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ENZYMATIC REDUCTION OF *O,O*-(4-NITROPHENYL) PHOSPHOROTHIOATE, *O,O*-DIETHYL *O*-(4-NITROPHENYL) PHOSPHATE, AND *O*-ETHYL *O*-(4-NITROPHENYL) BENZENE THIOPHOSPHONATE BY TISSUES FROM MAMMALS, BIRDS, AND FISHES*†

MARGARET HITCHCOCK and SHELDON D. MURPHY

Department of Physiology, Harvard University School of Public Health, Boston, Mass. U.S.A.

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Abstract—The reduction of the 4-nitro group of *O,O*-(4-nitrophenyl) phosphorothioate (parathion), *O,O*-diethyl *O*-(4-nitrophenyl) phosphate (paraoxon), and *O*-ethyl *O*-(4-nitrophenyl) benzene thiophosphonate (EPN) to the corresponding amino group was studied *in vitro* in tissues from mammalian, avian, and piscine species. The properties of the enzyme system that catalyzed the reduction of these insecticides were similar to those of previously reported enzyme systems which reduce *p*-nitrobenzoic acid. When NADPH and FAD were used as cofactors, the mitochondrial, microsomal, and soluble fractions contained, respectively, 34, 38, and 27 per cent of the parathion nitroreductase activity of rat liver whole homogenate. The capacities of liver homogenates from various species of the three classes of animals to reduce parathion and EPN were compared. Considerable variation in enzyme activity among a given class of animals as well as among the three classes was observed. For all species, the amount of EPN reduced was less than 25 per cent of the amount of parathion. Enzymatic activity was also present in mammalian kidneys, spleens, hearts, lungs, and erythrocytes, and also in avian kidneys. The contribution of reduction to the total inactivation of paraoxon, as measured by loss of anticholinesterase activity, was compared in rat, guinea pig, and chicken liver homogenates. Formation of aminoparaoxon by rat and chicken livers accounted for over half and guinea pig livers for a quarter of the total loss of anticholinesterase activities of the incubates.

THE ACUTE toxicity of the insecticides *O,O*-(4-nitrophenyl) phosphorothioate (parathion) and *O*-ethyl *O*-(4-nitrophenyl) benzene thiophosphonate (EPN) is due to the metabolic formation of powerful cholinesterase inhibitors, which are believed to be the corresponding oxygen analogues.[1–4] The accumulation of these toxic metabolites can be modified by alternative metabolic reactions resulting in degradation of the parent compound or its oxygen analogue. Enzymatic hydrolysis of the phosphorus nitrophenyl linkage of the oxygen analogues appears to be a major pathway of detoxication of these insecticides in mammals.[5–7]

It has been shown that chemical reduction of the 4-nitro group of parathion and paraoxon, *O,O*-diethyl (*O*-4-nitrophenyl) phosphate, to the corresponding amino derivatives greatly reduced their biological activity.[8] The acute toxicities in rats of the

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amino derivatives were 100- to 300-fold less than the nitro compounds, and the anticholinesterase activity *in vitro* of the amino derivative of paraoxon was about 13,000-fold less than the parent compound. The signs of poisoning in rats treated with aminoparathion and aminoparaoxon were slower in appearing compared with parathion and paraoxon and were not typical of anticholinesterase action.

It has been demonstrated that bovine rumen fluid is capable of reducing parathion and EPN to their amino derivatives *in vitro*.[9] Administration of parathion to a cow resulted in urinary excretion of aminoparathion accounting for 30 per cent of the dose, whereas in rats only 0·5 per cent of the total urinary excretion products was aminoparathion.[8]

The metabolic conversion *in vivo* of several other organic nitro compounds to their amino derivatives is well known,[10] and the presence and properties of a nitroreductase system in mammalian tissues has been studied in detail by Fouts and Brodie[11] and by Kamm and Gillette.[12] Adamson *et al.*[13] and Buhler[14] have recently shown that tissues of fish also contain an enzyme system which catalyzes the reduction of *p*-nitrobenzoic acid.

