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INACTIVATION *IN VITRO* OF MICROSOMAL OXIDASES DURING PARATHION METABOLISM*

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Abstract—The inactivation *in vitro* of microsomal oxidases during parathion metabolism was examined in relation to the binding to the microsomal membrane of sulfur resulting from desulfuration. Modulation of sulfur binding with sulfhydryl compounds revealed that the majority of bound sulfur is unrelated to inactivation. It was concluded that inactivation was related to either the binding of a very small fraction of detached sulfur to a high-affinity component of the oxidase system or a mechanism other than the binding of a reactive sulfur.

Metabolic studies of phosphorothionate insecticides have emphasized the fate of the toxic metabolites of desulfuration and the immediate degradation of parent compounds. However, certain toxicological effects of phosphorothionates, such as the inhibition of microsomal oxidation, may be unrelated to their antiesterase action, as others have suggested [1–4]. To a degree this effect is probably due to competitive interaction, since the administered compounds are alternative substrates for microsomal oxidases. At least some of the inhibitions of microsomal oxidases, however, seem to be linked to the microsomal oxidation of the parent compound.

Inhibition of parathion oxidase (measured by paraoxon production) itself during parathion metabolism *in vitro* was first indicated for cockroach fat body microsomes. The rate of the oxidation rapidly decreased, apparently due to the reaction itself or something that occurred under the reaction conditions [5, 6]. Improper reaction conditions, enzyme instability and inhibition by the accumulation of inhibitory metabolites were experimentally discounted [5]. Such non-linear reactions have been noted for rat liver, brain and lung microsomal preparations [7, 8], but the cause has not been examined in detail. Rapid inactivation has also been found in recent work with the reconstituted system; the enzyme was totally inactive within 5 min [9]. Pre-incubation of rat liver microsomes with parathion in the presence, but not in the absence, of NADPH apparently caused inhibition of benzphetamine metabolism and reduced cytochrome P-450 content [10]. The decrease in cytochrome P-450 was not attributed to lipid peroxidation or conversion to the inactive form, cytochrome P-420 [11]. Recent reports on the desulfuration of the thiono-sulfur structure of various compounds have tentatively implicated the covalent binding of a reactive sulfur atom in the inactivation of microsomal oxidases and centrilobular necrosis [8, 10, 12–14]. Although the original finding of inactivation during parathion metabolism led to the discovery of the binding of sulfur [6], there was and still is no direct evidence to link sulfur binding with inactivation.

The purpose of this research was to investigate the inactivation phenomenon in rat liver microsomes and examine its relationship to the binding of detached sulfur arising from parathion desulfuration.

MATERIALS AND METHODS

[^{35}S]parathion (*O,O*-diethyl *O*-4-nitrophenyl phosphorothionate, 3–9 mCi/m-mole) purchased from Amersham/Searle Corp., Arlington Heights, IL, was purified by Silica gel column chromatography, using chloroform as solvent. Pooled eluates containing radioactive material were evaporated and re-chromatographed with hexane to remove further impurities followed by a hexane–ethyl acetate (10:1) mixture to elute the pure material. Thin-layer chromatography using hexane–ethyl acetate (10:1) and hexane–ethyl acetate (1:1) systems failed to reveal any impurities. The final purified product was stored at −20° as a 0.1 M solution in acetone. Its chemical concentration was verified by gas chromatography by comparison to a non-radioactive standard (American Cyanamid, Princeton, NJ). To prepare aqueous emulsions of [^{35}S]parathion, the original acetone solvent was evaporated and replaced by an equivalent volume of acetone containing 1% Triton X-100 as emulsifier.

[^{35}S]DEPTA (diethyl phosphorothioic acid) was prepared by the alkaline hydrolysis of [^{35}S]parathion with 1 N KOH at 60° for 24 hr in the presence of 33% ethanol. Unreacted parathion was removed by extraction with hexane and 4-nitrophenol from acidified medium (pH 5) with hexane–ether (1:1). Product was extracted with ether after acidification to a pH of less than 1. Ether extracts were evaporated and the residue was dissolved in distilled water. Purification was accomplished by anion-exchange chromatography with Dowex 1-X8 [15] and DEPTA in the eluates was extracted with ethyl acetate. The purity of the final product was 90.5% confirmed by re-chromatography.

Phenyl-[1-^{14}C]*n*-propyl paraoxon had been prepared in this laboratory by coupling sodium [1-^{14}C]4-nitrophenate (22.5 mCi/m-mole, 99% pure radio-

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chemically, Amersham/Searle Corp.) with di-*n*-propyl phosphoryl chloride (a gift of Dr. J. E. Boyd, American Cyanamid Co., Princeton, NJ).

Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*, *exo*-5,8 dimethanonaphthalene) and dieldrin(1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-4,7-epoxy-1,4-*endo*, *exo*-dimethanonaphthalene) were gifts of Shell Chemical Co., New York, NY. The following chemicals were purchased from the suppliers indicated in parentheses: NADPH (P-L Biochemicals, Inc., Milwaukee, WI), glutathione and cysteine (Sigma Chemical Co., St. Louis, MO), dithiothreitol (Cleland's Reagent) and taurocholic acid (sodium salt) (CalBiochem, LaJolla, CA) and 2,5-diphenyl oxazole (PPO) and 1,4-bis-2-[4-methyl-5-phenyloxazolyl]benzene (dimethyl POPOP) (Packard Instrument Co., Inc., Downers Grove, IL).

Male Sprague–Dawley strain rats, weighing 285–380 g, were purchased from Taconic Farms, Germantown, NY, no statistically significant difference in enzyme activity was noted among rats of different weight. Rats were sacrificed by decapitation and livers immediately excised and chilled in ice-cold 0.25 M sucrose. After blotting excess solution, the organ was weighed and then homogenized in 0.25 M sucrose in a Sorvall Omni-mixer (Ivan Sorvall, Newton, CT) followed by six passes with a Teflon pestle in a glass homogenizer. Homogenates were strained through two layers of cheesecloth and made up to a volume corresponding to a 20% suspension. The suspension was centrifuged at 10,000 *g* for 15 min and microsomes were prepared by centrifugation of the post-mitochondrial supernatant at 230,000 *g* for 30 min using a Beckman L2-65B ultracentrifuge. The pellets thus obtained were separated from the underlying glycogen and washed with 1.15% KCl to remove residual hemoglobin. Microsomal pellets were stored at −50° and used within 3 weeks of preparation.

Standard incubation mixtures (pH 7.9) contained the following final concentrations of components: 2.5×10^{-5} M substrate, 5×10^{-4} M NADPH, 1×10^{-4} M EDTA, 4.6×10^{-2} M K_2HPO_4, 0.4×10^{-2} M KH_2PO_4 and 0.1 ml of enzyme in a total volume of 0.4 ml. Microsomes were resuspended to an equivalent of 4–20% homogenate. Enzyme concentration in terms of mg protein/ml for each experiment is given in Results and Discussion. Reactions were carried out at room temperature (23°) without shaking and stopped by the addition of 0.1 ml of 0.5 M trichloroacetic acid (TCA) for *n*-propyl paraoxon, parathion and aldrin assays and 2 ml of a 0.2 M TCA–acetone (1:1) mixture for the bound-sulfur assay. Assays for each independent experiment were performed in duplicate. Reaction time courses were followed by sampling 0.4 ml in duplicate from a larger incubation mixture at specified times.

The oxidative cleavage of phenyl-[1-^{14}C]*n*-propyl paraoxon [16] was assayed by separating the metabolite, [1-^{14}C]4-nitrophenol, from the remaining substrate by vortexing the sample with 2 ml of chloroform and 1 ml of 0.2 M sodium borate buffer, pH 9.5, and determining the radioactivity in the aqueous phase. Essentially all 4-nitrophenol was present in the aqueous phase and all substrate was in the chloroform phase. Dieldrin, the product of aldrin epoxidation, was measured gas-chromatographically after extraction with 1 ml of nanograde hexane (Mallinckrodt Chemical Works, St. Louis, MO).

For the analysis of [^{35}S]DEPTA, the TCA-stopped incubation mixture was first washed three times with 2 ml of analytical grade hexane to remove the remaining [^{35}S]parathion. DEPTA was then extracted into 1.2 ml ethyl acetate after the addition of 0.1 ml of 5 N HCl (extraction efficiency, 96 per cent). For the bound-sulfur assay samples were centrifuged at 11,000 *g* for 15 min in heavy-walled Corex tubes (Corning Glass Works, Corning, NY) using an International Centrifuge model HT (International Equipment Co., Needham Heights, MA) fitted with adapters. The supernatant was discarded and the pellet washed with 2 ml TCA–acetone mixture by vortexing for 30 sec and centrifugation. Preliminary experiments indicated that no less than 99.7 per cent of parathion and 90 per cent of DEPTA were removed by this procedure and that further washing failed to remove any more radioactivity. The pellet was dried with a gentle stream of air and solubilized by the addition of 0.1 ml of distilled water and 0.5 ml Soluene 350 (Packard Instrument Co., Inc., Downers Grove, IL).

