Biochemical Pharmacology, 1963, Vol. 12, pp. 1241–1252. Pergamon Press Ltd., Printed in Great Britain.

CHOLINESTERASE ACTIVITY IN *HAEMONCHUS CONTORTUS* AND ITS INHIBITION BY ORGANOPHOSPHORUS ANTHELMINTICS

R. M. LEE and MARGARET R. HODSDEN

Cooper Technical Bureau, Berkhamsted, Herts.

(*Received* 9 *May* 1963; *accepted* 12 *July* 1963)

Abstract—An enzyme hydrolysing the acetyl, acetyl-β-methyl and butyryl esters of choline has been demonstrated in homogenates of the parasitic nematode, *Haemonchus contortus*. It is concluded that there is no evidence for the existence of more than one cholinesterase in this species. The effects of temperature, pH, substrate concentration and inhibitors on the cholinesterase of *H. contortus* have been described. The reactions of Di-(2-chloroethyl) (3-chloro-4-methylcoumarin-7-yl) phosphate (Haloxon) with the cholinesterases of *H. contortus* and sheep erythrocytes have been studied. The results are discussed with regard to the fact that Haloxon is toxic to *H. contortus* but not to the sheep host. The cholinesterase of another helminth parasite, *Trichuris ovis*, is much less susceptible to inhibition by Haloxon than that of *H. contortus*. The inability of Haloxon to remove *T. ovis* from infected sheep is attributed to this reduction in cholinesterase inhibition.

ALTHOUGH various organophosphorus compounds have been shown to possess anthelmintic properties when dosed to livestock, there are few accounts of the action of these compounds on the enzyme systems of the parasites involved. Nimmo-Smith and Keeling[1] have described a non-specific esterase in *Trichuris muris*, which is very sensitive to inhibition by paraoxon, but the reaction between a cholinesterase of a worm parasite and an organophosphorus anthelmintic has not been reported. Indeed, the only experiments published which reveal the characteristics of a helminth cholinesterase are those of Bueding[2] for *Schistosoma mansoni*.

The present work was undertaken to gain some knowledge of the properties of the enzyme(s) which hydrolyse acetycholine in the economically important species, *Haemonchus contortus*, and to compare these properties with those of certain mammalian cholinesterases, having especial regard to the action of organophosphorus inhibitors on these enzymes.

A comparison of the anti-cholinesterase activity of certain anthelmintic and non-anthelmintic organophosphorus compounds was made in the hope that differences in their specificity for host or parasite cholinesterase would be revealed. Any such differences might explain the observation that some organophosphorus compounds are safe and efficient anthelmintics, whereas others are inefficient or too toxic to the host animal for use.

EXPERIMENTAL

Materials

Adult *Haemonchus contortus* and *Trichuris ovis* were obtained from the stomach and caecal contents of sheep, placed in 0·9% NaCl and stored at −20° until required.

Under these conditions, the cholinesterase of the helminth parasites was stable for several weeks, whereas considerable loss of activity occurred if the worms were stored at 0°.

Third stage larvae of *H. contortus* were obtained by infecting lambs (reared worm-free) with a few larvae of this species and culturing the faeces when the infection became patent. After 10–14 days incubation at 27°, the larvae were obtained from the cultures by extraction in a modified Baermann apparatus.

For the estimation of enzyme activity, the helminth material was homogenised in distilled water in an all-glass apparatus and used immediately, as 80 per cent or more of the enzyme activity was lost if the homogenate was stored at 0° overnight.

Plasma from sheep and guinea-pigs was obtained by centrifuging blood at 2000 rev/min for 20 min. Red blood cells were obtained by centrifuging and washing the erythrocyte layer three times with 0·9% NaCl to remove the plasma and leucocytes.

Estimation of esterase activity

Esterase activity was measured manometrically in a conventional Warburg apparatus using the technique of Ammon,[3] and colorimetrically by the Fleisher, Pope and Spear[4] modification of the method described by Hestrin.[5]

Measurements were made in duplicate, at 37° and corrected for non-enzymic hydrolysis of the substrate.

Manometric estimations were carried out in 0·025 M $NaHCO_3$, 0·1 M NaCl solutions saturated with 5% CO_2 in N_2 (pH 7·4). This method was used for most of the experiments not involving a change in pH, and evolution of CO_2 was followed for 60 min after the addition of a substrate.

In the colorimetric estimations, changes in optical density were measured by means of a spectrophotometer (Unicam, SP 600). Esterase activity is expressed throughout as μM of substrate hydrolysed per gram fresh tissue per hour (μM/g/hr).

