Vitamin B6 metabolism in chronic alcohol abuse The effect of ethanol oxidation on hepatic pyridoxal 5'-phosphate metabolism.

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Research Article

Individuals with chronic alcohol abuse frequently exhibit lowered plasma levels of pyridoxal 5'-phosphate, the coenzyme form of vitamin B6. Because the liver is the primary source of this coenzyme in plasma and also the principal organ that oxidizes ethanol, the effect of ethanol on hepatic pyridoxal phosphate metabolism was studied in the rat. The chronic feeding of ethanol (36 percent of the total dietary calories) for 6 wk significantly decreased the hepatic pyridoxal phosphate content both in animals given a sufficient amount of vitamin B6 in their diet and in those rendered vitamin B6 deficient. In isolated perfused livers, the addition of 18 mM ethanol lowered the pyridoxal phosphate content of livers from vitamin B6-sufficient animals and deceased the net synthesis of pyridoxal phosphate from pyridoxine by the livers of vitamin B6-deficient animals. Ethanol also diminished the rate of release of pyridoxal phosphate into the perfusate by the livers of vitamin B6-deficient rats. These effects of ethanol, in vitro, were abolished by 4-methyl pyrazole, an inhibitor of alcohol dehydrogenase. Thus the derangement of pyridoxal phosphate metabolism produced by ethanol is dependt upon its oxidation. These data support previous findings whic indicate that acetaldehyde is the responsible agent which acts by accelerating the degradation of intracellular pyridoxal phosphate.

Vitamin B_6 Metabolism in Chronic Alcohol Abuse

THE EFFECT OF ETHANOL OXIDATION ON HEPATIC PYRIDOXAL 5′-PHOSPHATE METABOLISM

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ABSTRACT Individuals with chronic alcohol abuse frequently exhibit lowered plasma levels of pyridoxal 5′-phosphate, the coenzyme form of vitamin B_6. Because the liver is the primary source of this coenzyme in plasma and also the principal organ that oxidizes ethanol, the effect of ethanol on hepatic pyridoxal phosphate metabolism was studied in the rat. The chronic feeding of ethanol (36% of the total dietary calories) for 6 wk significantly decreased the hepatic pyridoxal phosphate content both in animals given a sufficient amount of vitamin B_6 in their diet and in those rendered vitamin B_6 deficient. In isolated perfused livers, the addition of 18 mM ethanol lowered the pyridoxal phosphate content of livers from vitamin B_6-sufficient animals and decreased the net synthesis of pyridoxal phosphate from pyridoxine by the livers of vitamin B_6-deficient animals. Ethanol also diminished the rate of release of pyridoxal phosphate into the perfusate by the livers of vitamin B_6-deficient rats. These effects of ethanol, in vitro, were abolished by 4-methylpyrazole, an inhibitor of alcohol dehydrogenase. Thus the derangement of pyridoxal phosphate metabolism produced by ethanol is dependent upon its oxidation. These data support previous findings which indicate that acetaldehyde is the responsible agent which acts by accelerating the degradation of intracellular pyridoxal phosphate.

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INTRODUCTION

Vitamin B_6 deficiency is frequently associated with chronic alcohol abuse. Recent studies have shown that abnormally lowered plasma concentrations of pyridoxal 5′-phosphate (PLP),[1] the coenzyme form of vitamin B_6, may occur in alcoholic patients both with (1) and without (2) evidence of hepatocellular disease. Although inadequate dietary intake may be a contributing factor, Hines and Cowan have observed that even the parenteral administration of pyridoxine fails to elevate the subnormal plasma levels of PLP as long as alcohol consumption continues (1). The finding suggests that ethanol or its oxidation may interfere directly with the metabolism of vitamin B_6. In support of this hypothesis, studies from our laboratory with human erythrocytes have shown that the oxidation product of ethanol, acetaldehyde, impairs the net synthesis of PLP in these cells by enhancing its hydrolysis (2).

