ACTION OF CERTAIN ANTHELMINTICS ON ASCARIDIA GALLI (SCHRANK, 1788) AND ON HETERAKIS GALLINARUM (SCHRANK, 1788)

by

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INTRODUCTION

Discovery that one is infected with a worm parasite usually prompts one to use substances which will remove the unwelcome guest. Aversion to the parasites in farm animals may not be as intense, but a farmer or rancher may be eager to use anthelmintic compounds especially if the animals show unthriftiness or retarded growth associated with parasitosis.

Many anthelmintic substances have relatively little therapeutic value against a specific parasite, they are too expensive to justify mass treatment, or they possess toxic properties which can be more deleterious than the original worm burden. Therefore, specific anthelmintic efficacy and toxicity of a compound should be determined before it is widely used.

The purpose of this investigation was to test the relative effectiveness and toxicity of various compounds and combinations of compounds against Ascaridia galli, the large roundworm of chickens. This parasite is responsible for considerable "hidden" loss in chicken flocks, especially when it is present in the tissue phase of its life cycle.

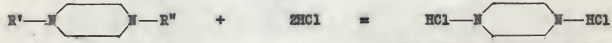
Piperazine citrate was used at various levels and in combination with nicotine, phenothiazine, and a piperazine derivative CL 16147. Vermizym, a compound not related to piperazine was also tested against A. galli. This anthelmintic contains as its active ingredient, papain, a proteolytic enzyme.

Effects of the piperazine compounds against Heterakis gallinarum, the cecal worm of chickens, were also studied. Pathological effects of this parasite on its host are not clearly known or understood, but it has been incriminated as a means of maintaining and spreading enterohepatitis (blackhead) in chickens and turkeys. Therefore, a drug which shows anti-heterakid activity would be of value to the poultry industry and, ultimately, to the consumer.

REVIEW OF LITERATURE

Piperazine, a drug formerly used as a treatment for gout and uric acid calculi, was recently found to be highly effective as an anthelmintic in man and animals. Hewitt, et al. (1947) discovered activity of piperazines in the chemotherapy of filariasis and Hewitt, et al. (1948a) demonstrated high efficacy of a piperazine in the treatment of ascariasis in dogs.

Hewitt, et al. (1948b) found that neither the piperazine nucleus nor any of its components were anthelmintically active. The piperazine nucleus acquires anthelmintic qualities when various chemical groups are added at certain points on its heterocyclic ring. For example, certain groups can be attached at the R' and R" positions in the ring:



Piperazine nucleus — Piperazine dihydrochloride

However, the toxicity of a piperazine compound increases and its efficacy decreases as the length of the alkyl chains increase.

Piperazine compounds appear to have a narcotic effect on helminths. Various workers have noted that worms which were expelled in the feces following piperazine treatment were immobilized. These worms recovered when placed in physiological solutions. Goodwin and Standen (1954) observed immobilization of pig ascarids in six to eighteen hours after they had been immersed in Ringer's solution containing piperazine citrate in concentrations of 1:500 or more. They suggested that this slow immobilization is advantageous to the treated individual because the worms lose their ability to make violent movements which can result in occlusion or perforation of the gut.

Piperazine compounds have relatively low oral toxicity. For example,

the LD_{50} of piperazine dihydrochloride for rats is approximately 4.92 gm per kg body weight, 3 gm per kg body weight for guinea pigs, 4 gm per kg body weight for rabbits, and 8 gm per kg body weight for chickens. The LD_{50} of this compound for animals capable of emesis is unknown (Dow Chemical Company, 1956).

Piperazine derivatives may not show equal anthelmintic action against a specific parasite. For example, Riedel (1950) found Caricide to be most effective (69.4 per cent) against Ascaridia galli in an oral dose of 1.0 gm per bird followed by a 0.5 gm redose several hours later. Riedel (1951) also reported that 2.0 per cent Caricide in feed was 81.0 per cent effective against A. galli. Subsequent studies have shown that lower levels of piperazine citrate are more effective against this parasite than the stated levels of Caricide.

Shumard and Eveleth (1955) subjected 16 chickens infected with A. galli and Heterakis gallinarum to various treatment levels of piperazine citrate. They found that levels of 8,000 and 16,000 mgm per gallon of drinking water, given free-choice for 24 hours, were 100 per cent effective against A. galli. However, some of the birds still harbored H. gallinarum.

These authors also supervised a test in which a poultryman treated a flock of 750 birds with 10,000 mgm piperazine citrate per gallon of drinking water. The poultryman reported that he found ascarids on the dropping boards. Two birds submitted for post-mortem examination were negative for A. galli. At a later date an additional 12 treated birds were found to be negative for A. galli, but seven of them were positive for H. gallinarum.

Bradley (1955) used piperazine citrate against A. galli in two commercial broiler flocks of 15,600 and 17,900 respectively. The flock of 15,600 8-week-old birds given 8,000 mgm piperazine citrate per gallon of drinking water

free-choice for 60 hours contained no mature worms and an average of 0.24 immature worms as compared with averages of 6.87 mature and 2.42 immature worms in the untreated controls. The other flock, treated with 6,000 mgm piperazine citrate per gallon of drinking water for 24 hours, averaged 0.14 mature worms and 0.85 immature worms, whereas, the controls harbored an average of 2.53 mature and 2.44 immature worms.

Horton-Smith and Long (1956) compared anthelmintic efficacies of some commonly used vermifuges with those of the piperazines against A. galli. Recovery of worms from the feces following treatment and also post-mortem examination showed that oil of chenopodium given at the rate of four drops in 2 ml liquid paraffin and phenothiazine, 0.5 gm per bird, removed 4.5 and 39 per cent of the worms, respectively. Carbon tetrachloride, piperazine adipate, and piperazine carbon bisulphide were 100 per cent effective at a dosage of 200 mgm or more per bird. These drugs were also tested against 14 day old A. galli larvae in chickens and results indicated that none of these were very effective, but there probably was a reduction of larvae in the chickens treated with carbon tetrachloride and the piperazines.

It is interesting to note that other workers likewise found phenothiazine to be relatively ineffective against A. galli. Roberts (1940) used 1 gm phenothiazine per pound body weight and found it only 56.2 per cent effective against this parasite. Reid (1946) indicated that phenothiazine had poor activity against this worm, but it was highly effective against H. gallinarum.

Horton-Smith and Long (1956) investigated effects of piperazine adipate, piperazine citrate, and piperazine carbon bisulphide on 14, 17, and 21 day old larvae and on adult A. galli. Piperazine citrate and piperazine adipate at 300 mgm per kg body weight did not remove all 14 and 17 day old worms, but these compounds removed 100 per cent of the larvae at a level of 600 mgm per

kg body weight. Piperazine carbon bisulphide was not markedly effective at 200 mgm per kg body weight against either 14 or 17 day old larvae.

Piperazine citrate and piperazine adipate removed all 21 day old larvae when used at levels of 300 mgm per kg body weight. Piperazine carbon bisulphide at 100 mgm per body weight was 100 per cent effective against 21 day old larvae.

The three drugs were more effective in removing adult worms. At a level of 300 mgm per kg body weight, piperazine citrate removed 100 per cent, piperazine adipate removed 92 per cent, and piperazine carbon bisulphide removed 80 per cent of the worms. In tests using low level piperazine citrate and piperazine adipate continuously, these authors found levels of 300 mgm drug per 100 gm wet mash and 300 mgm drug per 200 ml water were 80 to 100 per cent effective.

Shumard and Eveleth (1956) tested low levels of piperazine citrate in drinking water against A. galli in chickens. They reported that over a 24-hour period this drug given free-choice in the drinking water at levels of 2000, 1000, and 500 mgm per gallon removed 82.4, 74.7, and 38.5 per cent of the worms, respectively.

