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Biochemical Genetics of Resistance to Organophosphorus Acaricides in Three Strains of the Cattle Tick, *Boophilus microplus*

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Abstract

Three aspects of the biochemical genetics of resistance to organophosphorus compounds in the Biarra (B), Mackay (M) and Ridgelands (R) strains of the cattle tick *B. microplus* were studied. These were: decreased acetylcholinesterase (AChE) activity in adult brains of strains B and M; decreased AChE sensitivity to inhibitors in adult brains and in larvae of strains B, M and R; and increased detoxication in larvae and adult females of strain M. Comparisons were made with a susceptible reference strain (S).

Microspectrophotometric estimations of AChE activity in histochemical preparations of whole brains showed that hybrids had levels of activity approximately intermediate between those of the parental strains. Homogenates of brains from hybrids assayed biochemically gave similar but more precise results which indicated that decreased brain AChE activity was neither recessive nor dominant (degree of dominance, $D = +0{\cdot}02$) in strain B and incompletely recessive ($D = -0{\cdot}26$) in strain M. The proportions of brains showing decreased AChE activity in testcross and F_2 progenies indicated that decreased AChE activity in strains B and M is controlled by single autosomal genes.

Inhibition of AChE at diagnostic concentrations of coroxon in brains of B, B × S hybrid and S types suggested that decreased sensitivity of AChE in strain B is incompletely dominant ($D = +0{\cdot}10$). Kinetic studies on coroxon inhibition of AChE in brain homogenates of B, B × S hybrid and S types revealed the presence of each parental AChE component in hybrids in equal amounts and the absence of a hybrid enzyme. Dimethoxon inhibition of AChE in brains, their homogenates and larval homogenates of B, M and R types showed that decreased AChE sensitivity was a major mechanism of resistance to dimethoate strongly expressed in B × S and M × S hybrids. The proportion of brains showing decreased AChE sensitivity to coroxon in testcross and F_2 progenies indicated that decreased AChE sensitivity in strain B is controlled by a single autosomal gene.

The degree of dominance of increased degradative metabolism of coumaphos in strain M was variable; the hydrolytic rate in all M × S hybrids was similar to that of M but the overall detoxication rate in hybrids was lower. Genetic control of detoxication is discussed.

Introduction

Resistance to organophosphorus compounds in the Biarra (B) strain of *Boophilus microplus* (Roulston and Wharton 1967) is due to a marked decrease in acetylcholinesterase (AChE) sensitivity to inhibition; this was associated with a decrease in total AChE activity (Roulston and Wharton 1967; Roulston *et al.* 1968). An even greater decrease in total AChE activity in the Mackay (M) and Ridgelands (R) strains was accompanied by increased detoxication in strain M but not in strain R (Schuntner *et al.* 1968; Roulston *et al.* 1969); these reports showed that there was no decrease in AChE sensitivity in M strain ticks but there is more recent evidence (Schnitzerling *et al.* 1974) of the presence in strain M ticks of AChE of decreased

sensitivity of R type (Lee and Batham 1966; Schuntner *et al.* 1968). Diazinon resistance in strain B and dimethoate resistance in strain M are each controlled by an incompletely dominant autosomal gene (Wilson *et al.* 1971; Stone *et al.* 1973).

In strain R, decreased brain AChE activity was shown (Stone 1968*b*) to be controlled by an incompletely recessive autosomal gene, which is either closely linked to the gene for formothion resistance or is the same gene. This paper reports on similar studies on the inheritance of decreased brain AChE activity in strains B and M, on the inheritance of decreased brain AChE sensitivity in strain B and of increased detoxication in strain M.

Materials and Methods

Ticks

Strain B was established in culture as described by Roulston and Wharton (1967) and Wilson *et al.* (1971); strain M was cultured by Roulston *et al.* (1969) and selection for decreased brain* AChE activity gave rise to the homogeneous substrain MM_1 (Stone *et al.* 1973). This latter substrain was selected further for improved viability but is referred to here simply as strain M. The field history and laboratory purification of strain R was outlined by Stone (1968*a*). The acaricide-susceptible Yeerongpilly reference strain (strain S) of normal wild-type brain AChE activity and sensitivity has been cultured without contact with acaricides for over 18 years.

Acaricides

Coumaphos [3-chloro-4-methyl-7-coumarinyl diethyl phosphorothionate] and coroxon, its oxygen analogue, as well as dimethoxon, the oxygen analogue of dimethoate [dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolothionate] were used. These chemicals were at least 98% pure and the common names were recommended by the International Organization of Standardization (ISO/R 1750-1970).

Crossing Procedure

The crossing procedure described by Stone (1962, 1968*a*) consisted of the removal of engorged nymphs which were allowed to moult in isolation and then mated in single pairs in organza-covered Perspex rings attached by means of contact cement to the backs of steers. After engorgement the females were maintained at 27°C and 80–90% R.H. for oviposition. Eggs and larvae were maintained under the same conditions.

The following notation was used to identify the progeny of crosses by their parentage, the female parent being given first: F_1 and F_2 BS, SB MS, SM, MR; testcrosses† B/BS, B/SB, MS/M, SM/M, M/SM.

