hepatectomized rats, liver carboxylesterase and cholinesterase activities were significantly more inhibited in the later animals but were still very evident. This finding had brought additional evidence for the great capacity of rat liver for soman detoxification and the method used was convenient for quantitative measurement of liver involvement in such a process.

Military Technical Institute MILAN JOKANOVIĆ
Medical Department
Katanićeva 15
11000 Belgrade
Yugoslavia

REFERENCES

1. Aldridge WN, Hoskin FCG, Reiner E and Walker, CH, Suggestions for a nomenclature and classification of enzymes hydrolysing organophosphorus compounds. In: *Enzymes Hydrolysing Organophosphorus Compounds* (Eds. Reiner E, Aldridge WN and Hoskin FCG), pp. 246–253. Ellis Horwood Ltd, Chichester, 1989.
2. Sterri SH, Lyngaas S and Fonnum F, Toxicity of soman after repetitive injection of sublethal doses in rat. *Acta Pharmacol Toxicol* **46**: 1–7, 1980.
3. Bošković B, Jokanović M, Maksimović M and Radović Lj, Soman and sarin detoxicating enzymes in different animal species. In: *International Meeting on Esterases Hydrolysing Organophosphorus Compounds, Dubrovnik, Yugoslavia, 24–27 April 1988, Book of Abstracts*, p. 10, 1988.
4. Jokanović M, Role of carboxylesterase in soman, sarin and tabun poisoning in rats. *Pharmacol Toxicol*, **65**: 181–184, 1989.
5. Sterri SH, Lyngaas S and Fonnum F, Toxicity of soman after repetitive injection of sublethal doses in guinea-pig and mouse. *Acta Pharmacol Toxicol* **49**: 8–13, 1981.
6. Sterri SH, Valdal G, Lyngaas S, Odden E, Malthe-Sørensen D and Fonnum F, The mechanism of soman detoxification in perfused rat liver. *Biochem Pharmacol* **32**: 1941–1943, 1983.
7. Clement JG, Role of aliesterase in organophosphate poisoning. *Fundam Appl Toxicol* **4**: S96–S105, 1984.
8. Bošković B, The influence of 2-(*o*-cresyl)-4H-1:3:2-benzodioxaphosphorin-2-oxide (CBDP) on organophosphate poisoning and its therapy. *Arch Toxicol* **42**: 207–216, 1979.
9. Maxwell DM, Brecht KM and O'Neill BL, The effect of carboxylesterase inhibition on interspecies differences in soman toxicity. *Toxicol Lett* **39**: 35–42, 1987.
10. Gupta RC, Petterson GT and Dettbarn W-D, Mechanisms involved in the development of tolerance to DFP toxicity. *Fundam Appl Toxicol* **5**: S17–S28, 1985.
11. Waynforth HB, Hepatectomy. In: *Experimental and Surgical Technique in the Rat*, pp. 136–142. Academic Press, London, 1980.
12. Higgins GM and Anderson RH, Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. *Arch Pathol* **12**: 186–194, 1931.
13. Weil CS, Tables for convenient calculation of median-effective dose (LD_{50} and ED_{50}) and instructions in their use. *Biometrics* **8**: 249–263, 1952.
14. Ellman GL, Courtney KD, Andres V and Featherstone RM, A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* **7**: 88–95, 1961.
15. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **15**: 265–275, 1951.
16. de Jong LPA, van Dijk C and Benschop HP, Hydrolysis of the four stereoisomers of soman catalysed by liver homogenate and plasma from rat, guinea pig and marmoset, and by human plasma. *Biochem Pharmacol* **37**: 2939–2948, 1988.
17. Jokanović M, Role of skin enzymes in detoxification of soman and sarin in rat. *Iugoslav Physiol Pharmacol Acta* **25** (Suppl. 7): 67–68, 1989.

Biochemical Pharmacology, Vol. 39, No. 4, pp. 799–801, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Desulfuration of the insecticide parathion by human placenta *in vitro*

(*Received* 20 *April* 1989; *accepted* 29 *August* 1989)

Despite the existence of multiple forms (at least five) of cytochrome P-450 within human placenta, only a limited number of xenobiotics have been documented thus far as substrates for placental cytochrome P-450-dependent oxidation [1, 2]. Furthermore, these reported activities occur at extremely low rates [2], except for aryl hydrocarbon hydroxylase activities in placentae of smokers which can approach those activities found in adult rat livers [3–6].

