PROJECT

ANTICHOLINESTERASES: THEIR EFFECTS ON RABBITS

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D E D I C A T I O N

This work is dedicated to all those that are dear to me, both in memory and otherwise, for giving my life a purpose and a direction and for making all my endeavours worthwhile.

C O N T E N T S

ABSTRACTS

The percutaneous absorption of three anticholinesterases was studied. All three, Malathion, Ethylparathion and Carboryl were found to be absorbed percutaneously. From the depression of Cholinesterase activity caused by the three compounds it appears that Ethylparathion is a more potent poison than the other two.

An attempt was made at studying the retationship between depression of Cholinesterase activity and the extent of poisoning, but a more extensive study would be required before such a relationship can be shown either to exist or to be absent altogether. Only in one compound, ethylparathion, did results obtained indicate such a relationship.

A comparison of the extents of absorption, subsequent extent of poisoning after administration of a drug by two routes was made. Although the doses used in these two routes were not the same, the drug caused symptoms of poisoning when administered orally although no such symptoms of poisoning were observed when the drug was administered percutaneously. The drug, Carboryl, was administered in increasing doses, and it was observed that the rate of onset of toxic effects was funtion of dose. Increasing the dose was observed to enhance the rapidity with which toxic effects occured.

The effects of a dithio carbamate, dithane on rabbits was studied. The drug was found to have little of any effect on Cholinesterase activity after oral administration of relatively high doses. No symptoms of poisoning were observed.

It would be of great help if more was known about the ways in which the balance between enzyme activity and levels of Acetyl Choline determine the local response at the periphery and in the central nervous system.

INTRODUCTION

LITERATURE SURVEY

Increased use of Organophosphates and Carbamates as insecticides constitue a wide spread problem in that they are often responsible for human poisoning. The compounds are highly toxic and sublethal doses of the organophosphates have been shown to cause inhibition of Cholinesterases. Since toxic reactions could result from oral, inhibition and percutaneous absorption it is important to understand the factors influencing the toxicity of these compounds.

Poisoning with the anticholinesterases may be accidental or it could result from suicidal or homicidal intents. In Kenya, in a period of five years, 1973-1977, of the 1279 cases submitted to the Government Chemists' Laboratory, where death was allegedly caused by poisoning, 152 (11.9%) were due to organophosphates while 3(0.138%)were due to carbomates (appendix I).

Clinical cholinesterase assay may be used in diagnosis of suspected cases of poisoning. Persons working from day to day with cholinesterase inhibitors are at continous risk of accidental poisoning and should have a pre-exposure assay of blood cholinesterase levels to establish their individual normal enzyme levels. This should be followed by periodic assays during the working period.

Understanding the mechanisms of cholinesterase inhibition by the various inhibitors would help in interpretation of the results obtained from determinations of enzyme activity. This would help in selecting the most appropriate course of treatment, because the margin between death and survival can be very narrow in a rapidly chaning situation.

Acetyl Choline is the natural substrate of acetylcholinesterase, an enzyme of molecular weight 80,000, which exists in aggregates of two or three tetramers.

It is found in erythrotytes but its presence has not been associated with any of the functions of erythrocytes. It is also found in the parasympathetically unnervated effector organs, skeletal muslce and plates and in the central nervous system. Acetyl Choline is also hydrolysed by butyryl Cholinesterase, an enzyme present in blood.

Acetylcholine combines with the cholinesterases to form an enzyme substrate complex which rearranges to liberate choline and the acetylated enzyme. The acetylated enzyme is hydrolysed in presence of water to liberate the enzyme, and acetic acid. The hydrolysis of the acetylated enzyme is the rate limiting step. The entire hydrolysis of acetylcholine takes only 80 microseconds (Appendix II).

Cholinesterase inhibitors may be divided into three groups, i.e. reversible, "less" reversible, and irreversible inhibitors. This is based on the rates of hydrolysis of the enzyme-inhibitor complex. The reversible inhibitors interact with both the anionic site as well as the imidazole nitrogen of the esteratic site (Appendix II). The enzyme-inhibitor complex is readily hydrolysed by water.

The "less" reversible cholinesterase inhibitors include a very important group of compounds, i.e. the carbamates. These compounds are used in clinical medicine and also as insecticides. The carbamates used in clinical medicine include physostigmine and neostigmine. The former is obtained from the plant, Physostigma venenosum while the latter is a synthetic analogue. Both these compounds owe their therapeutic efficacy to their ability to inhibit cholinestrases and cause accumulation of acetylcholine.

The carbamates used as insecticides, unlike those used in medicine do not owe their insecticidal activity solely to anticholinesterase effect. OBRIEN (1967) has suggested that insects do not utilize cholinesterase at the neuromuscular function, but may utilize it in the central nervous system, subsequently carbamates used in clinical medicine being either ionized or readily ionizable cannot penetrate the blood brain barrier and do not show any insecticidal activity.

