THE EFFECTS OF CARICIDE AND OTHER ANTHELMINTICS ON THE TISSUE PHASE LARVAE OF ASCARIDIA GALLI (SCHRANK, 1788)

by

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A. B., The College of Wooster, Wooster, Ohio, 1951

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

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INTRODUCTION

The use of anthelmintic agents to control parasite damage in domestic animals probably originated in ancient times. The need for combating helminth infections was recognized long before it was possible to accurately evaluate the more covert implications of host-parasite relationships. Certainly, the dearth of effective antiparasitic substances during the past has limited the extent to which many animal diseases could be controlled.

In recent years, the synthesis of many new chemical compounds has made available a much larger selection of drugs with anthelmintic potentialities. An important part of the applied research in the field of parasitology has been directed toward the testing of these compounds with the use of experimental animals maintained under controlled laboratory conditions. Various aspects of Ascaridia galli infections in chickens have received considerable attention from a number of workers. At the present time, nicotine compounds are considered to be the drug of choice for this nematode. However, Hansen et al. (1954c) showed that, while this compound was effective at some levels in removing adults and the older larval stages, it was relatively ineffective against the young migratory larvae. Since the studies of Ackert (1923), Guberlet (1924), Ackert and Herrick (1928) and Todd et al. (1949b) had indicated that the greatest host injury due to A. galli was incurred during the first 21 days after infection with embryonated ova, the primary objective of this study was to determine the effect of several anthelmintics on this parasite during this deleterious larval period in its life cycle.

REVIEW OF LITERATURE

The use of compounds of piperazine as anthelmintics was first reported in 1947. At that time, as the result of extensive screening of a variety of drugs in the search for an effective antifilarial agent, the relative effectiveness of the compound was originally recognized. The search for such a substance had been initiated during World War II, when the necessity for maintaining our armed forces in endemic areas had emphasized the lack of a suitable compound for use in human filariasis (Kanegis, 1948). Among the major studies concerning piperazine were those of Hewitt and his associates (1947a, 1947b, 1947c, 1948a, 1948b) in which they experimentally evaluated the antiparasitic action of approximately 67 piperazine compounds, using the cotton rat and the dog as laboratory hosts. As a result of these tests, 49 of the compounds tested were found to have no filaricidal activity, and further testing of them was abandoned. Eighteen of the piperazine derivatives showed some degree of anthelmintic activity; of these, 1-carbethoxy-4-methylpiperazine was effective against microfilariae of Litomosoides carinii in the cotton rat. However, in other experiments against Dirofilaria immitis infections in dogs, the drug was found to be toxic at effective levels. The symptoms shown by the treated dogs were nausea, muscular weakness, salivation, and prostration. A closely related substance, 1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate[1], appeared to be more promising, not only because of its effectiveness in removing worms, but also because of its lower toxicity.

As a result of these preliminary studies, further research was undertaken on the toxicological, chemical, and pharmacological properties of certain of

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[1]Commercially available from Lederle Laboratories Division, American Cyanamid Company, as Hetrazan or Caricide.

the more promising piperazine compounds. Kushner (1948) reported on the chemical structure of the piperazines in general, and attempted to relate their molecular structure with their efficiency as chemotherapeutic agents. Harned et al. (1948a, b) studied the pharmacodynamic aspects of Hetrazan. The chemical preparation of piperazine derivatives was described by Stewart et al. (1948).

Subsequent to the preliminary laboratory trials in which the effectiveness of Hetrazan was demonstrated for filarial infections in the cotton rat and the dog, further tests were conducted which indicated its probable application in the treatment of a variety of helminth infections in other hosts. Hewitt et al. (1948b) successfully treated dogs experimentally infected with ascarids, and Kanegis (1948) reported similar results after treating Ascaris-infected cats and dogs with Caricide. Both papers reported that the drug approached 100 per cent efficacy in worm removal when administered at therapeutic levels. In another study, Oliver-Gonzalez and Hewitt (1947) investigated its effectiveness against trichinosis in white rats. Colglazier and Ensie (1951) observed that Caricide removed 98 per cent of the ascarids from 11 cats which they treated. Guilhon and Groulade (1951), working with Toxacara canis and T. mystax infections in dogs and a cat, respectively, found that Hetrazan treatments at optimum dosage levels were effective in reducing fecal egg counts. In a comprehensive study, Brown et al. (1954) evaluated the activity of 32 piperazine compounds against Syphacia obvelata, a mouse pinworm. Their results with Hetrazan indicated that it was ineffective against Syphacia in doses of 250 mgm/kg host body weight for two days. In another study, Guilhon (1951) described his work on experimental infections of Ascaridia columbae in pigeons. He found a complete absence of eggs in the feces resulted after repeated doses of a 3-5 per cent sugar solution of piperazine.

The use of Caricide as a treatment for Ascaridia galli, the large intestinal roundworm of the fowl, has been reported two different times in the literature. (Table 1). In 1950, Riedel published the results of a study from which he concluded that a low level of anthelmintic efficiency in addition to substantial host toxicity rendered Caricide undesirable for treating A. galli infections in chickens. In one series of tests, 55 Rhode Island Red Chickens were divided into three groups of approximately equal size. The first group constituted an untreated control. The second group was treated once with 1 gm of Caricide per bird, and Group III birds were given a 1 gm dose, then redosed with 0.5 gm four hours later. Results were as follows: Group I birds eliminated 0.75 per cent of their worms; Group II, 36.5 per cent; and Group III, 69.4 per cent. There was no appreciable difference in average weight gained per chicken in any of the three groups. In another series of tests in which dosages and related treatment techniques were varied, the best results were obtained by using a large initial dose of Caricide followed by a four hour fast, then a second dose of Caricide, with a purgative added to the feed after treatment. However, this treatment regime was considered unsatisfactory due to a marked weight loss sustained by the treated birds coupled with poor results in removal of worms.

The erratic results obtained in these earlier tests with single and double dose treatments administered individually prompted Riedel (1951) to study Caricide's anthelmintic effect when added to the feed mixture of test birds for an extended period of time. The drug was added in proportions of 0.25 per cent, 0.5 per cent, 1.0 per cent, and 2.0 per cent to the feed of four groups of chickens for seven consecutive days. Reduction in worm burden resulting from this type of treatment was as follows: those birds receiving 0.25 per cent Caricide feed ration eliminated 12.0 per cent of the Ascaridia

harbored; those given 0.5 per cent eliminated 28.6 per cent; those birds on the 1.0 per cent mixture passed 50.0 per cent of their worms; and a 2.0 per cent concentration was 81.0 per cent effective in reducing worm numbers. The level of infection remained constant in a group of control birds.

In another test in which 30 heavily infected chickens received a feed mixture containing 1 per cent Caricide for a two-week period and were purged with Epsom Salts at the end of the first and second weeks, 89.2 per cent of the worms were removed. In 30 unpurged but otherwise similarly treated birds the percentage of worm reduction was 72. In 15 untreated and unpurged controls the percentage of infection remained constant.

Concurrent with the recognition of the potential value of Caricide as an anthelmintic in certain animals, investigations were undertaken to evaluate its application to helminth infections in man. Santiago-Stevenson et al. (1947) reported a high rate of worm removal after treating six ascarid-infected patients with Hetrazan. On the other hand, Colbourne (1950) considered the results that he obtained with Hetrazan in treating 24 cases of ascariasis to be unsuccessful. In 24 additional cases which were treated with Oil of Chenopodium, he obtained a higher rate of cure. Etteldorf and Crawford (1950) published the results of a clinical study on the value of Hetrazan in treating children for Ascaris lumbricoides. Corcos et al. (1951) reported a complete cure in 20 of 22 cases of ascariasis, and concluded that Hetrazan was particularly effective against that type of infection. Similarly, Loughlin et al. (1951) estimated that Hetrazan had achieved a 91-94 per cent reduction in Ascaris infections in their patients. Hoekenga (1951) reported no toxic effects with varying dosages of Hetrazan, but of 15 patients that had received what he considered to be the optimum dosage three times a day for three days,

only 9 were cured. A later study of his (1952) likewise indicated this piperazine compound was ineffective in removing ascarids in man. However, Singh et al. (1952), using fecal egg counts as an index of infection, found that Hetrazan treatment reduced the number of ova in the feces of 7 infected children by 98 per cent. Colbourne (1952) reported skin irritation in 25 per cent of the ascariasis cases which he treated with Hetrazan in syrup form. In a preliminary report, Mojumdar and Biswas (1953) observed that an eight-day treatment regime had resulted in worm removal in 5 of 6 patients, but usually not until on or after the fifth day. While reporting toxic reactions in many patients following the administration of Hetrazan at a dosage level of 10 mgm per kg of host body weight, Brumpt and Sang (1954) concluded that it was effective against A. lumbricoides. Elsewhere in the literature, Ghanem (1954) reported that Hetrazan was not appreciably toxic to 125 patients with which he worked. A cure in 7 of 10 cases of ascariasis was reported by Basnuevo and Fontao (1954). Brown and Sterman (1954) considered Hetrazan to be highly effective against Ascaris lumbricoides when administered at specified dosage rates on seven consecutive days. Another worker, Blum-Gayet (1954) reported cures in 16 of 20 cases of ascariasis after one treatment with Hetrazan, and in 2 more cases following a second treatment.

The demonstration in recent years of the value of Hetrazan (Caricide) as a medical and veterinary anthelmintic has been reported in numerous publications, some of which have been cited in this review of literature. More recently the attention of some workers in this field has been turned toward the testing of other compounds of piperazine. Davies et al. (1954) tested some forty piperazine derivatives for activity against nematodes in various domestic animals. Their most extensive work involved the in vivo screening of piperazine adipate in dogs, cats, pigs, equines, and poultry. Their report

of the effects of this compound in treating 109 cockerels infected with Ascaridia galli revealed that, when incorporated in the feed mixture, it was 97 per cent effective in worm removal. These treatments were carried on for periods varying from one to three days. Single dose treatments administered orally by capsule varied in efficiency from 73 to 100 per cent. Leiper (1954) reported that he was able to remove all of the Ascaris from experimental pigs which he treated individually with polymeric piperazine-1-carbodithioic acid. Field testing of this drug was then carried out to check the apparent activity demonstrated under artificial conditions in the laboratory. Results there indicated that in a wet or dry mash feed mixture, at dosage levels of 100-150 mgm/kg body weight, this drug resulted in 83-100 per cent reduction in egg counts in the treated animals.