Substrates

(1) Acetylcholine perchlorate (ACh) (British Drug Houses, Ltd.).
(2) Acetyl-β-methylcholine chloride (MeCh) (L. Light & Co. Ltd.).
(3) *n*-Butyrylcholine chloride (BuCh) (L. Light & Co. Ltd.).
(4) Methyl *n*-butyrate (British Drug Houses, Ltd.).
(5) Glycerol tributyrate (tributyrin) (British Drug Houses, Ltd.).
(6) *p*-Nitrophenyl acetate (Cooper Technical Bureau).

Inhibitors

(1) Diethyl *p*-nitrophenyl phosphate (paraoxon) (Cooper Technical Bureau).
(2) Di-(2-chloroethyl) (3-chloro-4-methylcoumarin-7-yl) phosphate (Haloxon) (Cooper Technical Bureau).
(3) Dimethyl 1-hydroxy-2-trichloroethylphosphonate, (trichlorphon), (Farbenfabriken Bayer A.G.).
(4) N,N^{I},N^{II},N^{III} Tetra*iso*propylpryrophosphoramide, (TIPA), (Cooper Technical Bureau).
(5) Physostigmine, (Eserine), (British Drug Houses, Ltd.).
(6) 1,5-Bis-(4-trimethylammoniumphenyl)pentan-3-one di-iodide, (62.C.47), (a gift from Dr. A. McCoubrey of the Wellcome Research Laboratories, Beckenham).

All substrates and inhibitors were dissolved in distilled water where this was possible. Methyl butyrate, glycerol tributyrate, eserine and Haloxon were taken up in a small quantity of acetone before dilution with water.

RESULTS

The relative rates of hydrolysis of choline esters

A comparison of the relative rates of hydrolysis of ACh, MeCh and BuCh by *Haemonchus controlus* homogenates showed that all three choline esters were hydrolysed, the rates for MeCh and BuCh being about 50 per cent of that for ACh (Table 1).

TABLE 1. RELATIVE RATES OF HYDROLYSIS OF ESTERS BY *H. contortus* AND MAMMALIAN TISSUES

Enzyme source	Hydrolysis rate, (μM/g per hr) of:					
	ACh	MeCh	BuCh	Methyl butyrate	Tributyrin	p-nitrophenyl acetate
H. contortus (larvae)	56	30	29	0	0	17
H. contortus (adults)	39	22	22	—	—	—
Sheep:–						
erythrocytes	64	26	2	—	44	—
plasma	0	0	0	—	—	—
Guinea-pig:–						
erythrocytes	48	24	2	—	—	—
plasma	40	2	78	105	—	—

(Activity was measured manometrically over 0–30 min. Substrate concentration 10^{-2} M).

Sheep erythrocytes and plasma were also tested for hydrolysis of these esters; only the erythrocytes possessed activity, MeCh being hydrolysed at 40 per cent, and BuCh at 3 per cent of the rate for ACh.

The results obtained with guinea-pig erythrocytes and plasma demonstrated that the erythrocyte cholinesterase (ChE) had a low hydrolysis rate for BuCh, whereas the plasma enzyme exhibited the highest rate of hydrolysis with BuCh, and had little activity against MeCh.

TABLE 2. EFFECT OF CENTRIFUGATION ON THE HYDROLYSIS OF ACh AND BuCh BY *H. contortus* HOMOGENATES

Preparation	Rate of hydrolysis, μM/ml/hr				Activity as percentage of whole homogenate activity			
	ACh		BuCh		ACh		BuCh	
	1	2	1	2	1	2	1	2
Whole homogenate (10% fresh tissue)	3·4	2·8	1·9	1·6				
Supernatant, after centrifuging at 3000 rev/min for 20 min	1·7	1·4	1·0	0·8	50	50	53	50
Residue, resuspended in water to original volume of homogenate	1·7	1·4	0·9	0·8	50	50	47	50

The relative rates of hydrolysis of non-choline esters

No hydrolysis of methyl-butyrate or tributyrin could be demonstrated in homogenates of *H. contortus*, but *p*-nitrophenyl acetate was hydrolysed.

Under the same conditions, sheep erthrocytes hydrolysed tributyrin and guinea-pig plasma hydrolysed methyl butyrate (Table 1).

The possibility of more than one enzyme hydrolysing choline esters in H. contortus *homogenates*

Since a considerable hydrolysis of BuCh was found in homogenates of *H. contortus*, the possibility that more than one ChE was present in these homogenates was examined further.

Homogenates of *H. contortus* were centrifuged at 3000 rev/min for 20 min. Hydrolysis of ACh by the supernatant accounted for 51 per cent of the activity of the original homogenate, the remaining 49 per cent being found in the residue (Table 2).