The carbamates combine with the enzyme to form an enzyme-inhibitor complex (Appendix III). This rearranges to produce the carbamoylated enzyme liberating the alcohol. The carbamoylated-enzyme is hydrolysed to liberate the enzyme and the carbamic acid. Hydrolyses of the carbamoylated enzyme is the rate limiting the step and regeneration of the enzyme takes about 30 minutes. One compound belonging to this group was used in the present investigation.

The third group of anticholinesterases, the irreversible inhibitors include an important class of compounds that have been used in warfare in the past, and are used as insecticides in agriculture. This class of compounds is the organophosphates. Most of the organophosphates widely used as insecticides are dialkyl phosphates.

SHELLENBERGER et al (1965) have shown that inhibition of cholinesterase by dialkylphosphates is due to direct phosphosphorylation of the active esteratic site of the enzyme through an electrophilic mechanism. This may be followed by spontaneous enzyme reactivation or conversion to a non-reversible "aged" enzyme. GOUGH and SHELLENBERGER (1977) have shown that the extent of spontaneous reactivation and "aging" are apparently dependent on the alkyl substitution of the organophosphate and also of the enzyme source.

The organophosphates malathion and ethyparation are converted in the body to their oxo derivatives by liver microsomal oxidase enzymes. The oxo derivatives are more electrophilic than the thio forms and are therefore more potent cholinesterase inhibitors. The rates of oxidation vary from species to species and hence the selective toxicity of these compounds.

Spontaneous reactivation of enzymemmay partly be due to reversal of enzyme inhibitor complex as well as to hydrolysis of dialkyl-phosphorylated enzyme.

The rate of hydrolysis is extremely slow and ranges from 1½ hours to weeks. Recovery of enzyme activity is therefore almost entirely due to synthesis of new enzyme protein in some cases. Strong nucleophiles have been used to reactivate inhibited enzyme. The nucleophile attacks the electrophilic phosphate radical which is followed by a rearrangement in which the (free) enzyme is liberated. Dirnhuber and Cullumbine (1955) have shown that the "aged" enzyme is not sensitive to nucleophilic reactivation. "Aging" of the enzyme has been attributed to dealkylation of the dialkylphosphorylated enzyme apparently decreasing the electrophilicity of the phosphate radical.

Acetylcholine and the cholinesterases as mentioned earlier occur at the synapses and nerve endings of peripheral nerves as well as in some synapses in the brain and the spinal cord. Bajgar (1971) has shown that signs of poisoning after different doses of inhibitors could be best correlated with chnages in cholinesterase activity in the pons and the medullar than those in other parts of the brain. These findings are in agreement with those of De Candole et al (1953) who have shown that death in organophosphate poisoning is usually due to failure of the respiratory centre, indicating the importance of enzyme levels in the brain stem.

In producing acutre effects the rate of inhibition of cholinesterase may be just as important as the degree to which inhibition procedes. This is clearly evident with both the carbmates and the organophosphates. The carbamates do not require in vivo activation and produce symptoms of poisoning rapidly after ingestion (of poison). The severity of the toxic effects in carbamate poisoning depend on the speed of reactivation of the enzyme, and death is unlikely if the rate of inhibition is offset by speedier reactivation. It has been shown in patients suffering from myasthenia gravis treated with small repeated doses of organophosphates that the blood enzyme could be reduced to zero without the patients developing symptoms.

The aim of the present work was to investigate whether some of the anticholinesterases used in as insecticides are absorbed orally and percutaneously, and whether such absorption p produces toxic effects. The investigation was also hoped reveal any reletionship that may exist between extent of poisoning and whole blood cholinesterase activity.

METHODS

MATERIALS AND CHEMICALS

ANIMALS: Rabbits weighing between 1085 g. and 2110 grammes.

Stomach tube
Scissors
Syringes
Dessicator
Thermometer
pH meter
Pipettes 5 ml and 10 ml.
Measuring cylinders
Volumetric flasks, beakers and sample tubes

Propan-1, 2-diol
Toluene
Heparin 1% solution
Buffer solutions for standardizing the pH meter i,e. pH4 and pH 9 buffer solutions
Sodium barbitone
Potassium dihydrogen orthosphate
Sodium Chloride
Hydrochloric Acid 0.1M.
Acetyl Choline bromide 0.2% w/v prepared freshly

Malathion 9.65% w/v
Ethylparathion 9.9% w/v
Carbaryl 2.64% w/v, 5.28%, 8.5% w/v
Dithane 8.0% w/v, and 16% w/v

PROCEDURE FOR THE ORAL ADMINISTRATION OF DRUGS

A sample of blood, 2 ml, was withdrawn from the marginal ear vein of a rabbit weighing 1.6 kg, using a heparinized syringe, into a sample tube containing one drop of a 1% heparin solution. This was used later as the pretreatment control.

25 ml of a 2.64% w/v carbaryl solution was administered to the rabbit using a stomach tube and a syringe (Dose 0.42 g. per Kg.). Two samples of blood were withdrawn from the marginal ear vein as described above, one sample 2 hours (after administration of the drug) and the other 3 hours after administration of the drug.

