Booth, A. N., Wilson, R. H. & De Eds, F. (1953). *J. Nutr.* **49**, 347.
Buschke, W. (1943). *Arch. Ophthal., Chicago*, **30**, 735.
Dische, Z. (1959). *Amer. J. Ophthal.* **48**, 500.
Glock, G. E. & McLean, P. (1953). *Biochem. J.* **55**, 400.
Lerman, S. (1959*a*). *Science*, **130**, 1473.
Lerman, S. (1959*b*). *Nature, Lond.*, **184**, 1406.
Lerman, S. (1960*a*). *A.M.A. Arch. Ophthal*, **63**, 128.
Lerman, S. (1960*b*). *A.M.A. Arch. Ophthal.* (in the Press).
Mitchell, H. S. & Dodge, W. M. (1935). *J. Nutr.* **9**, 37.
Pirie, A., van Heyningen, R. & Boag, J. W. (1953). *Biochem. J.* **54**, 682.
Strehler, B. L. & Totter, J. R. (1952). *Arch. Biochem. Biophys.* **40**, 28.
van Heyningen, R. (1959). *Biochem. J.* **73**, 197.

Biochem. J. (1961) **79**, 229

The Effect of SKF 525A (2-Diethylaminoethyl 2:2-Diphenylvalerate Hydrochloride) on Organophosphate Metabolism in Insects and Mammals

BY R. D. O'BRIEN*
Pesticide Research Institute, London, Ontario, Canada

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The organophosphates are of two classes: those which are toxic directly, and those which require 'activation' in the animal body to produce the actual toxic compound. The directly acting compounds include the phosphates and phosphorothiolates, most of which contain the $-P(O)(OR)_2$ grouping, where R is an alkyl group. The other class includes the phosphorothionates, most of which contain the $-P(S)(OR)_2$ grouping, and the phosphorodiamidates that contain the $-P(O)(NR_2)$ grouping.

'Activation' is an oxidative reaction, involving the conversion in phosphorothionates of P(S) into P(O) (Gage, 1953) or, in phosphorodiamidates, of an alkyl into a hydroxyalkyl group (Heath, Lane & Park, 1955; Spencer, O'Brien & White, 1957). 'Activation' is accomplished in mammals almost exclusively by liver microsomes (Davison, 1955), and in insects principally by gut and fat body (Metcalf & March, 1953; O'Brien & Spencer, 1953).

It has been shown that, in mammalian-liver preparations, SKF 525A (2-diethylaminoethyl 2:2-diphenylvalerate hydrochloride) blocks the 'activation' of schradan (octamethylpyrophosphoramide; tetramethylphosphorodiamidic anhydride), Guthion [*OO*-dimethyl *S*-(4-oxo-3H-1:2:3-benzotriazine-3-methyl) phosphorodithioate] and parathion (diethyl *p*-nitrophenyl phosphorothionate) (Davison, 1955; Murphy & DuBois, 1957). *In vivo*, SKF 525A is a potent antagonist of poisoning by schradan and Guthion, but not of poisoning by parathion (O'Brien & Davison, 1958). The present paper examines this paradox further.

* Present address: Department of Entomology, Cornell University, Ithaca, New York, U.S.A.

The effects of SKF 525A on the toxicity or metabolism of organophosphates in insects have not been reported previously. They are of interest because of the possibility that SKF 525A might be useful as an adjuvant in the treatment of livestock by systemic organophosphates for internal parasites. They may also help to elucidate the nature of the differences between the activating systems of mammals and insects.

METHODS

The mice were female albinos (20–35 g.) from Rolfsmeyer Farms (Madison, Wis.). The houseflies (*Musca domestica*) were 1-day-old adult females of a normal CSMA strain. The cockroaches (*Periplaneta americana*) were adult females, about 28 days moulted.