Since, theoretically, nitroreduction could be a pathway of detoxication for parathion and EPN in animals, it seemed important to determine if these insecticides could serve as substrates in a nitroreductase test system *in vitro*. It was felt that studies of the capacities of various tissues from several species of animals to reduce parathion and EPN might be useful in understanding the causes of species variation in toxicity of these compounds. Failure to demonstrate significant enzymatic reduction of the insecticides by rat tissues *in vitro* would be consistent with the small amount of urinary aminoparathion for this species that was reported by Ahmed *et al.*[8]

This report describes the results of experiments which demonstrated that appropriately fortified homogenates of tissues from mammals, birds, and fishes catalyze the reduction of EPN, parathion, and paraoxon.

MATERIALS AND METHODS

Chemicals

The samples of parathion (99·8 per cent pure) and paraoxon were supplied by the American Cyanamid Co., Princeton, N.J., and recrystallized EPN was furnished by the E. I. DuPont de Nemours Co., Wilmington, Del. Nicotinamide adenine dinucleotide phosphate (NADP), reduced nicotinamide adenine dinucleotide phosphate (NADPH), glucose 6-phosphate, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) were obtained from the Sigma Chemical Co.

Animals

Mammals used in this study were adult male and female Holtzman rats (200–250 g), adult male and female Swiss–Webster mice (26–40 g), and adult male guinea pigs (300–400 g). Avian species, obtained from the Massachusetts Audubon Society, were chickens (leghorn cockerels, 300–350 g) and male and female English sparrows (22–28 g). The sparrows were held for 5–10 days after they were captured and were fed a commercial bird seed. Sunfish (*Lepomis gibbosus*), bullheads (*Ictalurus melas*), large mouth bass (*Micropterus salmoides*), alewife (*Alosa pseudoharengus*), bluegill (*Lepomis*

machrochirus), and sucker (*Catostomus commersoni*) were furnished by the Massachusetts Department of Fish and Game and were taken from a local fresh-water pond. Sunfish, bluegills, and bullheads weighed 40–80 g; alewife, suckers, and bass weighed 100–300 g. The salt-water fish were winter flounder (*Pseudopleuronectes americanus*, 220–325 g) and sculpin (*Myoxocephalus scorpius*, 260–360 g). These were taken from Narragansett Bay. All fish were used within 2–3 days of their capture.

Preparation of tissue samples

Animals were decapitated, exsanguinated, and the tissues immediately removed. For routine assays all tissues were homogenized at 4° in nine parts of 1·15% KCl containing 0·25% nicotinamide with a Potter–Elvehjem all glass homogenizer.

For cell fractionation studies, liver from adult male rats was homogenized in nine parts of ice-cold 0·25 M sucrose. The nuclei and cellular debris were sedimented by centrifugation at 600 *g* for 10 min. The mitochondrial fraction was prepared by centrifuging the 600 *g* supernatant at 8500 *g* for 10 min. The mitochondrial pellet was washed by resuspending it in cold 0·25 M sucrose and centrifuging at 8500 *g* for 10 min. The microsomal fraction was then prepared by centrifuging the 8500 *g* supernatant at 80,000 *g* for 60 min. The supernatant from this centrifugation was considered as the soluble fraction. The microsomal pellet was washed by resuspending it in sucrose at 0° and centrifuging at 80,000 *g* for 60 min. The mitochondrial and microsomal fractions were prepared in a refrigerated Beckman model L ultracentrifuge. After washing and separation, the mitochondrial and microsomal pellets were kept frozen for a maximum period of 24 hr, during which time no loss of activity occurred. For incubation, both mitochondrial and microsomal pellets were resuspended in a sufficient volume of KCl nicotinamide solution so that 0·25 ml was equivalent to 25 mg whole homogenate.