All three samples were assayed for cholinesterase activity using the Michel pH method described later.

The procedure was repeated using the following doses 0.53 g. per kg and 0.72 grammes per kg. In all four animals were used, using two animals for every dose given.

Similarly the experiment was repeated using 8.0% w/v dithane, (dose 1.23 g. per kg.) and 16% w/v (dose 2.46 grammes per kg.). However only three animals were used in this case.

PERCUTANEOUS ADMINISTRATION OF DRUGS

Four animals weighing between 1085 and 1600 grams were shaven on a patch of the skin of approximately 9 cm in diameter. A 2ml sample of blood was withdrawn from each animal from the marginal ear vein, using a heparinized syringe, into a sample tube containing one drop of 1% heparin solution. This was used as the pretreatment control.

A 9.65% w/v solution of malathion in propylene glycol was applied to the shaven area of each animal for not less than 2 hours, after which a sample of blood (2ml) was withdrawn from each animal as described above. Another sample of blood was withdrawn from each animal 3 hours after administration of the drug.

All twelve samples of blood were assayed for cholinesterase activity according to the Michel pH method to be described later.

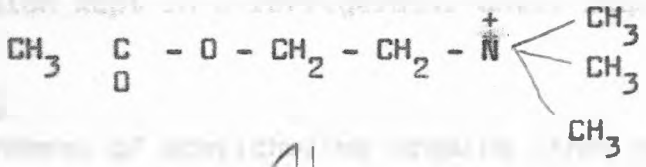
The experiment was repeated using ethyl parathion 9.9% w/v in propylene glycol. As with malathion four animals were used weighing between 1540 and 2110g. For each rabbit three blood samples were obtained. These were the control (pretreatment) and the experimental samples 1.5 and 2.5 hours after administration of the drug.

Similarly the experiment was repeated using carbaryl solution, 8.5% w/v. Four rabbits weighing between 1249 and 1725 grammes were used. The rabbits were observed for symptoms of poisoning but since no significant effects were observed in the first two hours the first blood sample was taken 4 hours after the administration of the drug and the second sample 6 hours after administration of drug

THE PROCEDURE FOR CHOLINESTERASE DETERMINATION - MICHEL, H.O. (1949)

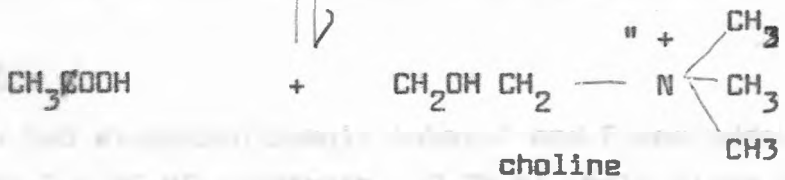
Theoretical basis for cholinesterase assay

Acetylcholine is hydrolysed by the cholinesterases to produce acetic acid and choline. The acetic aaid produced dissociates to form the acetate ion and the proton (hydrogen ion). The pH method of cholinesterase determination is based on the concentration of hydrogen ion. The reactions taking place may be represented by the following equations:



acetyl choline

Cholinesterase



choline

$$CH_3COOH \rightleftharpoons CH_3COO^- + H^+$$

$$Ka = \frac{[CH_3COO^-][H^+]}{[CH_3COOH]}$$

Ka = Dissociation Constant.

$$[H^+] = Ka \frac{[CH_3COOH]}{[CH_3COO^-]}$$

$$pH = pKa + \log \frac{[CH_3COO^-]}{[CH_3COOH]}$$

BUFFER SOLUTION

1.237 grams of sodium barbitone, 0.136 g. of potassium dihydrogen phosphate and 17.535 g of .sodium chloride were dissolved in 900 ml of distilled water, made up to nearly one liter with distilled water and the pH adjusted to 8 with 0.1M Hydrochloric acid. The volume was then accurately made up to 1 liter. A few drops of toluene were added as a preservative and the solution kept in a refrigerator until required for use.

SUBSTRATE

2 grammes of aceylcholine bromide (that had been dried in a dessicator for at least 4 days) was dissolved in 100ml of water. This solution was freshly prepared for every assay.

THE ASSAY

Into two stoppered sample tubes A and B was added 2.5 ml of buffer and 2.5 ml of substrate. 0.05 ml whole blood was added into tube A. The pH of both tubes was determined immediately and again after incubation for one hour. The pH of tube B, "Control" should not alter by more than 0.05 units. The difference in pH between tube A and tube B "Control" after incubation for one hour multiplied by a 100 gives the cholinesterase activity in pH units.

STANDARDIZATION OF pH METER

The pH meter was standardized before and during every experiment using pH 4 and pH 9 buffer solutions.

RESULTS

MALATHION

The results of this experiment are summarized in table 1. It can be seen that cholinesterase activity awas depressed in all four rabbits after 2 hours of administration of the drug percutaneously. The activity was still depressed after 3 hours, and the rate of depression appeared to be progressive.

