THE EFFECT OF PARATHION AND DDT ON CHOLINESTERASE ACTIVITY IN THE ROACH (*PERIPLANETA AMERICANA* L.)

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INTRODUCTION

It is known that several insecticides have a strong inhibitory effect on cholinesterase (ChE) activity, *in vivo* as well as *in vitro* [1,2]. Their toxic action may be at least partially due to this effect, since from experiments carried out by ROEDER, KENNEDY, AND SAMSON[3] and ROEDER[4] we know that inhibition of ChE activity interferes with nerve conduction by facilitating and finally blocking it.

As to the physiological action of 0,0'-diethyl-*p*-nitrophenyl thiophosphate (parathion) only little is known, but the data available render it probable that this action is at least partially based on inhibition of ChE activity. This was pointed out by KARCZMAR[5], HARRESTRUP ANDERSEN AND JERSILD[6], and VAN LUYT[7], who reported that the effect of parathion in the dog and in man shows signs of poisoning with acetylcholine (ACh) brought about by inhibition of ChE. In insects too, parathion seems to inhibit ChE activity; it is reported by METCALF AND MARCH[8] that in the brains of bees dead after poisoning with parathion, ChE activity has completely disappeared. According to these authors ChE from bee brains is also inhibited by parathion *in vitro*. ALDRIDGE[9] reports an inhibiting effect of parathion *in vitro* on the specific ChE from goat blood.

As to the action of 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT), the data available are often contradictory. DDT *in vitro* seems not to affect ChE activity, according to TOBIAS *et al.*[10]. These authors also report unchanged ChE activity in the ventral nerve cord of *Periplaneta* after DDT poisoning, though in the last stages of poisoning a considerable increase in ACh was found. On the other hand, METCALF AND KEARNS[11] suppose DDT to act as an anti-ChE, causing a lowering of synaptic resistance (facilitation). DRESDEN[12] also assumes a facilitating effect of DDT on nerve conduction but denies that this would be brought about by inhibition of ChE.

Some authors have attempted to correlate ChE inhibition and symptoms of poisoning. CHADWICK AND HILL[1] reported a direct relation between ChE inhibition and percentage mortality in studying the effect of several anti-ChE agents on the roach. METCALF AND MARCH[8] correlated strong hyperactivity after parathion poisoning with 48%, total paralysis with 90% inhibition of ChE in the central nervous system of *Musca*

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domestica L. It is, however, very difficult to draw any conclusion from these experiments and observations since from NACHMANSOHN[13] and ROEDER[4] it is known that nerve conduction is not affected at all unless ChE inhibition amounts to about 90%. The suggestions of YEAGER AND MUNSON[14] and of ROEDER AND WEIANT[15] that in the first instance DDT acts on the peripheral nerves led to examination of ChE activity in the peripheral nerves after parathion poisoning.

The present investigation has been carried out in order to ascertain the effect of DDT and parathion on ChE activity and to try to correlate the rate of inhibition of ChE with poisoning symptoms.

MATERIALS AND METHODS

As test animals adult specimens of *Periplaneta americana* L. were used. ChE preparations were obtained from the central nervous system (CNS), *i.e.*, brain and ventral nerve cord, and from meso- and metathoracic leg muscles. Extracts were made from these tissues as indicated in a former report (STEGWEE[16]). In one series of experiments haemolysate from cow erythrocytes was used as a ChE preparation.

ChE determinations were carried out using the manometric Warburg technique. The reagent mixture contained 0.025 *M* sodium bicarbonate, 0.0056 *M* ACh, extract from 15 mg of tissue (or 2 ml of haemolysate), and water to make up a volume of 1.6 ml. When the effect of parathion *in vitro* was studied the reagent mixture contained 0.2 ml of an acetone solution of parathion more or less diluted with water to obtain the required concentration. The standard acetone solution contained $8 \cdot 10^{-3}$ *M* parathion. Because of the high insolubility of parathion in aqueous solutions and of the effect of high concentrations of acetone, the concentration of parathion in the reagent mixture was not made higher than 10^{-5} *M*. The air above the reagent mixture contained 95% N_2 and 5% CO_2, the pH of the mixture thus being 7.4. The reaction temperature was kept constant at 27° C and readings were taken at 5 minute intervals for at least 30 minutes. Carbon dioxide production was plotted against time and the slope of the curve was determined mathematically using the method of the least squares. ChE activity was expressed as b_{30}, *i.e.*, the amount of CO_2 in μl evolved in 30 minutes by the quantity of enzyme present in 100 mg of tissue, the course of CO_2 production being linear.

The insecticides used were:

DDT: pure or as Shell Arkotine dust, containing 5% of technical DDT, both kindly provided by Dr S. LEEFMANS of the Laboratory of Applied Entomology.

