In Vitro Studies of Larvicidal Agents Against the Larvae of *Anaylostoma caninum*

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An investigation was conducted on larvicidal agents using a modification of the Baermann procedure for culture of the test larvae. *In vitro* tests against the larvae of *Ancylostoma caninum* indicated that *p*-chloro-*m*-xylenol is an effective larvicidal agent. The drug's activity is reduced by blood, plasma, and certain solubilizers.

THE LARVAL STAGES of helminths produce several diseases in man and animals. The most prominent such disease of man is larva migrans, a condition characterized by prolonged migration of a larval parasite in the skin or internal organs.

The term "cutaneous larva migrans," commonly known as "creeping eruption," refers to a migration of the larvae in the skin and is commonly caused by the larvae of the dog and cat hookworms, *Ancylostoma braziliense* and *A. caninum*.

Visceral larva migrans, the invasion of the internal organs, is primarily caused by the larvae of the cat and dog ascarid, *Toxocara canis*.

Many agents have been used in the treatment of larva migrans clinically, but an effective, safe, and highly satisfactory larvicidal agent is not at present available (1, 2).

METHOD

The larvae of *A. caninum* were isolated by a modified Baermann technique from fecal samples taken from infested dogs.

The Baermann procedure depends on the principle that nematode larvae will migrate out of fecal material into water maintained at a higher temperature than the fecal sample (3). A disadvantage of the method is that water when in contact with fecal material becomes soiled and turbid making examination difficult and shortening the life of the larvae unless a complete change of water can be effected. A high proportion of sluggish or inactive larvae may be produced. To overcome the difficulty, the fecal sample was suspended above the water level and kept moist with cloth wicks extending from the sample into the water. The larvae dropped into the water as they hatched, and contact of the larvae-bearing solution with the fecal sample was avoided.

The drugs tested were dissolved in water whenever feasible. If the agent was not water soluble, propylene glycol and/or Triton X-100[1] were used as solubilizing agents. The blanks used as controls consisted of water or an appropriate solution of the solubilizing agents in water. The drug concentrations referred to are the final concentrations after dilution with the larvae-bearing solution.

In vitro tests were conducted by placing 1 drop of the larvae-bearing solution in the well of the microscope slide and adding 1 drop of the solution to be tested. The slides were observed under the microscope at routine intervals.

Although normally lack of motility indicated death of the larvae, this was not true in all instances. Viable larvae which had become immotile would react visibly in a dilute solution of ethanol which appeared to act as a stimulant to them. One drop of ethanol-water (1:1) was added at the time of last observation. Larvae remaining immotile thereafter were considered dead.

EXPERIMENTAL

Initial studies tested the larvicidal properties of iodine in both free and combined forms.

In these tests only free iodine, as contained in Lugol's solution, was highly effective against the test larvae. Free iodine in Lugol's solution, diluted with water to a concentration of 0.55%, produced a 100% kill in 10 minutes. A concentration of 0.025% produced an 80% kill in 24 hours. These results were accepted as the standard for subsequent studies.

Screening tests were carried out on a series of compounds, and inactive agents were discarded. Results obtained with nicotine (alkaloidal), nicotine salicylate, phenol, creosote, and glaucarubin, all of which were less active than iodine, are recorded in Table I.

The chemical agent *p*-chloro-*m*-xylenol (PCMX) had a high level of larvicidal activity and was effective at much lower concentrations than was the standard. A concentration of 0.012% of PCMX produced 96% kill in 1 hour; 0.006% produced 100% kill in 24 hours (Table II).

PCMX has been reported to be an effective phenolic type germicidal and fungicidal agent. The compound is soluble in water only to the extent of 1 part in 3000, but is very soluble in acetone, ether, and alcohol. PCMX is reported to have an MLD in mice of 120 Gm./Kg. and an LD_{50} of 115 Gm./Kg. when injected as an aqueous suspension intraperitoneally (4).

It has been previously reported that the germicidal effectiveness of PCMX is partially destroyed by certain wetting or solubilizing agents (5). Further tests were conducted to determine the effects of solubilizing agents on the larvicidal properties of the drug. The most efficient solvent was a 5% aqueous solution of propylene glycol. Concentrations of propylene glycol of 10% or above were toxic to larvae, thus interfering with the tests. Other solubilizing agents used increased the survival time of the larvae, apparently through an inhibitory effect on the active agent. The results obtained with solubilizing agents are summarized in Table III.