The organophosphates used were: (1) Chlorthion (dimethyl *m*-chloro-*p*-nitrophenyl phosphorothionate; (2) Co-ral (diethyl 3-chloro-4-methylcoumarin-7-yl phosphorothionate); (3) Delnav [mixed isomers of 2:3-*p*-dioxandithiol *SS*-bis-(*OO*-diethyl phosphorodithioate)]; (4) Diazinon (diethyl 6-methyl-2-*iso*propylpyrimidin-4-yl phosphorothionate); (5) dimefox (tetramethylphosphorodiamidic fluoride); (6) dimethoate [*OO*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate]; (7) Dowco 109 (*O-p-tert.*-butyl-*o*-chlorophenyl *O*-methyl *N*-methylphosphoramidothionate); (8) EPN (ethyl *p*-nitrophenyl phenylphosphonothionate); (9) mipafox (*NN'*-di*iso*propylphosphorodiamidic fluoride; (10) paraoxon (diethyl *p*-nitrophenyl phosphate); (11) parathion; (12) ronnel (dimethyl 2:4:5-trichlorophenyl phosphorothionate); (13) schradan; (14) Systox (diethyl 2-ethylthioethyl phosphorothionate and its thiolo isomer); (15) TEPP (tetraethyl pyrophosphate; diethyl phosphoric anhydride); (16) Tetram [*OO*-diethyl *S*-(2-diethylaminoethyl)phosphorothiolate]; (17) Thimet [*OO*-diethyl *S*-(2-ethylthioethyl)phosphorodithioate].

We are indebted to Chemagro Corp. for (1), (2) and (14), Hercules Powder Co. for (3), Geigy Chemical Co. for (4), Fison's Pest Control Ltd. for (5), American Cyanamid Co. for (6) and (11), Dow Chemical Co. for (7) and (12), E. I. du Pont de Nemours Co. for (8), Dr G. Schrader for (10), Monsanto Chemical Co. for (13), which was further purified by Dr E. Y. Spencer according to Casida, Allen & Stahmann (1954), Dr J. R. Robinson for (15), and Chipman Chemical Co. for (16). Compound (8) was purchased from L. Light and Co. The SKF 525A was presented by Smith, Kline and French Ltd. Lilly 18947 (2:4-dichloro-6-phenylphenoxyethyldiethylamine) was kindly given by Dr H. H. Moorefield. For the synthesis of radioactive parathion, 15 mc of $H_3{}^{32}PO_4$ in HCl was placed in a 16 mm. × 100 mm. Carius tube and the water and HCl were removed by a current of dry N_2. Then 355 mg. of $PSCl_3$ was added and the tube was sealed and heated 18 hr. at 400° in a muffle furnace (Vigne & Tabau, 1958). Subsequent synthesis of parathion was according to Krueger, O'Brien & Dauterman (1960). The yield was 71% based on $PSCl_3$, the activity 5700 counts/min. per μg./ml., in a liquid-counting Geiger tube.

Paraoxon (diethyl *p*-nitrophenyl phosphate) was prepared by passing N_2O_4 for 5 min. at room temperature through a solution in 10 ml. of methylene chloride of a second batch of ^{32}P-parathion prepared as above. The solvent was removed, the mixture taken up in benzene and the solution added to an alumina column (Woelm, acid, activity grade II). The excess of parathion (239 mg.) was washed through with 200 ml. of benzene, and the paraoxon then eluted off with chloroform. The yield was 20%, based on the original $PSCl_3$.

Toxicity determinations. The dose of SKF 525A used was the highest which, when injected alone, produced no symptoms. For mice, the SKF 525A (0·2% in 0·9% NaCl) was injected intraperitoneally at a dose of 20 mg./kg. Immediately after, the organophosphate in 0·2 ml. of propylene glycol/20 g. mouse was injected intraperitoneally. Mortalities were determined 24 hr. thereafter. For houseflies, the SKF 525A in 0·9% NaCl (1·3 μl./fly) was injected into the thoracic muscle, at 100 mg./kg. The organophosphates were dissolved in acetone and applied topically at 1·3 μl./fly to the thorax, immediately after the SKF 525A. With cockroaches, the SKF 525A in 0·9% NaCl (5 μl./g. wt.) was injected intra-abdominally at 300 mg./kg. The organophosphate, dissolved in the same volume of propylene glycol or ethanol–propylene glycol (1:9, v/v), was then injected intra-abdominally.

