J. Pharm. Pharmac., 1970, **22**, 26–36 *Received June* 9, 1969

Some pharmacodynamic effects of the nematocides: methyridine, tetramisole and pyrantel

P. EYRE*

Department of Veterinary Pharmacology, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh 9, U.K.

Methyridine, tetramisole and pyrantel affect several cholinergic mechanisms. In the rabbit, methyridine and tetramisole cause hypotension which is partially antagonized by atropine. In the cat, tetramisole causes an increase in blood pressure and causes prolonged contraction of the nictitating membrane. The evidence indicates that tetramisole may release catecholamines from the adrenal medulla. Pyrantel contracts the nictitating membrane; an effect which is abolished completely by hexamethonium. The three anthelmintics block neuromuscular transmission in a way which is characteristic of non-competitive depolarizing agents. They also inhibit cholinesterases. These pharmacological data correlate closely with the reported clinical signs of the toxicity of these agents.

The compounds methyridrine, tetramisole and pyrantel are of relatively recent introduction to antinematodal chemotherapy. Methyridine [2-(2 methoxyethyl) pyridine], a colourless water-miscible liquid, was introduced in 1961. It was the first anthelmintic to possess the then novel characteristic of being more efficient when given subcutaneously than when given by mouth (Broome & Greenhalgh, 1961). The drug is moderately well-tolerated at approximately 200 mg/kg (Walley, 1961) Dullness and anorexia have been reported but the most marked effect is local oedema at the site of injection, followed by necrosis (Thorpe, 1962). Broome (1961) described the distribution of the drug in the body and also presented evidence of depolarizing neuromuscular block in the parasite. Catarsini & Gagliano (1963) observed that methyridine inhibited blood cholinesterases in sheep. In view of these reports it was of interest to study further the actions of methyridine on cholinergic mechanisms.

Tetramisole {(±)-1,2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole HCl}, a more recently introduced nematocide (Thienpont, Vanparijs & others, 1966), is a white water-soluble powder, most frequently given orally but may be injected subcutaneously. Its efficacy was established by Walley (1966). The therapeutic dosage (15 mg/kg orally or 12·5 mg/kg subcutaneously) produces depression of the central nervous system and occasional "muscular twitching" (Forsyth, 1966; Walley, 1966). Doses greater than this produced salivation and more marked and frequent muscular tremors. Kaemmerer & Budden (1966) demonstrated that small doses of tetramisole produced a pressor effect, while larger doses were depressor in the cat. In cattle and sheep there was lachrymation, salivation, increased gut activity and clonic muscular spasms at doses from 15–80 mg/kg subcutaneously. A feature in cattle

* Present address: Section of Pharmacology, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

was spastic tail erection similar to the morphine "Straub" phenomenon in mice. In view of these side-effects it seemed important to investigate the action of tetramisole on autonomic mechanisms.

Another nematocide, pyrantel tartrate {*trans*-1,4,5,6-tetrahydro-1-methyl-2-[2-(thienyl)vinyl]pyrimidine}, has been used in gastrointestinal parasitism in sheep (Austin, Courtney & others, 1966; Cornwell, 1966; Cornwell, Berry & others, 1966a, b). It is a water-soluble white powder and is administered orally at a dose of 25 mg/kg. Cornwell (1966) did not report any untoward side-effects at the dose used.

EXPERIMENTAL

Guinea-pig isolated ileum

Short lengths of guinea-pig terminal ileum were set up in the usual way in aerated Tyrode solution at 35° in a 15 ml organ bath. Isotonic contractions were recorded with a linear motion transducer and a "Grass" polygraph. Acetylcholine, histamine and the anthelmintics were added to the bath for 30 s every 2 min.

Cat nictitating membrane preparation

Experiments were made on 6 cats anaesthetized with sodium pentobarbitone (30 mg/kg, i.v. or i.p). The cats were prepared as described by Bowman, Callingham & Cuthbert (1964). Semi-isometric contractions of the nictitating membrane were elicited by stimulating supramaximally the preganglionic sympathetic trunk with 10 rectangular shocks/s (0·5 ms duration). Contraction of the membrane, femoral arterial blood pressure and respiratory volume were recorded with appropriate electronic transducers and a Grass polygraph. Drugs, dissolved in isotonic saline were injected via a cannula in the femoral vein.

Two cats were pre-treated with reserpine (CIBA) intramuscularly. Cat No. 1 received 0·5, 1·0, 2·0 and 3·0 mg/kg, respectively, 6, 5, 4 and 3 days before the day of experiment. Cat No. 2 received the same doses but, in addition, 3 mg/kg of reserpine was injected intramuscularly 12h before the experiment.