The use of piperazine hydrate as a medicinal for ascariasis in man was reported by White (1954). All three cases which he treated resulted in complete disappearance of Ascaris ova from the feces.

The use of carbon disulfide for Ascaridia galli infections in chickens has been reported by Roberts (1937), and by Knapp and Hansen (1954). Roberts treated three birds ranging in weight from one to two pounds with individual oral doses of 0.3 ml of carbon disulfide in gelatin capsules. Treatment was preceded by 17 hours of fasting. This procedure resulted in the removal of 85.7 per cent of the total worms harbored by the three birds, but was accompanied by definite toxic symptoms lasting up to five days after treatment.

The work of Knapp and Hansen (1954) consisted of four experiments with a total of 155 artificially infected chickens. In each test carbon disulfide was administered orally after a 12 hour fast. 0.3 ml of anthelmintic per bird removed 88 per cent of the ascarids. However, this treated group showed an average weight loss of 29.3 gms per chicken, after medication. A second

group received 0.6 ml of carbon disulfide per chicken. This dosage was 100 per cent effective in worm removal, but resulted in 33.3 per cent host mortality, and an average weight loss of 88.8 gms per chicken in those that survived the test. A third dosage level of 0.15 ml per chicken reduced the worm burden of the group by 65 per cent and a normal weight increase followed treatment. In vitro tests with adult A. galli indicated that gaseous carbon disulfide in an enclosed tube was fatal to the test worms in doses as small as 0.05 ml.

Table 1. Previous experimental tests with Caricide for Ascaridia galli infections in chickens.

Number of Chickens Used in Experiment	Caricide Dosage	Percentage of Worms Eliminated	Toxic Symptoms	Reference
15	0 gms (control)	0.75	none	Riedel (1950)
20	1 gm/bird	36.5	host weight retarded	"
20	1 gm/bird, plus 0.5 gm redose	69.4	host weight retarded more severely	"
5	1 gm/bird	13.3	host weight retarded somewhat	"
5	1 gm/bird after overnight fast	8.2	"	"
5	1 gm/bird after overnight fast, then 0.5 gm per bird 4 hrs. after first dose	7.5	"	"
5	1 gm/bird, then 0.5 gm/bird 4 hrs. after first dose, then $MgSO_4$ in feed 6 hrs. after second dose	43.5	severe loss of weight	"
40	0.25% concentration in feed for 1 week	12	none	Riedel (1951)
40	0.50%	28.6	none	"
40	1.0%	50.0	none	"
40	2.0%	81.0	none	"
40	5.0%	0	unpalatable	"
15	0 (control)	0	-	"
30	1% for 2 weeks with purge at end of first and second weeks	89.2	-	"
30	1% for two weeks	72.0	-	"

MATERIALS AND METHODS

Anthelmintics

In the present study the following three anthelmintics were used: Caricide or Hetrazan (1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate), Compound 180-C (1-carbethoxy-4-methylpiperazine hydrochloride), and carbon disulfide. The Caricide (Hetrazan) and Compound 180-C were obtained from the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y. Caricide was supplied in two forms: tablets, for the individual treatment of experimental animals; and as a dry powder, for addition to the test birds' feed ration. In all experiments, the drugs were administered per os. The dosage level and method of administration varied from test to test, as follows: In Test 1, a single dosage of 25 mgm of Caricide per bird was used; in Test 2, 12.5 mgm of Caricide was given in tablet form for eight consecutive days; Test 3, approximately 12.5 mgm of Caricide per bird, as a powder incorporated in the feed mixture; Test 4, an aqueous solution of 25 mgm of Compound 180-C per bird for eight consecutive days; and Test 5, a single dose of 0.05 ml of carbon disulfide administered in No. 4 gelatin capsules. The capsules were individually filled with reagent grade carbon disulfide with the aid of a tuberculin syringe, and were stored at 38° F. until used. Dosage rates, method of administration, and length of treatment with the various drugs tested are summarized more completely in succeeding sections of this thesis.

Toxicity

Riedel (1950, 1951) has reported some toxic effects of Caricide on chickens when administered at certain dosage levels. His work with birds averaging from 750 to 1050 gms in weight indicated that single or double doses of 0.5 gm

of Caricide per bird, or less, had no detrimental effects on the host. However, single or repeated 1.0 gm doses resulted in either a weight loss or failure to make normal weight gains. Other observable symptoms were loss of appetite, incoordination, droopy wings, mucoid droppings, and listlessness in the treated birds. Autopsies of birds exhibiting these tendencies in some cases revealed a dehydrated condition of the tissues, distended kidneys, and the crop filled with mucus. Riedel's report on group treatment experiments (1951) indicated that 0.25, 0.50, 1.0, and 2.0 per cent concentrations of Caricide were palatable to eight-week old chicks, but that a 5 per cent mixture was both toxic and unpalatable.

No visible toxic effects were noticeable in the present tests with Caricide or Compound 180-C at the levels which were used. In some cases, however, a slight initial decrease in feed consumption was noted in tests in which the medication was mixed with the feed mash.

Roberts (1957) observed that considerable distress and lack of appetite resulted following treatment of three 1.0 to 1.5 pound birds with 0.3 ml of carbon disulfide. In the present experiment, some transient symptoms of toxicity were evident immediately following the administration of 0.05 ml of this compound. These symptoms included gasping, listlessness, and inactivity for several hours subsequent to treatment. There were no permanent weight losses resulting from treatment with any of the three anthelmintic substances.

Mode of Action

In seeking an explanation for the variability in anthelmintic efficacy exhibited by Caricide (Hetrazan) when used in a number of different host species for a variety of parasitic infections, several factors must be considered. Most of them, admittedly, are merely speculative at the present

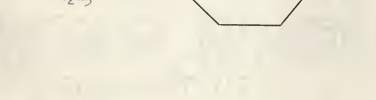
time. They may be considered under three broad categories: factors relating to the host, factors relating to the parasite, and factors inherent in the drug.

Within the first category might be included such variables among host species as age, diet, body temperature, and general physiological variations from one species to another. The potential role of natural and acquired immunity in host resistance to parasitism may play an important part in determining whether or not the host ultimately becomes infected. This applies to experimental infections as well as to those contracted through natural circumstances. The work of Ackert and his associates (1931a, 1931b, 1932, 1933a, 1933b, 1935a, 1935b, 1935c) provides considerable evidence in support of this idea. The combined influence of immunological factors, and of diet as indicated by Hansen et al. (1933) may help to explain the wide variation in infection within treated groups of experimental animals which received a constant number of infective ova and equal amounts of an anthelmintic.

Under factors relating to the parasite, such variables as morphological variation within the different species of ascarids, nutritional fluctuations, and differences in life cycles should be considered. Worm size may be important in that it would influence the amount of surface area which would be exposed to the toxic action of the anthelmintic. In previous studies Hetrazan therapy was directed against adult Ascaris lumbricoides residing in the lumen of the host's intestine, whereas, the present tests were directed against Ascaridia larvae during a stage of their life cycle in which they were partially imbedded in the mucosa of the intestine. This habitat would seem to render the parasite less accessible to the action of the anthelmintic. In the instances where Hetrazan was tested against filarial worms, the parasite was located in the circulatory or lymphatic system of the host, and the action of

the drug depended, among other things, upon its absorbtion into the circulation. Hetrazan is highly soluble in water (Harned et al., 1948a, b), which may partially account for its proven effectiveness against filarial parasites. Likewise, a high degree of solubility may also contribute to its lack of ability to act against worms whose habitat is the lumen of the intestine. Accordingly, a relatively insoluble compound would remain longer and exert a prolonged toxic effect on the worms in that situation (Chopra and Chandler, 1928). This idea was also propounded by Hall and Shillinger (1924), after tests of a large number of anthelmintics. They reported that an increase in drug solubility was almost always accompanied by a diminished anthelmintic efficacy. Woodruff (1951), by comparing liver biopsies made before and after Hetrazan treatment for Loiasis, concluded that the microfilariae of this nematode were removed from the blood stream and phagocytized in the liver as a result of this therapy. Johri (1953), while investigating the effect of Hetrazan on A. lumbricoides in children, found that treatment resulted in paralysis of those worms which were expelled.

Among the factors inherent in the nature of the drug that might influence its mode of action would be its chemical properties, and such physical characteristics as solubility, volatility, and osmotic properties. Caricide is known chemically as 1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate, and its structure is represented graphically as follows:



After conducting in vivo tests in cotton rats and dogs, Hewitt et al. (1947a, b, c) stated that the piperazine nucleus alone showed no anthelmintic activity. Therefore, at least against those parasites for which he conducted tests, the activity appears to be due to the side chain groups attached to the nucleus. That the HCl group (located in Caricide on the end of the chain attached to position 1) may be of some importance has been postulated by several workers. Caius and Mhaskar (1923), in attempting to correlate the chemical composition and therapeutic value of some anti-hookworm drugs, observed that the value of carbon tetrachloride was related to the cumulative effect of the halogen fraction of the compound. Hall and Shillinger (1924) stated that the apparent efficacy of both carbon tetrachloride and ethylene dichloride was due to their halogen concentration. Likewise, Wright and Schaffer (1932) observed that as the halogen content of certain drugs increased, so increased the anthelmintic efficacy.