Incubation procedure

For routine assays, the incubation mixture contained: 0·3 ml of 0·2 M phosphate buffer, pH 7·6; NADP, 3·9 μmole; glucose 6-phosphate, 8·4 μmole; FAD, 1·23 μmole; substrate, 1·2 μmole; tissue equivalent to 25 mg (fresh weight); and sufficient distilled water to make a final volume of 2·4 ml. The reactions were carried out at 37° for 30 min in evacuated Thunberg tubes. Tissue, substrate, and cofactor blanks were incubated at the same time. At the end of the incubation the tubes were placed in a boiling water bath for 2 min.

Assay of p-*amino derivatives of EPN, parathion and paraoxon*

Protein was precipitated by addition of 0·2 ml of 10% zinc sulfate to each incubate. The incubates were transferred to centrifuge tubes, the Thunberg tubes were rinsed with 2 ml distilled water, and the washings were added to the incubates. After centrifugation the supernatants were decanted off and extracted with 2 ml of chloroform. One ml of the chloroform extract was added to 2 ml of 0·5% *p*-dimethylaminobenzaldehyde in ethanol–glacial acetic acid (1:1, v/v). After 5 min the resulting yellow color was measured at 454 mμ with a Beckman DB spectrophotometer with a reagent blank as the reference cell. The amount of *p*-amino-EPN, *p*-aminoparathion or *p*-aminoparaoxon present was determined by reference to calibration curves, prepared by reducing known amounts of the parent compounds with 0·5% titanous chloride in

1 *N* HCl, extracting into chloroform, and estimating as described. In most cases results were expressed in micromoles of reduced product per 100 mg tissue per 30 min.

Total inactivation of paraoxon was measured by assaying for loss of anticholinesterase activity. Tissue incubates and incubates with substrate and cofactors but no tissue were diluted with sufficient distilled water so that an aliquot of 0·6 ml produced between 20–80 per cent inhibition of cholinesterase (equivalent to between 0·02 and 0·06 mμmoles paraoxon). The cholinesterase assays were performed by the manometric method of DuBois and Mangun[15] with 50 mg of homogenized rat brain as the enzyme source and 0·01 M acetylcholine chloride as substrate. The amount of paraoxon that was inactivated by tissue was calculated by subtracting the quantity remaining in the tissue incubates from the known quantities present in the reagent-blank incubates.

Identification of metabolites

The reduced products of parathion, paraoxon, and EPN were identified by subjecting the chloroform extracts to descending chromatography on Whatman no. 4 paper. The solvent systems used and the R_f values obtained are shown in Table 1.

TABLE 1. R_f VALUES OF PARATHION AND EPN AND THEIR POSSIBLE METABOLITES*

	A	B	C
Parathion	1·0	1·0	1·0
Aminoparathion	1·0	0·8	0·7
Paraoxon	1·0	1·0	0·8
Aminoparaoxon	0·9	0·9	0·9
EPN		0·9	0·05
Amino-EPN		0·9	0·9
p-NO_2 phenol	1·0	0·65	0·65
p-NH_2 phenol	0·75	0·50	0·31
$CHCl_3$ extract 1†	1·0	1·0, 0·8	1·0, 0·7
$CHCl_3$ extract 2‡	1·0, 0·9	1·0, 0·9	0·8, 0·9
$CHCl_3$ extract 3§		0·9	0·05, 0·9

* Solvents were run on Whatman no. 4 paper until the front had run 16 inches. Solvents used were: (A) butan-1-ol–acetic acid (glacial)–water (4:1:5, v/v); (B) butan-1-ol–ammonia (sp. gr. 0·88)–water (4:1:5, v/v); (C) ether–hexane–acetic acid (glacial) (100:50:1, v/v).

† 1 Extract from parathion incubation.

‡ 2 Extract from paraoxon incubation.

§ 3 Extract from EPN incubation.

Aromatic nitro compounds were detected by $TiCl_3$ reduction and coupling with *p*-dimethylaminobenzaldehyde. Amino compounds were coupled with the latter reagent only. In all three cases only the parent compound and its reduced product were detected in the chloroform extracts.

