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DEGRADATION OF PARATHION IN THE RAT*

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Abstract—Examination of urinary metabolites of ^{32}P- and ^{35}S-parathion orally administered to rats confirmed that initial metabolism of parathion involved activation to paraoxon and degradation to diethyl phosphorothioic acid. Sulfur detached in the activation reaction was excreted as inorganic sulfate. Experiments *in vitro* with ^{35}S-parathion indicated that the aryl-phosphate cleavage of parathion was mostly effected by liver microsomal oxidases; a minor portion was catalyzed by nonoxidative soluble enzymes requiring reduced glutathione.

PLAPP and Casida[1] showed that *O,O*-diethyl phosphorothioic acid (DEPTA) is the major degradation metabolite of parathion (*O,O*-diethyl *O*-4-nitrophenyl phosphorothioate) *in vivo* in rats and cockroaches. This implies enzymatic mechanisms for the cleavage of parathion at the aryl–phosphate bond. Microsomes contain enzymes for such a reaction.[2, 3] The reaction *in vitro* is evidently an oxidative one because $NADPH_2$ and O_2 are required. The cleavage, however, could be brought about by other types of enzymes, such as phosphatases and transferases. Metcalf *et al.*[4] reported more hydrolysis of parathion than of paraoxon in the homogenate of honey bees, although Krueger and Casida[5] could not reproduce their results. Matsumura and Hogendijk[6] studied hydrolytic enzymes in resistant and susceptible strains of houseflies; parathion and diazinon were hydrolyzed to DEPTA. These insect enzymes required no cofactors and seem to fall under the general category of esterases or phosphatases. According to Fukami and Shishido,[7] diazinon [*O,O*-diethyl *O*-(2-*iso*-propyl-4-methyl-6-pyrimidinyl] phosphorothioate and diazoxon [*O,O*-diethyl *O*-(2-*iso*-propyl-4-methyl-6-pyrimidinyl] phosphate were hydrolyzed to DEPTA and *O,O*-diethyl phosphoric acid, respectively, by the supernatant fractions of rat liver and cockroach fat body homogenates, presumably in the presence of reduced glutathione (GSH). This suggests that aryl–phosphate cleavage may also be catalyzed by GSH-dependent enzymes similar to those for desmethylation of methyl parathion and Sumithion.[7] Thus, there are three possible mechanisms for degradation of parathion to DEPTA: (1) microsomal oxidation; (2) hydrolysis by esterases or phosphatases; and (3) desarylation by GSH-dependent enzymes.

The present studies were done to re-examine the metabolism of parathion in the

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light of recent findings and to assess the significance of microsomal oxidation in the aryl–phosphate cleavage of parathion.

MATERIALS AND METHODS

Radioactive chemicals. ^{35}S- and ^{32}P-Parathion, prepared at the Radiochemical Centre, Amersham, Bucks., England, were purchased from Nuclear-Chicago Corp., Des Plaines, Ill. Initial specific activity of ^{35}S-parathion was 7·9 mc/m-mole and that of ^{32}P-parathion was 4·2 mc/m-mole. The labeled materials were purified by chromatography on silica gel columns with chloroform as the solvent. After evaporation of the solvent, acetone solutions (0·03–0·1 M) of the purified chemicals were stored at −15°. Concentration of ^{35}S-parathion was determined colorimetrically.[2]

Labeled DEPTA was prepared by hydrolyzing the purified ^{35}S- or ^{32}P-parathion with 1 N NaOH. Unhydrolyzed parathion was removed by chloroform extraction and most of the 4-nitrophenol was extracted with ethyl acetate after pH of the solution was adjusted to about 6 with HCl. The solution was further acidified to less than pH 1 with HCl and DEPTA was extracted with ethyl acetate. The labeled acid was stored as aqueous solutions (2×10^{-3} to 4×10^{-3}M) after evaporation of ethyl acetate.

^{35}S-Desethyl parathion (*O*-ethyl *O*-hydrogen *O*-4-nitrophenyl phosphorothioate) was prepared according to Plapp and Casida[1] by the hydrolysis of ^{35}S-parathion with equimolar KOH in 95% ethanol. Ethanol was evaporated and unhydrolyzed parathion was extracted with chloroform from an aqueous solution. After neutralization of the solution with HCl, hydrolysis products were separated on a Dowex 1-X8 column[8] and desethyl parathion was extracted from the eluted fractions with chloroform. Chloroform was evaporated and desethyl parathion was stored as an aqueous solution (approximately 7×10^{-5}M). A portion of the sample was rechromatographed on a Dowex 1-X8 column to check that the compound had not been decomposed during the separation on the column.

^{35}S-Sulfate was prepared by digesting the purified ^{35}S-parathion with concentrated HNO_3 for 8 hr. Most of the HNO_3 was boiled off and the trace of HNO_3 remaining was removed by heating to dryness with concentrated HCl. Distilled water was added to the residue and the turbid solution was centrifuged to obtain a clear supernatant solution containing approximately 5×10^{-4}M ^{35}S-sulfate.