Histochemical Assays

Brains dissected from adult ticks (engorged females unless indicated otherwise) were fixed in A.R. acetone at −15°C for at least 1 h. Incubation followed at 27°C for 6 h in 5-bromoindoxyl acetate medium [indoxyl acetate method (Pearse 1960)], or at 30°C for 2·5 h in 'direct colouring' thiocholine medium [thiocholine method (Karnovsky and Roots 1964)].

Brain esterase activity, shown to be due principally to AChE (Stone 1968*b*), was initially rated by eye according to the intensity of the coloured deposit of copper ferrocyanide (thiocholine method) or of indigo (indoxyl acetate method) and ranged from the most positive (5) to completely negative

* The 'brain' of the tick is more correctly a synganglion resulting from fusion of the ganglia of the central nervous system and includes the optic, cheliceral, palpal, pharyngeal, pedal and opisthosomal ganglia together with commissures such as the stomodeal pons (Fig. 1). The synganglion is invested by a thin neurilemma and is surrounded by a sheath which forms the periganglionic sinus of the dorsal aorta (Binnington and Tatchell 1973). The whole structure will be subsequently referred to in this paper as a brain and unless otherwise stated was taken from female ticks.

† BS/B, SB/B and M/MS unavailable for testing.

(0). This rating was adequate for distinguishing between 'high' and 'low' phenotypes but quantitative assessments were also made by means of a Leitz MPV microspectrophotometer on brains mounted whole on microscope slides. The absorbances at the plateau wavelengths of 525 nm (thiocholine method) or 650 nm (indoxyl acetate method) of coloured deposits in optic, cheliceral, palpal and pedal ganglia corrected for background absorbance were estimated using a 10× objective, a 10× eyepiece and a convenient small measuring field (10 by 10 μm). Brains were also scanned by means of a moving stage driven at 16 μm/s using a narrow measuring slit (500 by 4 μm) and 6× objective. Areas under absorbance curves were measured using a planimeter and compared.

Biochemical Assays

The AChE activities of individual or groups of brains (acetone-fixed or fresh, unfixed) or of larval homogenates were determined biochemically by a modification of the method of Ellman *et al.* (1961). After homogenizing in ice-cold deionized water (5–8 μl per brain), dilution with pH 7·0 buffer to 120–150 μl and incubation with substrate (acetylthiocholine iodide, final concentration $1 \cdot 5 \times 10^{-3}$M) and DTNB (5,5,-dithiobis-2-nitrobenzoic acid, Aldrich) at 27 or 37°C for 20 or 100 min, absorbances were read at 412 nm.

Inhibition Studies

In inhibition studies, acetone-fixed brains were immersed for 10 min in an aqueous (pH 6·0) buffered solution of $2 \cdot 5 \times 10^{-4}$ M coroxon containing 0·02% Triton X-100 and 1% ethanol before transferring to thiocholine medium. Homogenates of fresh, unfixed brains or larvae were similarly inhibited at various concentrations of coroxon or dimethoxon at 27°C prior to incubation at the same temperature for 20 min, thus enabling residual AChE activities and bimolecular rate constants (k_i, Aldridge 1950) to be determined essentially as described by Nolan *et al.* (1972).

Detoxication Studies

In detoxication studies batches of larval progenies (broods) or the individual unfed female progeny from matings were immersed in [^{14}C]coumaphos suspensions for 5 min, drained and incubated for 5 h at 30°C and 80–90% R.H. Unabsorbed coumaphos was washed off ticks with acetone before homogenization in methanol for separation of water-soluble and chloroform-soluble metabolites. Coroxon and coumaphos were separated and identified in the latter fraction by paper chromatography (Roulston *et al.* 1969). The detoxication index was calculated as the percentage of water solubles divided by the percentage of coroxon.

Dominance

Degree of dominance (D) of a mutant-type allele controlling AChE activity or sensitivity over the corresponding wild-type allele was calculated by means of the expression given by Stone (1968*c*) based on the formula of Falconer (1964) except that an arithmetic scale appeared more appropriate than a logarithmic scale for enzyme activities.

Results

(*a*) *Decreased Brain AChE Activity*

(i) *Distribution of AChE activity in the brain*

The optic ganglion had the most AChE activity of any single ganglion but the cheliceral ganglion and superimposed stomodeal pons formed an area in the flattened brain with the greatest concentration of AChE activity (Fig. 1, Table 1).

(ii) *F_1 progeny of reciprocal crosses*

The AChE activity in all structures in M brains was much lower than in corresponding structures in S brains but in F_1MS or F_1SM brains was only slightly lower (Table 1).