A further indication that the anthelmintic activity of Caricide resides in the side chain is found in work reported by Doak and Eagle (1951). In correlating the chemical structure and biological activity of some arsenobenzenes, they reported that the activity and toxicity of the substituted compounds tested depended upon the nature of the terminal groupings of the side chains. The length of the chains did not influence their anthelmintic activity.

Available experimental evidence, therefore, seems to indicate that the effectiveness of Hetrazan (Caricide) for some types of parasitic infection is due in part to its high solubility, and to the presence of the side chain terminating in a halogen compound.

Experimental Animals

The chickens used in the tests were obtained from an approved commercial hatchery as non-sexed day old birds. This precluded the likelihood of any outside source of Ascaridia infection. White Rocks were used in the Caricide experiments, and New Hampshires in Tests 4 and 5. On receipt at the laboratory, the chicks were vaccinated (intranasally, or with an additive in the drinking water) for Newcastle's disease, and were placed in electrically heated brooder batteries. At fourteen days of age they were weighed, wing-banded, and divided into groups of approximately equal weight by the technique of Gardiner and Wehr (1950). This facilitated maintaining the different groups of birds on approximately equal daily rations of food and water. A standard commercial feed in mash and pellet form was used in the first two tests, and a mash-type ration exclusively in the remaining work. After the chicks were experimentally infected, daily records were kept of their weights in grams. An attempt was made to weigh the birds at about the same time each day, so as to obtain an accurate indication of the day-to-day gains.

Infection Technique

In all five tests, the method of preparing Ascaridia egg cultures was similar to that described by Ackert (in Needham et al., 1937, p. 171). This technique consisted essentially of excising the anterior end of live female ascarids, and withdrawing the internal organs from the worm. After separating the uteri from the other viscera, only the portion of the uterus proximal to the genital pore was retained as an egg source. This seemed to increase the proportion of viable ova. These uterine sections were transferred to a petri dish filled with tap water, and the uteri dissected to allow the eggs to

spread out into the water. The covered petri dishes containing the egg cultures were maintained in a standard laboratory incubator at 28° C. for at least two weeks before being used. All cultures older than four weeks were considered to have passed the point of maximum viability, and were discarded. A variation in the Ackert technique for preparation of ova for incubation was followed in Test 4. After separation of the uteri from the worm in the usual manner, an artificial digestive juice containing 0.5 per cent HCl and 1 per cent pepsin was used to remove the remnants of uteri from the culture fluid. Since this digestion process removed immature eggs as well as uterine remnants (Hansen et al. 1954b), it facilitated the preparation of cultures with a high percentage of viable ova. Following incubation, the preparation of a stock solution of Ascaridia ova for administration to the chickens was patterned after another technique developed by Hansen et al. From the egg culture a random quantity of incubated ova was pipetted into a straight-sided shell vial containing 15 ml of water. After thoroughly agitating this suspension, one drop of fluid was removed with a calibrated pipette and examined under a compound microscope. When the addition of sufficient ova from the original culture to the shell vial had resulted in a count of 33 embryonated ova per drop of stock fluid from the vial, enough sucrose was added to the 15 ml egg suspension to form a 1.25 Molar solution. A sugar solution of this concentration gave the most permanent egg suspension in the tests conducted by Hansen et al. Their work in this connection had indicated that a water-egg suspension method of administering Ascaridia ova tended to result in an increase in the eggs per dose as the volume of the suspension decreased. Sugar-egg suspensions, in their tests, resulted in a relatively constant rate of infection regardless of the order in which the birds were infected. The same pipette was used for administering the infective ova to the birds that had

been used in preparing the stock solution. As an additional precaution to prevent any bias that might have arisen in spite of this infection technique, administration of the ova was alternated from a treated to an untreated group of birds. All infective eggs were given per os, in doses of either 75 ± 5 or 100 ± 10 infective ova.

Recovering Worms

In each experiment, the chickens were infected at fourteen days of age. From that point, two variations were followed in test procedure. In Tests 1, 2, and 3, two treated (Group A) and two infected but untreated (Group B) birds were sacrificed every 24 hours from the eleventh day post-infection until day 25 post-infection. This procedure was followed to test the action of the anthelmintic during the progression of the tissue phase in the Ascaridia life cycle. The pioneer work of Ackert (1925) on the life history of this worm established the fact that it went through a semi-migratory stage later designated as the "tissue phase", during which it buried its head in the mucosa of the intestine. Ackert and Herrick (1928), after additional work, discovered that the time at which the most injury to the host resulted was centered on about the fourteenth day after experimental infection. Ackert et al. later (1931) more definitely delimited this tissue phase as extending from the tenth to the seventeenth day after infection.

On the twenty-fifth day post-infection, an entire group of treated (Group C) and an equal number of control birds (Group D) were sacrificed. This procedure was established to ascertain the effect of the anthelmintic on the post tissue phase larvae which at that time theoretically would be located in the lumen of the small intestine.

The alternative experimental schedule carried out in Tests 4 and 5 was

followed as a result of information acquired during the earlier experiments. After reports of the first three tests were tabulated, it was evident that any significant activity of the anthelmintic being tested had been best indicated by the results obtained in Groups C and D, and that the information theretofore obtained from Groups A and B had been quite limited. Therefore, in Tests 4 and 5, the use of experimental groups A and B was tentatively eliminated, until the activity of the anthelmintic on the later stages of the larvae could be evaluated. Thus, only one treated and one control group of chickens were utilized in the last two tests. All birds were sacrificed twenty-one days after infection.

The methods employed in autopsying chickens to enumerate their worm burden were essentially the same as those described by Hansen et al. (1954c). A longitudinal incision was made through the abdominal wall of freshly killed birds. Through this opening the portion of the small intestine extending from the gizzard posteriorly to the remnant of the yolk sac diverticulum was excised with scissors and removed to a pint glass jar. (Ackert, 1951, considered this region to be the usual habitat of A. galli). The removal of worms contained within the intestinal sections was then accomplished by the flushing technique described by Ackert and Nolf (1929). Water under pressure from a rubber tube was directed into one end of the intestine through a small nozzle. This flushing action resulted in the washing of all unattached ascarids (i.e., those free in the lumen) from the intestine into the glass jar. In experiments 1, 2, and 3, in which a count of the tissue phase larvae was desired, a second procedure was followed, after flushing. Each section of intestine was cut into small pieces and placed in an individual quart glass jar containing about 600 ml of artificial digestion medium (0.5 per cent HCl and 1.0 per cent pepsin). This method for recovery of the tissue phase

larvae of Ascaridia was originated by Tugwell and Ackert (1952). The jars were then immersed in a water bath at 38° C. for about five to eight hours, with continual agitation. A modification of the apparatus used by Hansen et al. (1954c), with glass stirring rods driven electrically from rubber V-belts (Fig. 1) was utilized to facilitate the digestion process.

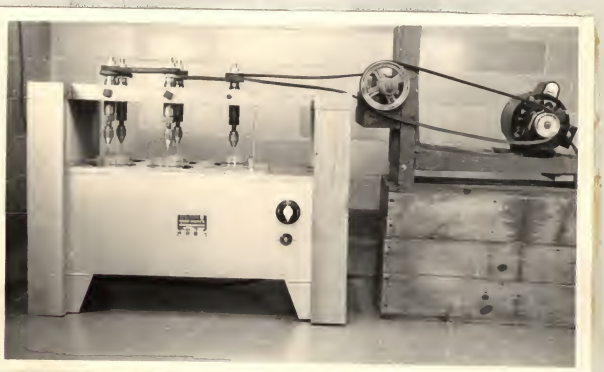


Fig. 1. Artificial digestion apparatus for recovering tissue phase larvae of Ascaridia galli from the walls of chicken intestines.

After digestion of the intestines was completed, the residue and digesting fluid remaining in the container with the worms was removed in this manner. The jars were removed from the water bath and were filled to the top with water, thus diluting the fluid present. After a short wait to allow the worms (which had a higher specific gravity than the detritus) to settle to the bottom of the jar, a J-shaped glass tube attached to an aspirator was

used to remove the supernatant fluid from the top of the jar. Three or four repetitions of this operation resulted in a clear, concentrated fluid containing the larvae which had been imbedded in the walls of the digested intestine. To both the flushed and digested specimen jars was added enough 10 per cent formalin to preserve the worms.

Counting and Measuring Worms

In counting the number of ascarids recovered, a wide-field binocular microscope was used to examine the contents of each jar. The preserving fluid containing the worms was poured, a little at a time, into a petri dish which had a specially designed grid on the bottom dividing it into a series of microscope fields. A fine dissecting needle was used to transfer the worms recovered under the microscope to screw-cap specimen vials containing 10 per cent formalin. A Veeder counter was used to record the number of ascarids as they were recovered.

Since worm length was considered as one criterion of the effect of the anthelmintic on worms exposed to it, measurements were taken of the lengths of samples of all worms recovered. This was done with the aid of a Leitz projection apparatus somewhat similar to a darkroom enlarger. The worms to be measured were placed on a 2" X 3" glass slide on a stage on which the lens of the viewer was focused. A magnified image of the worm was then projected onto a ground glass plate. Tracings of the enlarged images of the worms were made on onionskin paper, at a magnification of eight times. A Dietzgen planimeter was used to measure the length (in centimeters) of the traced lines, and conversion of this number gave the actual worm length.

EXPERIMENTAL RESULTS

Test 1

Each treated bird in this test received a single 25 mgm dose of Caricide. Beginning on the eleventh day after treatment, and continuing for ten days thereafter, two Group A and two Group B chickens were killed for autopsy each 24 hours. Groups C and D were sacrificed on the eleventh day after treatment. Results are recorded in Tables 2 and 3, and in Fig. 2.

An average of 2.2 tissue phase larvae and 8.6 lumen larvae were recovered from Group A birds, and an average of 2.9 tissue phase and 8.2 lumen larvae from Group B chickens. No statistically significant[1] differences existed between the numbers of worms recovered.