For studies on the metabolic balance of [^{35}S]parathion, a standard incubation was stopped with TCA. [^{35}S]parathion and [^{35}S]DEPTA were removed with three washes each of hexane and ethyl acetate, respectively, as described in the preceding paragraph and the remaining aqueous phase containing the majority of protein was transferred to a Corex tube. The original incubation tube was rinsed with 1 ml of distilled water which was then added to the Corex tube. One ml acetone was also added to the Corex tube to aid the sedimentation of denatured protein and then the entire mixture was centrifuged at 11,000 *g* for 30 min. The supernatant was decanted and the radioactivity of a 0.4-ml sample was determined after removing acetone by blowing with air to prevent quenching of scintillation. The radioactivity of this supernatant is referred to as soluble sulfur. The protein precipitate in the Corex tube and the residual protein in the original incubation tube were solubilized as described in the preceding paragraph, combined and analyzed for bound sulfur. Values for bound sulfur using this procedure were comparable to those derived by the assay of the preceding paragraph.

For those experiments requiring the recovery of pre-incubated microsomes, incubation mixtures were centrifuged in the presence of 25% glycerol at 230,000 *g* for 30 min. The use of glycerol during centrifugation reduced the loss of cytochrome P-450 and enzyme activity for all four microsomal reactions reported herein (an average of 51.6 per cent loss without glycerol, 15.1 per cent with glycerol). The initial incubation mixtures contained glycerol-washed microsomes so that valid comparisons could be made between microsomal reactions containing residual glycerol.

Radiometric analyses were accomplished by dissolving 0.2 ml of aqueous sample or 0.4 ml of ethyl acetate or hexane sample in 10 ml scintillant consisting of 5 g PPO, 0.2 g dimethyl POPOP, 500 ml toluene and methanol to 1 liter. The solution was modified for basic and acidic samples by including

4 ml acetic acid and 6.5 ml ethanolamine respectively. Samples were counted in a Packard Tri-Carb liquid scintillation spectrometer, model 3375. Internal standardization was done when necessary by using [1-^{14}C]hexadecane (1.10 μCi/g, Packard Instrument Co., Inc., Downers Grove, IL).

Gas chromatographic analyses were performed with a Packard gas chromatograph, Series 7400 using glass columns [2 mm (i.d.) × 1200 mm] containing Chromosorb G, 70–80 mesh, acid-washed, silylated and coated with the appropriate stationary phases: 3% Apiezon N for organophosphates, 3% Carbowax 20 for DEPTA and 1.5% OV-1 for dieldrin. Operating temperatures for inlet, column and detector were 195°–195°–200° (organophosphates), 150°–150°–200° (DEPTA) and 185°–185°–205° (dieldrin). Nitrogen was used as a carrier gas at flow rates of 45 ml/min (organophosphates), 33 ml/min (DEPTA) and 35 ml/min (dieldrin). Typical retention times were 3.6 min (parathion), 1.7 min (paraoxon), 2.5 and 5.2 min (*O*- and *S*- methyl DEPTA) and 4.7 min (dieldrin). Phosphorus-containing compounds were detected with a Melpar flame photometric detector (Tracor, Inc., Austin, TX) equipped with a 526-nm filter. Gas flow rates were H_2, 120; air, 70; O_2, 10 ml/min. A ^{63}Ni electron-capture detector was used for dieldrin determinations. Injections of 4-μl aliquots were made and sample concentrations computed by comparison to standards. DEPTA was methylated with diazomethane for gas chromatographic analysis as previously described [15].

Protein was measured according to the method of Lowry *et al.* [17], modified by the addition of 0.07% taurocholic acid (final concentration) to facilitate the solubilization of membrane-bound proteins. Cytochrome P-450 was measured according to the method of Omura and Sato [18] on a Beckman DK-2A spectrophotometer. Calculations of cytochrome P-450 content assumed a value of 91 $cm^{-1}\,mM^{-1}$ for the molar extinction increment between 450 and 490 nm. Measurements were conducted at room temperature, about 23°.

RESULTS AND DISCUSSION

Proper conditions for the assay of DEPTA and bound sulfur were established as a requisite to the investigations. Both the oxidative dearylation and desulfuration of parathion are greater with higher ionic strength up to $\mu \cong 0.2$ beyond which a broad optimum range exists. An ionic strength of 0.142 which approximates that of mammalian Ringer solution ($\mu = 0.163$) gave 96 per cent of the activity that was obtained at $\mu = 0.2$ and was used for all enzymatic assays. The range of pH optimum was between 7.6 and 8.2 and enzyme activity was nearly proportional to concentrations between 0.03 and 0.63 mg microsomal protein/ml.