The AChE rating of M and B brains was 0–1 (female) or 1–2 (male), F_1MS, F_1SM, F_1BS, and F_1SB brains rated 3–4 (female) or 4–5 (male) and S brains (female or male) rated 5 by the indoxyl acetate method; M, F_1MS and F_1SM brains rated the same by the thiocholine method but B brains rated 3–4 and F_1BS and F_1SB brains

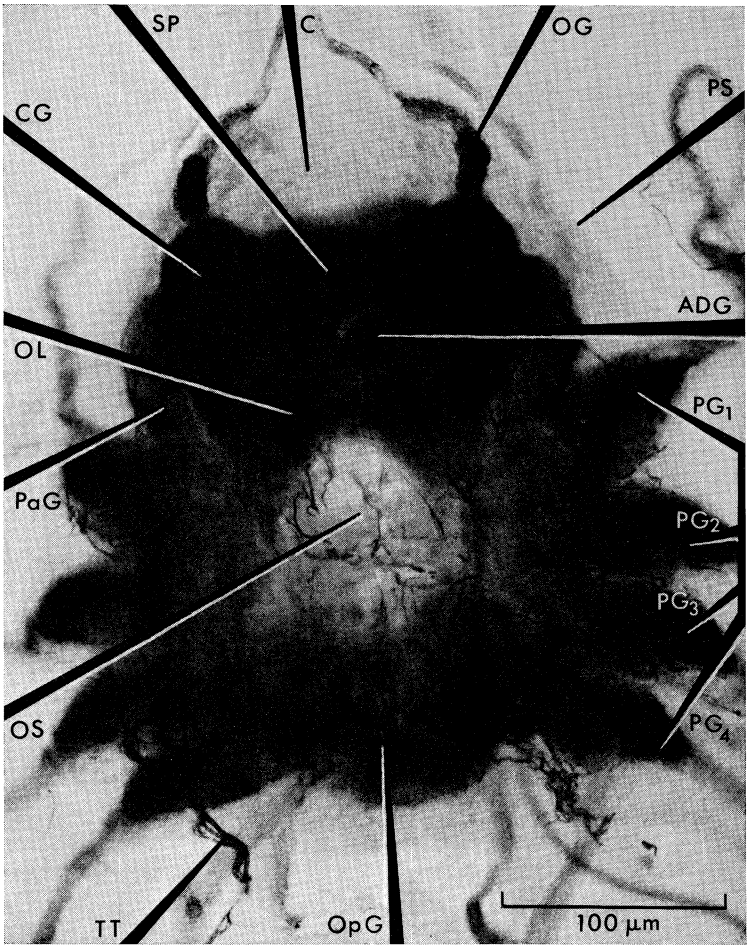

Fig. 1. Principal ganglia of the brain of *B. microplus* showing areas of AChE activity. *ADG*, anterior dorsal glomerulus; *C*, cortex; *CG*, cheliceral ganglion; *OG*, optic ganglion; *OpG*, opisthosomal ganglion; *OL*, olfactory lobe; *OS*, oesophageal space; PG_1–PG_4, pedal ganglia 1–4; *PaG*, palpal ganglion; *PS*, periganglionic sheath; *SP*, stomodeal pons; *TT*, tracheal trunks.

rated 4–5 by that method. As F_1MS and F_1BS male brains did not exhibit decreased AChE activity, sex linkage was absent. These ratings were checked where possible by means of the microspectrophotometric system and the results obtained by both methods were in approximate agreement (Table 2).

The relative activities of acetone-fixed M, F_1MS and F_1SM brains expressed as a percentage of that of S brains remained similar whether determined by the histo-

chemical or biochemical method (Tables 2 and 3); F_1SM brains were lower in activity than F_1MS brains by either method. Acetone fixation caused an appreciable loss of AChE activity varying from 45 to 68% (Table 3) and this had an effect on relative activity of M brains but not B brains.

Decreased AChE activity in strain M was incompletely recessive ($D = -0{\cdot}26$ and $-0{\cdot}15$ for unfixed brains and larvae respectively by biochemical assay) but in strain B could be regarded as neither dominant nor recessive ($D = +0{\cdot}02$).

Table 1. Histochemical estimation of AChE activity in ganglia of *B. microplus* brains of various genotypes by the thiocholine method

Absorbance of copper ferrocyanide deposit measured microspectrophotometrically on small stationary fields for 2–3 brains per genotype

Brain type	Ganglionic AChE activity (% of highest value)					
	Optic ganglion	Cheliceral ganglion + stomodeal pons	Pedal ganglia			
			1	2	3	4
S	92	100	62	62	71	75
M	12	13	6·6	7·2	7·2	7·2
F_1MS	64	81	46	50	49	54
F_1SM	75	68	43	45	39	39

Table 2. Histochemical estimation of AChE activity of *B. microplus* brains of various genotypes by the thiocholine method and by the indoxyl acetate method

Absorbance of copper ferrocyanide deposit (thiocholine method) and indigo deposit (indoxyl acetate method) was measured microspectrophotometrically by scanning an average of five brains per genotype using a narrow slit

Brain type	AChE activity determined by:			
	Thiocholine method		Indoxyl acetate method	
	Area under curve[A] (arbitrary units)	Percentage of S	Area under curve[A] (arbitrary units)	Percentage of S
S	235 (177–295)	100	189 (142–243)	100
M	54 (30–67)	23	35 (20–49)	19
F_1MS	131 (107–151)	56	149 (131–167)	79
F_1SM	101 (63–134)	43	114 (80–138)	60
B			49 (35–61)	25
F_1BS			176 (127–225)	93
F_1SB			136 (90–181)	72

[A] Range shown in parentheses.

(iii) *Testcross progeny*

MS/M, M/SM and SM/S brains were rated either 3–4 or 0–1 in AChE activity (thiocholine method) and there was no significant departure from the expected 1:1 ratio of these types (Table 4). B/BS and B/SB brains were rated similarly (indoxyl acetate method) into two types in the approximate ratio of 1:1.

