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EFFECTS OF HIGH DOSES OF VITAMIN E ON DIMETHYLNITROSAMINE HEPATOTOXICITY AND DRUG METABOLISM IN THE RAT

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Abstract—The administration of relatively high doses of vitamin E [55 mg/kg/day, intramuscular (i.m.)] to rats for 3 days resulted in a significant decrease in the acute hepatotoxicity of dimethynitrosamine (DMN). This decrease in toxicity was associated with a decrease in the hepatic metabolism of DMN. Since the metabolism of DMN is mediated by the liver microsomal mixed function oxidase (MFO), the effect of high doses of vitamin E on hepatic MFO was investigated. Rats were treated daily for 3 days with 10, 21, 45 or 100 mg/kg of vitamin E (i.m.), and various parameters of MFO activity were studied in liver cell fractions. DMN demethylase and ethylmorphine demethylase activities and cytochrome P-450 concentration were decreased in animals pretreated with 45 or 100 mg/kg of vitamin E (i.m.). Benzo(a)pyrene hydroxylase activity and cytochrome b_5 concentration were decreased only by pretreatment with the highest dose of vitamin E. NADPH cytochrome *c* reductase activity was unaffected by vitamin E pretreatment. Inhibition of drug metabolism in the rat was also demonstrated *in vivo*. Pretreatment of rats with vitamin E (100 mg/kg/day, i.m., 3 days) resulted in a significant prolongation of hexobarbital sleeping time. The effect of pretreatment of rats with vitamin E (100 mg/kg/day, i.m., for 3 days) on ethylmorphine demethylase was shown to be reversible after cessation of vitamin E administration. It is concluded that large doses of vitamin E inhibit hepatic microsomal MFO, and thus decrease the hepatotoxicity of DMN by inhibiting its metabolism to a presumed active metabolite.

The use of vitamins for protection against compounds which will produce acute hepatonecrosis has been reported. Kamm *et al.* [1] and Mirvish *et al.* [2] have shown that ascorbic acid prevents the formation of dimethylnitrosamine (DMN) from the reaction of sodium nitrite with aminopyrine in the acidic environment of the mammalian stomach. Recent studies have also shown that vitamin E also prevents this reaction in the rat stomach [3].

The present communication describes experiments which suggest that pretreatment of rats with vitamin E may protect them from hepatonecrosis resulting from the administration of preformed DMN. The protective effect of vitamin E was associated with a decrease in the rate of hepatic metabolism of DMN. Since DMN is metabolized by an enzyme of the hepatic mixed function oxidase (MFO) system [4, 5], we also investigated the effect of pretreatment with vitamin E on hepatic drug metabolism by the rat. A preliminary report of this work has been presented [6].

MATERIALS AND METHODS

Animal treatment. Male Sprague–Dawley rats (250–300 g) were caged individually and maintained with food (Purina rat chow) and water *ad lib.* for the duration of the experiments. The injectable vehicle and injectable vitamin E (*dl*-α-tocopherol)* were obtained from Hoffmann–La Roche Inc. For studies on the effects of vitamin E on the acute hepatotoxicity of DMN and on the blood level of DMN, rats were treated with vehicle [1.0 ml/kg, intramuscular (i.m.)] or vitamin E (55 mg/kg, i.m.) for 3 consecutive days. For studies on the effects of vitamin E on the hepatic MFO system and on hexobarbital sleeping time, rats were treated with vehicle (1.0 ml/kg, b.i.d., i.m.) or vitamin E (0–50 mg/kg, b.i.d., i.m.) for 3 consecutive days.

Determination of acute hepatotoxicity of DMN and DMN serum levels. Acute hepatotoxicity or an elevation in serum glutamic–pyruvic transaminase activity (SGPT) is defined in the present study as an increase in SGPT which exceeds twice the S.D. of the mean value obtained in vehicle-treated animals. DMN was given 18–20 hr after the third dose of vitamin E, and a fourth dose of vitamin E was administered following DMN treatment. SGPT levels were determined 24 hr after treatment of rats with DMN, and 18–20 hr after the last dose of vitamin E.

SGPT assays were carried out on blood obtained by cardiac puncture from rats which were lightly anesthetized with carbon dioxide. Serum was separated by centrifugation and the SGPT activity was estimated the same day by use of a commercial diagnostic kit (Determatube-SGP, Worthington Biochem. Corp., Freehold, NJ).