Since the liver is the principal organ responsible for the oxidation of ethanol, the nature of the derangement in PLP metabolism can be most directly elucidated by studies with hepatic tissue. The liver also plays a central role in the metabolism of vitamin B_6: a major portion of the pyridoxine absorbed by the intestine is accumulated by liver (3) and PLP in plasma is derived principally from the liver (4). In this communication, we report both the effect of chronic ethanol administration upon the hepatic PLP content of rats and the acute effect of ethanol oxidation upon PLP metabolism in isolated perfused rat livers.

Isolated perfused livers have not been previously employed to study PLP metabolism. In the course of

[1] *Abbreviation used in this paper:* PLP, pyridoxal 5′-phosphate.

these studies, it was found that the state of vitamin B_6 nutrition of the donor animals significantly affected hepatic PLP metabolism. For this reason, the effect of ethanol was examined with preparations from animals both sufficient and deficient in the vitamin.

METHODS

Experimental animals. All animals were male Sprague-Dawley rats (Laboratory Supply Co., Indianapolis, Ind.), housed in wire-bottomed cages and in a controlled temperature environment with fixed day–night cycles. To study the chronic effect of ethanol on hepatic PLP content in vivo, rats were pair-fed the liquid diets as described by Lieber and DeCarli (5). The alcohol diet contained 18% of the calories as protein, 35% as fat, 11% as carbohydrate, and 36% as ethanol. The control diet was identical except that ethanol was isocalorically replaced with dextrin-maltose. Both diets contained 0.725 μg pyridoxine-HCl/ml. Rats weighing 150–170 g were pair-fed these diets for 52±3 days and were considered to be vitamin B_6 sufficient. Another group of rats was rendered vitamin B_6 deficient. They were weanling littermates pair-fed for 42±3 days the alcohol and control liquid diets identical to those above, except that vitamin B_6 was omitted from both (diet 711, pyridoxine omitted, Bio-Serv, Inc., Frenchtown, N. J.).

Rats weighing 150–190 g and maintained on water and Purina Laboratory Chow ad libitum were used for the isolated perfused liver preparations. This diet contained 3.8 μg pyridoxine-HCl/g. These animals were estimated to consume a minimum of 50 μg pyridoxine-HCl daily, an amount generally considered to be sufficient for the growing rat. They were fasted 48 h before use. Vitamin B_6 deficiency was induced in weanling rats by feeding ad libitum water and a solid diet nutritionally complete except that vitamin B_6 was omitted (pyridoxine-deficient diet, ICN Pharmaceuticals, Inc., Cleveland, Ohio) for 40 days. These animals were also fasted 48 h before use.

Isolated perfused liver preparations. The method and apparatus of Miller, Bly, Watson, and Bale (6) were employed. The glassware was autoclaved and the tubings were chemically sterilized with 0.5% (vol/vol) formaldehyde. During perfusion, the pH of the medium was controlled between 7.35 and 7.50 with 1 M $NaHCO_3$ or 1 N HCl. The portal pressure was maintained at 16–18 cm H_2O and the portal flow rate, in excess of 8 ml/min per g liver. Two semisynthetic perfusion media were employed. An erythrocyte-free medium (7) contained Krebs-Henseleit bicarbonate buffer (pH 7.4); heparin, 1.6×10^4 U/liter; tetracycline, 15 mg/liter; dialyzed bovine serum albumin (essentially fatty acid-free, fraction V, Sigma Chemical Co., St. Louis, Mo.), 2.6 g/100 ml; and Na lactate, 10 mM. This medium was filtered through a 0.45-μm filter (Millipore Corp., Bedford, Mass.) before use. A second perfusion medium was identical to that described but contained 2.5 g/100 ml hemoglobin as bovine erythrocytes. The erythrocytes were prepared from heparinized (10^5 U/liter) fresh bovine blood which had been washed four times with sterile 154 mM NaCl at 4°C. The cells were stored at 4°C in a solution containing 143 mM NaCl, 5.7 mM KCl, 3.1 mM $CaCl_2$, 1.4 mM $MgSO_4$, and 5.6 mM glucose. The erythrocytes, used within 18 days of collection, were warmed to 37°C on the day of the study and washed three times with a solution identical to that described above except that glucose was omitted.