Group C birds contained, on the average, 9.05 lumen larvae, compared to 5.7 for Group D. This difference was not significant.

A random sample of worms recovered from Group A chickens indicated that they averaged 0.35 cm in length, compared to an average length in the Group B birds of 0.66 cm. However, in view of the fact that the variation between worm lengths from Group C and D hosts was not significant, the end effect of the Test 1 Caricide dosage on worm length is still obscure.

The average terminal weight (i.e., the final weight before sacrifice) of Group C chickens was 268.5 gms, compared to an average of 248.6 gms for Group D birds. This variation was not significant.

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[1] A level of significance of 5 per cent or less was considered to be of statistical significance in analyzing the data reported in this thesis.

Table 2. Results of daily examinations of chickens receiving a single 25 mgm dose of Caricide and their untreated controls.

Group	Chicken Number	Number of Days Post-Infection When Examined	Worms Recovered: Tissue Phase Number	Lumen Number	Lumen Length
	598	11	18	18	
	624	11	4	24	
	608	12	4	7	
	615	12	6	11	
	643	13	0	6	
	593	13	3	5	
	620	14	0	12	
	616	14	0	3	
A	599	15	4	18	
(Treated)	600	15	4	5	——
	646	16	0	6	
	570	16	0	0	
	601	17	0	0	
	564	17	0	28	
	596	18	0	4	
	559	18	0	13	
	626	19	0	2	
	618	19	0	0	
	625	20	0	2	
Total:			43	164	
Average:			2.2	8.6	0.33 cm
	629	11	11	4	
	630	11	13	33	
	611	12	2	8	
	638	12	6	22	
	578	13	15	12	
	621	13	3	17	
	554	14	2	4	
	617	14	0	19	
B	592	15	1	5	
(Control)	563	15	3	4	——
	675	16	1	10	
	552	16	0	0	
	642	17	0	7	
	567	17	0	2	
	647	18	1	6	
	622	18	0	4	
	568	19	0	1	
	674	19	0	3	
	595	20	0	2	
Total:			58	164	
Average:			2.9	8.2	0.66 cm

Table 3. Comparative data on weight gained after treatment and numbers and a statistical estimate of the lengths of lumen larvae recovered from a treated group of chickens and its untreated control eleven days after infection.

Group	Chicken Number	Weight Increase After Treatment Date (Gms)	Lumen Larvae Recovered Number	Length
C (Treated)	550	161	12	
	553	128	8	
	556	155	2	
	561	92	41	
	562	109	5	
	576	115	5	
	580	126	6	
	585	106	6	
	586	140	5	
	603	132	10	——
	604	107	8	
	609	112	4	
	610	123	1	
	623	119	18	
	632	142	1	
	644	104	14	
	649	88	8	
Total:		2059	154	
Average:		121.1	9.05	0.24 cm
D (Control)	551	102	10	
	557	88	6	
	558	77	5	
	565	96	1	
	569	160	4	
	573	110	8	
	575	116	1	
	582	130	7	
	584	73	10	——
	588	87	10	
	589	86	6	
	594	90	3	
	606	76	8	
	627	95	12	
	633	54	1	
	641	102	1	
	650	110	5	
Total:		1652	98	
Average:		97.1	5.7	0.21 cm

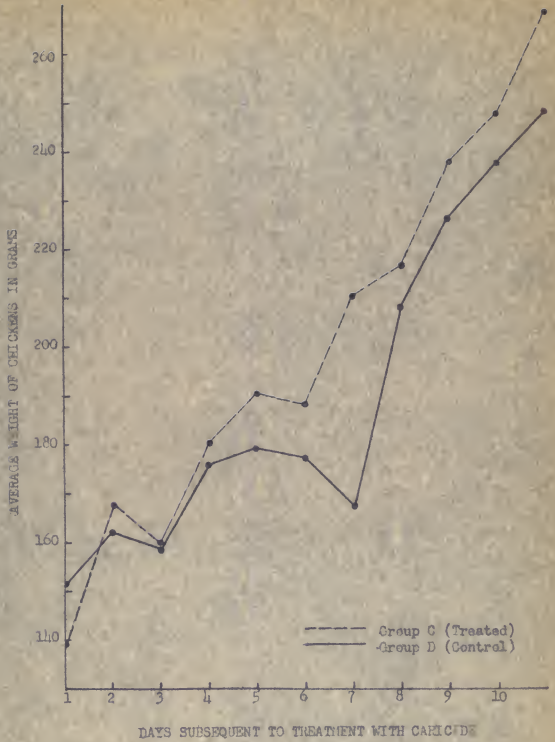

Fig. 2. Comparison of growth curves of chickens receiving a single 25 mgm dose of Caricide and their untreated controls.

Test 2

The treatment schedule for this test consisted of 12.5 mgm of Caricide given for eight consecutive days beginning the fourth day post-infection. Two Group A and two Group B birds were killed each twenty-four hours over a ten-day period beginning the eleventh day post-infection. Groups C and D were sacrificed the eleventh day after treatment. Results are summarized in Tables 4 and 5, and in Fig. 3.

Group A birds contained an average of 3.5 tissue phase larvae and 12.2 lumen larvae, while Group B birds were infected, on the average, with 4.3 tissue phase and 10.3 lumen larvae. No significant differences existed here.

Group C chickens were infected with an average of 6.3 lumen larvae compared to 6.1 worms per bird in Group D. This difference was not significant.

Ascarids recovered from Group A hosts averaged 1.48 cm in length, compared to an average worm length of 0.39 cm in Group B birds. This variation was significant at the 1 per cent level. Group C chickens carried worms that averaged 1.0 cm in length, compared to an average of 0.29 cm in the chickens comprising Group D. This degree of variation was also significant at the 1 per cent level.

Terminal weights of Group C and Group D birds were 309.8 gms and 294.1 gms, respectively. No significant variation existed here.

Table 4. Results of daily examinations of chickens receiving 12.5 mgm of Caricide for eight consecutive days and their untreated controls.

Group	Chicken Number	Number of Days Post-Infection When Examined	Worms Recovered: Tissue Phase Number	Worms Recovered: Lumen Number	Worms Recovered: Lumen Length
A (Treated)	753	11	12	40	
	702	11	9	36	
	739	12	0	6	
	735	12	5	23	
	751	13	23	16	
	756	13	14	10	
	787	14	1	4	
	712	14	0	10	
	709	15	0	5	
	717	15	1	0	
	800	16	3	13	—
	797	16	1	3	
	801	17	0	4	
	755	17	0	2	
	776	18	0	7	
	719	18	0	54	
	765	19	1	4	
	699	19	0	1	
	706	20	0	4	
	766	20	0	3	
Total:			70	245	
Average:			3.5	12.2	1.48 cm
B (Control)	723	11	10	22	
	708	11	16	21	
	793	12	5	33	
	768	12	4	9	
	808	13	15	3	
	746	13	12	12	
	789	14	13	7	
	806	14	0	0	
	745	15	1	2	
	715	15	0	13	
	731	16	5	7	—
	730	16	0	7	
	743	17	1	13	
	713	17	2	20	
	761	18	1	5	
	805	18	1	4	
	771	19	0	23	
	781	19	0	1	
	741	20	0	1	
	803	20	0	3	
Total:			86	206	
Average:			4.3	10.3	0.39 cm

Table 5. Comparative data on weight gained after treatment and numbers and a statistical estimate of the lengths of lumen larvae recovered from a treated group of chickens and its untreated control twenty-one days after infection.

Group	Chicken Number	Weight Increase After Treatment Date (Gms)	Lumen Larvae Recovered Number	Length
	703	125	1	
	704	108	17	
	705	78	6	
	707	143	4	
	714	90	6	
	718	158	0	
	727	131	6	
	728	132	22	
	734	151	5	
	740	154	1	
C	747	88	14	
(Treated)	757	114	11	——
	772	147	2	
	773	82	5	
	778	150	7	
	780	166	7	
	782	174	7	
	786	99	0	
	788	176	4	
	796	103	8	
	799	147	1	
	804	108	5	
Total:		2824	139	
Average:		128.3	6.3	1.00 cm
	710	96	6	
	711	93	7	
	716	104	24	
	721	123	15	
	724	94	11	
	725	125	0	
	726	124	8	
	732	108	9	
	737	128	4	
	742	102	0	
D	750	121	0	
(Control)	754	151	13	——
	758	108	7	
	759	148	0	
	760	112	9	
	762	116	3	

Table 5. (Cont')

Group	Chicken Number	Weight Increase After Treatment: Date (Gms)	Lumen Larvae Recovered: Number	Length
	764	110	1	
	774	130	4	
	775	141	5	
	791	105	5	
	794	138	1	
	748	110	2	
Total:		2587	134	
Average:		117.5	6.1	0.29 cm

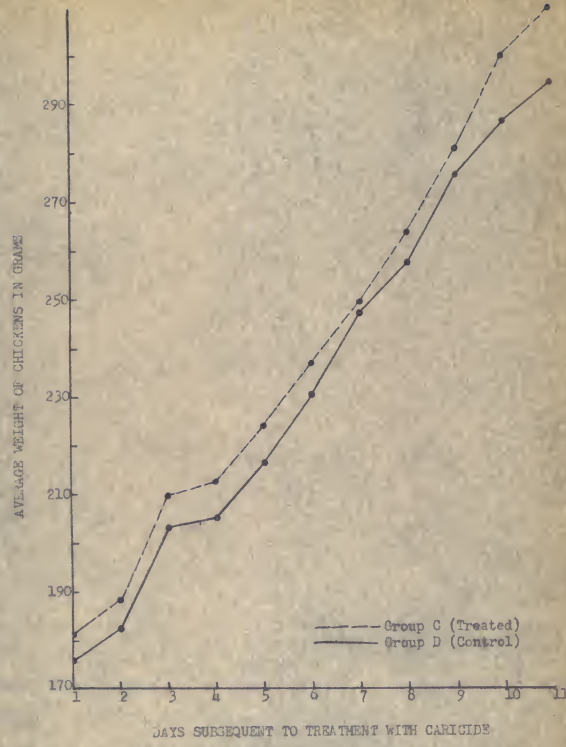

Fig. 3. Comparison of growth curves of chickens receiving 12.5 mgm of Caricide for eight consecutive days and their untreated controls.

