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THE ESTIMATION OF SERUM CHOLINESTERASE IN THE PRESENCE OF ANTI-CHOLINESTERASE INSECTICIDES

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Summary

Six methods have been employed for the assay of serum cholinesterase in the presence of a number of anti-cholinesterase insecticides, of which three were carbamates and two organophosphorus compounds. Inhibition of cholinesterase activity was demonstrated in every case, but four of the methods underestimated the degree of inhibition.

It was found that if serum samples to which four of the five insecticides had been added were left at room temperature for 24 hours there was almost complete recovery of the cholinesterase activity. This did not occur if the samples were stored at −15°.

On the basis of these studies two methods are recommended for the estimation of serum cholinesterase in the presence of anti-cholinesterase insecticides.

Introduction

Assays to determine circulating levels of serum cholinesterase (EC 3.1.1.8) are affected by the presence of a number of insecticides. Indeed the decrease in enzyme activity is of value in the diagnosis of poisoning by anti-cholinesterase insecticides and is also used to detect excessive absorption of such insecticides before clinical evidence of poisoning is apparent [1,2]. Anti-cholinesterase insecticides are of two types, organophosphorus compounds which, in general, behave as irreversible inhibitors of cholinesterase, and carbamates, which act as reversible inhibitors, although the mechanism of this reversibility is not of the usual type [3]. Witter [4] has pointed out that the reversibility of the reaction between the enzyme and inhibitor may lead to marked changes in the apparent cholinesterase activities found.

The present study was designed to determine the magnitude of such effects by assessing the influence of five insecticides on the results obtained using six different methods for the estimation of cholinesterase, chosen because of their likely use in clinical chemistry laboratories.

Materials and Methods

Insecticides

The following insecticides were studied:

Organophosphorus compounds

1. Diethyl-*S*-(2-(ethylthio) ethyl)phosphorothiolothionate (Disulfoton).
2. Dimethyl-2-dichlorovinylphosphate (Dichlorvos).

Carbamates

1. 1-naphthyl-*N*-methylcarbamate (Carbaryl).
2. 3:5-Dimethyl-4-thioethylphenyl-*N*-methylcarbamate (Methiocarb).
3. 1-Isopropyl-3-methyl-5-pyrazolyldimethylcarbamate (Isolan).

In order to simulate samples from a patient poisoned with an insecticide, one volume of the compound in acetone was added to 100 volumes of normal serum and left at room temperature until maximum cholinesterase inhibition had been achieved, as measured by Method 1. The concentration of the insecticide used was chosen so that the minimum enzyme activity obtained was between 6 and 14% of the uninhibited value.

Assays

The assays used for the estimation of serum cholinesterase levels and the conditions under which they were carried out, are summarised in Table 1.

Certain additional observations were made, namely:

1. For each method, the addition of one volume of acetone to 100 volumes of normal serum was shown to have no appreciable effect on the cholinesterase level.

2. To investigate the effect of storage, samples of the serum plus insecticide were stored at −15°, 4° and 23° and the cholinesterase estimated at intervals by Method 1.

TABLE 1

METHODS USED FOR THE ESTIMATION OF SERUM CHOLINESTERASE

Method No.	Substrate	Concentration of substrate (mM)	Dilution of serum	Temperature	Incubation time (min)
1	Benzoyl choline [5]	0.05	1 in 200	26°	3
2	Phenyl benzoate [6]	0.14	1 in 4,400	37°	60
3	Acetyl choline [7]	45	1 in 11	37°	60
4	Acetyl choline [8, 9]	30	1 in 45	37°	30
5	Acetyl thiocholine [10]	5	1 in 156	25°	1
6	Acetyl thiocholine [11]	2	1 in 551	37°	5

Results

In order to compare the methods listed, the cholinesterase was estimated on the samples of serum plus insecticide, and on the uninhibited serum, by Method 1 and by each of the other methods in turn. The results are shown in Table II.

