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THE ACUTE TOXICITY OF DICHLOROALKYL ARYL PHOSPHATES IN RELATION TO CHEMICAL STRUCTURE

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Abstract—The acute toxicity of di-alkyl 3-chloro-2-methyl coumarin-7-yl phosphates varies with basic group structure in the order $(EtO)_2P(O)$ > $(EtO)_2P(S)$ > $(Bu^nO)_2P(O)$ > $(PR^nO)_2P(O)$ > (4-chlorobutyl-*O*)$_2$P(O) > (3-chloropropyl-*O*)$_2$P(O) > (2-chloropropyl)$_2$P(O) > (2-chloroethyl)$_2$P(O) > (2, 3-dichloropropyl-*O*)$_2$P(O). A similar order was obtained with *p*-nitrophenyl esters. Poor correlation between rates of hydrolysis and inhibition of acetylcholinesterase suggests that the electronic effects of these structural variations on affinity for cholinesterase are outweighed by other considerations. Increased susceptibility to hydrolysis consequent upon chlorine substitution did not completely account for the low toxicity of the di-(2-chloroethyl) coumarin ester, which hydrolysed faster than the other members of the series.

Chlorine substitution increases the reactivation rate of phosphorylated calf erythrocyte acetylcholinesterase. This effect is greatest when the substitution is close to the phosphorus atom. The conversion of unstable phosphorylated enzyme to a stable form is also accelerated in the propyl compounds, particularly when the substitution is terminal. However, ageing rates following inhibition by butyl and 4-chlorobutyl compounds are essentially similar. These properties are shown to be relevant *in vitro* by comparing the recovery of brain acetylcholinesterase activity in poisoned chicks with the success of 2PAM therapy.

The potencies of the coumarin phosphates were assessed by incubating them with acetylcholinesterase under standard conditions. The order of potencies corresponded well with the order of toxicity with the exception of ethyl and 2-chloroethyl compounds.

THE DISCOVERY of a new class of anthelmintics was reported by Brown *et al.*[3] and one of these, di-(2-chloroethyl) (3-chloro-4-methyl-coumarin-7-yl) phosphate, is known as haloxon. Lee and Hodsden[8] and Lee[7] produced convincing evidence that the favourable therapeutic index of haloxon and of several other di-(2-chloroethyl) aryl phosphates stemmed from the differing reactions between these compounds and the cholinesterases of sheep and several worm species. The low acute toxicity to sheep arose, at least partially, from high rates of reactivation of inhibited cholinesterase.

The purposes of this communication are to examine the reactions of certain other di-chloroalkyl aryl phosphates with esterases, particularly with acetylcholinesterase, and to relate these, as far as possible, to the acute toxicity of the compounds to a mammal and a bird.

MATERIALS AND METHODS

Chemical

The materials used throughout the work are shown in Table 1 together with their structures. They will be referred to, henceforward, by code numbers or common names only.

Biological

Female Sprague–Dawley rats were used in these experiments. The test materials were dissolved in polyethylene glycol 200 or glycerol formal and they were dosed by oral catheterization.

The hens were, for the most part, Rhode Island Reds aged 2 yr, but on the occasions indicated in the text, Thornber '606' hens of a similar age were employed. The cockerel chicks used for experiments on oxime therapy were also '606' strain. Polyethylene glycol 200 was used to dissolve most material, but the less soluble materials were administered as wettable powders. The di-(2-chloroethyl) phosphates are neurotoxic (i.e. they cause a delayed ataxia (Malone[9])) and the domestic fowl is a convenient test animal for a direct comparison of their acute and delayed effects. Data on the delayed effects of the chloroalkyl phosphates to poultry will be published in due course.

