TRICHURICIDAL ACTIVITY OF PHTHALOFYNE[1, 2] AND CERTAIN RELATED COMPOUNDS

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Trichuriasis in humans and dogs has been regarded for several decades as a clinical disease entity. However, the inadequacy of available oral chemotherapeutic agents and techniques for the therapy of this disease in man is recognized (Faust, 1951; Brown, 1954; Jung, 1954). Therapy in dogs is also largely unsatisfactory. In addition, reports of cecectomy indicate that the surgical hazard is appreciable and that reinfection occurs (Bratt, personal communication, 1953; Bartlow, personal communication, 1954). Furthermore, the efficiency of a potentially useful trichuricide in dogs may be hindered by such factors as wide variations in cecal conformation, tonus of the ileocecal valve, presence of hard fecal masses, gas, and/or mucus (Chopra and Chandler, 1928; Enzie and Colglazier; 1953).

The present report deals with the *in vivo* determination of the trichuricidal activity in the dog of certain esters of phthalic acid and possible products of their hydrolysis. In addition, other pharmacologic properties of phthalofyne, the only compound in this study found to be an effective and safe trichuricide, are presented. The details of its clinical effectiveness have been reported elsewhere (Burch, 1954; Magrane, 1954; Green and Gruesser, 1954).

METHODS. Anthelmintic activity was determined in adult mongrel dogs, unselected as to sex, weighing 7–12 kgm. The animals were maintained on Borden's dog meal with water *ad libitum* except on the day prior to drug administration, when milk alone was fed.

Adequate parasite burdens were demonstrated twice each in all dogs prior to drug testing. The method used was a stool examination utilizing a flotation procedure as follows: (1) a stool sample was mixed with water, strained through 0.5 mm. wire gauze to fill a 15 ml. conical centrifuge tube; (2) the filtrate was centrifuged for 4 minutes at 800 r.p.m. and the supernatant decanted; (3) the supernatant was replaced by an equal volume of sodium dichromate solution (specific gravity 1.36) and recentrifuged for 15 seconds; (4) the top 3 mm. of this solution was removed by 3 dips of a section of glass tubing (8 cm. by 8 mm.) and transferred to a slide to make a field 22 mm. square. The number of ova found under a magnification of 100 diameters was counted and classified as light (3–100) or moderate (100–400). Other details of this method have been described elsewhere (Ehrenford, 1954). Animals which exhibited ova on the two occasions were considered to be positive and were used for the evaluation of the test drugs. Previous data (Ehrenford and Ehrenford and Burch, unpublished) on 80 untreated dogs with positive ova counts always showed the presence of adult *Trichuris vulpis* although no direct quantitative correlation existed between

[1] Preliminary report: Ehrenford, F. A.: J. Parasitol., **40**: 481, 1954.

[2] Generic name for 3-methyl-1-pentyn-3-yl acid phthalate, marketed under the trademark Whipcide by Pitman-Moore Company.

ova passed and the number of adult *T. vulpis* found present at necropsy. However, 50 of the 80 control dogs passing ova in numbers within the range of those used in the study reported herein showed the average number of adult *T. vulpis* to be 44 (range 3–260) as determined at necropsy. It should be emphasized that adult *T. vulpis* were always present at necropsy in untreated dogs when the ova count was positive and were occasionally observed in animals that were not found passing ova.

Compounds investigated in the dog were administered intragastrically in hard gelatin capsules. Presumptive evidence of drug effectiveness was obtained prior to necropsy by observing a 50 to 100 per cent reduction of *T. vulpis* ova in stools examined on one or two occasions following drug administration. Drug effectiveness was established by observing the presence or absence of adult *T. vulpis* in the cecum and colon examined five to seven days after treatment.

The Clearance Dose$_{50}$ (CD_{50}), the quantity of drug completely clearing 50 per cent of the dogs of adult *T. vulpis* as determined at necropsy, and the Toxic Dose$_{50}$ (TD_{50}), that amount of agent producing minimal toxic effects in 50 per cent of the dogs, were calculated by the method of Litchfield and Wilcoxon (1949). Toxicity was considered to be present when the dogs showed anorexia, emesis, ataxia, and somnolence, either alone or in combination. Since the Lethal Dose$_{50}$ (LD_{50}) could not be determined intragastrically in dogs (see RESULTS and DISCUSSION), such studies on another species were considered important. The LD_{50} (Miller and Tainter, 1944) was determined intragastrically in male albino mice (Webster-Swiss), 18–24 gm., starved for 24 hours, with water *ad libitum*.

RESULTS AND DISCUSSION. Anthelmintic activity and the incidence of toxic manifestations in dogs are summarized in table 1. It is evident that phthalofyne was the only one of the compounds investigated in which trichuricidal activity was present at a dose level below that producing minimal toxic effects. Indeed, the margin of safety or protective index (P.I. = TD_{50}/CD_{50}) of this compound was 3.7. This unexpected anthelmintic action of phthalofyne against *T. vulpis* is not readily explainable.

