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STUDIES ON THE METABOLISM OF THE PNEUMOTOXIN *O,S,S*-TRIMETHYL PHOSPHORODITHIOATE—I

LUNG AND LIVER MICROSOMES

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Abstract—The metabolism of *O,S,S*-trimethyl phosphorodithioate (OSSMe), a pneumotoxic impurity in some organophosphorus insecticides, was investigated in rat lung and liver microsomal preparations, using OSSMe labelled with ^{3}H or ^{14}C on one of its thiolo-methyl (CH_3S-) groups. Production of *O,S*-dimethyl phosphorothioate ($OSMeO^-$) and binding of radioactivity to protein were NADPH-dependent and were shown to be, at least partly, cytochrome P-450-dependent processes in both lung and liver microsomes. Incubation with reduced glutathione prevented the binding of radioactivity without affecting $OSMeO^-$ production. The K_m for the conversion of OSSMe to $OSMeO^-$ was 15-fold lower in lung (0.30 ± 0.07 mM) than in liver (4.63 ± 2.42 mM) microsomes. These results show that cytochrome P-450-dependent mixed-function oxidase is implicated in at least part of the metabolic activation of OSSMe, and suggest that the pulmonary isozyme(s) are more active at metabolizing OSSMe than hepatic isozymes. It is speculated, on the basis of literature data on other sulphur-containing chemicals, that the metabolic activation of OSSMe involves oxidation of a thiolo-sulphur, with subsequent formation of CH_3-*S*-*S*-protein disulphides.

O,S,S-trimethyl phosphorodithioate (OSSMe) is one of several pneumotoxic trialkyl phosphorothioates which can be found as impurities in commercial organophosphorus insecticides [1]. The reasons for the peculiar lung toxicity of OSSMe and the other trialkyl phosphorothioates are not known, but several factors, most notably the alterations of toxicity with modulators of xenobiotic metabolism, indicate that metabolic activation, probably in the lung itself, is necessary for toxicity [2–4]. The objectives of the present studies were to investigate whether lung tissue is capable of metabolic activation of OSSMe and, if so, to determine the characteristics of this metabolism, particularly the contribution of the cytochrome P-450 system. The underlying working hypothesis for these studies was that the existence of qualitative or quantitative differences between the metabolism of OSSMe by lung and liver tissue might explain the susceptibility of the lungs to this class of compounds.

As in many other studies of metabolically activated toxins [5], our experimental approach consisted of using radiolabelled OSSMe in order to assess water-soluble metabolite production on the one hand, and reactive metabolite formation measured as protein-bound radioactivity on the other hand. This article describes studies in which rat lung and liver microsomal preparations were incubated with OSSMe, and the accompanying paper [6] describes studies in which lung and liver slices were used.

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MATERIALS AND METHODS

Experimental animals. All rats used were female Porton-derived Wistar rats (LAC-P), weighing 180–200 g and housed in animal facilities with controlled temperature ($20 \pm 2°$) and a 12-hour light–dark cycle. The animals were allowed food and drink *ad libitum* until the day of the experiment.

Chemicals. All reagents used were of analytical quality or of the highest grade available. Tris, EDTA, NADH, NADP, NADPH, D-glucose-6-phosphate, glucose-6-phosphate dehydrogenase, reduced glutathione (GSH), methimazole (*N*-methyl-2-mercaptoimidazole) and *n*-octylamine, were obtained from Sigma (Poole, Dorset, U.K.); sodium phenobarbitone was obtained from BDH (Poole, Dorset, U.K.); piperonyl butoxide was obtained from Fluka (Buchs, Switzerland); SKF 525A (β-diethylamino-ethyl diphenylpropyl acetate) was obtained from Smith, Kline & French (Welwyn Garden City, Herts, U.K.); metyrapone was obtained from CIBA (Horsham, Sussex, U.K.).

