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THE ROLE OF LIVER MICROSOMAL ENZYMES IN THE METABOLISM OF PARATHION

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Abstract—The involvement of microsomal enzymes in the metabolism of the organophosphorus insecticide diethyl *p*-nitrophenyl phosphorothionate (parathion) by rat liver was studied. The effect of parathion on the activity of NADPH- and NADH-cytochrome *c* reductases in hepatic microsomes isolated from untreated and phenobarbital pretreated rats was determined.

The results demonstrate that both NADH- and NADPH-linked (microsomal) electron transport components are involved in the oxidative metabolism of parathion. Phenobarbital pretreatment increased the rate of parathion metabolism.

The activity of microsomal NADH-cytochrome *c* reductase was significantly higher than that of NADPH-cytochrome *c* reductase. Parathion had an inhibitory effect on both enzymes when applied directly to the microsomal suspension.

THE WORK of Neal,[1,2] Nakatsugawa *et al.*[3–5] and Wolcott *et al.*[6,7] demonstrated that many organophosphates were degraded by mixed function oxidases. The results indicated that the system for metabolism of organophosphates is closely associated with the microsomal electron transport chain involved with the oxidation of NADPH.[1,8] The experiments presented here are concerned with the metabolism of parathion by microsomal NADPH and NADH oxidizing systems. The effect was compared with that of mitochondrial oxidative enzymes. The effect of parathion on the activity of microsomal NADH- and NADPH-cytochrome *c* reductases was examined using liver from untreated and phenobarbital pretreated animals.

MATERIALS AND METHODS

Female albino rats, body wt 200–250 g were used. All animals were reared under the same conditions. Phenobarbital (50 mg/kg) in distilled water was given intraperitoneally in a volume of 2 ml/kg to half of the experimental animals, 72 and 48 hr before they were killed. The animals were starved for 24 hr before they were sacrificed.

Liver mitochondria and microsomes were isolated according to Remmer *et al.*[9] except that the 18,000 *g* spin was omitted. The procedure was carried out at 0–5°.

Protein was estimated by the colorimetric method of Lowry *et al.*[10]

For electron microscopy the microsomal preparation was fixed directly in 1% OsO_4 in Milloning's buffer pH 7·2. After dehydration in a series of graded acetone solutions the microsomal preparation was embedded in araldite. The material was stained with 0·05% aqueous solution of uranyl acetate and lead nitrate according to Reynolds[11] during dehydration. Siemens electron microscope Elmiskop I was used.

The reaction mixture for a study of parathion metabolism contained in 2·5 ml: 120 μmoles Tris buffer, pH 7·2, indicated amounts of microsomal or mitochondrial protein, 1·5 μmoles NADH and 2 μg parathion. The mixture was incubated for 15 min at 37° and the reaction was stopped by the addition of 3 ml acetone.

Parathion and paraoxon residues in the acetone extract from the reaction mixture were determined by gas chromatography. Column stationary phase was 6% QF-1 on chromosorb G (AW-DMCS) as a carrier. The temperature of the column was 200°, the temperature of the injector 230° and the temperature of the detector was 225°. The continuous flow of nitrogen was applied at a rate of 15 ml/min.

The activities of NADH- and NADPH-cytochrome *c* reductases were determined according to Masters *et al.*[12] The reaction mixture contained in 2·8 ml: 15 μg microsomal protein, 150 μmoles Pi buffer, pH 7·8, and 150 μmoles ferri cytochrome *c*. The reaction was started by the addition of 100 μmoles NADH or 100 μmoles NADPH. To study the effect of parathion, microsomes were preincubated with 2–100 μg parathion in Pi buffer, pH 7·8, for 10 min at 37°.

RESULTS AND DISCUSSION

The electron micrograph (Fig. 1) shows the microsomal fraction containing membranes completely enclosing a vesicular space which is characteristic of liver microsomes.[13] Parathion metabolism by different cell fractions is presented in Table 1.

TABLE 1. PARATHION METABOLISM BY DIFFERENT CELL FRACTIONS*

	Control rats		Phenobarbital injected rats	
	Parathion (μg)	Paraoxon (μg)	Parathion (μg)	Paraoxon (μg)
Mitochondria (3·6 mg protein)	1·23	0·00	1·30	0·00
Microsomes (without NADH) (2·32 mg protein)	1·44	0·00	1·35	0·00
Microsomes (2·32 mg protein)	0·26	0·34	0·10	0·10
(0·023 mg protein)	1·30	0·00	1·37	0·00
Supernatant (105,000 *g*)	1·51	0·00	1·40	0·00

* Indicated amounts of various fractions of rat liver were incubated with 2 μg parathion for 15 min at 37° in the respence of NADH. The data indicate the amounts of insecticides found after the reaction was stopped.

