ethanolic DPN solutions is the same as in ethanol solutions without DPN.

6. It has not been possible to demonstrate any interaction between free thiol radicals and $DPN^+$ or DPNH.

7. The experimental evidence supports an ionic rather than a free-radical mechanism for dehydrogenase action.

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# The Conversion of Schradan (OMPA) and Parathion into Inhibitors of Cholinesterase by Mammalian Liver

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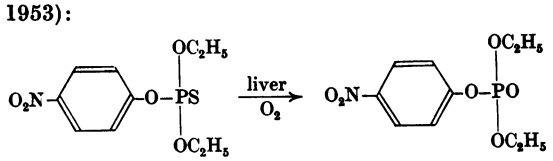
Schradan [$(Me_2N)_2PO.O.PO(NMe_2)_2$, OMPA, octamethylpyrophosphoramide, bis-*NNN'N'*-tetramethylphosphorodiamidic anhydride] and parathion (*OO*-diethyl *O*-*p*-nitrophenyl phosphorothioate) are weak inhibitors of cholinesterase *in vitro*, but when administered to animals the cholinesterase levels of the blood and tissues drop, and typical signs of acetylcholine poisoning follow (Dubois, Doull & Coon, 1950; Aldridge & Barnes, 1952; Kilby & Gardiner, 1952). It has been shown that in the presence of oxygen parathion is oxidized by mammalian liver slices to paraoxon (*OO*-diethyl *O*-*p*-nitrophenyl phosphate), a highly active inhibitor of cholinesterase (Myers, Mendel, Gersmann & Ketelaar, 1952; Gage, 1953; Metcalf & March, 1953):




Similarly, schradan is converted by mammalian liver slices in the presence of oxygen into the phosphoramide oxide and other products which are powerful inhibitors of cholinesterase (Dubois *et al.* 1950; Aldridge & Barnes, 1952; Kilby & Gardiner, 1952; Casida, Allen & Stahmann, 1954). This conversion has also been shown to occur in the liver *in vivo* (Cheng, 1951). However, the conversion does not occur in liver suspensions in the presence of oxygen. It therefore seemed interesting to investigate the difference between rat-liver slices and suspensions and to study the nature of the systems involved in the metabolism of parathion and schradan.

Since at 37° the conversion *in vitro* of both organophosphorus compounds is stopped after freezing, heating or dispersing the liver, it is concluded that enzymes probably participate in the process. It seemed possible that on dispersing the liver in buffer either an enzyme or a coenzyme was inactivated. It has now been found that the

reaction is enzymic and requires the presence of oxygen. Liver suspensions can be activated by preparing the liver in solutions containing nicotinamide with addition of a boiled liver extract or diphosphopyridine nucleotide. By differential centrifuging it has been possible to show that this oxidative conversion occurs in the liver microsomes with the addition of fortified liver supernatant. Preliminary investigations suggest that parathion and schradan are converted by similar systems in mammalian liver.

## METHODS AND MATERIALS

*Purification of parathion and schradan.* Schradan was obtained from Dr G. S. Hartley, of Pest Control Ltd., Cambridge. Traces of anti-cholinesterase impurities were removed by shaking a solution of schradan in chloroform with 1% (w/v) NaOH; after washing with water and drying, the chloroform was distilled off under reduced pressure.

Parathion was obtained from Dr H. Coates, of Albright and Wilson Ltd. The parathion was purified by shaking a chloroform solution with 1% (w/v) $Na_2CO_3$, washing, drying and distilling off the chloroform. In order to remove last traces of inhibitor a suspension of parathion (1 mg./ml.) was then prepared in 20% (w/v) gum acacia, and before use one part of this suspension was incubated for 1 hr. at 37° with an equal part of rat serum (Aldridge, 1953). The mixture was then heated to 80° for 1 min. and was then practically free from anti-cholinesterase activity.

*Pyridine nucleotides.* Diphosphopyridine nucleotide (DPN) was prepared by the method of Kornberg & Pricer (1953). The DPN preparation was found to be of 70% purity, when assayed by the methods of Gutcho & Stewart (1948) and Racker (1950). Chromatographic analysis (method of Burton & Pietro, 1954) showed that there were only traces of triphosphopyridine nucleotide (TPN) present.