A quantitative estimate of the acute hepatotoxicity of DMN in control and vitamin E-pretreated rats was obtained by calculation of the ED_{50} for DMN by probit analysis [7]. The ED_{50} is defined as the dose of DMN which elevates SGPT in 50 per cent of the animals.

DMN in serum, obtained as described above, was assayed by the procedure of Kamm *et al.* [8].

* Each ml of injectable vitamin E consisted of: Emulphor EL 620, 0.1 cm^3; alcohol anhydrous, 0.1 cm^3; propylene glycol, 0.1 cm^3; benzyl alcohol, 0.01 cm^3; sodium acetate, 0.3 mg; glacial acetic acid, 2.5 mg; sodium chloride, 9 mg; and disodium edetate, 0.1 mg.

Mixed function oxidase activity. Hepatic mixed function activities were determined on the same day the animals were killed. A 20% homogenate of each liver was prepared in 0.05 M sodium phosphate buffer, pH 7.4, containing 1.15% potassium chloride. The homogenate was centrifuged at 9000 *g* for 20 min at 4° and the 9000 *g* supernatant fraction was used as the source for DMN demethylase, ethylmorphine demethylase, and benzo(a)pyrene hydroxylase.

Ethylmorphine demethylase or DMN demethylase activity was assayed in an incubation mixture which contained phosphate buffer, pH 7.4 (50 mM), 1.0 ml of the 9000 *g* supernatant fraction (about 20 mg protein), glucose-6-phosphate (1.12 mM), NADP (0.17 mM), magnesium chloride (2.0 mM), nicotinamide (24 mM), semicarbazide (8.0 mM), ethylmorphine (1.0 mM) or DMN (40 mM), and distilled water to a final volume of 5.0 ml. The reaction was started by addition of the substrate and the samples were incubated aerobically at 37°. The incubation times were 10 and 30 min for the assay of ethylmorphine demethylase and DMN demethylase respectively. The reaction was stopped by adding 2.0 ml of cold 18% perchloric acid. Formaldehyde in the protein free supernatant fraction was estimated by a modified Nash procedure [9].

Benzo(a)pyrene hydroxylase activity was assayed in an incubation mixture which contained phosphate buffer, pH 7.4 (50 mM), 0.1 ml of the 9000 *g* supernatant fraction (about 2 mg protein), NADPH (0.9 mM), magnesium chloride (30 mM), bovine serum albumin (0.2 mg), and benzo(a)pyrene (76 μM), in a final volume of 1.0 ml. The reaction was started by the addition of substrate and the mixtures were incubated aerobically at 37° for 10 min. The reaction was terminated by the addition of 4.25 ml hexane–acetone (3.25: 1). Total hydroxylated metabolites in the organic phase were estimated by the method of Wattenberg *et al.* [10], as modified [11]; 3-hydroxybenzpyrene was used as a standard.

Hepatic microsomal cytochrome and heme content. Microsomes were isolated by homogenizing livers with 2 or 4 parts of buffer per g of liver. A 9000 *g* supernatant fraction was prepared and was centrifuged for 60 min at 105,000 *g* at 4°. The resulting microsomal pellet was resuspended in sodium phosphate buffer (0.05 M, pH 7.4) and centrifuged for 60 min at 105,000 *g* at 4°. Washed microsomes were resuspended in the homogenization medium to give a suspension containing from 5 to 15 mg protein/ml. Microsomal cytochrome *c* concentration [12] and cytochrome b_5 concentration [13] were determined spectrophotometrically. NADPH cytochrome *c* reductase activity was also determined [14]. The microsomal heme content was measured by the pyridine hemochromogen method [15].

Protein concentration. The protein concentration of suitably diluted aliquots of 9000 *g* supernatant and microsomal suspensions were assayed by the method of Sutherland *et al.* [16] using bovine serum albumin as the standard.

Hexobarbital sleeping time. Hexobarbital-induced sleep was measured in rats following the administration of 100 mg/kg of hexobarbital sodium (i.p.). The duration of sleep was taken to be the interval between the loss and the return of the righting reflex.

Statistics. Statistical analyses of the data from enzyme assays and the hexobarbital sleep time experiment were performed by a computer program for Student's *t*-test, which incorporates an analysis of variance; $P < 0.05$ was considered significant.