Rat isolated phrenic nerve-diaphragm

Hemidiaphragms, prepared as described by Bülbring (1946), were suspended in a 50 ml bath containing Krebs (1932) solution at 35°, gassed with 5% carbon dioxide in oxygen. The muscle was supramaximally stimulated, using the modified electrode of Cooper & Marshall (1962), with 8 rectangular impulses/min; alternately 4 shocks through the nerve and 4 directly. Isometric contractions were recorded kymographically.

Quantitative comparisons between the anthelmintics and tubocurarine were made. Log molar concentration-response curves were constructed and the molar concentration of each compound producing 50% neuromuscular block (EC50) was estimated graphically (Blackman & Ray, 1964).

Chick isolated biventer nerve-muscle preparation

The chick biventer was prepared according to Ginsborg & Warriner (1960). The nerve-tendon was stimulated in similar conditions to those described for the rat diaphragm, and semi-isometric contractions were recorded.

Chick isolated semispinalis muscle

The muscles, obtained from chickens less than 7 days old, were set up according to Child & Zaimis (1960) in a 5 ml organ bath containing Krebs solution at 35°, gassed with 5% carbon dioxide in oxygen, and isotonic contractions recorded.

In vivo: *peroneal nerve-digital extensor muscles, respiration and carotid blood pressure of the rabbit*

Experiments were made on rabbits of mixed breed and sex, weighing 1 to 2·5 kg, anaesthetized with urethane described by Eyre & Goff (1968). The centrally ligated peroneal nerve was supramaximally stimulated with 8 rectangular impulses/min at 5 to 10 V. Isometric muscle contractions were recorded kymographically, simultaneously with carotid blood pressure and respiratory movement (Eyre, 1967). Drugs were administered intravenously.

Cholinesterase activity

Cholinesterase activity was measured colorimetrically as described by Katsch (1955) and Eyre (1966). Blood was collected with heparin, by jugular venepuncture of sheep and horses. Horse plasma was used as the source of butyrylcholinesterase and sheep erythrocytes (washed three times with equal volumes of isotonic sodium chloride solution) were the source of acetylcholinesterase.

Log molar concentration-response (% inhibition) curves were constructed for each anthelmintic agent and eserine. The relative potency of the compounds was determined by estimating pI50 values graphically. The pI50 value is the logarithm of the reciprocal molar concentration of a drug which reduces enzyme activity by 50% (Blaschko, Bülbring & Chou, 1949).

Drugs

The drugs used were acetylcholine chloride, histamine acid phosphate; atropine sulphate; hexamethonium bromide; eserine (physostigmine) salicylate; tubocurarine chloride; decamethonium iodide; dibenamine hydrochloride, propranolol hydrochloride; tetramisole hydrochloride; pyrantel tartrate. Drug-solutions for injection were made in isotonic sodium chloride solution; other solutions were in distilled water.

RESULTS

Guinea-pig isolated ileum

Methyridine (10 to 20 μg/ml) caused slight spontaneous movement of the ileum. Tetramisole (5 to 10 μg/ml) produced more pronounced irregular spontaneous activity (Fig. 1a). Pyrantel (5 μg/ml) elicited a marked sustained contraction of the ileum which was followed by a number of spontaneous "spikes" for some 5 to 10 min after washing the preparation with fresh Tyrode (Fig. 1b).

Contractions produced by acetylcholine (0·02 to 0·20 μg/ml) were potentiated following treatment with pyrantel (Fig. 1b) whereas pretreatment with methyridine or tetramisole did not cause cholinergic potentiation. The responses due to histamine (0·03 to 0·20 μg/ml) were unaffected by the anthelmintics at the concentrations employed.

Hexamethonium (1·0 μg/ml) blocked the activity of pyrantel on the ileum (Fig. 1c) but did not antagonize the action of tetramisole. Atropine (0·1 to 0·2 μg/ml)