*Preparation of liver suspensions and subsequent fractionation.* Fractionation of a 10% (w/v) rat-liver suspension in cold 0·25M sucrose or in cold 0·15M-NaCl, 0·015M-$MgSO_4$ and 0·008M nicotinamide was achieved at 0° by the differential centrifuging technique of Schneider (1948). Microsomes were separated from the soluble clear fraction of liver by centrifuging at 18 000 *g* for 1 hr. at 0°. The material for an active preparation was prepared for routine use by centrifuging a cold suspension of rat liver (25%, w/v) in NaCl, $MgSO_4$ and nicotinamide solution at the maximum speed in a Minor (MSE) centrifuge for 10 min. at room temp.

*The formation and estimation of the anti-cholinesterase inhibitors from schradan and parathion.* Since the amounts of the organophosphorus compounds that are oxidized are very small, it proved impossible to measure the oxygen taken up in the conversion process. Inhibition of horse-serum pseudocholinesterase has therefore been used throughout this work.

The conversion of organophosphorus compounds was effected in air at 37° in Warburg flasks. Cofactors and inhibitors were added to 2 ml. of a liver preparation in the centre well of a flask and the organophosphorus compound was placed in the side arm. After 5 min. shaking at 37°, the contents of the side arm were tipped into the well of the flask. Controls were run: (1) by adding the organophosphate from the side arm at the end of the experiment, (2) by boiling the liver preparation or (3) by gassing with $CO_2 + N_2$ (5:95) in place of air. The contents of the flasks were suitably diluted with buffer and 0·5 ml. added to 1 ml. of horse serum (diluted 15-fold with buffer) in a Warburg flask together with 2 ml. of bicarbonate buffer (0·0357M-$NaHCO_3$, 0·04M-$MgCl_2$ and 0·164M-NaCl). After gassing with $CO_2 + N_2$ (5:95) and incubating at 37° for 30 min. the cholinesterase activity was determined manometrically by addition of butyrylcholine (Davison, 1953) and the activity was expressed as a percentage of control. The various inhibitors and cofactors added to the liver preparations were found not to interfere with this estimation. The units of inhibitor formed were expressed as 10 (2 − log% activity)/10 mg. of protein (determined by the biuret method), 10 units being equivalent to approximately $3 \times 10^{-6}$ mole metabolite from schradan/10 mg. of protein (Casida, Chapman, Stahmann & Allen, 1952) and to $6 \times 10^{-8}$ mole paraoxon/10 mg. of protein.

## RESULTS

### *The failure of rat-liver suspensions to convert schradan*

In agreement with others we have found that rat-liver suspensions oxygenated in Krebs–Ringer phosphate, in saline or bicarbonate–saline do not convert added schradan, although intact cell preparations will do so readily (see Table 2). Fleisher & Jandorff (1952) have claimed that this is due to the destruction of schradan by the liver suspension. However, if the supernatant obtained by centri-

Table 1. *The stability of schradan in liver suspensions*

Cooled rat-liver slices (2 g.) were put into a Warburg flask (1) containing 4 ml. of 0·0357M-$NaHCO_3$, 0·164M-NaCl buffer; 6 ml. of a 33·3% (w/v) rat-liver suspension in the same buffer was pipetted into a second flask (2). Schradan was added to each flask to give a final concentration of 0·5 mg./ml. After shaking at 37° in air for 30 min., the flask contents were centrifuged down; 0·5 ml. of the supernatant of each was incubated for 30 min. with horse serum (diluted 15-fold) and the inhibition of cholinesterase determined. Liver (1 g.) was then suspended in 3 ml. of supernatant from flask (1) and 1 g. of liver slices added to 3 ml. of supernatant from flask (2). The two preparations were placed in Warburg flasks (3 and 4) and shaken in air at 37° for 30 min. The anti-cholinesterase activity was again determined. Chloroform-soluble organic phosphate was also determined (method of Aldridge, 1954).

| Flask no. | Preparation | Inhibition of cholinesterase (%) | Chloroform-soluble phosphorus (μg./ml.) |
|---|---|---|---|
| 1 | Slices + schradan | 100 | 54 |
| 2 | Suspension + schradan | 0 | 60 |
| 3 | (1) + suspension | 100 | — |
| 4 | (2) + slices | 100 | — |
| 5 | Schradan alone | 0 | 60 |

fuging a liver suspension, previously incubated for 30 min. in air with schradan, is added to fresh rat-liver slices an inhibitor is readily formed (Table 1). Furthermore, the anti-cholinesterase produced by incubation of liver slices and schradan is itself not destroyed by liver suspensions. It therefore seems probable that the results obtained by Fleisher & Jandorff are due to the presence of impurities in technical schradan, which are in fact destroyed by incubating with liver suspensions (see also Casida & Stahmann, 1953).

