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ANTHELMINTICS, USED FOR THE TREATMENT OF FASCIOLIASIS AS UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION IN WARM BLOODED ANIMALS

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Dinitrophenol and liverfluke anthelmintics such as niclofolan, nitroxynil, bromophenophos and hexachlorophene increased ATP-ase activity of rat-liver mitochondria and caused a contracture of striated muscle *in vitro*. After lethal doses, rigidity occured soon after death. It is postulated that these anthelmintics are potent uncouplers of oxidative phosphorylation in warm-blooded animals.

Liver fluke anthelmintics
2,4-dinitrophenol
Uncoupling agents
ATP-ase liver mitochondria
Contracture of striated muscle
Post-mortem rigidity

1. INTRODUCTION

Recently newer anthelmintics, such as niclofolan (Bilevon® = 2,2′-dihydroxy-3,3′dinitro-5,5′dichloro diphenyl), nitroxynil (Trodax®, = *N*-methylglucamine salt of 4-cyano-2-iodo-nitrophenol) and bromophenophos (Acedist® = 4,4′, 6,6′-tetrabromo-2,2′-biphenyldiol monophosphate) have been shown to be effective against immature and mature liverflukes in doses with a greater margin of safety than previous drugs (Boray, Happich and Andrews, 1967; Boray and Happich, 1968). Nevertheless, these drugs are too toxic for treatment of acute outbreaks of Fascioliasis in sheep. Since although there is a reasonable safety margin against mature flukes, side effects can sometimes be observed under field conditions. After toxic doses, undesirable symptoms occur of which hyperthermia is one of the most striking. The animals become apathetic and the respiration rate increases (Flucke, Seltkamp and Wirtz, 1969; Kruyt and van der Steen, 1969). These symptoms prompted a further investigation of the mode of action of these drugs in laboratory animals.

2. METHODS

2.1. *Lethality test*

The anthelmintics used were dissolved in propylene glycol. Lethal doses of these drugs were injected i.p. into Swiss mice of either sex with an average body weight of 25 g.

2.2. *Diaphragm preparation*

The phrenic nerve diaphragm preparation, as described by Bülbring (1946), was used. Twitches of the striated muscle produced by electrical stimulation of the phrenic nerve or by direct stimulation of the muscle were recorded with a light spring loaded lever on a smoked kymograph drum. The nerve was stimulated supra maximally at 15 shocks per minute.

2.3. *Induction of ATP-ase activity of freshly prepared rat-liver mitochondria*

The preparation was similar to that described by Myers and Slater (1957). Inorganic phosphate was measured by the method of Summer (1944) and the protein content of the enzyme preparation by the

Table 1
Latency of onset of rigor mortis in mice after lethal doses of anthelmintics.

Test substance	Number of animals	Dose i.p. mg/kg b.w.	Time after which the animals died (min)	Time after which rigor mortis occurred (min)
Decapitation	8	–	< 1	60–90
Sodium pentobarbital	8	250 (i.v.)	< 1	60–90
Hexachlorophene	8	150	2–5	< 1
2,4-dinitrophenol	8	50	8–11	5–8
Niclofolan	8	50	7–11	< 1
Nitroxynil	8	50	13–19	3–5
Bromophenophos	8	150	24–30	5–10

method of Cleland and Slater (1953).

In all experiments 2,4-dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, was used for comparison.

3. RESULTS AND DISCUSSION

Post-mortem rigidity occurred within 5 to 8 min of an intraperitoneal injection of a lethal amount of DNP to mice and rats (table 1). This effect is due to a decrease in the ATP cencentration of striated muscle cells and not to a direct action on the contractile mechanism. Following decapitation or a lethal dose of sodium pentobarbital (Nembutal®), rigor mortis occurs after 60 to 90 minutes.