The addition of any of the insecticides to normal serum lowers cholinesterase activity, irrespective of the method used. The concentration of each insecticide needed to reduce enzyme activity to between 6 and 14% of the uninhibited value varies considerably as does the period of incubation at room temperature necessary to produce this effect. Isolan was the most powerful of the anti-cholinesterases tested.

Different methods of cholinesterase assay give different apparent levels, because of reactivation of the enzyme during the assay. Those using high dilutions of serum, high temperature and high substrate concentrations produce the greatest degree of reactivation, and this is seen especially with Method 2. Methods 1 and 5, which use short incubation periods and low temperatures gave the least degree of reactivation. Irrespective of the method, the degree of reactivation is greater with the carbamates than with the organophosphorus insecticides.

The results in Table III show that recovery of cholinesterase activity occurs during storage at 4° or room temperature. With the three carbamates, this was almost complete after storage for 24 hours at room temperature. The two organophosphorus compounds showed marked differences. In the case of Disulfoton, no increase in cholinesterase was found after storing the sample at room temperature for 48 hours. With Dichlorvos, however, considerable recovery of enzyme activity was found after storage at room temperature for as little as 4 hours. There was no appreciable change in apparent cholinesterase levels in those samples stored at −15°.

Discussion

It is clear from the present data that several of the published methods for

TABLE II

INSECTICIDES USED AND CHOLINESTERASE VALUES FOUND

The figures refer to the cholinesterase activity found in the presence of the insecticide, using the method shown, expressed as a percentage of the uninhibited activity.

Insecticide	Concentration in serum (M)	Time of preincubation (h)	Method					
			1		2	3	4	5	6
			Mean	Range					
Disulfoton	$5 \cdot 10^{-4}$	20	10	7–12	17	10	29	16	17
Dichlorvos	$5 \cdot 10^{-7}$	1	7	7–8	20	15	27	10	13
Carbaryl	$5 \cdot 10^{-4}$	1	9	6–14	56	21	32	10	33
Methiocarb	$7.5 \cdot 10^{-4}$	0.5	11	9–14	56	57	39	15	35
Isolan	10^{-7}	1.5	10	8–11	38	32	34	15	20

TABLE III

THE EFFECT OF STORAGE OF SERUM PLUS INSECTICIDE

Values of serum cholinesterase activity are expressed as a percentage of uninhibited activity, using Method I.

Insecticide	Temperature of storage	Time of storage (h)			
		0	4	24	48
Dichlorvos	23°	7	32	77	80
	4°		7	35	61
	- 15°		5	8	9
Carbaryl	23°	7	13	92	97
	4°		12	22	45
	- 15°		10	14	17
Methiocarb	23°	7	52	91	100
	4°		17	36	60
	- 15°		9	14	16
Isolan	23°	10	10	91	100
	4°		10	15	30
	- 15°		11	11	18

estimating serum cholinesterase can give gross errors in the presencc of some insecticides, and are quite unsuitable for detecting excessive absorption of these substances. This applies not only to the carbamate insecticides tested but also to the organophosphate Dichlorvos, and to a lesser degree, Disulfoton. It should, however, be noted that the addition of Disulfoton to serum probably does not reflect the situation in a patient who has absorbed this compound, since it may be metabolised to a more active anti-cholinesterase [3].

Method 1 appeared to give the best measure of cholinesterase activity in the presence of insecticide in that:

1. it gave the lowest results of the methods tested.

2. it uses a low temperature, short reaction time, and low substrate concentration.

3. the reaction rate was constant during the period of the test irrespective of the insecticide used.

Method 5 gave results only slightly higher than Method 1 and is a suitable alternative if an ultra-violet spectrophotometer is not available.

Other workers, using whole blood or erythrocytes, observed effects similar to those described in this paper; for example, the quantitative effects of dilution and substrate concentration on erythrocyte cholinesterase in the presence of carbaryl have been reported [12].

It has also been shown that even if a suitable method for estimating serum pseudocholinesterase in the presence of insecticides is used, a delay in carrying out the estimation will lead to considerable errors, unless the sample is stored deep-frozen. This means that transport of such samples at ambient temperature, to a reference laboratory, is out of the question.

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