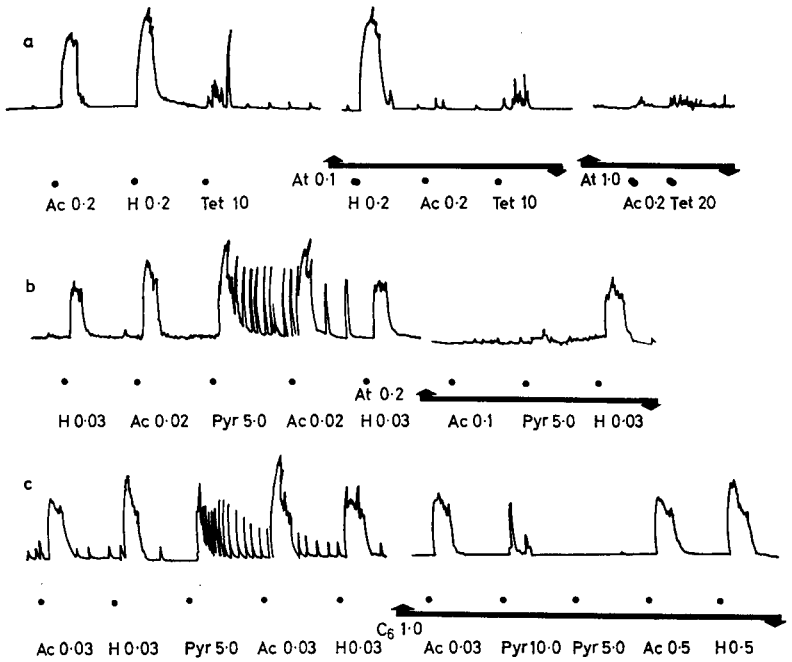

FIG. 1. Contractions of guinea-pig isolated ileum in 15 ml aerated Tyrode at 35°. H = histamine. Ac = acetylcholine. Tet = tetramisole. Pyr = pyrantel. At = atropine and C_6 = hexamethonium present between the arrows. Doses in μg/ml.

antagonized pyrantel completely and tetramisole partially. Atropine (1·0 μg/ml) abolished the response due to tetramisole (Fig. 1a).

Cat nictitating membrane preparation

Pyrantel (2 mg/kg, i.v.) contracted the nictitating membrane with a delay less than 10 s. At the same time there was a pronounced rise in blood pressure and transient dyspnoea. Hexamethonium (5 mg/kg) abolished both the contraction of the membrane due to preganglionic electrical stimulation and also the membrane contraction and the rise in blood pressure due to pyrantel (Fig. 2). Tetramisole (2 mg/kg, i.v.), after a delay of 20 s, produced a strong contraction of the nictitating membrane which was accompanied by a small brief fall in blood pressure followed by a sustained rise that persisted for up to 20 min. Respiration was affected only minimally, usually brief hyperventilation (Fig. 3). The actions of tetramisole were similar to, but more sustained than those of adrenaline (2 to 5 μg/kg, i.v.). Hexamethonium (5 to 10 mg/kg), while abolishing the effect of electrical stimulation of the membrane, did not inhibit the actions of adrenaline or tetramisole. Dibenamine (5 mg/kg), in contrast, reversed the pressor responses of adrenaline and tetramisole and partly inhibited their action on the nictitating membrane (Fig. 3). Bretylium (1 mg/kg) or propranolol (1 mg/kg) did not antagonize tetramisole.

In two reserpinized cats, tetramisole (2 mg/kg) and adrenaline (2 to 5 μg/kg) elicited a greater contractile response in the membrane than in non-reserpinized animals.

Methyridine was not examined.

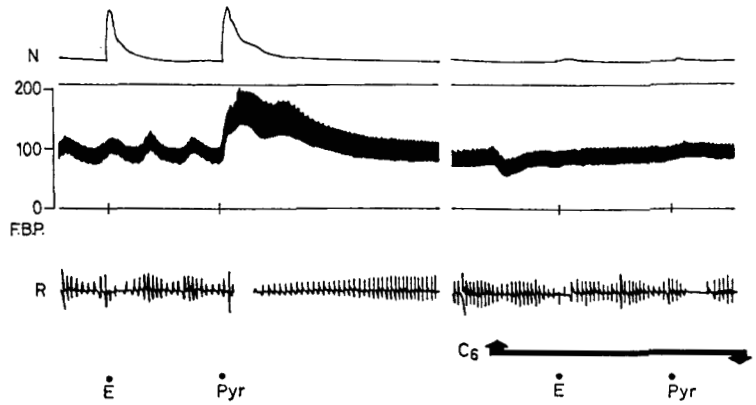

FIG. 2. Cat anaesthetized with sodium pentobarbitone. Top tracing = contraction of the nicitating membrane (N). Middle tracing = femoral arterial blood pressure (F.B.P.) mm Hg. Bottom tracing = respiratory volume (R) taken from a tracheal cannula. E = preganglionic stimulation, Pyr = pyrantel 2 mg/kg i.v. Hexamethonium (C_6, 5 mg/kg i.v. present between the arrows) abolishes the effects of electrical stimulation and those of injected pyrantel.