OSSMe was synthesized to a purity of 99.7% by Dr P. B. Farmer, MRC Toxicology Unit, Carshalton, and stored at −40°. [^{3}H]-OSSMe and [^{14}C]-OSSMe were also synthesized by Dr P. B. Farmer, by the reaction of *O,S*-dimethyl phosphorodithioate with [^{3}H]- and [^{14}C]-dimethyl sulphate respectively. Therefore, in both instances the radiolabel was on one of the thiolo-methyl groups of OSSMe. The reaction products were purified by column chromatography, fractions containing OSSMe were checked by capillary gas chromatography with nitrogen/phosphorus detection [7] and shown to be pure. The specific activity of [^{3}H]-OSSMe was 357 μCi/μmol

Table 1. *O,S,S*-trimethyl phosphorodithioate and dimethyl phosphoro(di)thioates

Chemical formula	Name	Abbreviation
$(CH_3O)(CH_3S)_2P(O)$	*O,S,S*-trimethyl phosphorodithioate	OSSMe
$(CH_3O)(CH_3S)P(O)S^-$	O,S-dimethyl phosphorodithioate	OSMeS
$(CH_3S)_2P(O)O^-$	*S,S*-dimethyl phosphorodithioate	SSMeO
$(CH_3O)(CH_3S)P(O)O^-$	*O,S*-dimethyl phosphorothioate	OSMeO

and that of [^{14}C]-OSSMe was 6 μCi/μmol. For all experiments radioactive OSSMe was diluted with excess unlabelled OSSMe to obtain final specific activities of 70–400 nCi/μmol, or 150–900 dpm/nmol (except for the kinetic studies in which specific activities at the highest concentrations were lower). Solutions of OSSMe were always made on the morning of the experiment. Various dimethyl phosphorothioates supplied by Dr J. Miles and Mr D. Mount, CDC (Atlanta, GA), were available from a previous study [8]. The diesters used are shown in Table 1 with their abbreviations. Aqueous solutions of these diesters were made at concentrations of approximately 0.1 M to serve as standards for the analysis of metabolites by thin-layer chromatography (TLC).

Preparation and incubation of microsomes. The method for preparing lung microsomes was developed from the work of Hook *et al.* [9], Matsubara *et al.* [10] and Boyd *et al.* [11]. All experiments were done on freshly-prepared microsomes. Six to twelve rats were used for each experiment. They were killed by decapitation, and in the experiments carried out with [^{14}C]-OSSMe the lungs were perfused via the pulmonary artery with 10 ml heparinized saline at room temperature. The lungs were then removed and sliced with a McIlwain tissue slicer [12] in order to facilitate homogenization. All subsequent steps were carried out at 0–4°. The slices from three (or four) pairs of lungs were homogenized in 12 ml (or 16 ml) of 0.154 M KCl in a 20 mM Tris–HCl buffer (pH 7.4 at room temperature) with a Potter–Elvehjem type Teflon–glass homogenizer [13] (0.125 mm clearance, 16 passes at 1900 rpm). A 3 g (or 4 g) portion from the liver of every third (or fourth) rat was similarly homogenized.

After centrifugation of the homogenates at 10,000 *g* for 10 min, the supernatants were spun at 100,000 *g* for 60 min. The resulting pellet was resuspended in the same buffer and recentrifuged at 100,000 *g* for 60 min. The resulting pellets were resuspended in 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA (further referred to as phosphate buffer) and pooled to give the lung and liver microsomal suspensions (further referred to as microsomes). The volumes of phosphate buffer were adjusted so as to achieve protein concentrations in lung and liver microsomes of between 1.5 and 2.5 mg/ml suspension.

First 0.1 ml of NADPH (to give a final concentration of 1 mM), or 0.1 ml of a NADPH generating system (NADPH 0.13 mM, NADP 0.2 mM, glucose-6-phosphate 4.9 mM, $MgCl_2$ 5 μM, glucose-6-phosphate dehydrogenase 0.2 U/ml, final concentrations), or 0.1 ml phosphate buffer (incubations without NADPH) were added to 0.8 ml of the microsomal suspension and incubated at 37° in a shaking water bath for 30–45 sec. Then 0.1 ml of the radiolabelled OSSMe (in phosphate buffer) were added and the incubation continued for variable lengths of time. In some experiments all these quantities were halved in order to use less labelled compound. Particular incubation conditions, such as concentrations of inhibitors, are given in the figure legends. The reaction was stopped by the addition of 5 vol. of ethyl acetate. The solvent phase was removed after spinning for 2 min at about 1500 *g*. In some instances a second similar extraction with ethyl acetate was done.

