THE EFFECT OF SOME ORGANOPHOSPHORUS AND CHLORINATED HYDROCARBON INSECTICIDES ON THE TOXICITY OF SEVERAL MUSCLE RELAXANTS

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Several investigators[1–14] have shown that certain insecticides, particularly organophosphorus and possibly chlorinated hydrocarbon compounds, are inhibitors of cholinesterases both *in vivo* and *in vitro*. It has been shown that the levels of cholinesterase in the body determine the rate of destruction of at least one muscle relaxant[15,16], and thus low levels of cholinesterase might result in a prolongation of action and enhancement of toxicity of some relaxants. The following experiments were performed to determine the effects of acute and chronic exposure of rats to some insecticides on the toxicity of representative muscle relaxants.

Experimental

Part I. Effect of Acute Exposure to Insecticides

Methods

The insecticides parathion and malathion were chosen as representatives of the organophosphorus group and aldrin as a representative of the chlorinated hydrocarbon group. Malathion and parathion were administered to male and female Wistar rats by intraperitoneal injection, and aldrin was administered by stomach tube. Doses of the insecticides corresponding to approximately ¼ of the LD50 were given as follows: aldrin—25 mg./kg., malathion—1 ml./kg. and parathion (diluted 1:500)

TABLE I

Muscle relaxants used

Muscle relaxant	Vehicle	Route of administration
Suxamethonium chloride	water	intramuscular
Mephenesin	propylene glycol, 40 per cent	intraperitoneal
Gallamine triethiodide	water	intraperitoneal
Benzimidazole	ethanol, 95 per cent	intramuscular
Tubocurarine chloride	water	intramuscular
Decamethonium iodide	water	intraperitoneal

—1 ml./kg. Parathion and aldrin were dissolved in peanut oil and corresponding control groups received peanut oil only. Malathion was obtained in two strengths, both in liquid form: 95 per cent malathion and commercial malathion containing 57 per cent of the 95 per cent product. There was no appreciable difference volume for volume in the toxicity of the two malathion preparations. The parathion preparation was also obtained in liquid form and contained 97·76 per cent of parathion.

After pretreatment with the insecticides, the rats received the muscle relaxants by injection eighteen to twenty-four hours later. Table I

shows the relaxants used in this study, the vehicle and the route of administration.

Three to five different doses of each relaxant were administered to groups of ten to twenty rats in both the pretreated and control groups. The per cent mortalities were converted to probits and plotted against the logarithm of the dose of muscle relaxant. The resultant linear dose responses were analysed statistically by methods proposed by Bliss[17,18], and Miller, Bliss and Braun[19], and the final results were recorded in the following Tables as toxicity of the relaxant to the pretreated rats in terms of the controls, expressed as a percentage. The results were considered significant when $P < 0.05$.

TABLE II

THE EFFECT OF PARATHION PRETREATMENT ON THE TOXICITY OF MUSCLE RELAXANTS

Muscle relaxant	Sex of rats	Pre-treated or control	Dose of relaxant (mg./kg.)	Mortality (per cent)	Toxicity to pretreated rats in terms of controls (per cent ± S.E.M.)	S* or NS
Suxamethonium chloride	F	P	1·96 2·26 2·61	60·0 53·3 71·4	141·0 ± 12·1	S
		C	2·61 3·00 3·45	26·7 40·0 93·5		
	M	P	1·70 1·96 2·26	53·4 73·3 93·0	136·0 ± 7·0	S
		C	2·26 2·61 3·00	33·3 80·0 93·3		
Gallamine triethiodide	F	P	15·1 17·0 19·0 21·4	20·0 8·3 40·0 38·5	85·4 ± 8·3	NS
		C	17·0 19·0 21·4	25·0 66·6 66·6		
	M	P	17·0 18·0 19·0 21·4	18·2 33·3 91·0 91·0	90·3 ± 4·7	NS
		C	13·5 15·1 17·0 21·4	8·3 54·6 63·6 81·8		

* Significant or not significant from 100 per cent.

Results

Table II shows the effect of pretreatment of male and female rats with parathion followed by suxamethonium chloride or gallamine triethiodide.

There was a significant increase in the toxicity of suxamethonium to rats pretreated with parathion, but no increase in the toxicity of gallamine to the pretreated animals. There was no apparent difference in response between male and female rats. Table III demonstrates the effect on rats of pretreatment with malathion followed by the administration of several muscle relaxants.

The increase in toxicity of the relaxants to the pretreated rats was particularly striking with suxamethonium. There were also significant increases in the toxicity of mephenesin and gallamine, and a decrease in the toxicity of benzimidazole to the rats pretreated with malathion. There was no change in the toxicity of decamethonium and tubocurarine.