Test 3

A continuous daily low-level dosage of Caricide at the rate of approximately 12.5 mgm per bird was added as a supplement to the feed of all treated chickens in this test. Beginning the eleventh day after infection, two Group A and two Group B birds were sacrificed each day, for ten days. Groups C and D were sacrificed, in their entirety, on the twenty-first day after infection. Results are recorded in Tables 6 and 7, and in Fig. 4.

The average infection in Group A birds was 1.2 tissue phase and 17.0 lumen larvae, compared to 2.5 tissue phase and 13.1 lumen larvae for the Group B chickens. These figures did not differ significantly.

Group C chickens carried an average worm burden of 7.3 lumen worms, while the Group D average was 17.1 worms per chicken. This difference in infection was not significant; however, it approached significance at the 5 per cent level.

The lumen larvae recovered from Group A hosts averaged 0.40 cm in length, compared to an average worm length of 0.70 cm in the Group B birds. This variation was significant at the 1 per cent level. Group C birds were infected with lumen larvae averaging 1.7 cm, while those from Group D hosts averaged 1.9 cm in length. This variation was of no significance at the 5 per cent level.

Terminal weights of Group C birds averaged 220.8 gms, while those of Group D averaged 188.0 gms. This degree of variation was of borderline significance at the 5 per cent level.

Table 6. Results of daily examinations of chickens receiving a continuous daily low-level dosage of Caricide at the rate of approximately 12.5 mgm per bird and their untreated controls.

Group	Chicken Number	Number of Days Post-Infection When Examined	Worms Recovered: Tissue Phase Number	Worms Recovered: Lumen Number	Worms Recovered: Lumen Length
A (Treated)	878	11	1	39	
	880	11	3	27	
	872	12	5	21	
	906	12	1	11	
	888	13	1	0	
	871	13	1	10	
	897	14	1	13	
	891	14	2	4	
	869	15	2	6	——
	890	15	0	50	
	909	16	1	1	
	893	16	1	14	
	910	17	1	10	
	874	17	0	4	
	882	18	0	18	
	915	18	1	24	
	905	19	0	42	
	901	19	0	12	
Total:			21	306	
Average:			1.2	17.0	0.40 cm
B (Control)	946	11	7	20	
	951	11	11	3	
	972	12	3	5	
	958	12	16	15	
	942	13	0	18	
	923	13	2	36	
	949	14	3	5	
	931	14	0	58	
	934	15	0	3	
	925	15	0	29	——
	950	16	0	9	
	974	16	0	1	
	927	17	0	8	
	973	17	0	8	
	952	18	0	4	
	965	18	0	13	
	967	19	0	0	
	975	19	3	1	
Total:			45	236	
Average:			2.5	13.1	0.70 cm

Table 7. Comparative data on weight gained after treatment and numbers and a statistical estimate of the lengths of lumen larvae recovered from a treated group of chickens and its untreated control twenty-one days after infection.

Group	Chicken Number	Weight Increase After Treatment Date (Gms)	Lumen Larvae Recovered Number	Length
C (Treated)	868	109	29	
	875	94	5	
	879	104	1	
	883	74	4	
	896	130	4	
	898	80	0	
	900	102	2	———
	902	90	0	
	907	114	1	
	908	70	17	
	912	74	23	
	913	97	12	
	918	108	3	
	919	78	1	
Total:		1324	102	
Average:		94.5	7.3	1.7 cm
D (Control)	924	78	14	
	928	80	3	
	930	58	7	
	933	66	60	
	939	38	24	
	941	91	15	
	944	82	2	
	953	42	1	
	954	54	44	———
	957	84	1	
	960	44	32	
	961	86	20	
	962	97	1	
	963	67	5	
	969	46	18	
	970	77	2	
	971	42	41	
Total:		1132	290	
Average:		66.5	17.1	1.9 cm

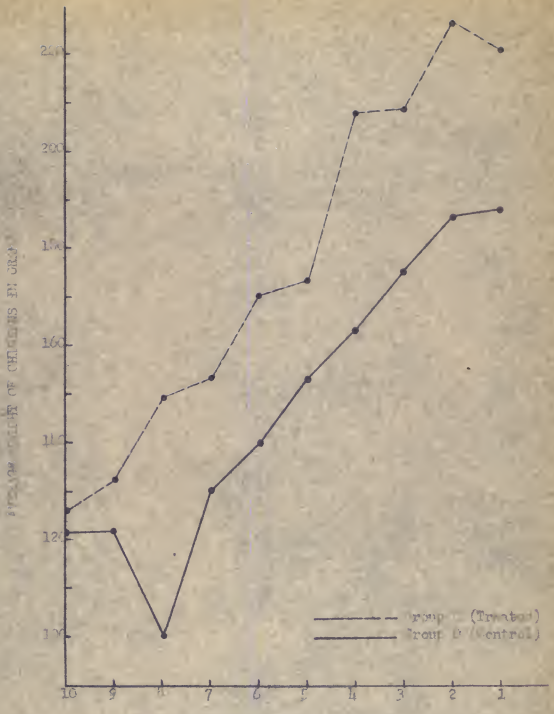

Fig. 4. Comparison of growth curves of chickens receiving a continuous dosage of approximately 12.5 mg. of furicide per day and their untreated controls.

Test 4

The anthelmintic used in this test was Compound 180-C. Each Group C bird received a daily dosage of 25 mgs, for eight consecutive days, beginning the tenth day post-infection. Group D birds were held as infected, untreated controls. Both groups were killed for autopsy twenty-one days after infection. Tables 8 and 9 and Fig. 5 depict the data obtained in this experiment.

The average number of lumen larvae recovered was 23.5 in Group C, and 15.1 in Group D. This variation was of no statistical significance.

Randomly chosen ascarids removed from Group C hosts averaged 1.00 cm in length, while worms recovered from Group D birds were, on the average, 1.4 cm long. This variation was significant at the 1 per cent level.

Terminal weights attained by the treated chickens averaged 236.1 gms, while for the untreated birds the average was 247.8 gms. These figures did not vary significantly.

Table 8. Data on weight gained after treatment and numbers and a statistical estimate of the lengths of lumen larvae recovered from a treated group of chickens twenty-one days after infection.

Group	Chicken Number	Weight Increase After Beginning of Treatment (Gms)	Lumen Larvae Recovered	
			Number	Length
	978	97	12	
	979	82	16	
	980	70	0	
	981	85	8	
	993	55	68	
	994	81	30	
	998	94	39	
	1001	84	9	
	1003	80	19	
	1005	44	84	
	1011	72	20	
	1013	27	83	
	1014	58	1	
	1021	92	208	
	1023	84	8	
	1024	80	13	
	1027	86	17	
	1036	75	132	
	1040	65	12	
	1041	86	1	
	1049	47	6	
0	1058	93	2	
(Treated)	1062	52	3	
	1064	78	1	
	1067	99	3	
	1074	62	25	
	1081	96	12	
	1089	67	2	
	1092	68	5	
	1095	90	49	
	1099	86	30	
	1100	72	19	
	1102	92	3	
	1110	67	6	
	1116	61	15	
	1117	84	14	
	1124	73	17	
	1128	86	8	
	1131	97	8	
	1135	82	18	
	1139	93	20	
	1142	86	4	
	1149	83	15	
	1158	76	9	
	1160	49	9	
	1161	80	1	
Total:		3516	1084	
Average:		76.4	23.5	1.00 cm

Table 9. Data on weight gained after treatment of Group C birds and a Statistical estimate of the lengths of lumen larvae recovered from a control group of chickens twenty-one days after infection.

Group	Chicken Number	Weight Increase After Beginning of Group C Treatment (Gms)	Lumen Larvae Recovered	
			Number	Length
	982	106	9	
	986	120	1	
	987	118	14	
	999	93	0	
	1000	92	4	
	1002	85	14	
	1004	50	3	
	1012	74	5	
	1015	111	2	
	1017	104	2	
	1020	82	8	
	1025	91	130	
	1046	76	15	
	1054	78	18	
	1056	92	7	
	1063	108	9	
	1066	71	0	
	1073	73	6	
	1079	75	5	
	1086	76	73	
D	1087	61	31	
(Control)	1090	89	16	----
	1101	70	72	
	1104	106	14	
	1105	73	7	
	1175	25	37	
	1109	79	2	
	1112	78	1	
	1115	60	5	
	1118	47	7	
	1119	70	11	
	1120	42	9	
	1121	88	3	
	1133	83	8	
	1134	95	2	
	1140	79	21	
	1141	101	4	
	1148	116	26	
	1150	84	11	
	1152	91	0	
	1162	71	15	
	1164	58	9	
	1165	103	9	
	1166	75	35	
	1167	66	5	
	1171	91	12	
Total:		3776	697	
Average:		82.08	15.1	1.4 cm

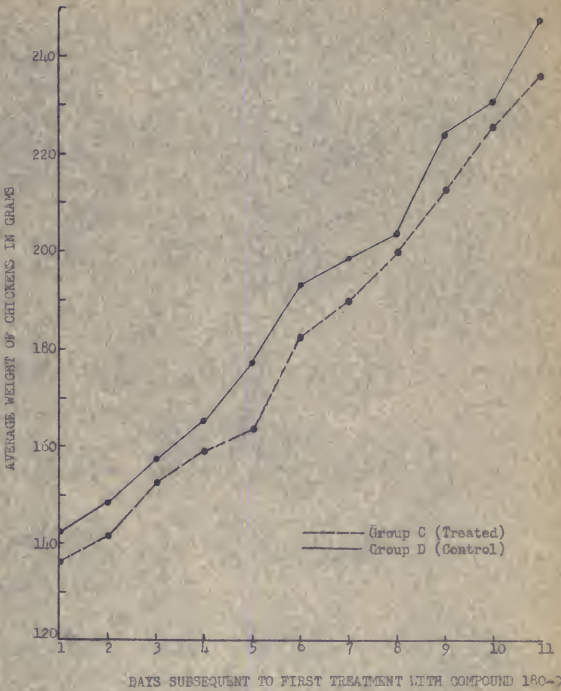

Fig. 5. Comparison of growth curves of chickens receiving 12.5 mgm of Compound 180-C for eight consecutive days and their untreated controls.