RESULTS

Effect of vitamin E on DMN hepatotoxicity. The effect of pretreatment with vitamin E on the acute hepatotoxicity of DMN was studied in rats which were treated as described in Materials and Methods. The dose–response curves for the acute hepatotoxicity of DMN in vehicle and vitamin E-pretreated rats are presented in Fig. 1. The ED_{50} for DMN in 59 vehicle-treated rats was 10.1 ± 1.4 mg/kg and that for 60 vitamin E-treated rats was elevated to 19.0 ± 2.3 mg/kg; the difference is significant ($P < 0.05$).

Effect of vitamin E on serum levels of DMN. In order to determine whether pretreatment with vitamin E affected the metabolism of DMN, rats were treated for 3 consecutive days with vitamin E (55 mg/kg, i.m.) and on day 4 a single dose of DMN (70 mg/kg, p.o.) was administered. Two hr after the dose of DMN, the serum levels of DMN in the vitamin E-pretreated rats were 25 per cent greater than those in the vehicle-treated animals, and at 6 hr the nitrosamine levels were more than 2-fold greater than in the vehicle-treated group (Table 1). These results suggested that vitamin E interferes in the metabolism of DMN.

Effect of vitamin E on DMN demethylase activity. To demonstrate an effect of pretreatment with vitamin E on DMN metabolism, rats were treated for 3 days with various doses of vitamin E (0–100 mg/kg/day, i.m.). Eighteen to 20 hr after the last dose of vitamin E, the animals were killed and hepatic DMN demethylase activity in the 9000 *g* supernatant fraction was determined. The data in Table 2 show that pretreatment with vitamin E resulted in a decrease in the DMN demethylase activity of the rat liver 9000 *g* supernatant fraction, and that the decrease was related to the dose of vitamin

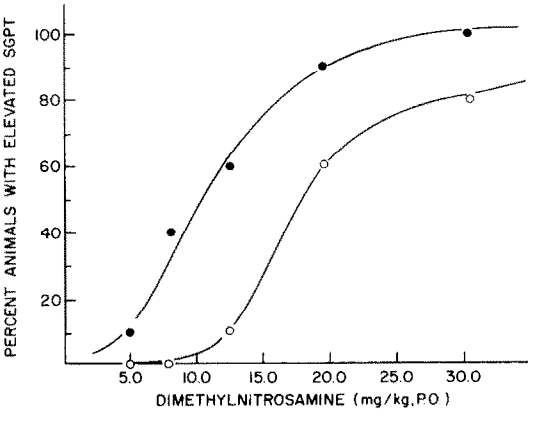

Fig. 1. DMN hepatotoxicity dose–response curves in vehicle- and vitamin E treated rats. Rats were pretreated with vehicle (1.0 ml/kg, i.m.) or vitamin E (55 mg/kg, i.m.) for 3 days, and on day 4 the animals were given DMN (0–30.4 mg/kg, p.o.) followed immediately by a fourth dose of vehicle (ml/kg, i.m.) or vitamin E (55 mg/kg, i.m.). Hepatotoxicity was estimated by determining SGPT levels. The ED_{50} values were calculated by probit analysis. Key: (●) vehicle control; (○) vitamin E (55 mg/kg, i.m.).

Table 1. Effect of pretreatment with vitamin E on serum levels of dimethylnitrosamine (DMN)

Treatment*	Serum levels of DMN after DMN administration† (μg/ml) 2 hr	6 hr
Vehicle (1.0 ml/kg, i.m.)	58.2 ± 2.4 (6)	25.2 ± 5.5 (6)
Vitamin E (55 mg/kg, i.m.)	73.7 ± 3.3‡ (6)	59.1 ± 1.9§ (6)

* Rats were treated for 3 consecutive days with vehicle or vitamin E; on day 4 DMN (70 mg/kg, p.o.) and a final dose of vehicle or vitamin E were given.

† Values are the mean ± the standard error of the mean for the number of animals indicated in parentheses.

‡ Different from vehicle-treated: $P < 0.01$.

§ Different from vehicle-treated: $P < 0.001$.

Table 2. Effect of pretreatment with vitamin E on hepatic dimethylnitrosamine demethylase activity

Treatment*	Dimethylnitrosamine demethylase Specific activity†,‡	% Decrease
Vehicle (1.0 ml/kg)	13.8 ± 1.2 (6)	
Vitamin E (10 mg/kg)	12.2 ± 0.2 (6)	10.9
Vitamin E (21 mg/kg)	12.2 ± 0.6 (6)	11.6
Vitamin E (45 mg/kg)	8.5 ± 0.8§ (6)	38.4
Vitamin E (100 mg/kg)	3.9 ± 0.4§,‖ (6)	71.7

* Rats were treated (i.m.) daily for 3 days, and enzyme activity was estimated 18 hr after the last dose.