Statistical

Median lethal doses to rats and hens were estimated using the method of Weil.[11]

Biochemical

Haloxon and related esters yield, on acid hydrolysis, substituted umbelliferones which are strongly fluorescent in alkaline solutions. To determine the absorption of haloxon from the gut of the fowl, unchanged haloxon was extracted from faeces in acetone which was then evaporated. The extract was refluxed for 2 hr with 5N perchloric acid to bring about the complete hydrolysis of haloxon. The fluorescent derivative, 3-chloro-4-methyl umbelliferone, is stable under these conditions and is a direct measure of the haloxon originally present. Urine samples were subjected to hydrolysis by 15N sulphuric acid for half an hour at 100° to recover 3-chloro-4-methyl umbelliferone from various conjugates. This treatment would also break down most of any haloxon present. Suitable aliquots were rendered alkaline by adding N/10 potassium carbonate before reading off the fluorescence using an EIL type 27A fluorimeter, employing 365 mμ Hg line for excitation and selecting fluorescent light having a wavelength greater than 480 μm.

The 4-nitrophenyl esters of di-chloralkyl phosphoric acids gave 4-nitrophenol on alkaline hydrolysis which was read at 410 mμ using a spectrophotometer (Unicam SP 600).

Acid hydrolysis of 20° of the 3-chloro-4-methyl umbelliferone phosphates was carried out in phosphate buffer (pH 6·0); acid conditions were preferred because in alkaline media ring opening leads to a progressive loss of fluorescence. Samples were made alkaline using N/10 potassium carbonate immediately before reading.

Enzymic hydrolysis of various di-alkyl aryl phosphates at 37° in phosphate buffer (pH 7·2) was estimated colorimetrically or fluorometrically.

Hen brain homogenates were prepared in phosphate buffer (pH 7·2) containing 0·5% gelatin.

Cholinesterase activity was estimated colorimetrically by the Fleisher *et al.*[4] modification of the method due to Hestrin[6] or manometrically in a conventional Warburg apparatus using the technique of Ammon.[2] Manometric estimations were carried out in 0·025 M sodium bicarbonate, 0·1 M NaCl solutions saturated with 5% carbon dioxide in nitrogen (pH 7·4).

The removal of excess inhibitor from calf erythrocytes during reactivation and ageing studies was accomplished by repeated centrifugation at 2° and washing in chilled saline. The erythrocyte suspension was made up to the original blood volume with saline. The Warburg flasks contained 0·5 ml of erythrocyte suspension in one side-arm and 30 mg acetylcholine perchlorate in the body of the flask. The total volume of liquid was 4 ml. When P2AM* was used to reactivate phosphorylated enzyme, it was added from a side-arm to give a final concentration of 5×10^{-3} M. Carbon dioxide evolution at 37° was followed until no further reactivation could be detected and the difference between this maximum activity and that in control flasks was taken as a measure of ageing.

Rates of ageing and reactivation were estimated by plotting the logarithms of the ratio (enzyme recoverable initially/enzyme recoverable at the time of observation) and the ratio (enzyme inhibited initially/enzyme inhibited at the time of observation) respectively, against time.

RESULTS

Toxicity in relation to chemical structure

Estimates of median lethal doses are given in Table 1.

Absorption, detoxification and excretion of haloxon by the domestic fowl

Haloxon has a very low oral acute toxicity (LD50 > 7000 mg/kg) to adult chickens and it was desirable to establish initially whether a substantial proportion of the dose had been absorbed. This was done by observing the elimination of haloxon *in vivo* (see Table 2).

A high proportion of the dose given was absorbed (between 70 and 90 per cent in 5 of the cases) as judged by the amount of unchanged haloxon in the faeces and around half of the absorbed dose appeared as umbelliferone conjugates in the urine collected from the ureters within four days of treatment.

Relatively little 3-chloro-4-methyl umbelliferone was found in the urine of birds treated orally with haloxon but, on acid hydrolysis, fluorescent material was recovered. A large part of this fluorescence was due to 3-chloro-4-methyl umbelliferone present as conjugates in the urine. The recoveries of haloxon and derivatives from the urine and faeces of birds dosed with the material are shown in Table 2.

