

PRELIMINARY TRIALS WITH PIPERAZINE ADIPATE AS A VETERINARY ANTHELMINTIC

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INTRODUCTION

DURING the last few years we have examined some 40 piperazine compounds for evidence of anthelmintic activity against the nematodes commonly found in domestic animals. Our trials were begun following the publication of the good results obtained when diethylcarbamazine acid citrate (hetrazan) was used to treat filariasis (Hewitt *et al.*¹), and to control ascarids in dogs and cats (Hewitt *et al.*² and Kanegis³). The high cost of hetrazan makes it impracticable for treatment of farm animals and attention was directed to the anthelmintic properties of cheaper piperazine derivatives.

A high level of toxicity or lack of therapeutic effect rendered the majority of compounds tested quite unsuitable for practical use. Piperazine hexahydrate, recently shown to be of value for treatment of *Enterobius* in man (White and Standen⁴) was found to be an effective ascarifuge in dogs and cats at an early stage in our trials. However, the physical nature and nauseating taste of this compound seriously detracted from its value for treatment of these animals. At this stage we were invited to evaluate the veterinary applications of piperazine adipate⁵ which has none of the disadvantages associated with the other compounds tested and it was rapidly established that besides being effective in cases of *Enterobius* in man, it showed very useful properties as a veterinary anthelmintic. The initial trials made against the ascarids of dogs were so successful that the work was extended to include tests against ascarids in horses, pigs and poultry. This paper briefly records the good results obtained against these and other species of nematodes in the various hosts.

MATERIALS AND METHODS

Pure piperazine adipate was used in these experiments. 3 horses were drenched with a suspension of the drug in water and 2 ate it mixed in bran mash. On 13 other occasions the drug was administered to these animals by stomach tube. It was given to pigs mixed in wet mash and to poultry in dry mash and occasionally in wet mash or in capsules. The majority of the dogs and cats were treated with piperazine adipate in gelatine capsules, the remainder received it in the form of tablets or ate it as powder mixed with their food.

No preliminary fastings, except in 4 dogs, nor post-dosing treatment with purgatives, etc., was carried out. Normal feeding procedure was followed and water was always freely available.

Evaluation of anthelmintic efficiency required different procedures according to the host species. A direct critical test—worms expelled/worms

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remaining—was possible in all cases with poultry as these could be slaughtered. A similar critical figure was obtained for 6 dogs. The remaining dogs and the cats could not be sacrificed, but all worms expelled by treatment were collected and comparisons made between pre-dosing and post-dosing faecal egg counts. A similar procedure was used for the horses and pigs. Where possible individual dogs, pigs, and horses received 2 or 3 successively larger doses at weekly intervals, the final dose being co-ordinated with the toxicity observations. By this means worms not removed by the small dose at the first treatment were expelled by a much bigger one the week after and a maximum percentage efficiency figure for the initial small dose could be established.

The toxicity of piperazine adipate has been investigated by Cross *et al.*,⁶ who found the anthelmintic to be practically non-toxic. Our toxicological observations were therefore designed to ascertain if a toxic response could be elicited by the oral administration of as much of the anthelmintic as its bulk would allow, using the conventional methods of dosing which obtain in practice. These amounts were from 3 to 5 times the therapeutic dose. All the animals under test were examined clinically, their food intake and behaviour observed, and blood tests completed. In cases where animals were sacrificed, a post-mortem examination was made, and sections of liver and kidney were microscopically examined.

EXPERIMENTAL RESULTS

1. DOGS

Ascarids. *Toxocara canis* and *Toxascaris leonina*.

13 dogs, mostly infected with *T. leonina*, were dosed once with piperazine adipate at rates varying from 25 to 200 mg./kg. of body weight. 193 ascarids were expelled after treatment and subsequent faecal examinations over a period of several weeks revealed no ascarid eggs. A post-mortem examination was made on one dog which had expelled ascarids after dosing, no further worms remained. An anthelmintic efficiency of 100 per cent. is indicated.