Determination of protein bound radioactivity. After extraction the remaining microsomal material was washed at least 3 times with ethanol/diethyl ether/water (7/10/3) and then solubilized with 1% NaOH at 60°. An aliquot was taken for protein determination, and 10 ml scintillant ReadySolv MP® (Beckman) containing 1% acetic acid were added to another aliquot for subsequent scintillation counting for at least 10 min in a Packard Tri-Carb® 460 CD scintillation counter, with counting efficiency determined by the external standard-channels ratio method.

It was assumed that the radioactivity remaining after successive washings represented covalently-bound material. It is unlikely, in view of the high aqueous solubilities of OSSMe and its diester metabolites, that any parent compound or diesters remained trapped. Moreover, "biological blanks" (e.g. incubations without NADPH) were always included in the experimental protocols.

Separation and quantitation of diester metabolites by thin layer chromatography and scintillation counting. The dimethyl phosphorothioate diesters resulting from the metabolism of radiolabelled OSSMe were separated by TLC on aluminium-backed sheets (20 cm × 20 cm, Merck) precoated with 0.2 mm thick Silicagel 60 F_{254}. Samples of the incubation medium were removed from under the protein plug created by the ethyl acetate extraction(s) and were applied on the TLC plates by means of 25–200 μl microlitre syringes in 5 μl aliquots, with hot air drying between each aliquot. On all spots 1 μl aliquots of unlabelled $OSMeO^-$ and $SSMeO^-$ (approx 0.1 M) were also applied. The total volume of medium thus applied varied from 10 to 50 μl.

Initially, the samples still contained variable amounts of protein and this sometimes seemed to cause unsatisfactory large spots. Later, sample application and spot quality were improved by adding two volumes of acetone to the medium removed for analysis and centrifuging this for 10 min in an Eppendorf bench centrifuge.

Samples from different tissues or different incubation conditions (e.g. with and without NADPH)

were always present on the same plate, so as to avoid spurious effects due to differences in sample application or elution of the plates. The elution systems were taken from a manuscript kindly sent by Dr. A. J. Gray prior to its publication [14]. The solvent used in all experiments consisted of acetonitrile/methanol/acetic acid (80/20/5), which led to a good separation of $OSMeO^-$, $SSMeO^-$ and OSSMe. In this system $OSMeS^-$ produced a streak and its R_f appeared to be close to that of OSSMe. However, $OSMeS^-$ could be separated from OSSMe and the other two diesters by eluting with the solvent system isopropanol/benzene/ammonium hydroxide (16/2/3). The latter system was only used in preliminary experiments, because it was found that no detectable amounts of $OSMeS^-$ were ever produced.

Visualisation of the diesters was obtained by spraying the plates with $PdCl_2$ (Sigma) 0.1% (w/v) in acetone/HCl (9/1). The plates were cut in rectangles or squares of 16 mm width and variable lengths up to 20 mm, depending on the sizes of the spots. The areas cut around the visualized diesters were placed in scintillation vials and 1 ml of 1 N HCl was added to help the silicagel disintegrate and separate from the aluminium sheet. When this was completed, 10 ml scintillant (Ready Solv MP®, Beckman) were added and radioactivity later measured by scintillation counting for at least 10 min. Numerous preliminary experiments established that counting was not affected by the presence of the aluminium plate in the bottom of the vial.