The origin of free umbelliferone in the faeces was not determined. The most reasonable explanation is non-enzymic hydrolysis of haloxon in the gut lumen, but the bile ducts may have transported the metabolite from the liver to the duodenum.

The extent of absorption and of detoxification were confirmed by observations on the fate of ^{32}P-labelled haloxon (see Table 3) administered in the feed to hens.

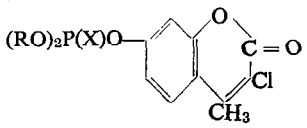
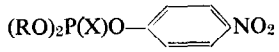
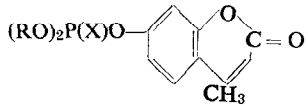
Investigation of the hydrolysis of the 3-chloro-4-methyl umbelliferonyl phosphates under acid conditions (pH 6·0) showed that haloxon was more susceptible to hydrolytic attack than coroxon (Table 4). However, the hydrolysis of haloxon by hen liver, brain and plasma under alkaline conditions (pH 7·2) (Table 5) was not rapid.

Inhibition of cholinesterase activity by di-alkyl and di-(chloroalkyl) phosphates

Di-alkyl aryl phosphates react with cholinesterase according to first order kinetics

* Pyridinealdoxime methiodide.

TABLE 1. TOXICITY OF CERTAIN DIALKYL ARYL PHOSPHATES TO RATS AND HENS

Structure	Compound	R	X	Rats	Hens	
				Oral LD50 (mg/kg) (with 95% confidence limits)	Dose Route	LD50 (mg/kg)
	Coroxon	$-C_2H_5$	O	9·8 (7·5–12·8)	Oral	2·2
	Haloxon	$-C_2H_4Cl$	O	895·9 (623–1288)	Oral IP*	>7000 800
	I	$-CH_2CH_2CH_3$	O	79·6 (34·3–184·4)	—	—
	II	$-CH_2CH(Cl)CH_3$	O	686·3 (569–828)	IP	>800
	III	$-CH_2CH_2CH_2Cl$	O	211·4 (167–267)	IP	300†
	IV	$-CH_2CH(Cl)CH_2Cl$	O	>1200	—	—
	V	$-CH_2CH_2CH_2CH_3$	O	63·4 (51·5–78·8)	—	—
	VI	$-CH_2CH_2CH_2CH_2Cl$	O	>100 < 200	IP	<188
	Coumaphos	$-C_2H_5$	S	56·0 (42·3–74·3)	—	—
	Paraoxon	$-C_2H_5$	O	1·8 (0·8–3·9)	Oral	2·0
	VII	$-C_2H_4Cl$	O	37·4 (26–54)	Oral	>100 < 200
	VIII	$-CH_2CH(Cl)CH_3$	O	173·0 (141–212)	IP	>120
	IX	$-CH_2CH(Cl)CH_2Cl$	O	—	IP	120
	X	$-CH_2CH_2CH_2Cl$	O	32·7 (25–43)	IP	>60 < 120
	Parathion	$-C_2H_5$	S	2·7 (1·6–4·6)	—	—
	XI	$-C_2H_5O$	O	29·0	—	—
	XII	$-C_2H_4Cl$	O	141·3 (102–196)	Oral	>1250

* = intraperitoneal; † = severe acute toxic symptoms, but no deaths at this dose.