The only symptoms of poisoning observed with these drugs were, tremor under the skin, and difficulties in breathing. In addition the animals appeared to be drowsy and relatively inactive as compared to animals that were not administered with the drug.

ETHYLPARATHION

The results of this experiment are summarized in table II Ethylparathion caused depression of cholinesterase activity in all four animals in less than 1.5 hours in all animals. The symptoms of poisoning, indicative of depression of cholinesterase activity and hence accumulation of acetylcholine were observed in all four animals in less than one hour.

The symptoms observed included the following: tremor of the facial muscles, uritability and increased motor activity which was followed by drowsiness and decrease movement, laboured and shallow breathing, excessive sativation, excretion of watery faeces, prostration, and convulsions followed by death, which in most cases occured in not less than three hours after administration of the drug.

CARBARYL

The results of both iexperiments where the drug was administered percutaneously and orally are summarized in table III and IV respectively.

The cholinesterase activity was depressed slightly in the case of percutaneous administration of the drug but no significant effects were observed within the first two hours of administration of the drug

However when the same drug was given orally in various doses as indicated in the experimental section, symptoms of poisoning indicative of accumulation of acetylcholine at the various cholinergic receptors were observed. The levels of cholinesterase activity did not appear to be depressed at all in most of the animals.

Symptoms of poisoning observed after oral administration of Carbaryl included the following, tremor under the skin surface, slight miosis, shallow and noisy laboured breathing, excessive salivation, extretion of watery faeces, and lacrimation. The symptoms of poisoning were observed to occur within 45 minutes of administration of the drug, and death occured within 2 hours in the group of animals that received a dose of 0.72 g kg^{-1} (grammes per kilogramme). In the two animals that received this dose, the blood cholinesterase activity did not appear to be deppressed at all.

DITHANE

The results of this experiment are recorded in tabble V. Although the drug was administered in relatively high doses, of 1.23 g per kg and 2.46g per kg, the cholinesterase activity was not depressed to any significant extent in most cases, except in one animal where it was depressed significantly. No symptoms of poisoning indicative of acetylcholine accumulation were observed.

TABLE I

MALATHION

"Control" = Blank sample containing no blood

pH_c = pH of blank sample containing no blood

pH_2 pH of incubated sample containing blood after incubation for one hour at 25°C

	$pH_c - pH_2$ ($\triangle$pH)	Cholinesterase activity (pH units)	% of Pretreatment Control
Rabbit 1			
Pretreatment control	0.325	32.5	
1st test sample	0.275	27.5	84.68
2nd Test sample	0.240	24.0	73.84
"Control" Blank	-	-	
Rabbit 2			
Pretreatment control	0.425	42.5	
1st test sample	0.375	37.5	88.23
2nd Test sample	0.275	27.5	64.70
"Control" Blank	-	-	
Rabbit 3			
Pretreatment control	0.300	30.0	
1st test sample	0.225	22.5	75.00
2nd test sample	0.200	20.0	66.67
"Control" Blank	-	-	
Rabbit 4			
Pretreatment control	0.450	45.0	
1st test sample	0.350	35.0	77.7
2nd test sample	0.30	30.0	66.67
"Control" Blank	-	-	

TABLE III

CARBARYL : PERCUTANEOUS

"Control" = Blank sample containing no blood

pHc is the pH of sample containing no blood

pH_2 = pH of incubated sample containing blood.

	$pHc - pH_2$ (pH)	Cholinesterase activity (in pH units)	% of pretreatment control
Rabbit 1			
Pretreatment control	0.250	25.0	
1st test sample	0.200	20.0	80.00
2nd test sample9	0.200	20.0	80.00
"Control"	-	-	
Rabbit 2			
Pretreatment control	0.250	25.0	
1st test sample	0.250	25.0	100.00
2nd test sample	0.200	20.0	80.00
"Control"		-	
Rabbit 3			
Pretreatment control	0.250		
1st test sample	0.250	25.0	100.00
2nd test sample	0.250	25.0	100.00
"Control"		-	
Rabbit 4			
Pretreatment control	0.300	30.0	
1st test sample	0.250	25.0	83.33
2nd test sample	0.250	25.0	83.33
"Control"			

TABLE IV

CARBARYL ORAL

"Control" = Blank sample containing no blood

pHc = pH of control (Blank sample without blood)

pH_2 = pH of sample containing blood after incubation for one hour at 25°C

	pHc - pH_2 (△ pH)	Cholinesterase activity (pH units)	% of pretreatment control
Rabbit 1			
Pretreatment control	0.375	37.5	100.00
1st test sample	0.375	37.5	100.00
2nd test sample	0.325	32.5	86.66
"Control" Blank	0.000	-	
Rabbit 2			
Pretreatment control	0.325	32.5	100.00
1st test sample	0.275	27.5	84.61
2nd test sample	0.275	27.5	84.61
"Control" Blank	0.000	-	
Rabbit 3			
Pretreatment control	0.300	30.0	100.0
1st test sample	0.300	30.0	100.0
2nd test sample	-	-	-
"Control" Blank	0.000	-	
Rabbit 4			
Pretreatment control	0.400	40.0	100.0
1st test sample	0.400	40.0	100.0
2nd test sample	0.400	40.0	100.0
"Control" Blank	0.000	-	