Test 5

A single dose of 0.05 ml of carbon disulfide was given each treated bird in this test. Here, as in Test 4, only one treated and one control group of chickens were used. Both were sacrificed twenty-one days post-infection. Data showing numbers and lengths of worms recovered and weight increases in the host birds are presented in Tables 10 and 11 and Fig. 6.

The average numbers of lumen larvae were 14.05 and 19.9, for Groups C and D, respectively. The difference between these two averages was not significant.

Lumen larvae from Group C hosts averaged 1.5 cm in length, compared to an average of 1.9 cm in the Group D chickens. This difference was significant at the 1 per cent level.

The terminal weights of Group C birds averaged 243.3 gms, and those of Group D, 239.6 gms. Again, there was no significant difference between these figures.

Table 10. Data on weight gained after treatment and numbers and a statistical estimate of the lengths of lumen larvae recovered from a treated group of chickens twenty-one days after infection.

Group	Chicken Number	Weight Increase After Treatment (Gms)	Lumen Larvae Recovered: Number	Length
	976	89	23	
	984	64	1	
	985	80	14	
	991	44	27	
	996	62	24	
	1007	94	7	
	1008	68	17	
	1010	64	12	
	1016	78	15	
	1018	86	4	
	1028	91	18	
	1031	54	51	
	1035	92	2	
	1039	80	23	
	1043	69	1	
	1044	102	13	
	1047	74	9	
	1048	99	6	
	1052	53	5	
	1053	63	6	
	1055	83	6	
G (Treated)	1071	55	8	——
	1073	85	21	
	1076	68	9	
	1077	60	7	
	1083	86	38	
	1084	84	9	
	1088	84	14	
	1094	76	10	
	1098	65	9	
	1111	79	29	
	1114	78	4	
	1123	78	11	
	1130	68	3	
	1132	80	10	
	1143	109	7	
	1144	70	33	
	1155	109	30	
	1157	99	82	
	1168	74	5	
	1169	66	12	
	1170	87	12	
	1172	69	3	
	1174	70	8	
Total:		3388	618	
Average:		77.0	14.05	1.5 cm

Table 11. Data on weight gained after treatment of Group C birds and a statistical estimate of the lengths of lumen larvae recovered from a control group of chickens twenty-one days after infection.

Group	Chicken Number	Weight Increase After Treatment of Group C (Gms)	Lumen Larvae Recovered	
			Number	Length
	977	52	4	
	988	57	5	
	989	68	31	
	990	71	82	
	992	79	8	
	995	63	1	
	997	64	3	
	1006	93	0	
	1009	80	1	
	1022	79	15	
	1026	45	19	
	1029	64	3	
	1030	78	23	
	1032	76	0	
	1034	43	2	
	1037	71	139	
	1038	78	19	
	1045	72	6	
	1050	73	5	
	1051	66	36	
	1059	102	85	
D	1065	71	1	
(Control)	1072	63	3	—
	1078	48	11	
	1080	76	30	
	1082	74	5	
	1085	70	0	
	1091	86	30	
	1093	49	1	
	1096	74	14	
	1097	76	0	
	1103	75	0	
	1106	59	22	
	1108	63	3	
	1125	75	8	
	1126	50	5	
	1127	79	38	
	1136	88	65	
	1137	76	20	
	1138	62	36	
	1145	66	35	
	1147	78	3	
	1153	61	49	
	1156	53	2	
	1159	84	45	
	1163	76	8	
	1173	75	18	
Total:		3518	939	
Average:		70.5	19.9	1.9 cm

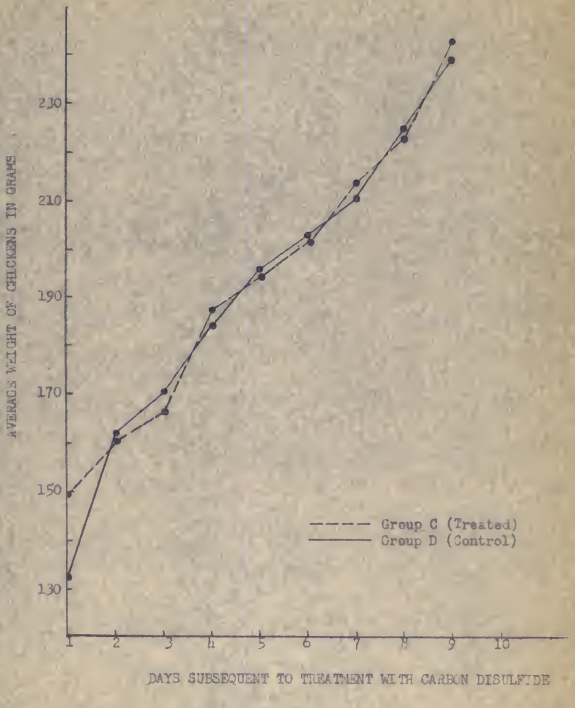

Fig. 6. Comparison of growth curves of chickens receiving a single 0.05 ml dose of carbon disulfide and their untreated controls.

DISCUSSION

In this thesis, an attempt has been made to demonstrate the effects of several anthelmintics on the larval stages of the nematode Ascaridia galli through the use of three quantitative criteria: a comparison of worm numbers, worm lengths, and host weight gains in infected, treated, versus corresponding infected, untreated, groups of chickens. The general method of anthelmintic screening practiced here has also been followed by Hansen et al. (1954c) and by other current workers. A known number of helminth eggs was fed to laboratory animals, and by comparing the relative number of worms harbored by treated and untreated hosts at autopsy, the ability of the test substance to remove the worms in question was ascertained. Riedel (1950, 1951), Sloan et al. (1954), and others have used a somewhat different experimental design to test anthelmintics in vivo when the host animals could not be sacrificed for autopsy, or when an undetermined level of infection was present. This procedure, however, required almost continuous observation of the test animals in order that all fecal material which they passed could be recovered and examined for worms. A comparison of the number of worms expelled by treated and untreated individuals was used as an indication of the efficacy of the anthelmintic.

All data in this thesis was subjected to statistical analysis in order that a uniform system might be followed for comparing results. The primary tool used for analyzing data was the t-test for group comparisons. Where the value of t obtained from this process was on or close to a significant level, a modified t-test using a square-root transformation[1] was also computed, as

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[1]The use of this technique was suggested by Prof. Henry Tucker, Dept. of Mathematics.

a means of checking the original statistical results.

A new procedure for estimating worm lengths by the use of a statistical sampling technique was followed throughout the course of the study. This method was based on an analysis of variance comparing the differences in lengths of worms found (a) within randomly selected chickens, and the variation existing (b) between the lengths of worms from one chicken and those from other chickens. The F ratio obtained from this analysis indicated that there was no statistically significant variation between lengths of worms recovered from any one bird and those taken from any other randomly chosen birds. Therefore, a sampling procedure was devised for estimating average worm length, based on the assumption that the lengths of worms taken from a few birds selected by chance from each group would furnish an accurate estimate of the average lengths of all the A. galli found within all the chickens from that particular group. Measurements were taken of all the worms from a sample of 10 per cent of the total number of birds from each group. The relationship between anthelmintic treatment and worm length, as evidenced by the experimental results of this study, appears to be uncertain.

Statistical analysis of both the data concerning worm number and terminal weights in Groups C and D of Test 3 indicated that the anthelmintic tended to be effective in removing lumen larvae under the conditions in which it was used, and that either this reduction in worm burden or some other factor resulted in a definitely recognizable weight advantage in the treated birds as compared to the untreated group. It would be very difficult, however, to establish a definite correlation between numbers of lumen larvae and amount of weight gained. Chicken #868, which made the third greatest weight gain (109 gms) of any bird in the group, had the heaviest Ascaridia infection, 29 worms. Chicken #883, which made the third smallest weight increase after

treatment, contained only 4 worms, much less than the average group infection rate of 7.3 worms. On the other hand, Chicken #912, which showed a weight gain equal to that of bird #683, contained 23 larval ascarids, or more than three times as many worms as a bird which showed an equal weight increase.

In Group D in Test 3, the average number of lumen larvae recovered per bird was 17.1, coupled with an average weight gain of 66.3 gms. Of the 7 birds which were infected with more than the mean number of worms, 6 made less than the average weight increase. In this group, the chicken with the heaviest worm infection (60) made a weight gain only 0.3 gm less than the average increase of 66.3 gms. Of the 10 birds whose weight gains exceeded the average, 8 had less than the average worm burden for the group.

Since it appears doubtful, in the light of these data, that the nearly significant weight increase that the Test 3 treated birds established over their controls was due directly to the quantitative severity of their worm infection, the source of this variation remains to be explained. One possibility is that the chemical constituents of the Caricide acted as a growth stimulant in the treated birds, independent of any anthelmintic activity it might have had. Another explanation might be that the continual presence of the drug in the intestine of the treated birds (the digestive tract of chickens which have food available is never entirely empty) acted in some way to disrupt or to completely prevent the entrance of the larvae into the tissues, which has been definitely defined as the time at which the greatest weight losses occurred in the test chickens in the experiments of other workers.