Measurements of radioactivity over the whole trajectory of the eluting solvent showed recoveries to vary from 70 to 120% of the total applied radioactivity, with no systematic difference in recovery between lung and liver. With the methods utilized and the specific activities of OSSMe used, the levels of radioactivity found were low (generally below 500 dpm), and there was some variation in baseline results between different experiments. However, the consistency of replicates within each experiment was good and the experimental protocols were such that it was valid to make internal comparisons between, e.g. lung and liver, NADPH and no NADPH, control and inhibitor.

Other measurements. Protein concentrations were measured by the method of Peterson [15], leaving out the precipitation step, using bovine serum albumin (Sigma) as a standard, and allowing for the quantities of NaOH used to solubilize the protein pellets.

Cytochrome P-450 content was only determined [16] twice, because this required a considerable quantity of lung microsomes which were needed for the metabolic studies. These measurements gave values of 0.454 and 1.231 nmol/mg microsomal protein for the liver, and respectively 0.050 and 0.156 nmol/mg microsomal protein for the lung. Although these contents differed two- to three-fold between successive measurements (possibly due to differences in protein concentrations of the suspensions), they confirm the findings of many others [17, 18], that the content of cytochrome P-450 per microsomal protein is 5- to 20-fold lower in the lung than in the liver.

Presentation of results. Results are presented as means with standard deviations, and statistical comparisons were made using Student's *t*-test, adopting a level of $P < 0.05$ as statistically significant.

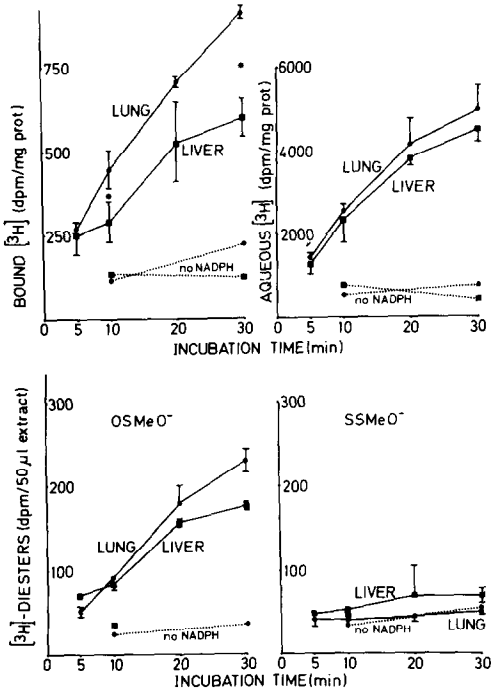

Fig. 1. Metabolism of *O,S,S*-trimethyl phosphorodithioate (OSSMe) by rat lung and liver microsomes. Lung microsomes from 9 female rats and liver microsomes from 3 of these rats were incubated at 37° with 1 mM [^{3}H-CH_3S]-OSSMe (specific activity 153 dpm/nmol) with or without NADPH (1 mM). Bound radioactivity is the radioactivity remaining in the microsomes after extensive washing; aqueous radioactivity is the radioactivity remaining in the medium after two ethyl acetate extractions; *O,S*-dimethyl phosphorothioate ($OSMeO^-$) and *S,S*-dimethyl phosphorodithioate ($SSMeO^-$) were determined by TLC of 50 µl samples of these extracts followed by scintillation counting of the appropriate areas. Values are means ± SD (N = 3 or 2) for incubations with NADPH and means of 2 determinations for incubations without NADPH. * $P < 0.05$ for lung vs liver.

RESULTS

Figure 1 shows that binding of radioactivity to microsomal protein and production of ethylacetate insoluble radioactivity were NADPH-dependent and increased with time of incubation in both lung and liver microsomes. At the concentration of OSSMe of 1 mM, binding of radioactivity was slightly higher with lung microsomes, but ethylacetate insoluble metabolite production was quantitatively (when expressed per mg microsomal protein) and qualitatively similar in lung and liver microsomes. In both instances the predominant diester found was $OSMeO^-$, with levels of $SSMeO^-$ being barely above those obtained in the absence of NADPH. When NADH (1 mM) was used instead of NADPH, the binding of radioactivity and the levels of diesters were only marginally increased above background (Fig. 2).