TABLE 2. ESTIMATES OF HALOXON AND METABOLITES IN URINE AND FAECES OF RHODE ISLAND RED HENS DOSED ORALLY WITH HALOXON

	Group 1 (dose 1000 mg)			Group 2 (dose 225 mg)		
	Hen 1	Hen 2	Hen 3	Hen 4	Hen 5	Hen 6
Total conjugated 3-chloro-4-methyl umbelliferone in urine samples as haloxon equivalents (mg)		Not estimated		133·4	58·0	78·4
Total free 3-chloro-4-methyl umbelliferone in faeces as haloxon equivalents (mg)	20·8	83·7	79·8	5·1	44·5[1]	106·8*
Unchanged haloxon in faeces (mg)	272·9	532·7	162·2	21·2	33·3	38·3
Total haloxon recovered or traced (mg)		Not estimated		159·7	140·8	223·5

* = Samples contaminated with urine.

and, when the alkyl groups are CH_3CH_2- or with longer chains, the reaction is slowly reversible or irreversible. Inhibition of di-(2-chloroethyl) compounds is not progressive and is rapidly reversible (Lee[7]) at a rate independent of the aryl moiety.

TABLE 3. RECOVERY OF ^{32}P-LABELLED HALOXON OVER A PERIOD OF 4 DAYS FROM THORNBER'S '606' HENS FED ON TREATED MASH FOR 24 HR

Bird	Estimate of haloxon ingested (mg)	Dose (mg/kg)	Total ^{32}P (%) recovery in droppings	Haloxon (%) recovery in droppings	Recovery (%) of metabolites
1	166	76·6	88·5	14·4	74·1
2	144	52·8	89·0	6·9	82·1
3	201	85·2	69·6	11·8	57·8
4	121	74·2	60·2	4·1	56·1
5	192	82·6	60·4	9·2	51·2
6	110	51·9	66·6	7·0	59·6

TABLE 4. HYDROLYSIS OF DIALKYL COUMARIN PHOSPHATES AT pH 6·0 AND 20°

Compound	Rate of hydrolysis min^{-1}
Coroxon	$5·6 \times 10^{-6}$
Haloxon	$2·1 \times 10^{-5}$
I	$8·4 \times 10^{-8}$
III	$1·1 \times 10^{-6}$
II	$7·5 \times 10^{-7}$
IV	$1·0 \times 10^{-6}$
V	$1·0 \times 10^{-8}$
VI	$1·0 \times 10^{-6}$

The anticholinesterase potency of the various 3-chloro-4-methyl umbelliferonyl phosphates is shown in Table 6. The residual cholinesterase activity in chick brain homogenates (2·5 per cent) is after 10 min exposure to the inhibitor at 10^{-6} M at 37°.

The hydrolysis of acetylcholine by calf erythrocyte cholinesterase, which had been partially inhibited by di-(chloroalkyl) phosphates, was followed after the excess inhibitor had been removed. Spontaneous reactivation occurred fairly rapidly with chloropropyl compounds, but not with the single di-(4-chlorobutyl) compound examined (Fig. 1). Cholinesterase inhibited by coroxon, I, V or VI did not reactivate spontaneously but showed recovery at varying rates (see Table 7) when subjected to the oxime P2AM at 5×10^{-3} M (Fig. 2).

It was further found that reactivation of cholinesterase inhibited by compounds I, II, III, V and VI was limited in that a certain proportion of the activity was not recoverable. Estimates of the rates at which this conversion of unstable inhibited enzyme (EP_u) to the stable form (EP_s) occurred (Fig. 3) and, also, of rates of reactivation are recorded in Table 7.

No stable phosphorylated cholinesterase was formed by incubation of the enzyme with haloxon or IV at 10^{-6} M for 90 min at 37°.

The influence of ageing on acute toxicity

The effects of organophosphates on the peripheral nervous system contribute appreciably to their toxicity (Heath[5]). The reactivating agent P2AM, which is effective peripherally, can thus be used to indicate the role of enzyme reactivation *in vivo*, and to demonstrate the influence of ageing on toxicity.

