Review

Modes of Action of Anthelmintic Drugs

R. J. MARTIN

Department of Preclinical Veterinary Sciences, R.(D.)S.V.S., Summerhall, University of Edinburgh, Edinburgh EH9 1QH, UK

SUMMARY

Modes of action of anthelmintic drugs are described. Some anthelmintic drugs act rapidly and selectively on neuromuscular transmission of nematodes. Levamisole, pyrantel and morantel are agonists at nicotinic acetylcholine receptors of nematode muscle and cause spastic paralysis. Dichlorvos and haloxon are organophosphorus cholinesterase antagonists. Piperazine is a GABA (γ-amino-butyric acid) agonist at receptors on nematode muscles and causes flaccid paralysis. The avermectins increase the opening of glutamate-gated chloride (GluCl) channels and produce paralysis of pharyngeal pumping. Praziquantel has a selective effect on the tegument of trematodes and increases permeability of calcium. Other anthelmintics have a biochemical mode of action. The benzimidazole drugs bind selectively to β-tubulin of nematodes, cestodes and fluke, and inhibit microtubule formation. The salicylanilides: rafoxanide, oxyclozanide, brotianide and closantel and the substituted phenol, nitroxynil, are proton ionophores. Clorsulon is a selective antagonist of fluke phosphoglycerate kinase and mutase. Diethylcarbamazine blocks host, and possibly parasite, enzymes involved in arachidonic acid metabolism, and enhances the innate, nonspecific immune system.

KEYWORDS: Mode of action; anthelmintics; levamisole; dichlorvos; piperazine; avermectins; praziquantel; benzimidazoles; salicylanilides; clorsulon; diethylcarbamazine.

INTRODUCTION

Parasitic nematodes affect animals and man causing considerable suffering and poor growth. Effective anthelmintic drugs, used to treat and control these infestations, must have selective toxic effects on these parasites. Unfortunately with the increased use of these compounds, anthelmintic resistance has appeared and increased in frequency (Prichard, 1994). If resistance to a particular anthelmintic has occurred, it is likely that another anthelmintic with the same mode of action will also be ineffective although other anthelmintics with another mode of action, may still be effective. Clearly then, it is important to have an understanding of the mode of action of anthelmintics in order to inform the selection of effective therapeutic agents. This review describes modes of action of anthelmintic drugs (Table I).

NICOTINIC AGONISTS

Levamisole, butamisole, pyrantel, morantel, oxantel, bephenium and thenium

Fig. 1 shows the chemical structures of nicotinic anthelmintics. There are the imidazothiazoles (levamisole and butamisole); the tetrahydropyrimidines (pyrantel, morantel and oxantel); the quaternary ammonium salts (bephenium and thenium) and the pyrimidines (methyridine). These compounds act selectively as agonists at synaptic and extrasynaptic nicotinic acetylcholine receptors on nematode muscle cells (Fig. 2) and produce contraction and spastic paralysis. The electrophysiological effects of levamisole, pyrantel, morantel and oxantel have been studied in greatest detail.

Table I.
Summary table of the modes of action of anthelmintic drugs

	Generic drug name	Examples of some U.K. & U.S.A. Trade Names
Nicotinic agonists	levamisole	Nilverm
	butamisole	Styquin
	pyrantel	Strongid
	morantel	Paratect
	bephenium	
	thenium	Canopar
	methyridine	
Acetylcholinesterase inhibitors	haloxon	Mulitwurma
	dichlorvos	
GABA agonist	piperazine	Endorid.
GluCl potentiators	ivermectin	Ivomec
	abamectin	Enzec
	doramectin	Dectomax
	moxidectin	Cydectin
	milbemycin D	
Calcium permeability increase	praziquantel	Droncit
β-tubulin binding	thiabendazole	Thibenzole
	cambendazole	Equiben, Noviben
	oxibendazole	Loditac
	albendazole	Valbazen
	albendazole sulphoxide	Rycoben
	fenbendazole	Panacur
	oxfendazole	Systamex
	mebendazole	Telmin
	flubendazole	Flubenol
	febantel	Bayverm
	netobimin	Hepadex
	thiophanate	Wormalac, Nemafax
	triclabendazole	Fasinex
Proton ionophores	closantel	Flukiver
	rafoxanide	Flukanide, Ranide
	oxyclozanide	Zanil
	brotianide	In Flukombin and Vermadex
	nitroxynil	Trodax, Dovenix
	niclopholan	Bilevon, Distolon
	hexachlorophene	Coopaphene, Distodin
	dibromosalan	
	niclosamide	Yomesan
Inhibition of malate metabolism	diamphenethide	Coriban
Inhibition of phosphoglycerate kinase and mutase	clorsulon	In Ivomec Super
Inhibitor of arachidonic acid metabolism and stimulation of innate immunity	diethylcarbamazine	Caricide,Filaricide

Electrophysiological effects of nicotinic anthelmintics in nematodes

Intracellular recordings made with micropip-ettes from *Ascaris suum* body muscles have shown that bath application of tetramisole, the D/L racemic mixture of levamisole, produces mem-
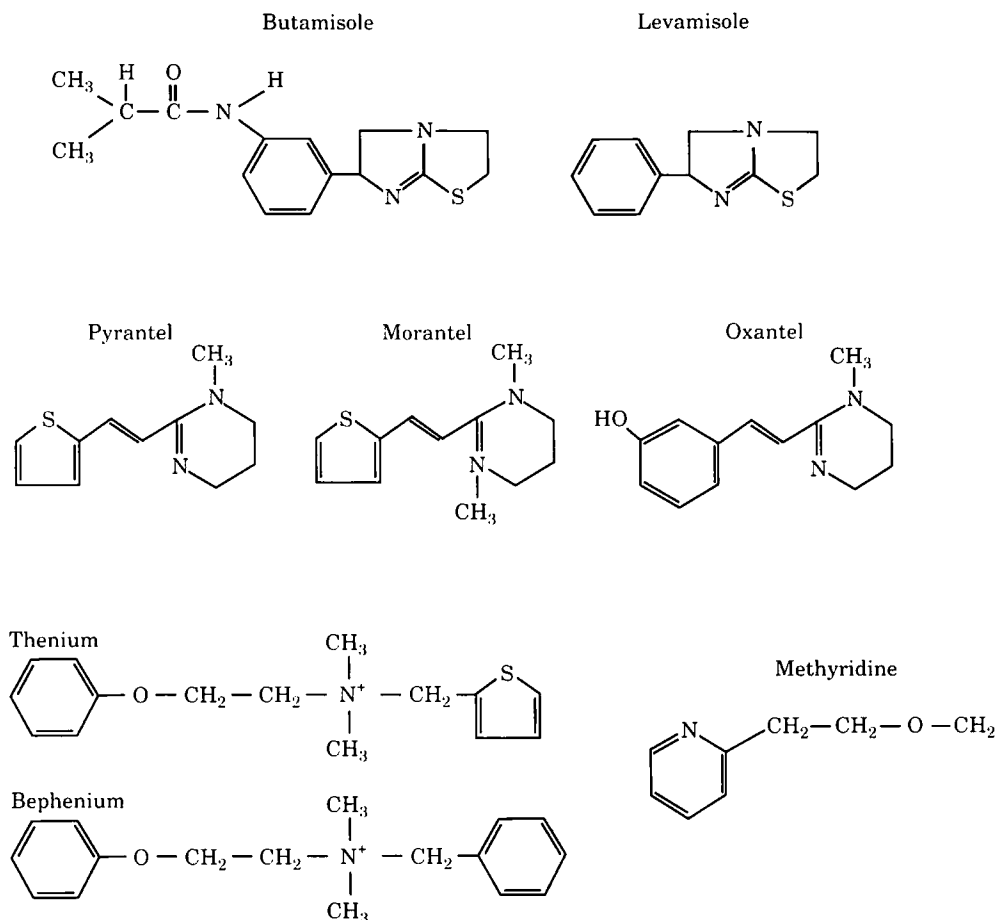
Butamisole   Levamisole

Pyrantel   Morantel   Oxantel

Thenium   Methyridine

Bephenium

Fig. 1. Chemical structures of nicotinic anthelmintics.

brane depolarization, an increase in spike frequency and contraction (Aceves *et al.*, 1970). Pyrantel and its analogues also produce depolarization, increased spike activity and contraction when applied to *Ascaris* muscle (Aubry *et al.*, 1970) suggesting that these compounds have a common mode of action.

The two-microelectrode current-clamp and voltage-clamp techniques have been used to examine muscle membrane conductance changes in *Ascaris* produced by acetylcholine, levamisole, pyrantel and morantel (Martin, 1982a; Harrow & Gration, 1985). These anthelmintics have been shown to increase the membrane conductance and depolarize the membrane by opening non-selective cation ion-channels that are permeable to both Na⁺ and K⁺. Simultaneous application of acetylcholine and pyrantel showed that both agonists acted on the same nicotinic receptors, and the relative potency of the anthelmintics in bath application exper-

iments was: morantel=pyrantel>levamisole>acetylcholine (Harrow & Gration, 1985).