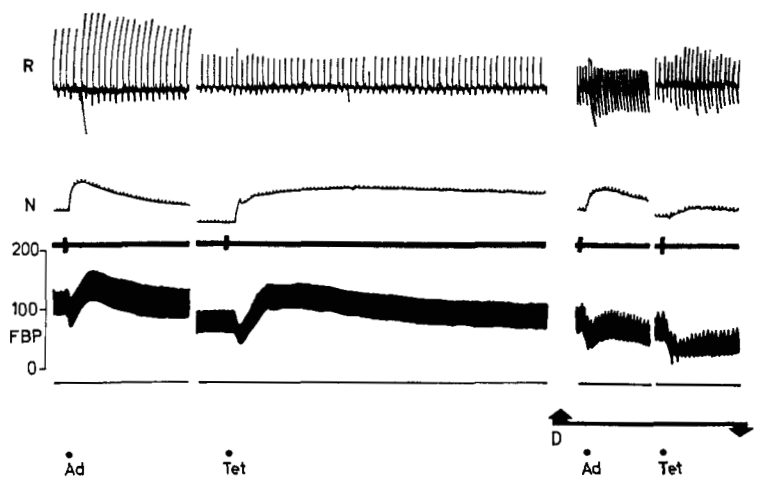

FIG. 3. Cat anaesthetized with sodium pentobarbitone. Top tracing = respiratory volume (R) recorded from a tracheal tube. Middle tracing = contraction of the nictitating membrane (N). Bottom tracing = femoral arterial blood pressure (F.B.P.) mmHg. Ad = adrenaline 3 μg/kg, i.v. Tet = tetramisole 2 mg/kg, i.v. Dibenamine (D, 5 mg/kg, i.v. present between the arrows) partially inhibits the actions of adrenaline and almost abolishes those of injected tetramisole.

Rat isolated phrenic nerve-diaphragm

Tetramisole (10^{-5} to 10^{-4}M) and pyrantel (10^{-6} to 10^{-5}M) augmented the twitch responses of both the directly and indirectly stimulated muscle, whereas methyridine did not (Fig. 4a).

Methyridine (10^{-3}M), tetramisole (10^{-4}M) and pyrantel (10^{-5}M) produced partial neuromuscular block which was not affected by a tetanus or by potassium or eserine. The anthelmintics reduced slightly the responses to direct stimulation (Fig.4b.) Tetramisole (10^{-5} to 10^{-4}M) or pyrantel (10^{-6} to 10^{-5}M) added during a partial neuromuscular block by tubocurarine (3×10^{-6}M) partly relieved the block (Fig. 4c).

Quantitative comparison showed that the molar EC/50 values were: decamethonium

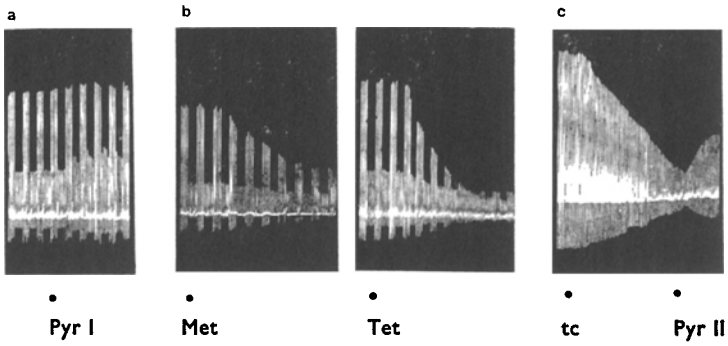

FIG. 4: a, b. Contractions of the isolated rat hemidiaphragm in 50 ml O_2 + CO_2—aerated Krebs at 35°. Muscle stimulated supramaximally with 8 rectangular shocks/min—alternately 4 shocks through the nerve (larger twitches) and 4 directly into the muscle (smaller twitches). c. Muscle stimulated 8 shocks/min. indirectly through the nerve.
Pyr = pyrantel I 9×10^{-6}, II 1×10^{-5}M. Met = methyridine 7×10^{-3}M. Tet = tetramisole 3×10^{-4}M tc = (+)-tubocurarine 3×10^{-6}M.

8×10^{-5}, tubocurarine $1{\cdot}6 \times 10^{-6}$, tetramisole $1{\cdot}4 \times 10^{-4}$, pyrantel $1{\cdot}2 \times 10^{-4}$ methyridine $5{\cdot}7 \times 10^{-3}$.