PCMX, combined with agents listed in Table III in the concentrations indicated, was only one-half to one-fourth as effective as the same concentration of drug in a 5% aqueous solution of propylene glycol.

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[1] Marketed by Rohm and Haas Co., Philadelphia, Pa.

TABLE I.—RESULTS OF TESTS OF LARVICIDAL ACTIVITY OF VARIOUS AGENTS

Drug	Concn., %	Percentages of Larvae Dead or Immotile 10 Min.	30 Min.	1 Hr.	2 Hr.	24 Hr.
Iodine (as free iodine in Lugol's solution)	0.055	100	100	100	100	100
	0.05	82	94	98	[b]	88
	0.025	53	65	75	75	80
	Control[a]	0	0	0	0	0
Nicotine	0.25	0	0	0	0	100
	0.1	75	75	63	75	92
	Control	0	0	0	0	0
Nicotine salicylate	0.16	44	57	75	82	[b]
	Control	0	0	0	0	[b]
Phenol	0.05	13	0	0	63	100
	0.025	0	0	0	50	0
	Control	0	0	0	0	0
Creosote	0.05	0	38	[b]	[b]	50
	Control	0	0	[b]	[b]	10
Glaucarubin	0.05	0	7	7	43	0
	Control	0	0	0	10	0

[a] Larvae in aqueous suspension. [b] Readings not taken.

Additional tests were performed to determine the effects of blood constituents on the activity of PCMX. Results obtained with *in vitro* studies reveal that the agent's effectiveness is reduced by these substances.

TABLE II.—RESULTS OF TESTS OF LARVICIDAL ACTIVITY OF *p*-CHLORO-*m*-XYLENOL USING PROPYLENE GLYCOL 5% AS SOLUBILIZING AGENT[a]

Concn. of PCMX, %	10 Min.	30 Min.	1 Hr.	2 Hr.	24 Hr.
0.05	83	100	100	100	100
0.025	80	90	90	[b]	[b]
0.012	29	67	96	98	100
0.006	0	0	0	10	100
Control	0	0	0	0	10

[a] Results expressed as percentages of larvae dead or immotile. [b] Readings not taken.

TABLE III.—EFFECTS OF SOLUBILIZING AGENTS ON THE ACTIVITY OF PCMX

Solubilizing Agent	Concn. of Solubilizing Agent, %	Effect on Percentage Kill
Polysorbate 80[a]	0.5	Reduced
Dioctyl sodium sulfosuccinate	0.125	Reduced
Sodium lauryl sulfate	0.25	Reduced
Castile soap	0.25	Reduced
Triton X-100	0.50	Reduced
Polyethylene glycols 400, 1540, 4000, 6000	10.0	Reduced
Propylene glycol	10.0	Increased
Propylene glycol	5.0	No effect

[a] Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.

The external use of PCMX as a germicidal agent has been without reported ill effects to man or animals. Blood and tissue concentrations, up to 4 mg. per cent, have been recorded by Zondek and Finkelstein following administration to humans by intramuscular and percutaneous routes (6). In view of the reported low toxicity of the drug, it appears desirable to obtain data on blood levels required for larvicidal activity in the body. In our preliminary *in vivo* studies in dogs, doses of 200 to 400 mg./Kg., by oral and intramuscular administration, were nephrotoxic. Further work on systemic use of PCMX in dogs and guinea pigs is in progress.

In view of the drug's high rate of absorption by percutaneous administration, the use of PCMX in treatment of cutaneous larva migrans is a possibility. Also of interest is application of the compound as an area spray where man and animals may be exposed to infectious larvae of parasites.

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Fluorometric Study of Antihistamines

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The reactions of 16 antihistamines with hydrogen peroxide and the fluorescent characteristics of the products are reported.

ANTIHISTAMINES containing an alkylamino substituent in the 2-position on a pyridine nucleus react with cyanogen bromide to yield fluorescent products (1). This reaction is the basis of a fluorometric method for the detection and determination of some of these antihistamines.

In the present work, the reactions of 16 antihistamines with a series of oxidants were studied. The fluorescence characteristics of the products formed upon treatment with hydrogen peroxide form the basis of this report. The corresponding studies on the cyanogen bromide systems were carried out for comparative purposes.

Excitation and fluorescence wavelengths and

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