While parathion and malathion are well-known inhibitors of cholinesterases, aldrin has been reported to exert pharmacological activity suggestive of an anticholinesterase compound[10]. Table IV shows the effect of pretreatment of rats with aldrin on the toxicity of several muscle relaxants.

As before, suxamethonium showed the most marked increase in toxicity to the pretreated animals. This increase appeared to be more marked

in the male rats, although the difference between males and females was not significant. Gallamine and decamethonium also showed increased toxicity to the pretreated rats, but the significance of the increase was small in the case of decamethonium.

TABLE III

THE EFFECT OF MALATHION PRETREATMENT ON THE TOXICITY OF MUSCLE RELAXANTS

Muscle relaxant	Sex of rats	Pre-treated or control	Dose of relaxant (mg./kg.)	Mortality (per cent)	Toxicity to pretreated rats in terms of controls (per cent ± S.E.M.)	S* or NS
Suxamethonium chloride	F	P	1·43 1·70 1·96	46·7 80·0 85·7	169·6 ± 10·0	S
		C	2·26 2·61 3·00	33·3 40·0 100·0		
	M	P	1·43 1·70 1·96	66·7 86·7 86·7	189·2 ± 15·9	S
		C	2·26 2·61 3·00	13·3 53·3 86·7		
Mephenesin	M	P	290 325 364	13·6 54·5 72·7	132·3 ± 9·0	S
		C	364 436 488	27·3 45·4 54·5		
Gallamine triethiodide	F	P	10·8 12·1 13·6 15·2	9·1 63·7 54·5 63·7	118·4 ± 7·4	S
		C	13·6 15·2 17·0 19·0	27·3 45·4 63·7 90·9		
	M	P	15·2 17·0 19·0	18·2 59·1 72·7	119·5 ± 7·4	S
		C	17·0 19·0 21·2 23·7	18·2 36·4 63·6 81·8		
Benzimidazole	F	P	394 442 496 556	9·0 0·0 40·0 63·6	78·0 ± 7·0	S
		C	394 442 496 556	45·4 70·0 80·0 72·7		
Decamethonium iodide	M	P	3·0 3·4 3·8 4·3	35·0 50·0 55·6 70·0	99·2 ± 7·6	NS
		C	3·4 3·8 4·3	30·0 80·0 80·0		
Tubocurarine chloride	M	P	0·300 0·330 0·363 0·400	15·0 30·0 40·0 90·0	100·4 ± 3·5	NS
		C	0·300 0·330 0·363 0·400	5·0 40·0 60·0 80·0		

* Significant or not significant from 100 per cent.

TABLE IV

THE EFFECT OF ALDRIN PRETREATMENT ON THE TOXICITY OF MUSCLE RELAXANTS

Muscle relaxant	Sex of rats	Pre-treated or control	Dose of relaxant (mg./kg.)	Mortality (per cent)	Toxicity to pretreated rats in terms of controls (per cent ± S.E.M.)	S* or NS
Suxamethonium chloride	F	P	2·26 2·61 3·00	33·3 66·7 93·4	111·5 ± 5·8	S
		C	2·26 2·61 3·00	20·0 40·0 73·2		
	M	P	1·76 2·26 2·61	60·0 86·7 93·4	141·8 ± 11·7	S
		C	2·26 2·61 3·00	26·7 53·3 73·3		
Mephenesin	F	P	286 321 360	13·3 66·7 73·3	102·1 ± 3·3	NS
		C	300 321 360	6·6 46·6 93·4		
	M	P	272 305 385 432	40·0 60·0 70·0 73·7	107·1 ± 28·4	NS
		C	272 305 385 432 485	50·0 65·0 50·0 50·0 80·0		
Gallamine triethiodide	F	P	12·1 13·6 15·2	26·7 73·3 73·3	118·1 ± 6·2	S
		C	15·2 17·0 19·0	46·6 73·3 86·7		
	M	P	15·2 17·0 19·0	33·3 60·0 85·8	123·8 ± 7·4	S
		C	17·0 19·0 21·2	21·4 46·7 53·3		
Benzimidazole	F	P	413 467 525 594	0·0 18·2 54·5 45·4	78·4 ± 10·1	NS
		C	467 525 594 670	54·5 72·7 91·0 100·0		
Decamethonium iodide	M	P	3·0 3·4 3·8	40·0 65·0 70·0	121·6 ± 10·4	S
		C	3·0 3·4 3·8 4·3	20·0 40·0 50·0 60·0		

* Significant or not significant from 100 per cent.