In addition to effects of the anthelmintics on the membrane conductance of the muscle, Harrow and Gration (1985) described the conductance dose–response curves for pyrantel and morantel as being 'bell shaped'. The effect of this concentration–effect relationship is that the conductance-response increases then decreases as the concentration of the anthelmintics rises. One explanation for this phenomena is that of open channel block (Colquhoun & Sakmann, 1985) by the anthelmintic. Levamisole, pyrantel, morantel and oxantel are large organic cations, and could enter the nicotinic ion-channel from the outside and try to pass through the channel like Na⁺ or K⁺ ions but produce the block at the narrow region of the channel, the selectivity filter. This block would be voltage-sensitive and increase with hyperpolarization of the membrane and concen-
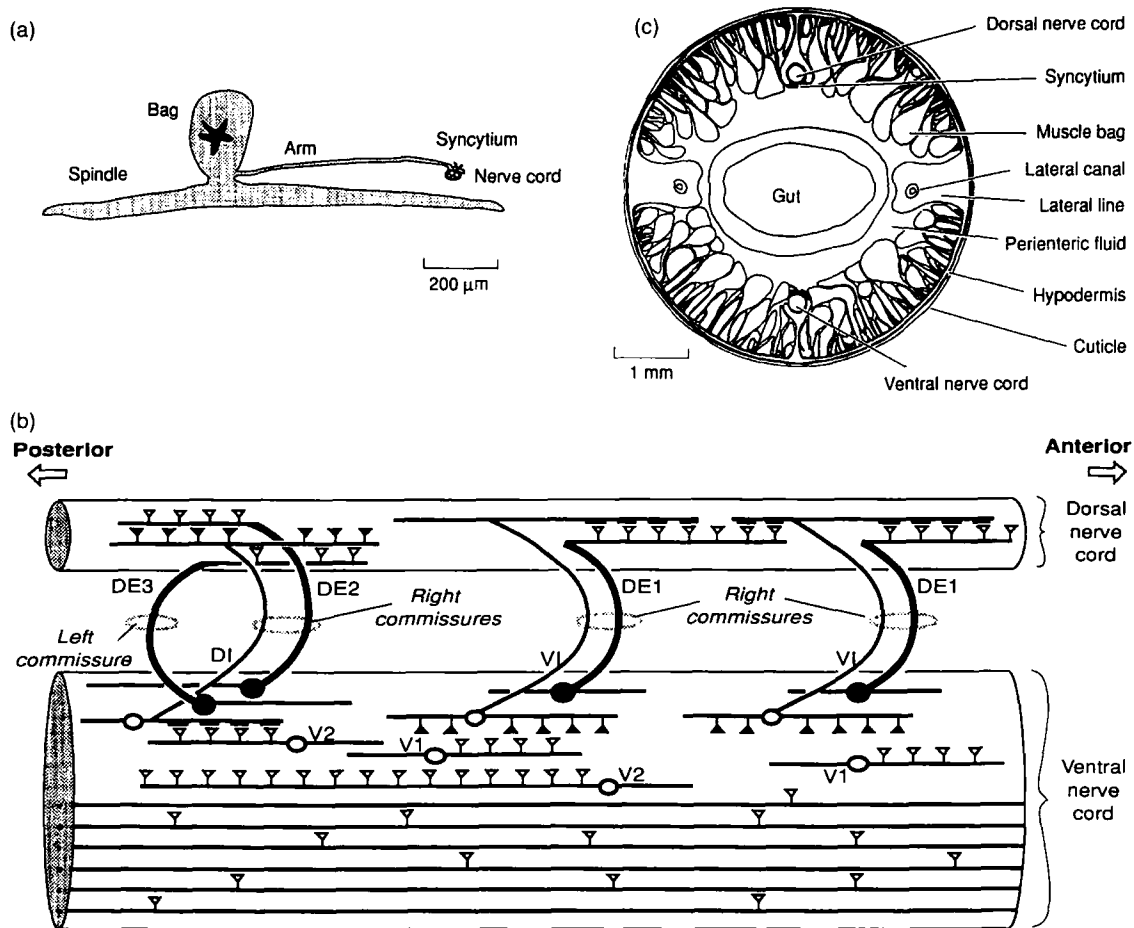
Fig. 2. Diagrams of the neuromuscular organization of nematode somatic muscle. (a) Diagram of a somatic muscle cell showing: the contractile spindle region; the balloon-shaped bag that contains the nucleus and glycogen granules; the arm which is a process of the muscle and reaches to one of the nerve cords where it divides into fingers that form an electrically-coupled complex with the fingers of adjacent muscle cells and collectively is known as the syncytium. The muscle cell possesses synaptic nicotinic acetylcholine receptors, and synaptic GABA receptors at the syncytial region and extrasynaptic acetylcholine and GABA receptors over the surface of the muscle. (b) Diagram of the dorsal and ventral nerve cord showing the cell represented in a segment. All the cell bodies of the motor neurones are contained in the ventral nerve cord. Each segment has three right-hand commissures and one left-hand commissure. Each commissure is made up of one or two axons or dendrites of the motor neurones that pass round the body to connect the ventral and dorsal nerve cord. Within each segment there are 11 motor neurones that are divided into seven anatomical types (DE1, DE2, DE3: dorsal excitatory motor neurones. DI: dorsal inhibitory motor neurones. VI: ventral inhibitory motor neurone. V1 & V2: ventral excitatory motor neurones. The remaining axons in the ventral nerve cord are intersegmental neurones. The excitatory motor neurones are cholinergic and the inhibitory motor neurones are GABAergic. (c) Diagram of a cross section of the body just caudal to the pharyngeal muscle. It shows the relative locations of the nerve cords, lateral lines, gut and muscle cells.

tration of the anthelmintic. This property of pro-ducing open channel block has been established using single-channel recording techniques (Robertson & Martin, 1993).

Single-channel currents activated by nicotinic anthelmintics

Initially, levamisole-activated channel currents [Fig. 3(a)] were recorded from the muscle vesicle

preparation (Robertson & Martin, 1993) using the patch–clamp technique. The ion-channel currents activated by low levamisole concentrations were shown to carry cations and to have similar kinetics to acetylcholine-activated channels. The levami-sole activated channels have a mean open time of 1.34 ms and a conductance in the range 20–45 pS. At higher concentrations of levamisole, a flicker-ing open channel block that increases on hyper-

polarization is observed [Fig. 3(b)]. At −50 mV the dissociation constant for the channel block was 123 μM. In addition to the flickering block, clusters of openings were also observed at high levamisole concentrations where openings were separated by long (seconds) closed times: this clustering behaviour is similar to that assumed to be desensitization in vertebrates but was more voltage-sensitive and more prominent at hyperpolarized potentials in *Ascaris*.

Pyrantel-activated channels [Fig. 3(c)] have also been recorded using the same preparation (Robertson *et al.*, 1992). Pyrantel-activated channel showed at least two distinguishable conductance levels: the main conductance level was near 40 pS and the smaller conductance level was near 22 pS, suggesting the presence of more than one type of nicotinic receptor in the membrane. Again, the channels opened by pyrantel were shown to be permeable to monovalent cations and channel-block occurred at hyperpolarized potentials. The channel block occurred more readily with pyrantel than with levamisole as shown by the fact that the dissociation constant, K_B, for levamisole was higher than that of pyrantel (levamisole K_B at −50 mV 123 μM; pyrantel K_B at −50 mV 37 μM).

Table II summarizes the channel blocking properties of levamisole and pyrantel and, in addition, morantel and oxantel. Some general points may be made by examining Table II. It can be seen that all the nicotinic anthelmintics produce open channel-block, a form of self antagonism, and that levamisole is the least potent at blocking its own channel. The significance of the open channel block may relate to the ability of some nematodes to resist the effects of nicotinic anthelminitics: channel block may occur at such low anthelmintic concentrations that normal concentrations of anthelmintic are ineffective.

In *Ascaris*, the channel-blocking ability of pyrantel is greater than levamisole, but the greatest channel-block is produced by morantel

with oxantel. occupying an intermediate position between morantel and oxantel. The least potent agonist in *Ascaris* is oxantel which produces the lowest probability of channel opening even at high concentrations (Dale & Martin, 1995). Interestingly, oxantel is not effective therapeutically against ascariasis but is used instead to treat *Trichuris* infections. The efficacy of oxantel against *Trichuris* but not against *Ascaris* may be due to differences in the nicotinic receptors of the two species of nematode: oxantel may be effective and produce opening of the *Trichuris* nicotinic acetylcholine receptor but not so effective on the *Ascaris* receptor. The concentrations of oxantel and pyrantel along the intestine may also be a factor affecting efficacies of the drugs.

Non-nicotinic or 'muscarinic' cholinergic receptors in nematodes and resistance to anthelmintics

In a number of vertebrate preparations, acetylcholine is able to alter the probability of opening of voltage-dependent channels by acting via G-protein-coupled muscarinic receptors. An action in nematodes at acetylcholine receptors analogous to vertebrate muscarinic receptors, modulating voltage-sensitive ion channels has yet to be fully reported.

There is biochemical evidence for the presence of 'muscarinic' receptors in *Ascaris* muscle:

(1) Donahue *et al.* (1982) have observed that effects of cholinergic stimulation includes increases in levels of cyclic-AMP;

(2) Arevalo and Saz (1992) have observed that acetylcholine increases levels of phosphorylcholine, 1,2-diacylglycerides and phosphatidic acid, and demonstrated the presence of phospholipase C activity. Donahue *et al.* (1982) and Arevalo and Saz (1992) did not distinguish between 'muscarinic' or 'nicotinic' cholinergic receptors.

Previous studies on nematodes have shown that resistance to nicotinic anthelmintics may take two forms: (1) The selection of genetically resistant

Table II
Voltage sensitivity of dissociation constants of open channel block by the nicotinic anthelmintics

Membrane	KB Levamisole	$2K_B$ Pyrantel	K_B Morantel	K_B Oxantel
−50 mV	123 μM	37 μM	12 μM	18.5 μM
−75 mV	46 μM	20 μM	1 μM	7.5 μM
Voltage-sensitivity of K_B	e-fold every 20 mV	e-fold every 40 mV	e-fold every 22 mV	e-fold every 29 mV

K_B is the dissociation constant: the lower the concentration of this constant the more potent it is. At the concentration of the KB the channel is blocked for 50% of its open time so the response would be reduced by a half.

(a) Levamisole

(b)

(c) Pyrantel
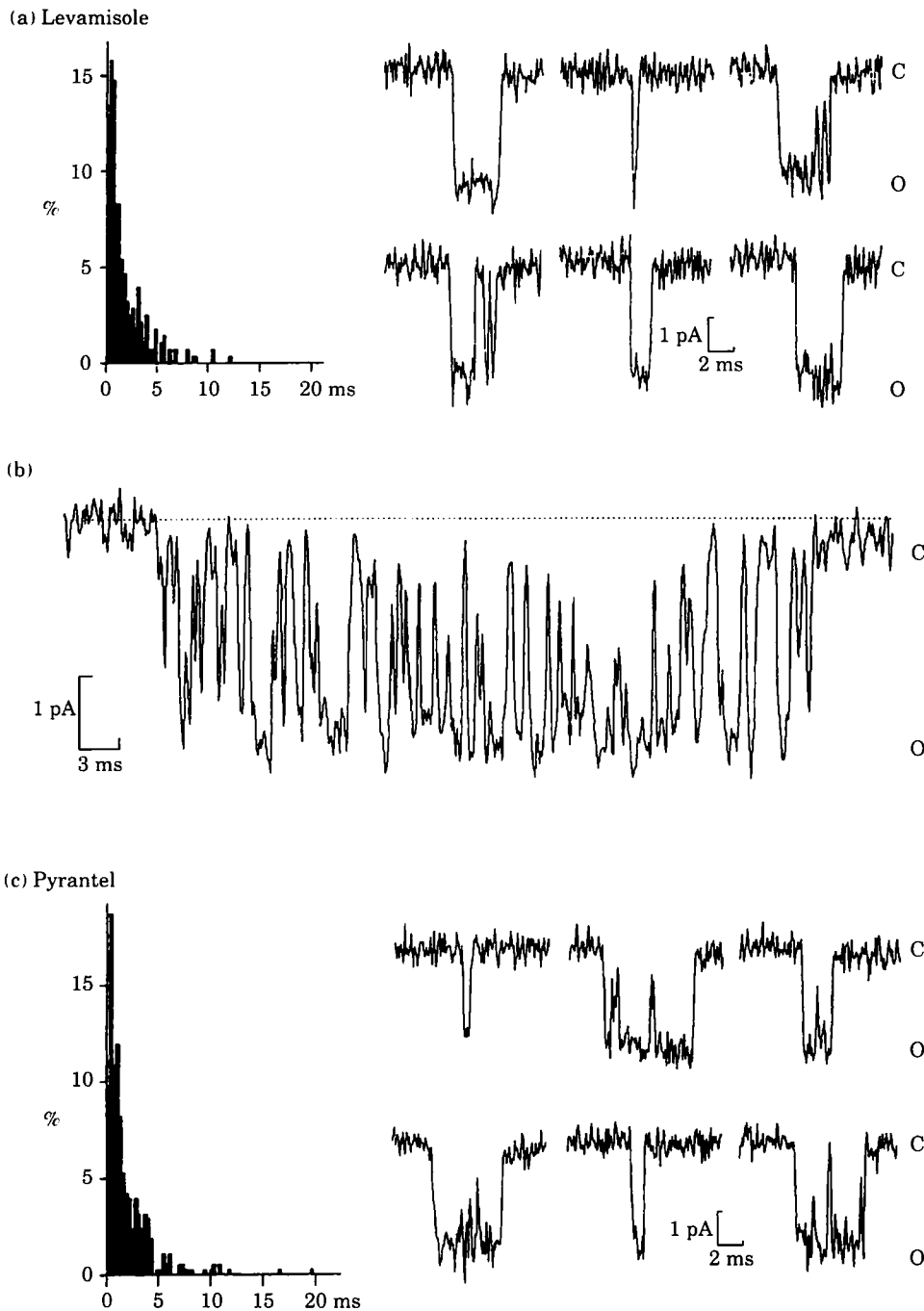
Fig. 3.   Patch-clamp records of single-channel currents activated by levamisole. The patch-clamp technique allows the activity of single ion-channels in the membrane of the cell to be recorded as the channel opens and closes. Small (1×10^{-12} A) current pulses are recorded with this technique. The currents are rectangular in shape; C is the closed state and O is the open state. (a) Histogram of open-times and records of levamisole-activated single-channel currents. Cell-attached patches. Openings downward. Patch potential –75 mV; 10 μM levamisole in the patch pipette. Mean open time: 1.34 ms. (b) A flickering burst demonstrating channel block produced by 30 μM levamisole at –75 mV, cell-attached patch. The comb effect of the channel current is characteristic of a drug moving into and blocking the ion-channel. (c) Histogram of open-times and records of pyrantel-activated single-channel currents. Cell-attached patch; openings downward; patch-potential –75 mV; 0.1 μM micromolar pyrantel in the patch-pipette. Mean open-time: 1.09 ms. The pyrantel channel open times are on average slightly shorter than those of levamisole.

mutants with modified nicotinic acetylcholine receptors on muscle (Lewis *et al.*, 1980; Lewis *et al.*, 1992). (2) Accommodation and recovery of parasites after long periods of exposure to the anthelmintics (Lewis *et al.*, 1980; Lewis *et al.*, 1992), and which may be explained by nicotinic receptor desensitization.