The phrenic nerve-diaphragm preparation of the rat can be used for studying the effects of uncoupling agents *in vitro* on contractions of the striated muscle of the diaphragm evoked by either stimulation of the phrenic nerve or direct stimulation of the muscle (Barnes, Duff and Threlfall, 1955). DNP (0.0465 μmol/ml) caused a decrease in contractions, those produced by phrenic nerve stimulation disappearing first followed by the gradual onset of a characteristic contracture. All the anthelmintics test-

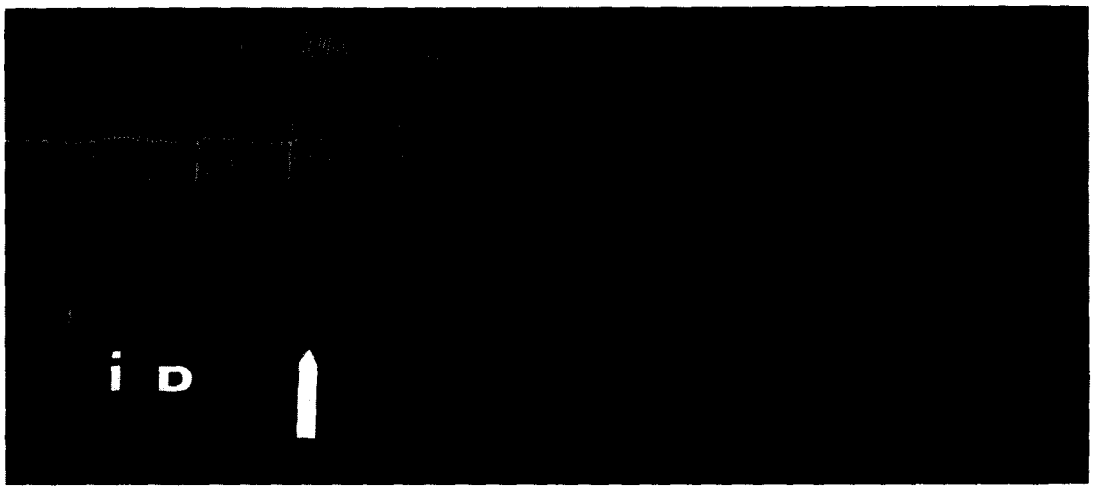

Fig. 1. Effect of nitroxynil (Trodax®, 0.067 μmol/ml), at arrow, on the rat isolated diaphragm preparation. One stimulation/4 sec supra maximally, with alternate groups of indirect stimulation of the phrenic nerve (i) and direct stimulation of the muscle (d).

Table 2
Induction of ATP-ase activity of rat-liver mitochondria in relation to concentration of the test substances at pH = 7.4. Mean ATP-ase activity is expressed as μmol Pi per mg N per hour. Determinations were performed in triplicate.

Test substances	Number of experiments	Concentration in Molar		
		10^{-4}	10^{-5}	10^{-6}
2,4-dinitrophenol	5	50.9	–	–
Niclofolan	5	57.7	32.2	12.2
Nitroxynil	5	51.5	23.2	13.4
Hexachlorophene	2	46.8	39.8	15.3
Bromophenophos	5	30.8	8.5	1.8

ed produced contracture. The potency of nitroxynil (fig. 1), niclofolan and hexachlorophene were similar to that of DNP. Bromophenophos was less active since the effects were only observed with concentrations greater than 0.14 μ mol/ml (fig. 2). In a third experiment, the ability of the anthelmintics to induce ATP-ase activity of freshly prepared rat-liver mitochondria was compared with that of DNP (table 2). At pH 7.4 the activities of niclofolan and nitroxynil were similar to that of DNP, bromophenophos was less active.

4. CONCLUSIONS

Although the rate of oxygen consumption after toxic doses of these anthelmintics was not measured it seems reasonable to conclude that these drugs are active uncouplers of oxidative phosphorylation in warm-blooded animals. The toxic symptoms described and those observed under field conditions, can be easily explained by this mode of action.

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Fig. 2. Effect of bromophenophos (Acedist®, 0.14 μmol/ml), at arrow, on the rat isolated diaphragm preparation. One stimulation/4 sec supra maximally, with alternate groups of indirect stimulation of the phrenic nerve (i) and direct stimulation of the muscle (d).

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