Kerr (1956) administered piperazine in feed at continuous low levels and found that levels of a tenth or less of the therapeutic dose were ineffective in preventing infection with A. galli. The piperazines were not consistently effective against H. gallinarum and with a dosage effective for A. galli, maximum removal of H. gallinarum was about 40 per cent.

Shumard and Eveleth (1956) used piperazine citrate in drinking water against Ascaridia galli in turkeys. In a field test, 600 turkeys were treated for 24 hours with 4,000 mgm piperazine citrate per gallon of drinking water. The owner noticed expulsion of many worms. Four days later, the owner submitted six birds for examination. Fecal examinations were negative

and at necropsy three immature worms were found in one bird, one each in two birds, none in the others. Thus an average of 0.66 worms were found per bird.

Mann, et al. (1955) treated dogs and cats with piperazine citrate at the rate of 100 mgm per kg body weight for a period of 10 days. Toxacara cati and Toxascaris leonina were removed during the first four days of treatment. There was some effect against Ancylostoma caninum and Taenia taeniaeformis, but piperazine citrate at the level used was ineffective against Dipylidium caninum and Trichuris vulpis.

Bradley, et al. (1956) also studied anthelmintic action of piperazine citrate in dogs. He used three medication levels: 10 doses of 80 mgm per pound body weight, a single dose of 160 mgm per pound body weight, and one dose of 80 mgm per pound body weight. Piperazine citrate at these levels removed all ascarids, some hookworms, and a few whipworms.

Shumard and Eveleth (1956) stated that within the limits of their experiment using piperazine citrate against Ascaris lumbricoides in swine, this drug appeared to be effective against ascarids in swine. Eight pigs weighing from 20 to 34 pounds were used. Fecal examinations were positive for ascarid eggs in all the animals. Drug dosage per animal varied from 170 to 730 mgm per kg body weight. The drug was given directly to the animals or in wet feed. Treated pigs harbored no mature ascarids and they had fewer immature worms than did the controls.

Poynter (1956) tested the action of piperazine adipate, piperazine citrate, piperazine phosphate, and carbodithioic acid against parasites of the horse. He found that 200 mgm piperazine base per kg body weight reduced ascarid egg counts to zero. There was also a temporary reduction in strongyle egg counts.

As a result of tests using piperazine citrate against cattle parasites,

Swanson, et al. (1957) stated that this drug will not replace phenothiazine as the drug of choice against these parasites, but it should be an effective supplement to phenothiazine. Drug dosages equivalent to 7.1 and 14.2 gm anhydrous piperazine per 100 pounds body weight were given to two groups of five calves each. Recovery of worms from feces following treatment and post-mortem examinations were used to determine drug efficacy. This drug in the doses given was equally as effective as phenothiazine against Oesophagostomum radiatum and was more effective than phenothiazine against Cooperia sp. and Trichuris discolor. The drug was found to be less effective than phenothiazine against stomach worms especially Haemonchus contortus and Trichostrongylus axei. Larval stages of the parasites were found on necropsy, few were eliminated after treatment. Neither of the drug levels was effective against Fasciola hepatica, Dictyocaulus viviparus, Capillaria bovis, or Moniezia sp.

Piperazine citrate, also known as "antepar" citrate, has been used in human medicine against Ascaris lumbricoides and Enterobius vermicularis. Goodwin and Standen (1954) discovered that a dose equivalent to 3 gm piperazine base removed most roundworms and was the most satisfactory dose for all individuals except children weighing less than 20 kg. They were given a dose equivalent to 2 gm piperazine base.

Brown and Sterman (1954), Brown (1955), Shafei, et al. (1955), and Swartswelder (1955) obtained best results with piperazine citrate against human ascarids when it was used for a period of several days. However, many cases were cured after one day of treatment. The minimum effective dose reported was 0.151 gm per kg body weight.

In treatment against Enterobius vermicularis, Brown and Chan (1955) used piperazine citrate in daily doses ranging from 22 mgm to 75 mgm piperazine base per kg body weight and determined that treatment for 14 consecutive days

was more effective than treatment for 10 days or for two 7-day periods with an intervening seven days of no treatment.

Piperazine dihydrochloride is another piperazine which has proved to be an effective anthelmintic especially against ascarids. Dosage recommended for chickens is 0.5 gm piperazine dihydrochloride per bird at six weeks of age or older. For swine and dogs, 1 gm piperazine dihydrochloride per 10 pounds of body weight was found to be effective. Chickens, swine, and dogs can also be treated with 0.4 per cent piperazine dihydrochloride in feed or 0.2 per cent piperazine dihydrochloride in water. Chickens and swine are treated at these levels in feed or water for 24 hours; however, dogs should be treated for three consecutive days (Dow Chemical Company, 1956).

Worley, et al. (1957) treated a group of 52 chickens infected with Ascaridia galli with 25 mgm piperazine dihydrochloride daily from the seventh to the fourteenth day following infection. This treatment period which supplied a total drug dosage of 200 mgm piperazine dihydrochloride per kilogram body weight significantly reduced the number of lumen larvae in the treated birds as compared with 54 control birds.

Nicotine, like the piperazines, appears to have a narcotic effect on nematodes (Kerr and Cavett, 1952). Herms and Beach (1916) found nicotine effective against A. galli when they steeped tobacco stems in water and then used this solution in wet mash which they fed to chickens.

Disadvantages of nicotine as an anthelmintic include toxic effects at high levels, poor anthelmintic action at low levels, and activity against mature worms only. A dosage of 50 mgm alkaloid per bird will remove 90 per cent or more of the Ascaridia galli (Kerr, 1956).

German workers recently reported favorable anthelmintic action of Vermixym, a proteolytic anthelmintic, in the treatment of human and small

animal helminthiasis. Vermizym is specifically active against nematodes and literally dissolves these parasites in the intestinal lumen.

Papain, the active principle in Vermizym, is obtained from the papaya tree, Carica papaya, especially the green fruit. It is a proteolytic enzyme in the class of papainase enzymes. Other enzymes in the group are ficin, extract of fig tree latex; and bromelin, a constituent of fresh pineapple juice.

Nematodes immersed in solutions of these enzymes soon disintegrate. The explanation of this phenomenon is that these enzymes attack keratin and, according to Chitwood (1936), the external cortical layer of body wall of nematodes such as Ascaris lumbricoides is composed of keratin. When this layer has been dissolved, the rest of the body soon disintegrates.

Disintegration of nematodes by these enzymes is especially spectacular under in vitro conditions. Berger and Ansenjo (1940) placed Ascaris lumbricoides specimens in 0.7 per cent papain solution with M/18 phosphate-phthalate buffer at pH 5 and noted that the worms were almost completely digested in 17 hours. A 0.07 per cent papain solution also showed some proteolytic action.

Liebmann (1953) used various nematodes for in vitro experiments using 2 to 4 per cent Vermizym suspensions. Toxocara canis, Strongylus vulgaris, Dictyocaulus filaria, Protostrongylus rufescens, Protostrongylus nigrescens, Haemonchus contortus, and Heterakis gallinae gradually dissolved in these Vermizym solutions.

Vermizym will not dissolve nematode ova. Ammon and Debusmann-Morgenroth (1953) found no effect of Vermizym solutions on Enterobius vermicularis, Ascaris lumbricoides, and Trichuris trichiura ova. Nematode ova contain chitin and this substance is not affected by the papainase enzymes.

Boch (1954a) made histological studies to investigate effects of Vermizym

on the mucous membranes of the small intestine in mice, cats, and rabbits. Doses of 1 gm Vermizym per kg body weight did not show any harmful effects after the second or third dose, but after the fifth dose, some epithelial desquamation occurred. There were no clinical reactions and the desquamation disappeared after five days.