*The activation of liver suspensions by cofactors*

The failure of liver suspensions to convert schradan would therefore appear to be due either to lability of an enzyme system or to dilution or destruction of cofactors normally present in intact cells. Since the addition of boiled yeast or liver extracts to liver suspensions resulted in some conversion being obtained, it seemed probable that on dispersion of the liver an essential cofactor was lost.

Addition of adenine nucleotides did not enhance conversion, but if nicotinamide and diphosphopyridine nucleotide (DPN) were added, schradan was effectively converted into its active metabolite (Table 2). A boiled liver suspension plus DPN did not oxidize schradan. It was also found that the addition of $Mg^{2+}$, and to a lesser extent $Co^{2+}$, increased the ability of liver suspensions to produce the anti-cholinesterase. Addition of $Fe^{3+}$, $Mn^{2+}$, $Ca^{2+}$, $K^+$ or $PO_4^{3-}$ had no such effect. Since it was found that addition of $Mg^{2+}$ to liver suspensions did not affect the stability of any anti-cholinesterase formed from schradan, it seems that $Mg^{2+}$ is a participant in the enzyme system. A further improvement in the metabolism of schradan could be obtained by centrifuging suspensions for 10 min. at 8500 *g* (Table 2). This improvement is possibly due to the separation of a nucleotidase in the whole liver suspension (see Carruthers & Suntzeff, 1954). Experiments on the stability and rates of reversal of inhibited cholinesterase, using the metabolites from slices and suspensions, indicate that the same compound is produced by each preparation (Table 3).

Table 2. *The conversion of schradan by liver preparations*

Different liver preparations were shaken in air with schradan (0·5 mg./ml.) at 37°. The amount of inhibitor produced was determined on the supernatant after centrifuging by the method described in the text.

| Liver preparation | Inhibitor units/10 mg. protein produced in 30 min. at 37° |
|---|---|
| 1. Rat-liver slices (1 g.) in 4 ml. of saline mixture | 12 |
| 2. Rat-liver (1 g.) squeezed through a glass syringe in 4 ml. of saline | 25 |
| 3. Liver suspension (25%, w/v) in Krebs–Ringer phosphate | 0·01 |
| 4. As (3) + 0·008 M nicotinamide | 6·3 |
| 5. Liver suspension (25%, w/v) in 0·15 M-NaCl, 0·015 M-$MgSO_4$ + 0·008 M nicotinamide | 9·5 |
| 6. As (5) + DPN (0·4 mg./ml.) after suspending | 30 |
| 7. As (6) but centrifuged 10 min. at 8500 *g* with DPN (0·4 mg./ml.) added afterwards | 48 |

Table 3. *Constants for the metabolites of schradan*

Schradan (0·5 mg./ml.) was added to rat-liver slices (1 g.) in 4 ml. of 0·15 M-NaCl, 0·015 M-$MgSO_4$ solution or to a rat-liver suspension (25%, w/v) in the same solution together with 0·008 M nicotinamide and DPN (0·4 mg./ml.). After shaking in air for 30 min. at 37°, the metabolite formed was extracted by shaking 4 ml. of the centrifuged supernatant with 4 ml. of chloroform. The chloroform layer was separated and the chloroform distilled off under reduced pressure; the residue was dissolved in 10 ml. of Sørensen's 0·067 M phosphate buffer (pH 7·6). Buffer solutions containing metabolite from slices or suspensions were kept at 37°, and the rate of loss of anti-cholinesterase activity determined at intervals by incubating 0·5 ml. with horse serum for 30 min. as described in the text. The rate of reversal of cholinesterase inhibition was determined by the method of Davison (1953) after inhibition of 4·5 ml. of rat serum by 0·5 ml. of the supernatant from slices or suspension.

| | Slices | Suspension |
|---|---|---|
| Rate of hydrolysis of schradan metabolite | $3 \times 10^{-2}$ min.$^{-1}$ | $3 \times 10^{-2}$ min.$^{-1}$ |
| Half-life for reversal of inhibition by the schradan metabolite | 28 hr. | 28 hr. |