Recovery under the present experimental conditions was 73 per cent.

The results show that NADH stimulated oxidative metabolism of parathion. Parathion metabolism in the absence of NADH is negligible. The activity of the microsomal fraction, on a protein basis, was 0·51 μg parathion metabolized per mg protein per 15 min, while the activity of the mitochondrial fraction was, in comparable conditions, 0·06 μg parathion metabolized per mg protein per 15 min. The microsomes are therefore the active components involved in the metabolism of parathion in the

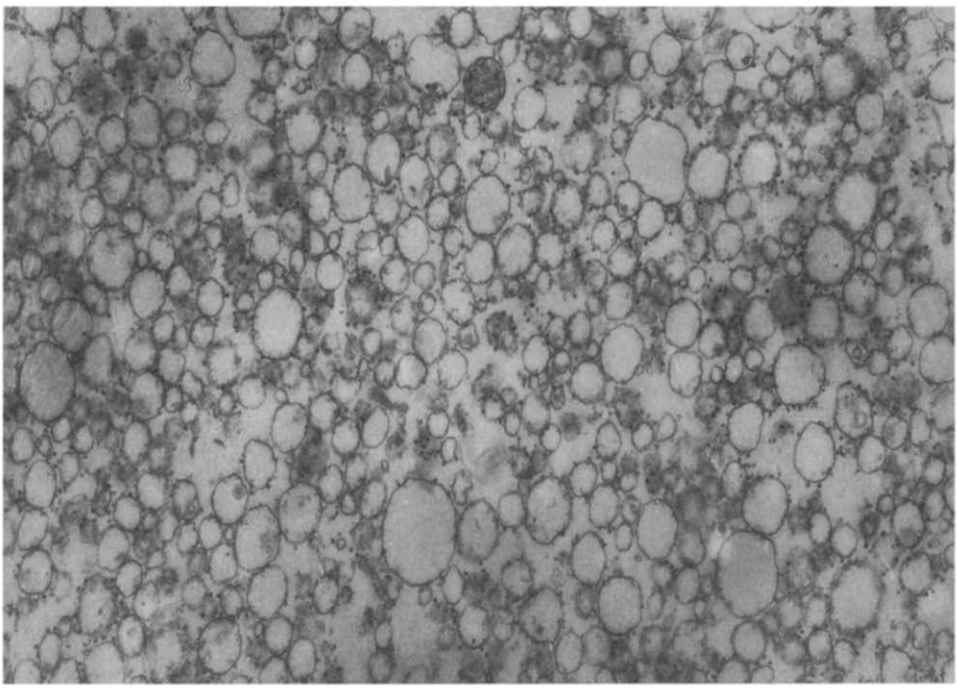

FIG. 1. Electron micrograph of microsomal fraction (× 41,250)

presence of NADH. When 2·3 mg of microsomal protein were preincubated with 2 μg parathion in the presence of NADH, 82 per cent of parathion was metabolized in 15 min incubation. From this amount 24 per cent was either metabolized by another pathway, or paraoxon underwent degradation as well.

The rate of parathion degradation was higher in the presence of microsomes isolated from phenobarbital pretreated rats and the amount of paraoxon detected was smaller in comparison with the untreated animals.

According to Neal[1] NADH neither inhibits nor stimulates parathion metabolism in the presence of NADPH. Kamin[14] stated that the activity of NADH-cytochrome b_5 reductase was not increased by drugs.

As there was an increase in NADPH-cytochrome *c* reductase and cytochrome P-450 activity, it appeared that NADPH-cytochrome *c* reductase and cytochrome P-450 were closely involved in the hydroxylation of drugs, whereas the NADH and cytochrome b_5 system served a different biological role, unfortunately still unknown.[14]

In general, many of the mixed function oxidations demonstrate a marked preference for NADPH with NADH being only about 1/7 to 1/3 as effective as NADPH.[15] According to our results, the activity of the microsomal NADH oxidizing system in the metabolism of parathion appears to be significant.