TABLE 3. HYDROLYSIS RATES OF MIXED SUBSTRATES BY *H. contortus* AND MAMMALIAN TISSUES

Enzyme source	Hydrolysis rate, μM/g/hr, ACh $2{\cdot}5 \times 10^{-3}$ M	BuCh $2{\cdot}5 \times 10^{-3}$ M	ACh + BuCh Found	ACh + BuCh Calc.	Summation %
H. contortus	55	19	30	74	40
Sheep blood	68	2	24	70	34
Guinea-pig blood	40	48	71	88	80

TABLE 4. EFFECT OF INHIBITORS ON THE HYDROLYSIS OF ACh AND BuCh BY *H. contortus* AND MAMMALIAN TISSUES

Name	Molar concentration	Percentage inhibition after 15 min: *H. contortus* ACh	*H. contortus* BuCh	Sheep erythrocyte ACh	Guinea-pig erythrocyte ACh	Guinea-pig plasma ACh	Guinea-pig plasma BuCh
Paraoxon	10^{-7}	28	21	45	40	47	46
Paraoxon	2×10^{-7}	61	58	—	—	—	—
Haloxon	5×10^{-7}	89	81	34	37	50	52
62.C.47	10^{-7}	0	—	80	82	1	—
62.C.47	10^{-6}	10	6	91	87	1	—
62.C.47	10^{-5}	49	46	96	95	3	2
62.C.47	10^{-4}	84	77	96	96	23	20
62.C.47	10^{-3}	95	—	94	96	74	75
TIPA	10^{-7}	0	0	—	1	0	0
TIPA	10^{-5}	0	0	—	7	75	75
TIPA	10^{-3}	0	0	20	15	87	84
Eserine	10^{-6}	48	—	87	82	80	—
Eserine	10^{-5}	76	73	96	87	92	—
Eserine	10^{-4}	100	—	100	99	100	—

(Inhibition was carried out at 37°, pH 7·4 in double side-arm Warburg flasks. The final substrate concentration was 10^{-2} M).

When BuCh was the substrate, approximately the same division of activity between the supernatant and the residue was found.

The hydrolysis of a mixture of equimolar concentrations of ACh and BuCh was compared using *H. contortus* homogenates, sheep blood and guinea-pig blood as sources of ChE enzymes. The results of these experiments showed that the rate of hydrolysis of this mixture by *H. contortus* and sheep blood was lower than that for ACh alone, but higher than that for BuCh alone. Using guinea-pig blood, the mixture of substrates produced a greater release of CO_2 than with either substrate alone, but not as great as the sum of the individual hydrolysis rates of ACh and BuCh (Table 3).

The same sources of ChE enzymes were used to compare the effects of various inhibitors on the rate of hydrolysis of ACh and BuCh (Table 4).

TABLE 5. VELOCITY CONSTANTS FOR THE REACTIONS OF *H. contortus* AND SHEEP ERYTHROCYTE CHOLINESTERASE WITH PARAOXON, AND OF *H. contortus* CHOLINESTERASE WITH HALOXON

Cholinesterase source	Inhibitor	Velocity constant k (l. mol $^{-1}$ min $^{-1}$)
H. contortus	Haloxon	$7{\cdot}3 \times 10^5$
H. contortus	Paraoxon	$1{\cdot}9 \times 10^5$
Sheep erythrocytes	Paraoxon	$1{\cdot}2 \times 10^6$

The results demonstrate that none of the inhibitors used against *H. contortus* ChE was able to inhibit the hydrolysis of one choline ester while leaving the hydrolysis of the other unaffected. In general, the inhibition of BuCh hydrolysis was slightly less than that of ACh under the same conditions, except in the case of TIPA, which had no effect on the hydrolysis of either substrate at concentrations up to 10^{-3} M.

By comparison, the guinea-pig plasma ChE was strongly inhibited by TIPA at concentrations greater than 10^{-5} M.

Both Haloxon, which showed a preference for inhibiting the plasma butyrylcholinesterase of the guinea-pig, and 62.C.47, which was specific for erythrocyte acetylcholinesterase at 10^{-5} M, produced a similar degree of inhibition of *H. contortus* ChE when BuCh was substituted for ACh as the substrate.

The hydrolysis of both ACh and BuCh by homogenates of *H. contortus* was inhibited by eserine, although this inhibitor was more effective against the mammalian cholinesterases.

The effect of temperature on the cholinesterase activity of H. contortus

The maximum ChE activity of *H. contortus* homogenates was observed at 39°. Inactivation of the enzyme was rapid above 40°, but temperature dependence below the optimum was not marked. The activation energy (E) of the reaction was calculated to be 5,800 cal/mole, using the Arrhenius equation,

$$\log \frac{k}{k_1} = -\frac{E}{2{\cdot}303\,R} \cdot \left(\frac{1}{T} - \frac{1}{T_1}\right)$$

where k and k_1 are the velocity constants at temperatures T and T_1. In Fig. 1 are plotted the hydrolysis rates of ACh by *H. contortus* against temperature.