DISCUSSION

Results obtained in experiments using malathion clearly indicate that it is absorbed percutaneously. This is in agreement with observations recorded in literature by Gleason et al (1969) who have reported that malathion is significantly absorbed percutaneously in rabbits, and that such absorption may produce toxic effects. However, whereas malathion causes considerable depression of cholinesterase activity, results obtained from the present work do not indicate that it causes significant accumulation of acetylcholine at the myoneural junction, since no symptoms clearly indicative of such an accumulation were observed.

The depression of blood cholinesterse activity was progressive in all four animals used in the experiments as can be seen in table I. There appeared to be considerable individual variation with respect to extent of depression of cholinesterase activity, and hence susceptibility to anticholinesterases.

The data obtained in these experiments does not indicate that there is any relationship between the extent of poisoning and depression of whole blood cholinesterase activity but a more extensive study of the factors underlying cholinesterase inhibition by malathion is necessary before a concrete conclusion can be drawn.

The rate of inhibition of cholinesterase may be a function of absorption, percutaneously in this case, but it is clearly evident that other factors play a major role in determining the extent of poisoning. From the observation that no symptoms of poisoning.were observed despite considerable depression of whole blood cholinesterase activity, it appears that there might be a difference in susceptibility to malathion, among the various cholinesterases and that acetylcholinesterase found at the myoneural junction may be less susceptible to malathion than whole blood cholinesterase.

More research is required to determine the susceptibility of each of the various cholinesterases to inhibition by anticholinesterases as this may be of great importance in determining the extent of poisoning.

Ethylparathion, unlike malathion, is a potent cholinesterase inhibitor and therefore a dangerous poison, to both man and animals. From the results obtained in experiments using the drug, the depression of blood cholinesterase activity may be correlated with the extent of poisoning. The depression of cholinesterase activity was found to be related to symptoms of poisoning, indicative of accumulation of acetylcholine at the myoneural junction.

The central nervous system observed clearly indicate that ethylparathion crosses the blood brain barrier, and that in addition to any other effects that it may have, it causes depression of the central nervous system cholinesterase. The effects observed were initial central nervous system stimulation manifested as increased movement and irritability which was followed by drowsiness. It was also observed to cause convulsions which may be due to cerebral hypoxia. These were always followed by death in all the animals used.

The other symptoms indicative of acetylcholine accumulation at the various cholinergic receptor sites were observed in not more than one hour from the time of administration of the drug. The most prominent symptoms of poisoning observed were those involving the gastro intestinal tract, and the exogenous glands. These were excessive salivation, and watery diarrhoea.

As with malathion, ethylparathion was observed to cause progressive depression of cholinesterase activity. This was seen both from the determination of blood cholinesterase levels and also from the symptoms of poisoning.

However, the depression of cholinesterase activity proceeded at a faster rate with ethylparathion. This was in agreement with the findings of Gough and Shellenberger (1977) who claimed that inhibition of cholinesterases by dialkylphosphates may be considered to be due to direct phosphorylation of the active esteratic site of the enzyme through an electrophilic mechanism, which may be followed by spontaneous reactivation or "aging" i.e. conversion to a non-reversible enzyme unhibitor complex. These workers suggested that the extent of spontaneous reactivation or aging depends on the alkyl groups substituted on the phosphate radical. The susceptibility, of the hydroxy group of serine (appendix III) of the esteratic site of the enzyme depends on the electrophilicity of the phosphate radical. The phosphate radical of ethylparathion being more electrophilic than that of malathion makes ethylparathion a more potent inhibitor of cholinesterase. The rate of spontaneous reactivation of the phosphorylated enzyme may be greater in the case of malathion than in the case of ethylparathion. This could not be clearly demonstrated in the present work because the method of cholinesterase determination used does not allow for rapid and simultaneous assay of blood samples, and that in the case of ethylparathion, the animals did not survive for long enough periods to allow for the appropriate observations.

Carbaryl has been classified by some authorities as a potent cholinesterase inhibitors. Gleason et al (1969). However, results obtained in experiment where carbaryl was administered percutaneously, and orally, there was only slight depression of cholinesterase activity in some of the animals. Whereas no symptoms clearly indicative of acetylcholine accumulation at the various cholinergic receptor sites were observed when carbaryl was administered percutaveously, the blood cholinesterase activity was slightly depressed.

This indicates that carbaryl is absorbed percutaneously but only to a limited extent. The relatively low toxicity of carbaryl when applied percutaneously could be attributed to this limited extent of absorption, but it could in addition be due to the rate of hydrolyses of the carbamoylated enzyme, to regenerate the enzyme being greater than the rate of absorption (percutaneously).