A balance sheet of parathion and its sulfur-containing metabolites for a 90-min period clearly indicated the production of at least three sulfur-containing metabolites: DEPTA, bound sulfur and what has been termed soluble sulfur (Fig. 1). These metabolites were not detected when reactions were carried out with boiled microsomes or in the absence of NADPH.

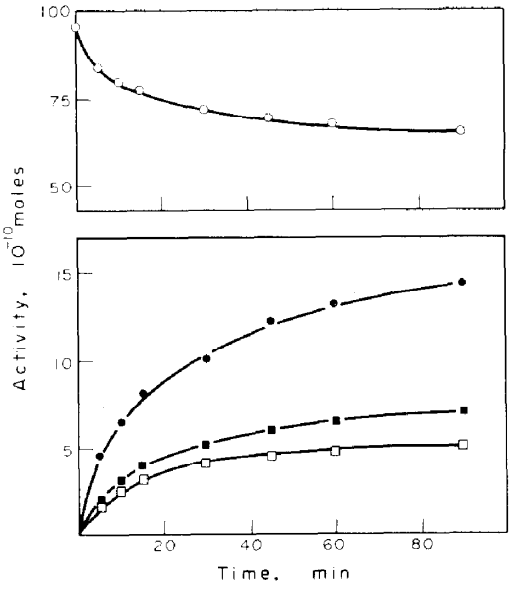

Fig. 1. Balance sheet of [^{35}S]parathion and its sulfur-containing metabolies. The reaction time course was followed in an incubation with 0.39 mg of microsomal protein/ml and initial parathion concentration of 2.31×10^{-5} M, see Materials and Methods. The [^{35}S]parathion remaining was estimated by radiometric analysis of hexane extracts. All other metabolites were analyzed as described in Materials and Methods. The average recovery of radiolabel at each time point was 98.9 per cent. Key: (○) parathion, (●) DEPTA, (■) bound sulfur and (□) soluble sulfur.

To ensure that the soluble sulfur component was not the result of solubilization of lipid-bound sulfur by acetone used in the procedure, 30-min incubations from which parathion and DEPTA had been extracted were centrifuged at 300,000 *g* for 30 min with or without the addition of 1 ml acetone. No difference in the amount of soluble sulfur was detected. A comparative preliminary experiment with [^{14}C-ethyl]- and [^{35}S]parathion indicated that the soluble sulfur fraction contained 15.6 per cent of the ^{35}S added as parathion, but only 1.4 per cent of the ^{14}C after a 30-min incubation. While the identity of the soluble sulfur was unknown, it was probably derived from desulfuration. This conclusion is based on the facts that for a 30-min reaction (1) neither bound nor soluble sulfur alone could account for the paraoxon produced (13.6×10^{-10} moles), (2) the total concentration of the two sulfur metabolites nearly matched the paraoxon concentration, and (3) these sulfur metabolites plus DEPTA equaled the decrease in hexane-soluble radioactivity which presumably represented parathion (Fig. 1). These results argue against the use of bound sulfur as a measure of paraoxon production without prior verification by a balance sheet.

Figure 1 also shows a rapid deceleration of the production of the three ^{35}S-metabolites. This is clearly seen in a plot of reaction rates vs time as measured by the slope of the curves at various time points in Fig. 1 (Fig. 2). The change in enzyme rate during parathion metabolism is contrasted with that of the linear metabolism of *n*-propyl paraoxon which shows deceleration only after 7 min of reaction possibly due to substrate and/or cofactor depletion. The initial rate

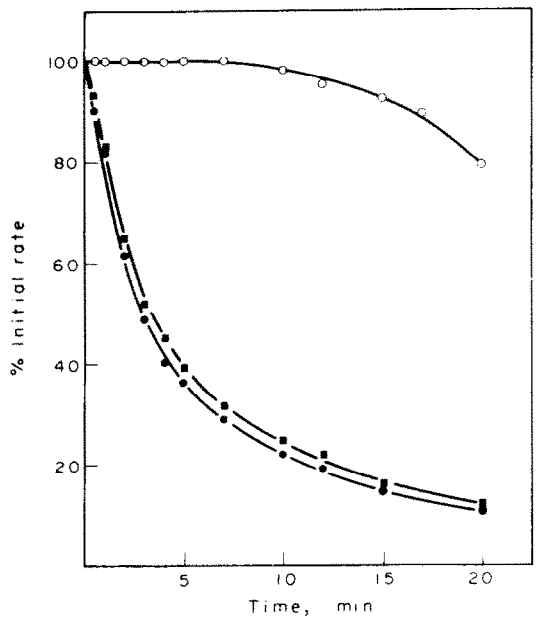

Fig. 2. Enzyme rate vs time. Enzymatic rates were computed manually from the fitted curves of Fig. 1 and a separate time course of *n*-propyl paraoxon metabolism using 0.34 mg of microsomal protein/ml. The amount of substrate consumed at 10 min and 20 min was 20.9 and 38.2 per cent for *n*-propyl paraoxon, and 14.1 and 18.8 per cent for parathion. Key: (●) DEPTA, (■) bound sulfur and (○) 4-nitrophenol.