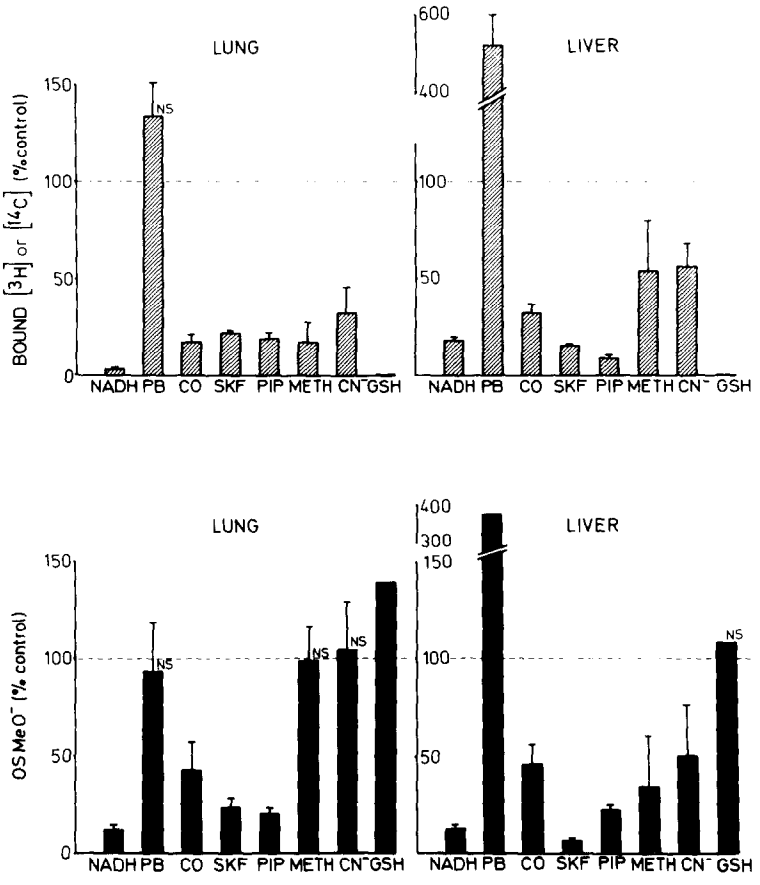

Fig. 2. Effects of phenobarbitone pretreatment and various *in vitro* treatments on the metabolism of *O,S,S*-trimethyl phosphorodithioate (OSSMe) by rat lung and liver microsomes. In different experiments, lung and liver microsomes of female rats were incubated at 37° with 1 mM [^{3}H-CH_3S]-OSSMe or 1 mM [^{14}C-CH_3]-OSSMe (specific activities from 280 dpm/nmol to 785 dpm/nmol) with or without NADPH (1 mM or a NADPH generating system) for 10 or 15 min. Bound radioactivity, aqueous radioactivity and diesters were determined as indicated in the legend of Fig. 1, except that in some instances TLC was carried out on 50 μl samples containing 33 μl acetone + 17 μl aqueous extract of the medium. Abbreviations: NADH = 1 mM NADH used instead of 1 mM NADPH; PB = 500 mg phenobarbitone/l drinking water for 7 days until the day before the experiment; CO = 15 min pre-exposure of microsomes to CO/O_2 80/20 (at 0°, no NADPH present); SKF = 1 mM SKF525A; PIP = 1 mM piperonyl butoxide (in methanol, 1% v/v final concentration); METH = 1 mM methimazole; CN^- = 1 mM NaCN; GSH = 5 mM glutathione. Values are means + SD expressed in per cent of appropriate control after subtraction of values obtained without NADPH (N = 3 or 4, except for GSH where N = 2). Differences from control are significant, except where indicated (NS).