Experiments in vivo Male albino rats weighing 250–350 g were used. An acetone solution of parathion was diluted with corn oil for oral administration. One to 2 ml of this solution, containing less than 10% acetone, was introduced into the stomach. For i.p. administration of parathion or its derivatives, 1–2 ml of an aqueous emulsion or solution was injected. At least two rats were used for each treatment. The rats were individually held in metabolism cages without food; water was given *ad libitum*. Urine was collected in test tubes immersed in a dry ice–acetone mixture and kept frozen until analysis.

Experiments in vivo. For homogenate experiments, tissues of male albino rats were ground in ice-cold 0·25 M sucrose in an Omni-mixer and then homogenized with a glass homogenizer and a Teflon pestle. The homogenates were strained through four layers of cheesecloth. Fractionation of 10 per cent liver homogenates was done by centrifuging at successively higher speeds in a Spinco L2-65 centrifuge at about 4°. Fractions obtained were: F-1, pellet from the 1600 *g*, 10 min centrifugation; F-2,

pellet from the 10,000 *g*, 10 min centrifugation of the supernatant above F-1; F-3, pellet from the 105,000 *g*, 60 min centrifugation of the supernatant above F-2; and F-4, supernatant above F-3. The pellets were resuspended in 0·25 M sucrose to make the original volume of the homogenate from which the pellets were prepared. When soluble enzymes were studied, the supernatant above the F-2 fraction was centrifuged at 269,000 *g* for 60 min. The supernatant thus obtained was referred to as the soluble fraction.

To remove endogenous cofactors from the soluble fraction, "desalting" was done at about 4° with a column of Sephadex G-25 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J., U.S.A.). A 2 cm (inside diameter) × 30 cm column of the Sephadex was packed in 0·02 M phosphate buffer, pH 7·4. The soluble fraction was concentrated approximately 3-fold by adding dry Sephadex G-25 and filtering. Eight ml or less of the sample was loaded on the column. Elution was done with the phosphate buffer and fractions were collected. Location of enzyme activities matched that of the red color of hemoglobin measured by average of absorbances at 540 and 578mμ. Preliminary experiments had established that this procedure completely separates blue dextran 2000 (Pharmacia) and ^{35}S-sulfate.

Metabolism of parathion *in vitro* was studied usually at room temperature (approximately 25°) by using ^{35}S-parathion. Unless otherwise stated, an incubation mixture contained 1 ml of 10% homogenate or its equivalent subcellular fraction in 0·25 M sucrose to make a final enzyme concentration of 2·5%. The following compounds were added to make a total volume of 4 ml (final concentrations indicated): ^{35}S-parathion ($2{\cdot}5 \times 10^{-5}$M), $NADPH_2$ (P-L Biochemicals, Inc., Milwaukee, Wisc.) (10^{-3}M) or GSH (Sigma Chemical Co., St. Louis, Mo.) (10^{-3}M), nicotinamide (0·01M), KCl (0·15 M), Na_2HPO_4 (8×10^{-3}M), and KH_2PO_4 (2×10^{-3}M). When the desalted soluble fraction was studied, 1 ml of an enzyme sample in 0·02 M phosphate buffer was incubated with other components just described except that nicotinamide was omitted. The pH of the incubation mixture was 7·2–7·3. The incubation was done without shaking, usually for 1 hr, and terminated by addition of 1 ml of a solution containing 10% trichloroacetic acid and 3 N HCl.

Analyses. Each urine sample containing radioactive metabolites was passed through filter paper and made to a constant volume (usually 25 ml) for estimation of total radioactivity. In some experiments involving ^{35}S-parathion, 4-ml aliquots were acidified with 1 ml of 3 N HCl and shaken twice with 5 ml of ethyl acetate. Radioactivity was determined in ethyl acetate extracts and aqueous samples.

Metabolites *in vitro* were determined as follows. Unmetabolized parathion was removed from the acidified incubation mixture with 10 ml of *n*-hexane. The remaining aqueous phase was washed with 10 ml of *n*-hexane, and traces of *n*-hexane were removed by a stream of N_2. When the F-4 and soluble fractions were studied, three hexane extractions wered one instead of two to ensure removal of parathion. Metabolites were then extracted with 5 ml of ethyl acetate.

Anionic metabolites were separated by ion-exchange chromatography of Plapp and Casida[8] as before by using a 2·5 cm (inside diameter) × 29 cm column of Dowex 1-X8 anion exchange resin. A typical solvent system was (capital letters correspond to eluting solutions shown in Fig. 1): A, elution gradient (600 ml) pH 2 to pH 1 HCl; B, elution gradient (600 ml) pH 1 to 1 N HCl; C, water (115 ml); D, elution gradient (400 ml) pH 1 HCl plus methanol (1:3) to 1 N HCl plus methanol (1:3); E, elution

gradient (400 ml) 1 N HCl plus methanol (1:3) to 6 N HCl plus methanol (1:3); and F, 6 N HCl plus methanol (1:3, 120 ml). In the latter part of the experiments, columns of 1·0 cm (inside diameter) × 21 cm were used with smaller volumes of eluents to compensate for lower radioactivities.