These resistant or recovered nematodes no longer respond to the nicotinic anthelmintics, but interestingly, may still respond to acetylcholine (Coles *et al.*, 1975). One explanation for these results is that in addition to the nicotinic receptor on nematode muscle, there are other non-nicotinic cholinergic receptors present on muscle not stimulated by the nicotinic anthelmintics. Such receptors may facilitate contraction by being coupled via G-proteins to voltage-activated channels.

Genetics of resistance to nicotinic anthelmintics

The genetics of resistance to the anthelmintic levamisole has been studied in the laboratory using the small free-living soil nematode, *Caenorhabditis elegans*. Several hundred levamisole-resistant alleles have been identified that were isolated in several screens (Lewis *et al.*, 1980; Lewis *et al.*, 1992). The genes responsible for this resistance included: *lev-1*, *unc-29* and *unc-38* that encode the proteins subunits that make up the nicotinic ion channels of the nematode. In addition, other genes including: *unc-50*, *unc-63* and *unc-74* that are believed to be involved in receptor biosynthesis have been identified.

Only two strains of *lev-1* (×21 and ×61) were dominant when crossed over with wild-types (non-resistant) (Fleming *et al.*, 1994). The *lev-1* (×21) strain only involves mutation at a single amino-acid, replacing glutamic acid in the ion pore of the receptor ion channel (in alpha-7 at position 237) with a positively-charged lysine. This change is believed to be sufficient to change the ion channel from cationic to anionic, i.e., to convert the receptor from an excitatory channel to an inhibitory one. The *lev-1* (×61) strain contains the amino-acid leucine in the pore of the ion-channel (in alpha-7 at position 247): this point mutation is expected to produce increased desensitization and reduced affinity for levamisole, rendering levamisole less potent as an agonist.

The genetics of levamisole resistance in parasitic nematodes remains to be studied.
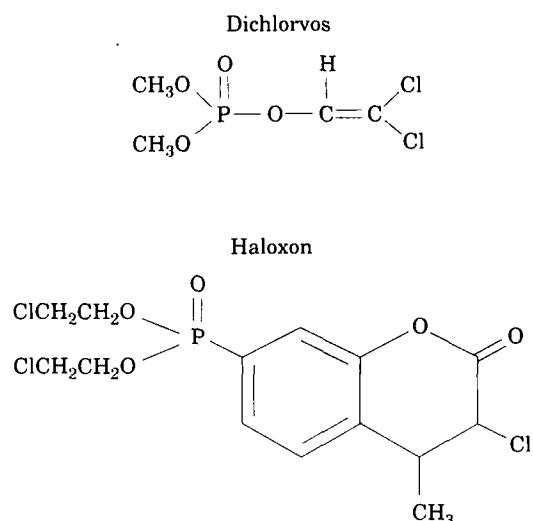

Fig. 4. Chemical structure of dichlorvos and haloxon.

ORGANOPHOSPORUS CHOLINESTERASE INHIBITORS

Dichlorvos, haloxon

Compounds like dichlorvos and haloxon are selective organophosphorus anti-cholinesterases (Fig. 4), and have an anthelmintic action, as well as an insecticidal, action. Dichlorvos and haloxon can control insect parasites, as well as helminth parasites. The mode of action of these compounds is to block the action of the parasite enzyme, acetylcholinesterase, leading to the excessive build up of the neurotransmitter, acetylcholine. This mode of action also predisposes towards toxicity in the host animal where acetylcholinesterase enzymes are also present. Because more selective combined anthelmintic+insecticidal agents (avermectins and milbemycin) are available, the organophosphorus compounds are now used less frequently.

The existence of cholinesterase, the enzyme that breaks down acetylcholine, was first described in *Ascaris* by Bueding (1952). The distribution of cholinesterase was described by Lee (1962) who used histochemical techniques. In the head region of *Ascaris*, most of the enzyme activity is associated with the contractile spindle region of the muscle and is in the extracellular matrix. Lee (1962) also described cholinesterase activity on the muscle arms near their endings on the nerve cords but not on the bag region of the muscle.

In *C. elegans*, three classes of acetylcholinesterase are recognized: class A, class B, and class C,
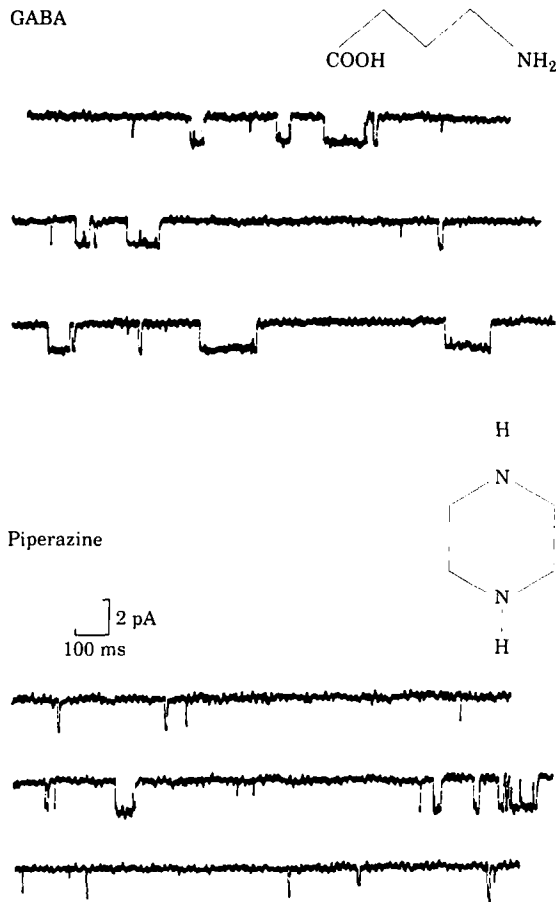
Fig. 5. The chemical structures of GABA and piperazine (left) and single-channel currents activated by them (right). Openings downward. Cell attached patches: -75 mV GABA channel mean open-times 32 ms. Piperazine mean-open times 18 ms. GABA 3μM. Piperazine 500 μM.

and are known to be products of three separate genes: *ace-1*, *ace-2 and ace-3* (Opperman & Chang, 1992). The three classes of acetylcholinesterase are separable by their solubility in Triton, their temperature stability and sensitivity to cholinesterase antagonists. In *C. elegans*, the major form is class B which is distributed in the head and body; classes A and C have a distribution biased towards the head. Extraction of the different classes for biochemical characterization has produced evidence that the enzyme may exist as a monomer, dimer and tetramer. Defects in the *ace-1, ace-2 and ace-3* genes suggest that the functions of the different classes are supplementary but that if there are defects in all of the genes, then elevated levels of acetylcholine occur in *C. elegans* and there is motor incoordination (Opperman & Chang, 1992).

Although acetylcholinesterase is responsible for the breakdown of acetylcholine and involved in motor action in nematodes, it is also secreted into the external environment in large quantities by *A. suum* and other parasitic nematodes. The function of the secreted acetylcholinesterase may be to reduce the effects of host acetylcholine in the intestine, perhaps decreasing mucosal glandular secretion by the host. The original hypothesis of a biochemical 'hold-fast' effect (reduced acetylcholine in the host intestine blocking peristalsis of the gut) has now been rejected. The identification of the function of secreted cholinesterase by parasitic nematodes, and the ability to antagonize this enzyme, may lead to the increased use of anticholinesterases in the future to facilitate removal of gut nematodes.

GABA AGONIST

Piperazine

Piperazine has a heterocylic ring structure (Fig. 5), and unlike γ-amino-butyric acid (GABA), lacks a carboxyl group. However, these two compounds act on the same receptor that is a ligand-gated Cl⁻ channel found on the synaptic and extrasynaptic membrane of nematode muscle (Martin, 1980; Martin, 1982). Bath application of GABA or piperazine increases the opening of the muscle membrane Cl⁻ channels, hyperpolarizes the membrane potential, increases the membrane conductance and produces spastic paralysis. Piperazine appears to have a selective effect on large nematodes of the gastrointestinal tract, perhaps because the high CO_2 environment allows CO_2 to mimic the missing carboxyl group of piperazine. Piperazine appears less active against nematodes that occupy a high O_2 environment.

Piperazine- and GABA-activated channel currents (Fig. 5) have been recorded using cell-attached and isolated outside-out patches (Martin, 1985). Although the channels may have more than one conductance level, the most frequently observed is 22 pS for both agonists. However, the mean open time of the channels for piperazine was shorter, 14 ms, than that produced by GABA, which produced channels that had mean open times of 32 ms. GABA is more than 10–100 times more potent than piperazine when conductance dose–response relationships are examined during bath application of the agonists (Martin, 1985).
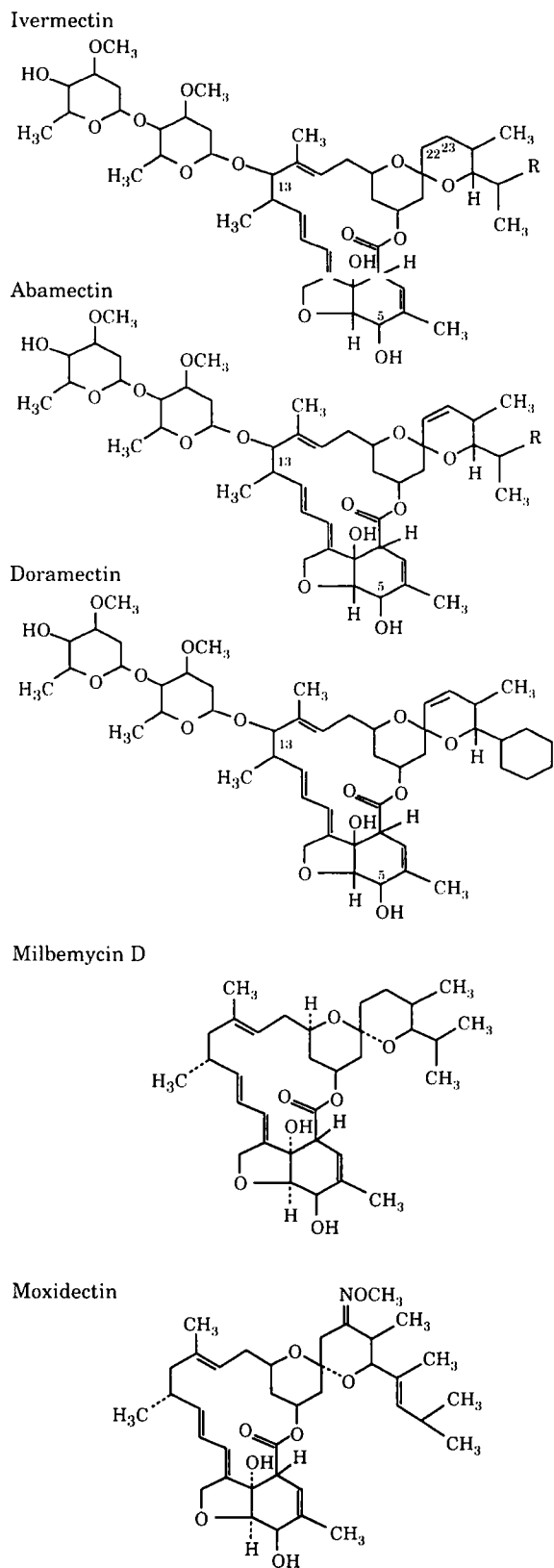
Ivermectin

Abamectin

Doramectin

Milbemycin D

Moxidectin

Fig. 6.