RESULTS

Properties of the enzyme system in rat liver which catalyzed reduction of EPN and parathion

The properties of the enzyme system responsible for reducing parathion, paraoxon, and EPN were found to be similar to the nitroreductase system described by Fouts and Brodie.[11] Experiments with rat liver whole homogenates showed that NADP,

glucose 6-phosphate, and FAD were required as cofactors (Table 2). Enzyme activity was 27 per cent less when nicotinamide was absent. The reaction was inhibited by the presence of 100 per cent oxygen, but no increase in the amount of reduced product was observed when the tubes were flushed with nitrogen as compared with evacuating them. The effect of varying the tissue levels and incubation times on the reduction of parathion was studied in whole homogenates of male rat liver. Fig. 1 shows that a

TABLE 2. COFACTOR REQUIREMENTS FOR ENZYME ACTIVITY*

System	Aminoparathion (μmole formed/100 mg/30 min)
Tissue	0·0
Tissue + 8·4 μmole glucose 6-phosphate	0·0
Tissue + 8·4 μmole glucose 6-phosphate + 3·9 μmole $NADP^+$	<0·03
Tissue + 8·4 μmole glucose 6-phosphate + 3·9 μmole $NADP^+$ + 1·23 μmole FAD	1·380
Tissue + 1·23 μmole FAD	0·00
Tissue + 8·4 μmole glucose 6-phosphate + 1·23 μmole FAD	0·00
Tissue + 3·9 μmole $NADP^+$ + 1·23 μmole FAD	0·00

* Male rat liver, 25 mg, homogenized in 1·15% KCl containing 0·25% nicotinamide incubated for 30 min with 1·2 μmole parathion.

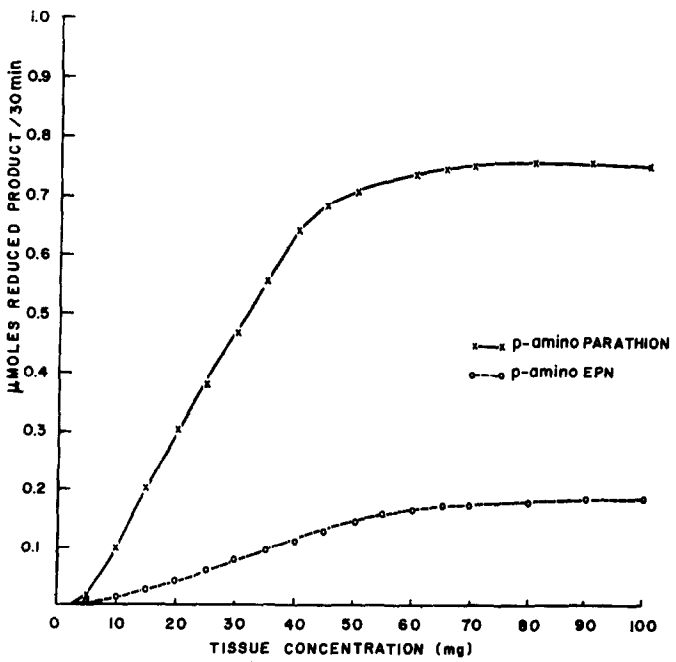

FIG. 1. Reduction of parathion and EPN by various quantities of rat liver.

linear relationship was obtained between tissue concentration and enzyme activity with quantities of liver ranging from 10–40 mg. Fig. 2 shows that a linear relationship was also obtained between enzyme activity and length of incubation time between 5 and 55 min with 25 mg of liver homogenate. Optimum concentrations of NADP, glucose 6-phosphate, and substrate were determined for rat and guinea pig liver

homogenates and were found to be the same. These concentrations were used for all tissue and species comparisons.