Radioactivity was measured in a Packard model 3003 Tri-Carb Scintillation Spectrometer. Counting mixtures consisted of 0·5 ml of a sample and 20 ml of a scintillation solution 50 g of PPO (2,5-diphenyloxazole), 2 g of dimethyl POPOP 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene, 65 ml of ethanolamine, 5 l. of toluene, and methanol to make 10 l. Quenching was checked by internal standards.

RESULTS

Parathion metabolism in vivo

Urinary excretion of parathion metabolites was studied after oral administration of 0·2, 1 or 5 mg/kg of ^{35}S- or ^{32}P-parathion. No rats developed signs of poisoning. Ion-exchange chromatography of urine samples showed six metabolites (Fig. 1).

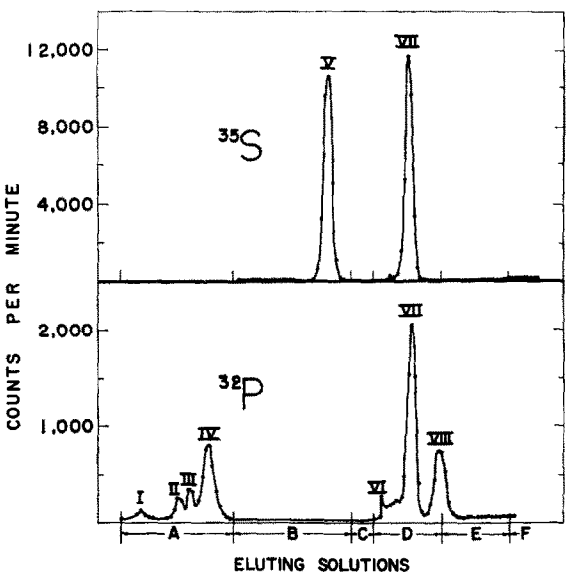

FIG. 1. Ion-exchange chromatograms of urinary metabolites of ^{32}P- and ^{35}S-parathion. Rats received 5 mg/kg of ^{32}P- or ^{35}S-parathion orally. Urine samples collected over 24 hr were chromatographed on Dowex 1-X8 columns by using eluting solutions described in Materials and Methods. Only those fractions that gave more radioactivity than twice the background are represented by dots.

DEPTA (peak VII) was a major metabolite with both labelings as expected from Plapp and Casida's report.[8] The identity of this metabolite was confirmed by comparing the chromatogram of a urine sample with that of the authentic sample, potassium *O,O*-diethyl phosphorothioate (supplied by American Cyanamid Co., Princeton, N.J.).

Peak VI was observed with some samples. It was evidently the result of decomposition of DEPTA and did not represent a separate metabolite, as Matsumura and Hogendijk[6] also reported. This peak was less conspicuous when a larger amount of DEPTA was chromatographed. The use of a larger volume of eluent HCl before the HCl–methanol mixture resulted in increased decomposition. The degree of

decomposition varied also with batches of the resin samples. These results showed that the decomposition of DEPTA was catalyzed by the Dowex 1-X8 resin under acidic conditions.

^{32}P-Parathion gave five other metabolites that were not labeled with ^{35}S. Comparison of the chromatograms with those published by other workers[6, 8] indicated that these were phosphoric acid (peak II), *O*-ethyl phosphoric acid (peak III), *O,O*-diethyl phosphoric acid (peak IV) and desethyl paraoxon (*O*-ethyl *O*-hydrogen *O*-4-nitrophenyl phosphate) (peak VIII). Peak I was not identified.

One metabolite (peak V) was labeled with ^{35}S, but not with ^{32}P, indicating that this metabolite was derived from the sulfur detached by the activation of parathion to paraoxon. Because inorganic sulfate was suspected, aliquots of eluates were mixed with a non-radioactive K_2SO_4 solution and sulfate ions were precipitated with benzidine hydrochloride as described before.[2] Precipitates of benzidine sulfate were filtered and dissolved in 20 ml of scintillation solution. Counting showed that approx. 90 per cent of the radioactivity was recovered in the benzidine sulfate after correction for quenching. Peak V and the peak for benzidine sulfate were similar and could be overlapped. Benzidine sulfate obtained with eluates containing ^{35}S-DEPTA had little, if any, radioactivity. The metabolite in peak V could also be precipitated with $BaCl_2$ after addition of non-radioactive K_2SO_4. The radioactive sulfate chromatographed on the Dowex 1-X8 column appeared at the same position of the chromatogram as peak V. When 0·5 ml of 10^{-4}M ^{35}S-sulfate was co-chromatographed with a urine sample containing approximately the same radioactivity of the peak V metabolite, a single peak appeared at the position of peak V with approximately twice the peak height of ^{35}S-sulfate chromatographed alone. These results indicated that the ^{35}S-metabolite was inorganic sulfate. Relative amounts of the urinary metabolites of parathion are listed in Table 1.

