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ANTHELMINTIC ACTIVITY OF METHYRIDINE AGAINST EXPERIMENTAL NEMATODE INFECTIONS IN MICE

BY

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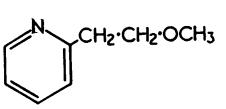
Critical studies using mice infected experimentally with nematodes (*Nematospiroides dubius, Nippostrongylus muris* and *Heterakis spumosa*) have shown methyridine to be an extremely effective anthelmintic when administered orally or subcutaneously. Comparisons of the two treatment regimes show subcutaneous administration to be the more efficient in terms of absolute dose, but neither treatment has a significant advantage in therapeutic safety margin. Methyridine has a more uniform anthelmintic action than either phenothiazine or bephenium against adult forms of the three test nematodes. In addition the drug possesses activity against all stages of certain immature nematode infections.

Although certain pyridine alkaloids possess anthelmintic properties, no simple pyridine derivative had shown useful activity until the recent paper of Broome & Greenhalgh (1961) describing the effects of 2-(β-methoxyethyl)pyridine (methyridine)



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(I) (active principle of " Promintic," Imperial Chemical Industries). It was shown that methyridine has considerable anthelmintic activity against experimental nematode infections of mice.

Methyridine may be prepared by a method similar to that used for the corresponding ethyl homologue by Doering & Weil (1947). It is a colourless, sweet-smelling liquid (b.p. = 94 to 96° C/17 mm, sp. gr.$^{20°}$ = 0.988, $n_D^{20°}$ = 1.4975) and is completely miscible with water and common solvents in all proportions. In addition the compound is a weak base (pK_a approximately 5.5) which forms a water-soluble hydrochloride (m.p. 104 to 105° C), hydrobromide (m.p. 84 to 86° C) and picrate (m.p. 130 to 131° C).

In the present paper detailed anthelmintic studies with nematode infections of mice are described, including comparative tests with other anthelmintics and some toxicological work.

METHODS

Nematodes. All tests were carried out using one or more of the three nematodes *Heterakis spumosa, Nippostrongylus muris* and *Nematospiroides dubius.* Cultures of *H. spumosa* and *N. muris* were maintained in rats by the methods of Smith (1953) and Brackett & Bliznick (1949) respectively. *N. dubius* was cultured in exactly the same way as *N. muris* except that mice, instead of rats, were used as host animals and they were infected orally.

Trials with adult nematode infections in mice. The methods used for these trials were essentially those developed earlier in these laboratories by Dr D. G. Davey. They have not previously been described and a full account of them is therefore included. To conserve animals, time and materials, each mouse received two nematode infections consisting of *H. spumosa* in the large intestine and either *N. muris* or *N. dubius* in the small intestine. Care was taken to ensure that all species reached maturity together so that none were being naturally eliminated at the time of the trial. This was achieved by infecting newly weaned mice (13 to 15 g and reared under pathogen-free conditions) with 350 embryonated *H. spumosa* eggs given orally 28 days before the experiment was due to commence. Sixteen days later half the mice were infected with 100 *N. dubius* larvae orally and seven days before the commencement of the experiment the remaining mice received 500 *N. muris* larvae together with 1.25 mg cortisone acetate (both given subcutaneously). Thus at the commencement of the experiment the infections of *H. spumosa* were 28 days old, those of *N. dubius* 12 days old and *N. muris* 7 days old, and all were at the early adult stage.

Experimental design. Three separate balanced factoral experiments involving over 800 mice were used to compare the anthelmintic activity of methyridine (oral and subcutaneous injection), bephenium (a mixture of 33% bephenium embonate+67% bephenium hydroxynaphthoate) and fine-particle phenothiazine. The first two trials were needed to compare the dose/parasite mortality relationships of the three drugs, using doses graded from those having no activity against the parasite to those producing toxic effects in the host. The third experiment was concerned solely with providing additional information on the relative activity of methyridine when administered orally and subcutaneously.