† Values are the mean ± the standard error of the mean for the number of animals indicated in parentheses.

‡ n moles of formaldehyde formed/mg of protein/30 min.

§ Different from vehicle-treated: $P < 0.001$.

‖ Different from vitamin E (45 mg/kg)-treated: $P < 0.001$.

E. Thus, the increased DMN serum levels described above were probably related to the inhibition of hepatic DMN metabolism.

Effects of vitamin E on drug metabolism. In order to determine if the effect of vitamin E was specific for the metabolism of DMN, we studied a variety of drug metabolic reactions in liver cell fractions from rats which had been pretreated with vitamin E. The parameters that were measured included: ethylmorphine demethylase, benzo(a)pyrene hydroxylase activity, microsomal NADPH cytochrome *c* reductase activity, and cytochrome P-450, cytochrome b_5 and total heme concentrations.

Ethylmorphine demethylase and benzo(a)pyrene hydroxylase activities in the rat liver 9000 *g* supernatant fraction were inhibited following pretreatment with vitamin E (Table 3). The activity of ethylmorphine demethylase was inhibited at a pretreatment dose of 45 mg/kg/day ($P < 0.001$), but significant inhibition of benzo(a)pyrene hydroxylase was observed only at a pretreatment dose of 100 mg/kg/day ($P < 0.01$).

The microsomal cytochrome P-450 content of rat liver microsomes was estimated from the absorbance of the reduced carbon monoxide complex in hepatic microsomes. The apparent decrease in cytochrome P-450 content was not due to the induction of an anomalous P-450 spectrum following pretreatment with vitamin E, since the shape of the spectrum from vitamin E-pretreated rats did not differ significantly from that of vehicle-pretreated animals (data not shown).

Table 3. Effect of pretreatment with vitamin E on hepatic benzo(a)pyrene hydroxylase and ethylmorphine demethylase activities

Treatment*	Benzo(a)pyrene hydroxylase Specific activity†,‡	% Decrease	Ethylmorphine demethylase Specific activity†,§	% Decrease
Vehicle (1.0 ml/kg)	1.79 ± 0.28 (6)		15.4 ± 0.9 (6)	
Vitamin E (10 mg/kg)	1.40 ± 0.77 (6)	21.8	13.2 ± 1.7 (6)	14.3
Vitamin E (21 mg/kg)	1.27 ± 0.09 (6)	29.0	13.2 ± 1.3 (6)	14.3
Vitamin E (45 mg/kg)	1.09 ± 0.14 (6)	39.1	8.5 ± 0.7‖ (6)	44.8
Vitamin E (100 mg/kg)	0.33 ± 0.03¶ (6)	81.6	2.6 ± 0.4‖,**(6)	83.1

* Rats were treated (i.m.) daily for 3 days, and enzyme activity was estimated 18 hr after the last dose.

† Values are the mean ± the standard error of the mean for the number of animals indicated in parentheses.

‡ nmoles of hydroxybenzpyrene formed/mg of protein/10 min.

§ nmoles of formaldehyde formed/mg of protein/10 min.

¶ Different from vehicle-treated: $P < 0.01$.

‖ Different from vehicle-treated: $P < 0.001$.

** Different from vitamin E (45 mg/kg)-treated: $P < 0.001$.

Table 4. Effect of pretreatment with vitamin E on components of hepatic microsomes

Exp.	Treatment*	NADPH cytochrome *c* reductase† (nmoles/mg protein/min)	Cytochrome P-450† (nmoles/mg protein)	Cytochrome b_5† (nmoles/mg protein)	Heme content† (nmoles/mg protein)
1	Vehicle (1.0 ml/kg)	43.3 ± 2.1 (6)	1.15 ± 0.11 (6)		
	Vitamin E (10 mg/kg)	41.6 ± 2.4 (6)	1.06 ± 0.11 (6)		
	Vitamin E (21 mg/kg)	46.3 ± 4.6 (6)	0.97 ± 0.00 (6)		
	Vitamin E (45 mg/kg)	49.6 ± 4.6 (6)	0.84 ± 0.08‡ (6)		
	Vitamin E (100 mg/kg)	40.9 ± 4.2 (6)	0.56 ± 0.01§,‖ (6)		
2	Vehicle (1.0 ml/kg)		1.19 ± 0.06 (5)	0.46 ± 0.02 (5)	1.56 ± 0.13 (5)
	Vitamin E (100 mg/kg)		0.65 ± 0.09‡ (5)	0.34 ± 0.02¶ (5)	0.71 ± 0.07¶ (5)

* Rats were treated (i.m.) daily for 3 days, and enzyme activity and cytochrome content were estimated 18 hr after the last dose.