TABLE 1. URINARY EXCRETION OF DEGRADATION PRODUCTS OF PARATHION*

	% Degradation products				
	^{32}P			^{35}S	
Parathion dose (mg/kg)	0·2	1	5	0·2	5
Phosphoric acid	—	3	4	—	—
Ethyl phosphoric acid	—	4	5	—	—
Diethyl phosphoric acid	30	22	19	—	—
Inorganic sulfate	—	—	—	44	50
DEPTA	39	43	45	48	46
Desethyl paraoxon	—	12	21	—	—
Unknown	31	16	6	8	4

* Various doses of ^{32}P- or ^{35}S-parathion were given orally to rats and urine samples were collected over 24 hr. Degradation products were separated by ion-exchange chromatography as in Fig. 1 and percentages were calculated from radioactivities and volumes of eluate fractions. Figures are given only where a clear peak was observed. Data in each column are averages from two rats.

Because sulfate could be produced by desulfuration of DEPTA or desethyl parathion, urine was analyzed by ion-exchange chromatography after intraperitoneal injection of 1·2 mg/kg of ^{32}P-DEPTA, 1·6 mg/kg of ^{35}S-DEPTA or 0·04 mg/kg of ^{35}S-desethyl parathion. Almost all the radioactivity in the urine samples collected

24 hr after injection of DEPTA was due to unchanged DEPTA. With ^{32}P-DEPTA, a very small peak appeared around the position of *O,O*-diethyl phosphoric acid in the ion-exchange chromatogram. This peak contained less than 2 per cent of the total radioactivity eluted from the column. With ^{35}S-DEPTA, more than 98 per cent of the radioactivity was extractable with ethyl acetate from urine acidified to below pH 1. A very small peak was found at the position of sulfate in the chromatogram, representing less than 1 per cent of the total radioactivity eluted. While excretion of ^{35}S-material continued over 3 days at lower levels, excretion of sulfate did not increase. No ^{32}P- or ^{35}S-peaks other than those mentioned were found. These results showed that desulfuration of DEPTA occurred very little, if at all. Desulfuration of desethyl parathion was also low, although ion-exchange chromatograms indicated two or three minor metabolites, other than sulfate, in addition to the original desethyl parathion. Radioactivity of the sulfate peak in the chromatogram, from urine samples collected at 22 hr after injection, was less than 5 per cent of the total radioactivity eluted. When ^{35}S-sulfate was injected i.p. at the dose of 0·03 mg/kg (as free acid), excretion of

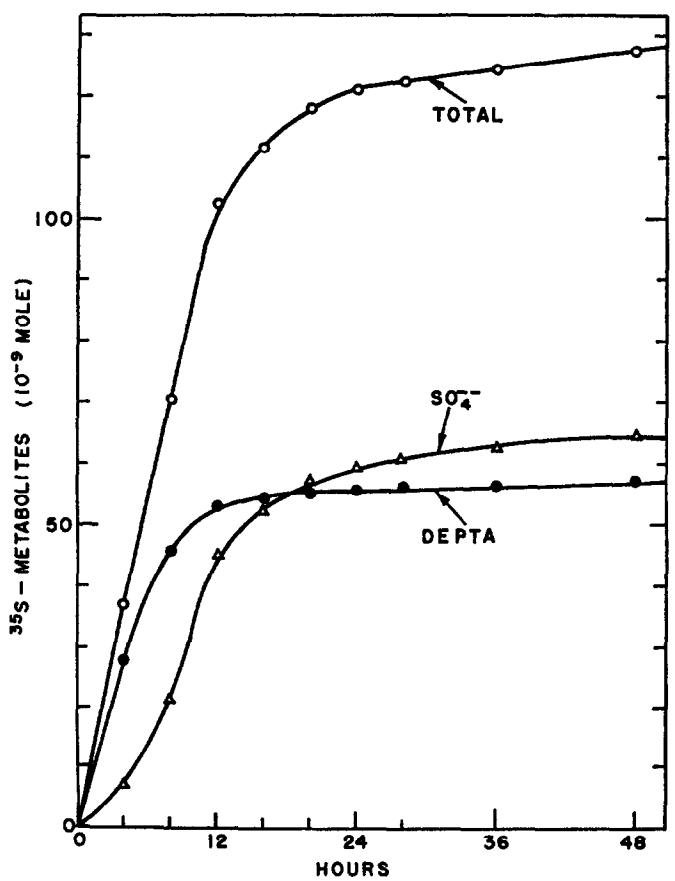

FIG. 2. Time course of urinary excretion of ^{35}S-parathion metabolites. Urine was collected at various time intervals. The DEPTA was extracted with ethyl acetate from acidified urine. The aqueous layer contained inorganic sulfate. The data are average values from three rats given 0·2 mg/kg of ^{35}S-parathion orally.

radioactivity nearly reached a plateau in 34 hr. About 65 per cent of the dose was excreted at 24 hr and 70 per cent at 48 hr. Therefore, some of the sulfate produced by parathion metabolism could have been excreted into feces or incorporated into other forms not readily excreted.

