spermine and spermidine in the herpes simplex virion. *Proc Natl Acad Sci USA* **68**: 2818–2821, 1971.
24. Ehresmann DW, Deig EF and Hatch T, *Anti-viral Properties of Algal Polysaccharides and Related Compounds*. Walter de Gruyter, Berlin, 1979.
25. Baba M, Pauwels R, Balzarini J, Arnout J, Desmyter J and De Clercq E, Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus *in vitro*. *Proc Natl Acad Sci USA* **85**: 6132–6136, 1988.
26. Schols D, Baba M, Pauwels R and De Clercq E, Flow cytometric method to demonstrate whether anti-HIV-1 agents inhibit virion binding to T4$^+$ cells. *J Acquired Immune Deficiency Syndromes* **2**: 10–15, 1989.
27. Nakashima H, Yoshida O, Baba M, De Clercq E and Yamamoto N, Anti-HIV activity of dextran sulphate as determined under different experimental conditions. *Anti-viral Res* **11**: 233–246, 1989.

Biochemical Pharmacology, Vol. 39, No. 4, pp. 797–799, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Liver esterases and soman toxicity in the rat following partial hepatectomy

(*Received* 17 *May* 1989; *accepted* 20 *September* 1989)

It is well established that soman (1,2,2′-trimethylpropyl methylphosphonofluoridate) is detoxificated through hydrolysis catalysed by the unspecific enzyme somanase (EC 3.1.8.1, organophosphorus compound hydrolase [1]) and binding to serine esterases other than acetylcholinesterase (EC 3.1.1.7) such as carboxylesterase (EC 3.1.1.1; carboxylic ester hydrolase) and cholinesterase (EC 3.1.1.8). These enzymes are widely distributed in the body. In rat liver, which possesses a high capacity of soman detoxification [2] as indicated by great activity, sensitivity and affinity of carboxylesterases and somanases towards the agent [3], the carboxylesterase and cholinesterase activities were inhibited to a lesser extent than in other organs after soman poisoning [4, 5].

The importance of liver in soman detoxification had been previously investigated both by soman perfusion through rat liver [6] and by alteration of liver carboxylesterase and cholinesterase activities after pretreatment with various agents such as phenobarbital [4, 7], triorthocresyl phosphate (TOCP) [4] or 2-(*o*-cresyl)-4H-1:3:2-benzodioxaphosphorin-2-oxide (CBDP) [7–9], tetraisopropyl pyrophosphoramide (iso-OMPA) [7, 10] and *N,N′*-diisopropylphosphordiamidofluoridate (mipafox) [10]. So far, no data has been available concerning the influence of partial hepatectomy on acute toxicity of organophosphorus compounds. The purpose of this study was to compare the abilities of intact and partially removed rat livers to detoxify soman as well as to examine the effect of the widely used method of patial hepatectomy on the acute soman toxicity in rats.

Materials and methods

Animals. All experiments were carried out on male Wistar rats weighing 170–190 g which had food and water *ad lib.* Rats under slight ether anesthesia underwent partial hepatectomy according to the procedure of Waynforth [11]. The technique (originally described by Higgins and Anderson [12]), based on removal of the main lobes (median and left lateral) which comprise approximately two-thirds of the liver, is tolerated well by the animals and simple to perform. However, the ablation of 85% of the liver was not tolerated as well.

Materials. Soman was obtained from the Chemistry Section at the Military Technical Institute. It was 98.8% pure as determined by nuclear magnetic resonance spectroscopy. Other chemicals were obtained from various commercial sources: tributyrin and physostigmine sulfate (Serva, Heidelberg, F.R.G.); methyl butyrate (Fluka, Buchs, Switzerland); ethyl butyrate and atropine sulfate (Sigma Chemical Co., Poole, U.K.); triorthotolyl phosphate (Eastman Kodak, Rochester, NY) and oxime HI-6 (pyridinium, 1-(((4-carbamoylpyridinio)-methoxy) methyl-2-(hydroxyiminomethyl) dichloride) (Institute SBS Sarajevo, Yugoslavia).

Toxicology. Acute toxicity of soman based on 24 hr mortalities was calculated by the method of Weil [13]. TOCP (250 mg/kg, s.c.) was administered 24 hr before performing partial hepatectomy.

Enzyme determinations. Rats were pretreated with physostigmine sulfate (0.2 mg/kg, s.c.) + atropine sulfate (20 mg/kg, i.m.) + HI-6 (50 mg/kg, i.m.) 20 min prior to the s.c. injection of soman. This treatment schedule was adopted to ensure that there were survivors at the 30 min sampling period. Such pretreatment did not affect normal carboxylesterase and cholinesterase activity in rat liver.

Liver homogenate (1:10) in saline was prepared in Potter–Elvehjem homogenizer and centrifuged at 3000 rpm for 10 min.

Carboxylesterase activities were assayed by the pH-stat technique using tributyrin (final concentration 6.5 mmol/L), ethyl (9 mmol/L) and methyl (5 mmol/l) butyrates as substrates. Butyric acid, liberated from enzymatic degradation of the substrates, is titrated with 0.01 N NaOH solution. To a reaction vessel 10 mL of substrate solution was added and then titrated to pH 7.60 at 25°. The amounts of liver homogenate supernatant added were: 0.2 mL for tributyrin and 0.05 mL for ethyl and methyl butyrate hydrolysis. Enzyme activities were calculated from the rate of addition of NaOH during the first 5 min after homogenate was added (and expressed as nmoles of substrate hydrolysed per min per mg of protein). All solutions were CO_2-free. Cholinesterase activity in liver was assayed towards acetylthiocholine iodide as substrate (and expressed as nmoles of substrate hydrolysed per min per mg protein) according to the method of Ellman *et al.* [14]. Protein content was determined by the method of Lowry *et al.* [15].