TABLE V

GROWTH OF MALE RATS ON A DIET CONTAINING MALATHION

Treatment	No. of days on test	No. of rats on test	Body weight mean ± S.E.M. (g.) Initial	Final	Gain	Food consumption (g./rat/day)	Food efficiency (g. gain/ g. food consumed × 100)
Control diet ..	31	60*	107·5 ± 1·7	188·4 ± 1·8†	80·9	14·7	17·7
Control diet + malathion (500 p.p.m.) ..	31	60*	107·0 ± 1·5	196·0 ± 1·9†	89·0	14·4	19·9

* In 4 groups of 15 each. † $t = 2{\cdot}90$; $P < 0{\cdot}01$.

There was some indication of a decrease in the toxicity of benzimidazole, but the difference was not significant. There was no increase in the toxicity of mephenesin to the rats pretreated with aldrin.

Part II. Effect of Chronic Exposure to an Insecticide

Methods

Sixty male rats were divided into four equal groups, and fed a proprietary brand of laboratory diet. A further sixty male rats were similarly divided and fed a similar diet containing in addition 500 parts per million of malathion. All of the animals received water *ad libitum* and their respective diets for a period of thirty-one days. The rats were weighed once weekly, and the amount of food consumed in this time was recorded.

TABLE VI

THE TOXICITY OF SUXAMETHONIUM CHLORIDE TO RATS ON A DIET CONTAINING MALATHION

Pretreatment	Sex of rats	No. of rats per dose	Dose of relaxant (mg./kg.)				Mortality (per cent)				Toxicity to pretreated rats in terms of controls (per cent ± S.E.M.)	S* or NS
Control diet + malathion (500 p.p.m.) ..	M	15	2·05	2·30	2·60	3·00	26·7	20·0	40·0	46·7	98·0 ± 7·7	NS
Control diet ..	M	15	2·05	2·30	2·60	3·00	13·3	20·0	33·3	73·4		

* Significant or not significant from 100 per cent.

The diet containing malathion was prepared by dissolving an appropriate amount of 57 per cent commercial malathion in corn oil and mixing this solution thoroughly with the diet. The malathion in the diet constituted 500 p.p.m. of 95 per cent technical malathion, which is present in the commercial product at a concentration of 57 per cent. The corn oil used in this diet amounted to 5 per cent of the total. No corn oil was added to the control diet.

After thirty-one days on these diets, all rats received graded doses of suxamethonium by intramuscular injection. The deaths that occurred within one hour were recorded, and the data again treated statistically.

Results

Table V shows the effect of malathion on the body weights and food consumption of the experimental animals, and Table VI shows the effect of feeding malathion to rats on the toxicity of suxamethonium.

The rats that received the diet containing malathion were significantly heavier at the end of the experimental period than the rats that received the control diet; however, the food consumption was equal in both groups, resulting in an increased food efficiency for the group that received the insecticide. It is very likely that the increase in body weight was due to the corn oil in the diet and not to the malathion; however, it is interesting to note that Ball, Kay and Sinclair[20] reported an increase in weight in rats fed aldrin, although in this case there was an increase in food consumed.

Chronic feeding of 500 p.p.m. of malathion to the rats did not increase the toxicity of suxamethonium. This particular muscle relaxant was chosen, since it was shown previously (Table III) that a single injection of malathion greatly increased the toxicity of this relaxant to male rats.

Discussion

The administration of sub-lethal doses of parathion, malathion and aldrin to rats increased the toxicity of some muscle relaxants to these animals, presumably due to a lowering of cholinesterase levels by the insecticides. Davison[3] presented evidence that the organophosphorus compounds phosphorylate both true and pseudocholinesterase. He suggested that these compounds combine with an amino group of pseudocholinesterase, but with some other group in true cholinesterase. Fraser[15] reported that suxamethonium was hydrolysed *in vitro* by pseudocholinesterase, but not by true cholinesterase; however, he suggested that *in vivo* there are factors other than the plasma levels of pseudocholinesterase that determine the final effect of suxamethonium on the muscle. The deaths observed were due to cessation of respiration, and any reduction of cholinesterase levels by the insecticides would enhance the paralysing properties of suxamethonium, by decreasing the rate of destruction of the latter.

While only indirect evidence[10] has been given that aldrin is a cholinesterase inhibitor, these experiments with aldrin and the muscle relaxants agree with this evidence.