Groups of 10 mice (all infected with *H. spumosa* but 5 containing *N. muris* and 5 *N. dubius* in addition) were randomly assigned to each dosage regime. A similar group of 10 mice served as control in each experiment to provide an estimate of the worm burden before treatment.

Administration of drugs. All drugs were administered in a single dose to provide information on the type of activity likely to be achieved under practical conditions in the field. The appropriate dose of drug was prepared in 0.5 ml. of a dispersing agent for those animals dosed orally but in 0.2 ml. for the subcutaneous treatments. The dispersing agent contained 0.1% Lissapol C, 0.1% Lissapol N.X. and 0.1% Dispersol O.G. All treatments were administered on Day 1 of the experiment.

Assessment of anthelmintic activity. Anthelmintic activity was assessed by comparing the worm burdens of treated and control animals at post-mortem examination 5 days after treatment. To facilitate worm-counting the animals were starved 24 hr prior to sacrifice. After killing, the abdomen was opened and the large and small intestines were removed to separate petri-dishes. Each large intestine was slit longitudinally, and all *H. spumosa* worms were scraped into warm water. They were then transferred to a transparent container (with transverse lines to facilitate counting) and counted over a black tile.

The small intestines containing *N. muris* were compressed between perspex plates and the nematodes were counted with the aid of an illuminated magnifying-glass over a white tile. To assess the number of *N. dubius* it was necessary to slit the upper part of the small intestine and to scrape the worms into warm water. They were then gently teased apart and counted in a transparent dish over a white tile.

Trials with immature nematode infections of mice. These trials were confined to the relatively rapid maturing nematodes *N. muris* and *N. dubius.* Equal numbers of clean mice were infected with either *N. muris* or *N. dubius* larvae, using the techniques previously

described. A single experiment involving about 250 mice was used to investigate the anthelmintic activity of graded doses of methyridine (subcutaneous injection) and bephenium (oral) against 1-, 2-, 4-, 6- and 8-day-old infections respectively. Groups of 10 mice (5 infected with *N. muris* and 5 with *N. dubius*) were randomly assigned to each treatment and 10 similar mice served as controls. All drugs were administered as previously described, and anthelmintic activity was assessed by worm counts made at post-mortem examination 12 days after infection.

Acute toxicity. The standard technique for estimating acute drug toxicity has been used in these experiments. At least 5 groups of 10 mice (of the same weight and infected with nematodes as in the anthelmintic tests) were used to assess the toxicity of graded doses of the three anthelmintic drugs.

Statistical techniques. All statistical analyses have been kindly carried out by our colleague Dr O. L. Davies, using the probit mortality/log dose relationship to assess the toxicity of these drugs to parasites and hosts.

RESULTS

The relative efficiency of methyridine, bephenium and phenothiazine against adult nematode infections in mice. The results of anthelmintic and toxicity trials are presented in Table 1 together with two indices of chemotherapeutic efficiency. These are based on the ratio between doses of drug killing 50% of the hosts and 50% of the parasites (LD50/ED50 ; Table 1a) and those killing 5% of the hosts and 95% of the parasites (LD5/ED95 ; Table 1b).

Of the drugs tested, only methyridine possessed useful anthelmintic activity when administered by subcutaneous injection. Any given dose of the drug was significantly more effective when given subcutaneously than orally (Table 1). Whether injected subcutaneously or administered orally, methyridine was a more efficient anthelmintic than either bephenium or phenothiazine in terms of absolute dose.

Since there are large differences in the relative toxicity of these three anthelmintic drugs it is necessary to compare the therapeutic ratios to evaluate their comparative merit. The data presented in Table 1 indicate that the therapeutic ratios for treating parasitized mice with methyridine are similar for all three test nematodes. By contrast, the other drugs vary widely in their effectiveness against the same test organisms. As a result methyridine is the only drug among those tested which will almost completely eliminate all test infections at a safe therapeutic dose.