Chick isolated biventer nerve-muscle

Tetramisole (10^{-5} to 10^{-4}M) and pyrantel (10^{-6} to 10^{-5}M) increased the twitch response of the preparation and reversed the blocking action of tubocurarine. Methyridine did not show these effects.

Tetramisole (10^{-4}M), pyrantel (2×10^{-5}M) and methyridine (1×10^{-3}M) produced reversible neuromuscular block and contracture of the muscle, indicative of depolarization (Fig. 5).

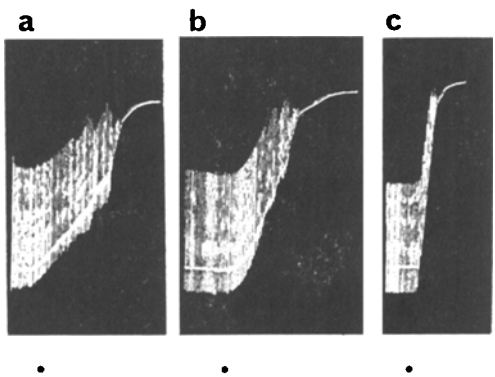

FIG. 5. Contractions of the chick isolated biventer nerve-muscle preparations in 20 ml O_2 + CO_2 —aerated Krebs at 35°. Muscle stimulated supramaximally with 8 rectangular shocks/min through the nerve. Effects of: a. ● Methyridine, $2{\cdot}4 \times 10^{-3}$M. b. ● Tetramisole. 1×10^{-4}M c. ● Pyrantel 1×10^{-5}M.

Chick isolated semispinalis muscle

Tetramisole (6×10^{-5}M), pyrantel ($1{\cdot}5 \times 10^{-5}$M) and methyridine ($2{\cdot}8 \times 10^{-3}$M) produced contractions of the semispinalis which were inhibited by tubocurarine ($1{\cdot}5 \times 10^{-7}$M) (Fig. 6). Pyrantel showed marked tachyphylaxis and inhibited slightly the responses to decamethonium and nicotine.

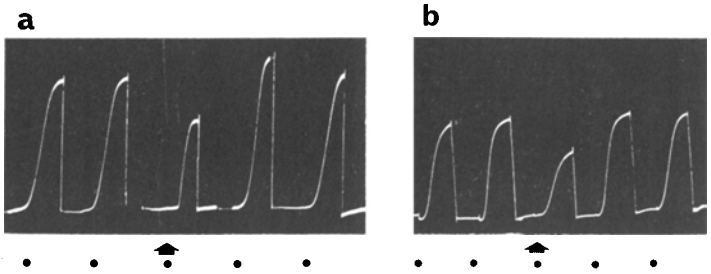

FIG. 6. Contractions of the semispinalis muscle of the chick in 10 ml O_2 + CO_2—aerated Krebs at 35°. a. ● Contractions caused by methyridine $2{\cdot}8 \times 10^{-3}$M. (+)-Tubocurarine ($1{\cdot}5 \times 10^{-7}$M) added at the arrow. b. ● Contractions due to tetramisole 6×10^{-5}M. Tubocurarine (5×10^{-7}M) added at the arrow.

In vivo *peroneal nerve-digital extensor muscles, respiration and carotid blood pressure of rabbit*

A small intravenous dose of methyridine (10–50 mg/kg) increased slightly the twitch-height of the extensor muscles and caused a precipitous fall in carotid blood pressure which was partially prevented by atropine 1 mg/kg (Fig. 7). Respiratory