The course of excretion with time was examined over 48 hr after oral administration of 0·2 mg/kg of ^{35}S-parathion (Fig. 2). Although excretion was not complete at the end of 48 hr, it nearly reached a plateau within 24 hr. Ion-exchange chromatography showed that the only ^{35}S-metabolite in the ethyl-acetate extract of acidified urine was DEPTA and that the only ^{35}S-metabolite in the aqueous phase was inorganic sulfate. Time course samples were, therefore, analyzed after ethyl-acetate extraction. No extraction with *n*-hexane was needed because urine contained no ^{35}S-labeled material extractable with *n*-hexane. The DEPTA:sulfate ratio varied with time. The rate of excretion of DEPTA was highest between 0 and 4 hr, whereas sulfate excretion reached the peak at 8 hr or slightly later.

Distribution of parathion degrading enzymes among tissues of rats

Degradation of ^{35}S-parathion by 2·5 per cent homogenates of nine tissues was examined in the presence of 10^{-3}M $NADPH_2$ or GSH, or in the absence of such cofactors (Table 2). Metabolites extractable with ethyl acetate from acidified media

TABLE 2. DEGRADATION OF PARATHION BY HOMOGENATES OF VARIOUS TISSUES*

Tissue	Degradation product (10^{-6}M)		
	No cofactor	$NADPH_2$	GSH
Liver	1·687	4·241	1·977
Lung	0·074	0·264	0·094
Kidney	0·090	0·230	0·090
Spleen	0·049	0·058	0·043
Pancreas	0·038	0·020	0·026
Heart	0·030	0·086	0·143
Brain	0·058	0·068	0·069
Small intestine	0·038	0·059	0·062
Large intestine	0·046	0·058	0·049

* A mixture containing 2·5% tissue homogenate, 2·5 % 10^{-5}M parathion, 10^{-3}M cofactor, 0·01 M nicotinamide, 0·15 M KCl, 8×10^{-3}M Na_2HPO_4 and 2×10^{-3}M KH_2PO_4 was incubated for 60 min. The reaction was terminated with 10% TCA–3 N HCl. After remaining substrate was extracted twice with hexane, the degradation product was extracted with ethyl acetate. The data are an average of two experiments.

were measured. Liver was by far the most active. Degradation by liver homogenates without added $NADPH_2$ was variable, possibly because of variation in the level of endogenous cofactors. The activity was greatly enhanced by $NADPH_2$. The effect of GSH was very slight. Lung and perhaps kidney also had some activity. Degradation activity of extrahepatic tissues, however, never reached 10 per cent of that of liver. Therefore, further studies were done on the liver enzymes.

Subcellular distribution of parathion degrading enzymes in the liver homogenates

On fractionation of the liver homogenate, the F-4 fraction showed the highest activity when no cofactor was added (Table 3). The sum of activities of the four

fractions, however, was much lower than the activity of the homogenate. $NADPH_2$ at 10^{-3}M enhanced the activity of particulate fractions, especially that of the F-3 or microsomal fraction. Activity of the F-4 fraction was only slightly increased by $NADPH_2$. GSH had a small enhancing effect on all fractions. Although the F-4 fraction gave a linear time course of reaction over a period of 1 hr, the reaction of the

TABLE 3. DEGRADATION OF PARATHION BY LIVER FRACTIONS*

Fraction	Degradation product (10^{-6}M)		
	No cofactor	$NADPH_2$	GSH
F-1	0·185	1·254	0·221
F-2	0·060	1·474	0·098
F-3	0·029	3·878	0·073
F-4	0·349	0·470	0·448
Whole homogenate	1·498	4·231	1·717

* Fractions were obtained from 10 per cent liver homogenates by differential centrifugation: F-1, pellet from the 1600 *g* 10 min centrifugation; F-2, pellet from the 10,000 *g* 10 min centrifugation; F-3, pellet from the 105,000 *g* 60 min centrifugation, and F-4, supernatant above F-3. Pellets were resuspended to original volume with 0·25 M sucrose. The incubation mixture contained 2·5% tissue suspension, $2{\cdot}5 \times 10^{-5}$M parathion, 10^{-3}M cofactor, 0·01 M nicotinamide, 0·15 M KCl, 8×10^{-3}M Na_2HPO_4 and 2×10^{-3}M KH_2PO_4. After 60 min, the reaction was terminated with 10% TCA–3 N HCl. After remaining substrate was extracted twice with hexane, the degradation product was extracted with ethyl acetate. The data are an average of two experiments.

microsomal fraction slowed down with time. The nonlinearity of the latter reaction may be due to the inactivation of the enzymes[2] and/or to the decrease of $NADPH_2$ concentration by oxidation.