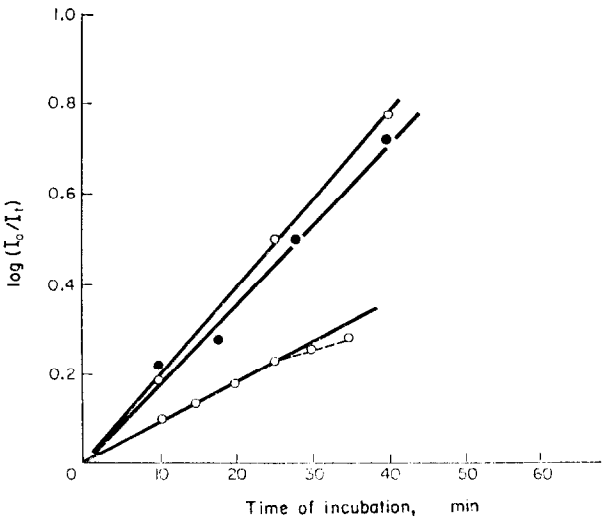


FIG. 1. Spontaneous reactivation of phosphorylated calf erythrocyte acetylcholinesterase, following inhibition with di-alkyl (3-chloro-4-methyl 7-yl-coumarin) phosphates. Key: I_t is inhibited enzyme at time t; ○—haloxon; ●—II; ⊙—III.

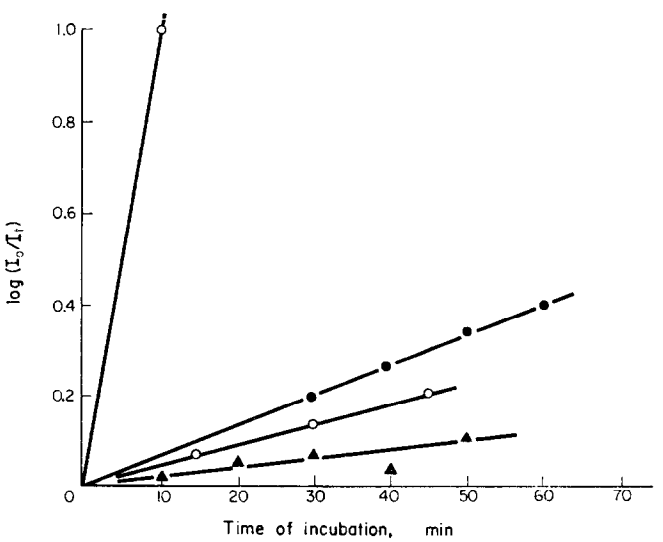


FIG. 2. Reactivation of phosphorylated calf erythrocyte acetylcholinesterase by 5×10^{-3} 2PAM following inhibition by di-alkyl (3-chloro-4-methyl 7-yl coumarin) phosphates. Key: I_t is inhibited enzyme at time t; ○—coroxon; ●—I; ⊙—VI; ▲—V.

One week-old chicks were given oral doses of the phosphates sufficient to kill a proportion of the recipients, and one half of each test group received P2AM by injection into the perivisceral cavity when toxic symptoms became apparent. The mortalities following these treatments are shown in Table 8. It was not possible to elicit a toxic response with IV.

TABLE 5. ESTIMATES OF ENZYMIC HYDROLYSIS OF CERTAIN DI-ALKYL ARYL PHOSPHATES BY HEN TISSUES

	Liver		Plasma		Brain	
Compound	Number of birds	Mean activity μM/g/hr	Number of birds	Mean activity μM/h/gr	Number of birds	Mean activity μM/g/hr
Coroxon[1]	1	0·04				
Haloxon	5	0·30 ± 0·05	5	0·08 ± 0·01	2	0
Haloxon[1]	1	0·29				
XII	5	0·24 ± 0·04	5	0·02 ± 0·01	2	0
Paraoxon[1]	1	0·02				
VII	1	0·10				
VIII	1	0·10				

[1] R. M. LEE, personal communication.