The difference in potency may be explained by the fact that higher concentrations of piperazine are required to produce the same opening rate of the channel as that produced by GABA, and that the average duration of the channel openings produced by piperazine is much shorter. The same probability of channel opening can be achieved with lower concentrations of GABA than piperazine. Therapeutically, however, GABA would be ineffective because it is not selective like piperazine and is highly ionized and does not cross the cuticle.

## GLUTAMATE-GATED CHLORIDE (GLUCL) RECEPTOR POTENTIATORS

Ivermectin, abamectin, doramectin, milbemycin D, moxidectin

The avermectins (Fig. 6) are a group of broad-spectrum, macrocyclic, lactone antibiotic anthelmintics used to control nematode parasites in man and animals (Campbell & Benz, 1984), and assumed to have the same mode of action in both (Shoop *et al.*, 1995). They are used to control onchocerciasis (river blindness) in humans and gastrointestinal, cardiac and respiratory nematode parasites of domestic animals. The mode of action of the avermectins is to selectively paralyse the parasite by increasing muscle Cl⁻ permeability, but the identity of the channel targeted by the avermectins has been controversial, see Arena (1994) for a recent review. Cloning of a GluCl-α1 and a GluCl-β subunit from the model soil nematode *C. elegans*, and co-expression of the subunits in *Xenopus* oocytes, has led to the identification of an avermectin-sensitive GluCl ion-channel (Cully *et al.*, 1994). The effects of avermectins, at low concentrations, are to potentiate the effect of glutamate, and at higher concentrations, the avermectins open the glutamate-gated channel directly. The selective therapeutic effects of the avermectins could be explained by an action on a Glu Cl⁻

Fig. 6. Chemical structure of avermectin and milbemycin anthelmintics. Ivermectin is at least 80% 22,23 dihydroavermectin B1a (R is sec butyl) and not more than 20% 22,23 dihydroavermectin B1b (R is isopropyl). Abamectin is at least 80% avermectin B1a (R is sec butyl) and not more than 20% avermectin B1b (R is isopropyl).
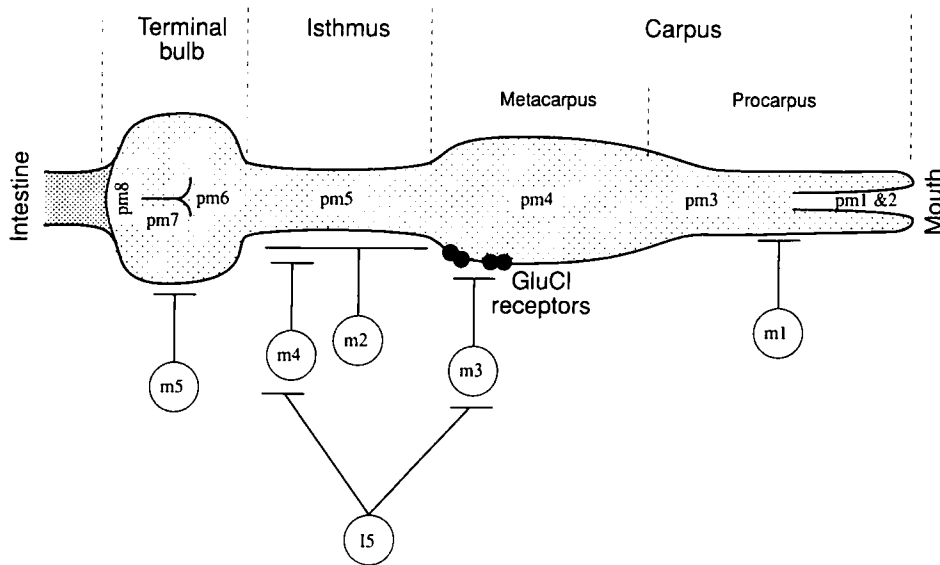
Fig. 7. Diagram of the pharyngeal muscle of *C. elegans* showing the location of the GluCl receptors on the *pm4* muscle cells that are innervated by the *m3* motor neurones. Also illustrated is the division of the pharyngeal muscle into the regions: terminal bulb; isthmus; carpus (subdivided into metacarpus and procarpus). The other motor neurones (*m1*, *m2*, *m4* & *m5*) that innervate the muscle cells (*pm3*, *pm5*, *pm6*, *pm7*) are shown. The interneurones *I5* that inhibits *m3*, as well as *m4*, is shown. Adapted from Avery (1993).
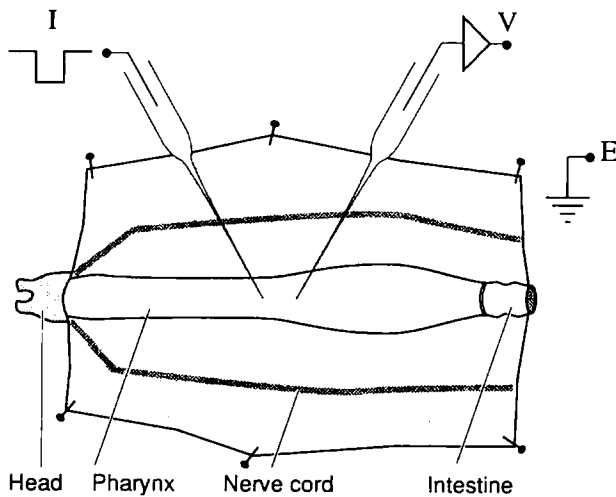
Fig. 8. Diagram of the preparation used to record the electrophysiological effect of glutamate and milbemycin D on the pharyngeal membrane potential and input conductance. Two micropipettes were inserted into the pharyngeal muscle: one was used to record membrane potential (V); the other was used to inject current (I).

ion-channel that is present in parasitic nematodes but not present in the host animal.

Molecular experiments using the *lacZ* marker suggest that the GluCl-β subunit of the glutamate

channel is expressed in the pharynx of *C. elegans* pM4 muscle (Fig. 7) (Laughton *et al.*, 1995). The location of the GluCl-α1 subunit is not known. The pharyngeal muscle is required for feeding, and is known to receive an inhibitory motor neurone, M3 (Fig. 7), that is not likely to be GABA-ergic (Laughton *et al.*, 1995) but glutamatergic (Avery, 1993). The location of an avermectin-sensitive GluCl channel, has been identified using a two microelectrode current clamp technique (Martin, 1996) in the parasitic nematode, *A. suum* (Fig. 8). Experiments show that the pharyngeal muscle of this parasite possesses glutamate receptors that gate chloride channels and that are sensitive to the avermectin analogue milbemycin D.

Effect of glutamate

Fig. 9 shows effects of the application of milbemycin D and glutamate on *Ascaris* pharyngeal muscle input conductance and membrane potential: glutamate [Fig. 9(b)] produces a transient small hyperpolarization of 1 mV associated with an increase in membrane conductance. The effect of glutamate but not GABA [Fig. 9(c)] in pharyngeal *Ascaris* preparations is to produce a reversible increase in input conductance associated with a small change in membrane potential and to

(a)

5 mV

30 s

890 nM Milbemycin

(b)

100 µM Glut.

(c)

5 mV

30 s

300 µM GABA
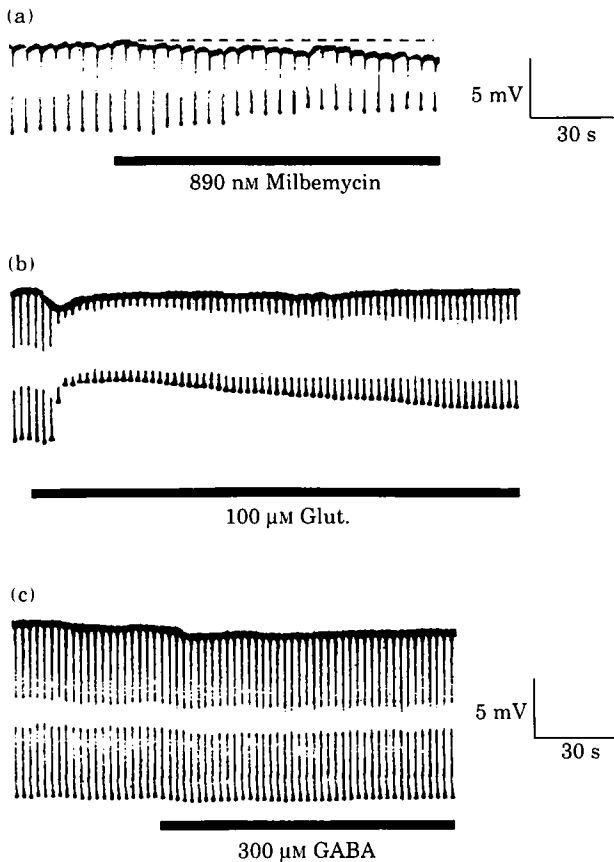
Fig. 9. (a) Effect of 890 nM milbemycin D on the membrane potential and input conductance. Milbemycin slowly produced an increase in input conductance that was not reversed on washing (not shown). (b) Effect of application of 100 µM L-glutamate on membrane potential and input conductance. Different preparation from (a). Glutamate (applied during horizontal bar) produced a transient small hyperpolarization of 1 mV associated with an input conductance change from 157 µS to a peak of 429 µS (ΔG: 272 µS) desensitizing to 231 µS (ΔG: 74 µS) after 4 min. (c) After washing the preparation the input conductance of the pharynx returned towards control levels (142 µS) and the effect of 300 µM GABA was tested without effect (applied during the horizontal bar). The lack of effect of GABA was not due to desensitization because subsequent application of 100 µM L-glutamate increased the input conductance again (not shown).