The effects of microsomal protein concentration differed between lung and liver microsomes: for incubation times of 5–15 min, there was a linear relationship between lung microsomal protein concentrations (up to 5 mg/ml) and binding of radioactivity or $OSMeO^-$ production, whereas in liver microsomes a plateau appeared starting around a protein concentration of 2 mg/ml (not shown).

Figure 2 summarizes the results of different experiments in which microsomes of phenobarbitone-pretreated rats were used, or in which microsomes were incubated in the presence of various inhibitors of xenobiotic metabolism. Phenobarbitone induction greatly increased the binding of radioactivity and production of $OSMeO^-$ (but not that of $SSMeO^-$) in liver microsomes, but did not modify these indexes in lung microsomes. Pre-exposure of microsomes to carbon monoxide led to substantial decreases (without complete abolition) in binding of radioactivity and $OSMeO^-$ production in both lung and liver microsomes. Incubation in the presence of 1 mM SKF525A or 1 mM piperonyl butoxide also led to reductions in binding and $OSMeO^-$ production. These indexes were similarly decreased when compared to solvent controls (1% acetone v/v) by 5 mM metyrapone (16–52%) and 5 mM *n*-octylamine (11–51%), but in pulmonary microsomes the exact extent of the reduction was difficult to ascertain, because 1% acetone in itself considerably affected binding (35% of control) and $OSMeO^-$ production (58% of

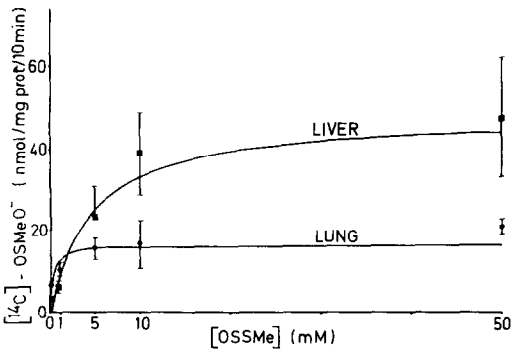

Fig. 3. Kinetics of the conversion of *O,S,S*-trimethyl phosphorodithioate (OSSMe) to *O,S*-dimethyl phosphorothioate ($OSMeO^-$) by rat lung and liver microsomes. Lung microsomes from 2 × 12 female rats and liver microsomes from 4 of these rats were incubated for 10 min at 37° with [^{14}C-CH_3S]-OSSMe in concentrations of 0.04 mM–50 mM (specific activities varying from 16 to 738 dpm/nmol) with and without 1 mM NADPH. $OSMeO^-$ was determined by TLC of 100 μl samples containing 67 μl acetone + 33 μl aqueous extract of the medium, followed by scintillation counting of the appropriate area; dpm-values were divided by half the corresponding specific activity of [^{14}C-CH_3S]-OSSMe. Values are means ± SD after subtraction of values obtained without NADPH (N = 3 for liver microsomes; N = 4 for lung microsomes except at 50 mM where N = 2).

control). Incubation with 1 mM methimazole reduced radioactive binding, but not $OSMeO^-$ production in lung microsomes, and both binding and $OSMeO^-$ in liver microsomes.

Incubations in the presence of 1 mM NaCN, resulted in a decreased binding of radioactivity in both lung and liver, and a decreased production of $OSMeO^-$ in liver microsomes only. In the presence of reduced glutathione (5 mM) the binding of radioactivity was completely abolished in both pulmonary and hepatic microsomes, whilst the amounts of $OSMeO^-$ were not affected or even slightly increased.

Figure 3 shows that the kinetics of the conversion of OSSMe to $OSMeO^-$ differed considerably between lung and liver microsomes. An iterative non-linear curve fitting procedure [19] using the mean data points weighted by their square yielded estimated values (± standard error of the estimate, 4 degrees of freedom) for K_m of 0.30 ± 0.07 mM in lung microsomes, and 4.63 ± 2.42 mM in liver microsomes, with corresponding V_{max} values of 15 ± 2 nmol $OSMeO^-$/mg lung microsomal protein/10 min incubation and 44 ± 15 nmol $OSMeO^-$/mg liver microsomal protein/10 min incubation. Using individual data points or other weighting factors gave essentially the same values.