Boch (1954b) also used doses of 1 gm Vermizym per kg body weight against nematode infections in 44 foxes. He found that this anthelmintic was effective against Toxocara, Trichuris, and Ancylostoma, but not against Strongyloides infections.

Schaper (1951), Hannak (1951), and Barrera Moncada (1953) reported varying success with Vermizym in human medicine. Treatment levels of Vermizym for adults were equivalent to 5 to 15 gm papain per individual and for children, Vermizym equivalent to 5.6 to 13 gm papain per individual. No toxic effects were observed. This anthelmintic was found to be from 50 to 70 per cent effective against Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, and Necator americanus.

MATERIALS AND METHODS

All chickens used in the tests were non-sexed White Rocks obtained as day-old chicks from a commercial hatchery. They were raised in electric brooders and battery cages and were fed a commercial ration containing 18 per cent protein.

Prior to experimental infection with Ascaridia galli or Heterakis gallinarum, the chicks used in each test were banded, weighed, and placed in groups of approximately equal weights according to the method of Gardiner and Wehr (1950).

Ascaridia galli eggs used in the tests were cultured using the methods

of Hansen, et al. (1954, 1956). The body contents of adult worms were stripped out and the uteri were placed in artificial digestive juice (1.0 per cent pepsin and 0.5 per cent hydrochloric acid) in a Petri dish. When the uterine walls had been digested, tap water was added and after the eggs had settled to the bottom of the dish, the supernatant solution was withdrawn. The digestive juice was removed in three to four additional washings with tap water.

Due to the small size of H. gallinarum, the following egg culture technique was devised. A group of H. gallinarum was placed on a small 30-mesh screen and pressure was applied thoroughly crushing the worms against the screen. The macerated worms were washed from the screen in a Petri dish containing artificial digestive juice. In four to five minutes the mixture was poured through an 80-mesh screen into another Petri dish. This screen retained the worm cuticula and other debris. Then the eggs were washed until the digestive juice was removed.

Dr. Uriel Rocha of Sao Paulo, Brazil, (personal communication) found that mold growth in egg cultures could be inhibited by the addition of merthiolate. Therefore, a drop of 1:1000 merthiolate solution was added to 10 cc water in each Petri dish culture. All egg cultures were incubated at 30° to 33°C. for 14 days.

Each chicken to be infected was given 100± 10 A. galli or H. gallinarum eggs per os. A calibrated micropipette was used in feeding the eggs to the birds. A variation of the egg administration technique of Hansen, et al. (1954) was used. All water was withdrawn from the Petri dish egg culture and 10 to 15 ml of a 1.25M sucrose solution was poured into the dish. After the eggs had been scraped from the bottom of the dish with a scalpel, the sugar-egg suspension was poured into a small bottle. A drop of the suspension

was placed on a glass slide and the eggs were counted under a compound microscope. When it was necessary to dilute the suspension, additional 1.25% sugar solution was added and the eggs in several drops of the new suspension were counted. The suspension was diluted until the micropipette would deliver 100± 10 eggs when filled to the calibration point.

The hydraulic method of Ackert and Nolf (1929) was used to recover A. galli from the small intestine lumen. The intestine from the gizzard to the yolk sac diverticulum was removed from the body cavity and was then attached to a small water hose and the contents flushed into pint jars. The flushings were poured through a 20-mesh sieve which held back the worms. The worms were counted and then preserved in a 10 per cent formalin solution.

Worms recovered from test groups which had been treated against tissue phase larvae of A. galli in Test 1 were measured. The image of each worm was projected through a lens in a photographic bellows. Tracings of the image were made and these were measured with a Dietzgen planimeter.

In Tests 1, 2, 3, and 4, A. galli which had been expelled with the feces following anthelmintic treatment were recovered and counted. From the time of treatment until 48 hours after treatment, all feces were collected from the dropping trays at 6-hour intervals and were placed in quart jars. The feces were treated with a 10 per cent formalin solution for 24 hours before processing to preserve the worms and to make them more resilient.

The feces were washed through two sieves: the first, a 10-mesh sieve, retained most of the worms and the other, a 20-mesh sieve, recovered the smaller specimens. This worm recovery method was an adaptation of Hall's critical test of anthelmintics (Hall and Foster, 1917).

Heterakis gallinarum were recovered from chicken ceca by using an adaptation of the Ackert and Nolf (1929) hydraulic method for worm recovery.

A longitudinal cut was made in the blind extremity of the cecum which was then connected, at the constricted end, to a small water hose. After the contents had been flushed into a pint jar, the cecum was opened and the lining scraped. These scrapings were added to the jar.

The jars were filled with water and a level teaspoonful sodium chloride was added to each jar. In this solution, which was approximately normal saline, the worms neither burst nor rose to the surface, but remained intact in the sediment at the bottom of the jar. In three to four hours most of the pasty material in the flushings was emulsified and suspended in the supernatant solution which was withdrawn. Water was added, and after the sediment had settled, the supernatant solution was withdrawn again. After several washings the sediment was poured into a Petri dish and examined with a wide-field binocular microscope.

The digestion method of Ackert and Tugwell (1948) was used to recover H. gallinarum from abnormal or enlarged ceca containing a hard cecal core. The ceca were cut in several pieces which were placed in jars with an artificial digestive juice. These jars were placed in a 37°C. water bath and the contents were agitated continually for two hours. The supernatant solution was withdrawn, undigested cecal cores were macerated, and the sediment was examined for worms.

Cecal feces were collected for a period of 48 hours following anthelmintic treatment from groups in which the efficacies of the piperazines were tested against adult H. gallinarum. These feces were washed through a 40-mesh sieve and the material retained by the sieve was examined with a wide-field binocular microscope.

All anthelmintics used in this study were furnished by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

Drugs were used in tablet form for Tests 1, 2, 3, and 4. Various levels of piperazine citrate and combinations of piperazine citrate with nicotine, phenothiazine, and piperazine derivative CL 16147 were used. Piperazine derivative CL 16147 was also tested at various medication levels in Test 3. Vermizym was used in Test 4.

Piperazine dihydrochloride, 52.1 per cent piperazine base, and piperazine citrate, 35.5 per cent piperazine base, were supplied in powder form for Tests 5, 6, and 7. These drugs were dissolved in water and a micropipette was used in treating each bird individually. Treatment levels for all tests are given in detail in the experimental results.

Statistical analysis of test results was done by the Department of Statistics, American Cyanamide Company, Pearl River, New York and by the Statistical Laboratory, Kansas State College, Manhattan, Kansas.

EXPERIMENTAL RESULTS

Test 1

Two levels of piperazine citrate, a combination of piperazine citrate and phenothiazine, and a combination of piperazine citrate, nicotine, and phenothiazine were tested against tissue phase larvae and adult Ascaridia galli. Each drug was tested across four experimental procedures as indicated in Table 1. Drug levels and experimental results are listed in Tables 2, 3, 4, and 5.

The birds were weighed at 11, 22, 27, 43, and 63 days of age (three days pre-infection and 8, 13, 29, and 49 days post-infection). The sex of birds in Procedures B, C, D, and F was determined at autopsy. Bird weight gains, number of worms recovered from intestinal lumen and feces, and average worm

lengths were used as criteria for determining drug activity.

A small number of birds was used for each treatment, therefore, in respect to weight gains, it is not possible to say that one treatment was significantly better than another. Calculation of the rank correlation coefficient for weight gains between cockerels and pullets gave a value of -.093 which is not significant. Analysis of variance of weight gains of treated versus controls for the cockerels and pullets gave F values of 1.269 and 0.149, respectively. These values are not significant.

Analysis of worm counts showed that in Procedure A, the piperazine citrate and phenothiazine significantly reduced the number of worms with respect to the control (Procedure E, Table 2). Similar results were not obtained in the other drug treatments given in Table 2.