Parathion metabolism. Each mouse was injected intraperitoneally with [^{32}P]parathion (0·1% in propylene glycol) at a dose of 5 mg./kg., by using an Agla micrometer syringe. At the required time thereafter, the mouse was homogenized with 250 ml. of 5% trichloroacetic acid in a Waring Blendor for 2 min., then filtered through a wire screen. Three such preparations were pooled and counted, then 500 ml. was extracted with 100 ml. of benzene. The phases were separated by centrifuging and the benzene was dried with sodium sulphate and counted; 50 ml. of the benzene solution was passed through a 15 cm. × 1 cm. column of Woelm alumina grade II, and eluted with a further 50 ml. of benzene. Another 10 ml. of benzene was then passed through to check for complete elution, then 50 ml. of chloroform was passed through. The benzene fraction contained the parathion, and the chloroform contained the paraoxon (Plapp & Casida, 1958). Duplicate samples were counted in a liquid counter. In recovery experiments, 80% of injected parathion was recovered in the aqueous and benzene fractions; 96% of parathion added to a trichloroacetic acid-treated and filtered homogenate was extracted into the benzene.

Cockroaches were injected with [^{32}P]parathion (0·1% in propylene glycol) at 5 mg./kg. by means of an Agla syringe. Groups of five were homogenized in 250 ml. of 5% trichloroacetic acid, which was then extracted with 100 ml. of benzene. Subsequent fractionation was as described for the mouse.

Paraoxon metabolism. Mice were treated and homogenized as above, except that 1 mg. of [^{32}P]paraoxon/kg. was injected. A portion (500 ml.) of the pooled homogenates was extracted with 100 ml. of chloroform, the phases were separated by centrifuging and the chloroform phase (containing unchanged paraoxon) was dried with Na_2SO_4 and counted. In experiments in which [^{32}P]paraoxon was added directly to the homogenate before filtering, subsequent recovery into the chloroform of only 35% was obtained, although the recovery into the chloroform of paraoxon added to the filtered homogenate was 98%.

Cockroaches were injected and homogenized as described under 'Parathion metabolism', except that 1 mg. of [^{32}P]-paraoxon/kg. was injected. The 250 ml. of homogenate was extracted with 100 ml. of chloroform, which was dried with Na_2SO_4 and counted. In experiments in which [^{32}P]-paraoxon was added directly to the homogenate before filtering, subsequent recovery into the chloroform was complete (103%).

Schradan activation. One gut from a female cockroach was incubated in a 10 ml. beaker with 1·5 ml. of 0·05M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 7·4, 0·25 ml. of 0·5% schradan and 0·25 ml. of water or SKF 525A solution. The beakers were shaken on the platform of a wrist-action shaker for 90 min. The solutions from four were pooled and duplicate 1 ml. samples added to a Warburg flask containing 1 ml. of a cholinesterase preparation (laked human erythrocytes at one-third their blood concentrations) and 0·5 ml. of 1·5% $NaHCO_3$. The standard Warburg assay was used at 25°, with 0·2 ml. of 2% acetylcholine bromide as substrate.

RESULTS

Studies on mammals and their tissues

Toxicity. Table 1 shows that in mice the only phosphorothionate against which SKF 525A provided substantial protection was dimethoate. Further work showed that the LD_{50} of dimethoate was raised from 80 to 195 mg./kg. by the SKF 525A. This 2·4-fold increase is comparable with the 1·6–2·0-fold increase previously reported for Guthion (O'Brien & Davison, 1958). It appears that protection against phosphorothionates is the exception rather than the rule. With Thimet a distinct synergism was noted. Previous work (O'Brien & Davison, 1958) had shown the SKF 525A did not protect mice from parathion poisoning. A brief study showed that neither did it synergize para-

thion poisoning, e.g. 10 mg. of parathion/kg. killed 10% of control mice or of mice treated with 20 mg. of SKF 525A/kg. (duplicate batches each of ten mice were used).

In a brief study on rats, involving 28 animals, little or no protective action against dimethoate was found, e.g. 350 mg. of dimethoate/kg. killed 89% without or 78% with SKF 525A.

Protection against all three phosphorodiamidic compounds was given by SKF 525A in mice. Further studies with mipafox indicated about a 1·6-fold increase in the LD_{50}; this may be compared with the fourfold increase reported for schradan (O'Brien & Davison, 1958). Table 1 indicates that for dimefox the increase in LD_{50} must be substantially less than twofold.