When subcellular fractions from rat liver, prepared as in Methods, were incubated with parathion as the substrate, the mitochondrial, microsomal, and soluble fractions contained, respectively, 38, 34, and 28 per cent of the total activity of the whole homogenate (Table 3). Centrifugation of the 8500 *g* supernatant at 80,000 *g* for 4 hr,

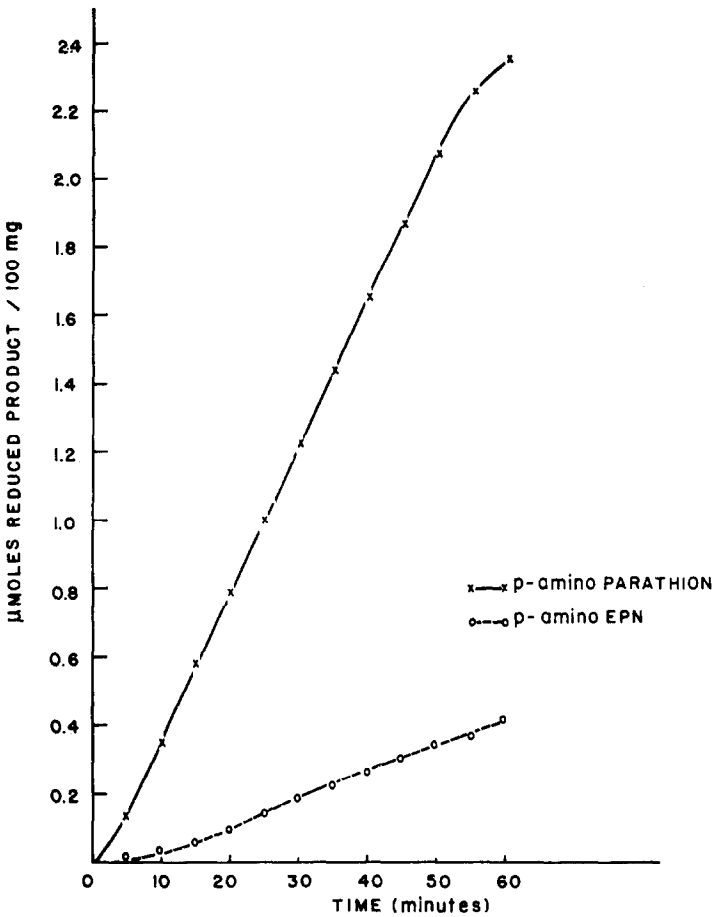

FIG. 2. Rate of reduction of parathion and EPN by rat liver (each incubate contained 25 mg of whole homogenate).

instead of the usual 1 hr, did not alter the intracellular distribution of the enzyme, which indicates that the activity found in the soluble fraction was not associated with the light microsomes. When NADP and glucose 6-phosphate were used as cofactors, activity in the microsomal fraction could be demonstrated only by addition of soluble fraction. When NADPH was substituted for NADP and glucose 6-phosphate, activity could be demonstrated in the microsomal fraction alone, thus showing that the enzyme is NADPH dependent. Under the conditions described in Methods, no measurable amount of reduced product was formed without the addition of FAD.

FAD is known to stimulate nitroreductase.[11, 13] With rat liver whole homogenate and parathion or EPN as substrate, it was found that concentrations of FAD up to $0{\cdot}5 \times 10^{-2}$ M stimulated the enzyme, but concentrations in excess of this amount caused inhibition. Adamson *et al.*[13] found that addition of equimolar concentrations

TABLE 3. INTRACELLULAR DISTRIBUTION OF ENZYME ACTIVITY*

Tissue fraction	(μmole Aminoparathion/100 mg/30 min)	
	$NADP^+$ and glucose 6-phosphate + FAD	NADPH + FAD
Whole homogenate	1·200	1·201
600 *g* supernatant	1·200	1·187
8,500 *g* supernatant	0·750	0·749
Soluble fraction	0·338	0·351
Mitochondria	0·00	0·409
Microsomal fraction	0·00	0·452
Mit. + soluble fraction	0·760	
Mic. + soluble fraction	0·803	

* Various cell fractions equivalent to 25 mg rat liver, 1·2 μmole parathion, 1·23 μmole FAD, and 3·9 μmole NADPH or 3·9 μmole $NADP^+$ and 8·4 μmole glucose 6-phosphate.