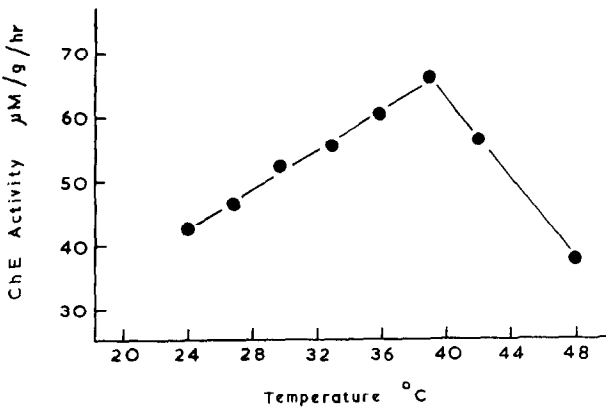

FIG. 1. The effect of temperature on the cholinesterase (ChE) activity of *H. contortus* homogenate. Substrate—Acetylcholine 2×10^{-3} M, pH 7·4

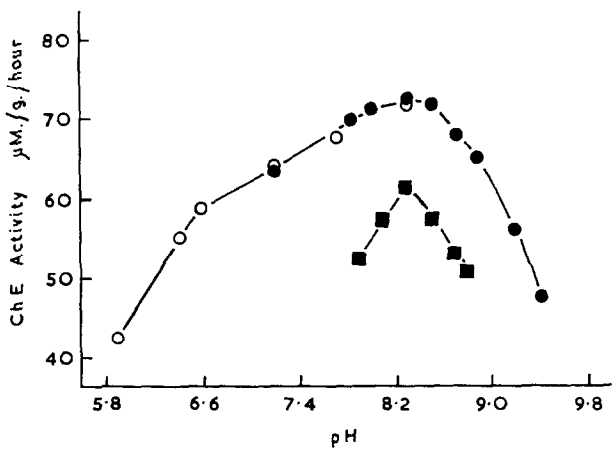

FIG. 2. The effect of changes in pH on the cholinesterase (ChE) activity of *H. contortus* homogenate. Substrate—Acetylcholine 2×10^{-3} M. Temp. 37°. ● = 0·1 M tris-HCl buffer; O = 0.15 M phosphate buffer, ■ = 0·1 M borate buffer.

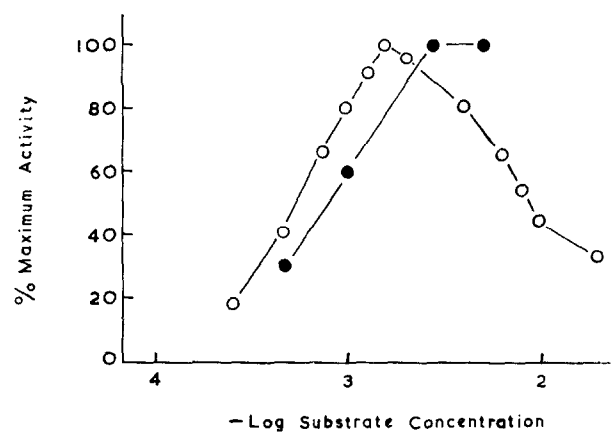

FIG. 3. The effect of substrate concentration on the cholinesterase activity of *H. contortus* homogenate. Temperature, 37°, pH 7·4. Substrate—O = Acetylcholine, ● = Butyrylcholine.

The effect of pH on the cholinesterase activity of H. contortus

The cholinesterase activity of *H. contortus* homogenates reached a maximum at pH 8·3 in phosphate, borate and Tris–HCl buffers (Fig. 2). Borate buffer was inhibitory and produced a sharper peak than the other buffers. In phosphate and Tris–HCl buffers the enzyme was active over a wide range of pH.

The Effect of substrate concentration on the cholinesterase activity of H. contortus

The ChE activity of *H. contortus* homogenates increased with increasing ACh concentration from 0·25 mM to 2·0 mM, above the latter concentration a slow decrease of activity occurred as the ACh concentration was increased.

When BuCh was the substrate, no inhibition by excess substrate was found, although the minimum concentration of BuCh necessary for maximum enzyme activity was also 2·0 mM (Fig. 3).

Rates of reaction of organophosphorus inhibitors with helminth and sheep cholinesterases

The rates of reaction of paraoxon and Haloxon with the cholinesterases of *Haemonchus contortus* and sheep erythrocytes have been determined.