The compound Lilly 18947 is an inhibitor of barbiturate degradation (Fouts & Brodie, 1956). Since it has been shown that such compounds antagonize schradan poisoning (O'Brien & Davison, 1958), Lilly 18947 was tested in the mouse in the same way as SKF 525A, by using the hydrobromide salt. It was an effective antagonist; 60 mg./kg. protected fully against the standard 25 mg./kg. dose of schradan which alone gave 67% mortality. However, it was less effective than SKF 525A, weight for weight: at 20 mg. of antagonist/kg. the standard schradan dose gave 31% mortality with Lilly 18947, compared with 0% for SKF 525A.

With phosphates and phosphorothiolates, no activation is required; consequently if SKF 525A acts solely by blocking activation, it should have no effect in these cases. This was confirmed with TEPP and paraoxon (Table 1). Tetram was studied because of the report of Scaife & Campbell (1959) that the degradation of this compound was accomplished by liver microsomes and was inhibited *in vitro* by SKF 525A. If this was also true *in vivo*, SKF 525A should synergize Tetram poisoning. This was confirmed (Table 1). Further examinations showed that 20 mg. of SKF 525A/kg. lowered the normal LD_{50} of Tetram (0·55 mg./kg.) to 0·20 mg./kg.

Metabolism. The essential paradox to resolve was: why does SKF 525A antagonize parathion 'activation' by liver homogenates, yet have no saving effect on parathion poisoning? The first point examined was the effect of SKF 525A on [^{32}P]parathion metabolism in the intact mouse. Fig. 1 shows that the production of paraoxon (the actual toxic compound) was substantially increased by SKF 525A *in vivo*, in contrast with the results expected from the report on liver homogenates (Davison, 1955). There was negligible effect on the levels of parathion or degradation products.

A study was then made of the production by liver slices of paraoxon from parathion. Table 2 shows that paraoxon production was substantially inhibited by mM-SKF 525A, the concentration used by Davison. However, Gaudette & Brodie (1959) found that high concentrations (5 mM) of SKF 525A caused non-specific inhibition of microsomal reactions, owing to protein denaturation; at 0·4 mM, this non-specific effect disappeared. The

Table 1. *Effect of SKF 525A on the toxicity of organophosphates to mice*

The appropriate dose was first established approximately with groups of three mice, then duplicate groups of ten mice were used for each dose. If agreement to the nearest 10% was not obtained, further groups of ten were tested until three consecutive groups agreed within 10%. The SKF 525A dose was 20 mg./kg.

	Organophosphate dose (mg./kg.)	Percentage mortality alone	Percentage mortality with SKF 525A
Phosphorothionates			
Chlorthion	140	80	60
Co-ral	24	67	83
Dimethoate	90	70	0
Delnav	55	70	90
Diazinon	15	20	30
Dowco 109	600	90	70
EPN	15	40	50
Ronnel	800	50	50
Thimet	3	30	90
Phosphorodiamidic compounds			
Schradan	25	78	10
Dimefox	1	20	0
Dimefox	2	100	100
Mipafox	30	70	10
Phosphates and phosphorothiolate			
Paraoxon	2·5	60	60
TEPP	6	0	0
TEPP	7	100	100
Tetram	0·45	0	100

Table 2. *Effect of SKF 525A on oxidation of parathion to paraoxon* in vitro

Results are given as total μg. of paraoxon produced by five cockroach guts or 0·5 g. of sliced mouse liver. Each figure is an average from two experiments.

Tissue	Alone	With SKF 525A (mM)	Inhibition (%)	Alone	With SKF 525A (0·4 mM)	Inhibition (%)
Liver slices	4·17	1·28	69	5·33	3·90	27
Whole cockroach guts	6·68	1·93	71	4·93	3·36	32

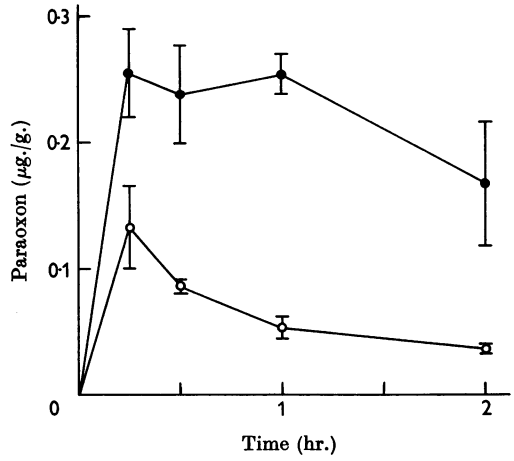

Fig. 1. Production of paraoxon after intraperitoneal injection of parathion (5 mg./kg.) into mice. Each point is an average of two experiments, each with pooled homogenates of three mice. Vertical lines show range. ○, Without SKF 525A; ●, with SKF 525A (20 mg./kg.).