Parathion: purified up to 99.5% (melting point 3° C), prepared by Mr H. R. GERSMANN and kindly provided by Professor J. A. A. KETELAAR of the Laboratory of General and Inorganic Chemistry.

These insecticides were applied in the following ways:

DDT by putting the animals in a tube of which the inner walls had been covered with a thin layer of DDT dust or by injecting intra-abdominally 50 μg of DDT (in rapeseed oil) per gram body weight.

Parathion by allowing the animals to walk on filter paper containing the residue of an acetone solution of parathion from which the acetone had been evaporated; or by injecting intra-abdominally 2 μg of parathion (in an aqueous 0.125% acetone solution) per gram body weight.

EXPERIMENTAL

1. *Effect of parathion on ChE activity in vitro*

To study the effect of parathion on ChE activity *in vitro* the enzyme was left in contact with parathion solution for one hour before adding the substrate. To eliminate the effect of acetone, blanks were run with acetone only.

The first series of experiments concerned the effect of parathion on ChE from the CNS of *Periplaneta*. As shown in Table I, parathion in concentrations varying from 10^{-5} to 10^{-8} *M* did not affect the ChE.

As it was not *a priori* impossible that at other substrate concentrations the ChE might be affected by parathion, some experiments were carried out with varying

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substrate concentrations, the parathion concentration being 10^{-6} *M*. The results are shown in Table II. It appears, that neither at lower nor at higher substrate concentrations is the ChE activity inhibited.

TABLE I

THE EFFECT OF PARATHION ON ChE FROM THE CNS OF *Periplaneta in vitro*

Substrate	$C_{parathion}$	b_{30}	% inhibition
ACh 0.0056 *M*	—	372	—
ACh 0.0056 *M*	10^{-5} *M*	378	0
ACh 0.0056 *M*	10^{-6} *M*	381	0
ACh 0.0056 *M*	10^{-7} *M*	368	0
ACh 0.0056 *M*	10^{-8} *M*	372	0

TABLE II

EFFECT OF PARATHION ON ChE ACTIVITY AT VARIOUS SUBSTRATE CONCENTRATIONS

C_{ACh}	b_{30}		% inhibition
	normal	with 10^{-6} *M* parathion	
0.125 *M*	171	171	0
0.025 *M*	240	234	0
0.005 *M*	382	382	0
0.0005 *M*	158	161	0

As for the effect of parathion on the specific ChE from cow erythrocytes, it appeared that this ChE too was not affected at all by 10^{-6} *M* parathion, which concentration is stated by some authors to bring about a 50% inhibition. It seemed of little importance whether the acetone concentration was high or low (the parathion possibly being more or less well dissolved). Table III gives the results of the experiments. It is evident that any inhibition is due to the effect of acetone and not of parathion.

TABLE III

THE EFFECT OF PARATHION IN AN AQUEOUS ACETONE SOLUTION ON THE ChE ACTIVITY OF 0.2 ml OF HAEMOLYSATE FROM COW ERYTHROCYTES

Substrate	$C_{parathion}$	$C_{acetone}$	$\mu l\ CO_2/30$ min	% inhibition
0.0056 *M* ACh	—	—	66	—
0.0056 *M* ACh	10^{-6} *M*	0.0125%	64	3
0.0056 *M* ACh	—	—	80	—
0.0056 *M* ACh	10^{-6} *M*	0.0125%	77	4
0.0056 *M* ACh	10^{-6} *M*	12.5%	48	40
0.0056 *M* ACh	—	0.0125%	76	5
0.0056 *M* ACh	—	12.5%	45	45
0.0056 *M* ACh	—	—	66	—
0.0056 *M* ACh	10^{-6} *M*	12.5%	39	40
0.0056 *M* ACh	—	12.5%	42	37

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2. Effect of parathion and DDT on ChE activity in vivo

The reason for comparing the effects of parathion and DDT was the striking resemblance of the outward symptoms of poisoning. These symptoms consist of strong initial hyperactivity and especially hypersensitivity to external stimuli, gradually followed by marked tremors of legs, wings, and finally the whole body, spasms (often accompanied by vomiting), and loss of equilibrium, until at last total paralysis occurs and the animal dies. These symptoms are also similar to those brought about by eserine, as is pointed out by TOBIAS *et al.*[10], so that it would be interesting to know whether the ChE is affected or not after DDT or parathion.

Applying DDT as a contact poison, in the last stage of poisoning, when the animals were almost completely paralyzed, it was found that ChE activity in the CNS had almost entirely disappeared.

Parathion, used as a contact poison, had the same effect as DDT. When injected it also caused a complete disappearance of ChE activity in the CNS.

When, however, DDT was administered by injection no decrease of ChE could be found, although clear symptoms of poisoning were visible. These rather surprising results will be discussed later on.