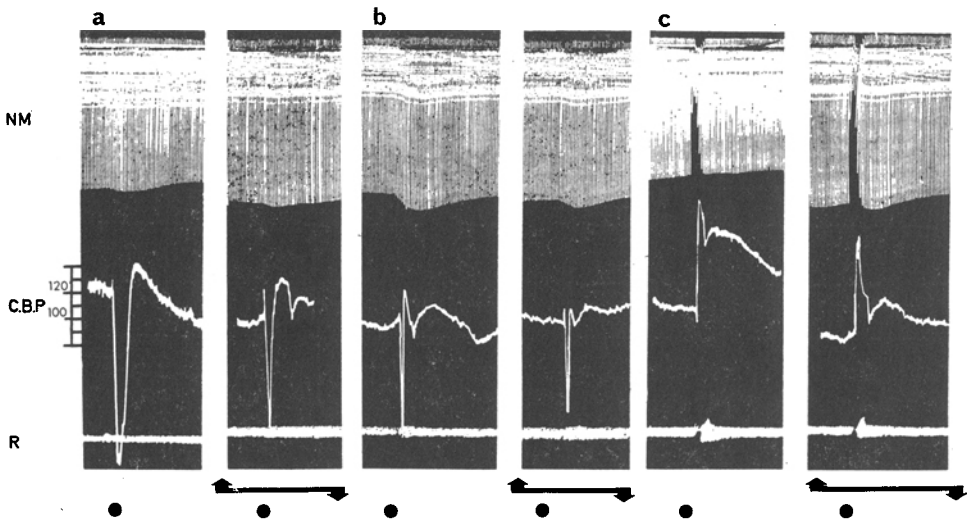

FIG. 7. Rabbit anaesthetized with urethane. Top tracing: (NM) isometric contractions of the digital extensor muscles due to peroneal nerve stimulation at 8 impulses/min. Second tracing: carotid blood pressure (C.B.P.) in mmHg. Bottom tracing: respiratory volume (R) from tracheal cannula. a. ● Methyridine 20 mg/kg, i.v.; between arrows—atropine 1 mg/kg, i.v. b. ● tetramisole 10 mg/kg, i.v.; between arrows—atropine 1 mg/kg, i.v. c. ● Pyrantel 2 mg/kg, i.v.; between arrows—hexamethonium 1 mg/kg, i.v.

volume and rate were not altered. A therapeutic dose of methyridine (200 mg/kg, s.c.) produced a sustained hypotension and partial neuromuscular block.

Tetramisole (5–10 mg/kg, i.v.) caused a sharp transient fall in blood pressure which was partially antagonized by atropine. Respiratory volume was either reduced briefly or unaffected. Neuromuscular transmission was briefly enhanced; an effect which diminished after repeated dosing in the same animal (Fig. 7) A therapeutic dose of tetramisole (10–25 mg/kg, s.c.) caused a prolonged fall in blood pressure.

Neuromuscular transmission was enhanced. Doses greater than 30 mg/kg caused profound, sustained hypotension and apnoea together with neuromuscular block. The animal could be kept alive only by means of a respiration pump.

Pyrantel (2–10 mg/kg, i.v.) caused an immediate brief increase in carotid blood pressure followed by a more sustained pressor response. The secondary hypertension was almost abolished by hexamethonium (1 mg/kg) (Fig. 7). Transient apnoea and neuromuscular block coincided with the first phase of hypertension.

A therapeutic dose of pyrantel (25 mg/kg, s.c.) produced a transient small hypotension followed by a sustained rise in blood pressure. Neuromuscular transmission was slightly enhanced but respiration was unaffected. Pyrantel (50–100 mg/kg) given subcutaneously caused total neuromuscular block and apnoea together with oscillating rises and falls of blood pressure. Animals died of cardiac arrest despite artificial respiration.

Cholinesterase activity

Acetylcholinesterase of sheep erythrocytes. The pI50 of eserine was 6·4. Tetramisole was the most active anthelmintic compound (5·0) and pyrantel was next (4·5). The activity of methyridine was very small (1·9).

Butyrylcholinesterase of horse plasma. The pI50 of eserine was 7·0. Tetramisole and pyrantel were weaker inhibitors of the plasma enzyme than of erythrocytes—having values respectively of 4·4 and 4·0. Methyridine had a greater inhibitory action on the plasma (2·3) enzyme than on erythrocytes (1·9).

DISCUSSION

The results show that the three nematocides evoke several pharmacological responses.

Tetramisole and pyrantel produced reproducible contractions of the ileum which were abolished by atropine; showing that muscarinic receptors were stimulated either directly or indirectly to induce contraction. Pyrantel caused more marked, more regular and more prolonged spontaneous contractions and also potentiated acetylcholine. Hexamethonium abolished the responses due to pyrantel but not those of tetramisole; which suggested that tetramisole may have a direct muscarinic action whereas pyrantel was acting by stimulating nicotinic receptors of the ganglionic synapses of the intestine.

This possibility was studied further using the cat superior cervical ganglion-nictitating membrane preparation, in which the membrane contracted strongly following intravenous injection of pyrantel. Pyrantel produced a pronounced pressor response which was confirmed in another group of experiments in the rabbit. Premedication with hexamethonium abolished the contractile effect of pyrantel and of preganglionic electrical stimulation on the nictitating membrane, whereas the action of adrenaline on the membrane was unchanged. Similarly, hexamethonium antagonized the hypertensive action of pyrantel in the cat and rabbit.