All isolated livers were perfused at 37°C. The volume of the perfusion medium for each experiment was 100 ml. All preparations remained viable for at least 4 h and they fulfilled the following criteria: perfusion rate in excess of 8 ml/min per g liver; absence of swelling or infarction; gluconeogenesis rate within 20% of 1.0 μmol/min per g liver with 10 mM lactate as substrate; and a linear rate of bile production. The rate of gluconeogenesis was not used as a criterion in perfusions with ethanol because of the known effect of ethanol on gluconeogenesis (8). All perfusions of livers from vitamin B_6-sufficient animals were performed with the erythrocyte-containing medium and all other perfusions with the erythrocyte-free perfusate. The erythrocytes were found to synthesize PLP from pyridoxine in an amount less than 8% of that released by the liver (data not shown). However, consistent with previous data (4, 9), they did not release PLP into the ambient medium. Hence, the release of PLP from the liver into the perfusate could be measured without interference after separation of the erythrocytes by centrifugation. Subsequent experiments employing erythrocyte-free perfusate showed no differences in hepatic PLP metabolism, rates of gluconeogenesis, or viability of the preparations provided that rats under 200 g in weight were used as donors and perfusion rates above 8 ml/min per g liver were maintained. These findings are consistent with those of Hems, Ross, Berry, and Krebs (10).

Analyses. PLP was measured enzymatically with tyrosine apodecarboxylase (2). Two separate segments of each liver were assayed for PLP content. Each segment was blotted dry, weighed, and disrupted at 4°C in 100 times its weight of 80 mM Na phosphate buffer (pH 7.4) with a Polytron tissue homogenizer (Kinematica GmbH., Luzern, Switzerland). Aliquots were taken for protein determination and duplicate PLP assays. Zero-time tissue samples of the isolated perfused livers were obtained by ligating minor lobes of the liver. The amount of PLP released into the perfusion medium was measured on 2-ml aliquots of the perfusate, sampled as a function of time. The samples were cooled to 0°C and clarified by centrifugation. Samples for PLP determination were deproteinized with 10% (wt/vol) trichloroacetic acid and processed as described previously (2).

The protein concentrations of the homogenized liver samples were measured by the Biuret method (11) or by the method of Lowry, Rosebrough, Farr, and Randall (12). The latter method was employed for all animals fed chronically the liquid diets because the increased triglyceride content of the livers from animals fed the alcohol-containing diet caused turbidity in the Biuret reaction.

The concentrations of ethanol and glucose in the perfusate were measured enzymatically (13, 14).

Data analyses. Experimental results are expressed as means±SD. The P values were obtained by the use of Student's t test for significance of the difference between two group means. However, where indicated, the t test for significance of the difference between the means of paired data was also employed.

RESULTS

Effect of chronic ethanol feeding on hepatic PLP content. Rats fed both the alcohol-containing ($n = 11$) and the control liquid diets ($n = 11$) consumed a mean of 50 μg pyridoxine-HCl daily. This amount of dietary pyridoxine-HCl is considered by most investigators to be sufficient for the growing male rat (15–

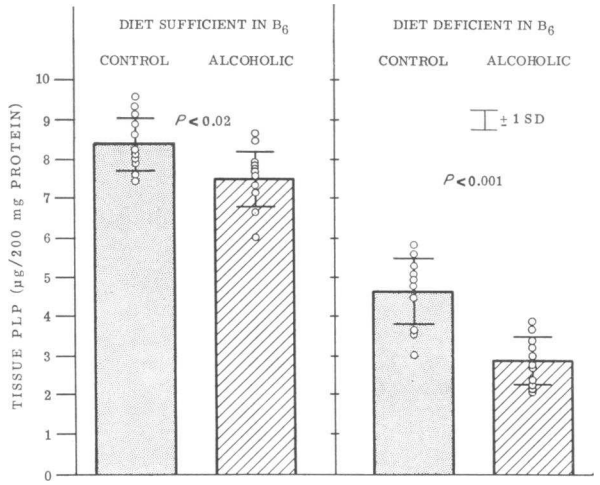

FIGURE 1 Effect of chronic ethanol ingestion on the hepatic content of PLP of pair-fed rats. Each point is the mean value of duplicate assays on two separate segments of liver.