In this connection, an analysis of the data concerning the number of days post infection at which tissue phase larvae were recovered, in the first three tests, suggested a negative answer to this question. The peak of the

tissue phase, i.e., the day on which the greatest number of worms were recovered from the intestinal walls by the digestion process, was eleven days post infection for both treated and control groups in Test 1, day 13 for both treated and control birds in Test 2, and day 12 post infection for both treated and control groups in Test 3. Thus, the presence of the drug appeared to have no influence, in either suppressing or accelerating the migratory cycle of the Ascaridia larvae in these tests.

Another possible indirect influence of anthelmintic treatment on the host which may have resulted in, or at least contributed to, the nearly significant weight gains demonstrated by treated Group C in Test 3, could be explained in the following manner. The presence of certain bacteria and/or protozoa in the alimentary tract of normal chickens could conceivably result in the release by these micro-organisms of toxic metabolic products which are regularly absorbed by the host. The action of the test drug may have destroyed these organisms, and thus contributed incidentally to the bird's well-being by removing the source of these toxic substances, which had been acting as a "drag" on the animal prior to the time of treatment. The treated host was then able to expend the extra energy, which formerly had been sidetracked, for its own anabolic processes. Todd and Hansen (1951) have postulated a similar theory in attempting to explain the commonly observed situation in which chickens with light worm infections often gained the least weight during experiments. They believed that the failure of these birds to make normal weight gains was due to the portion of their total energy expenditure which was lost in combatting their parasites. This explanation also may serve to clarify some of the disproportionate-appearing results which were cited in preceding sections of this discussion.

The end of the tissue phase cycle as indicated by the last day after

infection on which tissue phase larvae were present coincides in general with the stages of the cycle as described by Tugwell and Ackert (1952). They stated that the tissue phase was most prominent from the tenth to the seventeenth day after infection, but that it extended to the twenty-third day. The data from the present experiments show that in Test 1, the last mucosa larva was recovered on day 15 post infection in the treated birds, and on day 18 in the control. In Test 2, the last mucosa larvae were found on days 19 and 18 in the treated and control groups, respectively, and in Test 3, the last tissue larvae in Group A were present on day 18, and in the Group B birds, on day 19. Thus, the indication is that the end of the tissue phase is not influenced to any extent by the treatment schedules followed in the three Caricide tests. This consideration of the apparent influence of Caricide treatment on the tissue cycle has been included because the effects of the migratory stage on the health of the host are so pronounced. The addition to the feed of a drug which might be effective against adult Ascaridia might not only be useless for removing the larval stages, but could actually result in more than normal damage to young birds by the creation of a semi-toxic environment in the intestinal lumen, thereby influencing the larvae to migrate into the mucosa at an earlier than normal date. In addition, the drug might also influence them to delay their return to the lumen. However, since the migratory cycle does not seem to be influenced by Caricide, these possibilities are irrelevant in an evaluation of the activity of Caricide as used in the work described here.

In Test 5, carbon disulfide was chosen as the anthelmintic to test the action of a fumigant-type compound on the tissue phase larvae. This experiment seemed pertinent at this time because of the overall lack of activity against larvaforms demonstrated by Caricide, in which active principle was

not a fumigant. The inaccessibility of the mucosa larvae to the toxic action of an alkaloid in the form of nicotine was reported by Hansen et al. (1954c). The fumigating action of carbon disulfide also proved to be ineffective in removing larval ascarids in a 1-dose treatment.

A resume of the experimental results contained in this thesis indicate, therefore, that while Compound 180-C and carbon disulfide were not applicable, in the dosages used, as anthelmintics to combat the tissue phase of the Ascaridia life cycle, further investigation on the effects of continuous low-level Caricide treatment for this parasite is necessary before a final decision can be made on its applicability to A. galli infections in chickens. At the present time, a repetition of Test 3 is being conducted to attempt to resolve these questions.

SUMMARY

A series of five laboratory experiments involving approximately 420 chickens have been conducted to evaluate the effects of three anthelmintics on the lumen and tissue phase larvae of Ascaridia galli, the large roundworm of chickens. All birds used in this study were infected with 100 ± 10 or 75 ± 5 embryonated A. galli ova. In Tests 1, 2, and 3, the experimental birds were divided into four groups. Groups A (treated) and B (control) were further subdivided into groups of two birds each, which were slaughtered at consecutive intervals during the progression of the tissue phase of the Ascaridia life cycle. Groups C (treated) and D (control) were killed 21 days after infection. In Tests 4 and 5, all birds were killed 35 days post infection.

In Test 1, a single 25 mgm dose of Caricide (1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate) had no statistically detectable effects on either the number of lumen or tissue phase larvae, or on the growth of the host birds.

In Test 2, 12.5 mgm of Caricide given to the treated birds for eight consecutive days had no appreciable effects on either host weight or numbers of lumen or tissue phase larvae.

A continuous daily low level dosage of Caricide (approximately 12.5 mgm per bird per day) added to the feed of the treated groups in Test 3 resulted in a nearly statistically significant reduction in numbers of lumen larvae in the Group C chickens. In addition, the average weight gains of these birds were significantly greater than those of the corresponding control group.

A daily dosage of 25 mgm of Compound 180-C (1-carbethoxy-4-methyl piperazine hydrochloride) for eight consecutive days had no significant effect on either worm number or host weight.

A single 0.05 ml dose of carbon disulfide was ineffective in influencing either host worm populations or weight increases.

ACKNOWLEDGMENT

For the invaluable aid of Dr. M. P. Hansen, major professor, who not only provided encouragement and moral support during the preparation of this thesis, but helped with much of the routine experimental work involved in the project, my appreciation is expressed. The assistance of Prof. Henry Tucker, Dept. of Mathematics, for aid in the statistical analysis of some of the data, and Mr. B. R. B. Persaud, for help in numerous instances, is also gratefully acknowledged.

BIBLIOGRAPHY

Ackert, J. E.
On the habitat of Ascaridia perspicillum (Rud.). J. Parasit. 10: 101-103. 1923.

Ackert, J. E., and C. A. Herrick.
Effects of the nematode Ascaridia lineata (Schneider) on growing chickens. J. Parasit. 15: 1-13. 1928.

Ackert, J. E., and L. O. Nolf.
New technique for collecting intestinal roundworms. Science. 70: 310-311. 1929.

Ackert, J. E., M. F. McIlvaine, and N. Z. Crawford.
Resistance of chickens to parasitism affected by Vitamin A. Amer. J. Hyg. 13: 320-336. 1931.

Ackert, J. E., and L. O. Nolf.
Resistance of chickens to parasitism affected by Vitamin B. Amer. J. Hyg. 13: 337-344. 1931.

Ackert, J. E., D. A. Porter, and T. D. Beach.
Age resistance of chickens to the parasite, Ascaridia lineata. J. Parasit. 19: 157. 1932.

Ackert, J. E., L. L. Eisenbrandt, B. Gladding, and J. H. Wilmoth.
On the comparative resistance of six breeds of chickens to the nematode, Ascaridia lineata (Schneider). J. Parasit. 20: 127. 1933.

Ackert, J. E., and T. D. Beach.
Resistance of chickens to the nematode (Ascaridia lineata) affected by dietary supplements. Trans. Am. Micros. Soc. 52: 51-58. 1933.

Ackert, J. E., L. L. Eisenbrandt, J. H. Wilmoth, B. Gladding, and J. Pratt.
Comparative resistance of five breeds of chickens to the nematode, Ascaridia lineata (Schneider). J. Agr. Res. 50: 607-624. 1935.

Ackert, J. E., and L. L. Eisenbrandt.
Comparative resistance of bronze turkeys and white leghorn chickens to the intestinal nematode, Ascaridia lineata (Schneider). J. Parasit. 21: 200-204. 1935.

Ackert, J. E., D. A. Porter, and T. D. Beach.
Age resistance of chickens to the nematode Ascaridia lineata (Schneider). J. Parasit. 21: 205-213. 1935.

Adams, J. C. L., and A. W. Woodruff.
Diethylcarbamazine in the treatment of onchocerciasis. Trans. Roy. Soc. Trop. Med. Hyg. 47: 66-69. 1953.

Alley, J. C.
Caricide in the treatment of Dirofilaria immitis in Zanzibar. Vet. Record. 62: 522. 1950.

Almeida, C. G. L. de.
Ensaio terapeutico com o hetrazan em casos de infestacao par Acanthocheilonema perstans. Anais do Instituto de Medicina Tropical. 9: 127-144. 1952.

Ashburn, L. L., T. A. Burch, and F. J. Brady.
Pathologic effects of suramin, hetrazan and arsenamide on adult Onchocerca volvulus. Boletin de la Oficina Sanitaria Panamericana. 28: 1107-1117. 1949.

Basnuevo, J. G.
La piperazina en el tratamiento de la filariasis. Revista Kuba de Medicina Tropical y Parasitologia. 4: 126. 1948.

Basnuevo, J. G., and J. A. Fontao.
Ascariasis y dietilendiamina (piperacina). Revista Cubana de Laboratorio Clinico. 8: 19-21. 1954.

Berg, J. A. G. ten.
Filariasis loa; treatment with hetrazan. Documenta de Medicina Geographica et Tropica. 4: 107-111. 1952.

Berg, J. A. G. ten.
Filariasis loa. Behandeling met hetrazan. Nederlandsch Tijdschrift voor Geneeskunde. 96: 2411-2417. 1952.

Baylet, R.
Considerations sur le traitement local de l'onchocercose en particulier par injection de Notezine intrakystique. Bulletin de la Societe de Pathologie Exotique. 46: 335-338. 1953.

Belhommet, F.
Essai de therapeutique de la bilharziose par la carbilazine. Annales de la Societe Belge de Medecine Tropical. 33: 3-11. 1953.

Beye, H. K., J. F. Kessel, J. Heuls, G. Thooris, and B. Bambridge.
Nouvelles recherches sur l'importance, les manifestations cliniques, et la lutte contre la filariose a Tahiti, Oceanie francaise. Bulletin de la Societe de Pathologie Exotique. 46: 144-163. 1953.