Identity of the metabolites produced in the presence of 10^{-3}M $NADPH_2$ was examined by ion-exchange chromatography. Metabolites were extracted with ethyl acetate as described under Analyses and the ethyl acetate was evaporated. The residue was dissolved in distilled water and chromatographed on Dowex 1-X8 columns. With particulate fractions, F-1, F-2, and F-3, the only metabolite on the chromatograms was DEPTA. The supernatant fraction, F-4, gave another minor metabolite in addition to DEPTA. This minor metabolite appeared to be desethyl parathion and was produced slightly more when $NADPH_2$ was replaced with GSH. It accounted for roughly 10 per cent of the total radioactivity eluted from the column; the remaining 90 per cent was DEPTA. Oxygen requirement of the enzymes in the F-3 and F-4 fractions was examined by using Thunberg tubes. Anaerobic conditions suppressed the metabolism by the F-3 fraction to 33 per cent of the control but enhanced the activity of the F-4 fraction by 80 per cent in the presence of GSH or $NADPH_2$ and by 70 per cent in the absence of cofactors. This indicated that nonoxidative enzymes are responsible for the metabolism by the F-4 fraction.

Nature of the soluble enzymes

Although the F-4 fraction showed much lower activity than the particulate fractions, it was further studied because the activity was probably due to nonoxidative soluble enzymes, different from microsomal oxidases studied previously.[2, 3] Because the

activity of the F-4 fraction was somewhat enhanced by $NADPH_2$ as well as by GSH, contamination by lighter microsomes was suspected. Therefore, soluble enzymes were studied with the supernatant fraction from a faster centrifugation as described in Materials and Methods. The soluble fraction thus prepared showed an activity similar to the F-4 fraction with or without GSH.

Because the soluble fraction contains endogenous cofactors that may support enzymatic activities, the fraction was "desalted" by gel filtration as described in Materials and Methods (Fig. 3). The desalted enzyme preparation had only a slight

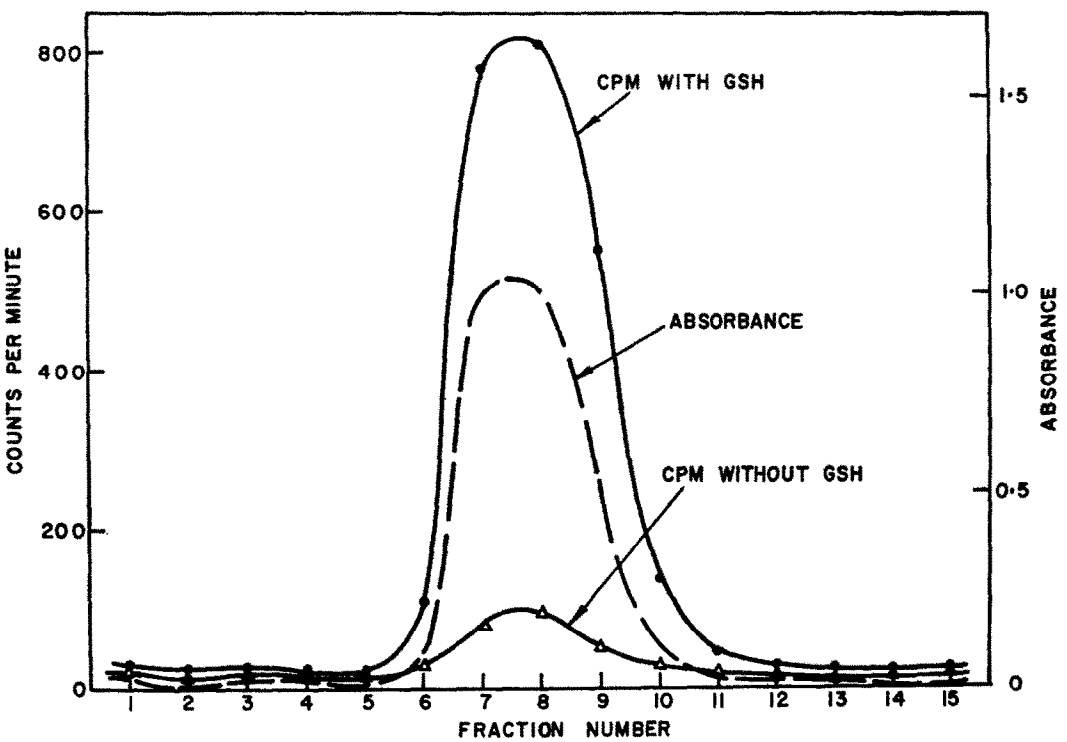

FIG. 3. Desalting of concentrated soluble fraction of liver homogenate by gel filtration. Enzymatic activity is indicated by formation of ^{35}S-metabolites that were extracted with ethyl acetate. The soluble fraction was desalted by passing through a Sephadex G-25 column. Averages of absorbances at 540 and 578 mμ were determined for red-colored fractions plus five fractions before and after the color appeared. Each fraction contained approximately 7·3 ml. Portions of each fraction were incubated with $2{\cdot}5 \times 10^{-5}$M parathion, 0·15 M KCl, 8×10^{-3}M Na_2HPO_4, and 2×10^{-3}M KH_2PO_4. The incubations were done either with or without 10^{-3}M GSH.