18). After 52±3 days, the hepatic PLP content of the control animals, 8.5±0.7 μg/200 mg protein, was significantly higher ($P < 0.02$) than that of the alcohol-fed animals, 7.6±0.8 μg/200 mg protein (Fig. 1). The PLP content per 200 mg tissue protein was employed as the basis for comparison because of differences in liver weight caused by variations in hepatic triglyceride content.

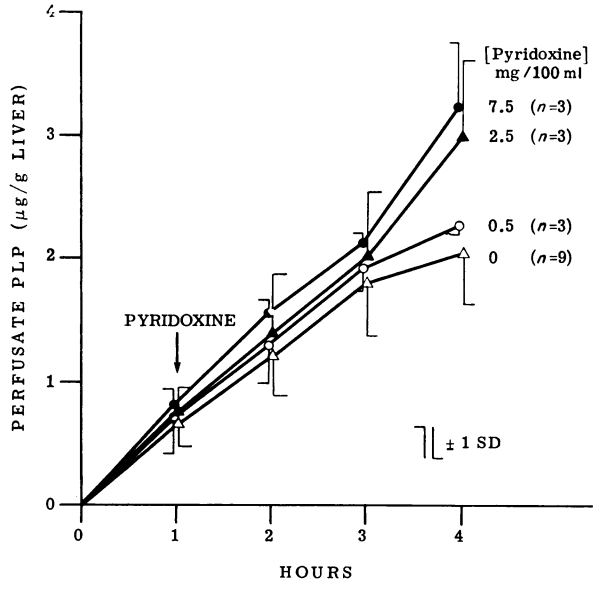

FIGURE 2 Effect of pyridoxine on the release of PLP into the perfusion medium by isolated livers from vitamin B_6-sufficient rats. Pyridoxine was added to the medium after 1 h of perfusion. The number of experiments for each group is given in parentheses, and each point represents the mean value.

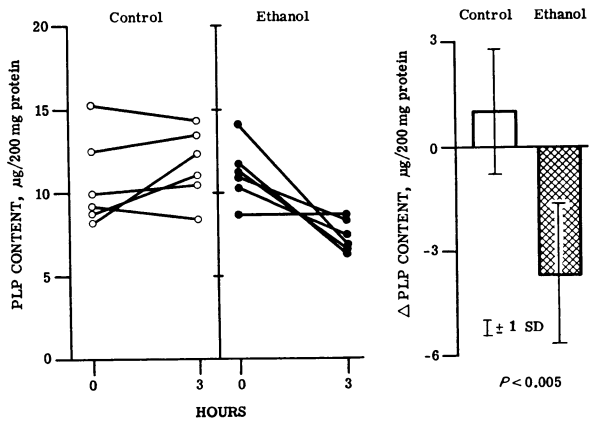

FIGURE 3 Effect of ethanol (18 mM) on the hepatic PLP content of isolated perfused livers from vitamin B_6-sufficient rats. Perfusions were carried out without added pyridoxine. The PLP content of livers perfused without (○—○) and with ethanol (●—●) was measured at the beginning and at the end of the perfusion. The change in hepatic PLP content for each perfusion was calculated, and the bar graph compares the mean changes in hepatic PLP content for the two groups.

A second group of weanling rats were fed the above liquid diets but devoid of vitamin B_6 for 42±3 days. At the time of sacrifice, physical signs of vitamin B_6 deficiency were observed: all animals exhibited dermatoses and many exhibited acrodynia. The hepatic PLP content was reduced in both groups. However, that for the pair-fed control animals ($n = 10$), 4.7±0.9 μg PLP/200 mg protein, was significantly higher ($P < 0.001$) than that of the alcohol-fed animals ($n = 11$), 3.0±0.6 μg/200 mg protein (Fig. 1).