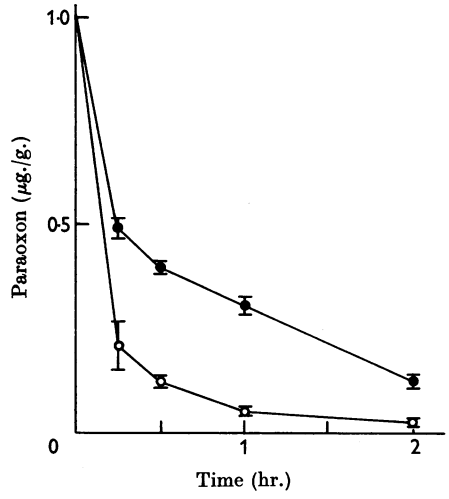

Fig. 2. Degradation of paraoxon after its intraperitoneal injection (at 1 mg./kg.) into mice. Details are as in Fig. 1. Results are corrected for partial recovery.

liver-slice work was therefore repeated with 0·4 mM-SKF 525A; as Table 2 shows, inhibition of paraoxon production was still found. Davison's results are therefore confirmed; but the results *in vivo* and *in vitro* are in conflict.

The only explanation that would resolve the conflict would be that paraoxon degradation *in vivo* was inhibited by SKF 525A. This rather improbable hypothesis was examined by injecting [^{32}P]paraoxon into mice and studying its degradation. As Fig. 2 shows, paraoxon degradation was indeed inhibited by 20 mg. of SKF 525A/kg.

Studies on insects and their tissues

Toxicity. As schradan is not toxic to the cockroach and housefly, it could not be tested in this study.

Against the cockroach, only Guthion, paraoxon, parathion and ronnel were tested. A clear-cut synergism, instead of the expected protection, was found with Guthion: the LD_{50} was lowered by SKF 525A treatment from 2·5 to 0·5 mg./kg. The plots of probit-of-mortality against organophosphate dose were not parallel for the two treatments (i.e. with and without SKF 525A), so that the LD_{85} was slightly increased by the SKF 525A, from 5·0 to 5·5 mg./kg. The toxicity of parathion was unaffected: the LD_{50} at 1 week was 0·9 mg./kg. with or without SKF 525A. However, the agent delayed the toxic effects; after one day, 1 mg. of parathion/kg. killed or prostrated an average of 80 %, but with SKF 525A this figure was reduced to 10 %. The toxicity of paraoxon was synergized: thus 0·5 mg. of paraoxon/kg. killed an average of 20 %, but with SKF 525A this figure was increased to 80 %. With ronnel, slight synergism was found: the LD_{50} was 59 mg./kg. without and 51 mg./kg. with SKF 525A.

The results with the fly are shown in Table 3. For Guthion and Diazinon distinct synergism was found; in the other four cases there was little effect.

Metabolism. The oxidation of [^{32}P]parathion to paraoxon was studied in the living cockroach. As Fig. 3 shows, although SKF 525A inhibited paraoxon formation to a small extent during the first 30 min., yet by 1 hr. it had almost doubled the paraoxon level. This second phase of the effect resembles that found in the mouse (Fig. 1).

Paraoxon production from cockroach gut *in vitro* was next studied, with the two SKF 525A concentrations which had been used for mouse liver. As Table 2 shows, SKF 525A substantially inhibited paraoxon production, to an extent remarkably similar to that found for liver.

Finally the degradation of injected [^{32}P]paraoxon was examined. Fig. 4 shows that paraoxon was degraded less rapidly in the cockroach than in the mouse, and that the degradation was inhibited by 20 mg. of SKF 525A/kg. As in the mouse, this finding accounts for the fact that SKF 525A causes increased concentrations of paraoxon after injection of parathion, in spite of its inhibitory effect on paraoxon synthesis.