The results of these experiments (Figs. 4 and 5) show that the reactions between *H. contortus* ChE and both paraoxon and Haloxon are time dependent, whereas only

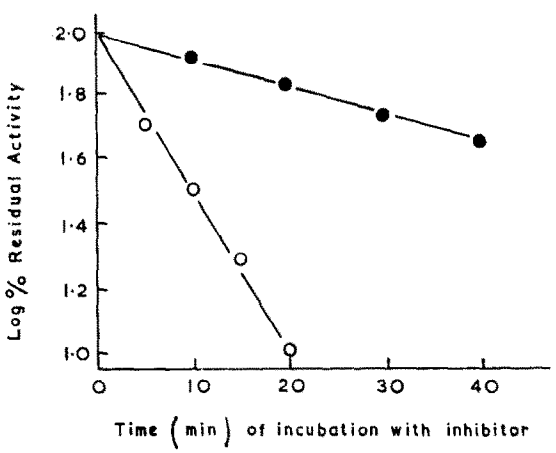

FIG. 4. The inhibition of sheep erythrocyte and *H. contortus* cholinesterases by 10^{-7} M paraoxon. Substrate—Acetylcholine, 10^{-2} M. Temp 37°, pH 7·4 ○ – Sheep erythrocyte cholinesterase, ● – *H. contortus* cholinesterase.

paraoxon gives a straight line relationship when log percentage activity is plotted against time for sheep erythrocyte ChE. The inhibition of sheep erythrocyte ChE by Haloxon is independent of time after the initial 10 min incubation with inhibitor at 37°.

A plot of the reciprocal of the inhibitor concentration against time for 50 per cent inhibition (Fig. 6) also gave straight lines for the reactions of *H. contortus* ChE with paraoxon and Haloxon, and of sheep erythrocyte ChE with paraoxon. Thus, in these

three instances, the reaction is bimolecular; the velocity constants (k) for these reactions have been calculated from

$$k = \frac{1}{tI} \ln \frac{100}{b}$$

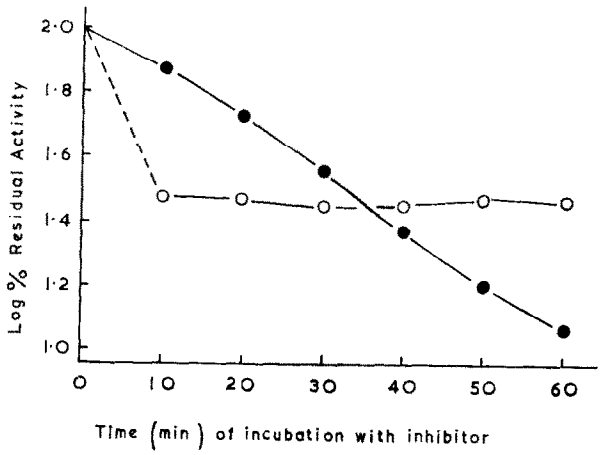

Fig. 5. The inhibition of sheep erythrocyte and *H. contortus* cholinesterases by Haloxon. Substrate—Acetylcholine 10^{-2} M; Temp 37°, pH 7·4. ○ = Sheep erythrocyte cholinesterase, 5×10^{-7} M Haloxon, ● = *H. contortus* cholinesterase 5×10^{-8} M Haloxon.

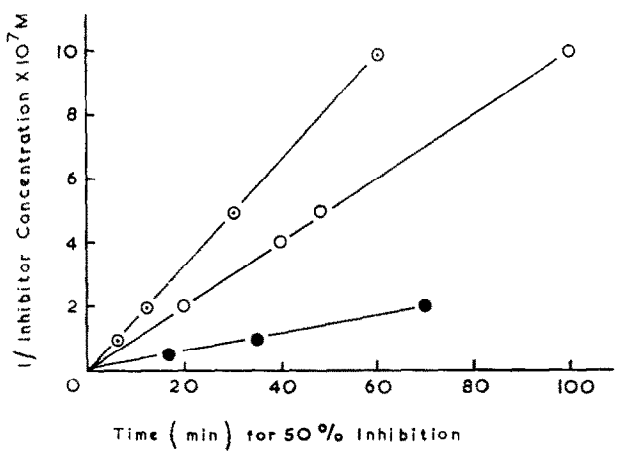

Fig. 6. The relationship between time of inhibition and concentration of inhibitor for the cholinesterases of sheep erythrocytes and *H. contortus*. Substrate—Acetylcholine, 10^{-2} M. Temp 37°, pH 7·4. ○ = Sheep erythrocyte cholinesterase with paraoxon, ● = *H. contortus* cholinesterase with paraoxon, ○ = *H. contortus* cholinesterase with Haloxon.