Table IV gives the data from the above experiments.

TABLE IV

EFFECT OF DDT AND PARATHION (AS CONTACT POISONS AND INJECTED) ON ChE ACTIVITY IN THE CNS OF THE ROACH

	Normal b_{30}	$DDT_{cont.}$		$DDT_{inj.}$		$Par._{cont.}$		$Par._{inj.}$	
		b_{30}	% inh.	b_{30}	% inh.	b_{30}	% inh.	b_{30}	% inh.
1	350	0	100	—	—	0	100	0	100
2	350	20	94	—	—	0	100	0	100
3	330	0	100	—	—	0	100	0	100
4	194	—	—	194	0	—	—	—	—
5	200	—	—	193	0	—	—	—	—
6	214	—	—	208	0	—	—	—	—

As it was suggested by LAUGER *et al.*[17] that narcotics had a therapeutic effect on DDT-poisoned rats, the effect of CO_2 narcosis on DDT and parathion-poisoned roaches was examined. For this purpose the animals were injected with DDT in rapeseed oil or entirely powdered with DDT dust and kept under CO_2 narcosis during poisoning. In the case of parathion this substance was injected or the animals were kept on a piece of filter paper covered with the residue of an acetone solution of parathion. The results of these experiments are shown in Table V.

It should be noted that the low values for b_{30} found in the experiments 4, 5, and 6 from Table IV and 3 and 4 from Table V are probably due to seasonal variations in the ChE content, since these experiments were carried out in the winter, whereas all the other experiments were done in the summer.

Table VI shows the results of experiments carried out in order to determine the ChE activity in the CNS and in peripheral nerves (those in the legs) from animals that had reached the hyperactive stage after parathion poisoning. To determine ChE in the

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peripheral nerves extracts were made from whole leg muscles. In the first experiments it was observed that in parathion poisoned animals that did not yet show any symptoms, the leg muscles had lost some 50% of their ChE activity, ChE activity in the CNS still having its normal value. The following experiments demonstrated that in the hyperactive stage of poisoning ChE inhibition in the leg muscles is much higher than in the CNS.

TABLE V

EFFECT OF CO_2 NARCOSIS ON ChE INHIBITION BY DDT AND PARATHION (AS CONTACT POISONS AND INJECTED) IN THE CNS OF THE ROACH

	Normal b_{30}	$DDT_{cont.}$		$DDT_{cont.}+CO_2$		$DDT_{inj.}$		$DDT_{inj.}+CO_2$	
		b_{30}	% inh.	b_{30}	% inh.	b_{30}	% inh.	b_{30}	% inh.
1	350	0	100	360	0	—	—	—	—
2	350	48	86	340	0	—	—	—	—
3	200	—	—	—	—	193	0	197	0
4	214	—	—	—	—	208	0	214	0
		$Par._{cont.}$		$Par._{cont.}+CO_2$		$Par._{inj.}$		$Par._{inj.}+CO_2$	
5	350	0	100	0	100	0	100	0	100
6	350	0	100	0	100	0	100	90	70
7	330	—	—	—	—	0	100	0	100

TABLE VI

ChE ACTIVITY IN THE CNS AND IN LEG MUSCLES OF *Periplaneta* IN THE HYPERACTIVE STAGE AFTER PARATHION POISONING

Central nervous system			Normal b_{30}	Leg muscle Hyperactive b_{30}	% inh.
Normal b_{30}	Hyperactive b_{30}	% inh.			
380	380	0	24	12	50
350	170	52	—	—	—
350	90	74	30	3	90
350	160	54	30	3	90
350	160	54	25	3	88
—	—	—	25	3	88
—	—	—	30	—	—

DISCUSSION

Experiments have demonstrated that *in vitro* parathion does not inhibit ChE from the CNS of *Periplaneta* or from cow erythrocytes. These results are in flat contradiction to those obtained by METCALF AND MARCH[8] and ALDRIDGE[9]. It is not very likely that this contradiction is due to the use of different test animals or different methods, for when we worked with TEPP (tetraethylpyrophosphate) our results confirmed absolutely the findings of METCALF AND MARCH. Probably the degree of purity of the parathion used has to be taken seriously into account. Our parathion had a very high degree of

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purity. The parathion used by METCALF AND MARCH was a technical product (possibly containing impurities), while ALDRIDGE does not mention any physical constant or degree of purity of his material. In a foot-note this author admits that his E 605 contains isomers. Besides, it seems to be uncertain whether E 605 is really identical with parathion, since KETELAAR[18] demonstrated that the German E 605, up to then considered to be the ethyl compound, was in fact the dimethyl compound*.

As for the effect of parathion *in vivo*, the experiments with injected parathion and with parathion as a contact poison entirely confirm the results obtained by METCALF AND MARCH[8] and KARCZMAR[5].