inhibit pharyngeal pumping that is part of the nematode parasite feeding process. Zero-Cl⁻ solutions were shown to abolish reversibly the glutamate-induced conductance responses (Martin, 1996) and were used to demonstrate that the glutamate effect was mediated by a Cl⁻ channels in *Ascaris.*
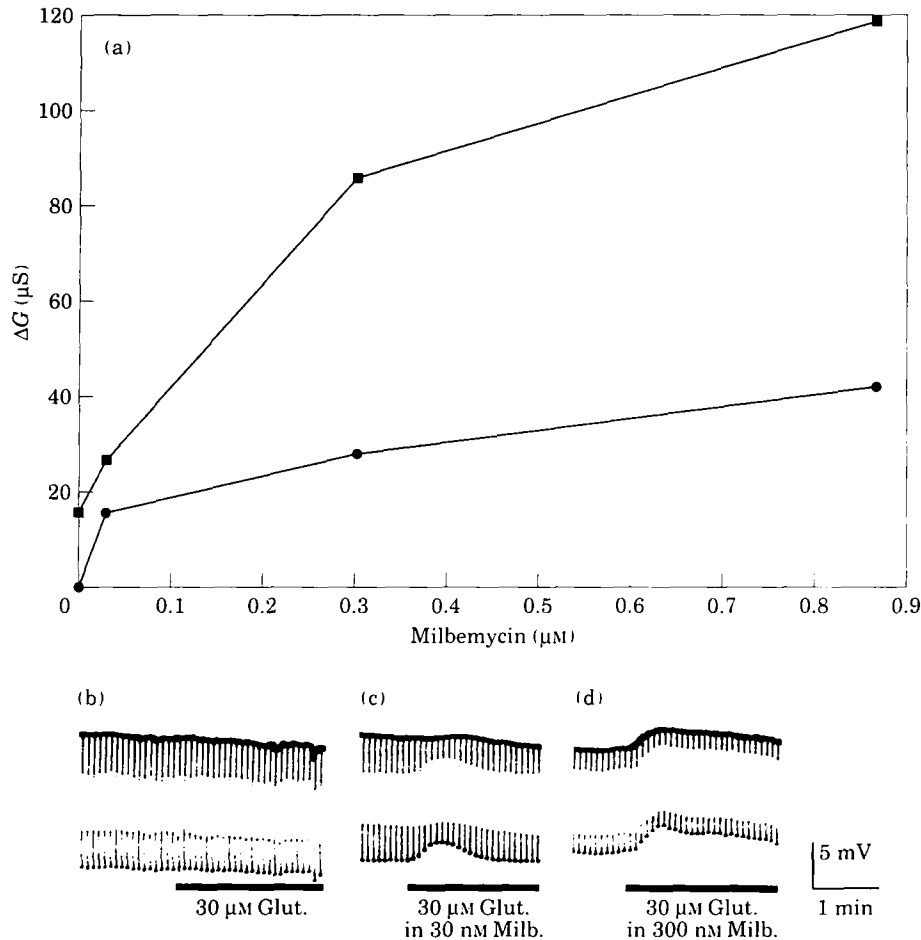
Effect of milbemycin D

The effect of ivermectin and milbemycin D, on the *C. elegans* avermectin-sensitive glutamate receptor expressed in *Xenopus* oocytes is to produce a potentiation of glutamate effects and to produce a slow irreversible increase in conductance of the membrane (Cully *et al.,* 1994). The effects of milbemycin D on the input conductance of the *Ascaris* pharyngeal preparation is to produce a small change in membrane conductance and to potentiate the effect of low concentrations of glutamate [Figs. (9a), 10(a), (b), (c) and (d)]. The location of the GluCl receptor or the avermectin binding α-subunit in the nematode remains to be explored fully. Recent molecular biological experiments with *C. elegans* on an ivermectin-resistant strain (*avr-15*) suggest that it is a GluCl-α2 subunit that is present in the pharyngeal muscle of *C. elegans* not the GluCl-α1 (review: Cully *et al.,* 1996). The location and function of the original GluCl-α1 subunit remains to be determined. However, the presence of more than one GluCl-α subunit indicates the presence of more than one site of action for the avermectins. It implies that the appearance of resistance to avermectins requires mutation of more than one gene.

In addition to effects on the pharyngeal muscle, ivermectin also has an effect on somatic muscle and opens non-GABA activated channels, and in addition, inhibits GABA-activated channels (Holden-Dye & Walker, 1990). Thus again it appears that the avermectins may have more than one site of action in parasitic nematodes.

INCREASED CALCIUM PERMEABILITY

Praziquantel

Fig. 11 shows the chemical structure of praziquantel. Praziquantel has a selective toxic effect on schistosome parasites, where its mode of action has been studied more extensively (Andrews *et al.,* 1983; Harnett, 1988) than in cestodes. Many actions of praziquantel may be explained by an increased Ca^{2+} permeability of parasite muscle and/or tegumental membranes. Fig. 12 is a diagram of the schistosome tegument and underlying muscle that illustrates the known sites where Ca^{2+} crosses into and between intracellular compartments, and therefore, the possible sites for praziquantel action.

Fig. 10. Glutamate potentiation effects of milbemycin D. (a) (■) Plot of peak ΔG produced by 30 μM glutamate against milbemycin concentration: (●) plot of resting input conductance of the pharynx against milbemycin concentration. Results from the experiment illustrated in (b), (c) & (d).(b) Control 30 μM glutamate response, peak ΔG, is small, 16 μS. (c) In the presence of 30 nM milbemycin (D) the peak ΔG produced by 30 μM glutamate increased to 27 μS. (d) 300 nM milbemycin D increased the peak ΔG to 86 μS. In this particular preparation the glutamate response was a small depolarizing potential, indicating that the Cl-reversal potential was slightly depolarized relative to the membrane potential.
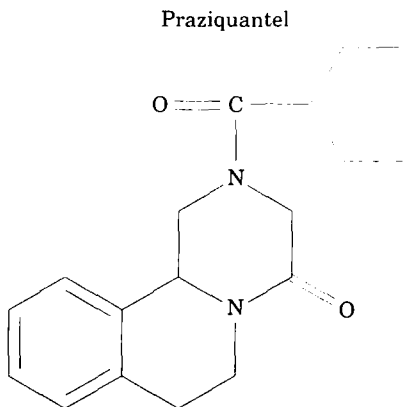
Praziquantel

Fig. 11. Chemical structure of praziquantel.

Effect on Ca^{2+} permeability

Praziquantel is known to increase [45]Ca^{2+} influx across the schistosome tegument and to cause rapid muscle contraction of the parasite (Mehlhorn et al., 1981). It is known that bathing the parasites in Ca^{2+}-free medium blocks the praziquantel induced contraction (Fetterer et al., 1980; Wolde-Mussie et al., 1982; Thompson et al., 1984) but the effect of bathing the preparation in Ca^{2+}-free bath solution is not immediate, requiring more that 10 min to be effective. The effect of praziquantel on Ca^{2+} influx suggests that the sites of action are Ca^{2+} permeable ion channels in the membrane of the tegument and muscle cell (Blair et al., 1994). The contractions in shistosomes are reversed if praziquantel is removed and may be
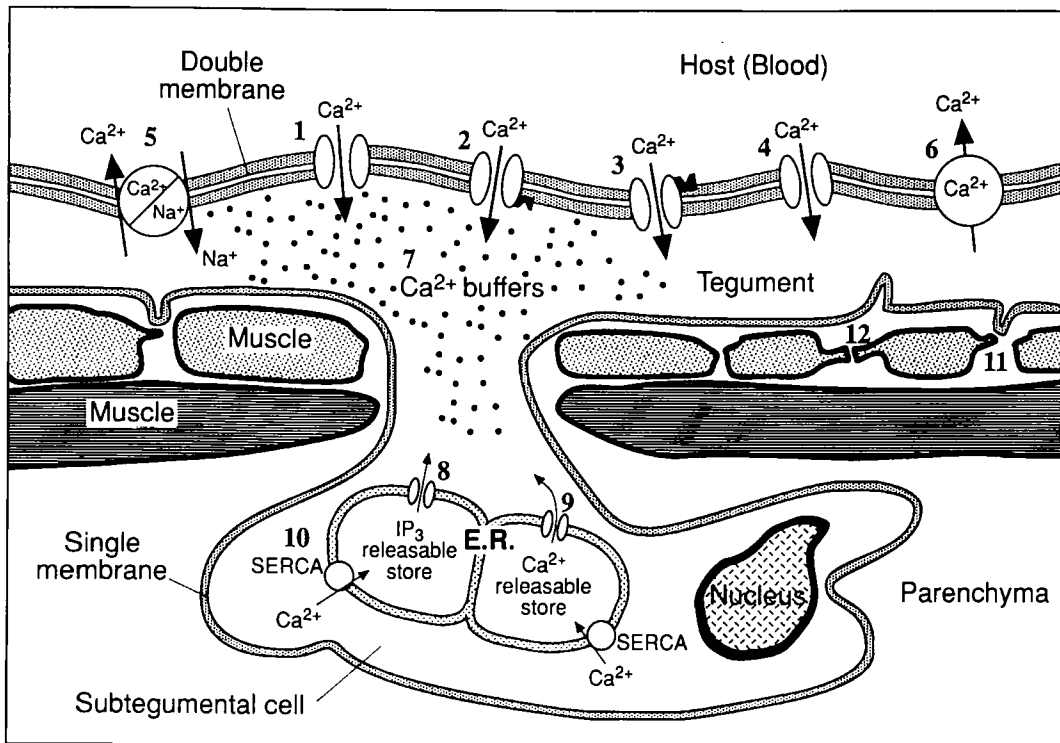
Fig. 12. Diagram of the structure of the body wall of *Schistosoma mansoni* showing the possible sites of action of praziquantel and mechanisms of Ca^{2+} transport into and out of the tegument. 1: Voltage-activated Ca^{2+} channel. 2: Intracellular messenger activated Ca^{2+} channel. 3: Extracellular receptor operated Ca^{2+} channel. 4: Non-selective cation channel also allowing entry of Ca^{2+}. 5: Na^+/Ca^{2+} exchanger. 6: Ca^{2+} ATPase pumping out Ca^{2+}. 7: Intrategumental Ca^{2+} buffers. 8: IP_3 releasable store from the sarcoplasmic reticulum. 9: Ca^{2+} induced Ca^{2+} release channel (CICR channel). 10: Ca^{2+} ATPase pump: sarcoplasmic endoplasmic reticulum Ca^{2+} (SERCA). 11: Electrical junction between muscle cell and tegument. 12: Electrical junctions between muscle cells. If praziquantel acts like caffeine it would act on site 9. 13: A proton pump may set up a pH gradient across the tegument and be required for normal function. A proton ionophore would upset this gradient and lead to the demise of the organism.

blocked by Mg^{2+}, or La^{3+} but not by Ni^{2+} Co^{2+} or the calcium-channel blocker D-600 (Fetterer *et al.*, 1980; Wolde-Mussie *et al.*, 1982). The application of a high K^+ solution to depolarize the schistosome preparation results in a rapid depolarization, but the application of praziquantel resultsin a slower onset depolarization. From these observations, it may be concluded that praziquantel somehow results in an increase in Ca^{2+} permeability across parasite membranes via channels that are not activated by depolarization. These channels must be pharmacologically different from those of the host animal or the praziquantel would affect the host animal.

Experiments on a snail smooth muscle preparation (Gardner & Brezden, 1984) have illustrated that caffeine mimics the effect of praziquantel in producing contraction, and that prior treatment of the muscle preparation with caffeine

will eliminate the effect of praziquantel on the muscle preparation in Ca^{2+}-free solutions. These experiments suggest that caffeine and praziquantel share the same site of action.

In vertebrate smooth muscle preparations, caffeine acts to stimulate the opening of a large cation channel known as the CICR channel (calcium-induced calcium release channel) (Herrmann-Frank *et al.*, 1991) in the sarcoplasmic reticulum. This channel is activated by cytosolic rises in Ca^{2+}, adenosine triphosphate (ATP) and caffeine but is inhibited by Mg^{2+}. The CICR channel produces regenerative Ca^{2+} release from the sarcoplasmic reticulum following entry of Ca^{2+} through voltage-activated channels (Gregoire *et al.*, 1989). Large cation channels, which might be similar to the CICR channel, have been observed in the tegumental membrane of schistosomes (Day *et al.*, 1992).

