hydrochloric acid and pH value in the fasting state before administration of the base and before administration of the hydrochloride.

Furthermore, it should be stressed that the limited data presented in this report on DMCT cannot exclude the possibility that DMCT base can exert similar effect on the absorption to greater cumulative amounts of CTC base after oral administration to human subjects. More extensive studies are desirable with regard to absorption in this case.

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Effect of Fenitrothion on Hepatic Microsomal Components of Drug Metabolizing System in Mice[1]

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To examine the mechanisms of the inhibition of drug metabolizing enzyme activity by organophosphate insecticides, microsomal components of drug metabolizing system were determined after treatment with fenitrothion. Mice given intraperitoneal dose of fenitrothion exhibited the rapid decrease in cytochrome P-450 content and slight inhibition of NADPH-cytochrome c reductase activity. Administration of fenitrothion to phenobarbital-treated mice decreased in N-demethylation of aminopyrine as well as cytochrome P-450 content. The actual decrease in cytochrome P-450 content caused by fenitrothion in phenobarbital-treated mice was greater than non-treated mice.

The results presented here strongly suggest that the inhibition of drug metabolizing enzyme by fenitrothion was due to the decrease in cytochrome P-450.

In a preceding paper,[3] we reported that some organophosphate insecticides inhibit hepatic drug metabolizing enzymes both *in vivo* and *in vitro*, although the mechanism whereby organophosphates inhibit drug metabolizing enzymes has not been clarified except that the inhibition originated from microsomes other than soluble components. It must be noted, however, that the *in vivo* inhibition by these thiophosphate insecticides occurs very rapidly, that is, within a few hours after the administration of thiophosphates.

This paper deals with the *in vivo* effect of fenitrothion, O,O-dimethyl O-3-methyl-4-nitrophenyl phosphorothioate, on the components of microsomal drug metabolizing system of mouse liver.

Result and Discussion

Effect of fenitrothion on mouse liver microsomal cytochrome P-450 content and NADPH-cytochrome c reductase activity is shown in Table I. It is obvious that microsomes from fenitrothion-treated mice showed the lowered cytochrome P-450 content in comparison with that of control mice.

1) This work was presented at the Sixth Symposium on Drug Metabolism and Action, Tokyo, November 1974.
2) Location: *Aobayama, Sendai.*
3) M. Uchiyama, T. Yoshida, K. Homma and T. Hongo, *Biochem. Pharmacol.*, **24**, 1221 (1975).

Table I. Effect of Fenitrothion on Microsomal Cytochrome P-450 Content and on NADPH-cytochrome c Reductase Activity in Mice

Treatment	Dose (mg/kg)	Cytochrome P-450 (nmole/mg protein)	NADPH-cyt c reductase (unit/mg protein/min[a])
Control	—	1.008±0.044[b]	2.691±0.108
Fenitrothion	100	0.653±0.039	2.298±0.027
	200	0.569±0.003	

Mice were injected intraperitoneally with fenitrothion 2 hr prior to sacrifice.
a) One unit is defined as an absorbance change of 1.0 per minute at 550 mμ and at 25°.
b) Each value is the mean±S.E. of at least six mice.

The magnitude of the decrease in cytochrome P-450 content was somewhat dose-dependent, namely 36% of control value was obtained by the administration of 100 mg/kg of fenitrothion, 44% by 200 mg/kg. NADPH-cytochrome c reductase activity was only slightly reduced by the administration of fenitrothion (100 mg/kg).

The administration of fenitrothion to mice which had been pretreated with phenobarbital is shown in Table II.