of the parathion reaction could be re-established by additional enzyme which had been standing at room temperature (Fig. 3). Thus, the deceleration was not due to (1) the depletion of substrate or cofactor, (2) enzyme instability, or (3) rapid reversible inhibition by accumulated reaction products or inhibitors in the medium. Separate experiments had shown that parathion oxidase loses only 14 per cent of its initial activity while suspended in buffer at room temperature for 6 hr.

Microsomes were pre-incubated with parathion (5×10^{-5} M), NADPH (10^{-3} M), DEPTA (10^{-6} M), paraoxon (10^{-6} M) or 4-nitrophenol (10^{-6} M). After 30 min the missing reaction components were added to each pre-incubation and a reaction time course for DEPTA was followed for 20 min. No inhibition was observed for the pre-incubations of microsomes with parathion, NADPH, DEPTA, and paraoxon when compared to microsomes pre-incubated in buffer. Although 4-nitrophenol caused a 14–16 per cent diminution in enzyme activity, this could not account for the dramatic decrease in activity during the parathion oxidation. Thus, the progressive inhibition was not caused by these metabolites.

To determine whether inhibition was irreversible, aliquots were removed from the complete reaction system at 15 and 20 min to determine the reaction rates of bound sulfur and DEPTA production during this time interval. At 20 min the remaining incubation mixture was centrifuged in the presence of 25% glycerol to recover microsomes, as described in Materials and Methods. After resuspending the pre-incubated microsomes, the initial reaction rates of parathion metabolism during the first 5 min were determined (Fig. 4) and compared to the final rates of the preceding reaction. The initial rates for the pre-incubated enzymes (10^{-10} moles/mg of protein/min) were

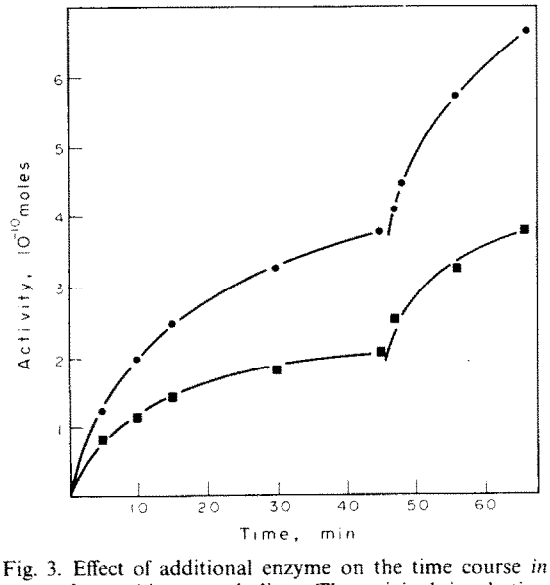

Fig. 3. Effect of additional enzyme on the time course *in vitro* of parathion metabolism. The original incubation mixture contained 1.524 mg of microsomal protein in a total volume of 12 ml. At 46 min, 0.2 ml of a concentrated microsomal suspension (5.08 mg of microsomal protein/ml) which had been standing at room temperature was added to the remaining 8 ml of incubation mixture. Analyses were performed as described in Materials and Methods. Key: (●) DEPTA, and (■) bound sulfur.

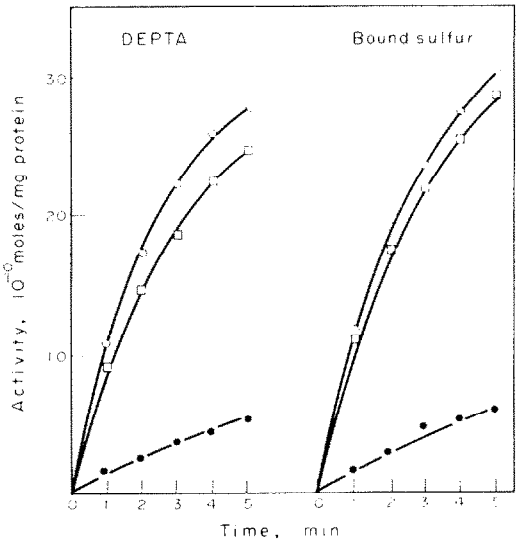

Fig. 4. Irreversibility of inhibition by parathion metabolism. Glycerol-washed microsomes were pre-incubated in a standard or appropriately modified incubation mixture: (●) pre-incubation with parathion plus NADPH, (□) pre-incubation with NADPH, (○) pre-incubation with parathion. After 20 min the incubation mixture was centrifuged, as described in Materials and Methods. Microsomes were resuspended in 0.05 M phosphate buffer, pH 7.9 (0.385 mg of microsomal protein/ml) and a time course of activity was performed using a standard incubation mixture. Each value is the average of two independent experiments.