*Distribution of the enzyme system converting parathion and schradan*

Separation of rat-liver suspensions in 0·25 M sucrose or in the NaCl–nicotinamide–$MgSO_4$ solution was effected by differential centrifuging. It was found that neither the cells and nuclei nor the mitochondria converted either schradan or parathion, although the mitochondria could oxidize added glutamate, choline or tyramine. The major part of the activity remained in the supernatant after removal of the mitochondria. This fraction was further separated into microsomes and a clear supernatant, but neither fraction was as efficient alone as the two fractions together (Table 4). It is interesting to note that the enzyme converting parathion and schradan shows a similar

distribution. It has also been found that tri-*o*-cresyl phosphate (TOCP) (Aldridge, 1954) and *OO*-dimethyl *O*-*p*-nitrophenyl phosphorothioate are converted by fortified liver suspensions from weak inhibitors into active anti-cholinesterases.

Table 4. *The distribution of the converting enzyme in rat-liver fractions*

Liver suspensions (10%, w/v) in 0·15M-NaCl, 0·015M-$MgSO_4$ and 0·008M nicotinamide were separated by differential centrifuging at 0° (Schneider, 1948). DPN (0·4 mg./ml.) was added to each fraction together with schradan (1 mg./ml.) or parathion (200 μg./ml.) in suspension. After 30 min. shaking in Warburg flasks in air at 37°, the anti-cholinesterase activity of suitable dilutions of the different preparations was determined as described in the text. The mitochondria used were shown to be active against glutamate, choline and tyramine.

| Liver fractions | Units of inhibitor produced by Schradan | Parathion |
|---|---|---|
| Cells and nuclei | 0 | — |
| Mitochondria | 0 | — |
| Microsomes | 2 | 160 |
| Clear supernatant | 17 | 130 |
| Microsomes + clear supernatant | 50 | 910 |

Table 5. *Conversion of schradan and parathion by male and female rat liver*

Chilled liver was squeezed through a glass syringe into the nicotinamide–NaCl–$MgSO_4$ solution (25%, w/v). Schradan (1 mg./ml.) or parathion (200 μg./ml.) was added. The organophosphate and liver brei were shaken in air at 37° for 30 min. and the number of units of inhibitor produced determined. Values for $LD_{50}$ for parathion are those of Dubois *et al.* (1949) and for schradan are those observed in this laboratory.

| | Males | Females |
|---|---|---|
| $LD_{50}$ of schradan (mg./kg.) | 7 | 27 |
| Units obtained *in vitro* from schradan | 35, 40, 40 | 15, 16, 20, 20 |
| $LD_{50}$ of parathion (mg./kg.) | 7 | 4 |
| Units obtained *in vitro* from parathion | 7·5, 7·5 | 69, 48 |

*The distribution of the converting system in rat tissues and the effect of sex differences*

Liver, brain, kidney or spleen was forced through an all-glass syringe (Aldridge, personal communication) to give a tissue brei in the saline mixture. Only the liver brei converted added schradan into an active anti-cholinesterase.

Since schradan is more toxic to male rats and parathion more toxic to female rats, it seemed interesting to see if the tissue brei preparation of the liver of male and female rats differed in converting ability. It was found that the liver preparation of male rats was about twice as efficient as the female in converting schradan (Table 5). Female rat livers were much more efficient than male in converting parathion.

*Requirement for added DPN*

It has been shown that addition of nicotinamide is necessary for the conversion of schradan and parathion by rat-liver suspensions. This conversion is considerably enhanced if DPN is added (Table 2). The rate of production of an anti-cholinesterase from schradan by rat-liver microsomes and supernatant with added DPN was proportional to time (Fig. 1), but, if a brain suspension containing a

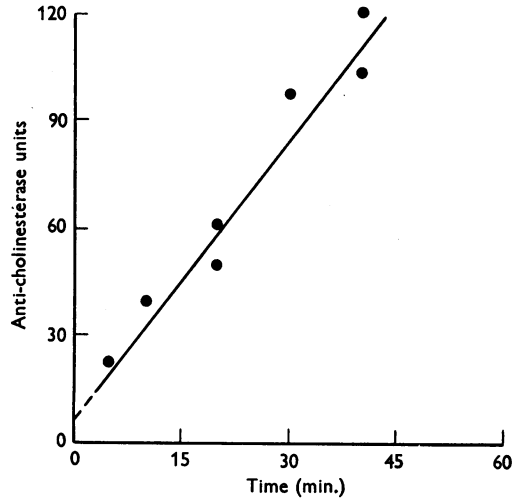



Fig. 1. The rate of production of inhibitor from schradan by rat liver. An active preparation of rat-liver microsomes was made as described in the text. Schradan (1 mg./ml.) and DPN (0·4 mg./ml.) were added and the whole was shaken in air at 37° for varying times.