The present study, by quantifying covalently bound [^{35}S] after incubation of placental homogenates with [^{35}S]parathion [*O,O*-diethyl-*O*-(4-nitrophenyl)phosphorothioate], demonstrates the capacity of human placentae from nonsmokers to desulfurate this organothiophosphate insecticide. Parathion is known to undergo oxidative desulfuration by hepatic cytochrome P-450-dependent monooxygenases, leading to formation of the potent cholinesterase inhibitor paraoxon [*O,O*-diethyl-*O*-(4-nitrophenyl)phosphate], diethyl phosphorothioate, diethyl phosphate, and atomic sulfur [7, 8]. Atomic sulfur is a highly reactive electrophile, which binds covalently to tissue macromolecules following administration of [^{35}S]parathion *in vivo* [9] and upon incubation of this insecticide with hepatic microsomes *in vitro* [10]. As a result, covalent binding of [^{35}S] following incubation of tissues with [^{35}S]parathion can be utilized as a sensitive assay to document the occurrence of desulfuration of this insecticide.

Methods

[^{35}S]Parathion (20–50 mCi/mmol) was obtained from the Amersham Corp. (Arlington Heights, IL). Unlabeled parathion was purchased from Chem Service Inc. (West Chester, PA), while $NADP^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from the Sigma Chemical Co. (St Louis, MO). Placentae were obtained following repeat Cesarean sections from nonsmokers who were not receiving any medications chronically. Four patients were anesthetized by epidural injection of lidocaine hydrochloride; two other patients underwent general anesthesia with thiopental sodium, succinylcholine

chloride, atracurium besylate, and isoflurane. Placentae were immersed in ice-cold buffer (50 mM sodium phosphate, pH 7.4) as soon as possible in the delivery room. Tissues were transported to the laboratory within 15 min following delivery. A portion of each placenta (40–60 g) was rinsed, excised free from connective tissue, and homogenized in 4 vol. of buffer. The homogenate was filtered through cheesecloth and the volume noted. Incubation volumes were 3 mL, containing an NADPH-generating system [11], homogenate equivalent to 500 mg tissue, and, unless otherwise indicated, a total parathion concentration of 20 μM (including 5×10^5 dpm). Reactions were carried out at 37° in a Lab Line Orbit Shaker Bath, and were terminated by quick-freezing. Samples were slowly thawed the next day and the proteins precipitated by addition of an equal volume of 20% trichloroacetic acid. In certain instances when enzymatic activity was extremely low, several incubations were pooled to increase total radioactivity present. The precipitated pellets were washed with ethyl acetate, followed by methanol at 50°, and then by ethanol:water (50:50). Each wash was repeated until radioactivity was no longer detected in the wash (usually 10–15 washes per solvent were required with wash volumes of 12 mL). Although it is not possible to state unequivocally that the remaining radioactivity was covalently bound, such washing yielded results highly suggestive of covalently bound radioactivity. Pellets were dissolved in 2 mL of NCS tissue solubilizer (Amersham Corp.), neutralized by addition of glacial acetic acid, and counted on a scintillation counter following addition of 10 mL Ecolume (ICN, Irvine, CA).

Results and discussion

Biotransformation of parathion by rat hepatic microsomes leads to polysulfides and/or hydrodisulfides formed by the combination of reactive sulfur with cysteine residues of microsomal protein [12]. Consequently, reactive sulfur likely binds to a variety of proteins, including cytochrome P-450 [12, 13]. In the present study, incubation of human placental homogenate with parathion resulted in limited desulfuration of this insecticide, as evidenced by the presence of covalently bound sulfur to placental proteins (Figs. 1 and 2). It should also be noted that determination of covalently bound sulfur will underestimate the amount of atomic sulfur produced should reactive sulfur covalently bind to cellular macromolecules other than proteins. Production of covalently bound sulfur required an NADPH-generating system and was inhibited by carbon monoxide (Fig. 3). Considering what is currently known about the biotransformation of parathion by cytochrome P-450 [13, 14], these data suggest that a placental cytochrome P-450-dependent monooxygenase(s) catalyzes the oxidative desulfuration of parathion. Parathion has been reported to undergo oxidative desulfuration by several different forms of cytochrome P-450 [8, 15], including $P\text{-}450_{\beta NF\text{-}B}$ which can catalyze the hydroxylation of polycyclic aromatic hydrocarbons. Therefore, that form(s) of cytochrome P-450 in human placenta which mediates aryl hydrocarbon hydroxylase activity [3–6] may also catalyze the oxidation of parathion.

Neal and Halpert [15] have shown that sulfur binding to hepatic cytochrome P-450 as a result of biotransformation of parathion leads to a loss of cytochrome P-450 detectable as its carbon monoxide complex and a loss of cytochrome P-450-dependent monooxygenase activity. Therefore, deviation from linearity of sulfur binding with time (Fig. 1) likely resulted from inactivation of cytochrome P-450.