† Values are the mean ± the standard error of the mean for the number of animals in parentheses.

‡ Different from vehicle-treated: $P < 0.05$.

§ Different from vitamin E (45 mg/kg)-treated: $P < 0.05$.

‖ Different from vehicle-treated: $P < 0.001$.

¶ Different from vehicle-treated: $P < 0.01$.

Microsomal cytochrome P-450 content and NADPH cytochrome *c* reductase activity were estimated in hepatic microsomes isolated from livers of rats 18–20 hr after 3 days pretreatment with vitamin E (10–100 mg/kg/day). The data in Table 4 show that the vitamin E had no effect on NADPH cytochrome *c* reductase activity in rat liver microsomes. In contrast, vitamin E pretreatment caused a dose-related decrease in microsomal cytochrome P-450 content. The hepatic cytochrome P-450 content in rats pretreated with 45 and 100 mg/kg/day of vitamin E decreased 27 and 49 per cent respectively. In another experiment, hepatic microsomal cytochrome b_5 content was decreased significantly after 3 days of pretreatment with 100 mg/kg/day of vitamin E (Table 4). The data in Table 4 also show that the total microsomal heme content of vitamin E-pretreated rats (100 mg/kg/day) was 46 per cent less than that of vehicle-treated rats. The total heme content in both vehicle- and vitamin E-pretreated rats was about the same as the amount of heme represented by cytochrome P-450 plus cytochrome b_5.

Effect of vitamin E on hexobarbital sleeping time. The effect of pretreatment with vitamin E on *in vivo* drug metabolism was estimated by determining the effect on hexobarbital-induced sleeping time in rats. The animals were pretreated with various doses of vitamin E for 3 days, and 18–20 hr after the last dose, hexobarbital (100 mg/kg, i.p.) was administered. The data in Table 5 show that vitamin E pretreatment prolonged hexobarbital sleeping time and that the amount of prolongation increased with increasing doses of vitamin E.

Table 5. Effect of pretreatment with vitamin E on hexobarbital sleeping time

Treatment*	Hexobarbital sleeping time	
	Minutes†	% Increase
Vehicle (1.0 ml/kg)	18 ± 4 (8)	
Vitamin E (21 mg/kg)	25 ± 5 (9)	39
Vitamin E (45 mg/kg)	33 ± 6‡ (10)	83
Vitamin E (100 mg/kg)	61 ± 5§,‖ (10)	239

* Rats were treated (i.m.) daily for 3 days; 18 hr after the last dose they were given hexobarbital (100 mg/kg, i.p.) and sleeping time was recorded.

† Values are the mean ± the standard error of the mean for the number of animals indicated in parentheses.

‡ Different from vehicle-treated: $P < 0.05$.

§ Different from vitamin E (45 mg/kg)-treated rats: $P \pm 0.01$.

‖ Different from vehicle-treated: $P < 0.001$.

Recovery of ethylmorphine demethylase activity in vitamin E-treated rats. In order to show the reversibility of MFO inhibition in vitamin E-treated rats, ethylmorphine demethylase activity in rat liver was monitored for several days following the cessation of vitamin E treatment. About 48 hr after the final dose of vitamin

Table 6. Recovery of ethylmorphine demethylase activity following treatment with vitamin E

Treatment*	Ethylmorphine demethylase activity† (nmoles formaldehyde/mg protein/10 min)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Vehicle	23.8 ± 1.7 (6)	21.4 ± 1.5 (6)	25.0 ± 0.6 (6)	23.7 ± 1.5 (6)	19.1 ± 1.0 (6)	23.8 ± 1.5 (6)
Vitamin E	10.6 ± 0.8‡ (6)	5.8 ± 0.9‡ (6)	6.8 ± 0.6‡ (6)	9.0 ± 0.7‡ (6)	12.6 ± 1.2‡ (6)	19.0 ± 1.6§ (6)

* Vitamin E (100 mg/kg/day, i.m.) or vehicle (1.0 ml/kg/day, i.m.) was administered on days 0, 1 and 2; ethylmorphine demethylase activity was measured on days 1 through 6.