The metabolism of PLP in isolated perfused livers of vitamin B_6-sufficient rats and the effect of ethanol. Since PLP in the circulation is derived principally from liver (4), the ability of the isolated perfused liver to release PLP into the perfusate was examined. When livers from vitamin B_6-sufficient rats were perfused without added pyridoxine in the perfusion medium, PLP appeared in the perfusate at a nearly linear rate, 0.6±0.1 μg/h per g wet wt, during the first 3 h (Fig. 2). The rate tended to decrease only during the 4th h. Presumedly the content of endogenous vitamin B_6 precursors in the liver became limiting at this point. The addition of 0.5, 2.5, or 7.5 mg pyridoxine to the perfusate (100 ml) did not significantly alter the initial rate of appearance of PLP in the perfusate. However, with 2.5 and 7.5 mg of pyridoxine in the medium, linearity of the rate of PLP release was sustained for the entire 4-h period. Increasing the pyridoxine concentration to 12.5 mg/100 ml did not further augment PLP release.

The effect of perfusion on the PLP content of isolated livers from vitamin B_6-sufficient donor animals is

shown in Fig. 3. In the absence of added pyridoxine, the hepatic content of PLP, 10.6±2.5 μg/200 mg protein, was not changed significantly (paired t test, $P < 0.30$) after 3 h of perfusion. In other experiments where pyridoxine in concentrations as high as 12.5 mg/100 ml was added to the perfusate, there was also no significant change in hepatic PLP content. These findings are apparently characteristic of the vitamin B_6-sufficient state and are in agreement with similar studies with isolated hepatocytes (19).

Because the addition of pyridoxine affected neither the initial rate of PLP release nor hepatic PLP content substantially, the effect of ethanol was examined without exogenous pyridoxine in the medium (Fig. 3). Perfusion of the isolated livers with 18 mM ethanol for 3 h lowered the hepatic PLP content significantly (paired t test, $P < 0.02$). Comparison of the mean change in tissue PLP induced by ethanol, a decrease of 3.8±2.0 μg/200 mg protein, with that for the control perfusions, an increase of 1.0±1.7 μg/200 mg protein, indicated that the effect of ethanol is highly significant ($P < 0.005$). However, ethanol was found not to alter the rate of release of PLP during the course of 3 h of perfusion.

The metabolism of PLP in isolated perfused livers of vitamin B_6-deficient rats and the effect of ethanol. In contrast to the above findings, when isolated livers from vitamin B_6-deficient rats were perfused without added pyridoxine, almost no PLP was released into the medium in 3 h (Fig. 4). The hepatic PLP content of these animals (2.5±1.0 μg/200 mg liver protein), lowered markedly as a result of the vitamin B_6-deficient diet, remained essentially unchanged at the end of 3 h of perfusion. The addition of 0.12, 0.6, and 1.2 mg of pyridoxine to the medium (100 ml) induced a striking, time- and dose-related increase in the appearance of PLP in the perfusate (Fig. 4). Furthermore, the hepatic content of PLP increased. Thus, a mean rise of 4.7± 1.2 μg PLP/200 mg liver protein was observed 2 h after 1.2 mg of pyridoxine was added to the medium (Fig. 5). A higher concentration of pyridoxine, 2.4 mg/100 ml, did not further increase either the rate of PLP release or the hepatic PLP content.