DISCUSSION

Three main conclusions may be drawn from these studies on the metabolism of OSSMe by lung and liver microsomes.

First, in both lung and liver microsomes the main metabolite produced was $OSMeO^-$.

Secondly, in both lung and liver microsomes cytochrome P-450 dependent mixed-function oxidase is implicated in at least part of the metabolic conversion of OSSMe to $OSMeO^-$ and the generation of metabolites capable of binding to macromolecules.

Thirdly, these experiments also gave evidence that pulmonary microsomes despite their lower cytochrome P-450 content, are more active at metabolizing OSSMe than hepatic microsomes.

(i) *Microsomal metabolism of OSSMe*

Contrary to the situation with slices, in which lung and liver tissue were shown to release different proportions of $OSMeO^-$ and $SSMeO^-$ [6], both lung and liver microsomes produced predominantly the $OSMeO^-$ diester. Small and variable amounts of $SSMeO^-$ were found above background levels and "no NADPH" controls, thus suggesting that oxidative *O*-dealkylation is a possible, but minor pathway for the metabolism of OSSMe. This discussion will therefore focus on the biotransformation of OSSMe to $OSMeO^-$, since this pathway was not only quantitatively more important, but probably also more relevant with regard to toxicity.

(ii) *Involvement of cytochrome P-450*

Several classically-required conditions for establishing the involvement of cytochrome P-450 in the biotransformation of foreign compounds [20, 21] were met. Thus, production of the $OSMeO^-$ metabolite and binding of radioactivity were found to be strictly NADPH-dependent and to be significantly inhibited by mixed-function oxidase inhibitors such as carbon monoxide, SKF525A, piperonyl butoxide, *n*-octylamine and metyrapone. Induction of monooxygenase by phenobarbitone pretreatment led to higher levels of both indexes in liver microsomes, but not in lung microsomes. It is indeed known that phenobarbitone induction is specific for hepatic mono-oxygenases, and hardly affects extrahepatic mono-oxygenase activities [17, 22, 23].

Involvement of the FAD-dependent mono-oxygenase was also envisaged in view of the partial inhibition found with the cytochrome P-450 inhibitors and in view of the known role of this monooxygenase in the biotransformation of several sulphur-containing chemicals [24–26]. In lung microsomes, methimazole, a good and possibly specific substrate for the FAD-containing mono-oxygenase [27], had no influence on the production of $OSMeO^-$, but markedly reduced the binding of radioactivity to protein (conceivably by quenching reactive intermediates), whereas in liver microsomes both $OSMeO^-$ and binding were significantly decreased in the presence of methimazole. Therefore, participation of FAD-dependent mono-oxygenase in the activation of OSSMe could not be demonstrated in lung microsomes, and could not be excluded in liver microsomes.

Cyanide is not usually included in drug metabolism studies, but here NaCN had been used as a "negative control", as in the studies of Boyd *et al.* [11] on the metabolism of 4-ipomeanol, in which a similar 1 mM CN^- concentration had no effects on covalent binding. Cyanide is classically considered not to inhibit cytochrome P-450-mediated drug metabolism

[27]. However, cyanide does interact with cytochrome P-450 forming a "modified type II spectrum" with relatively high spectral dissociation constant, and 10 mM cyanide has been noted to inhibit aminopyrine demethylation [28]. The differential effect of 1 mM CN^- found here between hepatic and pulmonary microsomes for the conversion of OSSMe to $OSMeO^-$ may reflect differences in the affinity of the respective cytochromes for OSSMe and CN^-. The decrease in covalent binding in the lung and liver microsomes may have resulted from a non-enzymatic reaction of the reactive metabolites with CN^- (cf. [29]).