Hookworms. *Ancylostoma caninum* and *Uncinaria stenocephala*.

16 dogs infested with *A. caninum* were dosed at rates ranging from 100 mg./kg. to 300 mg./kg. on 3 successive days. 372 hookworms were removed by the dosing, but in no case did the faeces become negative for hookworm eggs even after repeated treatments. Post-mortem examination of 4 dogs showed that dosing had removed only 22/81 worms. Taking all our data together it is concluded that an average efficiency of more than 40 per cent. cannot be obtained against this species by the use of single doses up to 300 mg./kg. of body weight. The northern hookworm, *U. stenocephala*, which is a less sturdy parasite than *A. caninum*, appears to be more susceptible to piperazine adipate. 2 dogs were given 100 mg./kg. of body weight and expelled 38 worms. A post-mortem examination showed that all the worms present in both dogs had been removed by the treatment, indicating an efficiency of 100 per cent. 5 more dogs infected with

Uncinaria were treated with one dose of 100 mg./kg. Hookworm eggs completely disappeared from the faeces of 4 of them and in the fifth, which is known to have expelled at least 94 worms, the faecal egg count fell from 2000 to 150 eggs per g.

Cestodes. *Tania hydatigena* and *Dipylidium caninum*.

9 of the dogs were infected with *T. hydatigena* and 2 with *D. caninum* in addition to their nematode infestations. Piperazine adipate showed no apparent anthelmintic effect against these common tapeworms.

Toxicity

2 dogs, 18 months old, and weighing 12 and 15 kg., received a total of 26 and 27 g. of piperazine adipate over a period of 18 weeks, which represented an intake of 1800 and 2170 mg./kg. This dosing routine was finished by the administration of 6 and 9 g. of piperazine adipate in 72 hours, which is equivalent to 500 mg./kg. The faeces of both dogs were softer than normal, although the shape was maintained. No ill effects were detected, except that one observer, who knew both dogs well, was of the opinion that there was some evidence of slight hypersensitivity for 24 to 48 hours after dosing with 500 mg./kg. of piperazine adipate.

2. FELINES

Ascarids. *Toxocara mystax* and *Toxascaris leonina*.

3 cats infected with ascarids were dosed at the rate of 100 mg./kg. of body weight. All the post-dosing faecal material could not be collected, but it is known that many worms were expelled. Subsequent faecal examinations remained negative and a 100 per cent. efficiency seems probable.

One lion and 4 lionesses infected with *T. leonina* were dosed with ca. 100 mg./kg. of body weight, of the drug given concealed in gutted rabbits which were readily eaten. At least 496 worms were expelled by the treatment and probably more which could not be collected. Subsequent faecal examinations indicated that 3 of the beasts were cured and that the infestations in the other 2 were markedly reduced.

Palatability.

4 dogs and 1 cat ate doses of the drug equivalent to 100 mg./kg. of body weight quite readily in their ordinary meat or fish food. They did not vomit afterwards and throughout the dosing trials too, there was a complete absence of this undesirable feature which so commonly accompanies anthelmintic treatment in these species.

3. PIGS

Ascaris lumbricoides.

7 lightly infected store pigs were treated with doses of 150 to 600 mg./kg. of body weight in wet mash. The lower dose rate was ineffective, but higher rates led to removal of 31 worms and subsequent faecal examina-

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tions remained negative for ascaris eggs. A dose rate of 300 to 400 mg./kg. to a maximum of 40 g. seems to be necessary for a 100 per cent. cure.

Æsophagostomum spp.

21 pigs carrying light to moderate infestations of nodular worms were dosed at an estimated rate of 300 to 400 mg./kg. of body weight. 1010 worms were expelled from 1 group of 12 lightly infested animals. Only 2 of these ceased to pass eggs in the faeces after treatment, the remainder retaining small residual infestations. Complete post-dosing samples could not be obtained from the 9 other pigs, but 1025 worms were recovered from the relatively small quantity available and the total number passed must have reached several thousands. Comparison of mean pre- and post-dosing faecal worm-egg counts showed a drop from 1100 to less than 50 eggs per g. Efficiency against these worms is probably 80 to 90 per cent. at practicable dose rates.