The anthelmintic activity of methyridine and bephenium against immature nematode infections of mice. The results of these studies indicate that subcutaneously administered methyridine possesses marked anthelmintic activity against certain immature nematode infections of mice. Comparisons with bephenium show methyridine to be the more active drug in terms of absolute dose. Thus the ratios for doses of methyridine and bephenium needed to produce approximately the same degree of anthelmintic activity were 1/3.2 (*N. muris*) and 1/4.0 (*N. dubius*) respectively. This compares with the corresponding ratios of 1/1.3 and 1/6.3 for the adult forms of these nematodes (Table 1a).

The anthelmintic activity of the two drugs administered in doses of equal toxicity to the host (approximately 50% of LD5) is shown in Fig. 1. The results obtained from the previous experiment with 12-day-old *N. dubius* infections have been included for completeness.

TABLE 1A

THE ANTHELMINTIC ACTIVITY AND TOXICITY OF METHYRIDINE (SUBCUTANEOUS AND ORAL), BEPHENIUM AND PHENOTHIAZINE

LD50's, ED50's and their standard errors

	Toxicity	*Heterakis spumosa*		*Nippostrongylus muris*		*Nematospiroides dubius*	
Anthelmintic drug	LD50 (mg/25 g)	ED50 (mg/25 g)	Therapeutic ratio	ED50 (mg/25 g)	Therapeutic ratio	ED50 (mg/25 g)	Therapeutic ratio
Methyridine (subcutaneous injection)	41±2·5%	2·7±10%	15±10·5%	4±8·5%	9·5±9%	7±8·5%	6±9%
Methyridine (oral)	70±17%	5±10·5%	13·5±20·5%	5±9·5%	13·5±20%	9±10%	7·5±20·5%
Bephenium	197±13%	12±12·5%	16±18·5%	5·5±13%	34·5±19%	43±12·5%	4·5±18·5%
Phenothiazine	458±7%	8·5±12·5%	53·5±14·5%	21±33%	22±34%	No action	—

TABLE 1B

THE ANTHELMINTIC ACTIVITY AND TOXICITY OF METHYRIDINE (SUBCUTANEOUS AND ORAL), BEPHENIUM AND PHENOTHIAZINE

LD5's, ED95's and their standard errors

	Toxicity	*Heterakis spumosa*		*Nippostrongylus muris*		*Nematospiroides dubius*	
Anthelmintic drug	LD5 (mg/25 g)	ED95 (mg/25 g)	Therapeutic ratio	ED95 (mg/25 g)	Therapeutic ratio	ED95 (mg/25 g)	Therapeutic ratio
Methyridine (subcutaneously)	21·5±6%	8±9·5%	2·5±11·5%	9·5±8·5%	2·5±10·5%	15±8%	1·5±10%
Methyridine (oral)	23±24%	16±9%	1·5±26%	11·5±8%	2·0±26%	20±10%	1±26·5%
Bephenium	64·5±23%	36±13·5%	1·5±27·5%	12·5±11%	5±26%	93±12%	0·5±27%
Phenothiazine	221·5±12·5%	25±15·5%	8·5±20·5%	50·5±44%	4·5±47%	No action	—