Adult worms challenged with high level piperazine citrate (Procedure C and D), piperazine citrate and phenothiazine (Procedure C and D) and piperazine citrate, nicotine, and phenothiazine (Procedure C) were effectively removed from the host. These groups also demonstrated 100 per cent treatment efficacies (Tables 4 and 5).

Analysis of variance of worm lengths in Procedure A (Table 2) gave an F value of 8.46; the L.S.D. at the 5 per cent level was 8.23. Thus worms from birds treated with piperazine citrate, low level, and the piperazine citrate and phenothiazine combinations were significantly shorter than those of the other groups. Analysis of variance of worm lengths in Procedure B (Table 3) gave an F value of 1.67 indicating that variation in worm lengths under the different treatments was no greater than the variation in worm lengths within the same treatment.

Test 1 demonstrated that the drug treatments did not influence weight

Table 1. Test 1 experimental design.

Age of birds (days)	14	21-29	44	60	64
Procedure	Infection of birds with A. galli	Treatment period A at 7-15 days post-infection	Sacrifice time A at 30 days post-infection	Treatment period B at 46 days (1-day) post-infection	Sacrifice time B at 50 days post-infection
A	+*	+	+	-	-
B	+	+	-	-	+
C	+	+	-	+	+
D	+	-	-	+	+
E	+	-	+	-	-
F	+	-	-	-	+

*(+) = test performed; (-) = test not performed.

Table 2. Effects of various drug treatments at 7-15 days post-infection. Test 1 Procedure A.

Drug treatment (12 birds/treatment)	Average weight gain (gms)	Total number of *A. galli* recovered (lumen)	Average worm lengths (mm) (30 days post-infection)
Piperazine citrate 25-40 mgm base/kg/day	314	14	37.1*
Piperazine citrate 50-80 mgm base/kg/day	323	41	47.6
Piperazine citrate 25-40 mgm base/kg/day Phenothiazine 300-500 mgm/kg/day	290	9*	38.0*
Piperazine citrate 12.5-20 mgm base/kg/day Nicotine 15-25 mgm/kg/day Phenothiazine 150-250 mgm/kg/day	317	44	52.0
Non-medicated control (Procedure E)	304	85	48.9

*Significant at 5 per cent level.

Table 3. Effects of various drug treatments at 7-15 days post-infection. Test 1 Procedure B.

Drug treatment (12 birds/treatment)	Average weight gain (gms)	Total number of A. galli recovered (lumen)	Average worm length (mm) (50 days post-infection)
Piperazine citrate 25-40 mgm base/kg/day	635	19	60.9
Piperazine citrate 50-80 mgm base/kg/day	634	23	64.7
Piperazine citrate 25-40 mgm base/kg/day Phenothiazine 300-500 mgm/kg/day	662	4*	62.0
Piperazine citrate 12.5-20 mgm base/kg/day Nicotine 15-25 mgm/kg/day Phenothiazine 150-250 mgm/kg/day	729	13*	70.3
Non-medicated control (Procedure F)	618	36	68.2

*Significant at 5 per cent level.

Table 4. Effects of various drug treatments at 7-15 days post-infection and at 46 days post-infection. Test 1 Procedure C.

Drug treatment (12 birds/treatment)		Average weight gain (gms)	Total number of *A. galli* recovered from test groups		Period B treatment efficacy** (%)
Period A	Period B		Lumen	Feces	
Piperazine citrate 25-40 mgm base/kg/day	Piperazine citrate 35 mgm/kg	629	6*	15	71
Piperazine citrate 50-80 mgm base/kg/day	Piperazine citrate 70 mgm/kg	638	0*	6	100
Piperazine citrate 25-40 mgm base/kg/day Phenothiazine 300-500 mgm/kg/day	Piperazine citrate 35 mgm/kg Phenothiazine 430 mgm/kg	692	0*	7	100
Piperazine citrate 12.5-20 mgm/base/kg/day Nicotine 15-25 mgm/kg/day Phenothiazine 150-250 mgm/kg/day	Piperazine citrate 35 mgm/kg Nicotine 40 mgm/kg Phenothiazine 430 mgm/kg	664	0*	11	100
Non-medicated control (Procedure F)	Non-medicated control (Procedure F)	618	36	0	0

*Significant at 5 per cent level.

**Treatment efficacy: $\frac{\text{Number worms recovered from feces}}{\text{Total number worms recovered from intestine and feces}} \times 100 = \text{Per cent efficacy.}$

Table 5. Average weight gain of birds and anthelmintic activity of drugs against A. galli at 46 days post-infection. Test 1 Procedure D.

Drug treatment (12 birds/treatment)	Average weight gain (gms)	Total number of A. galli recovered from test groups		Treatment efficacy (%)
		Lumen	Feces	
Piperazine citrate 35 mgm base/kg	657	4*	88	95.6
Piperazine citrate 70 mgm base/kg	676	0*	10	100
Piperazine citrate 35 mgm base/kg Phenothiazine 430 mgm/kg	631	0*	38	100
Piperazine citrate 35 mgm base/kg Nicotine 40 mgm/kg Phenothiazine 430 mgm/kg	657	1*	50	98
Non-medicated control (Procedure F)	618	36	0	0

*Significant at 5 per cent level.

gain of the experimental birds. Combinations of piperazine citrate and phenothiazine or piperazine citrate, nicotine, and phenothiazine showed an increase in anthelmintic activity over piperazine citrate alone when tested against tissue phase larvae of A. galli. However, piperazine citrate alone was as active as these drug combinations against adult A. galli.

Test 2

Test 2 compared the anthelmintic activity of two piperazine citrate levels with those of a piperazine citrate and phenothiazine combination against adult A. galli. Table 6 presents the experimental design of this test.

Table 6. Test 2 experimental design.

Age of birds (days)	21	67	77
Procedure	Infection of birds with A. galli	Treatment at 46 days post-infection	Sacrifice at 49 days post-infection
A	+	+	+
B	+	+	+
C	+	+	+
D	+	-	+

Each bird in Procedures A and B received piperazine citrate approximately equivalent to 30 or 60 mgm base per kilogram body weight. Procedure C birds were given a combination of piperazine citrate and phenothiazine approximately equal to 30 mgm base and 375 mgm phenothiazine per kilogram body weight.

Data of worm numbers recovered from the test groups and group treatment efficacies are given in Table 7. In view of results obtained in Test 1, weight gains within the groups were not related to drug effects, thus only

Table 7. Treatment efficacies of drugs used in Test 2.

Procedure and Treatment	Group (12 birds/group)	Total number of *A. galli* recovered from test groups: Lumen	Feces	Treatment efficacies: Group (%)	Average (%)
A Piperazine citrate 30 mgm base/kg	1	3	38	93	94.5
	2	2	40	95	
	3	0	51	100	
	4	4	29	88	
B Piperazine citrate 60 mgm base/kg	1	0	56	100	100
	2	0	36	100	
	3	0	31	100	
	4	0	16	100	
C Piperazine citrate 30 mgm base/kg Phenothiazine 375 mgm/kg	1	4	90	94.5	95.8
	2	2	17	89.5	
	3	0	27	100	
	4	1	26	96.3	
D Non-medicated control	1	93	0	0	0
	2	81	0	0	
	3	67	0	0	
	4	52	0	0	

worm numbers were used as a criterion of anthelmintic activity.

Group efficacy determination indicates that levels of about 30 mgm piperazine base per kilogram body weight removed from 85 to 100 per cent of the worms and levels of about 60 mgm piperazine base per kilogram body weight removed all worms. A combination of the lower level of piperazine citrate

with phenothiazine ranged from 89.5 to 100 per cent effective. The lower level of piperazine citrate by itself averaged 94.5 per cent effective and the combination of this level with phenothiazine averaged 95.8 per cent effective. These results indicate that the addition of phenothiazine to the reported level of piperazine citrate did not increase anthelmintic activity.