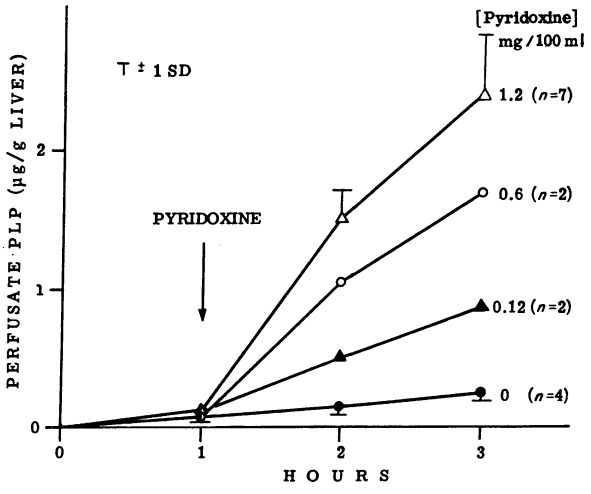

FIGURE 4 Effect of pyridoxine on the release of PLP into the perfusion medium by isolated perfused livers from vitamin B_6-deficient rats. Pyridoxine was added to the medium after 1 h of perfusion. The number of experiments in each group is given in parentheses, and each point represents the mean value.

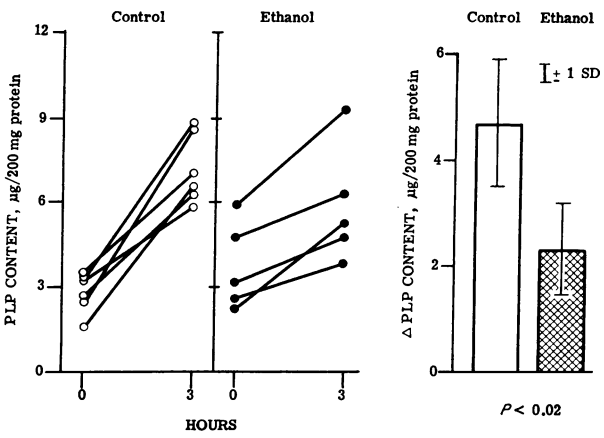

FIGURE 5 Effect of ethanol (18 mM) on the hepatic PLP content of isolated perfused livers from vitamin B_6-deficient rats. Perfusions were carried out with 1.2 mg pyridoxine in the perfusion medium (100 ml). The PLP content of livers perfused in the absence (○—○) and in the presence of ethanol (●—●) was measured at the beginning and at the end of the perfusion. The change in hepatic PLP content for each perfusion was calculated, and the bar graph compares the mean changes in hepatic PLP content for the two groups.

Because of this dependence of isolated livers from vitamin B_6-deficient rats upon exogenously supplied pyridoxine, the effect of ethanol (18 mM) was examined with 1.2 mg pyridoxine added to the perfusate. The presence of ethanol significantly ($P < 0.02$) decreased the rise in hepatic PLP content from 4.7±1.2 to 2.4± 1.0 μg PLP/200 mg tissue protein (Fig. 5). Moreover, ethanol decreased the rate of PLP release by the livers of B_6-deficient rats almost 50%, from 1.5±0.4 μg PLP/h per g liver to 0.8±0.2 μg PLP/h per g liver (Fig. 6A). These differences are highly significant, $P < 0.001$ at 2 h after the addition of ethanol and $P < 0.005$ at 3 h.

Obliteration of the effect of ethanol by 4-methylpyrazole in isolated perfused livers from vitamin B_6-deficient rats. 4-Methylpyrazole is a relatively nontoxic and potent, competitive inhibitor of liver alcohol dehydrogenase (20, 21) and has been shown to inhibit ethanol oxidation in the isolated perfused rat liver (22). Livers from vitamin B_6-deficient rats were perfused

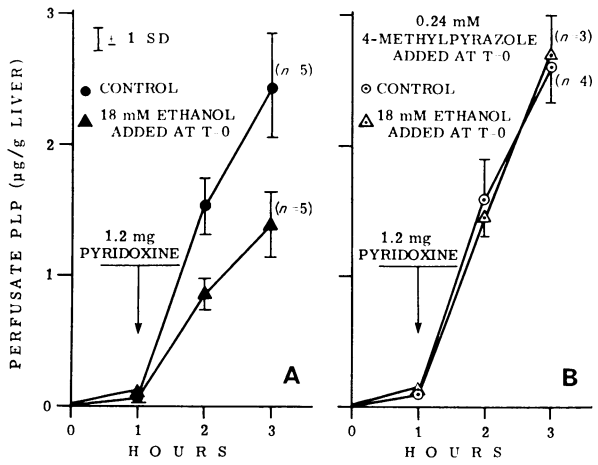

FIGURE 6 Effect of ethanol (18 mM) and 4-methylpyrazole (0.24 mM), on the release of PLP into the perfusate by isolated livers from vitamin B_6-deficient rats. The number of experiments for each condition is shown in parentheses and each point represents the mean value. Ethanol and 4-methylpyrazole were added to the medium (100 ml) at the start of the perfusion, and pyridoxine, 1.2 mg, was added after the 1st h of perfusion. (A) Perfusions without 4-methylpyrazole. (B) Perfusions with 4-methylpyrazole.