The present study does not definitively establish the metabolic activation of parathion to the potent cholinesterase inhibitor paraoxon by human placenta since no

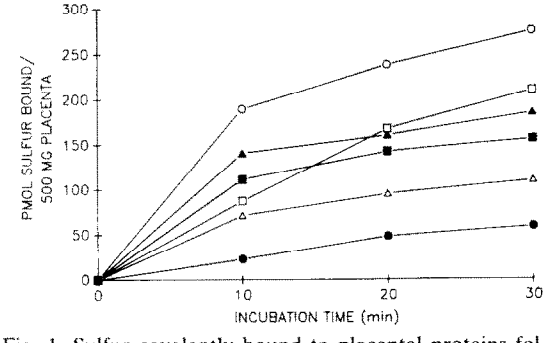

Fig. 1. Sulfur covalently bound to placental proteins following incubation of parathion (20 μM) with placental homogenates *in vitro*. Each line represents a single placenta.

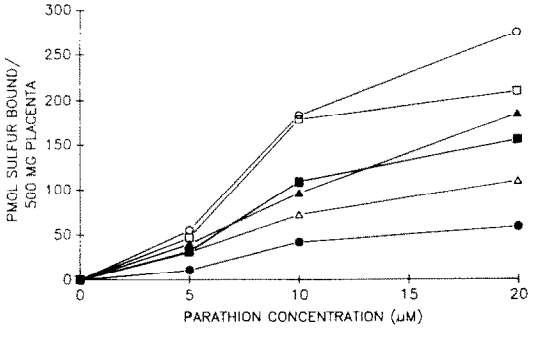

Fig. 2. Sulfur covalently bound to placental proteins following incubation of various concentrations of parathion with placental homogenates *in vitro*. Incubation times were 30 min. Each line represents a single placenta.

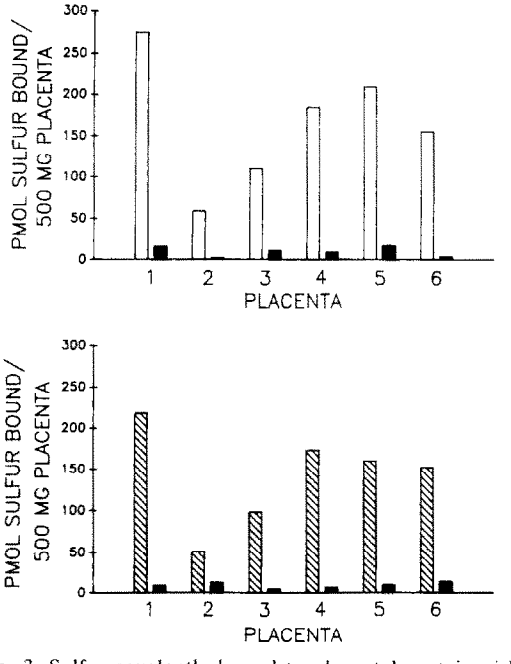

Fig. 3. Sulfur covalently bound to placental proteins following incubation of parathion (20 μM) with placental homogenates *in vitro* with ■ and without □ exposure to carbon monoxide (upper panel) or in the presence ▨ or absence ■ of an NADPH-generating system (lower panel). Samples utilized for experiments in the lower panel were dialyzed for 6 hr in 50 mM sodium phosphate buffer (pH 7.4) at 4° before incubation. Incubations were performed at 37° for 30-min.

attempts at metabolite identification (other than sulfur) were made. However, the assumption that at least some paraoxon was produced is not unreasonable since oxidative desulfuration of parathion by rat hepatic cytochrome P-450 leads predominantly to production of paraoxon with little diethyl phosphoric acid [7, 8].

Although most organothiophosphate insecticides are considered to be only mildly teratogenic (if at all), these chemicals are fetotoxic to mice and rats, resulting in decreased litter sizes as well as increased stillbirth rates, although at doses also toxic to the mother [16–21]. Similarly, maternal exposures to organothiophosphate insecticides frequently lead to decreased survival rates following birth [20, 22, 23]. However, one must cautiously consider the toxicological significance (if any) of the results of the present study because of the extremely low rates of desulfuration, as indicated by covalent binding of sulfur to placental protein.

In conclusion, the present study documents the capacity of human placenta to desulfurate the insecticide parathion. This activity, which requires NADPH and is inhibited by carbon monoxide, is likely catalyzed by a cytochrome P-450-dependent monooxygenase(s).

Acknowledgements—This work was supported by Grant ES04335 from NIEHS.

LESTER G. SULTATOS*†
CAROL L. GAGLIARDI‡

**Department of Pharmacology and Toxicology; and*
‡Department of Obstetrics and Gynecology
The University of Medicine and Dentistry of New Jersey, Newark, NJ 07103–2757, U.S.A.