Test 3

Anthelmintic efficacies of various levels of piperazine citrate and piperazine derivative CL 16147 were compared with those of a combination of piperazine citrate and piperazine derivative CL 16147. Table 8 presents the experimental design of this test.

Table 8. Test 3 experimental design.

Age of birds (days)	14	60	62
Procedure	Infection of birds with *A. galli*	Treatment at 46 days post-infection	Sacrifice at 48 days post-infection
A	+	+	+
B	+	+	+
C	+	+	+
D	+	+	+
E	+	+	+
F	+	+	+
G	+	+	+
H	+	-	+
I	-	-	+

Drug levels of piperazine citrate and piperazine derivative were

approximately equivalent to 30, 15, and 7.5 mgm base per kilogram body weight. The drug combination contained these drugs in levels approximately equal to 7.5 mgm base each per kilogram body weight. Data of worm numbers recovered from the test groups and also group treatment efficacies are given in Table 9.

A method of analysis by Berkson (1953) was used in comparing effects of piperazine citrate and the piperazine derivative. The potency of piperazine citrate, relative to the derivative, was estimated to be 1.62 or 62 per cent more effective than the derivative. Approximate 95 per cent confidence limits were 1.48 to 1.76.

A combination of the two drugs at the lower level appears to be more active than either of the compounds alone at that level. This increased activity could have been due to a synergistic relationship or it may have been due only to an increase in drug quantity. However, even this increased efficacy is at such a low level as to have no usefulness to poultrymen in treating their flocks.

Test 4

Anthelmintic activity of Vermizym against tissue phase larvae and adult A. galli was investigated in Test 4. The experimental design of this test is given in Table 10.

The birds were fasted 28 hours during the treatment periods to prevent dilution of the proteolytic principle in the anthelmintic. No toxic effects were noted during the treatment periods. Experimental results for this test are included in Table 11.

As far as weight gains are concerned, there was little difference between the treated and the control birds. T-test values for worm numbers were .93 for Procedures A and B and 1.75 for Procedures C and D. These values are

Table 9. Treatment efficacies of drugs used in Test 3.

Procedure and Treatment	Group (8 birds/group)	Total number of *A. galli* recovered from test groups		Treatment efficacies	
		Lumen	Feces	Group (%)	Average (%)
A	1	0	58	100	98.4
Piperazine citrate 30 mgm base/kg	2	2	68	97.1	
B	1	13	34	72.3	72.5
Piperazine citrate 15 mgm base/kg	2	18	48	72.7	
C	1	78	8	9.3	12.3
Piperazine citrate 7.5 mgm base/kg	2	36	8	18.1	
D	1	3	20	86.9	86.7
Piperazine derivative (CL 16147) 30 mgm base/kg	2	7	46	86.4	
E	1	43	5	10.4	11.1
Piperazine derivative (CL 16147) 15 mgm base/kg	2	61	8	11.4	
F	1	42	0	0	1.4
Piperazine derivative (CL 16147) 7.5 mgm base/kg	2	29	1	3.3	

Table 9. (concl.)

Procedure and Treatment	Group (8 birds/group)	Total number of *A. galli* recovered from test groups		Treatment efficacies	
		Lumen	Feces	Group (%)	Average (%)
G Piperazine citrate 7.5 mgm base/kg	1	44	15	25.4	43.6
	2	9	26	75.7	
Piperazine derivative (CL 16147) 7.5 mgm base/kg					
Non-medicated infected control	1	18	0	0	0
	2	38	0	0	
Non-medicated non-infected control	1	0	0	0	0
	2	0	0	0	

not significant.

After treatment time B, the feces were examined for worms. The control birds expelled 9 worms during the fasting period, whereas, treated birds expelled 7 worms. These and other experimental results indicate that Vermizym, at the levels used in this test, was not active against A. galli.

Table 10. Test 4 experimental design.

Age of birds (days)	23	34	51	55
Procedure	Infection of birds with A. galli	Treatment time A: (1-day) at 11 days post-infection	Treatment time B: (1-day) at 28 days post-infection	Sacrifice at 30 days post-infection
A	+	+	-	+
B	+	-	-	+
C	+	-	+	+
D	+	-	-	+

Table 11. Effects of Vermizym obtained in Test 4.

Procedure and Treatment (19 birds each)	Average weight gain (gms)	Total number of A. galli recovered from test groups: Lumen	Feces	Treatment efficacy (%)
A Vermizym 0.5-1.0 gm/kg	334	93	0	-*
B Fasted control	359	73	0	-
C Vermizym 0.5-1.0 gm/kg	342	47	7	12.9
D Fasted control	343	58	9	-

* (-) = no drug treatment against adult worms.

Test 5

Test 5 investigated activity of high level piperazine dihydrochloride against Heterakis gallinarum, cecal worm, larvae in the intestine and ceca. The experimental design of this test is presented in Table 12.

Table 12. Test 5 experimental design.

Age of birds (days)	21	21-22	52
Procedure	Infection of birds with H. gallinarum	Treatment at time of infection	Sacrifice at 31 days post-infection
A	+	+	+
B	+	-	+

Each bird in the treated groups was given piperazine dihydrochloride approximately equivalent to 125 mgm base per kilogram body weight at the following intervals: 1 hour pre-infection and 1, 3, 5, 7, 24, 26, 30, and 32 hours post-infection respectively. The total quantity of piperazine dihydrochloride given each bird during the treatment was approximately equivalent to 1250 mgm piperazine base per kilogram body weight. Experimental results are given in Table 13.

Some of the birds were droopy for a short time following treatment, but this condition may have been due to a respiratory disease (CRD) present in the groups.

Occurrence of abnormal ceca in certain birds complicated the study of anthelmintic activity in this test. These ceca had thickened walls and contained a cecal core which was composed of concentric layers of material. Cecal contents and cecal plugs were examined in the search for the etiological agent of the condition, but none was found.

Table 13. Effects of piperazine dihydrochloride on the larvae of H. gallinarum and on a pathological condition of the ceca of the experimental chickens. Test 5.

Procedure and Treatment	Groups (12 birds/group)	Total number of H. gallinarum recovered		Number of birds parasitized by H. gallinarum	Number of birds with abnormal ceca
		Normal Ceca	Abnormal Ceca		
A Piperazine dihydrochloride 1250 mgm base/kg	1	91	-*	10	0
	2	86	-	10	0
B Non-medicated Infected control	1	159	1	4	5
	2	5	2	2	6

*(-) = No abnormal ceca.

Approximately half of the control birds had abnormal ceca. Only rarely were worms recovered from abnormal ceca; however, all treated birds had normal ceca. Medicated groups had higher ratios of birds parasitized by H. gallinarum than did non-medicated groups.

There were too few non-medicated control birds parasitized by H. gallinarum for an adequate comparison between weight gains in parasitized and non-parasitized birds. However, no overt effects of parasitism were noted in any birds.

It is difficult to determine direct effects of the drug on H. gallinarum, but there are quite high numbers of worms in the treated groups. This may indicate that the presence of a high drug concentration with the larvae did not have a pronounced effect on them.

Test 6

Various levels of piperazine dihydrochloride were tested against H. gallinarum larvae in the intestine and ceca. The experimental design of this test is given in Table 14.

Table 14. Test 6 experimental design.