In order to study the effects of SKF 525A upon 'activation' of schradan by cockroach guts, schradan was incubated with the guts and samples were taken after (usually) 60 min. for anticholinesterase assay of the 'activation' product. Fig. 5 shows for schradan that activation was roughly linear for 1·75 hr., and also demonstrates the considerable variation with individual guts. Consequently in subsequent work quadruplicate treatments were pooled and duplicate samples then taken for assay.

Table 3. *Effect of SKF 525A on the toxicity of phosphoro-thionates and -dithoates to houseflies*

The procedure was as in Table 1, except that groups of 20 houseflies were used.

Insecticide	LD_{50} alone (mg./kg.)	LD_{50} (mg./kg.) with 10 mg. of SKF 525A/kg.
Co-ral	1–2	1–2
Dimethoate	0·4–0·8	0·4
Diazinon	1	0·5
Dowco 109	Approx. 8	Approx. 6
Guthion	4	2
Ronnel	1	1

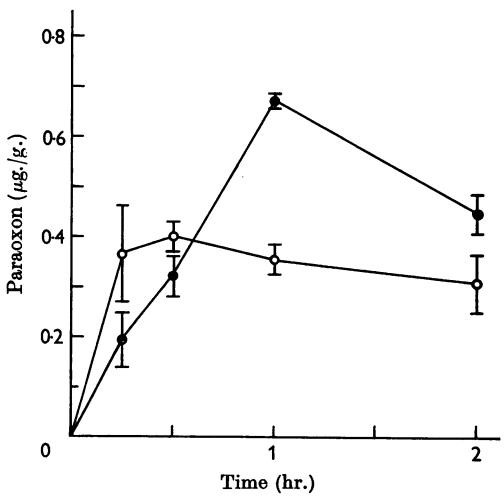

Fig. 3. Production of paraoxon after injection of parathion (5 mg./kg.) into cockroaches. Each point is an average of two experiments, each with a homogenate of five insects. Vertical lines show range. ○, Without SKF 525A; ●, with SKF 525A (20 mg./kg.).

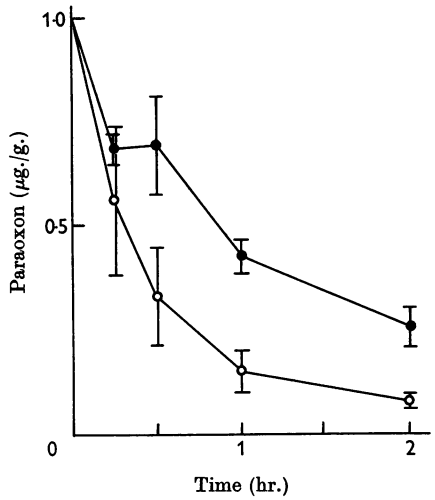

Fig. 4. Degradation of paraoxon after its injection (at 1 mg./kg.) into cockroaches. Each point is an average of three or five experiments, each with a homogenate of five insects. Vertical lines show standard deviation. Results are corrected for partial recovery. ○, Without SKF 525A; ●, with SKF 525A (20 mg./kg.).

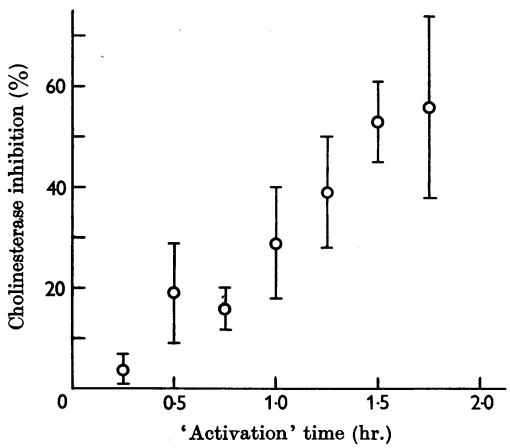

Fig. 5. Effect of period of contact between cockroach gut and schradan *in vitro* on schradan 'activation', as assayed by inhibition of erythrocyte cholinesterase by the activated schradan. Each point is an average of four experiments, each with a single gut. Vertical lines show standard deviation.

Another problem was that SKF 525A has anticholinesterase activity. The I_{50} (concentration for 50% inhibition) was 6·3 μM for human plasma, 0·32 mM for housefly head and 2·5 mM for laked human erythrocytes. The last-named were therefore used subsequently with SKF 525A. With plasma it was shown that inhibition was independent of time of incubation with the plasma, between 20 and 70 min. The inhibition is thus not progressive as with organophosphates, but time-independent.