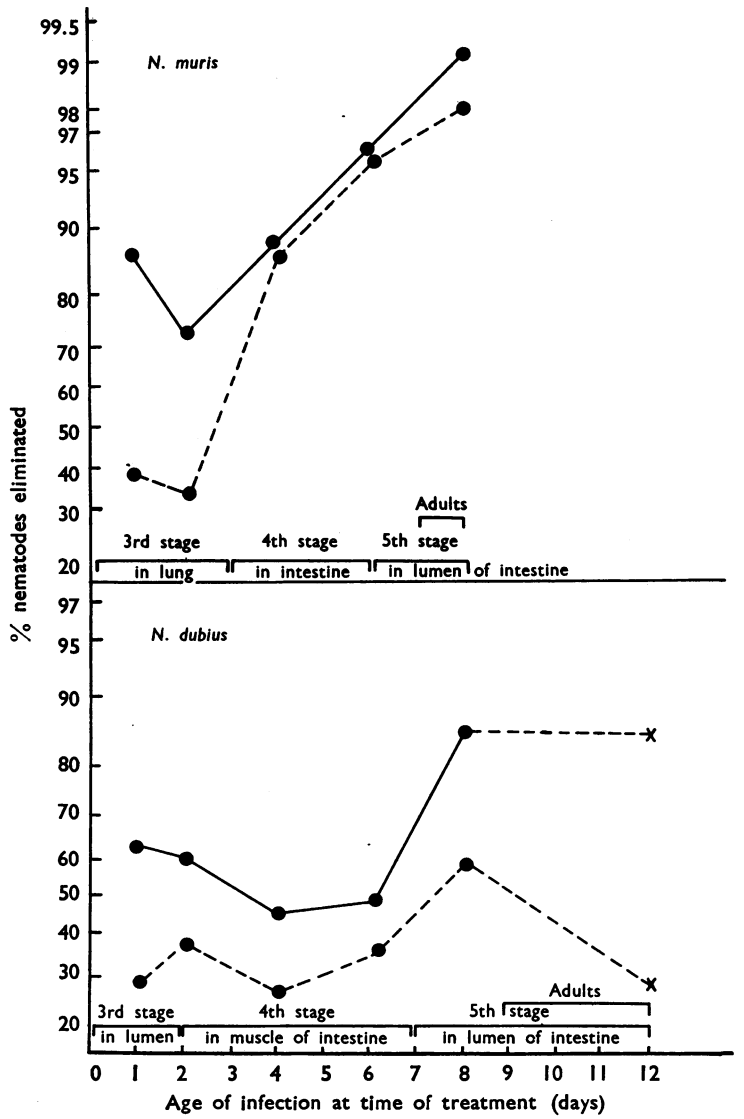

Fig. 1. Effect of methyridine subcutaneously and bephenium (oral) on immature nematode infections in mice. ●——● methyridine (10 mg/25 g). ● - - - ● bephenium (32 mg/25 g).

Fig. 1 shows that methyridine tends to give slightly better results than the equivalent dose in terms of toxicity of bephenium against immature *N. muris*, particularly when administered soon after infection. In addition, methyridine appears clearly more efficient than bephenium against immature *N. dubius* of all ages. However, these conclusions can only be regarded as indications when the standard errors attached to the toxicity data (Table 1b) are considered.

DISCUSSION

The anthelmintic studies described in this report indicate clearly that methyridine possesses considerable activity against both adult and immature nematode infections of mice. Examination of the results shows the drug is generally more active against adult worms than either phenothiazine or bephenium, two of the anthelmintics most commonly employed in veterinary practice. The margin of safety between toxic and therapeutic doses of methyridine is not so great as that for the other drugs when considered in relation to selected individual nematode species. However, considering the three test nematodes as representing a mixed natural infection, methyridine is the only compound (of those tested) which will almost completely cure mice at a safe therapeutic dose. It is this uniformity of action against the three nematode species examined, irrespective of their habitat or location in the alimentary canal, which makes the drug unique in our experience.

Another interesting feature associated with the anthelmintic action of methyridine is the fact that it is more effective in terms of absolute dose when administered subcutaneously than when administered orally. However, it is also more toxic by subcutaneous injection, so that there is little to choose between the two methods of treatment on the grounds of safety.

In addition to possessing activity against adult nematodes, a good anthelmintic must also be active against the immature forms. The comparative trials indicate that methyridine is at least as good as bephenium against immature nematode infections. It appears that differences in the relative activity of the two drugs against adult worms are reflected in their relative activity against the corresponding immature forms. However, certain exceptions do arise, as illustrated by the trials with 1- and 2-day-old *N. muris* infections; here methyridine appears to be clearly the more effective drug, and it should be noted that the larvae of *N. muris* are actually migrating through the lung at this time. Thus methyridine is able to affect nematode larvae which are not even in the gut. This feature is unlikely to be shared by any of the conventional relatively insoluble anthelmintics which are poorly absorbed by the mammalian host.

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