The experiments with injected DDT showed similar results to those obtained by TOBIAS *et al.*[10], who also reported unchanged ChE activity in the CNS of the roach after DDT injection. When, however, DDT was used as a contact poison, poisoning symptoms developed much sooner and ChE appeared to be inhibited to a large extent. The question of whether, in the case of contact, DDT is taken up by the nerve endings (and transported through the nerves, as suggested by ECKART[19], the nerves being otherwise impermeable to DDT, is under investigation, but cannot be answered at present.

As for the effect of CO_2 narcosis on the action of DDT as a contact poison, no satisfactory explanation has been found. It does not seem very probable that the DDT uptake through the cuticula is inhibited by CO_2, since after the narcosis the animals showed clear symptoms of poisoning, as was demonstrated with control animals. Perhaps the above-mentioned transport through the nerves is blocked by narcosis, but to verify this we need to know much more about the action of CO_2 on nerves than we do now.

It seems rather difficult to correlate the symptoms of parathion poisoning with the rate of ChE inhibition. In the introduction it has already been pointed out that conduction in the CNS is not affected by inhibition of ChE unless this inhibition amounts to at least 90%. Thus, hyperactivity cannot be explained by assuming a partial inhibition of the ChE in the CNS, as METCALF AND MARCH did. This fact, in connection with some of the symptoms of poisoning during the hyperactive stage (such as strong hypersensitivity to external stimuli), gave rise to the supposition that the cause of hyperactivity might be found in the peripheral nerves, myoneural junctions or sense organs. The experiments have demonstrated that during hyperactivity after injection of parathion, when in the CNS 26–48% of the normal ChE activity is still present, ChE in the leg muscles, including the peripheral nerves, is inhibited for about 89%. Because of the low ChE content of the muscles this inhibition may be sufficient to produce one or more of the following effects, that may be regarded as responsible for hyperactivity: (a) improved afferent conduction, (b) improved efferent conduction, (c) improved myoneural transmission, and (d) increased sensitivity of sense organs. Which of these effects are produced is still uncertain. According to ROEDER AND WEIANT[15] DDT in low concentrations produces effect (a) and possibly also (d). If these effects are due to inhibition of ChE (the anti-ChE effect of DDT has been demonstrated in this report), parathion possibly acts in the same way*.

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* *Note added in proof.* Recently DIGGLE AND GAGE (*Biochem. J.*, 48 (1951) XXV) have shown that ChE inhibition by parathion is directly proportional to the S-ethyl isomer content.

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SUMMARY

1. *In vitro*, parathion does not inhibit cholinesterase from the central nervous system of *Periplaneta americana* L. or from cow erythrocytes. Injected parathion causes in *Periplaneta* a strong inhibition of cholinesterase. In the course of poisoning, the cholinesterase in the leg muscles, including the sensory organs and nerves, is first affected. Arguments are put forward to suggest that the hyperactive stage of parathion poisoning is due to improved peripheral conduction.

2. DDT as a contact poison causes inhibition of cholinesterase in *Periplaneta*. This effect is abolished by CO_2 narcosis. Injected DDT does not affect ChE activity.

RÉSUMÉ

1. *In vitro* le parathion n'inhibe pas la cholinestérase du système nerveux central de *Periplaneta americana* L. ou d'érythrocytes de vache. Sous forme d'injection le parathion provoque une forte inhibition de cholinestérase. Au cours de l'intoxication la cholinestérase du muscle de la jambe, de même que les organes sensoriels et les nerfs est affectée la première. Nous avons avancé des arguments en faveur de l'idée que la phase hyperactive de l'empoisonnement par le parathion serait due à une meilleure conduction périphérique.

2. Comme poison de contact le DDT inhibe la cholinestérase de *Periplaneta*. Cette action est abolie par la narcose au CO_2. Sous forme d'injection le DDT n'affecte pas l'activité cholinestérasique.

ZUSAMMENFASSUNG

1. *In vitro* übt Parathion keine hemmende Wirkung auf die Cholinesterase des Zentralnervensystems von *Periplaneta americana* L. oder von Erythrocyten (Kuh) aus. Wird Parathion injiziert, so hemmt es stark die Cholinesterase von *Periplaneta*. Im Laufe der Vergiftung wird die Cholinesterase der Beinmuskeln, der Sinnesorgane und Nerven zuerst angegriffen. Es werden Argumente angeführt, welche darauf hinweisen, dass das hyperaktive Stadium der Parathionvergiftung auf eine verbesserte peripherische Konduktion zurückgeführt werden könnte.

2. Als Kontaktgift hemmt DDT *Periplaneta*-Cholinesterase. Dieser Effekt wir durch CO_2-Narkose wettgemacht. Injiziertes DDT hat keine Wirkung auf die Cholinesterase-Aktivität.

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