Parasite antigen exposure

It has also been found that curative doses of praziquantel in infected animals results in antigens of schistosomes being exposed and binding and penetration of host antigen cells to the parasite after 17 h treatment (Harnett & Kusel, 1986; Brindley & Sher, 1987). The immune system of the host, therefore, plays an important role in the praziquantel-induced death of the parasite. One explanation for the exposure of the parasite antigen may be that increased cytosolic Ca^{2+} causes phospholipase C activation and then activation of protein kinase C (Berridge & Irvine, 1984; Sigiya & Furuyama, 1990). The phosphorylation of proteins by protein kinase C could then destabilize the tegument causing the observed vacuolation and antigen exposure (Wiest *et al.*, 1992). Once again the mode of action of praziquantel relates to an effect on Ca^{2+} permeability of the tegumental membranes. Praziquantel is used frequently to treat tapeworm infestations but less is known of the mode of action in this group of parasites. It is presumed that there is a common mode of action against trematodes and cestodes.

INHIBITION OF MICROTUBULE FORMATION, β-TUBULIN BINDING

Benzimidazoles: thiabendazole, cambendazole, mebendazole, fenbendazole, oxibendazole, oxfendazole, albendazole, albendazole sulphoxide, parbendazole, flubendazole, triclabendazole & prodrugs: netobimin, febantel, thiophanate

Fig. 13 illustrates the chemical structures of the benzimidazole anthelmintics and prodrugs. Prodrugs are converted to active benzimidazoles by metabolic processes in the host animal so that it is the active metabolites that are responsible for the anthelmintic action. Triclabendazole, the flukicidal compound, is assumed to act by the same mechanism as the other benzimidazoles but a clear explanation for its selective effect against fluke is not known (McKellar & Kinabo, 1991) but may be related to its high level of plasma protein binding.

β-tubulin binding

Mebendazole (Borgers *et al.*, 1975; Van den Bossche & De Nollin, 1973) and flubendazole (Van den Bossche & De Nollin, 1973) induce the loss of cytoplasmic microtubules of the tegumental and intestinal cells of cestodes and nematodes, and this is followed by loss of transport of

secretary vesicles, a decreased glucose uptake and an increased utilization of stored glycogen. In *Ascaris*, mebendazole is taken up by pharyngeal and intestinal cells where it is found in the cytoplasmic fraction bound to proteins with molecular weights of about 50 and 100 kDa that represent monomers and dimers of tubulin. Our current understanding of the mode of action of the benzimidazoles thus started when it was recognized that the effect of mebendazole on *Ascaris* was to disrupt the microtubules of the intestinal cells producing an inability to take up glucose (Van den Bossche, 1972; Van den Bossche *et al.*, 1982). It was found that benzimidazole anthelmintic competed with the binding site for [H³] colchicine on β-tubulin and that potency of the benzimidazole anthelmintics correlated with the dissociation constant for binding to nematode β-tubulin (Lacey, 1990; Sangster *et al.*, 1985).

Microtubules are intracellular organelles that serve a variety of functions including movement of chromosomes during cell division; providing the structural skeleton to the cell; movement of intracellular particles including energy metabolites; and exocytosis. They are found in both animal, plant, fungi and some bacterial cells (Stryer, 1995).

Microtubules are composed of two 450 amino-acid proteins known as α-tubulin and β-tubulin. Fig. 14 is a diagrammatic representation of β-tubulin. It is composed of three domains: domain 1 is 34 kDa in size; domain 2 is 19 kDa in size and the tail is 2 kDa in size. There are GTP binding sites present which bind to sites I, II, III, and IV on the β-tubulin. The β-tubulin is folded so that the GTP-binding sites form a pocket.

Formation of microtubules

The formation of microtubules is a dynamic process (Fig. 14.) Formation involves the polymerization of tubulin at one end (the positive pole) and the depolymerization at the other end (the negative pole). The microtubules that form are made up of 13 tubulin molecule rings (6 α-tubulin+7 β-tubulin alternating with 7 α-tubulin+6 β-tubulin rings). Seventy-five to 85% of the mass of microtubules is composed of the tubulin proteins but in addition, microtubule-associated proteins (MAPs) are present stabilizing the structure. A number of factors favour polymerization including GTP, Mg^{2+}, and an increase in temperature (to 37°C). A decrease in temperature (to 4°C), the presence of Ca^{2+} or calmodulin will favour depoly-
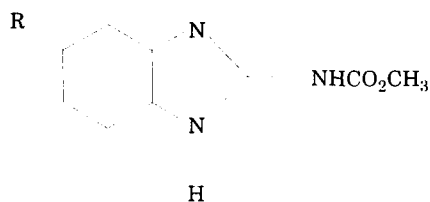
BENZIMIDAZOLES

PRODRUG
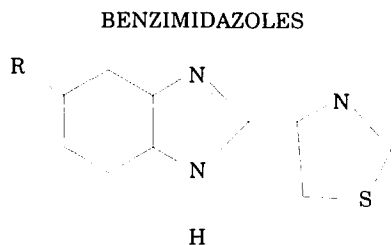
Thiabendazole: R = H—

Cambendazole: R = (CH₃)₂CHOCONH—
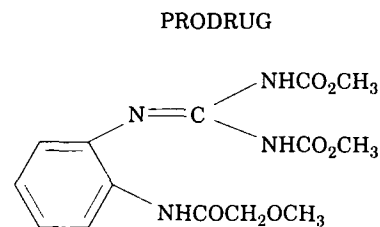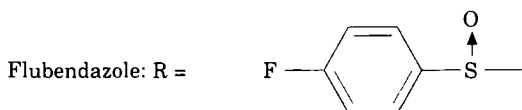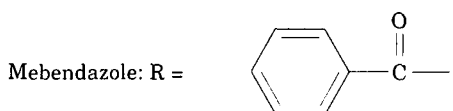
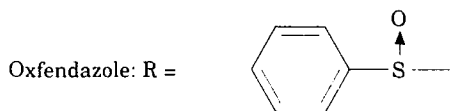Febantel: converts to fenbendazole

BENZIMIDAZOLE CARBAMATES

TRICLABENDAZOLE

Oxibendazole: R = CH₃CH₂CH₂O—

Albendazole: R = CH₃CH₂CH₂S—

Fenbendazole: R =

Oxfendazole: R =
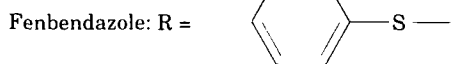
Mebendazole: R =
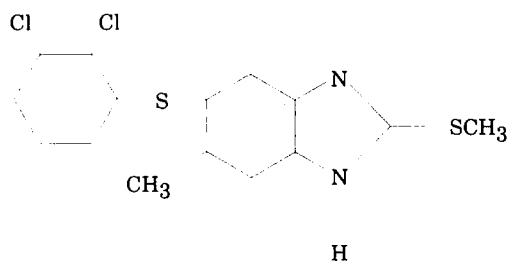
Flubendazole: R =

Fig. 13. Chemical structure of benzimidazoles, benzimidazole carbamates, prodrug febantel and the antifluke drug triclabendazole.

merization. Interestingly, α- and β-tubulin will polymerize into a number of shapes (rings and sheets) in addition to microtubules when *in vitro* preparations are made. The formation of microtubules may be inhibited by substances that bind to the leading edge (the positive pole) of polymerization. This process of inhibition is known as 'capping', and colchicine, vinblastine, vincristine,

the mitotic inhibitors and benzimidazoles can do this by binding to β-tubulin molecules.

Thus the mode of action of the benzimidazole anthelmintics is the selective binding to nematode β-tubulin, and consequent inhibition of microtubule formation. The effects are, therefore, slower in onset than the anthelmintics that act as neurotransmitter agonists, and include slow onset

β-Tubulin

Domain I: 34 KDa   Domain: II 19 KDa Tail: 2 KDa

IV I   II   III
100   200   300   400

Hinge

Microtubule formation from α- and β-tubulin

○ α-tubulin
+
● β-tubulin

13 membered
spiral made of
alternating α-
and β-tubulin

Vesicle
transport along
microtubules

Negative pole.
Microtubule
breakdown
increased by fall in
temperature and Ca^{++}.

Positive pole.
Microtubule
formation
increased
by GTP, Mg^{++},
temperature
inhibited by
benzimidazoles.

Fig. 14. Diagram of the 50 kDa β-tubulin protein. It consists of three domains that are separated by protease action. In the middle in a 'hinge' allowing the molecule to fold. The location of the amino acids 100, 200, 300 and 400 are shown. Below this is a diagram representing the formation of microtubules by α- and β-tubulin that polymerizes by forming a 13 membered hollow spiral. Each turn of the spiral allows the α- and β-tubulin to pack alternately along the length of the microtubule. Polymerization takes place at the positive pole where temperature and GTP Mg^{2+} and other factors favour microtubule formation. The formation of microtubules is inhibited by binding of benzimidazoles to β-tubulin to produce 'Capping' and inhibition of further microtubule formation. Breakdown occurs at the negative pole. The physiological function of the microtubules include intracellular transport via special proteins, kinesins or dynesins (here represented by the match-stick man).

starvation of the nematode (intestinal cell disruption) and an inhibition of egg production.

Genes for β-tubulin and resistance

In nematodes, two isotypes of β-tubulin have been identified: isotype I and isotype II which have separate genes (Guenette *et al.*, 1991; Guenette *et al.*, 1992; Lubega *et al.*, 1994). Each of these isotypes have alleles: up to six for isotype I; and up to 12 for isotype II. It is not known if the specific isotypes have different functions in the nematodes.

A reduction in the number of isotype alleles for β-tubulin is associated with the appearance of benzimidazole resistance (Roos et al., 1995). Resistance is associated with a progressive loss of alleles of isotype 1 and a total loss of isotype 2 from the nematode population. A resistant population of Haemonchus contortus has been characterized by only the presence of a single β-tubulin allele referred to as allele 200. The appearance of resistance can be explained by a loss of susceptible phenotypes and the survival of a resistant phenotype and its increased representation in the remaining population.

In fungi, benzimidazole resistance is associated with the appearance of a different form of β-tubulin (Fujimura et al., 1992; Jung et al., 1992) which is characterized by the appearance of tyrosine instead of phenylalanine in position 200. Because mammalian β-tubulins have also tyrosine present in the 200 amino acid position (Lewis et al., 1985) it is unlikely that benzimidazole resistance may be overcome by changes in drug chemistry. It would not be possible to design a selectively-toxic agent against the fungi because the fungi and host β-tubulins would both bind the benzimidazole to the same degree and so be toxic to both species. A similar phenomenon may occur with nematode parasites.

## PROTON IONOPHORES: SALICYLANILIDES AND SUBSTITUTED PHENOLS.

Salicylanilides: closantel, rafoxanide, oxyclozanide, brotianide and substituted phenols: nitroxynil, niclopholan, hexachorophene dibromsalan & niclosamide

The pharmacology of a range of anti-fluke drugs have been reviewed (McKellar & Kinabo, 1991). In this section, the mode of action of the proton ionophores (often called oxidative phosphorylase uncouplers) are considered. The chemical structure salicylanilides and nitroxynil illustrates that each anthelmintic molecule possesses a detachable proton (Fig. 15). These molecules are very lipophilic and may shuttle protons across membranes, particularly the inner mitochondrial membrane. Fig. 16 represents the process by which ATP production occurs in the mitochondria, that is coupled to the proton gradient across the inner mitochondrial membrane. Oxidative phosphorylation may be summarized as electrons from NADH or FADH being conveyed through a

series of protein complexes on the inner mitochondrial membrane. This process leads to protons being pumped out of the mitochondria matrix producing a proton motive force that is due to the pH gradient and transmembrane electric potential. ATP is synthesized when the protons flow back into the mitochondrial matrix through an enzyme complex. This process of oxidative phosphorylation takes place in the host animal (Stryer, 1995), as well as in the parasitic helminths (Van den Bossche, 1972; McKellar & Kinabo, 1991).