Table 4 shows that schradan 'activation' by cockroach gut was substantially inhibited by SKF 525A even at 10^{-5} M. Unfortunately the extent of inhibition cannot be quantitatively stated, since no figure is available for the anticholinesterase potency of the 'activation' product, hydroxymethylschradan, against erythrocyte cholinesterase. If it is substantially the same as for plasma cholinesterase (O'Brien & Spencer, 1955), one may calculate that 10^{-5} M-SKF 525A inhibited production of hydroxymethylschradan by about 65%.

DISCUSSION

These findings suggest (but do not prove) that SKF 525A fails to protect against parathion poisoning because it has two contrary actions: it inhibits the production of paraoxon from parathion (as judged by studies *in vitro*) but also inhibits the degradation of paraoxon. The same phenomena are observed in the cockroach and the mouse.

From the limited data available, SKF 525A appears to be an inhibitor of 'activation' of phosphorothionates in general. Yet it sometimes antagonizes, sometimes synergizes and sometimes has no effect upon phosphorothionate poisoning, according to the species and the phosphorothionate that are examined. Presumably this variation reflects variations in the relative potency of its inhibition of activation and degradation in each case.

Table 4. *Effect of SKF 525A on schradan activation by cockroach guts*

The figures were obtained by reference to a control treated with the same level of SKF 525A. The indicated concentration of SKF 525A during incubation with the gut was 2·7 times that in the subsequent incubation with cholinesterase. For details see Methods.

Concn. of SKF 525A (M)	Percentage erythrocyte-cholinesterase inhibition caused by 'activated' schradan
0	59
10^{-3}	14
10^{-4}	23
10^{-5}	29

The only non-metallic inhibitor of a paraoxon-degrading enzyme reported so far is ethylenediaminetetra-acetic acid (Main, 1960). SKF 525A should prove a valuable tool in assessing the importance of paraoxonase and related enzymes, and in distinguishing between the various enzymes that degrade organophosphates. Its use in studies of phosphorothionate activation must clearly be limited to those cases where appropriate degrading enzymes are absent; such conditions have not yet been achieved.

The activating systems of the cockroach and mouse are virtually identical in their sensitivity to SKF 525A (Table 3). The demonstration that the 'activation' of schradan by cockroach gut is inhibited by SKF 525A, as had been shown for 'activation' by mouse liver (Davison, 1955), is a further point of similarity. Nevertheless, there is evidence that the systems are in fact substantially different, particularly in their cofactor requirements when broken-cell preparations are used (O'Brien, 1956).

One feature of this study is the lack of correlation between toxicity and the total body level of paraoxon. For instance, Figs. 1 and 2 show that the agent SKF 525A has a marked effect upon the paraoxon level of mice poisoned either with parathion or paraoxon. Yet the agent has a negligible effect on the toxicity of these organophosphates. This finding throws some doubt on the validity of conclusions about toxicity drawn from observations on whole-body levels of toxic compound, as was extensively done by Krueger *et al.* (1960), and as is implicit in the present study. Instead one should perhaps study the concentration of toxic compound in, say, the nervous system. Compounds of this type are particularly likely to diffuse rapidly into lipoidal tissue, such as the nervous system, by virtue of their high lipid–water partition coefficients. We have observed partition coefficients between triacetin and water of 6900 for parathion, 37 for paraoxon and 39 for malathion.

SUMMARY

1. SKF 525A (2-diethylaminoethyl 2:2-diphenylvalerate hydrochloride) protects mice against poisoning by the three phosphorodiamidic compounds tested. Of the eight phosphorothionates tested, protection against dimethoate only was observed.

2. With houseflies and American cockroaches, SKF 525A gave no protection against any phosphorothionates.

3. SKF 525A inhibited the conversion of parathion into paraoxon by mouse-liver slices or cockroach guts.

4. SKF 525A inhibited the 'activation' of schradan by cockroach guts, as had previously been shown for rat-liver preparations.

5. With mice and cockroaches *in vivo*, SKF 525A increased the concentration of paraoxon caused by the injection of parathion, and reduced the degradation of injected paraoxon.