TABLE 6. RESIDUAL ACETYLCHOLINESTERASE ACTIVITY IN CHICK BRAIN HOMOGENATES (2·5%) FOLLOWING 10 MIN INHIBITION AT 37° WITH INHIBITOR AT 10^{-6} M

Compound	Percentage residual activity
V	1·0
I	2·6
Coroxon	4·2
VI	8·5
Haloxon	10·1
III	10·4
II	23·5
IV	76·1

TABLE 7. RATES OF REACTIVATION AND AGEING OF PHOSPHORYLATED CALF ERYTHROCYTE ACETYLCHOLINESTERASE AT 37°

Compound	Structure of alkoxy group	Rate of reactivation min^{-1}		Rate of conversion to stable phosphorylated enzyme min^{-1}
		Water	2PAM	
Coroxon	$CH_3{\cdot}CH_2{\cdot}O$—	—	$23{\cdot}0 \times 10^{-2}$	—
Haloxon	$CH_2(Cl){\cdot}CH_2{\cdot}O$—	$3{\cdot}8 \times 10^{-2}$	—	—
I	$CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}O$—	—	$1{\cdot}5 \times 10^{-2}$	$0{\cdot}43 \times 10^{-2}$
II	$CH_3{\cdot}CH(Cl){\cdot}CH_2O$—	$3{\cdot}7 \times 10^{-2}$	—	$0{\cdot}50 \times 10^{-2}$
III	$CH_2(Cl){\cdot}CH_2{\cdot}CH_2{\cdot}O$—	$2{\cdot}0 \times 10^{-2}$	—	$1{\cdot}89 \times 10^{-2}$
V	$CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}O$—	—	$0{\cdot}385 \times 10^{-2}$	$2{\cdot}4 \times 10^{-2}$
VI	$CH_2(Cl){\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}O$—	—	$1{\cdot}0 \times 10^{-2}$	$1{\cdot}7 \times 10^{-2}$

Chicks poisoned by coroxon, haloxon and I responded well to P2AM and there was possibly an effect in the two V groups although this was not statistically significant; otherwise there was little effect.

The brains of chicks receiving toxic doses of selected phosphates showed inhibited cholinesterase which could be reactivated to varying degrees by P2AM. Enzyme

TABLE 8. THE EFFECT OF P2AM ON THE ACUTE TOXICITY OF VARIOUS DI-ALKYL PHOSPHATES

Compound	Dose (mg/kg)	Number of chicks	Dose of P2AM (mg/kg)	Time intervals for P2AM dosing (min)	Number killed	Percentage mortality	Therapeutic effect*
Coroxon	2	20	—	—	7	35	Yes
		20	2 × 10	5, 10	0	0	
Haloxon	400	20	—	—	17	85	Yes
		20	2 × 10	10, 20	7	35	
I	5	20	—	—	14	70	Yes
		20	2 × 10	10, 20	4	20	
II	600	20	—	—	3	15	No
		20	2 × 10	10, 20	5	25	
III	100	20	—	—	18	90	No
		20	2 × 10	10, 15	17	85	
IV	1600	14	—	—	0	0	—
V	10	20	—	—	17	85	No
		20	2 × 10	10, 20	14	70	
	8·5	20	—	—	6	30	No
		20	2 × 10	10, 20	11	55	
VI	48	15	—	—	7	47	No
		15	2 × 10	10, 20	5	33	
P_2AM	20	20	—	—	0	0	—

* Therapeutic effect assessed by Fisher Exact Probability test.