Age of birds (days)	14	14-15	35
Procedure	Infection of birds with H. gallinarum	Treatment at time of infection	Sacrifice at 21 days post-infection
A	+	+	+
B	+	+	+
C	+	+	+
D	+	-	+
E	-	-	+

Birds in the treated groups were given piperazine dihydrochloride levels approximately equivalent to 250, 125, or 50 mgm base per kilogram body weight at the following intervals: 1 hour pre-infection and 1, 3, 5, 7, 24, 26, 28, 30, and 32 hours post-infection. The total amount given each bird was approximately equal to 2500, 1250, or 500 mgm base per kilogram body weight. Experimental results are given in Table 15.

In this test some of the birds showed pronounced droopiness following treatment. However, they also showed symptoms of a respiratory disorder and this may have caused a state of depression.

Abnormal ceca in certain birds, especially in the infected control groups, again complicated analysis of anthelmintic activity. All birds treated with 2500 mgm base per kilogram body weight had normal ceca. As the drug levels for the groups decreased, the number of birds with abnormal ceca increased. Half of the infected control birds had abnormal ceca, whereas, all birds in the non-infected groups had normal ceca.

Statistical analysis of variation in worm numbers in the treated groups gave an F value of 1.31 which is not significant. Total worm numbers in the infected control groups were high, but the number of worms per bird were very irregular.

The data may indicate some drug action in reducing worm numbers, but the piperazine dihydrochloride levels used in this test cannot be considered effective against H. gallinarum larvae.

Test 7

Two piperazine compounds were tested at various levels against H. gallinarum larvae in the intestine and ceca. These compounds were also tested against adult H. gallinarum. The experimental design of Test 7

Table 15. Effects of various levels of piperazine dihydrochloride on the larvae of H. gallinarum and on a pathological condition of the ceca of the experimental chickens. Test 6.

Procedure and Treatment	Groups (12 birds/group)	Total number of H. gallinarum recovered		Number of birds parasitized by H. gallinarum	Number of birds with abnormal ceca
		Normal ceca	Abnormal ceca		
A Piperazine dihydrochloride 2500 mgm base/kg	1	34	-	10	0
	2	26	-	6	0
B Piperazine dihydrochloride 1250 mgm base/kg	1	39	0	10	1
	2	19	-	8	0
C Piperazine dihydrochloride 500 mgm base/kg	1	81	0	8	2
	2	47	0	7	2
D Non-medicated Infected controls	1	237	3	5	6
	2	176	1	4	6
E Non-medicated Non-infected controls	1	0	-	0	0
	2	0	-	0	0

is given in Table 16.

Birds in the groups treated against larvae were given piperazine dihydrochloride or piperazine citrate approximately equivalent to 125, 62.5, or 25 mgm base per kilogram body weight at the following intervals: 1, 3, 5, 7, 9, 24, 26, 28, 30, and 32 hours post-infection. No pre-infection treatment was given in this test.

The total amount of piperazine base given each bird was approximately 1250, 625, or 250 mgm piperazine base per kilogram body weight. The levels used against adult H. gallinarum were 145 mgm piperazine base per kilogram body weight. Experimental results are given in Tables 17, 18, and 19.

Abnormal ceca were found in all groups under both piperazine dihydrochloride and piperazine citrate treatment. There is a significant correlation between number of birds parasitized and the size of drug dose. The number of birds parasitized by H. gallinarum increased as the drug levels were increased.

Analysis of variation in worm numbers between treatment groups gave the following F values: piperazine dihydrochloride, 1.00, piperazine citrate, 2.79, and a comparison between piperazine dihydrochloride and piperazine citrate treatments, 2.06. The F value of 2.79 is significant, however, variation in worm numbers from groups under each treatment was very great. This variation may have caused statistical analysis to indicate a difference in drug effect when none existed.

Differences in worm number in groups treated against adult H. gallinarum with either piperazine dihydrochloride or piperazine citrate were not significant. The experimental results in Test 7 indicate that neither piperazine dihydrochloride nor piperazine citrate showed activity, at the levels used, against either larvae or adult H. gallinarum.

Table 16. Test 7 experimental design.

Age of birds (days)	28	28-29	58	60
Procedure	Infection of birds with H. gallinarum	Treatment at time of infection	Treatment at 30 days post-infection	Sacrifice at 32 days post-infection
A	+	+	-	+
B	+	+	-	+
C	+	+	-	+
D	+	+	-	+
E	+	+	-	+
F	+	+	-	+
G	+	-	+	+
H	+	-	+	+
I	+	-	-	+
J	-	-	-	+

Table 17. Effects of various levels of piperazine dihydrochloride on the larvae of H. gallinarum and on a pathological condition of the ceca of the experimental chickens. Test 7.

Procedure and Treatment	Groups (12 birds/group)	Total number of H. gallinarum recovered		Number of birds parasitized by H. gallinarum	Number of birds with abnormal ceca
		Normal ceca	Abnormal ceca		
A	1	43	1	7	3
Piperazine dihydrochloride 1250 mgm base/kg	2	47	0	6	3
B	1	136	0	7	4
Piperazine dihydrochloride 625 mgm base/kg	2	90	0	5	3
C	1	54	0	3	4
Piperazine dihydrochloride 250 mgm base/kg	2	41	0	3	6
I	1	16	0	3	6
Non-medicated Infected controls	2	119	0	2	6
J	1	0	-	0	0
Non-medicated Non-infected controls	2	0	-	0	0

Table 18. Effects of various levels of piperazine citrate on the larvae of H. gallinarum and on a pathological condition of the ceca of the experimental chickens. Test 7.

Procedure and Treatment	Groups (12 birds/group)	Total number of H. gallinarum recovered		Number of birds parasitized by H. gallinarum	Number of birds with abnormal ceca
		Normal ceca	Abnormal ceca		
D Piperazine citrate 1250 mgm base/kg	1	37	0	7	2
	2	95	0	9	1
E Piperazine citrate 625 mgm base/kg	1	98	0	7	4
	2	161	0	9	3
F Piperazine citrate 250 mgm base/kg	1	80	1	4	4
	2	125	0	7	3
I Non-medicated Infected control	1	16	0	3	6
	2	119	0	2	6
J Non-medicated Non-infected control	1	0	-	0	0
	2	0	-	0	0

Table 19. Effects of piperazine dihydrochloride and piperazine citrate on adult H. gallinarum. Test 7.

Procedure and Treatment	Groups (12 birds/group)	Total number of H. gallinarum recovered*		Number of birds parasitised by H. gallinarum	Number of birds with abnormal ceca
		Normal ceca	Abnormal ceca		
G Piperazine dihydrochloride 145 mgm base/kg	1	13	0	5	5
	2	10	1	2	6
H Piperazine citrate 145 mgm base/kg	1	4	1	2	7
	2	68	0	3	5
I Non-medicated Infected control	1	16	0	3	6
	2	119	0	2	6
J Non-medicated Non-infected control	1	0	-	0	0
	2	0	-	0	0

*No worms were recovered from cecal feces expelled during 48 hours post-treatment.

DISCUSSION

Experimental results of tests in this investigation indicate successful helminth therapeusis with some compounds and limited anthelmintic activity of others. Apparently these results were influenced by factors inherent in parasite, drug, or host.

Ascaridia galli, the large roundworm of chickens, is found in the lumen of the small intestine. It is subjected to substances normally present in the intestine and also to all substances introduced into the alimentary canal. Under these conditions an anthelmintic substance fed to the chicken will contact this parasite. Conversely, such substances can be swept away in the peristaltic stream before quantities sufficient to harm the parasite can accumulate or they may be modified by chemical conditions in the intestine so that they become ineffective against the parasite.

Heterakis gallinarum, the cecal worm, is not subjected to the quantity nor variety of substances which challenge A. galli. Relatively small quantities of material pass from the intestine into the ceca. Many soluble substances perhaps do not even enter the ceca. However, a substance which is introduced into the ceca will remain there for a relatively long period of time.