REFERENCES

1. Juchau MR, Drug biotransformation in the placenta. *Pharmacol Ther* **8**: 501–524, 1980.
2. Juchau MR, Namkung MJ and Rettie AE, P-450 cytochromes in the human placenta: oxidations of xenobiotics and endogenous steroids. *Trophoblast Res* **2**: 235–263, 1987.
3. Welch RM, Harrison YE, Grommi BW, Poppers PJ, Finster M and Conney AH, Stimulatory effects of cigarette smoking on the hydroxylation of 3,4-benzpyrene and N-demethylation of 3-methyl-4-monomethylaminoazo-benzene by enzymes in human placenta. *Clin Pharmacol Ther* **10**: 100–109, 1969.
4. Nebert DW, Winker J and Gelboin HV, Aryl hydrocarbon hydroxylase activity in human placenta from cigarette smoking and nonsmoking women. *Cancer Res* **29**: 1763–1769, 1969.
5. Juchau MR, Human placental hydroxylation of 3,4-benzpyrene during early gestation and at term. *Toxicol Appl Pharmacol* **18**: 665–675, 1971.
6. Pelkonen O, Jouppila P and Karki NT, Effect of maternal cigarette smoking on 3,4-benzpyrene and *N*-methylaniline metabolism in human fetal liver and placenta. *Toxicol Appl Pharmacol* **23**: 399–407, 1972.
7. Kamataki T, Lee Lin MCM, Belcher DH and Neal RA, Studies of the metabolism of parathion with an apparently homogenous preparation of rabbit liver cytochrome P-450. *Drug Metab Dispos* **4**: 180–189, 1976.
8. Guengerich FP, Separation and purification of multiple forms of microsomal cytochrome P-450. *J Biol Chem* **252**: 3970–3979, 1977.
9. Poore RE and Neal RA, Evidence for extrahepatic metabolism of parathion. *Toxicol Appl Pharmacol* **23**: 759–768, 1972.
10. Norman BJ, Poore RE and Neal RA, Studies of the binding of sulfur released in the mixed-function oxidase-catalyzed metabolism of diethyl *p*-nitrophenyl phosphorothionate (parathion) to diethyl *p*-nitrophenyl phosphate (paraoxon). *Biochem Pharmacol* **23**: 1733–1744, 1974.
11. Sultatos LG and Murphys SD, Kinetic analyses of the microsomal biotransformation of the phosphorothioate insecticide chlorpyrifos and parathion. *Fundam Appl Toxicol* **3**: 16–21, 1983.
12. Davis JE and Mende TJ, A study of the binding of sulfur to rat liver microsomes which occurs concurrently with the metabolism of *O,O*-dietyl *O-p*-nitrophenyl phosphorothioate (parathion) to *O,O*-diethyl *O-p*-nitrophenyl phosphate (paraoxon). *J Pharmacol Exp Ther* **201**: 490–497, 1977.
13. Halpert J and Neal RA, Inactivation of rat liver cytochrome P-450 by the suicide substrates parathion and chloramphenicol. *Drug Metab Rev* **12**: 239–259, 1981.
14. Guengerich FP, Enzymology of rat liver cytochrome P-450. In: *Mammalian Cytochrome P-450* (Ed. Guengerich FP), pp. 1–54. CRC Press, Boca Raton, FL, 1987.
15. Neal RA and Halpert J, Toxicology of thiono-sulfur compounds. *Annu Rev Pharmacol Toxicol* **22**: 321–339, 1982.
16. Kalow W and Marin A, Second-generation toxicity of malathion in rats. *Nature* **192**: 464–465, 1961.
17. Tanimura T, Katsuya T and Nishimura H, Embryotoxicity of acute exposures to methyl parathion in rats and mice. *Arch Environ Health* **15**: 609–613, 1967.
18. FAO/WHO, *Evaluations of Some Pesticide Residues in Food*. WHO/Food Add/69.35, Geneva, 1969.
19. Fuchs V, Golbs S, Kuhnert M and Osswald F, Studies into prenatal toxic action of parathion methyl on Wistar rats and comparison with prenatal toxicity of cyclophosphamide and trypan blue. *Arch Exp Vet Med* **30**: 343–350, 1976.
20. Villeneuve DC, Willes RF, Lacroix JB and Phillips WEJ, Placental transfer of ^{14}C-parathion administered intravenously to sheep. *Toxicol Appl Pharmacol* **21**: 542–548, 1972.
21. Talens G and Wooley D, Effects of parathion administration during gestations in the rat on development of the young. *Proc West Pharmacol Soc* **16**: 141–145, 1973.
22. Barbes JM and Denz FA, The chronic toxicity of *p*-nitrophenyl diethyl thiophosphate (E605). *J Hyg* **49**: 430–441, 1951.
23. FAO/WHO, *1972 Evaluations of Some Pesticide Residues in Food*. WHO Pesticide Reidue Series. No. 2, Geneva, 1973.

† Address correspondence to: Lester Sultatos, Ph.D., Department of Pharmacology and Toxicology, University of Medicine and Dentistry of New Jersey, Newark, NJ 07103-2757.