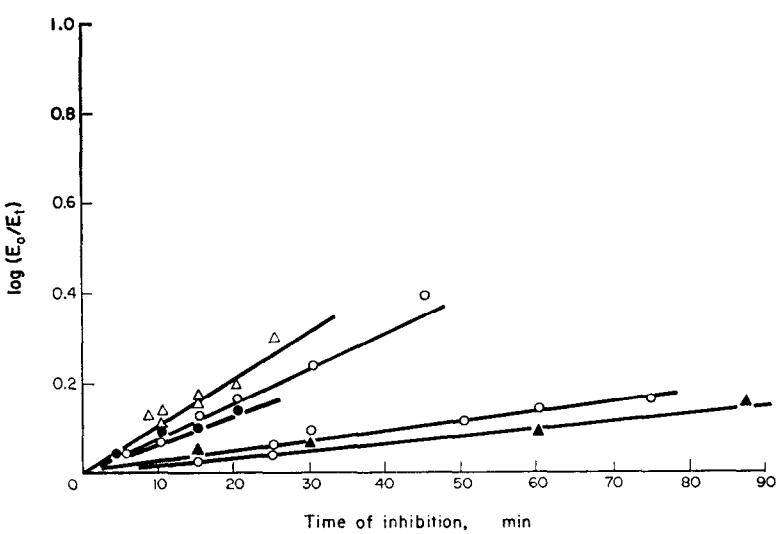


FIG. 3. Conversion of phosphorylated calf erythrocyte acetylcholinesterase to stable forms during inhibition by di-alkyl (3-chloro-4-methyl 7-yl coumarin) phosphates. Key: E_t is enzyme recoverable after inhibition for t min; ○—III; ●—VI; ⊙—II; △—V; ▲—I.

phosphorylated by II, III or VI and reactivated by P2AM did not approach the activity of the brains of untreated control birds, whereas those treated with coroxon or haloxon attained activities comparable with that of the controls. The results of this experiment are illustrated in Fig. 4, which shows reactivation following the introduction of P2AM.

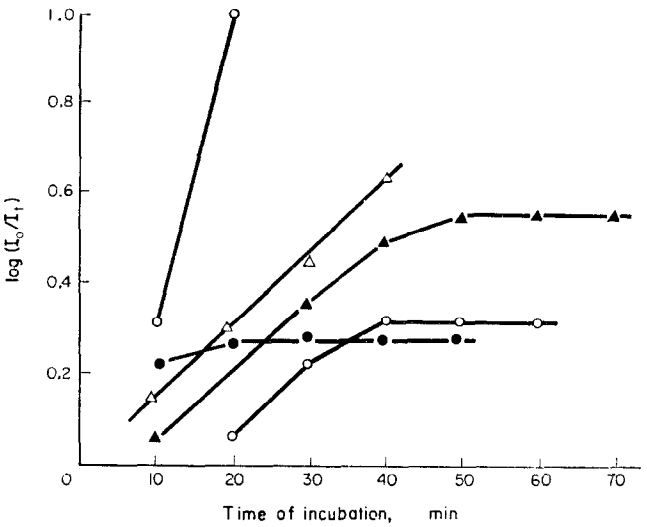


FIG. 4. Reactivation of phosphorylated chick brain cholinesterase by 5×10^{-3} M 2PAM, following oral administration of di-alkyl (3-chloro-4-methyl 7-yl coumarin) phosphates. Key: I_t is enzyme inhibited after t min incubation with 2PAM; ○—coroxon, 2 mg/kg; ⊙—VI, 8 mg/kg; ●—II, 400 mg/kg; △—haloxon, 500 mg/kg; ▲—III, 150 mg/kg.

DISCUSSION

The inhibitory activity of organophosphates is partially determined by (i) electron density in the region of the phosphorus atom and (ii) fit of the molecule on the enzyme surface (O'Brien,[10] Heath[5]). An increase in the number of carbon atoms in the alkyl chain decreases the charge on the phosphorus atom, and the nature of the alkyl group is, therefore, an important factor in the reactivity of the di-alkyl aryl phosphates. Chlorine, on the other hand, is strongly electro-negative and the introduction of chlorine into the alkyl groups of these compounds has a pronounced effect. In terms of the electronic theory, chlorine substitution, particularly when the substitution is close to the phosphorus atom, might be expected to weaken the esteratic bond by reducing the electron density on the phosphorus and rendering it more susceptible to attack by hydrolysis under alkaline conditions. For acid hydrolysis, the effects are not easily foreseen but the results suggest that compounds with chlorine substituted alkoxy groups hydrolyse faster than the corresponding unsubstituted compounds.