of FAD to liver homogenates of different avian and piscine species stimulated nitroreductase by the same order of magnitude in each species and concluded that differences in the basal level of enzyme activity *in vitro* were not due to the availability of flavoproteins. As a result of these conclusions, FAD at $0{\cdot}5 \times 10^{-2}$ M was used in all routine incubations in this study. When FAD was replaced by $0{\cdot}5 \times 10^{-2}$ M FMN or riboflavin, the rate of reduction of parathion was increased by 24 and 59 per cent and of paraoxon by 45 and 70 per cent, respectively. This observation is in agreement with the findings of Fouts and Brodie[11] and of Adamson *et al.*[13] on the relative stimulation of nitroreductase by these three flavins.

Kamm and Gillette[12] recently suggested a mechanism to explain the role of flavins in the reduction of *p*-nitrobenzoic acid by a solubilized microsomal enzyme. According to this mechanism, an enzyme (possibly NADPH–cytochrome c reductase,) prepared by treatment of microsomes with pancreatic lipase, catalyzes the transfer of H^+ from NADPH to FAD to form $FADH_2$. Under anaerobic conditions, $FADH_2$ then reduces the nitro compound to the amino derivative nonenzymatically. These workers incubated pancreatic lipase-treated microsomes with NADPH and FAD and then inactivated the enzyme which reduced FAD to $FADH_2$. When nitrobenzoic acid was added to the inactivated incubate under anaerobic conditions, aminobenzoic acid was formed. A similar experiment was carried out in which parathion, instead of nitrobenzoic acid, was incubated with rat liver whole homogenate or washed microsomes, NADPH, and FAD. No aminoparathion was detected in incubates in which the enzyme had been inactivated by heat before addition of parathion. Thus, a mechanism of nonenzymatic reduction of parathion did not appear to operate under the conditions used in this investigation.

Reduction of insecticides by tissues of various species

The reduction of parathion, paraoxon, and EPN was compared, under identical conditions, with liver homogenates from several species of mammals, birds, and

fishes. Table 4 shows the values obtained. The rate of reduction varied according to the nature of the substrate. Rat, guinea pig, and chicken livers were capable of reducing more paraoxon than parathion. For all species, the amount of EPN reduced was less than 25 per cent of the amount of parathion. Since under normal conditions the body temperatures of fish would be below the 37° used for these tests *in vitro*, the reduction of parathion at 21° by liver homogenates of alewife, bluegill, and flounder was compared with the activity at 37°. At the lower temperature there was 34, 66, and 37 per cent less reduction of parathion, respectively.

Comparison of the data obtained under identical conditions shows that there is considerable variation in enzyme activity among different species within a given class of animals as well as among the three classes studied. In all species studied the amount of EPN reduced was consistently low as compared with parathion.

TABLE 4. REDUCTION OF EPN, PARATHION AND PARAOXON BY LIVER HOMOGENATES OF VARIOUS SPECIES

Species	No. of animals	Aminoparaoxon (μmole formed/ 100 mg/30 min)	Aminoparathion (μmole formed/ 100 mg/30 min)	Amino-EPN (μmole formed/ 100 mg/30 min)
Rat	6	1·914 ± 0·540*	1·256 ± 0·321	0·254 ± 0·053
Mouse	6		1·191 ± 0·088	0·220 ± 0·031
Guinea pig	9	0·944 ± 0·164	0·455 ± 0·113	0·081 ± 0·010
Chicken	4	1·225 ± 0·138	0·518 ± 0·096	0·090 ± 0·014
English sparrows	8		0·196 ± 0·120	0·052 ± 0·012
Bullhead	6		1·431 ± 0·162	0·249 ± 0·027
Sucker	3		1·364 ± 0·063	0·237 ± 0·014
Flounder	5		0·839 ± 0·184	0·125 ± 0·015
Sculpin	5		0·544 ± 0·190	0·055 ± 0·018
Large mouth bass	4		0·913 ± 0·281	0·145 ± 0·032
Sunfish	6		0·954 ± 0·041	0·155 ± 0·038
Bluegill	4		1·093 ± 0·340	0·180 ± 0·046
Alewife	4		0·850 ± 0·116	0·136 ± 0·037

* Figures are means ± S.E.M.