(iv) *F_2 progeny*

F_2MS, F_2SM, F_2BS, F_2SB and F_2(BS+SB) brains were either 3–5 or 0–1 in AChE activity by the indoxyl acetate method, the two types being present in a ratio which did not differ significantly from 3:1 (Table 4). The absence of sex linkage was

Table 3. Biochemically determined AChE activity of fresh and acetone-fixed brains of *B. microplus* females

Brains were incubated for 20 min at 27°C (unfixed) or for 100 min at 37°C (acetone-fixed) in substrate of final concentration $1{\cdot}5\times10^{-3}$ M acetylthiocholine iodide

Brain type	AChE activity per brain: Unfixed brains			Acetone-fixed	Loss on acetone fixation (%)
	(μmol h^{-1})[A]	Percentage of S[B]	*D* of mutant-type allele	Percentage of S	
S	$1{\cdot}54\times10^{-2}$ [C]	100		100	45
F_1MS	$1{\cdot}00\times10^{-2}$	65	−0·26	58	
F_1SM				44	
M	$9{\cdot}2\times10^{-4}$	6·0		14	68
F_1BS	$1{\cdot}18\times10^{-2}$	77	+0·02		
B	$8{\cdot}5\times10^{-3}$	55		63	54

[A] Average of 130 brains per value.
[B] Corresponding values obtained under similar conditions for homogenates of larvae were S 100%, F_1SM 62%, M 9·5%, F_1MR 12%, R 11%. *D* for F_1SM was −0·15.
[C] Approximately equivalent to 254 μmol h^{-1} g^{-1} assuming an average brain weighs 60 μg. Values as high as $2{\cdot}2\times10^{-2}$ μmol h^{-1} per brain (368 μmol h^{-1} g^{-1}) have been recorded (Stone, unpublished data).

Table 4. Observed and expected numbers of *B. microplus* with normal and decreased brain AChE activity

AChE activity was determined histochemically in brains of engorged females by the thiocholine and indoxyl acetate methods

Brain type	Rating 3–4		0–1		χ^2 [A]	
	Observed	Expected	Observed	Expected		
MS/M[B]	7	9	11	9	0·50	n.s.
SM/M[B]	20	21	22	21	0·02	n.s.
M/SM[B]	8	8	8	8	0·00	n.s.
(B/BS+B/SB)[C]	13	19	25	19	3·18	n.s.
	3–5		0–1			
	Observed	Expected	Observed	Expected		
F_2MS[C]	33	36	15	12	0·69	n.s.
F_2SM[C]	30	36	18	12	3·36	n.s.
F_2BS	28	31·5	14	10·5	1·14	n.s.
F_2SB	11	12	5	4	0·08	n.s.
F_2(BS+SB)[C]	20	24	12	8	2·04	n.s.

[A] Yates correction for continuity used. Each (observed−expected) value brought closer to zero by 0·5.
[B] Acetylthiocholine iodide medium. [C] 5-Bromoindoxyl acetate medium.
n.s. Not significant.