Table II. Effect of Fenitrothion on Microsomal Cytochrome P-450 Content and on N-Demethylation of Aminopyrine in Phenobarbital-treated Mice

Pre-treatment	Treatment	Cytochrome P-450 (nmole/mg protein)	N-Demethylation[a] (nmole HCHO/ mg protein/30 min)
None	Control	1.066±0.026[b]	8.65±0.81[b]
Phenobarbital	Control	2.184±0.249	17.13±2.21
Phenobarbital	Fenitrothion (50 mg/kg)	1.298±0.226	7.00±0.63
Phenobarbital	Fenitrothion (100 mg/kg)	1.136±0.118	3.31±0.07
Phenobarbital	Fenitriothion (200 mg/kg)	1.026±0.088	4.02±0.70

Mice were injected intraperitoneally with sodium phenobarbital (80 mg/kg) daily for two days, and then received fenitrothion.
Mice were sacrificed 2 hr after intraperitoneal administration of fenitrothion.
a) Activity was determined at 9000×*g* supernatant as reported previously.[3]
b) Each value is the mean±S.E. of six mice.

There was a decrease in N-demethylation of aminopyrine as well as a decrease in cytochrome P-450 content. As can be seen, the actual decrease in cytochrome P-450 content caused by fenitrothion in phenobarbital-treated mice was greater than that of non-treated mice. It is suggested that the difference in the extent of the decrease in cytochrome P-450 content between non-treated and phenobarbital-treated mice may be related to the greater rate of metabolism of fenitrothion *in vivo* in the phenobarbital pretreated animal. However, additional works are required to clarify this point. However, the results presented here strongly suggest that the inhibition of drug metabolizing enzyme activity by fenitrothion was caused by the decrease in cytochrome P-450.

There are at least three mechanisms which may account for the decrease in cytochrome P-450 content brought about by fenitrothion. There are inhibition of heme and/or cytochrome P-450 synthesis, destruction of heme moiety or interference the characteristic spectrum of cytochrome P-450 with carbon monoxide. Because the levels of cytochrome P-450 were measured quite soon after the administration of fenitrothion, the results obtained here suggest that fenitrothion decreases cytochrome P-450 either by inhibiting the characteristic spectrum with carbon monoxide or by destroying the existing cytochrome P-450.

Although an acceptable explanation is obscure in the present state of knowledge, an interesting evidence was reported recently by Norman, *et al.*[4] that in the conversion of para-

4) B.J. Norman, R.E. Poore and R.A. Neal, *Biochem. Pharmacol.*, **23**, 1733 (1974).

thion to paraoxon *in vitro*, the sulfur atom is released in a reactive form and becomes covalently bound to microsomal membrane and suppressed both drug metabolizing enzyme activity and cytochrome P-450 content. If this reaction is also acceptable to *in vivo*, it may explain the results observed in this study, since the alteration of cytochrome P-450 microenvironment is very likely to be derived from thiophosphates.

The experiments are now under way to examine the various mechanisms described above.

Experimental

Materials——Pure fenitrothion was kindly furnished by Sumitomo Chemical Co., Ltd. All other materials were of reagent grade and were purchased commercially.

Animal and Treatment——All mice employed in this experiment were male of ddY strain weighing 25—28 g. Mice were fed and water *ad libitum*. Mice in the experimental group were injected intraperitoneally with various doses of fenitrothion dissolved in 0.1 ml of corn oil, and the control mice were injected the vehicle only. Sodium phenobarbital was given intraperitoneally at a concentration of 80 mg/kg daily for two days. All mice were sacrificed 2 or 4 hr after treatment with fenitrothion at which time microsomal drug metabolizing enzyme activity was significantly inhibited as reported previously.[3] Homogenization and subcellular fractionation of liver were performed as described previously except 1.15% KCl containing 1 mM EDTA was used as homogenizing medium.

Analyses——The activity of NADPH-cytochrome c reductase was measured as reported by Masters, *et al.*[5] Microsomal cytochrome P-450 content was determined by the method of Omura and Sato,[6] using extinction coefficient of 91 mM^{-1} cm^{-1} between 450 and 490 nm. Microsomal protein was measured by the method of Lowry, *et al.*[7] with bovine serum albumin as the standard.

5) B.S.S. Masters, C.H. Williams and H. Kamin, "Methods in Enzymology," Vol. 10, eds. by S.P. Colowick and N.O. Kaplan, Academic Press, New York, 1967, p. 565.
6) T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2370 (1964).
7) O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).