(iii) *Comparison between lung and liver microsomal metabolism of OSSMe*

When results were expressed per milligram of microsomal protein, lung and liver microsomes did not greatly differ in the production of $OSMeO^-$ or in the covalent binding at incubation concentrations of 1 mM OSSMe. However, the difference becomes considerable if the results were to be expressed per nanomole of cytochrome P-450 (this assumes that metabolism is entirely due to mixed-function oxidase). Indeed, the cytochrome P-450 content of pulmonary microsomes is 5–20 times lower than that of hepatic microsomes [17, 18].

The 15-fold K_m difference found between lung and liver microsomes (Fig. 3) is probably of more relevance with regard to the lung specificity of OSSMe toxicity, since it suggests that OSSMe is a much better substrate for pulmonary mixed-function oxidase. This situation resembles that described for another lung toxin, 4-ipomeanol [11]. Studies with 4-ipomeanol have shown that organ-dependent kinetic differences in the activation of chemicals are due to differences in the relative concentrations of cytochrome P-450 isozymes having different substrate specificities [18, 30–32]. Our results suggest that OSSMe is a specific substrate for the pulmonary cytochrome P-450 isozyme(s) and we think that this is a likely mechanism for the lung-specific toxicity of OSSMe. It would be important to determine whether this high activation rate of OSSMe is a specific feature of the rat lung, and the extent to which extrapolation to humans is warranted.

Nature of reactive metabolites

Several arguments lead us to speculate that the metabolic activation of OSSMe involves oxidation of a thiolo-sulphur.

Thus, indirect evidence has been provided for the sulphoxidation of *S*-alkyl phosphorothiolates, either by chemical oxidation [33], or by microsomal mixed function oxidases [34]. The resulting highly reactive phosphorothiolate-*S*-oxides were never isolated, as they probably rearrange to phosphinyloxysulphenates, possibly via a cyclic phosphoranoxide (see Fig. 4). The corresponding diester of phosphoric acid (here $OSMeO^-$) presumably results from further reaction with water, whilst reaction with protein cysteinyl groups conceivably results in the formation of CH_3-*S*-*S*-protein disulphides. The extremely efficient scavenging activity of GSH, as well as the effect of CN^- is consistent with such a possibility. The formation of CH_3-*S*-*S*-protein disulphides could, directly or indirectly, lead to alterations in essential biosynthetic and regulatory functions in some lung cells, as has been proposed to explain the cellular toxicity of paraquat [35, 36].

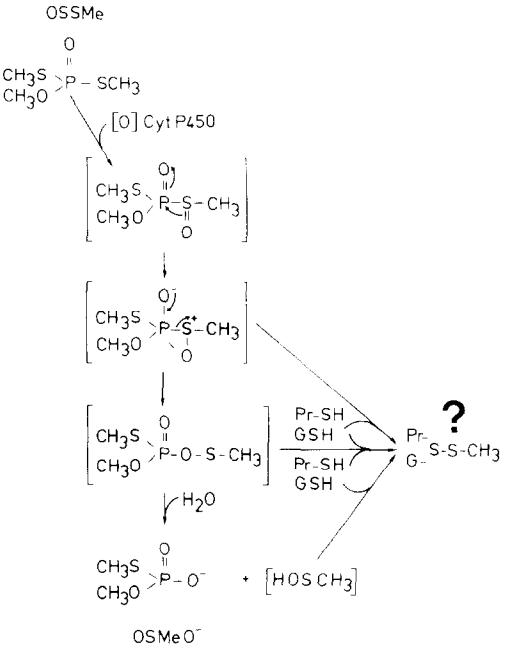

Fig. 4. Hypothetical pathway for the oxidative metabolism of *O,S,S*-trimethyl phosphorodithioate (OSSMe). Sulphoxidation of a thiolo-sulphur by cytochrome P-450-dependent mixed-function oxidase is hypothesized; the resulting phosphorothiolate-*S*-oxide rearranges via a cyclic phosphoranoxide to the phosphinyl oxysulphenate according to a model proposed by Segall and Casida [33]. In this scheme the putative reactive intermediate is $[CH_3S^+]$ which binds covalently to SH groups on proteins (Pr) or glutathione (GSH) forming mixed disulphides.

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