Toxicity.

A group of 6 pigs, 4 to 6 months old, received 450 g. of piperazine adipate daily in their food for 3 days, which represented a total individual intake of 1875 mg./kg. The medicated food was taken readily on the first day, but eaten reluctantly on the second and third days. No abnormality, apart from a loss in weight which approximated 2 pounds per pig during the period of the test, was observed. A normal weight gain was observed the week following the test.

4. EQUINES

6 yearling Dartmoor ponies were used in the trials. Their weights were approximately 100 to 120 kg. Initially they carried light infestations of ascarids, small redworms and pinworms and, after being given low-level doses of piperazine adipate to indicate if it had any possible potentialities in this host, their ascaris and pinworm burden was increased by administrations of infective eggs. When the infections had had time to proceed towards maturity the animals were dosed at the 250 to 400 mg./kg. of body weight level followed by 1250 to 1500 mg./kg. a week later. The very small number of worms removed by the big final doses and the fall to zero of the ascarid and strongyle faecal egg counts in all the 6 ponies at the end of the tests left little doubt that the doses of 250 to 400 mg./kg. given the previous week had been highly effective and already removed the great majority of parasites originally present.

Toxicity.

Some increase in the liquid content of the faeces was observed for 24 hours when the ponies received 1250 to 1500 mg./kg. of piperazine adipate administered in 5000 ml. of water by stomach tube. No other abnormality was detected.

Ascarids. Parascaris equorum.

Dose rates of 120 to 150 mg./kg. of body weight were ineffective, 250 mg./kg. apparently removed 14/16 worms and 400 mg./kg. apparently removed all of 51 worms, ranging from small 100 mm. stages to mature

adults, from 3 horses. Efficiency against equine ascarids was further confirmed when 2 young zebras given *ca.* 300 mg./kg. of body weight of piperazine adipate in their food expelled at least 75 adult ascarids and over 100 immature ones.

Pinworms. Oxyuris equi.

The anthelmintic effect against the adult females was similar to that against ascarids. 250 mg./kg., removed about 80 per cent. of some 800 of them and there was an apparent 100 per cent. cure in two horses at 400 mg./kg.—211 worms being removed and no more appearing after the subsequent 1250 to 1500 mg./kg. dose a week later. The males and larval stages proved more resistant—only 60 to 90 per cent. appeared to be removed at the 250 to 400 mg./kg. dose rates. 3636 were expelled altogether by the 18 dosings given.

Small strongyles. Mainly Trichonema spp., and Triodontophorus spp.

Doses below 250 mg./kg. of body weight were not very effective, but judging by the numbers expelled, 11,221 in all, and the drop in faecal egg counts, a minimum efficiency of 85 per cent. can be expected at a 400 mg./kg. dose rate in ponies of this size. A rather lower dose rate would probably be satisfactory in bigger horses in which the gut volume would be relatively smaller.

5. POULTRY

Ascarids. Ascaridia galli.

109 infected 14-week-old cockerels were available. 70 were treated with piperazine adipate and 39 kept as controls. All worms expelled were collected and each bird was then examined post-mortem, and the remaining worms counted. These trials are summarised in Table I. 1537 worms were expelled by treatment and 47 left, giving an anthelmintic efficiency of 97 per cent. When more than 150 mg./kg. of body weight was given or taken by the birds in wet or dry mash the efficiency was always greater than 96 per cent. (62 birds treated). After individual dosing *Ascaridia* began to appear in the faeces at 3 hours, and were nearly all expelled by 6 hours. After administration in the food the majority were expelled within 24 hours of commencing the trial. An additional test in 12 laying hens showed that treatment caused no fall in egg production compared with 12 control birds.