activity. The activity was restored by addition of 10^{-3}M GSH. Recovery of the activity through the column, as calculated by the activity times volume values, was 137 per cent of the original sample, possibly because of removal of interfering factors. About 80 per cent of the metabolites produced by the desalted enzyme preparation in the presence of GSH was eluted as DEPTA from the ion-exchange column.

A cofactor effect of a few compounds (10^{-3}M) was examined. Cysteine, mercaptoethanol and BAL (2,3-dimercaptopropanol) were 8, 35 and 11 per cent, respectively,

as effective as GSH. The oxidized form of GSH (GSSG), mercaptoacetic acid and benzenethiol were ineffective.

Various salts (10^{-3}M) added to the desalted enzymes in the presence of GSH gave the following metabolism percentages compared with no addition of such a salt: $CaCl_2$(103); $MgCl_2$(106); $MnCl_2$(96); $FeCl_3$(67); $FeCl_2$(127); $CuSO_4$(0).

The time course of metabolism by the desalted enzyme preparation in the presence of GSH was linear up to 30 min or longer. Measurement of reaction rate with various concentrations of ^{35}S-parathion indicated that the K_m value of the enzyme(s) was higher than $2{\cdot}5 \times 10^{-5}$M, although the relatively low activity of the enzyme preparation did not allow accurate determination.

DISCUSSION

Our experiments on metabolism *in vivo* of ^{32}P-parathion confirmed the report of Plapp and Casida[1] that DEPTA is the major urinary metabolite of parathion. However, some quantitative and qualitative differences among various metabolites (Fig. 1) were noted between the two studies. Plapp and Casida[1] found the following four metabolites in the urine of rats after oral administration of 1·5 mg/kg of parathion: diethyl phosphoric acid (16%), DEPTA (78%), desethyl paraoxon (2%), desethyl parathion (4%). We found that desethyl parathion was almost missing, while what appears to be desethyl paraoxon was an important metabolite at the doses of 1 and 5 mg/kg. Also, diethyl phosphoric acid, ethyl phosphoric acid and phosphoric acid were found in significant quantities (Table 1). Desethyl paraoxon, diethyl phosphoric acid, ethyl phosphoric acid and phosphoric acid were produced from paraoxon rather than from the corresponding P=S analogs because experiments with ^{35}S-labeled compounds showed that desulfuration of DEPTA and desethyl parathion was negligible. It is probable that these hydrolysis products of paraoxon appeared in the urine only after some paraoxon was produced. Therefore, the difference between the results of the two studies can be explained by the different length of urine collection period. It seems likely that Plapp and Casida's results represent a very early stage of metabolism, whereas we collected the urine over 24 hr.

The metabolite causing peak VIII (Fig. 1) was probably derived from paraoxon because it was not produced from DEPTA or desethyl parathion. Although the identification of this metabolite as desethyl paraoxon is only tentative, the result does show that paraoxon degradation is not solely through aryl–phosphate hydrolysis. Dealkylation (alkyl–phosphate cleavage) has been considered more important with dimethyl esters than with diethyl esters. But a considerable desethylation of coumaphos [*O*-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl) *O*,*O*-diethyl phosphorothioate] and/or its P=O analog has also been reported.[9] Desethylation may be more prevalent than expected from our current knowledge.

Metabolism of ^{35}S-parathion yielded only two major ^{35}S-metabolites, DEPTA and inorganic sulfate. Most of the sulfate is derived from desulfuration of parathion and DEPTA is an end product of parathion degradation. Therefore, these two metabolites represent two modes of initial metabolic attack on parathion, degradation and activation. The lag between excretion of DEPTA and sulfate suggests that more than one enzyme is involved in the formation of sulfate from parathion. Evidence from the experiments *in vivo* is summarized in Fig. 4.

Studies *in vitro* revealed that liver was the major tissue of parathion degradation.