When carbaryl was administered orally, symptoms of poisoning indicative of accumulation of acetylcholine at the myoneural function were observed. These effects were observed within the first 45 minutes in all the four animals used, indicating not only significant absorption of carbaryl from the gastro intestinal tract but also a rapid rate of absorption. Despite the observation of the various symptoms of poisoning with carbaryl, there was only slight or no depression of cholinesterase activity. Since the symptoms of poisoning indicate accumulation of acetylcholine and therefore inhibition acetylcholinesterase, it seems that the different cholinesterases may exhibit differences in susceptibility to anticholinesterases. Thus acetylcholinesterase, from the results obtained with oral administration of carbaryl, does appear to be more susceptible than whole blood cholinesterase.

Increasing the dose of carbaryl administered orally not only enhanced the severity of poisoning, but was also observed to shorten the duration of time between administration of the drug and onset of symptoms of poisoning. This clearly indicates that absorption of carbaryl from the gastrointestinal tract is a function of concentration of the drug therein and that its effect on acetylcholinesterase may be dependent on plasma concentration, and therefore concentrating at the receptor sites.

Results obtained when dithane, a dithiocarbamate, was administered orally indicate that it does not depress cholinesterase activity in rabbits.

Despite the relatively high doses administered no symptoms of poisoning indicative of acetylcholine accumulation were observed. The cholinesterase activity was unaffected in most of the animals used, and only in one case did there appear to be any depression of enzyme activity. This could only, in the light of results obtained and observations made, be attributed to idiosyncratic responces.

The fact that dithane although a carbamate does not depress cholinesterase activity indicates structural specificity of the enzyme. Dithane may not possess a carbon atom that is electrophilic enoggh to attack the hydroxy group of serine at the active esteratic site of the enzyme. Unlike ethylparathion and malathion, dithane does not appear to be activated by microsomal oxidases to an active Oxo form.

CONCLUSSION

Malathion, Carbaryl and Ethylparathion are all absorbed percutaneously but their relative potencies as cholinesterase inhibitors differ. Percutaneous absorption of malathion and carbaryl does not cause any significant effects on rabbits, but that of ethylparathion does produce severe toxic effects, indicating clearly that ethylparathion is a more potent poison.

Carbaryl is absorbed to a greater extent from the gastro-intestinal tract than percutaneously, Therefore toxicity of this drug is related to route of administration. Oral ingestion of carbaryl may not only rapidly produce symptoms of poisoning but could be fatal.

Dithane is either not absorbed from the gastrointestinal tract, or it has little or no anticholinesterase activity. It is in this respect safer as an agricultural chemical than the above anticholinesterases.