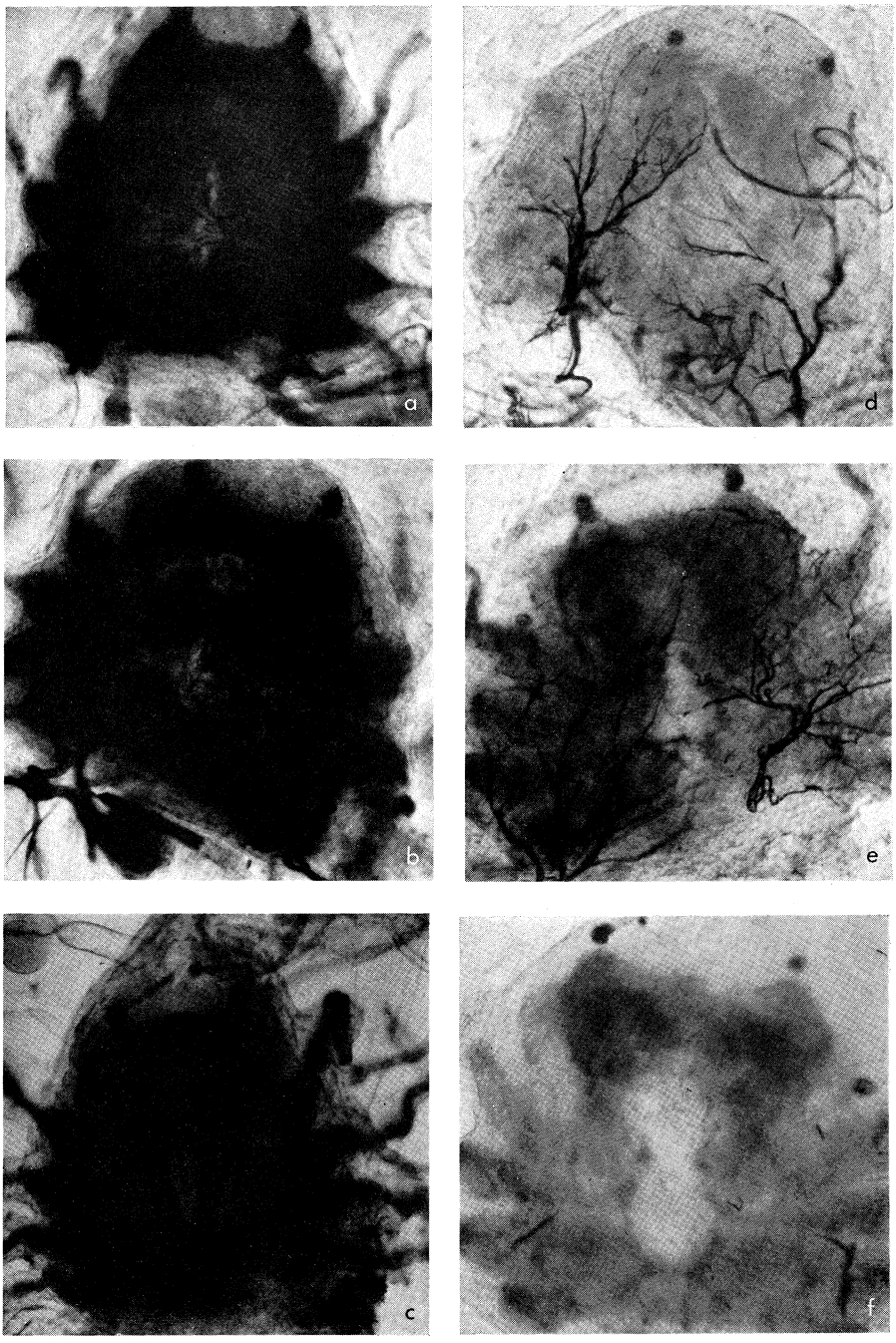

Fig. 2. AChE activity (thiocholine method) of whole mounts of brains of *B. microplus*. (*a*) S type, uninhibited. (*b*) B type, uninhibited. (*c*) B × S hybrid type, uninhibited. (*d*) S type, inhibited 10 min in $2{\cdot}5 \times 10^{-4}$ M coroxon. (*e*) B type, inhibited 10 min in $2{\cdot}5 \times 10^{-4}$ M coroxon. (*f*) B × S hybrid type, inhibited 10 min in $2{\cdot}5 \times 10^{-4}$ M coroxon.

confirmed as its presence would have required a 1:0 ratio for F_2SM and F_2SB and a 1:1 ratio for F_2MS and F_2BS female brains.

The results in section (*a*) provide strong evidence that decreased brain AChE activity in each of the strains B and M is controlled by a single autosomal genetic factor.

Table 5. Inhibition data determined histochemically on acetone-fixed brains and biochemically on homogenates of fresh unfixed brains of B, M, R, F_1BS and S types

Inhibitor concentration (M)	Method	Brain type	Residual activity[A] (%)
Coroxon $2{\cdot}5\times10^{-4}$	Histochemical[B]	B	53
		F_1BS	39
		S	18
Coroxon $3{\cdot}5\times10^{-5}$	Biochemical	B	100
		F_1BS	55
		S	0
Dimethoxon $2{\cdot}0\times10^{-3}$	Biochemical	B	70
		M	91
		R	89
		S	2

[A] Expressed as percentage of initial AChE activity present in uninhibited preparations.

[B] Determinations based on average absorbances measured on the principal brain ganglia and using small stationary fields; thiocholine method.

(*b*) *Decreased Brain AChE Sensitivity*

(i) *F_1 progeny of reciprocal crosses*

Inhibition of acetone-fixed brains with $2{\cdot}5\times10^{-4}$ M coroxon resulted in the activity of S brains declining from a rating of 5 to 0–1, of B brains from 3–4 to 2–3 and of F_1BS and F_1SB brains from 4–5 to 2–3 (Fig. 2). These ratings were confirmed by use of the microspectrophotometric technique (Table 5). The percentage of residual AChE after inhibition of both acetone-fixed whole brains and homogenized, fresh, unfixed brains (Table 5) from B × S hybrids was intermediate between those of the parental strains. The *D* value of $+0{\cdot}1$ showed decreased AChE sensitivity in unfixed brains to be incompletely dominant.

This phenotypic effect was clarified by measuring the rate constant (k_i) for the enzyme-inhibitor reaction (Fig. 3) and relative sensitivity [$k_i(S)/k_i$, see Table 6] of AChE in brain homogenates which more accurately quantitates decreased AChE sensitivity. The kinetic plots for brain AChE of B and S types were linear over the ranges tested and there was no intercept on the ordinate; this suggested that there was only one AChE component in each strain (Fig. 3). These components were similar in reactivity to the less sensitive components found in *B. microplus* larvae by Nolan *et al.* (1972) and correspond to component II of Nolan and Schnitzerling (1975). However the inhibition rates for F_1BS and F_1SB were non-linear and suggested the presence of two components (Figs 3 *b*, *c*, *e*, and *f*, Table 6). Component II_s had a sensitivity ($k_i = 6{\cdot}9\text{–}8{\cdot}2\times10^4$) of a similar order to that of S type AChE