In general, electronic theory fails to account for the changes in anticholinesterase activity when the basic groups are varied in organophosphorus compounds which are otherwise the same (Aldridge,[1] Heath[5]). The effects of basic groups on the electrophilic activity of the phosphorus atom are outweighed by other effects that they produce. This generalization is well illustrated by the (3-chloro-4-methyl coumarin-7-yl) phosphates, in which there is a poor correlation between inhibitory potency (Table 6)

and rates of hydrolysis. There is an agreement, however, between the order of toxicity to rats, coroxon > V > I > VI > III > II > haloxon > IV, and that of potency, V > I > coroxon > VI > haloxon > III > II > IV.

Compounds containing the group Cl CH_2 CH_2- hydrolyse faster than their CH_3 CH_2- analogues (Table 4) and, since hydrolysis is the principal mode of detoxification of the di-(2-chloroethyl) aryl phosphates, this property has a possible bearing on the acute toxicity of the class. The elimination of large doses of haloxon by hens *in vivo* is not sufficiently rapid in its own right to account for the very low toxicity of the compound. A high proportion of the haloxon given orally to hens was absorbed and only some 50–80 per cent (of the absorbed compound) was recovered in the form of metabolites during the next few days. Quite considerable quantities of haloxon were available, therefore, to affect cholinesterase systems but failed to kill the recipient animals.

Lee and Hodsden[8] suggested that the low toxicity of compounds of this type arises from rapid reactivation of phosphorylated cholinesterase. The rates of reactivation shown in Table 7 are biased since both ageing and reactivation processes affect the concentration of unstable phosphorylated enzyme. Nevertheless, they reflect the situation *in vivo* and may be used for comparative purposes. The rates of reactivation were in the order haloxon > II > III > coroxon > I > VI > V which shows reasonable agreement with the order of toxicity to rats, viz. haloxon < II < III < VI < I < V < coroxon. The compounds haloxon, II and III reactivated spontaneously; the remainder required a stronger hydroxylating agent than water.

The conversion of inhibited cholinesterase to a stable form at a fairly rapid rate also influences toxicity, primarily because of the ineffectiveness of therapy using reactivating agents. The oxime, P2AM, reduced the lethal effect of coroxon and haloxon but not of the propyl and butyl compounds. Stable phosphorylated enzyme appeared in the brains of chicks shortly after treatment with these last-mentioned materials. Where both ageing and reactivation occur, the relative rates of the two processes must partially determine the toxicity of the phosphate.

Certain conclusions regarding the influence of the structure of basic groups on the reactivity and toxicity of the di-alkyl (3-chloro-4-methyl coumarin-7-yl) phosphates may be drawn. Chlorine in the 2-position, i.e. relatively close to the phosphorus, is associated with reduced toxicity (haloxon, II) and the 2,3-di-chloropropyl compounds examined (IX, IV) both have a very low toxicity and poor inhibitory powers. Acetylcholinesterase phosphorylated by haloxon and II showed high rates of reactivation and it is not unreasonable to associate reduced potency and toxicity with this characteristic. The effect of chlorine substitution on reactivation is diminished as the chlorine becomes more remote from the phosphorus (compounds III and VI).

Ageing was observed in all compounds containing alkoxy groups larger than ethyl. Chlorine substitution enhanced ageing in the propyl compounds and the effect was pronounced in compound III with substitution on the terminal carbon. However, compound VI with terminal substitution in butyl groups was essentially similar to the unsubstituted parent compound and it is possible that the effects of large alkoxy groups override those of substituents remote from the phosphorus.

The affinity for acetylcholinesterase of compounds such as those under consideration can only be assessed by comparing their inhibitory powers under identical conditions. This potency which relates very well to the order of toxicity of the

compounds, is determined by the reactivity of the compounds and the processes of reactivation and ageing. However, other properties, such as increased susceptibility to hydrolytic attack, which influence the relationship between potency and toxicity as with, for example, the material haloxon, must not be ignored.

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