† Values are the mean ± the standard error of the mean for the number of animals in parentheses.

‡ Different from vehicle-treated rats: $P < 0.001$.

§ Not different from vehicle-treated rats.

E, ethylmorphine demethylase activity began to increase (Table 6). Enzyme activity increased steadily and approached vehicle-treated control activity 4 days after the last dose of vitamin E.

DISCUSSION

A variety of secondary and tertiary amines react with sodium nitrite at the acid pH of the mammalian stomach to form nitrosamines [17] which are toxic and/or carcinogenic [17, 18]. The toxicity and/or carcinogenicity of nitrosamines, particularly DMN, is mediated by metabolic transformation to an active intermediate by the hepatic MFO system [18]. We have shown previously that vitamin C [1, 6, 8, 19] and vitamin E [3] prevent the nitrosative cleavage of aminopyrine by sodium nitrite in the acidic environment of the rat stomach. In the course of these studies, we observed an apparent inhibition by vitamin E of the acute hepatotoxicity of preformed DMN. The present study was undertaken to explore this observation in further detail.

Studies reported in this communication demonstrate that, in rats pretreated with relatively high doses of vitamin E (55 mg/kg/day, i.m., 3 days), the dose–response curves for the acute hepatotoxicity of DMN are shifted to the right (Fig. 1). Since the hepatic toxicity of DMN is mediated presumably by a metabolic process [17], we studied the effect of pretreatment with vitamin E on the metabolism of DMN by the rat. When rats were pretreated with vitamin E, under the same conditions which decreased the toxicity of DMN, we observed an increase in serum levels of DMN 2 and 6 hr following the administration of 70 mg/kg (p.o.) of the nitrosamine (Table 1). More direct evidence suggesting that vitamin E pretreatment interferes with the metabolism of DMN was obtained from *in vitro* studies of the hepatic metabolism of DMN by liver preparations from rats which had been pretreated with varying doses of vitamin E. Our data show a decrease in hepatic DMN demethylase activity (Table 2) and support the suggestion of an inhibitory effect of relatively high doses of vitamin E on the hepatic metabolism of DMN by the rat.

The inhibition of hepatic DMN demethylase activity suggested that hepatic MFO activity, in general, might be inhibited following pretreatment with relatively high levels of vitamin E. Our results show that pretreatment of rats with vitamin E causes a decrease in the hepatic activities of ethylmorphine demethylase and benzo(a)pyrene hydroxylase, and a decrease in cytochrome P-450 and cytochrome b_5 concentrations (Tables 2, 3 and 4); vitamin E pretreatment had no effect on NADPH cytochrome *c* reductase activity (Table 4).

Although the carbon monoxide reduced spectrum of cytochrome P-450 from animals treated with high doses of vitamin E was altered quantitatively but not qualitatively (data not shown), it is possible that the decrease in hepatic MFO activity and the apparent decrease in cytochrome P-450 content were due to the binding of vitamin E to the terminal oxidase, thus resulting in an inhibition of activity; it is well known that vitamin E accumulates in the mammalian liver [20]. Therefore, we measured the total heme content of microsomes from vitamin E-treated rats and from control rats. The total heme content in vitamin E-treated rats was significantly less than in control rats (Table 4) but in both instances, the total heme was almost totally accounted for by the cytochrome P-450 and the cytochrome b_5 content. Thus, the inhibition of hepatic MFO activity appears to be more closely related to a decrease in cytochrome P-450 levels rather than to a competition for binding sites on the terminal oxidase.

The inhibitory effect of pretreatment with vitamin E on drug metabolism is also suggested by *in vivo* studies. Thus, we have demonstrated that hexobarbital sleeping times are prolonged in rats pretreated with relatively high doses of vitamin E (Table 5), and this effect is dose related. Finally, we have presented evidence which suggests that the inhibition of hepatic MFO activity by vitamin E is reversible following cessation of treatment (Table 6).

The results of this study show that vitamin E protects the rat from DMN-induced hepatotoxicity by inhibiting the hepatic metabolism of DMN to its presumed active intermediate. Furthermore, we have presented evidence which suggests that the hepatic metabolism of a variety of drug substrates may be inhibited by relatively large amounts of vitamin E and that the inhibitory effect is reversed in about 3 days following cessation of vitamin E intake. Skaare and Nafstad [21] have recently reported histological evidence which is consistent with our finding that vitamin E decreases the acute hepatotoxicity of DMN.

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