pyridine nucleotidase was added, the rate of conversion was considerably reduced. Addition of increasing amounts of three different preparations of DPN to the microsome preparation (Fig. 2) resulted in increased production of anti-cholinesterase, although addition of more than $1 \times 10^{-3}$M DPN resulted in less than the optimum production of inhibitor. Reduced DPN was about 90% as efficient in converting as DPN when added to the microsome-supernatant preparation plus schradan. In another series of experiments reduced DPN (Gutcho & Stewart, 1948) was added to the liver microsome-supernatant preparation. The oxidation of DPNH at 37° was followed by the decreased absorption at 340 mμ. in a Unicam spectrophotometer (SP. 500). Addition of schradan decreased the rate of oxidation. This suggests that some DPN

is reduced by the system when schradan is added and that oxidized DPN is therefore necessary for the system.

Results previously obtained suggest that TPN or TPNH cannot effectively replace DPN in the enzyme system converting schradan or parathion (Davison, 1954). Furthermore, it has so far been impossible to demonstrate that microsomes by themselves or with addition of oxidized or reduced pyridine nucleotide convert either schradan or parathion. Addition of DPN to liver suspensions without added nicotinamide resulted in a preparation about one-third as active as if nicotinamide had been present; however, the absence of nicotinamide in the microsome-supernatant preparation resulted in only slight loss of activity.

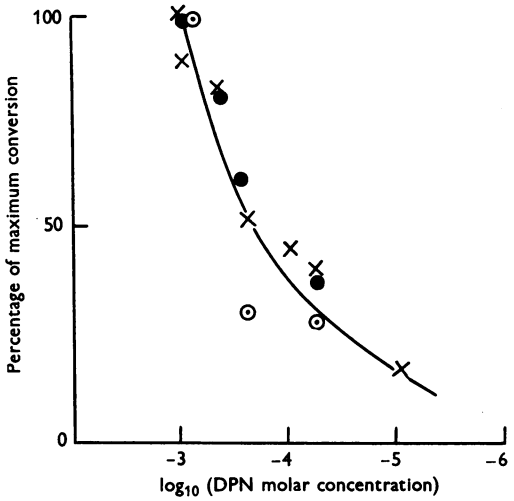



Fig. 2. The effect of addition of DPN on the conversion of schradan. Nicotinamide was added after centrifuging a rat-liver suspension (25%, w/v) in NaCl–$MgSO_4$ at 8500 *g* for 10 min. at room temp. Then DPN at varying concentrations and schradan (1 mg./ml.) were added to different flasks and the production of anti-cholinesterase on shaking in air for 30 min. at 37° was determined. Our own DPN (×), DPN from Light and Co. Ltd. (⊙) and from Boehringer and Soehne, Germany (●) were used.

*Properties of the active enzyme system*

The fortified liver microsome preparation was highly thermolabile. Thus, warming to 50° for 10 min. destroyed all its activity. The optimum pH for the conversion of both parathion and schradan is near neutrality (Fig. 3). The action of a number of different types of inhibitor on the conversion process has also been studied. It will be seen that the oxidation of the two organophosphorus compounds by rat-liver microsome preparations shows a similar sensitivity to inhibition (Table 6). The

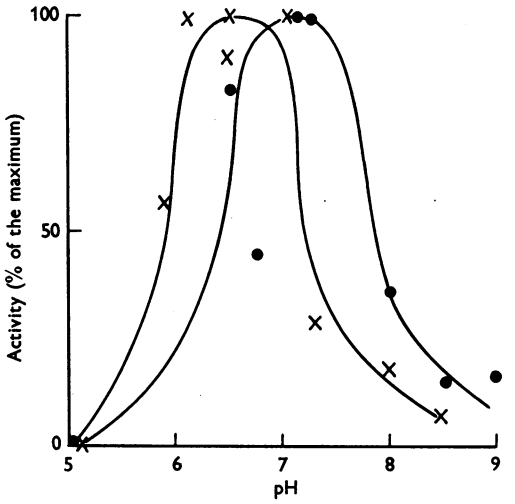



Fig. 3. The pH optimum curves for conversion of schradan and parathion. Equal volumes of the fortified microsome-supernatant preparation and either Sørenson's phosphate or McIlvaine's phosphate–citric acid buffers were added together with the organophosphate and the pH was determined. After shaking in air for 30 min. at 37° the anti-cholinesterase produced was measured. Concentrations of 1 mg./ml. schradan (●), and 200 μg./ml. parathion (×) were used.