Statistics. Significance of difference was examined by Student's *t*-test; $P < 0.05$ was considered statistically significant.

Results and discussion

The results presented in Table 1 show that partial hepatectomy markedly increased acute toxicity of soman in rats. Treatment with 250 mg/kg of TOCP, which caused complete inhibition of plasma and skin carboxylesterase and 85% of liver carboxylesterase activities [3], strongly potentiated the toxicity of soman. After TOCP treatment most soman binding sites are already occupied and cholinesterases are then widely exposed to the agent. Under these circumstances detoxification of this compound is greatly altered since the reduction of soman LD_{50} to 15% of its initial value (Table 1) cannot further be attributed to carboxylesterases but to enzymatic hydrolysis. Therefore,

Table 1. Effect of partial hepatectomy on soman toxicity in rats

% of liver removed	Soman LD_{50} (μg/kg)	95% limits	TOCP pretreated soman LD_{50}* (μg/kg)	95% limit	Relative toxicity‡
0	80.0	(71.0–91.0)	11.3	(10.1–12.7)	7.1
67	51.5	(45.8–58.7)	6.3	(5.6–7.1)	8.2
85	38.1	(36.6–41.2)	4.8	(4.3–5.5)	7.9

* Rats were treated with TOCP (250 mg/kg, s.c.) 24 hr before performing partial hepatectomy. Soman was given 24 hr later.

‡ Relative toxicity = $\frac{\text{soman } LD_{50}}{\text{soman } LD_{50} \text{ after TOCP pretreatment}}$.

Table 2. Effect of soman on liver carboxylesterase and cholinesterase activities in intact and partially hepatectomized rats

Rats	Dose soman ($\times LD_{50}$)	Carboxylesterase† Tributyrin	Ethyl butyrate	Methyl butyrate	Cholinesterase
Control	—	138 ± 38 (100)	1261 ± 241 (100)	854 ± 73 (100)	12.5 ± 1.2 (100)
		NS	NS	NS	NS
Intact	0.75	117 ± 6 (84.8)	1095 ± 152 (86.8)	828 ± 104 (97.0)	12.2 ± 2.0 (97.6)
		NS	NS	NS	NS
	5	107 ± 7 (77.5)	928 ± 120 (73.6)	729 ± 145 (85.4)	9.1 ± 2.3 (72.8)
		*	*	*	*
Partially hepatectomized	0.75	84 ± 12 (60.9)	725 ± 186 (57.5)	521 ± 95 (61.0)	7.9 ± 1.0 (63.2)
		*	*	*	*
	5	73 ± 15 (52.9)	374 ± 97 (29.7)	304 ± 69 (35.6)	4.9 ± 1.7 (39.2)

Partially hepatectomized rats received equitoxic doses of soman (i.e. 39 and 258 μg/kg) considering that the LD_{50} value was 51.5 μg/kg (Table 1).

Rats were pretreated with physostigmine (0.20 mg/kg, s.c.) + atropine (20 mg/kg, i.m.) + HI-6 (50 mg/kg, i.m.) 20 min prior to receiving soman. Soman was given s.c. 24 hr after two-thirds hepatectomy and enzyme activities (expressed as nmol substrate hydrolysed per min per mg protein) were determined 30 min later.

† Mean ± SE; (N = 6–8), numbers in parentheses indicate percentage change compared to controls (100%).

* $P < 0.05$; ** $P < 0.005$; (Student's t-test).

NS, not significant.

it appears that when doses close to LD_{50} of soman are given by the s.c. route only a minor part of the agent is detoxificated by somanases. This is in accordance with the results of de Jong *et al.* [16] who found that less toxic isomers of soman are preferentially degraded by somanases. However, the importance of these enzymes should be more expressed in poisonings with larger doses of soman. In addition, partial hepatectomy did not markedly influence potentiation of the soman toxicity by TOCP.

The effect of two doses (0.75 and 5 LD_{50}) of soman on carboxylesterase and cholinesterase activities in rat liver was examined in intact and two-thirds hepatectomized rats (Table 2). 0.75 LD_{50} of soman given to intact rats only slightly inhibited liver carboxylesterase activity. The other dose (5 LD_{50}) of the agent administered to intact rats significantly inhibited liver carboxylesterase activity towards ethyl butyrate as substrate. When equitoxic doses of soman were given to partially hepatectomized rats, liver carboxylesterase and cholinesterase activities were significantly more inhibited than in intact rats. These enzymes were not maximally inhibited in partially hepatectomized rats that received 5 LD_{50} of soman, indicating that the main part of the agent was detoxificated through hydrolysis and binding before it reached these enzymes. One-third of liver that was left in the rats appeared to be successful in detoxification of soman as evidenced by 30–53% of carboxylesterase activities that were preserved after administration of 5 LD_{50} of soman (Table 2).

Under the conditions of reduced liver size and inhibition of major part of liver carboxylesterase activity by TOCP, binding processes are not as important for soman detoxification and, thus, the significance of somanase activity which was not affected by such treatment seems to be crucial for survival of animals. Moreover, somanase and carboxylesterase activities from other tissues such as plasma and skin [17] that remained unaffected after partial hepatectomy may markedly contribute to this process.

In conclusion, the acute toxicity of soman in rats was greatly increased after partial hepatectomy. When equitoxic doses of soman were given to intact and two-thirds

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