Pure parathion does not have anticholinesterase activity *in vitro*[6,7], but is converted to a compound having this activity when administered *in vivo*[4,7,8]. Gage[8] reported that an extract of liver, from rats treated with parathion, contained a substance identical with paraoxan by chromatographic analysis. The total amount of active substance extracted from the rat liver was about 0·1 per cent of the parathion administered. Other organophosphorus compounds, such as tetraethyl pyrophosphate, have anticholinesterase activity without undergoing conversion *in vivo*[4]. The authors have not seen any evidence for conversion of malathion to another active anticholinesterase compound.

The organophosphorus compounds are gradually destroyed by enzymes in the body. Aldridge[21] reported that rabbit serum contains an esterase

that hydrolyses diethyl *p*-nitrophenyl phosphate (E 600) rapidly, while rat serum hydrolyses it slowly. This might be a possible explanation for the prolonged action of these compounds in rats, although the rate of recovery of cholinesterases must also be taken into consideration.

Lucas and Miles[16] performed experiments similar to those described herein. Sarin, an organophosphorus compound, increased the period of respiratory paralysis in monkeys induced by suxamethonium, and decreased the periods of respiratory paralysis induced by tubocurarine and gallamine[16]. The latter observations might be attributed to a lowering of the true cholinesterase levels. Sarin had no effect on the respiratory paralysis produced by decamethonium. Lucas and Miles[16] administered Sarin at a level of 2/3 of the LD50, whereas, in the experiments in this paper the insecticide was administered at a level of approximately $\frac{1}{4}$ of the LD50. This latter dose of insecticide is considerably less than the former; therefore, it is possible that only the pseudocholinesterase levels were lowered appreciably, and true cholinesterase levels were not affected. Hazleton[11] has reported that plasma cholinesterase and red blood cell cholinesterase (true cholinesterase) are affected by different amounts of insecticide, depending on the insecticide chosen. This might explain the discrepancy in results obtained in these two laboratories with tubocurarine and gallamine.

In the present series of acute experiments, there was some discrepancy between the toxicity of a muscle relaxant to rats pretreated with different insecticides. For example, the toxicity of gallamine was increased by pretreatment of the animals with malathion and aldrin, but not by pretreatment with parathion, and mephenesin was more toxic to rats pretreated with malathion than those pretreated with aldrin. A possible explanation is that cholinesterase levels were not lowered to the same extent by the three insecticides, even though they were given at a dose in the same ratio to the LD50. It is also very likely that other cholinesterases in the body, as well as other enzyme systems may be adversely affected to a different extent by these insecticides. Denny and Hagerman[22] extracted a cholinesterase from human leucocytes that behaved in a similar fashion to the true cholinesterase of red blood cells and brain. The direct action of the cholinesterases and other enzymes on the muscle relaxants is not too well known except in the case of suxamethonium[15].

The toxicity of benzimidazole was significantly reduced in rats pretreated with malathion but not in those pretreated with aldrin. No explanation is evident at present for this observation.

Chronic feeding of 500 p.p.m. of malathion to rats did not increase the toxicity of suxamethonium to the experimental animals. Hazleton[11] reported that chronic feeding of malathion to rats for two years at a level of 100 p.p.m. had no effect on cholinesterase levels of plasma, red cells and brain, but at 1000 p.p.m. these levels were moderately depressed. Therefore, it was felt that a level of 500 p.p.m. might have a slight effect on cholinesterase levels. However, in this experiment, this level of malathion was apparently not sufficient to increase the toxicity of this relaxant.

There is the possibility that excessive exposure to these insecticides,

sometimes experienced by spray applicators and formulators of the insecticides might be sufficient to augment the toxicity of some of the muscle relaxants, should they be used on the individual. Lucas and Miles[16] have warned of this possibility, and Hansson[23] has also warned of the possible hazard to farm animals exposed to a sprayed field, and subsequently treated in surgery with a relaxant. Gage[9] has suggested that a decrease in cholinesterase in man of more than 40 per cent of the population average should be regarded as a reason for taking appropriate action.

Summary

1. Parathion, malathion and aldrin were administered to rats in sublethal doses, followed by graded doses of various muscle relaxants eighteen to twenty-four hours later. It was found that pretreatment with these insecticides increased the toxicity of some of the relaxants to the animals, had no effect on the toxicity of some, and apparently decreased the toxicity of one relaxant.

2. The change in toxicity of the relaxants was believed to be due to lowered levels of cholinesterases and possibly other enzymes in the rats when pretreated with these insecticides.

3. Feeding malathion at a level of 500 p.p.m. in the diet to rats for thirty-one days had no effect on the toxicity of suxamethonium chloride to these animals.

4. The possible hazard of insecticide-relaxant interaction to humans and farm animals has been discussed.

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