Toxicity.

Groups of 12-week-old birds were dosed with piperazine adipate powder in capsules at the rate of 1000 mg./kg. and 2000 mg./kg. The lower rate is 5 times and the higher rate 10 times the therapeutic dose. No ill effects were observed at the lower dosage level. At the higher level the birds were lethargic and disinclined to feed for 1 to 3 hours after dosing, but had completely recovered by the fifth hour. Some scouring was apparent for a few hours, but the faeces became normal again within 24 hours. Other birds were fed for 24 hours on dry mash containing piperazine adipate

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powder at the rate of 1 part powder to 35 parts mash, which gives 10 times the therapeutic dose. At this concentration the medicated mash proved slightly unpalatable, but the birds achieved an intake of 4 g. of powder per head in the 24 hours. All the birds remained entirely normal.

TABLE I
DETAILS OF POULTRY EXPERIMENTS

Number of birds dosed	Number of control birds	Mode of administration of drug	Amount of drug taken by each bird in one day, mg./kg.	Worms removed	Worms removed by dosing, per cent.	Worms expelled naturally by controls, per cent.	
				Worms present pre-dosing			
4	4	Single dose in capsule	100	95/130	73	0	
4	4	" " " "	150	107/110	97	0	
4	4	" " " "	300	58/58	100	0	
6	5	" " " "	440	69/69	100	0	
10	4	" " " " or one 300 mg. tablet per bird	ca 400	248/248	100	0	
			Days fed				
4	0	1 part powder to 700 parts dry mash fed <i>ad. lib.</i>	3	138	67/71	94	-
6	6	1 part powder to 500 parts dry mash fed <i>ad. lib.</i>	3	202	103/103	100	7
8	4	1 part powder to 500 parts dry mash fed <i>ad. lib.</i>	3	220	174/175	99	5
12	0	1 part powder to 500 parts dry mash fed <i>ad. lib.</i>	3	264	440/442	99.5	-
4	4	1 part powder to 350 parts dry mash fed <i>ad. lib.</i>	2	297	29/29	100	0
4	4	1 part powder to 350 parts dry mash fed <i>ad. lib.</i>	1	330	72/72	100	0
4	0	1 part powder to 350 parts wet mash in two feeds	1	250	75/78	96	-

DISCUSSION

The preliminary trials with piperazine adipate which are summarised above indicate that this compound shows promise of proving a highly efficient ascaricide with such a wide margin of safety that it will be of considerable value in veterinary therapeutics. The facility with which it can be administered in both wet and dry food, the apparent lack of irritant effects on the gut, mucosa and other toxic manifestations, the absence of any need for supporting therapy such as purgatives, and an anthelmintic activity which is by no means restricted to members of the *Ascaroidea*, give this drug many advantages over those in current use.

The anthelmintic properties and lack of toxicity suggest that it would provide a valuable method of treatment for ascaris, redworm and oxyurid infections in horses, especially young thoroughbreds, which suffer severely from ascarids. In pigs, efficiency against ascarids and nodular worms would eliminate the necessity of using sodium fluoride for the former and phenothiazine for the latter and the possibility of giving the drug in wet food would obviate the risk of toxicity that is run when sodium fluoride is given in this way. The absence of vomiting after dosing is a valuable asset in treatment of dogs and cats for ascarids and the possibilities for administration in the food render it suitable for treatment of similar worm species frequently found in exotic carnivores. Further trials in progress

may show that it will also give useful control of at least some species of hookworms in those hosts in which use of carbon tetrachloride and similar compounds is not always possible or even advisable.

Virtually 100 per cent. control of *Ascaridia* is possible in poultry and piperazine adipate has advantages over carbon tetrachloride treatment in respect of likely toxicity and over phenothiazine-nicotine-bentonite in that it can be given in wet as well as dry mash and there is no subsequent feather staining by the excretory products derived from phenothiazine.

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