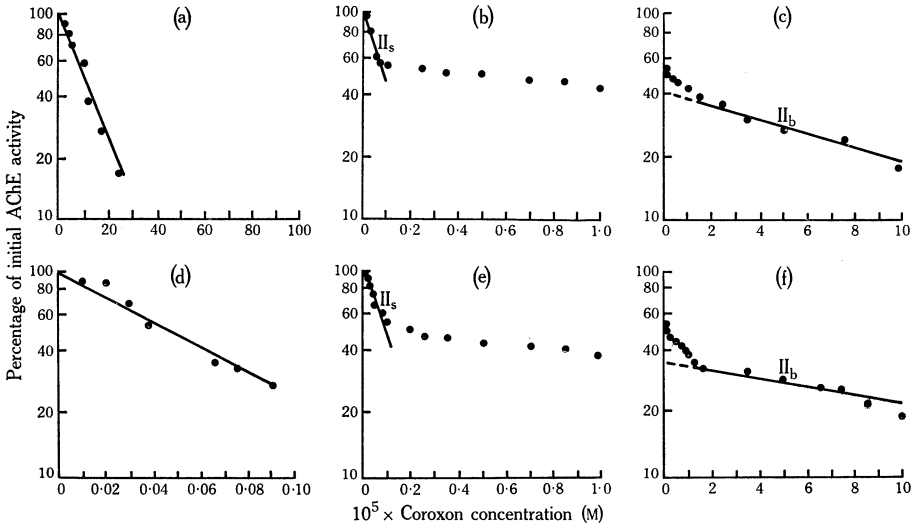

Fig. 3. Effect of coroxon inhibition of the AChE activity *in vitro* of B, B × S hybrid and S homogenates of brains from *B. microplus* females. (*a*) B, (*b*) and (*c*) F_1BS, (*d*) S, (*e*) and (*f*) F_1SB type.

Table 6. Bimolecular rate constants [k_i] for inhibition of AChE in homogenates of fresh brains or of larvae of B, M, R, F_1BS, F_1SB, F_1MS, F_1MR and S types

Inhibitor	Homogenate	Type	k_i (litre mol^{-1} min^{-1})	$k_i(S)/k_i$
Coroxon	Brains	B	$6{\cdot}9\times10^2$	218
		F_1BS II_b	$7{\cdot}7\times10^2$	181
		II_s	$6{\cdot}9\times10^4$	2
		F_1SB II_b	$5{\cdot}3\times10^2$	264
		II_s	$8{\cdot}2\times10^4$	1·7
		M	$4{\cdot}1\times10^4$	3·4
		F_1MS	$3{\cdot}4\times10^4$	4·1
		F_1MR	$2{\cdot}8\times10^4$	5·0
		R	$2{\cdot}5\times10^4$	5·6
		S	$1{\cdot}4\times10^5$	1
Dimethoxon[A]	Brains	B	$>3{\cdot}5\times10^1$	>100
		F_1BS	$>3{\cdot}5\times10^1$	>100
		M	$>3{\cdot}5\times10^1$	>100
		F_1MS	$>3{\cdot}5\times10^1$	>100
		R	$>3{\cdot}5\times10^1$	>100
		S	$3{\cdot}5\times10^3$	1
Dimethoxon	Larvae	B	$>6{\cdot}9\times10^1$	> 55
		M	$>6{\cdot}9\times10^1$	> 55
		R	$>6{\cdot}9\times10^1$	> 55
		S	$>3{\cdot}8\times10^3$	1

[A] Dimethoxon was insufficiently active at maximum solubility to inhibit the required 50% of AChE for calculation of k_i values.

($k_i = 1{\cdot}4 \times 10^5$). Component II_b comprised about 38–42% of the total AChE and had a sensitivity ($k_i = 5{\cdot}3$–$7{\cdot}7 \times 10^2$) similar to that of B type AChE ($k_i = 6{\cdot}9 \times 10^2$). This component would constitute most of the residual AChE in hybrid brains after inhibition at diagnostic concentrations.

In the 27 000 *g* residue obtained from homogenates of F_1BS and F_1SB larvae, component II_b was identified ($k_i = 5{\cdot}3 \times 10^2$) and constituted about 22–25% of the AChE. This result indicated that decreased AChE sensitivity was not sex-linked as, in that case, these two residues would have been expected to differ considerably in amount of component II_b.

A diagnostic concentration of 2×10^{-3} M dimethoxon almost completely inhibited AChE in homogenates of fresh S brains but left considerable levels of residual AChE in homogenates of B and M brains (Table 5). The sensitivity of AChE in the latter two homogenates and in those of F_1BS and F_1MS brains was $<1/100$ of that for S brains homogenates (Table 6). Thus, decreased AChE sensitivity is a major mechanism of resistance to dimethoate in the parental B and M strains and is also strongly expressed in their hybrids with strain S.

(ii) *F_2 progeny*

Twenty F_2BS and 23 F_2SB brains were inhibited in $2{\cdot}5 \times 10^{-4}$ M coroxon and residual AChE activity assessed histochemically by the thiocholine method; four brains of each were rated 0–1 whilst 16 and 19 respectively were rated 2–3. When 31 F_2(BS+SB) brains were similarly inhibited the ratio was 9:22. There was no significant difference from a 1:3 ratio of brain types in F_2BS, F_2SB and F_2(BS+SB) with strongly inhibited to slightly inhibited AChE. Thus the absence of sex linkage was confirmed, and evidence obtained of a single incompletely dominant, autosomal genetic factor controlling 'decreased cholinesterase sensitivity' in strain B.