The effect of tetramisole on the nictitating membrane was different. Tetramisole caused a contraction of the organ which was slower in onset and more prolonged in duration. The arterial pressure responded with a small sharp fall followed by a prolonged pressor phase. These responses of tetramisole resembled those of adrenaline. Hexamethonium, at the dosage used, did not inhibit the action of tetramisole

on the nictitating membrane—an effect which was partly inhibited by dibenamine but not by propranolol. It seemed, therefore, that tetramisole may not have been acting on nicotinic receptors in the ganglion, but in some way the anthelmintic caused stimulation of adrenoceptive mechanisms in the nictitating membrane. Bretylium failed to reduce the action of tetramisole on the membrane and in two cats which had been chronically treated with reserpine, there was no diminution of the action of tetramisole.

It is possible that tetramisole stimulates the cat nictitating membrane (a) by direct stimulation of adrenoceptive receptors or (b) by releasing adrenaline from the adrenal medulla; an effect which would be unaffected by bretylium and incompletely inhibited by reserpinization. The fact that the pressor effect of tetramisole is biphasic, and there is a delay in onset of responses followed by prolonged hypertension and membrane contraction, tends to support the probability of adrenaline release from the adrenal gland. This is further supported by the fact that dibenamine reverses the pressor responses of both adrenaline and tetramisole (since the adrenal gland contains principally adrenaline).

The failure of hexamethonium to block the effect of tetramisole in contrast to pyrantel, may be due to tetramisole being a more potent nicotinic stimulant of the adrenal medulla or to it having some direct action on adrenoceptive receptors. The present evidence does not allow the latter possibility to be ruled out.

Tetramisole and pyrantel both consistently augmented skeletal neuromuscular transmission and antagonized the myoneural blocking action of (+)-tubocurarine. These effects may be ascribed both to the depolarizing action and to cholinesterase inhibition by the anthelmintics. Methyridine, which is a weaker depolarizer and has a much smaller anticholinesterase action, had little effect on the ileum and showed little or no tendency to augment neuromuscular transmission or to antagonize curare. Increasing concentrations of the three drugs caused neuromuscular block *in vitro* and *in vivo*. In the rat diaphragm *in vitro*, this blocking action was not overcome by the addition of potassium or eserine, or by tetanic stimulation. Furthermore, when the anthelmintics were tested on the semispinalis and biventer muscles of the chicken, contractions were produced which were antagonized by tubocurarine. These results suggest strongly that the three nematocidal compounds cause neuromuscular paralysis which is of the non-competitive type and is accompanied by depolarization in a manner characteristic of nicotine, succinylcholine or decamethonium.

The myoneural actions of the three nematocides *in vitro* were confirmed *in vivo* in the rabbit. Methyridine caused a fall in blood pressure in rabbits. Tetramisole had a depressor action in the rabbit whereas in the cat the response was always pressor. Atropine partly inhibited the hypotension caused by methyridine and by tetramisole in the rabbit, and it would appear that methyridine and tetramisole act by exerting both muscarinic and nicotinic effects. Pyrantel appears to excite mainly nicotinic receptors.

The results of these experiments are consistent with the main features of the toxicity of the drugs which have been reported from the field. Broome (1961) showed that methyridine paralysed the parasitic helminth and suggested that depolarization may be a principal feature of the drug. This was confirmed. Catarsini & Gagliano (1963) showed some depression of circulating cholinesterase activity in

sheep. It seems likely that owing to the relatively small action of methyridine on cholinesterases, that direct depolarization may be more important.

The pharmacodynamics of tetramisole agree with the symptoms of salivation, defaecation and muscular contraction which have been described (Forsyth, 1966; Walley, 1966; Kaemmerer & Budden, 1966). Forsyth was the first to suggest a similarity between tetramisole intoxication and organophosphorus poisoning, but he did not investigate the implications of his observation. Some of the toxicity of this drug may be concerned with cholinesterase inhibition leading to manifestations of the muscarinic and nicotinic actions of acetylcholine; in addition to which there is depolarizing neuromuscular block which might explain Kaemmerer's observations of spastic tail erection and muscle tremors.

However, the actions of tetramisole on sympathetic elements have not been described hitherto and these effects do not "fit" the picture of toxicity of tetramisole which has become recognized. It must be assumed that in outward symptoms the cholinergic manifestations predominate.