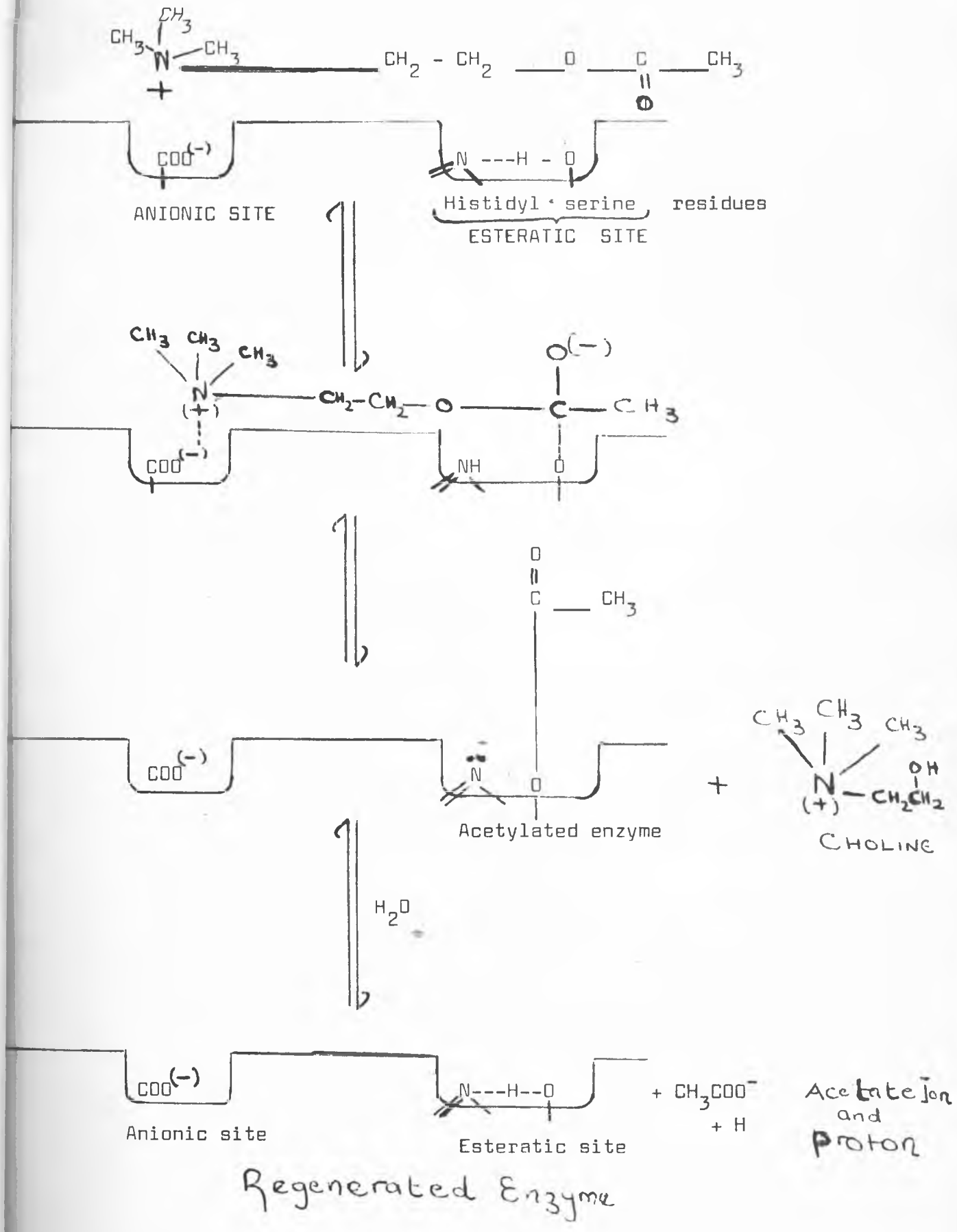
APPENDIX I

FREQUENCY OF POISONING DUE TO THE ORGANO PHOSPHATES (MALATHION AND PARATHION) AND THE CARBAMATES

YEAR	# OF CASES	OF DEATHS DUE TO ORGANOPHOSPHATES	OF DEATHS DUE TO CARBAMATES
1973	252	31	1
1974	255	19	2
1975	254	27	
1976	253	38	
1977	265	37	
	1279	152	3

