP supplementation ($413\cdot9 \pm 210\cdot4$ $\mu mol/24$ h and $188\cdot2 \pm 24\cdot8$ respectively).

Discussion

The high incidence of subnormal plasma levels of PLP in patients with severe liver disease, regardless of the aetiology, is clearly shown by the present findings. Many factors could contribute to this, including poor dietary intake and impaired absorption of the vitamin from the gastrointestinal tract. However, the greatly impaired response to intravenous supplements with vitamin B₆, particularly when given as pyridoxine hydrochloride, which is the form commonly present in the diet, suggests that abnormal handling of the vitamin may be an important cause of deficiency.

Previous workers have shown that phosphorylation of pyridoxine appears to be impaired in alcoholic patients (Hines *et al.*, 1970), the defect being more pronounced when alcohol was consumed during the period of investigation. In keeping with these findings, the same group (Hines 1969), have also reported decreased levels of erythrocyte pyridoxal kinase in chronic alcohol abuse, although other workers were unable to confirm this in a more recent study (Lumeng and Li, 1974). Furthermore, the present results would suggest that it is liver disease, rather than a direct effect of alcohol, which plays a role in the abnormal handling of vitamin B₆, as impaired response to supplementation was found whether or not the patients had a history of recent heavy drinking. This is in keeping with experimental evidence that the liver plays a central role in the metabolism of vitamin B₆ (Colombini and McCoy, 1970). Furthermore, in fulminant hepatic failure, where alcohol was not a factor, impaired utilisation of pyridoxine has also been found (Rossouw *et al.*, 1976).

The total daily dose of pyridoxine hydrochloride administered to patients in the present study (100 mg) was greatly in excess of estimated pool sizes of 22-27 mg (Tillotson *et al.*, 1967), and also the normal daily requirements for vitamin B₆ of 1-2 mg/day (Department of Health and Social Security, 1969). Thus, it is unlikely that the lack of a rise of plasma PLP was due either to an increased metabolic requirement for the vitamin, or to greatly increased uptake into depleted tissue stores. One possible explanation is that the phosphorylation of pyridoxine to PLP was impaired. But there must be another abnormality in addition to explain the smaller increase in plasma levels of PLP observed in the patients as compared with normal volunteers to supplementation with PLP itself. The most likely explanation is that elimination of PLP is markedly increased, possibly as a result of enhanced degradation. Experimental studies have shown that 4-pyridoxic acid is formed primarily from degradation of PLP (Contractor and Shane, 1970). Our results support this by showing significantly higher values for urinary 4-pyridoxic acid excretion after administration of PLP than pyridoxine hydrochloride. The higher quantities excreted by patients who showed no increase in plasma levels of PLP after pyridoxine hydrochloride, and patients who showed the least increase after PLP, would also be consistent with an increased rate of PLP degradation.

The subnormal plasma levels of PLP present in these patients may have important consequences, for pyridoxal-5′-phosphate is an important cofactor in many metabolic processes, including the synthesis of nucleic acids (Tratakellis *et al.*, 1964). A deficiency of vitamin B₆ has been shown to result in impaired hepatic incorporation of tritiated thymidine *in vitro* (Leevy *et al.*, 1965) and, because of this, hepatic regeneration may be adversely affected. Furthermore, PLP plays an important role in the metabolism of amino acids (Sauberlich, 1968), whose disordered

homeostasis may be important in the pathogenesis of hepatic encephalopathy. Subnormal levels of PLP may well exacerbate abnormalities, as well as leading to impaired utilisation of dietary protein. Supplementation with vitamin B₆ should therefore be included in the nutritional management of patients with decompensated cirrhosis, and the present study has demonstrated that high doses of pyridoxal-5'-phosphate, rather than the more readily commercially available pyridoxine hydrochloride are necessary.

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