(*c*) *Coumaphos Detoxication*

(i) *F_1 progeny of reciprocal crosses*

F_1MS and F_1SM larvae were almost as active as M larvae in their hydrolytic metabolism of coumaphos (Table 7) resulting in production of water-soluble metabolites at about the same overall rate and suggesting almost complete dominance. However more coroxon remained unmetabolized by F_1SM than by M larvae and this was reflected in the detoxication index which was intermediate between M and S resulting in a *D* value of $-0{\cdot}30$ for this hybrid. The only F_1MS brood available was similar to M in all respects in detoxication of coumaphos. Therefore it is clear that detoxication of coumaphos has a variable degree of dominance in strain M.

Both S and M unfed females (Table 8) were less active in metabolizing coumaphos than larvae (Table 7); the detoxication index was about the same for S larvae and females. This value was lower for M females than larvae but percentage water-soluble metabolites in strain M females was about twice that in strain S and thus females closely resembled larvae in this respect.

(ii) *Testcrosses*

Two out of eight* SM/S females clearly resembled their more resistant parent in their ability to metabolize coumaphos to water-soluble products and were classified

* This small sample was the largest practicable in this new type of assay.

as hybrid types. A third female was somewhat intermediate but its detoxication index lay outside the range of S parents and it was classified as a hybrid (Table 8). The three hybrids and five homozygous susceptibles present constitute a ratio which does not differ significantly ($\chi^2 = 0{\cdot}12$) from 1:1, as expected for a single gene with a D value

Table 7. Metabolism of [^{14}C]coumaphos by individual broods of *B. microplus* larvae obtained by single pair crossings

Ticks were immersed in 0·0002% suspensions and homogenized after 5 h incubation at 30°C

Type of progeny	Metabolites				Detoxication index[B]	
	Water soluble[A] (%)		Coroxon[A] (%)			
S	37·4	38·7	21·4	21·7	1·7	1·8
	40·1		22·1		1·8	
	38·6		21·5		1·8	
M	74·6	73·5	4·0	3·5	18·6	21·1
	70·4		3·6		19·6	
	75·4		3·0		25·1	
F_1SM	69·7	71·4	8·0	8·4	8·7	8·5
	75·4		8·0		9·4	
	69·0		9·2		7·5	
F_1MS	76·8		3·9		19·7	

[A] Expressed as percentage of total internal radioactivity.
[B] (Percentage water solubles)/(percentage coroxon).

Table 8. Genotype classification of individual unfed *B. microplus* females by metabolic activity towards [^{14}C]coumaphos

Ticks were immersed in 0·0001% suspensions and homogenized after 5 h incubation at 30°C

Tick type	Metabolites				Detoxication index[B]		Genotype
	Water soluble[A] (%)		Coroxon[A] (%)				
S	19·6	15·9	8·4	9·3	2·3	1·8	
	9·0		10·3		0·87		
	22·6		8·1		2·8		
	12·4		10·3		1·2		
M	37·9	34·0	4·6	4·3	8·2	7·8	
	29·0		4·3		6·7		
	37·2		4·1		9·1		
	31·2		4·2		7·4		
SM/S	10·6		3·6		2·9		S
	10·6		5·5		1·9		S
	14·0		5·8		2·4		S
	30·7		4·4		7·0		MS
	22·5		5·8		3·9		MS
	42·0		4·7		8·9		MS
	14·3		8·1		1·8		S
	15·4		5·4		2·8		S

[A] Expressed as percentage of total internal radioactivity.
[B] (Percentage water soluble)/(percentage coroxon).

of −0·3, and differs significantly ($\chi^2 = 4{\cdot}17$) from 3:1 as expected for two independent non-recessive genes. However as this ratio does not differ significantly from 1:3 ($\chi^2 = 0{\cdot}17$) or 1:7 ($\chi^2 = 2{\cdot}57$) as expected for two or three complementary genes respectively, these latter possibilities cannot be excluded.

Discussion

Decreased brain AChE activity in the B and M strains of *B. microplus* has now been shown to be controlled by single, autosomal, non-dominant genes similar to the gene *ch* in the R strain (Stone 1968*b*). The symbols dca^B, dca^M and dca^R are proposed for these genes in strains B, M and R respectively, so that *ch* now becomes dca^R.

The zero value obtained for degree of dominance of dca^B is consistent with a heterozygote producing a single dose of each parental-type gene product. This situation is to be expected according to Wagner and Mitchell (1964) but the point of no dominance depends on the scale used. The most appropriate scale usually results in a normal distribution of values obtained from metric characters (Falconer 1964) and it seems likely that an arithmetic scale would be suitable for enzyme activity measurements. A logarithmic scale, shown by Stone (1968*c*) to be most appropriate in calculating degree of dominance when the final expression of tolerance to chemicals was being measured, appears to be less appropriate in this instance.