Table 1. Effect of parathion metabolism on cytochrome P450 and microsomal oxidase levels*

	Treatment†			
	Parathion	NADPH	Parathion + NADPH	% Decrease
Cytochrome P-450	6.6	6.1	5.0	24.2
Parathion oxidase (DEPTA)	38.1	39.0	19.5	48.8
n-Propyl paraoxonase	49.9	117.9	15.4	69.1
Aldrin epoxidase	52.6	119.9	14.6	72.2

* Glycerol-washed microsomes were pre-incubated for 30 min under the conditions indicated in the table. The concentrations of parathion and NADPH were 2.5×10^{-5} M and 5×10^{-4} M respectively. After centrifugation in the presence of glycerol, microsomes were resuspended and assayed for enzymatic activity and cytochrome P-450 as described in Materials and Methods.

† Values are expressed as 10^{-10} moles/mg of protein/15 min reaction, and represent the average of two independent experiments. Each incubation contained an average of 0.21 mg of microsomal protein/ml.

1.12 for DEPTA and 1.03 for bound sulfur, in good agreement with the final rates of the preceding reactions (1.20 for DEPTA and 0.97 for bound sulfur). This showed that the microsomal enzymes remained inhibited even after resuspension in fresh incubation medium and excluded the possibility that inactivation was caused by progressive inhibition due to inhibitor accumulation. Control pre-incubations with the incomplete system confirmed that cofactor or substrate itself were not causing the inactivation (Fig. 4). Table 1 shows that pre-incubation with the complete system results in a high degree of cross-inactivation of the microsomal oxidases. A smaller, but distinct decrease of cytochrome P-450 was also found. The preceding experiments establish that the inhibition of rat liver microsomal oxidases during the oxidative metabolism *in vitro* of parathion is essentially an irreversible inactivation. Furthermore, the progressive inactivation of parathion oxidase was accompanied by a parallel increase in the binding of sulfur detached during the activation reaction.

Three thiol-containing compounds, cysteine, glutathione and dithiothreitol, were used to examine the relation of sulfur binding to enzyme inactivation. All three compounds enhanced DEPTA production and reduced sulfur binding (Fig. 5). Equimolar concentrations of the oxidized forms (except cystine which was not tested because of poor solubility) had much less effect. Since thiol compounds have been reported to alter the balance of DEPTA and paraoxon production [19], a balance sheet of ^{35}S-metabolites was generated for a 90-min time course in the presence