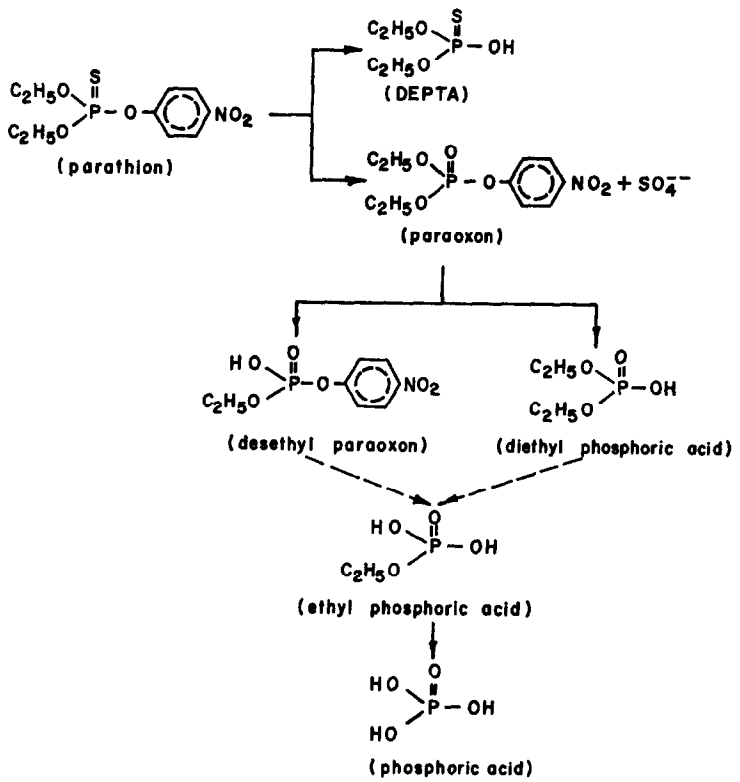

FIG. 4. Metabolism *in vivo* of parathion in the rat.

Fractionation of liver homogenates showed that the oxidative microsomal enzymes of the F-3 fraction were active in producing DEPTA as previously reported.[2, 3] The activity of the F-1 and F-2 fractions was probably attributable to contamination by microsomal enzymes of the F-3 fraction. However, DEPTA was produced also by soluble enzymes in the F-4 fraction. Most of the activity of the F-4 fraction was due to enzymes requiring GSH. These enzymes resemble the desmethylation enzymes described by Fukami and Shishido[7] in their requirement for GSH, indicating that aryl–phosphate cleavage by the F-4 fraction is catalyzed by transferase rather than phosphatase. A small fraction of the activity of the F-4 fraction was retained even after removal of endogenous cofactors by gel filtration. This suggests that a third type of enzyme, a true phosphatase, may also exist. It is more likely, however, that the apparent activity in the absence of GSH was due to radioactivity of residual ^{35}S-parathion which could not be completely removed from emulsified protein.

Degradation of $2{\cdot}5 \times 10^{-5}$M parathion by particulate fractions (F-1, F-2 and F-3, Table 3) was about 14 times as high as that by the F-4 fraction. Because the reaction by microsomal enzymes slowed down with time and soluble enzymes gave a linear time course of reaction, comparison of initial reaction rates would have given even a larger factor of difference. The activity of the soluble enzymes was not greatly enhanced by any of several cations tested. The K_m value of the soluble enzymes was evidently higher than $2{\cdot}5 \times 10^{-5}$M, whereas Neal[10] reported a K_m value of

$5{\cdot}33 \times 10^{-5}$M for the oxidases of rat liver microsomes that produced DEPTA from ^{32}P-parathion. Although determination of the K_m values for the two enzyme systems could not be made under the same conditions, evidence presented makes it unlikely that the soluble enzymes will become important even at considerably lower substrate concentrations or at low doses *in vivo*. Thus, the microsomal oxidases are probably most important in the production of DEPTA *in vivo* and the soluble enzymes play only a minor role in this metabolic process. True phosphatases are probably not involved in the direct degradation of parathion, although the possibility of their presence in the extrahepatic tissues at low levels has not been excluded. We may conclude, therefore, that the metabolism of parathion in the rat is mostly initiated by liver microsomal oxidases.

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REFERENCES

1. F. W. PLAPP and J. E. CASIDA, *J. econ. Ent.* **51**, 800 (1958).
2. T. NAKATSUGAWA and P. A. DAHM, *Biochem. Pharmac.* **16**, 25 (1967).
3. R. A. NEAL, *Biochem. J.* **103**, 183 (1967).
4. R. L. METCALF, M. MAXON, T. R. FUKUTO and R. B. MARCH, *Ann. ent. Soc. Am.* **49**, 274 (1956).
5. H. R. KRUEGER and J. E. CASIDA, *J. econ. Ent.* **54**, 239 (1961).
6. F. MATSUMURA and C. J. HOGENDIJK, *J. agric. Fd Chem.* **12**, 447 (1964).
7. J. FUKAMI and T. SHISHIDO, *J. econ. Ent.* **59**, 1338 (1966).
8. F. W. PLAPP and J. E. CASIDA, *Analyt. Chem.* **30**, 1622 (1958).
9. H. R. KRUEGER, J. E. CASIDA and R. P. NIEDERMEIER, *J. agric. Fd Chem.* **7**, 182 (1959).
10. R. A. NEAL, *Biochem. J.* **105**, 289 (1967).