where t = time of inhibition, I = molar concentration of inhibitor and b = percentage residual activity (Table 5). The velocity constant for the inhibition of sheep erythrocyte cholinesterase by paraoxon was found to be $1{\cdot}2 \times 10^6$ l.mol^{-1}min^{-1}, which is in excellent agreement with the value of $1{\cdot}1 \times 10^{-6}$ l.mol^{-1}min^{-1} given by Aldridge and Davison.[6]

The pattern of CO_2 evolution by the cholinesterases of *H. contortus* and sheep erythrocytes was followed after inhibition by paraoxon and Haloxon (Figs. 7 and 8). After inhibition of sheep erythrocyte ChE by Haloxon, it was found that the production of CO_2 per unit time increased with time after the addition of substrate (Fig. 8), demonstrating that, in the presence of excess substrate, the inhibition of sheep erythrocyte ChE by Haloxon is reversible. Forty minutes after the addition of ACh, the activity of the inhibited erythrocyte ChE was equal to that of the control.

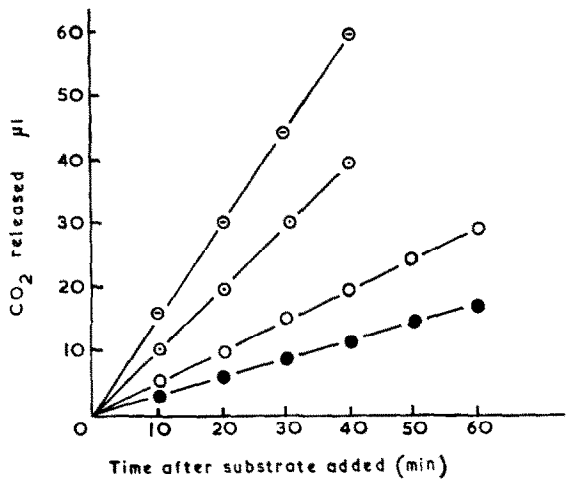

FIG. 7. The pattern of CO_2 evolution by sheep erythrocyte and *H. contortus* cholinesterases, in the presence of 10^{-2} M acetylcholine, after inhibition by paraoxon for 10 min. Temp 37°, pH 7·4. ⊖ = Sheep erythrocyte cholinesterase—uninhibited control, ○ = Sheep erythrocyte cholinesterase + 10^{-7} M paraoxon, ○ = *H. contortus* cholinesterase—uninhibited control, ● = *H. contortus* cholinesterase + 4×10^{-7} M paraoxon.

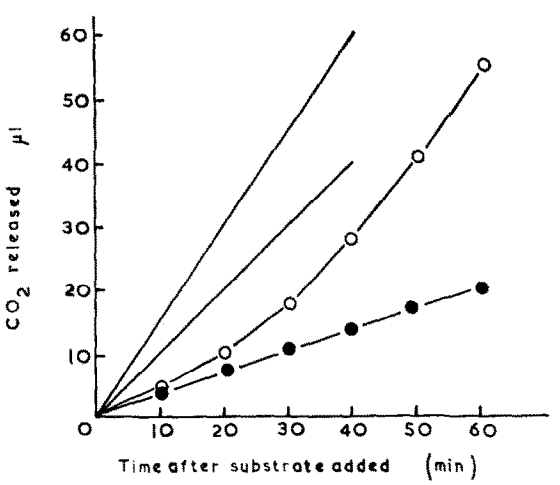

FIG. 8. The pattern of CO_2 evolution by sheep erythrocyte and *H. contortus* cholinesterases, in the presence of 10^{-2} M acetylcholine, after inhibition by 10^{-7} M Haloxon for 15 min. Temp 37°, pH 7·4. ○ = Sheep erythrocyte cholinesterase + Haloxon, ● = *H. contortus* cholinesterase + Haloxon. The controls shown in Fig. 7 are included for comparison.

After incubation of sheep erythrocyte ChE with paraoxon, and of *H. contortus* ChE with paraoxon and Haloxon, no reversibility could be demonstrated, the evolution of CO_2 with time by inhibited enzyme preparations being linear.