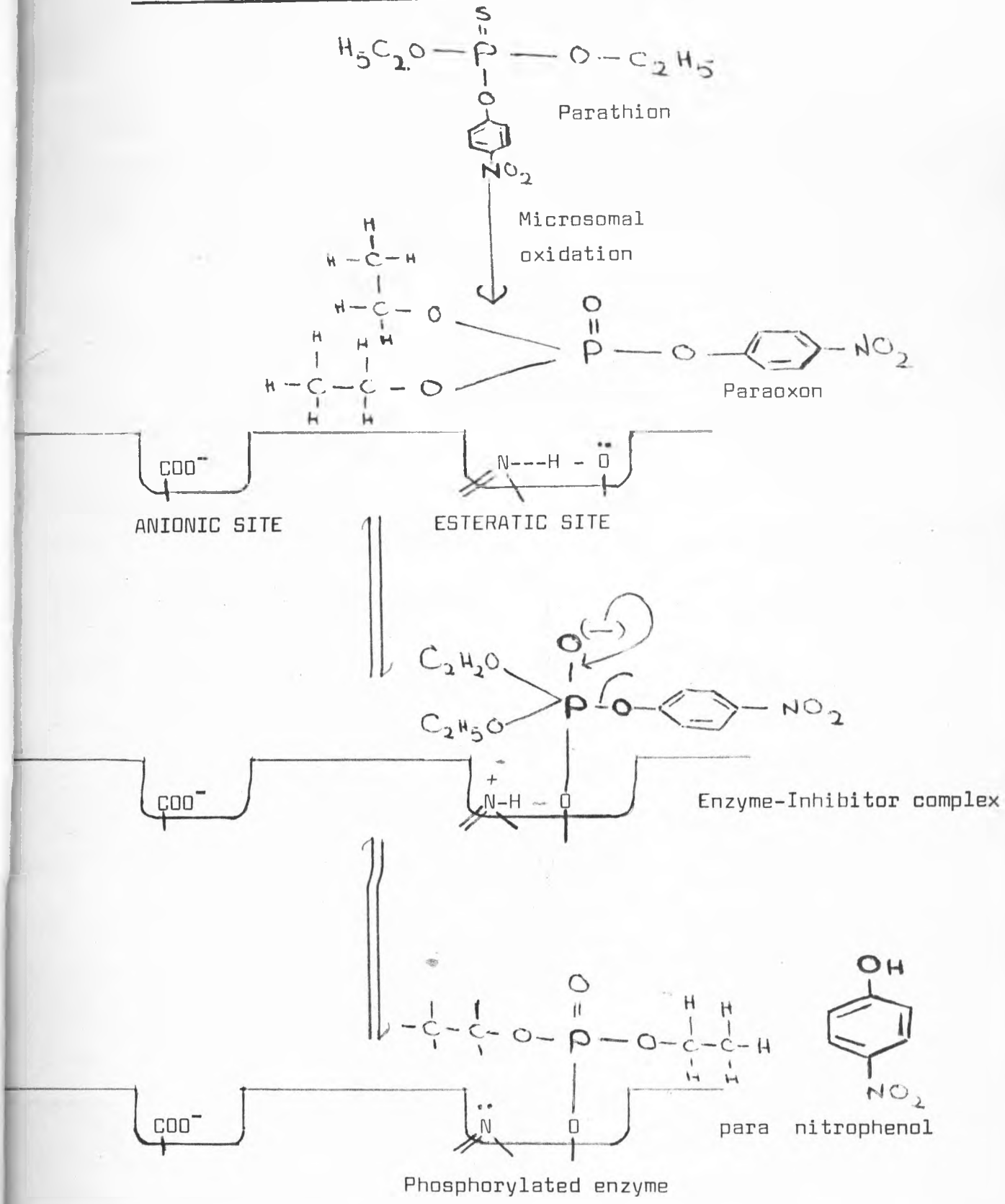
APPENDIX II

MECHANISM FOR THE ENZYMATIC HYDROLYSIS OF ACETYLCHOLINE



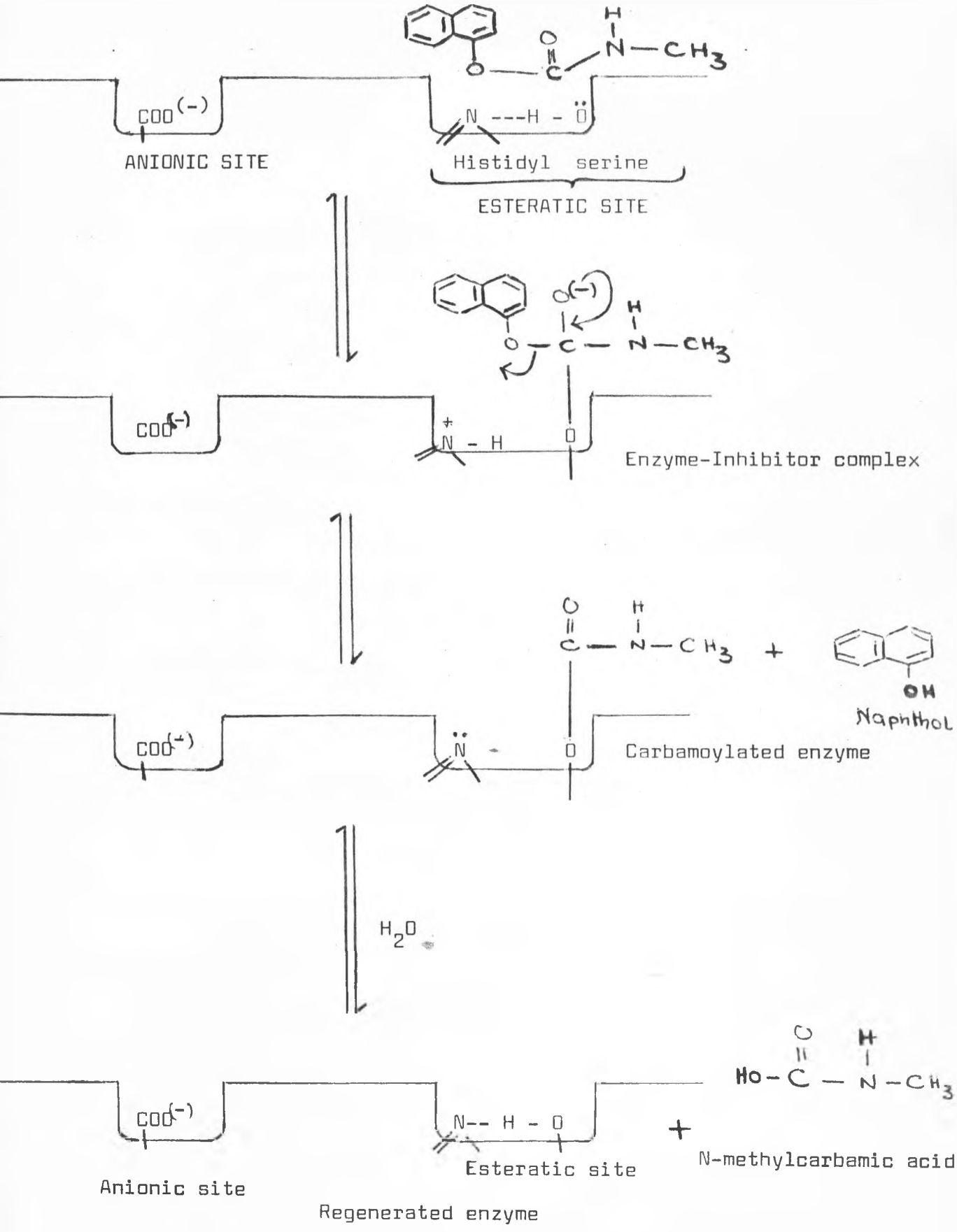
APPENDIX III

MECHANISM FOR THE INHIBITION OF CHOLINESTERASE BY PARATHION



APPENDIX IV

MECHANISM OF INHIBITION OF CHOLINESTRASE BY CARBARYL



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