Lipid soluble substances that can carry protons may shuttle across the inner membrane of the mitochondria, and remove the proton gradient and uncouple oxidative phosphorylation so that the oxidation of NADH and FADH is no longer linked to the production of ATP and may carry on without its production. Fig. 15 shows the position of the proton that is able to dissociate from a variety of compounds. 2,4-Dinitrophenol, carbonyl-p-trifluoro-methoxyphenylhydrazone, (Stryer, 1995) and hexachlorophene, nitroxynil, oxyclozanide (Corbett & Goose, 1971; Edwards et al., 1981b) are all lipophilic compounds that are capable of carrying protons across membranes, and therefore, may act to uncouple oxidative phosphorylation preventing the production of the proton gradient across the inner mitochondrial membrane.

The mechanism of action of the proton ionophores has been assumed to be to selectively uncouple oxidative phosphorylation in parasite mitochondria. Pax and Bennett (1989) have experimental evidence that the inner membrane of the parasite may not be the site of action of the proton ionophores. They observed that application of closantel to Schistosoma mansoni and Fasciola hepatica produces a fall in tegumental pH (6.8–6.5) when measured with an intracellular electrode, and that this change in pH occurred after 10 min and before any change in the production of ATP by the parasite. The intra-tegumental fall in pH was associated with a reduction in motility of the parasite and could explain the anti-parasitic action of closantel. It was concluded by Pax and Bennett (1989) that closantel was a membrane active molecule and that it may affect a number of biochemical and physiological processes. These processes are presumably sensitive to changes in intra-tegumental pH. Thus the site of action of the proton ionophores may include the tegument rather than just the mitochondria.

Oxyclozanide

Rafoxanide

Closantel

Brotianide
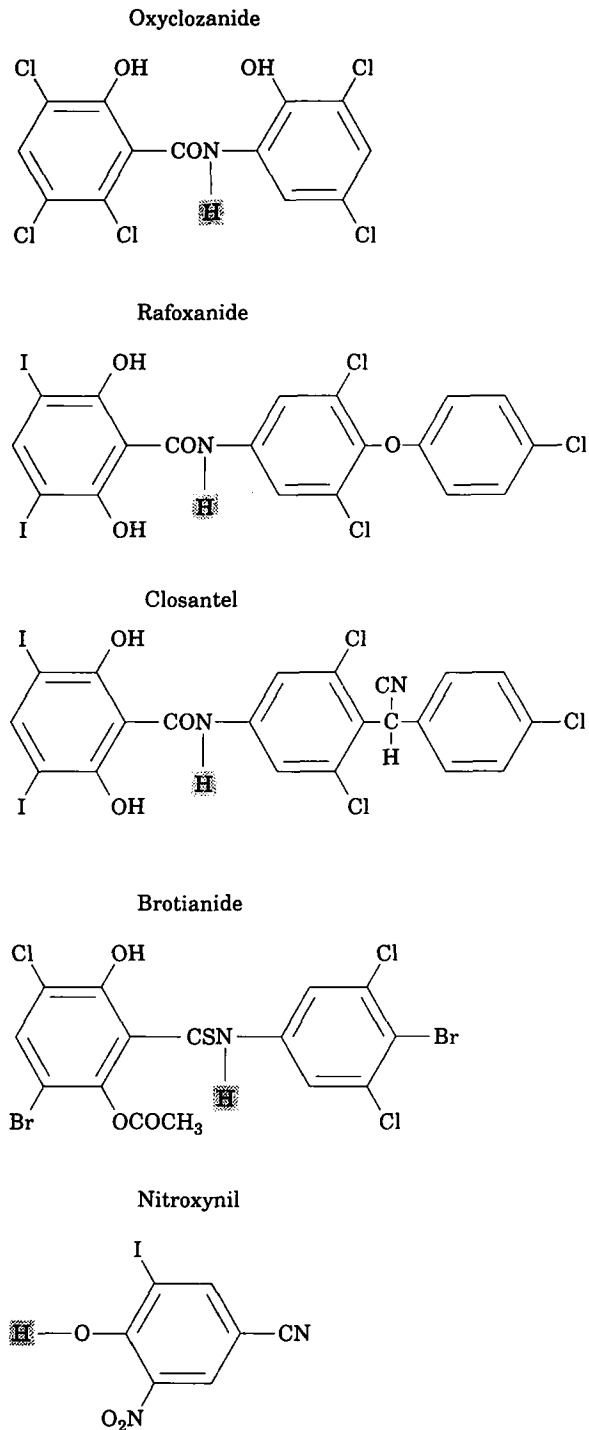
Nitroxynil

Fig. 15. Chemical structure of the salicylanilides oxyclozanide, rafoxanide & closantel and the DNP derivative nitroxynil. The location of the dissociating proton (H) is shown in the shaded box.

The anthelmintics that have the longest half-life in the body are the salicylanides and nitroxynil.
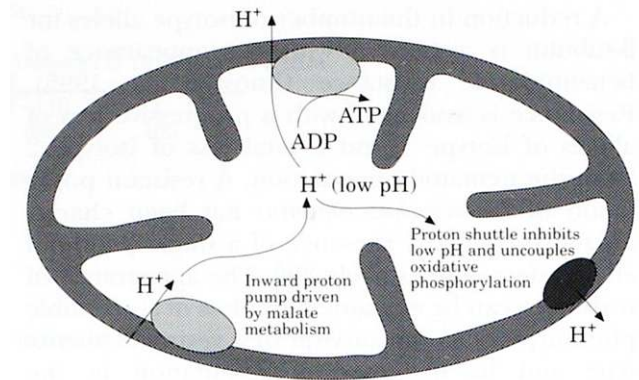
Fig. 16. Diagram of the mitochondria illustrating the inhibitory effects of an oxidative phosphorylase uncoupler on normal ATP production that is driven by the proton gradient inside the mitochondria and produced by tricarboxylic acid metabolism.

This is explained by their strong plasma protein binding which is more than 99% for the salicylanilides (Mohammed-Ali & Bogan, 1987) and 98% for nitroxynil (Alvinerie et al., 1995). The selective anthelmintic action of these highly protein-bound anthelmintics may be explained, in part, by their effect against blood-sucking parasites, concentrating the anthelmintic in the parasite without the high tissue levels being produced in the host. The high level of protein binding may explain the selective effect of these agents, and the fact that well bled out carcasses have low tissue residue levels (McKellar & Kinabo, 1991). Thus the mode of action of this group of anthelmintic involves the selective delivery of the proton ionophores to the parasite because of the high level of plasma-protein binding.

DIAMPHENETHIDE

Diamphenethide (Fig. 17) is more active against immature *Fasciola hepatica* in the liver than the adult *Fasciola* in the bile ducts (Kendall & Parfitt, 1973). Diamphenethide is a prodrug that is deacetylated in the host's liver to an active form which is the monoamine and the diamine (Coles, 1976). The effects of the diamphenethide amine (active form) on *Fasciola in vitro* have been studied by Edwards et al. (1981a). These authors described how diamphenethide amine produces an elevation of malate concentrations, an intermediary breakdown product of glucose metabolism. They were not able to identify an action of diamphenethide on a particular enzyme in the glycolytic pathway but suggested that the effect on malate

Diamphenethide

$$CH_3CONH - \langle \rangle - OCH_2CH_2OCH_2CH_2O - \langle \rangle - NHCOCH_3$$

Clorsulon

Cl

$Cl_2 = C$ NH_2

$H_2NO_2S - \langle \rangle - SONH_2$

Diethylcarbamazine

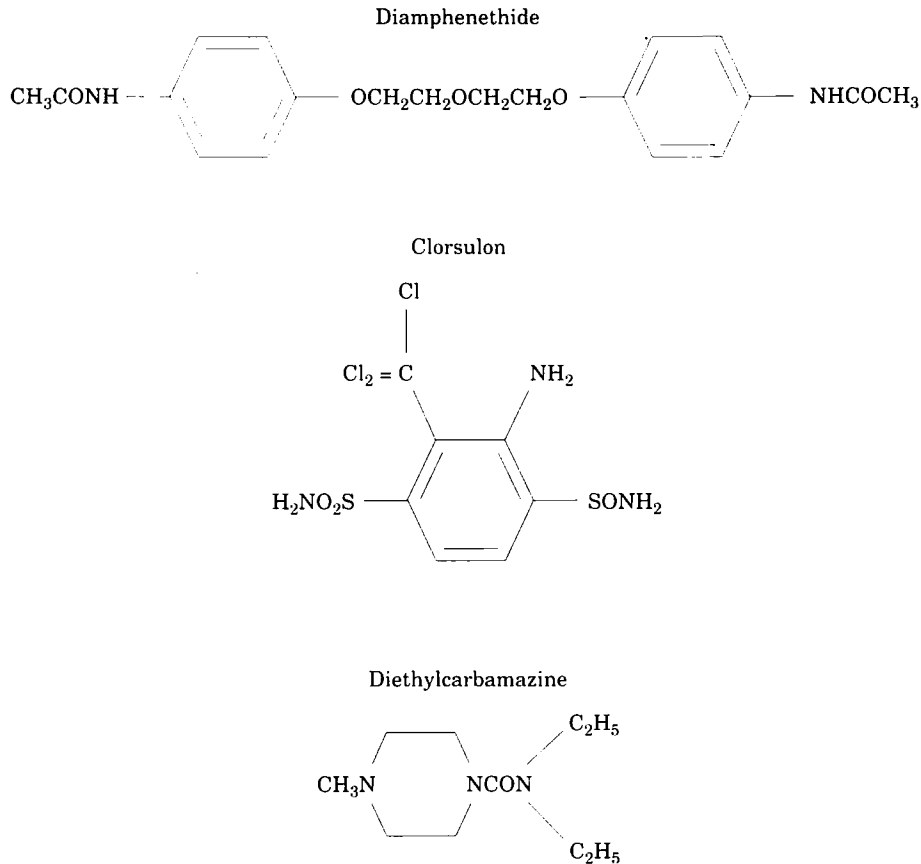$$CH_3N \langle \rangle NCON \begin{matrix} C_2H_5 \\ C_2H_5 \end{matrix}$$

Fig. 17.   Chemical structure of diamphenethide, clorsulon and diethylcarbamazine.

was likely to be a primary effect because it occurred early on and before the deterioration of the whole parasite. Interestingly, in the second paper of Edwards *et al.* (1981b) the effect of dopamine, a putative neurotransmitter in *Fasciola* had a protective effect against diamphenethide.

The action of diamphenethide remains to be defined in greater detail but the study of Edwards *et al.* (1981a) showed that its biochemical effects contrasted with the proton ionophore, oxyclozanide and appears to involve effects on malate metabolism in *Fasciola*.

INHIBITION OF PHOSPHOGLYCERATE KINASE AND MUTASE

Clorsulon

Clorsulon is 4-amino-6 trichloro ethenyl 1,3-benzenedisulphonamide (Fig. 17). Structurally, it is similar to 1,3-diphosphoglycerate (Schulman *et al.*, 1982) and consequently, inhibits the enzymes

phosphoglycerate kinase (Schulman *et al.*, 1982) and phosphoglyceromutase (Schulman & Valentino, 1982) of *Fasciola* and inhibits the Emden–Meyerhoff pathways in fluke. As a result, there is a selective inhibition of glucose utilization, and acetate and propionate formation. The inhibition of the *Fasciola* phosphoglycerate kinase is competitive (Fig. 18) with a K_i of 0.29 mM: clorsulon competitively inhibiting the binding of ATP and 3-phosphoglycerate to the phosphoglycerate kinase. Schulman *et al.* (1982) suggested that the large group on the six position of clorsulon prevented the conformational change in the kinase enzyme required for activity. There was also a good correlation between the K_i values for antagonism of *Fasciola* phosphoglycerate kinase for a range of compounds related to clorsulon and the potency of these compounds as antifluke agents. This evidence supports the suggested mode of action.