The various methods used to measure AChE activity in brains had previously given results indicating that decreased AChE activity is incompletely recessive in strain R (Stone 1968*b*) but in similar studies on strains B and M there is somewhat less agreement in this respect between the methods. The inconsistency lies in the comparatively low values for degree of dominance obtained for acetone-fixed brains assessed with acetylthiocholine iodide by both the histochemical method and the long-incubation (100-min) biochemical method. As acetone fixation caused a differential loss in AChE activity and the rate of hydrolysis of acetylthiocholine iodide by larval and brain homogenates declined on prolonged incubation (H. J. Schnitzerling, personal communication; B. F. Stone, unpublished data), it is possible that either or both of these factors could affect the relative activity of the AChE of hybrid and parental types and thus the degree of dominance indicated by such tests.

It seems likely that the initial reaction rate in homogenates of fresh brains would closely approximate the rate at which AChE functions in the live ticks, so that the results given by the short-incubation biochemical method may be a better estimate of overall AChE activity than those obtained from acetone-fixed brains by the 6-h histochemical methods or the 100-min biochemical method. Decreased AChE activity is therefore considered to be incompletely recessive in strain M and neither dominant nor recessive in strain B.

Decreased brain AChE sensitivity in strain B has been shown to be determined by a single incompletely dominant autosomal gene (Tables 5 and 6, Figs 2 and 3) for which the symbol Dcs^B is now proposed. Similar studies were not undertaken with strains R and M, which also have decreased AChE sensitivity as a resistance mechanism, because the existing techniques were not sensitive enough at the low levels of AChE present in these strains. Thus, the presence in strains R and M of a gene resembling Dcs^B can only be inferred at this stage.

It is of considerable interest that only one AChE component, the least sensitive in each case, appears to be present in adult brains of each parental strain as compared with two components in larval homogenates of each strain (Nolan *et al.* 1972), the most sensitive component I (Nolan and Schnitzerling 1975), common to larvae of both strains, being absent. As it would be surprising if larvae and adults differed qualitatively in distribution of AChE components, it appears possible that component I in larvae (and adults) may be derived from tissues other than the brain. Certainly, AChE is known to occur in other tissues in adults [haemolymph and salivary glands (J. Nolan and B. F. Stone, unpublished data)].

In strain S adult brains there is the less sensitive 'critical component II' of Nolan and Schnitzerling (1975) referred to here as component II_s, and in strain B the much less sensitive component II, referred to here as II_b. As brains from hybrid ticks possessed both components II_s and II_b the possibility was considered that, in addition, a hybrid enzyme may be present. If this were the case components II_s and II_b would each constitute 25% and the hybrid enzyme 50% of the AChE present. In terms of activity in F_1BS, component II_b would represent 25% of 55 units (B, Table 3) of activity, i.e. 13·8 units out of a total of 77 units (F_1BS, Table 3) which is 17·9%. This is not in agreement with the 40% shown (Fig. 1) which is close to the theoretical 35·7% (50% of 55 units out of a total of 77 units) calculated on the basis of a simple 1:1 mixture of II_b and II_s. The non-linear portions of the curves in Figs 3 *b*, *c*, *e*, and *f* can be explained as curvature due to the overlapping portions of the straight lines.

Similarly the value of 22–25% component II_b in the 27 000 *g* residue from homogenized F_1BS and F_1SB larvae is very close to the theoretical 25% (50% of 30 units out of a total of 60 units) calculated on the basis of a simple 1:1 mixture of II_b and II_s. This may be compared with the expected 12·5% II_b if a hybrid enzyme were present.

A marked decrease in sensitivity of AChE to dimethoxon in brain and larval homogenates of B, M, F_1BS and F_1MS (as well as R) types left no doubt as to their principal mechanism of resistance to dimethoate but detoxication studies would be required to determine if this is the sole mechanism of resistance to dimethoate in strain M.

The study of metabolism of coumaphos by individual broods of larvae showed clear differences between the progeny of M × M and S × S single pair matings and between those of M × S and S × S matings. The calculation of the ratio between percentage water solubles and the active toxicant, coroxon, was an attempt to find an expression to represent the balance between toxication and detoxication. Hybrids varied in their ability to detoxify from a level intermediate between that of their parents (F_1SM) to a level almost as high as their resistant parents (one F_1MS brood). However the limited data for the latter prevents any firm conclusions being reached in relation to sex linkage. This first attempt to classify individual unfed testcross females by metabolic activity as S × S or M × S progeny was reasonably successful despite the generally lower levels of metabolic activity in females than larvae, and shows some promise for this type of genetic study. Although it seems unlikely that two independent dominant genes are involved in detoxication, clearly larger numbers of testcross ticks would be essential for metabolic studies in order to distinguish between a single gene and two or three complementary genes. It would be desirable to investigate these latter possibilities in view of the theoretical proposal put forward

by Tsukamoto (1969) that detoxication of some insecticides may involve a series of metabolic steps under control of separate genes. Desulphuration of coumaphos to produce coroxon and hydrolysis of either to release water-soluble products may well be separate metabolic steps.

The studies reported herein on inheritance of coumaphos detoxication are limited but are included because useful conclusions can be reached; the information has not been reported previously and it is unlikely that further investigations to obtain more complete data will be attempted. Strain M is no longer homogeneous and now possesses a component which is resistant to chlorpyrifos possibly by detoxication (W. J. Roulston, personal communication). It is not known if the removal of this component is possible and clearly this would be an essential first step in any attempt to repeat these inheritance studies.

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