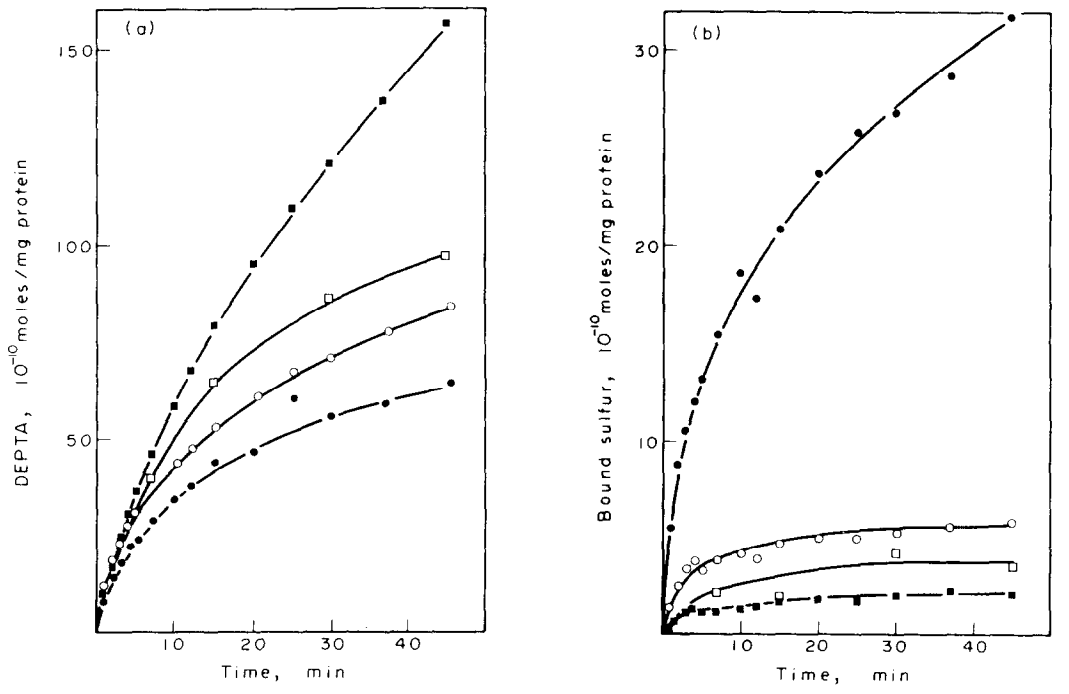

Fig. 5. Effect of SH compounds on parathion metabolism. A typical time course using 0.183 mg of microsomal protein/ml was obtained as described in Materials and Methods. The final concentration of dithiothreitol, glutathione and cysteine was 10^{-3} M. Key: (●) control, (○) glutathione, (□) cysteine and (■) dithiothreitol.

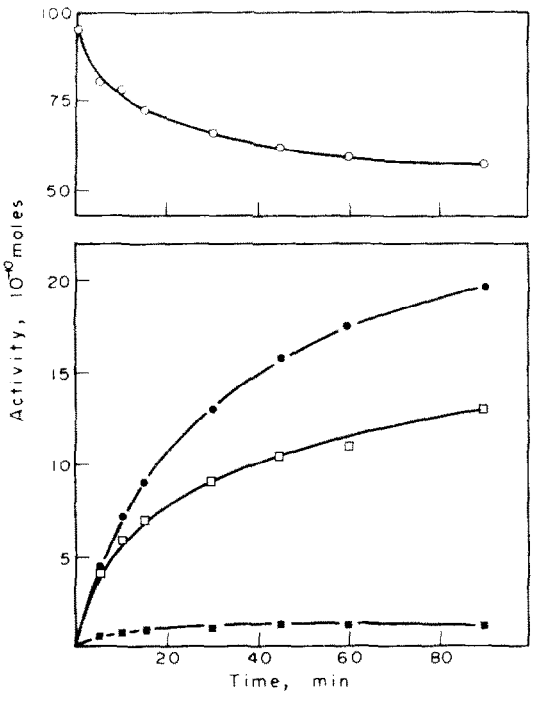

Fig. 6. Effect of glutathione on the [^{35}S]parathion balance sheet. A time course experiment was carried out with 0.35 mg of microsomal protein/ml in the presence of 10^{-3} M glutathione and an initial parathion concentration of 2.27×10^{-5} M [^{35}S]parathion, see Materials and Methods. The [^{35}S]parathion remaining was estimated by radiometric analysis of hexane extracts. All other metabolites were analyzed as described in Materials and Methods. The average recovery of radiolabel at each time point was 97.2 per cent. Key: (○) parathion, (●) DEPTA, (■) bound sulfur and (□) soluble sulfur.

of glutathione (Fig. 6). Glutathione enhanced the production of both total detached sulfur and DEPTA, the sum of which again matched the decrease in parathion. The enhancement appeared to favor the DEPTA pathway slightly over desulfuration. The drastic decrease in sulfur binding was accompanied by an increase in soluble sulfur which may reflect the ability of glutathione to trap detached sulfur. However, the presence of a ^{35}S-containing glutathione has not been confirmed. The data of Fig. 5 also indicate that glutathione prevents the majority of sulfur binding with only a modest protection of the enzyme, whereas further reductions in bound sulfur by dithiothreitol show greater improvements in enzyme activity. There is uncertainty as to the identity of the low level of bound sulfur in the dithiothreitol co-incubation. This uncertainty stems from the apparent instability of chemically prepared and chromatographically purified DEPTA. Use of this chemical source to determine DEPTA residues in the bound sulfur assay indicated that as much as 10 per cent of the DEPTA produced during parathion metabolism could contribute to the radioactivity in the bound sulfur fraction. However, the high residual value may be an artifact of DEPTA instability. An indirect indication that the contribution of DEPTA to the bound sulfur fraction is minimal is the leveling off of bound sulfur while the amount of DEPTA continues to increase during parathion metabolism in the presence of dithiothreitol. An alternative possibility that the low level of bound sulfur is of a different chemical identity than detached sulfur atoms cannot be eliminated.