Thus the location of these parasites in their host may have had a direct influence on the activity of anthelmintic drugs used in this investigation. For example, a piperazine citrate level of 30-35 mgm base per kilogram body weight removed 88 to 100 per cent of the adult A. galli, but this drug given at a level of 145 mgm base per kilogram body weight had no significant effect on adult H. gallinarum. These results may indicate that the drug did not reach the ceca.

Pathological conditions in the ceca also could influence the activity of

piperazines against adult H. gallinarum. Several workers have described lesions or nodules in the cecal wall which were associated with the presence of this parasite. Such conditions could modify normal functioning of the ceca. Thus deleterious effects of H. gallinarum could protect it from anthelmintic compounds. However, most workers agree that any pathological effect of H. gallinarum is moderate and would not alter functional activity of the ceca.

The tissue phase in the life cycle of A. galli also influences anthelmintic activity of compounds used against it. Research has revealed that drugs which are active against adult A. galli are often not effective against the tissue phase larvae. These larvae bury their anterior ends between intestinal villi and into the glands of Brunner. Such close contact is injurious to the host, but a parasite in this situation is not subjected to the full effect of substances present in the intestine. In this investigation a combination of piperazine citrate and phenothiazine was found to be more effective against tissue phase larvae of A. galli than piperazine citrate alone. Perhaps the combination with phenothiazine, an insoluble compound, remained at an effective anthelmintic level in the intestine for a longer period of time and thus showed a greater activity than the piperazine alone.

In this study the piperazines were not effective against larvae of H. gallinarum. Piperazine dihydrochloride and piperazine citrate were administered at high levels for a period of 32 hours at the time of infection. None of the treatments significantly reduced the number of worms. If these treatments coincided with the passage of worms into the ceca, the worms must have been in contact with the drugs at some time during the treatment period and yet they apparently were not effected by the drugs.

Though the piperazines did not seem to affect H. gallinarum larvae, they did prevent development of abnormal ceca or typhlitis in the treated birds. Abnormal ceca appeared in all non-medicated groups which were infected with H. gallinarum. All birds in non-medicated groups which had not been infected with H. gallinarum had normal ceca.

Cecal contents and cecal cores of abnormal ceca were examined microscopically in the search for an etiological agent of the typhlitis. No organism was found. However, many organisms can cause cecal lesions and inflammation in poultry. Table 20 reviews those which have caused typhlitis in chickens. All typhlitic conditions can be prevented in disease-free birds through good sanitation, nutrition, and housing. In this study cecal contents were examined at 21 to 30 days after infection with H. gallinarum. Perhaps the etiological agent had been destroyed in the cecal cores and older cecal lesions.

Smith and Graybill (1920) were the first workers to demonstrate that enterohepatitis (blackhead) developed following an infection with H. gallinarum. They found the etiological agent in the diseased ceca. Numerous workers have described abnormal ceca which appeared in chickens and turkeys following infection with this nematode. Thus circumstantial evidence indicates that the abnormal condition of ceca found in the present investigation was a manifestation of enterohepatitis.

If the disease in the abnormal ceca was enterohepatitis, then the experimental results may suggest new theories for the development of the disease. Connell (1950) discussed the time of release of metacyclic forms of the etiological agent of blackhead in the ceca from H. gallinarum and he postulated that it might be incident to either the second or third larval moults or both. According to Clapham (1933) these moults occur 48 to 96 hours

Table 20. Typhlitis in the chicken.

Disease	Etiological agent	Pathology
Avian Colibacillosis	Escherichia coli and other coliform bacteria	Hemorrhagic inflammation of the ceca.
Erysipelothrix septicemia	Erysipelothrix rhusiopathiae	Ceca enlarged with edematous mucosa containing numerous petechiae and small yellowish nodules visible through the serosa.
Pullorum	Salmonella pullorum	Cheesy core sometimes tinted with blood.
Tuberculosis	Mycobacterium avium	Irregular greyish-yellow or greyish-white nodules. (Seen in cases of long infection)
Cecal coccidiosis	Eimeria tenella	Mottled, thickened wall; bloody cheesy core; frank hemorrhage.
Enterohepatitis Histomoniasis "Blackhead"	Histomonas meleagridis	Early lesions small, raised pin-point ulcers. Later much enlarged and thickened with yellowish patches on the serosa. Adhesions of the ceca to other organs can occur. Ceca filled with tough, leathery yellowish white material tightly adherent to the mucosa. Cecal plug composed of concentric layers of material.
Trichomoniasis	Trichomonas gallinarum	Lesions essentially like those in enterohepatitis.

Table 20. (concl.)

Disease	Etiological agent	Pathology
Helminthic Typhlitis	Heterakis gallinarum	Ceca deep red in color, local congestion and punctate hemorrhages. Some blood and exudate present in lumen.
Helminthic Typhlitis	Strongyloides avium	Cecal walls greatly thickened; discharge thin and bloody.
Helminthic Typhlitis	Trichostrongylus tenuis	Cecal walls thickened, reddened. Small hemorrhages present.
Non-specific Typhlitis	Unknown	Opaque, whitish or yellow-tinged firm cecal cores. Appear during first weeks of life.

*Biester, 1948, Tyzzer, 1934, Morgan and Hawkins, 1949.

after infection. If the pre-infection treatment with piperazine was most important in the prevention of typhlitis, then the metacyclic forms may have been released at an earlier time than suggested by Connell. Possibly these forms were carried with the larvae and were affected in some way by the drug as it came into contact with them in the intestine or ceca.

Experimental results in this investigation also suggest an influence which abnormal ceca may have in anthelmintic tests. Fecal examinations are usually made before anthelmintic treatment to determine if test birds are infected with H. gallinarum. Ascaridia galli eggs are very similar to H. gallinarum eggs and when birds infected with A. galli are used, an infection with this parasite can be erroneously diagnosed as H. gallinarum. These birds may have abnormal ceca and therefore few or no H. gallinarum. If an anti-heterakid drug is used in such birds, the absence of H. gallinarum following treatment might cause the investigator to believe that the drug had 100 per cent efficacy against this parasite.

Experimental results obtained in this investigation cannot explain any effects the piperazines may have on the development of abnormal ceca when given at the time of infection with H. gallinarum. Further research to study relationships of this parasite to the development of abnormal ceca has been planned.

SUMMARY

Six drugs or drug combinations were tested against Ascaridia galli in four tests utilizing 628 chickens. Two drugs were tested against Heterakis gallinarum in three tests utilizing 408 chickens. Birds used in each test were separated into groups of approximately equal weight and each bird was given 100± 10 A. galli or H. gallinarum eggs per os.

All birds in a treatment group received the same drug level regardless of weight. Drug dosages of milligrams per kilogram body weight were estimated using the average bird weight at treatment time. Results of the tests are as follows:

Piperazine citrate at levels of 25-40 mgm base per kilogram body weight and 50-80 mgm base per kilogram body weight did not reduce worm numbers in tests against tissue phase A. galli. However, a combination of the lower level of piperazine citrate and phenothiazine, 300-500 mgm per kilogram body weight significantly reduced worm number when tested against tissue phase A. galli. Differences in average lengths of worms recovered from groups treated with the lower piperazine citrate level and the piperazine citrate and phenothiazine combination were significant in groups sacrificed at 30 days post-infection, but not significant in groups sacrificed at 50 days post-infection.

Significant reduction in worm numbers occurred in the group which was treated against tissue phase A. galli with piperazine citrate, 12.5-20 mgm base per kilogram body weight, nicotine, 15-25 mgm per kilogram body weight, and phenothiazine, 150-250 mgm per kilogram body weight and sacrificed at 50 days post-infection. No significant reduction occurred in the group sacrificed at 30 days post-infection.