Table 6. *The inhibition of the conversion of schradan and parathion*

An active microsome supernatant preparation was obtained by suspending chilled rat liver (25%, w/v) in 0·15 M-NaCl, 0·015 M-$MgSO_4$ and 0·008 M nicotinamide and centrifuging for 10 min. at 8500 *g* at room temp. DPN (0·4 mg./ml.) and 1 mg./ml. schradan or 200 μg./ml. parathion in suspension were added. The inhibitors were added to the conversion mixture 5 min. before addition of the organophosphorus compound. Percentage inhibition was calculated as the inhibition of anti-cholinesterase produced in 30 min. at 37° compared to a control without inhibitor.

| Inhibitor added | Final concentration (M) | Percentage inhibition of the conversion of: Schradan | Parathion |
|---|---|---|---|
| Mercuric chloride | $10^{-3}$ | 100 | 90 |
| Iodoacetate | $10^{-3}$ | 84 | 100 |
| *p*-Chloromercuribenzoate | $10^{-4}$ | 33 | 66 |
| Chloropicrin | $10^{-3}$ | 100 | 100 |
| Hydroxylamine | $10^{-3}$ | 100 | 90 |
| 2:4-Dinitrophenol | $10^{-3}$ | 100 | 100 |
| Potassium cyanide | $10^{-4}$ | 15 | 0 |
| Potassium cyanide | $10^{-5}$ | 0 | 0 |
| Choline | $10^{-3}$ | 52 | 93 |
| Trimethylamine | $10^{-3}$ | 39 | — |
| Dimethylamine | $10^{-3}$ | 38 | — |
| SKF 525A | $10^{-3}$ | 54 | 52 |

compound SKF 525A [2-(diethylamino)ethyl diphenylpropylacetate hydrochloride] also inhibits both conversions. This seemed particularly interesting, as La Du, Trousof & Brodie (1952) have shown that this substance inhibits the de-alkylation of aminopyrine and other alkylamines *in vitro* by a liver microsome preparation and *in vivo*. In view of these results the effect of SKF 525A has been examined *in vivo*. SKF 525A (100 mg./kg.) was given by intraperitoneal injection to six male rats of about 250 g. After 20 min. these animals, and a further six untreated rats, were given 8 mg./kg. of schradan by intraperitoneal injection. Within 40 min. all six rats of the control group had died with typical signs of acetylcholine poisoning and only one of the SKF group (this animal did not show signs of acetylcholine poisoning). The remaining rats survived with no signs of poisoning. Similar experiments with parathion (10 mg./kg.) resulted in signs of acetylcholine poisoning (red tears, etc.) lasting for 24 hr. in the pretreated group, although in this case four out of the SKF group died compared to three from the control group of six.

## DISCUSSION

It is now recognized that the liver is the site for the metabolism of a number of *N*-dimethyl compounds (Butler & Waddell, 1954). It is interesting therefore that not only is schradan metabolized by mammalian liver but also that entirely different compounds, parathion, tri-*o*-cresyl phosphate and *OO*-dimethyl *O*-*p*-nitrophenyl phosphorothioate, are oxidized by the same tissue. Several workers have shown that rat, rabbit or mouse-liver slices convert either schradan or parathion into powerful inhibitors of cholinesterase and that liver suspensions failed to convert either compound. Addition of nicotinamide and diphosphopyridine nucleotide has now been found to be necessary for the optimum production of an anti-cholinesterase from schradan by whole rat-liver suspensions.

Using the differential centrifuging technique of Schneider (1948), it has been found that conversion of both schradan and parathion occurs in the microsome plus supernatant fraction of the liver and DPN (Davison, 1954). Omission of nicotinamide in this preparation resulted in slight loss of activity.