with 18 mM ethanol and 0.24 mM 4-methylpyrazole, and 1.2 mg of pyridoxine/100 ml was added to the perfusate after 1 h. This concentration of 4-methylpyrazole inhibited more than 80% the oxidation of ethanol by the perfused livers. It also abolished almost completely the effect of ethanol on both the rate of PLP release (Fig. 6B) and the hepatic PLP content (Table I). In these experiments, 4-methylpyrazole itself did not alter the viability of the liver preparations.

DISCUSSION

The data clearly demonstrate that ethanol administration alters the hepatic metabolism of PLP in the rat, both acutely and chronically, regardless of the state of vitamin B_6 nutrition (Figs. 1, 3, 5, and 6A). Since 4-methylpyrazole completely abolishes the effects of ethanol in the isolated perfused liver (Table I and Fig. 6), it is apparent that the action of ethanol is associated with its oxidation. This result is consistent with our previous observations in human erythrocytes (2) and in isolated rat hepatocytes (23) which implicate acetaldehyde as the agent responsible for the deleterious effects of ethanol on PLP metabolism. Furthermore, it has been shown that acetaldehyde acts to enhance the enzymatic hydrolysis of PLP by cellular phosphatases (2). These findings, therefore, provide an explanation for previous observations that giving rats ethanol increases the urinary excretion of nonphosphorylated vitamin B_6 compounds (24) and that the perfusion of rat livers with ethanol increases the amount of nonphosphorylated vitamin B_6 compounds which appear in the perfusate (25).

Because plasma PLP concentrations are lowered in chronic alcohol abuse (2), it was of interest to study not only the effect of ethanol on hepatic PLP content but also on the release of PLP by the liver. In the course of these studies, it was found that the behavior of the perfused livers differed with the state of vitamin B_6 nutrition of the donor animals (Figs. 2–5). Thus the livers from the animals fed the laboratory chow (vitamin B_6-sufficient) released PLP into the perfusate even without exogenously added pyridoxine. The addition of large doses of pyridoxine failed to increase either the PLP content of the liver or the initial rate of appearance of PLP in the perfusate. By contrast, the livers from the vitamin B_6-deficient animals released very little PLP into the perfusate in the absence of exogenous precursors. However, when small amounts of pyridoxine were added, striking increases in both hepatic PLP content and PLP concentration of the perfusate were observed. In the light of our recent studies of the normal mechanism of control of hepatic PLP metabolism (19), these findings may be interpreted to be characteristic of the vitamin B_6-saturated and the B_6-deficient states, respectively. Apparently the laboratory chow contained sufficient vitamin B_6 to saturate the liver with nonphosphorylated vitamin B_6 compounds. Both tissue PLP content and the rate of PLP release by the liver are thus already at a maximum. On the other hand, the heightened responsiveness to pyridoxine observed with the vitamin B_6-deficient animals (Fig. 4) may be the result of increased PLP synthesizing capacity induced by vitamin B_6 deficiency, as has been previously demonstrated in brain tissue (26).