The inhibition of *Fasciola* phosphoglyceromutase by clorsulon was studied by Schulman and
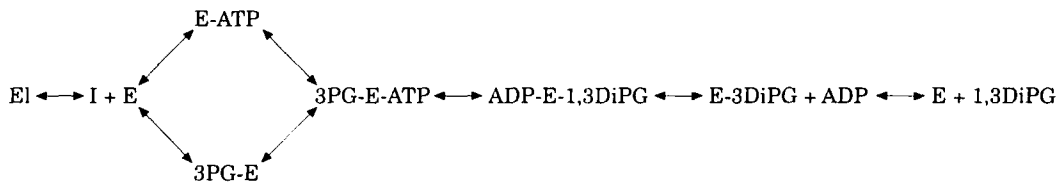
$$E\text{-}ATP$$

$$El \longleftrightarrow I + E \qquad 3PG\text{-}E\text{-}ATP \longleftrightarrow ADP\text{-}E\text{-}1,3DiPG \longleftrightarrow E\text{-}3DiPG + ADP \longleftrightarrow E + 1,3DiPG$$

$$3PG\text{-}E$$

Fig. 18. Diagram of the competitive antagonism of phosphoglycerate kinase (E) of *Fasciola hepatica* by clorsulon (I). Diphosphoryl glycerate: DiPG. 3-Phosphoryl glycerate: 3PG.

Valentino (1982) who pointed out that this enzyme requires 2,3-diphosphoglycerate for activation and the close similarity of clorsulon to diphosphoglycerates could explain its inhibition of the mutase enzyme. These authors purified the enzyme and compared some of its properties with mammalian phosphoglyceromutase that were not inhibited by clorsulon. There appears to be no recent studies on the mode of action of this series of compounds although clinical trials and efficacy studies have been made.

DIETHYLCARBAMAZINE

The chemical structure of diethylcarbamazine a piperazine derivative is shown in Fig. 17. Electrophysiological experiments following bath application to *A. suum* of high concentrations of diethylcarbamazine have shown that it does not mimic the action of piperazine (Martin, 1985).

Effects of diethylcarbamazine

A major indication for diethylcarbamazine at low doses is as an anti-filarial drug; it is a good microfilaricide (including microfilariae of the dog heartworm, *Dirofilaria immitis*) but has limited macrofilaricidal effects. The limited action of diethylcarbamazine against some adult helminths (e.g. cattle lungworm, *Dictyocaulus*) requires nearly 10 times the dose of the prophylactic anti-filarial dose, and may therefore, involve another mode of action. This review covers the mode of action of low doses of diethylcarbamazine; little is known of the mode of action of high doses of diethylcarbamazine.
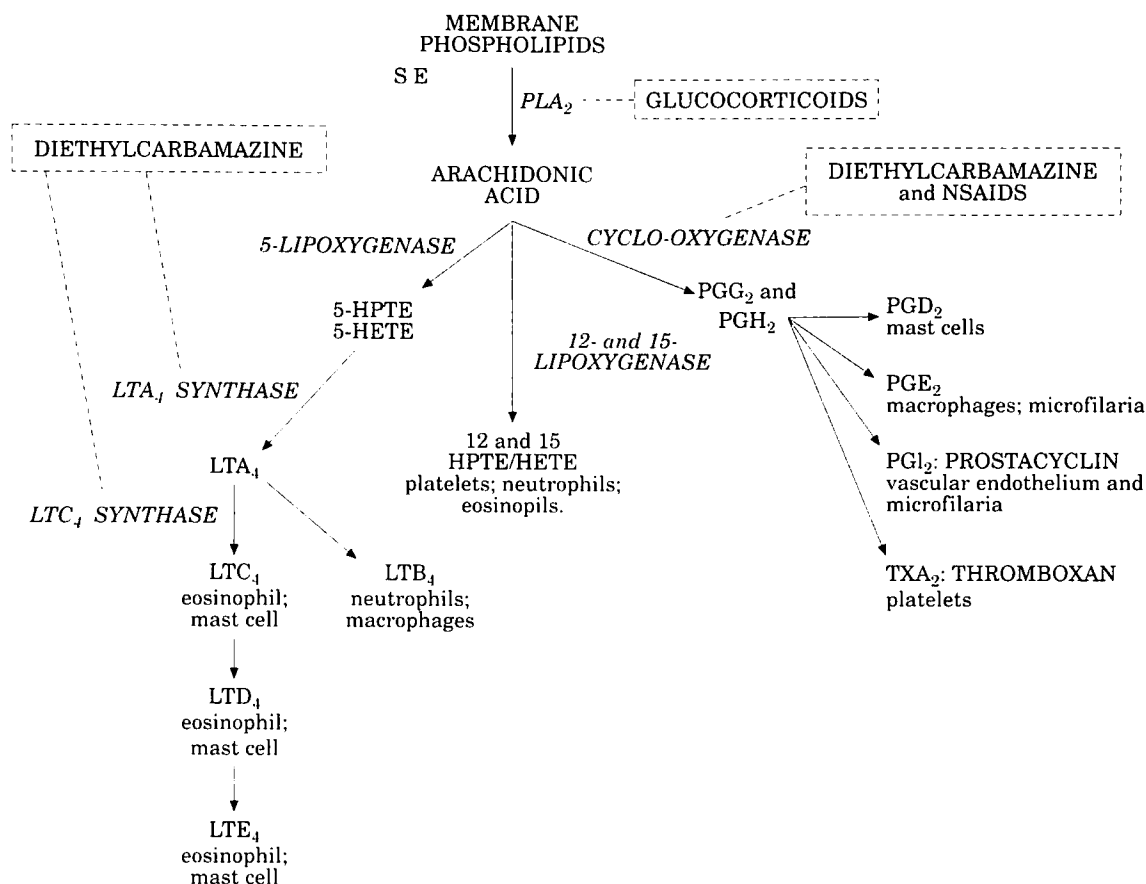
Most studies suggest that diethylcarbamazine has no direct action on filarial parasites (Johnson *et al.*, 1991). It appears to have no action at reasonable concentrations *in vitro*. In marked contrast to *in vitro* experiments, it is known that *in vivo* diethylcarbamazine is effective within 4 min following intravenous injection (Hawking & Laurie, 1949). The specific immune response

does not appear to be involved because nude, athymic mice, infected with *Brugia pahangi*, showed a marked reduction in microfilariae following diethylcarbamazine treatment (Vickery *et al.*, 1986). Thus T cells and T-dependent responses (IgG and IgE) do not appear to be involved. The involvement of complement also seems unlikely because clearing of microfilaria from the nude mice by diethylcarbamazine was not inhibited by prior treatment with cobra venom factor treatment (Vickery *et al.*, 1986).

Effects on arachidonic acid metabolism may be the mode of action of diethylcarbamazine

Diethylcarbamazine has an antagonistic action of the metabolic enzymes that metabolize arachidonic acid, the products released as a result of phospholipase A_2 action on cell membranes. Fig. 19 illustrates the sites of action of diethylcarbamazine proposed by Maizels and Denham (1992). Although 5-lipoxygenase is often being cited as being inhibited by diethylcarbamazine, this may not be the case. In a mast cell line, conversion of 5-HPETE to leukotriene (LT)A_4 by LTA$_4$ synthetase was blocked by diethylcarbamazine (Mathews & Murphy, 1982). Razin *et al.* (1984) found that in mouse, 5-lipoxygenase conversion of arachidonic acid to 5-HPETE was not inhibited but that LTC$_4$ and LTA$_4$ production was inhibited.

Diethylcarbamazine also appears (Kanesa-Thasan *et al.*, 1991) to inhibit endothelial production by cyclo-oxygenase of prostaglandin (PG)I_2 (prostacyclin) and PGE$_2$ production but does not affect platelet production of thromboxane (TX)A_2 by cyclo-oxygenase and the 12-lipoxygenase products 12- and 15-HPETE. Interestingly, microfilariae also produce their own PGI$_2$ and PGE$_2$ and like endothelial cell of blood vessels, diethylcarbamazine also inhibits the microfilarial production (Kanesa-Thasan *et al.*, 1991). The co-administration of glucocorticoids, that are known to block phospholipase A_2 activity and the production of arachidonic acid (Fig. 19), together

MEMBRANE
PHOSPHOLIPIDS

S E

PLA₂ - - - - GLUCOCORTICOIDS

DIETHYLCARBAMAZINE

ARACHIDONIC
ACID

DIETHYLCARBAMAZINE
and NSAIDS

5-LIPOXYGENASE   CYCLO-OXYGENASE

5-HPTE
5-HETE

PGG₂ and
PGH₂

PGD₂
mast cells

12- and 15-
LIPOXYGENASE

PGE₂
macrophages; microfilaria

LTA₄ SYNTHASE

LTA₄

12 and 15
HPTE/HETE
platelets; neutrophils;
eosinopils.

PGI₂: PROSTACYCLIN
vascular endothelium and
microfilaria

LTC₄ SYNTHASE

LTC₄
eosinophil;
mast cell

LTB₄
neutrophils;
macrophages

TXA₂: THROMBOXAN
platelets

LTD₄
eosinophil;
mast cell

LTE₄
eosinophil;
mast cell

Fig. 19. Diagram of the production of the leukotrienes and prostaglandins via lipoxygenase or cyclo-oxygenase from arachidonic acid that is produced from membrane phospholipids as a result of phospholipase A_2 activity. The inhibitory sites of action of glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs) and diethylcarbamazine are shown. Diethylcarbamazine is shown inhibiting the action of LTA_4 synthase that converts 5-hydroperoxyeicosatetraenoic acids (5-HPETE) and 5-hydroxyeicosatetraenoic acids (5-HETE) to the leukotrienes LTA_4 and LTA_4 to LTC_4 in mast cells and eosinophils. Diethylcarbamazine is also shown inhibiting the cyclo-oxygenase enzyme that converts arachidonic acid to the prostaglandins PGG_2 and PGH_2. The subsequent production of PGD_2, PGE_2, PGI_2 & TXA_2 is also inhibited in the blood vessels and microfilaria.

with diethylcarbamazine, reduces the effect of diethylcarbamazine (Stingl *et al.*, 1988). Thus, diethylcarbamazine seems to inhibit production of PGI₂ and PGE₂ by microfilaria and endothelial cells. The normal level of production of PGI₂ and PGE₂ that produces a tonic dilation of blood vessels and inhibits aggregation of neutrophils and eosinophils is reduced by administration of diethylcarbamazine.

A plausible explanation of the mode of action of diethylcarbamazine is that this compound alters the metabolism of arachidonic acids in host endothelial cell and also in microfilariae that are susceptible to the action of diethylcarbamazine. There is then a vasoconstriction, amplified endothelial adhesion and immobilization of the micro-

filariae and cytotoxic activity by the host platelets and granulocytes. It appears that diethylcarbamazine activates an innate immune response rather than an adaptive immune response (Maizels & Denham, 1992). This mode of action can explain why diethylcarbamazine has no action *in vitro* against microfilariae and is effective in non-immune animals.

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nicotinic anthelmintics and the GluCl receptors of *Ascaris*.

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