The activity of NADPH- and NADH-cytochrome *c* reductases in the presence of parathion was measured and the results are presented in Table 2.

TABLE 2. EFFECT OF PARATHION ON THE NADH-CYTOCHROME *c* REDUCTASE* AND NADPH-CYTOCHROME *c* REDUCTASE IN LIVER MICROSOMES OF INTACT AND PHENOBARBITAL TREATED RATS

					Phenobarbital-treated rats	
		Parathion (μg)	Enzyme activity	Inhibition (%)	Enzyme activity	Inhibition (%)
NADH	Control	—	851	—	953	—
	1	2	630	25·97	860	9·76
	2	10	553	35·02	745	21·83
	3	50	405	52·41	630	33·90
	4	100	303	64·40	403	57·72
NADPH	Control	—	90	—	135	—
	1	10	70	22·23	120	10·97
	2	100	60	33·34	90	33·34

* The enzyme activity was expressed in nmoles cytochrome *c* reduced/min per mg protein.

The activity of microsomal NADPH-cytochrome *c* reductase had only 10–20 per cent of the activity of NADH-cytochrome *c* reductase. The incubation of microsomes with parathion inhibited both enzymes; NADH-cytochrome *c* reductase was inhibited twice as much as NADPH-cytochrome *c* reductase. The activity of both enzymes was increased by about 12 per cent by phenobarbital pretreatment of animals. Parathion inhibition of enzymes in phenobarbital treated rats was smaller.

The results indicate that both NADH and NADPH oxidizing systems are involved in parathion metabolism. By studying the use of reduced NADP and/or reduced

NAD for the oxidative *N*-demethylation of aminopyrine and other drug substrates, Cohen and Estabrook[16] observed the ability of NADH to markedly stimulate the rate of aminopyrine metabolism in the presence of either NADPH or NADP plus a NADPH-generating system. They concluded that there were co-operative interactions between microsomal electron-transport reactions.[17] It is possible that, since the cell contains both NADPH and NADH, the co-operative effects studied *in vitro* have physiological significance.[17]

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REFERENCES

1. R. A. NEAL, *Biochem. J.* **103,** 183 (1967).
2. R. A. NEAL, *Biochem. J.* **105,** 289 (1967).
3. T. NAKATSUGAWA and P. A. DAHM, *Biochem. Pharmac.* **16,** 25 (1967).
4. T. NAKATSUGAWA, N. M. TOLMAN and P. A. DAHM, *Biochem. Pharmac.* **17,** 1517 (1968).
5. T. NAKATSUGAWA, N. M. TOLMAN and P. A. DAHM, *Biochem. Pharmac.* **18,** 1103 (1969).
6. R. M. WOLCOTT, W. K. VOUGHN and R. A. NEAL, *Toxic. appl. Pharmac.* **22,** 213 (1972).
7. R. M. WOLCOTT and R. A. NEAL, *Toxic. appl. Pharmac.* **22,** 676 (1972).
8. R. E. MENZER and W. C. DAUTERMAN, *J. Agr. Fd Chem.* **18** (b), 1031 (1970).
9. H. REMMER, H. GREIM, J. B. SCHENKMAN and R. W. ESTABROOK in *Methods in Enzymology* (Eds. R. W. ESTABROOK and M. E. PULLMAN), Vol. 10, pp. 703–708. Academic Press, New York, (1967).
10. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
11. E. S. REYNOLDS, *J. cell Biol.* **17,** 208 (1963).
12. B. S. S. MASTERS, C. H. WILLIAMS and H. KAMIN in *Methods in Enzymology* (Eds. R. W. ESTABROOK and M. E. PULLMAN), Vol. 10, pp. 565–573. Academic Press, New York (1967).
13. G. E. PALADE and P. SIEKEVITZ, *J. biophys. biochem. Cytol.* **2,** 171 (1956).
14. H. KAMIN and B. S. S. MASTERS in *Enzymatic Oxidation of Toxicants* (Ed. E. HODGSON), pp. 5–25. N.C. State University, Raleigh (1968).
15. B. S. COHEN and R. W. ESTABROOK, *Archs. Biochem. Biophys.* **143,** 37 (1971).
16. B. S. COHEN and R. W. ESTABROOK, *Archs. Biochem. Biophys.* **143,** 46 (1971).
17. B. S. COHEN and R. W. ESTABROOK, *Archs. Biochem. Biophys.* **143,** 54 (1971).