Replotting the DEPTA data of Fig. 5 in terms of reaction rates vs time (Fig. 7) shows, however, that all three SH compounds failed to protect enzyme activity. A plot of enzyme activity (in terms of DEPTA production) vs sulfur binding (Fig. 8) reveals that enzyme activity is not unequivocally defined by the level of bound sulfur. For example, for approximately 2×10^{-10} moles/mg of protein of bound sulfur, 90 per cent of the initial activity of the control reaction is retained, but only 20 per cent remains for the dithiothreitol co-incubation. Therefore, most, if not all, of the bound sulfur in the control reaction is unrelated to the inactivation of microsomal oxidases. Whether sulfur binding may have adverse effects on other microsomal enzymes is unknown at this time.

From these analyses it follows that the capacity of an SH compound to prevent sulfur binding does not necessarily give it the capacity to retard enzyme inactivation. Sulfhydryl compounds may reduce binding by combining with detached sulfur to form a hydrodisulfide (−SSH compounds). Hydrodisulfide formation with cysteine residues of microsomal proteins by a portion of the reactive sulfur released during carbon disulfide metabolism has been suggested [20]. On the other hand, the effects of SH compounds on enzyme activity may reflect an ability to restore the activity of inhibited enzymes, observed activity being the balance between inactivation and reactivation.

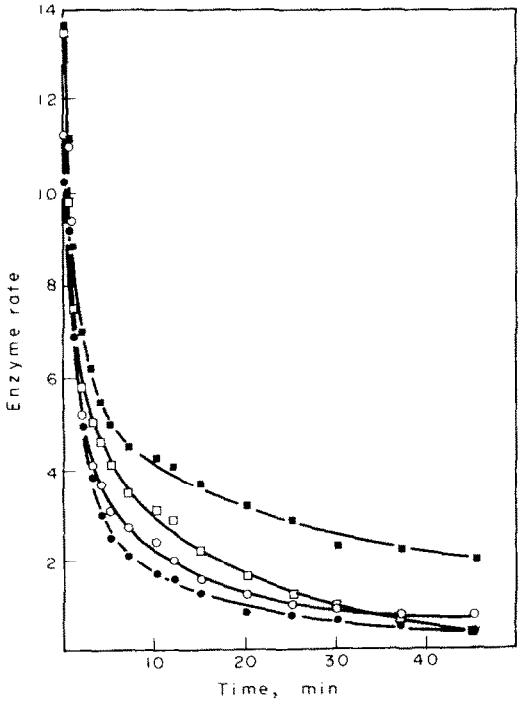

Fig. 7. Effect of SH compounds on enzyme rate. Enzymatic rates were computed manually from the fitted curves of Fig. 5 and expressed as 10^{-10} moles/mg of protein/min for the specified times. Key: (●) control, (○) glutathione, (□) cysteine and (■) dithiothreitol.

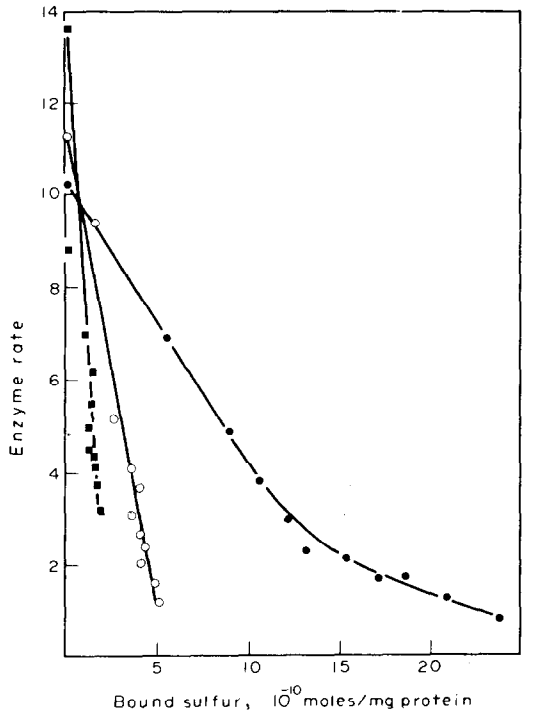

Fig. 8. Enzyme rate vs bound sulfur. The enzyme rate of Fig. 7 was plotted as a function of experimental values of bound sulfur at corresponding times for the first 20 min of the time course. Key: (●) control, (○) glutathione and (■) dithiothreitol.

Thus, enzyme inactivation due to a mechanism other than the binding of a reactive sulfur is a viable alternative hypothesis.

If the persistent low level of bound sulfur observed in the presence of dithiothreitol is derived from the detached sulfur, however, it may represent the critical binding responsible for enzyme inactivation. The data are consistent with the hypothesis that a vital enzyme component is more competitive than the SH compounds for the reactive sulfur.

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