Although ethanol lowered the PLP content of the livers from both the vitamin B_6-sufficient and the deficient rats, an effect on PLP release was discerned only with

TABLE I

Effect of Ethanol and 4-Methylpyrazole on the Rise in PLP Content of the Livers from Vitamin B_6-Deficient Rats Perfused with Pyridoxine, 1.2 mg/100 ml

Conditions	*n*	Increase in hepatic PLP content	*P* values
		µg/200 mg protein	
1. Control	5	4.35±1.3*	
2. Ethanol, 18 mM	5	2.35±0.8	<0.02‡
3. 4-Methylpyrazole, 0.24 mM	4	4.44±0.9	NS§
4. Ethanol + 4-methylpyrazole	3	5.31±0.8	<0.005‖

* Mean±SD.
‡ Difference between conditions 1 and 2.
§ Difference between conditions 1 and 3.
‖ Difference between conditions 2 and 4.

the livers from the deficient animals. The liver content of PLP has been shown to be controlled principally by the capacity of the intracellular proteins to bind PLP and the enzymatic hydrolysis of PLP synthesized in excess of this binding capacity; regulation of PLP metabolism at the enzymatic steps of PLP synthesis appears to be insignificant physiologically (19). The effect of ethanol on cellular PLP content can, therefore, be understood on the basis of the ability of acetaldehyde to displace PLP from protein binding, recently reported (23). However, the factors which govern the release of PLP from liver into its ambient medium are currently unknown. Understanding of the apparently discrepant effects of alcohol on PLP release thus awaits the elucidation of the mechanism of PLP transport, a process which probably is unique to the liver (4). The problem is presently under investigation in our laboratory. The results of this study suggest that the amount of endogenous stores of precursor compounds in liver and the PLP synthesizing capacity of the liver may be important dependent variables.

The difference in the response to exogenous pyridoxine administration between the vitamin B_6-sufficient and B_6-deficient preparations is worthy of comment from another viewpoint. Previous studies have shown that the oral administration of pyridoxine to man consuming a self-selected, regular diet (2) and the parenteral administration of pyridoxine to dogs fed a standard laboratory diet (4) produce prompt and sustained rises in the level of PLP in the plasma. Since the liver is the principal source of plasma PLP (4), it was initially surprising to find that in rats fed the laboratory chow, perfusion of liver with pyridoxine produced no increase in the rate of appearance of PLP in the perfusate. However, the subsequent finding that the livers from vitamin B_6-deficient rats did respond as expected suggested that perhaps it is the livers of man and the dog which are not fully saturated with vitamin B_6 precursors. In support of this hypothesis, we have found that the plasma PLP concentration of rats fed the standard laboratory diet is 150–220 ng/ml and it increases only slightly when the animals are given 4 mg pyridoxine/kg body wt intraperitoneally. In contrast, the plasma PLP content of vitamin B_6-deficient rats is 5–6.5 ng/ml and it increases more than 40-fold in 3 h when given the same dose of pyridoxine. The plasma PLP content of dogs fed their standard laboratory diet is only 8–20 ng/ml and that of man on a regular diet, 10–30 ng/ml. In man, the oral administration of 25–50 mg pyridoxine daily increases the plasma PLP concentration to a maximal plateau of 150–180 ng/ml within 4 days (2). Thus there may exist interspecies differences in the ease with which the liver can be saturated with vitamin B_6 precursors. Alternatively, the diets of man and the dog may contain only marginally sufficient amounts of vitamin B_6. The criteria for the determination of the "optimal" requirements of the water-soluble vitamins have been recently discussed (27).

It is pertinent to note the pathologic implications of the effect of ethanol on liver PLP content, particularly if the regular diet of man is only marginally sufficient in vitamin B_6. It has been shown previously in the rat that vitamin B_6 deficiency produces fatty liver (28) and in the monkey, cirrhosis (29). The clinical and biochemical alterations produced by vitamin B_6 deficiency in the rat are worsened by the concomitant administration of ethanol (28). The data here presented further emphasize this interaction between alcohol and vitamin B_6 metabolism. However, the effect of alcohol on the various PLP-dependent pathways may well not be proportional to the percentage reduction of hepatic PLP content. It is known that the enzymes differ in their affinity for PLP, apoenzyme stability and inducibility, and the ability to transfer PLP to other apoenzymes (30–32). A study to identify the pathways most susceptible to damage by ethanol oxidation is currently in progress.

ACKNOWLEDGMENTS

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