Correlation of anthelmintic efficiency with ability to inhibit cholinesterase in organophosphorus compounds

Trials of Haloxon have shown that this organophosphorus compound is an efficient anthelmintic for *H. contortus*, but is not effective in removing *T. ovis* from the intestine of sheep.[7] Trichlorphon (Dimethyl 1-hydroxy-2,2,2,-trichloroethylphosphonate), on the other hand, is an effective anthelmintic for both *T. ovis* and *H. contortus*.[8,9]

A comparison of the inhibition of the cholinesterases of these two helminth species by Haloxon and trichlorphon (Table 6) showed that the ChE of *H. contortus* was

TABLE 6. INHIBITION OF THE CHOLINESTERASE OF *H. contortus* AND *T. ovis* BY HALOXON AND TRICHLORPHON

Inhibitor		Percentage inhibition 15 min	
Name	Concentration	*H. contortus*	*T. ovis*
Haloxon	10^{-6}	90	24
Trichlorphon	10^{-5}	55	67

(Inhibition was carried out at 37°, pH 7·4 in double side-arm Warburg flasks. The substrate was acetylcholine 10^{-2} M).

slightly less susceptible to trichlorphon at 10^{-5} M than was that of *T. ovis*, whereas the *H. contortus* ChE was much more susceptible to Haloxon at 10^{-6} M than was the *T. ovis* enzyme.

DISCUSSION

The inability of homogenates of *H. contortus* to hydrolyse aliphatic esters such as methyl butyrate and tributyrin suggested that no ali-esterase was present. Hydrolysis of choline esters by *H. contortus* was demonstrated, and this hydrolysis could be inhibited by the addition of eserine, suggesting that the enzyme(s) responsible was a cholinesterase rather than a non-specific esterase.

The hydrolysis of BuCh by *H. contortus* homogenates was as rapid as that of MeCh (about 50 per cent of the ACh hydrolysis rate), and this finding posed the question of whether more than one cholinesterase was present in these homogenates, since high rates of BuCh hydrolysis by mammalian acetylcholinesterases are rare.[10–12] However, hydrolysis of MeCh by a butyrylcholinesterase is known to occur in chicken plasma,[13, 14] and Arthropod acetylcholinesterases are also known which hydrolyse both MeCh and BuCh.[15, 16]

Differentiation of two cholinesterases in *Schistosoma mansoni* homogenates was achieved by centrifugation at 18,000 rev/min;[2] the butyrylcholinesterase activity remained in the supernatant, whereas the acetylcholinesterase was entirely in the residue. Centrifugation of *H. contortus* homogenates at 3000 rev/min for 20 min

merely divided the cholinesterase activity into a supernatant fraction (50 per cent) and a residue fraction (50 per cent), no difference being found when BuCh was substituted for ACh as the substrate. If two cholinesterases were present in these homogenates, they were not so different in solubility as to be separated by low-speed centrifuging.

In experiments where ACh and BuCh were presented to the helminth enzyme system simultaneously, the combined hydrolysis rate was less than that observed with ACh alone. This result is similar to that found when using sheep blood, where an acetylcholinesterase in the red cells is the only cholinesterase present.[17] A comparison with guinea-pig blood, which has cholinesterase enzymes in the plasma and red cells, showed that where a high rate of BuCh hydrolysis existed the release of CO_2 in the presence of both ACh and BuCh was in excess of that produced with either substrate alone. This result again suggests that one acetylcholinesterase only is present in *H. contortus* homogenates.

An attempt to differentiate two cholinesterases in *H. contortus* homogenates by the use of selective inhibitors was equally unsuccessful.

TIPA, which is an accepted inhibitor of butyrylcholinesterases from mammalian tissues[18, 19] had no effect at 10^{-3} M on the hydrolysis of ACh or BuCh by *H. contortus* homogenates.

Compound 62.C.47, which is a specific inhibitor for acetylcholinesterases in mammals,[20] was ineffective as an inhibitor of *H. contortus* cholinesterase at concentrations which strongly inhibited guinea-pig erythrocyte cholinesterase. On the other hand, 62.C.47 was a better inhibitor of *H. contortus* cholinesterase than of the plasma cholinesterase of the guinea-pig.

A comparison of the hydrolysis rates of ACh and BuCh by *H. contortus* after inhibition by 62.C.47 showed that the hydrolysis of BuCh was slightly less affected. Paraoxon and Haloxon gave similar results, but the difference in inhibition never amounted to more than 8 per cent, and cannot be considered as good evidence of the existence of more than one cholinesterase in this helminth species.

These results provide the strongest evidence that ACh and BuCh are hydrolysed by the same cholinesterase in these worms, and it is concluded that there is no evidence for the existence of two cholinesterases in *H. contortus* homogenates.

The properties of the cholinesterase of *H. contortus* resembled those of a mammalian acetylcholinesterase in that it was inhibited by excess ACh, hydrolysed MeCh at a relatively rapid rate and was insensitive to TIPA. It could be distinguished from the acetylcholinesterase of most mammals by the fact that the helminth cholinesterase was capable of higher than usual rates of BuCh hydrolysis, and was less susceptible to inhibition by such compounds as 62.C.47 and paraoxon.