Evidence that phthalofyne acts as an unchanged molecule is suggested by the

TABLE 1

Anthelmintic activity and toxicity of phthalic acid esters and possible products of their hydrolysis in dogs

Compound	Mol. Wt.	*Trichuris vulpis* Anthelmintic Activity			Toxic Effects*		
		No. of Dogs†	mgm./kgm.	% Cleared	No. of Dogs†	mgm./kgm.	% Toxic
3-methyl-1-pentyn-3-ol (methylparafynol)	98.1	4	>50	0	8	50	75
3-methyl-1-pentyn-3-yl acid phthalate (phthalofyne)	246.3	30	CD_{50}= 120(97–149)	50	24	TD_{50}= 450(424–477)	50
1-pentyn-3-yl acid phthalate	232.4	3	>400	0	7	400	14.3
di-n-butyl phthalate	278.3	3	>450	0	7	450	14.3
di-ethyl-phthalate	222.2	4	>400	0	8	400	25
phthalic anhydride	148.1	3	>450	0	7	450	28.6

* See METHODS.

( ) 95% confidence limits.

† Represents only the number of dogs tested at the highest dose level; it does not represent the total number of animals tested with each agent except for phthalofyne.

observations that neither the possible hydrolysis products nor the related esters possessed trichuricidal activity although they were administered in increasing dosages until minimal toxic signs were observed (see table 1). Additional supportive evidence is to be found in studies on mouse lethality in which it was observed that the intragastric LD_{50} for phthalofyne was 1500 ± 95 mgm./kgm. (6.09 ± 0.38 mM./kgm.) and that for methylparafynol was 790 ± 85 mgm./kgm. (8.06 ± 0.76 mM./kgm.). Thus, mole for mole, phthalofyne was more toxic than methylparafynol in mice suggesting that phthalofyne is not acting as methylparafynol, a possible hydrolysis product.

The acute toxic manifestations for phthalofyne in mice appeared in the following order: ataxia, stupor, and loss of righting reflex accompanied by general depression which persisted in some cases for more than 24 hours. All fatalities occurred within 48 hours due to respiratory paralysis. In acute toxicity studies of phthalofyne in dogs, the signs observed were: emesis (450 mgm./kgm.), anorexia (475 mgm./kgm.), and somnolence (500 mgm./kgm.), which persisted for less than 24 hours. The acute toxic signs observed in the other compounds studied were as follows: methylparafynol (ataxia at 50 mgm./kgm.); 1-pentyn-3-yl acid phthalate (emesis at 400 mgm./kgm.); di-n-butyl phthalate (emesis at 450 mgm./kgm.); di-ethyl phthalate (emesis and anorexia at 400 mgm./kgm.); and phthalic anhydride (emesis at 450 mgm./kgm.).

Inasmuch as phthalofyne exhibited a desirable protective index (P.I. = 3.7), studies to determine its lethality in dogs were attempted. An LD_{50} in dogs was not obtainable at doses investigated (750, 1000, 1500, and 3000 mgm./kgm.) since emesis regularly occurred, usually within one hour after dosing. Transitory signs of tremor, somnolence and/or ataxia were observed at the 1000–3000 mgm./kgm. dose levels. From these data it would appear that emesis can act as a protective mechanism. Few anthelmintics show this interesting phenomenon, e.g., cadmium oxide in swine (Bunde *et al.*, 1954).

In subtoxic doses, phthalofyne was found to be ineffective against *Toxocara canis*, *Toxascaris leonina*, *Taenia spp.*, and *Dipylidium caninum*, and exhibited only slight activity against *Uncinaria stenocephala* and *Ancylostoma caninum*.

Observations at necropsy on test dogs revealed no gross signs of pathology in the lungs, liver, kidneys, gastrointestinal tract, pancreas, spleen, or bladder which were attributable to phthalofyne.

These data in dogs, indicating the relative infrequency of untoward effects at doses which are trichuricidal, recommend phthalofyne as a useful anthelmintic. Furthermore, it represents an addition to the helminthic chemotherapeutic armamentarium of another agent whose acute lethality is blocked by virtue of its emetic properties.

SUMMARY

A new anthelmintic compound, phthalofyne, 3-methyl-1-pentyn-3-yl acid phthalate, was found to be specific, effective, and safe against canine *Trichuris vulpis* in a single oral dose.

Other esters of phthalic acid (1-pentyn-3-yl acid phthalate, di-n-butyl phtha-

late, di-ethyl-phthalate) as well as methylparafynol and phthalic anhydride were not found to be effective trichuricidal agents.

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