On first consideration it is curious that no particular "clinical" syndrome of toxicity has been reported for pyrantel. This may be due to the fact that pyrantel is always given orally and is poorly absorbed from the intestine. Hence general systemic pharmacological effects will not occur in the normal conditions of usage of the drug Tetramisole on the other hand may be injected or, when given by mouth, is well-absorbed into the circulation, thus giving rise to the pharmacodynamic actions reported in the literature.

Acknowledgements

I am grateful to Professor F. Alexander of Edinburgh for his support and encouragement in the initiation of this study. Grateful acknowledgement is also due to Professor H. G. Downie for his interest and encouragement of the continuation and completion of the study at Guelph, and to Dr. D. J. Ecobichon for much helpful discussion. I.C.I. of Macclesfield, Cheshire, England gave generous supplies of methyridine and tetramisole, and Pfizer of Sandwich, Kent, England supplied the pyrantel. Thanks are also due to Mr. David Goff for indispensable technical assistance.

REFERENCES

AUSTIN, W. C., COURTNEY, W., DANILEWICZ, J. C., MORGAN, D. H., CONOVER, L. H., HOWES, H. L., LYNCH, J. E., MCFARLAND, J. W., CORNWELL, R. L. & THEODORES, V. J. (1966). *Nature, Lond.*, **212**, 1273.

BLACKMAN, J. G. & RAY, C. (1964). *Br. J. Pharmac. Chemother.*, **22**, 56–65.

BLASCHKO, H., BÜLBRING, E. & CHOU, T. C. (1949). *Ibid.*, **4**, 29–32.

BOWMAN, W. C., CALLINGHAM, B. A. & CUTHBERT, A. W. (1964). *Ibid.*, **22**, 558–576.

BROOME, A. W. J. (1961). *Ibid.*, **17**, 327–338.

BROOME, A. W. J. & GREENHALGH, N. (1961). *Nature, Lond.*, **189**, 59–60.

BÜLBRING, E. (1946). *Br. J. Pharmac. Chemother.*, **1**, 38–61.

CATARSINI, O. and GAGLIANO, I. (1963). *Atti. Soc. ital. Sci. vet.*, **17**, 690–695.

CHILD, K. J. & ZAIMIS, E. (1960). *Br. J. Pharmac. Chemother.*, **15**, 412–416.

COOPER, W. C. & MARSHALL, P. B. (1962). *J. Inst. Sci. Technol.*, **8**, 61–63.

CORNWELL, R. L. (1966). *Vet. Rec.*, **79**, 590–595.

CORNWELL, R. L., BERRY, J., LIGHT, T. C., MERCER, E. A. & PHILLIPS, G. (1966a). *Ibid.*, **79**, 626–629.

CORNWELL, R. L., BERRY, J., LIGHT, T. C., MERCER, E. A., PHILLIPS, S. & PULLEN, D. A. (1966b). *Ibid.*, **79**, 723–728.

EYRE, P. (1966). *Res. Vet. Sci.*, **7**, 161–167.

EYRE, P. (1967). *Br. J. Pharmac. Chemother.*, **32**, 624–634.

EYRE, P. & GOFF, D. (1968). *J. Inst. Sci. Technol.*, **14**, 56.

FORSYTH, B. A. (1966). *Austr. Vet. J.*, **42**, 412–419.

GINSBORG, B. L. & WARRINER, J. (1960). *Br. J. Pharmac. Chemother.*, **15**, 410–411.

HESTRIN, S. (1949). *J. biol. Chem.*, **180**, 249–261.

KAEMMERER, K. & BUDDEN, R. (1966). *D. Tierärtzl. Wschr.*, **73**, 235–243.

KATSCH, S. (1955). *J. appl. physiol.*, **8**, 215–219.

KREBS, H. A. & KENSELEIT, K. (1932). *Hoppe-Seylers Z. physiol. Chem.*, 210, 33–66.

THIENPONT, D., VANPARIJS, O. F. J., RAEYMAEKERS, A. H. M., VANDENBERK, J., DEMOEN, P. J. A., ALLEWIJN, F. J. N., MARSBOOM, R. P. H., NIEMEGEERS, C. J. E., SCHELLEKENS, K. H. L. & JANSSEN, P. A. J. (1966). *Nature, Lond.*, **209**, 1084–1086.

THORPE, E. (1962). *J. comp. Path. Ther.*, **72**, 29–32.

WALLEY, J. K. (1961). *Vet. Rec.*, **73**, 159–168.

WALLEY, J. K. (1966). *Ibid.*, **78**, 406–414.