Brodie and his associates have found that barbiturates, sympathomimetic amines and thiopentonal are all oxidized by liver microsomes plus the supernatant, $Mg^{2+}$ and TPN (Brodie & Cooper, 1954; Axelrod, 1954). The supernatant and TPN can be replaced by reduced TPN or any system that will generate TPNH. Since there are many similarities between the preparation of Brodie and our own, it seemed interesting to see if DPN could be replaced by TPN or TPNH. The results (Davison, 1954) suggest that TPN or TPNH will not replace DPN. However, this conclusion can only be provisional in that the sample of TPN used in those studies was grossly impure. Injection of the compound SKF 525A increases the survival time and prolongs the signs of acetylcholine poisoning in rats treated with schradan and possibly parathion; furthermore, *in vitro* SKF 525A inhibits the conversion process. It is interesting that SKF 525A also inhibits the oxidation of barbiturates both *in vivo* and *in vitro* (Kensler, Matchett & Bradley, 1954), while iodoacetate has been shown to inhibit the oxidation of barbiturates *in vitro* (Brodie & Cooper, 1954).

Mercuric chloride, iodoacetate, *p*-chloromercuribenzoate and chloropicrin inhibit the conversion of parathion and schradan. This suggests that SH groups are an essential part of the system. The absence of any inhibitory effect by cyanide shows that cytochrome oxidase does not participate in the reaction; it is therefore interesting to note that DPN–cytochrome *c*–reductase found chiefly in the liver microsomes (Brody, Bain & Wang, 1952) is sensitive to inhibition by *p*-chloromercuribenzoate but not by cyanide (Mahler & Elowe, 1954). Choline, trimethylamine, dimethylamine and SKF 525A also inhibit the conversion, possibly by competitive inhibition. The enzyme systems for conversion of schradan and parathion are sensitive to the same temperature changes and both have about the same optimum pH. These results suggest that the oxidation of schradan and parathion is effected by similar enzyme systems.

Despite these similarities at least one basic difference between the two systems has been found. The liver of male rats converts schradan better than does the liver of female rats and the reverse holds for parathion. The active metabolite from schradan is not very rapidly hydrolysed *in vitro* by liver suspensions and there is no evidence to suggest that the cholinesterase of female rats is more susceptible than that of males to inhibition by organophosphorus compounds. Thus male and female rats are equally sensitive to poisoning by two compounds which do not undergo conversion *in vivo*, tetraethyl pyrophosphate and *OO*-diethyl *O*-*p*-nitrophenyl phosphate (Aldridge & Barnes, 1952). This sex difference in the metabolism of the compounds, also seen in the oxidation of barbiturates (Quinn, Axelrod & Brodie, 1954), may account for the fact that schradan is more toxic to male rats than to female (Aldridge & Barnes, 1952), while parathion is more toxic to female rats than to male (Dubois, Doull, Salerno & Coon, 1949). In spite of these differences it can be concluded that the enzyme system responsible for the metabolism of schradan, parathion and probably

tri-*o*-cresyl phosphate is located in liver microsomes plus the supernatant and is active with the addition of nicotinamide, $Mg^{2+}$ and diphosphopyridine nucleotide. This metabolic system probably has a very broad specificity, for not only are barbiturates and amines (Brodie & Cooper, 1954; Axelrod, 1954) oxidized by a similar system, but also 4-dimethylaminoazobenzene (Mueller & Miller, 1948) and 4-aminoimidazole-5-carboxamide [4(5)-amino-5(4)-carbamoylglyoxaline] (Miller & Warren, 1953) are oxidized by rat-liver suspensions only after addition of nicotinamide, $Mg^{2+}$ and diphosphopyridine nucleotide. Furthermore, the enzyme system of insects which converts added parathion and schradan into anti-cholinesterase resembles the mammalian system in all respects so far investigated (Metcalf & March, 1953; O'Brien & Spencer, 1953; Kok & Walop, 1954). All these findings suggest that the microsome–supernatant system studied in this paper has an important role in fundamental metabolism.

## SUMMARY

1. Parathion, schradan, tri-*o*-cresyl phosphate and *OO*-dimethyl *O*-*p*-nitrophenyl phosphorothioate have been shown to be oxidized *in vitro* by a rat-liver preparation to powerful inhibitors of cholinesterase.

2. The enzyme system is located in the microsome-plus-supernatant fraction of liver suspensions.

3. Diphosphopyridine nucleotide and magnesium ions are necessary for the conversion process *in vitro*.

4. Cytochrome oxidase is not an essential participant in the reaction, but it has been shown that the system is sensitive to thiol group inhibitors.

5. The conversion of parathion and schradan is mediated by similar but not necessarily identical systems.

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