Because of the considerable sex differences that have been observed for other drug-metabolizing enzymes, the capacities of the livers from male and female rats and mice to reduce parathion were compared. No significant sex difference in the rate of formation of aminoparathion was found in either species.

The data in Table 5 show that parathion can be reduced by tissues other than liver. Rat kidney, spleen, heart, and lung contained reductase activity as did chicken and sparrow kidney. Rat erythrocytes had very low activity.

Comparison of nitroreduction and total destruction of anticholinesterase activity of paraoxon

Since aminoparaoxon is a much weaker inhibitor of cholinesterase than paraoxon,[8] reduction could be considered a pathway for inactivation of paraoxon. Measurements of the loss of anticholinesterase activity of paraoxon in the presence of tissue reflect the total amount of paraoxon inactivated by all mechanisms that are operative under a given set of conditions. When the incubation system described in methods was used but FAD omitted, no measurable amount of aminoparaoxon was formed.

TABLE 5. TISSUE DISTRIBUTION OF NITROREDUCTASE

Species	No. of animals	Tissue	Aminoparathion (μmole/100 mg/30 min)
Rat	6	Liver	1·256 ± 0·321*
	6	Kidney	1·004 ± 0·518
	6	Spleen	0·983 ± 0·059
	6	Heart	0·305 ± 0·099
	6	Lung	0·246 ± 0·101
	3	Erythrocytes	0·082 ± 0·009
	3	Plasma	0
English sparrow	8	Liver	0·196 ± 0·120
	8	Kidney	0·287 ± 0·144
Chicken	4	Liver	0·518 ± 0·096
	4	Kidney	0·601 ± 0·039

* Figures are means ± S.E.M.

Therefore, loss of anticholinesterase activity of the incubate was considered to include all mechanisms other than reduction. The results of experiments in which the inactivation of paraoxon by rat, guinea pig, and chicken liver homogenates was measured in the presence or absence of FAD are shown in Table 6. In all three

TABLE 6. CONTRIBUTION OF REDUCTION TO THE TOTAL INACTIVATION OF PARAOXON

Species	No. of animals	Paraoxon inactivated (μmole)			Aminoparaoxon (μmole formed with FAD/25 mg 30 min)	% of Total inactivation as aminoparaoxon
		With FAD	Without FAD	Difference		
Rat	6	0·848 ± 0·057*	0·793 ± 0·059	0·113 ± 0·016	0·452 ± 0·122	56·9 ± 13·8
Guinea pig	3	0·928 ± 0·029	0·740 ± 0·029	0·189 ± 0·040	0·214 ± 0·133	27·0 ± 6·33
Chicken	3	0·564 ± 0·042	0·450 ± 0·055	0·111 ± 0·021	0·294 ± 0·077	55·2 ± 3·70

* Figures are means ± S.E.M.

species less paraoxon was destroyed in the absence of FAD, that is, when reduction did not take place. The incubates that contained FAD were also assayed for accumulation of aminoparaoxon and Table 6 shows the contribution of reduction to the total inactivation of paraoxon by livers of the three species. In incubates containing FAD and rat or chicken liver homogenates, over half of the total loss of anticholinesterase activity was due to the formation of aminoparaoxon; whereas with guinea pig liver reduction could account for only a quarter of the total loss of anticholinesterase activity of paraoxon. The small difference between the total inactivation in the presence and absence of FAD suggests that, when FAD is present, the nitroreductase system competes with other metabolic pathways of inactivation of the substrate, paraoxon.

DISCUSSION

This investigation has shown that the organophosphate insecticides, parathion, paraoxon, and EPN, can serve as substrates for a nitroreductase system present in tissues of mammals, birds, and fishes. The cofactor requirements and intracellular

distribution of the enzyme system that catalyzes the reduction of these insecticides were similar to those of the nitroreductase system described by Fouts and Brodie.[11] Under the conditions used in this study reduction of EPN, parathion, and paraoxon by tissue homogenates required the addition of flavin cofactors.