6. It was concluded that the diverse effects of SKF 525A on organophosphate toxicity in various species were due to the variations in the importance of inhibition of 'activating' as opposed to degrading enzymes.

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REFERENCES

Casida, J. E., Allen, T. C. & Stahmann, M. A. (1954). *J. biol. Chem.* **210**, 607.
Davison, A. N. (1955). *Biochem. J.* **61**, 203.
Fouts, J. R. & Brodie, B. B. (1956). *J. Pharmacol.* **116**, 480.
Gage, J. C. (1953). *Biochem. J.* **54**, 246.
Gaudette, L. E. & Brodie, B. B. (1959). *Biochem. Pharmacol.* **2**, 89.
Heath, D. F., Lane, D. W. J. & Park, P. O. (1955). *Phil. Trans.* B, **239**, 191.
Krueger, H. R., O'Brien, R. D. & Dauterman, W. C. (1960) *J. econ. Ent.* **53**, 25.
Main, A. R. (1960). *Biochem. J.* **74**, 10.
Metcalf, R. L. & March, R. B. (1953). *Ann. ent. Soc. Amer.* **46**, 63.
Murphy, S. D. & DuBois, K. P. (1957). *J. Pharmacol.* **119**, 572.
O'Brien, R. D. (1956). *Canad. J. Biochem. Physiol.* **35**, 45.
O'Brien, R. D. & Davison, A. N. (1958). *Canad. J. Biochem. Physiol.* **36**, 1203.
O'Brien, R. D. & Spencer, E. Y. (1953). *J. agric. Food Chem.* **1**, 946.
O'Brien, R. D. & Spencer, E. Y. (1955). *J. agric. Food Chem.* **3**, 56.
Plapp, F. W. & Casida, J. E. (1958). *J. econ. Ent.* **51**, 800.
Scaife, J. F. & Campbell, D. H. (1959). *Canad. J. Biochem. Physiol.* **37**, 297.
Spencer, E. Y., O'Brien, R. D. & White, R. W. (1957). *J. agric. Food Chem.* **5**, 123.
Vigne, J. P. & Tabau, R. L. (1958). *Bull. Soc. chim. Fr.*, p. 1194.

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Mitochondria of the Ehrlich Ascites-Tumour Cell

2. DIPHOSPHOPYRIDINE NUCLEOTIDE- AND TRIPHOSPHOPYRIDINE NUCLEOTIDE-DEPENDENT OXIDATION OF *ISO*CITRATE*

BY A. O. HAWTREY† AND M. H. SILK‡
Liesbeek Cancer Research Clinic, Rosebank, Cape Town, South Africa

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Studies of oxidative phosphorylation in Ehrlich ascites-cell mitochondria with α-oxoglutarate, L-glutamate, succinate and ascorbate as substrates were reported by Hawtrey & Silk (1960*a*). The present paper describes studies of *iso*citrate oxidation in the same tumour mitochondria and amplifies the findings of a preliminary report (Hawtrey & Silk, 1960*b*).

The mechanism of *iso*citrate oxidation has been investigated in considerable detail in normal-cell mitochondria, but little is known about the pathways which operate in malignant cells.

In rat-liver mitochondria and rat-heart sarcosomes the cofactor of *iso*citric dehydrogenase has been shown to be triphosphopyridine nucleotide (Kaplan, Swartz, Frech & Ciotti, 1956; Purvis, 1958*a*, *b*), although Ernster (1959) maintains that a diphosphopyridine nucleotide-dependent *iso*citric dehydrogenase is also present. Vignais, Vignais & Bartley (1957) have reported P:O ratios for diphosphopyridine nucleotide- and triphosphopyridine nucleotide-dependent *iso*citric dehydrogenases in the presence of intact rat-liver mitochondria, and both diphosphopyridine nucleotide- and triphosphopyridine nucleotide-dependent enzymes have been demonstrated in ox-adrenal mitochondria (Grant & Mongkolkul, 1958).

The cofactor requirement of *iso*citric dehydrogenase in tumour-cell mitochondria does not appear to have been correspondingly well investi-

* Part 1: Hawtrey & Silk (1960*a*).

† Present address: Division of Biochemistry, National Chemical Research Laboratory, C.S.I.R., Pretoria, South Africa.

‡ Present address: S.A. Poliomyelitis Research Foundation, P.O. Box 1038, Johannesburg, South Africa.