A major difference between the cholinesterase of *H. contortus* and that of its host was found in their reaction with Haloxon. The reaction of *H. contortus* cholinesterase with Haloxon was bimolecular and irreversible, whereas the reaction between sheep erythrocyte cholinesterase and Haloxon, although probably bimolecular, was reversible. In symbolic terms, *H. contortus* cholinesterase reacts with Haloxon according to equation (1), sheep erythrocyte cholinesterase according to equation (2)

$$E + I_{A+B} \leftrightharpoons EI_{A+B} \rightarrow EI_A + B \qquad (1)$$

$$E + I_{A+B} \leftrightharpoons EI_{A+B} \rightarrow EI_A + B \rightarrow E + I_A \qquad (2)^*$$

* The evidence for assuming that sheep erythrocyte cholinesterase is phosphorylated will be published shortly.

where *A* and *B* are, respectively, dichloroethyl phosphate and 3-chloro-4-methyl-7-hydroxycoumarin, the two direct hydrolysis products of Haloxon.

In *H. contortus* homogenates, EI_1 is stable whereas in sheep erythrocyte preparations it is not, so that active enzyme is released. This reaction of sheep erythrocyte cholinesterase with Haloxon is very similar to that described by Aldridge[21] for the reaction between rabbit erythrocyte cholinesterase and methyl-paraoxon.

Thus, the efficiency of Haloxon as an anthelmintic can be explained by these findings that the cholinesterase of *H. contortus* is irreversibly inhibited by this organophosphorus compound, whereas the cholinesterase of the sheep host is only temporarily affected, recovery being rapid in the absence of a sustained high concentration of Haloxon. In this way, any effect that Haloxon has on the cholinesterase of the host animal is rapidly reversed as excretion of the drug takes place, but the cholinesterase of the helminth parasite is inactivated for a long period.

It has been shown that Haloxon is a much more effective inhibitor of the cholinesterase of *H. contortus* than of the same enzyme from *Trichuris ovis*, but that trichlorphon inhibits the cholinesterase from both species about equally. This finding offers an explanation of the fact that trichlorphon is a more efficient anthelmintic than Haloxon for *T. ovis*, and suggests that the inhibition of cholinesterases in helminths may be the mode of action of organophosphorus anthelmintics. This suggestion presupposes that the hydrolysis of choline esters is an essential activity in helminth parasites of the sheep alimentary canal. It is considered that the activities of many of these helminths are complex enough to require a relatively well-organised nervous system in which acetylcholine plays the usual role.

REFERENCES

1. R. H. NIMMO-SMITH and J. E. D. KEELING, *Exp. Parasitol.* **10**, 337 (1960).
2. E. BUEDING, *Brit. J. Pharmacol.* **7**, 563 (1952).
3. R. AMMON, *Pflug. Arch. ges. Physiol.* **233**, 486 (1933).
4. J. H. FLEISHER, E. J. POPE and S. F. SPEAR, *A.M.A. Arch. industr. Hlth.* **11**, 332 (1955).
5. S. HESTRIN, *J. biol. Chem.* **180**, 249 (1949).
6. W. N. ALDRIDGE and A. N. DAVISON, *Biochem. J.* **52**, 663 (1952).
7. J. ARMOUR, P. R. M. BROWN and J. E. N. SLOAN, *Vet. Rec.* **74**, 1454 (1962).
8. H. McL. GORDON, *Aust. vet. J.* **34**, 104 (1958).
9. R. P. LEE and R. A. O. SHONEKAN, *Vet. Rec.* **71**, 537 (1959).
10. A. N. DAVISON, *Biochem. J.* **54**, 583 (1953).
11. V. P. WHITTAKER, *Physiol. Rev.* **31**, 312 (1951).
12. G. B. KOELLE, *Biochem. J.* **53**, 217 (1953).
13. B. MENDEL, D. B. MUNDELL and H. RUDNEY, *Biochem. J.* **37**, 473 (1943).
14. C. J. EARL and R. H. S. THOMPSON, *Brit. J. Pharmacol.* **7**, 261 (1952).
15. N. N. SMALLMAN and L. S. WOLFE, *J. cell. comp. Physiol.* **48**, 197 (1956).
16. J. E. CASIDA, *Biochem. J.* **60**, 487 (1955).
17. R. M. LEE. Unpublished results (1962).
18. W. N. ALDRIDGE, *Biochem. J.* **53**, 62 (1953).
19. L. AUSTIN and W. K. BERRY, *Biochem. J.* **54**, 695 (1953).
20. B. J. BAYLISS and A. TODRICK, *Biochem. J.* **62**, 62 (1956).
21. W. N. ALDRIDGE, *Biochem. J.* **54**, 442 (1953).