Reduction of the nitro group to the amino group probably proceeds via the formation of the nitroso or the hydroxylamino derivatives, or both. Bueding and Jolliffe[16] found that xanthine oxidase reduced trinitrotoluene to the hydroxylamine derivative and that this reaction proceeded at a faster rate than the subsequent formation of the amino derivative. If this were the case in the present study the accumulation of intermediates would be expected. The data of Table 1 suggest that this was not so, although they could have escaped detection. However, Fouts *et al.*[11] found that highly purified xanthine oxidase, when incubated with xanthine, NADPH or NADH and FAD, did not detectably reduce the substrates used in their study. They concluded that xanthine oxidase had only a minor role, if any, in reducing aromatic nitro compounds. Since the enzyme system used in the present study was similar to that used by Fouts *et al.*, it is assumed that the same conclusions apply. The results of the experiments, using NADPH in which parathion was added after heat denaturation of the enzyme, indicated that the mechanism of coupled enzymatic and nonenzymatic reactions described by Kamm and Gillette[12] was not responsible for reducing parathion. Nonenzymatic transfer of the H^+ from $FADH_2$ to the nitro compound did not seem to take place under the conditions used in this study. The hydrogen donor was probably NADPH with a flavin acting as the prosthetic group.[11, 16] However, since nitroreductase is a flavoprotein, a limiting factor in determining the amounts of parathion and EPN that could be reduced *in vivo* might be the availability of flavins. In the absence of adequate quantities of these compounds, other pathways of metabolism would probably predominate. This was suggested by the experiments *in vitro*, which showed that the total inactivation of paraoxon in the absence of FAD was 80–90 per cent as great as when FAD was present, even though in the latter case as much as 50 per cent of the total loss of anticholinesterase activity could be accounted for by reduction of paraoxon to aminoparaoxon. Since Ahmed *et al.*[8] did not find appreciable quantities of aminoparaoxon as a urinary metabolite of parathion in rats, it appears that in this species conditions *in vivo* do not favor nitroreduction as a metabolic pathway, even though, under appropriate conditions *in vitro*, rat tissues have a relatively high parathion nitroreductase activity.

Guinea pig liver homogenates reduced only about one-half as much parathion and EPN as mouse and rat liver homogenates; however, rats and mice are equally or more susceptible than guinea pigs to acute poisoning by these insecticides.[17] For these mammalian species, therefore, the liver nitroreductase activity *in vitro* did not correlate with the species difference in toxicity.

Under the conditions used, reduction of parathion by chicken livers *in vitro* was less than for mouse and rat livers. In recent experiments (S. D. Murphy and R. L. Lauwerys, unpublished observations) it was found that chickens were more than twice as susceptible as mice to poisoning by this insecticide. On the basis of only these two criteria, it might be concluded that difference in nitroreductase activity contributed to the greater susceptibility of chickens to poisoning by parathion. However, other factors may be equally or more important. For example, the hydrolysis of paraoxon to *p*-nitrophenol by unfortified chicken liver homogenates was much less

than for mouse livers.[18] Sunfish livers also had very low hydrolytic activity, but the fish were less susceptible than mice to poisoning by parathion. In the present investigation it was found that the *in vitro* nitroreduction of parathion by sunfish and mouse livers was about equal.

In species where hydrolytic degradation of parathion or paraoxon does not occur or is very slow, alternative pathways of inactivation would be expected to assume a more important role as a detoxication mechanism than hydrolysis, because reduction occurs with the parent compound, while most currently available evidence indicates that hydrolysis occurs only after oxidation of parathion to paraoxon.[7, 19] In order for this theory to be valid, however, favorable conditions for reduction must be present *in vivo*. This is apparently not the case for rats. Whether nitroreduction of parathion occurs and is an important detoxication pathway in intact birds and fishes require additional qualitative and quantitative studies of metabolites that are formed in these classes of animals *in vivo*.

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