None of the drugs used in tests against tissue phase A. galli influenced weight gain of the experimental birds.

A combination of piperazine citrate, 35 mgm base per kilogram body weight, nicotine, 40 mgm per kilogram body weight, and phenothiazine, 430 mgm per kilogram body weight effectively removed adult A. galli. This level of piperazine removed 88 to 100 per cent of adult A. galli in another test as compared with 100 per cent efficacy of piperazine citrate levels of

60 to 70 mgm base per kilogram body weight. Addition of phenothiazine, 300-500 mgm per kilogram body weight to the lower piperazine citrate level did not increase anthelmintic activity of the piperazine.

Levels of piperazine citrate at 7.5, 15, and 30 mgm piperazine base per kilogram body weight were approximately 62 per cent more effective against adult A. galli than similar levels of piperazine derivative CL 16147. A combination of low level piperazine citrate and low level derivative resulted in a low increase in efficacy against adult A. galli.

Vermizym was not active against either tissue phase larvae or adult A. galli at levels of 0.5 to 1.0 gm per kilogram body weight. With respect to weight gains, there was no difference between groups treated with Vermizym and the non-medicated controls.

Treatment with piperazine dihydrochloride approximately equivalent to 1250 base per kilogram body weight at time of infection with H. gallinarum had no apparent effect on H. gallinarum larvae; however, this drug prevented development of typhlitis (abnormal ceca) in the treated birds. Treated groups had higher ratios of birds parasitized by H. gallinarum than did non-treated groups.

Piperazine dihydrochloride used in levels approximately equivalent to 600, 1250, and 2500 mgm base per kilogram body weight prevented development of abnormal ceca at the highest level and few at the lower levels, but differences in worm numbers of groups treated at these levels were not significant.

Abnormal ceca developed in groups treated with piperazine dihydrochloride and piperazine citrate at levels of 250, 625, and 1250 mgm base per kilogram body weight when the pre-infection treatment was omitted. Fewer abnormal ceca developed in the birds treated post-infection than in the non-treated controls. Differences in number of H. gallinarum recovered from

these groups were not significant.

Neither piperazine dihydrochloride nor piperazine citrate were found to be active at levels of 145 mgm piperazine base per kilogram body weight against adult H. gallinarum.

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Action of piperazine compounds on lumen and tissue phase larvae of Ascaridia galli (Schrank), a roundworm of chickens. Poult. Sci. (In press). 1957.

ACTION OF CERTAIN ANTHELMINTICS ON ASCARIDIA GALLI (SCHRANK, 1788) AND ON HETERAKIS GALLINARUM (SCHRANK, 1788)

by

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Ascaridia galli, the large roundworm of chickens, and Heterakis gallinarum, the cecal worm of chickens, are economically important especially when they are present in large poultry flocks. Under these conditions many birds are confined to a limited area and thus the probability of parasitic infection is increased.

The purpose of this investigation was to compare anthelmintic activity of a piperazine compound with the anthelmintic activity of other compounds against A. galli. The lowest effective dose of the piperazine compound was determined. Vermizym, a compound not related to piperazine, was also tested against A. galli. These drugs were tested against A. galli in four tests utilizing 628 chickens.

Two piperazine compounds were tested against H. gallinarum in three tests utilizing 408 chickens. Some reports indicate that these compounds may not be effective against the cecal worm, but laboratory controlled tests using piperazines against this parasite have not been reported.

Birds used in each test were separated into groups of approximately equal weight. Each bird was given 100± 10 A. galli or H. gallinarum eggs per os. Drugs were given orally either in tablet form or as a solution given with a calibrated micropipette.

In tests against tissue phase larvae of A. galli, nine daily drug treatments were given and in tests against H. gallinarum larvae, 10 treatments were given at the time of infection. In two tests against H. gallinarum, one pre-infection treatment and nine post-infection treatments were used. In the other, 10 post-infection treatments were given. The post-infection treatments were given from 1 to 32 hours post-infection. Only one treatment was used in tests against adult A. galli or H. gallinarum.

Average weight gains of birds and average lengths of worms recovered

were used as criteria of drug effects in the test using piperazine citrate and other drugs against tissue phase larvae of A. galli. In tests against adult worms, worm numbers were used as the criterion of drug effects.

Worms which had been expelled in the feces following treatment were recovered in tests against adult worms. Contents of the intestines or ceca were flushed out with water to recover worms which were not expelled. Results of the tests are as follows:

Piperazine citrate at levels of 25-40 mgm base per kilogram body weight and 50-80 mgm base per kilogram body weight did not reduce worm numbers in tests against tissue phase A. galli.

A combination of piperazine citrate at a level of 25-40 mgm base per kilogram body weight and phenothiazine, 300-500 mgm per kilogram body weight significantly reduced worm numbers in the test against tissue phase A. galli.

Average lengths of worms recovered from groups treated with the lower piperazine citrate level and the piperazine citrate and phenothiazine combination were significantly less in groups sacrificed at 30 days post-infection, but not significantly less in groups sacrificed at 50 days post-infection.

Significant reduction in worm numbers occurred in the group which was treated against tissue phase A. galli with piperazine citrate, 12.5-20 mgm base per kilogram body weight, nicotine, 15-25 mgm per kilogram body weight, and phenothiazine, 150-250 mgm per kilogram body weight and sacrificed at 50 days post-infection. No significant reduction occurred in the group sacrificed at 30 days post-infection.

None of the drugs used in tests against tissue phase A. galli influenced weight gain of the experimental birds.

A combination of piperazine citrate, 35 mgm base per kilogram body

weight, nicotine, 40 mgm per kilogram body weight, and phenothiazine, 430 mgm per kilogram body weight effectively removed adult A. galli.

A piperazine citrate level of 30-35 mgm base per kilogram body weight removed 88 to 100 per cent of the adult A. galli and a level of 60-70 mgm base per kilogram body weight effectively removed all adult worms.

Addition of phenothiazine, 300-500 mgm per kilogram body weight to a piperazine citrate level of 25-40 mgm base per kilogram body weight did not increase anthelmintic activity of the piperazine.

Levels of piperazine citrate at 7.5, 15, and 30 mgm base per kilogram body weight were found to be approximately 62 per cent more effective against adult A. galli than similar levels of piperazine derivative CL 16147.

A combination of low level piperazine citrate and low level piperazine derivative resulted in a low increase in efficacy against adult A. galli.

Vermizym was not active against tissue phase larvae and adult A. galli at levels of 0.5-1.0 gm per kilogram body weight. In respect to weight gain, there was no difference between birds treated with Vermizym and the non-medicated controls.

Treatment with piperazine dihydrochloride approximately equivalent to 1250 mgm base per kilogram body weight at time of infection with H. gallinarum had no apparent effect on H. gallinarum larvae; however, this drug prevented development of typhlitis (abnormal ceca) in the treated birds. Treated groups had higher ratios of birds parasitized by H. gallinarum than did non-treated groups.

Differences in number of H. gallinarum recovered from groups treated with piperazine dihydrochloride approximately equivalent to 500, 1250, and 2500 mgm base per kilogram body weight at time of infection were not significant, but as the drug levels for the groups decreased, the number of birds

with abnormal ceca increased.

When the pre-infection treatment was omitted, abnormal ceca developed in the groups treated with piperazine dihydrochloride and piperazine citrate at levels of 250, 625, and 1250 mgm base per kilogram body weight. Fewer abnormal ceca developed in the birds treated post-infection than in the non-medicated controls.

Differences in number of H. gallinarum recovered from groups treated at various levels of piperazine dihydrochloride or piperazine citrate were not significant.

Neither piperazine dihydrochloride nor piperazine citrate were found to be active at levels of 145 mgm base per kilogram body weight against adult H. gallinarum.