Binkley, K. L.
Treatment of ascariasis in zoo animals with 1-diethylcarbamyl-4-methylpiperazine hydrochloride. J. A. V. M. A. 125: 408-409. 1954.

Blum-Geyst, J.
Essai sur les nematodes antillais de deux anthelminthiques encore peu utilises dans les pays de langue francaise. Bulletin de la Societe de Pathologie Exotique. 47: 286-288. 1954.

Boley, L. E., N. D. Levine, W. L. Wright, and R. Graham.
Treatment of Percheron horses for gastrointestinal parasites with a phenothiazine-carbon disulfide mixture. J. A. V. M. A. 99: 408-411. 1941.

Bonnin, H., and G. F. Moretti.
Preuves clinique et biopsique de l'action lethale d'un derive de la piperazine sur la filaire Loa loa adult. Bulletin de la Societe de Pathologie Exotique. 43: 279-282. 1950.

Bozicevich, J., and W. H. Wright.
Carbon disulphide for the removal of stomach worms from swine. Vet. Med. 30: 390-393. 1935.

Briceno Rossi, A. L., and R. Hewitt.
Treatment of Bancroftian filariasis with hetrazan in Puerto Cabello, Venezuela. South. Med. J. 42: 978-981. 1949.

Briceno Rossi, A. L.
Resultados obtenidos con el hetrazan en diferentes modalidades patologicas o clinicas de la wuchereriasis bancrofti. Archivos Venezolanos de Patologia Tropical y Parasitologia Medica. 1: 86-91. 1949.

Brumst, G., and N. Naffah.
Un foyer de filariose au Liban. Traitement par le diethylcarbamazine. Les resultats d'un essai de depistage par l'intradermoreaction. Bulletin de la Societe de Pathologie Exotique. 46: 191-194. 1953.

Brown, H. W., K. F. Chan, and K. L. Hussey.
The efficacy of piperazine compounds against Syphacia obvelata, a pinworm of mice. Am. J. Trop. Med. Hyg. 3: 504-510. 1954.

Brown, H. W., K. F. Chan, and B. D. Ferrell.
A study of the activity of chemotherapeutic agents on infections of Syphacia obvelata and Aspiculuris tetraptera. Exper. Parasit. 3: 45-51. 1954.

Brown, H. W., and M. M. Sterman.
Treatment of Ascaris lumbricoides infections with piperazine citrate. Am. J. Trop. Med. Hyg. 3: 750-754. 1954.

Brumpt, L. C.
Le mode d'action de la diethylcarbamazine sur les filaires. Comptes Rendus des Seances de la Societe de Biologie. 146: 209-211. 1952.

Brumpt, L. C., and H. T. Sang.
Activite de la diethylcarbamazine (hetrazan, notezine, banocide) contre les nematodes intestinaux. Bulletin de la Societe de Pathologie Exotique. 47: 170-178. 1954.

Burch, T. A.
Experimental therapy of onchocerciasis with suramin and hetrazan. Boletin de la Oficina Sanitaria Panamericana. 28: 233-248. 1949.

Burch, T. A.
Prurito producido por el hetrazan como una prueba de diagnostico para la oncocercosis. Revista del Colegio Medico de Guatemala. 2: 53-57. 1951.

Burch, T. A., and L. L. Ashburn.
Experimental therapy of onchocerciasis with suramin and hetrazan; results of a three-year study. Am. J. Trop. Med. 31: 617-623. 1951.

Burch, G. R., and F. A. Ehrenford.
Canine strongyloidiasis. Vet. Med. 48: 417-420. 1953.

Brygoo, E.
Essai de traitement de la filariose (F. loa et F. perstans) par le 3.799 R. P. Bulletin de la Societe Pathologie Exotique. 42: 313-317. 1949.

Caius, J. F., and K. S. Mhaskar.
The correlation between the chemical composition of anthelmintics and their therapeutic values in connection with the hookworm inquiry in the Madras Presidency. XXII. Summary and conclusions. Indian J. Med. Res. 11: 371-375. 1923.

Canet, J., and P. Jahan.
Traitement de la filariose a W. bancrofti en Indochine par un nouveau filaricide: la 1-diethyl-carbamyl-4-methylpiperazine ou 3.799 R. P. Bulletin de la Societe de Pathologie Exotique. 42: 408-414. 1949.

Canet, J., and P. Jahan.
Essais de traitement de la filariose canine en Indochine par le 1-diethyl carbamyl 4-methylpiperazine. Bulletin de la Societe de Pathologie Exotique. 43: 482-489. 1950.

Chan, K. F., and H. W. Brown.
Treatment of experimental trichinosis in mice with piperazine hydrochloride. Am. J. Trop. Med. Hyg. 3: 750-754. 1954.

Chaudhuri, R. N.
Notes of some remedies. XXVII. Drugs in helminthic diseases, Part II. Indian Med. Gaz. 84: 105-106. 1949.

Chernin, E.
Diethylcarbamazine (hetrazan) in the treatment of strongyloidiasis. J. Parasit. 40: 589-590. 1954.

Chesterman, C. C.
Tropical diseases as an aftermath of war. J. Trop. Med. Hyg. 52: 155-157. 1949.

Chopra, R. N., and A. C. Chandler.
Anthelmintics and their uses. Baltimore: Williams and Wilkins, 1928.

Colbourne, M. J.
Unsuccessful treatment of ascariasis with hetrazan. Lancet. 1: 996. 1950.

Colbourne, M. J.
Treatment of ascariasis with hetrazan in the Gold Coast. Trans. Roy. Soc. Trop. Med. Hyg. 46: 662-665. 1952.

Colglazier, M. L., and F. D. Enzie.
Notes on caricide as an anthelmintic for cats and dogs. Proc. Helmin. Soc. Wash. 18: 50-52. 1951.

Corcos, A., R. Dupoux, and S. Abitbol.
Traitement des parasitoses intestinales a vers ronds par le diethyl carbamyl 4 methyl piperazine (notezine). Bulletin de la Societe de Pathologie Exotique. 44: 209-215. 1951.

Coutinho, J. O., J. Croce, R. Campos, and V. Amato Neto.
Resultados obtidos com o emprego da dietilcarbamazina (hetrazan) no tratamento da estrongiloidiase. Hospital (Rio de Janeiro). 42: 339-343. 1952.

Crosnier, R., A. Darbon, J. F. Dulac, and F. Quilicchini.
Filariose loa et amibiase. Incidences du traitement par la notezine. Bulletin de al Societe Pathologie Exotique. 46: 702-708. 1953.

Cross, E. G., A. David, and D. K. Vallance.
Piperazine adipate: a new anthelmintic agent. Part II. Toxicological and pharmacological studies. Brit. J. Pharmacy and Pharmacol. 6: 711-717. 1954.

Dassanayake, W. L. P.
A follow-up of 230 cases of bancroftian filariasis treated with hetrazan at the filariasis clinic at Dehiwala, Ceylon. Ann. Trop. Med. Parasit. 48: 123-126. 1954.

Davies, M. T., J. Forrest, F. Hartley, and V. Petrow.
Piperazine adipate: a new anthelmintic agent. Part I. Physico-chemical properties. Brit. J. Pharmacy and Pharmacol. 6: 707-710. 1954.

Deane, M. P., and O. R. DaCosta.
Relatorio preliminar de experiencias com o Hetrazan, feitas com o fim de verificar sua aplicabilidade no controle da transmissao da filariose em Belem. Revista do Servico Especial de Saude Publica. 2: 527-544. 1948.

Deschiens, R., M. Poirier, and L. Lamay.
Sur l'action anthelminthique des derives de l'ethylene-diamine et de la piperazine. Bulletin de la Societe de Pathologie Exotique. 47: 83-86. 1954.

Dijk, I. J. van.
Di-ethyl-carbamazine, een nieuw middel tegen ascariasis. Maandschrift voor Kindergeneeskunde. 22: 57-59. 1954.

Doak, G. O., and H. Eagle.
Correlations between the chemical structure and biological activity of arsenobenzenes. First Symposium on Chemical-Biological Correlation, pp. 7-45. Washington: National Research Council, National Academy of Sciences, 1951.

Ecker, J. A., L. L. Lovshin, and Alfred Reich.
Acanthocheilonema perstans. The persistent filaria: with a report of six cases. Ann. Inter. Med. 40: 611-615. 1954.

Enigk, K.
Die biologie von Capillaria plica (Trichuroidea, Nematodes). Zeitschrift fur Tropenmedizin und Parasitologie. 1: 560-571. 1950.

Etteldorf, J. N., and L. V. Crawford.
Treatment of ascariasis in children. Use of 1-diethylcarbamyl-4-methyl piperazine dihydrogen citrate (hetrazan). J. A. M. A. 143: 797-799. 1950.

Foley, R. V.
The treatment of canine filariasis. Vet. Med. 45: 485-489. 1950.

Foster, A. O.
Notes on veterinary parasitology. New treatment for dog heartworms. Vet. Med. 44: 460. 1949.

Galliard, H., and R. Mille.
Essais de traitement de la filariose a Wuchereria bancrofti var. pacifica par le 1-diethyl carbamyl, 4-methyl piperazine, a Tahiti. Bulletin de la Societe de Pathologie Exotique. 42: 304-313. 1949.

Galliard, H., and R. Mille.
Un nouveau medicament antifilarien: le 1-diethylcarbamyl-4-methyl-piperazine, experimente a Tahiti. Bulletin de l'Academie National de Medecine. 3e Serie, 133: 83, 85-87, 84. 1949.

Gardiner, J. L., and E. E. Wehr.
Selecting experimental groups of chicks by weight. Proc. Helmin. Soc. Wash. 17: 25-26. 1950.

Garin, C., and J. P. Garin.
Sur le traitement de la filariose a F. loa par le notezine. Jour. de Medecone de Bourdeaux et du Sud-Ouest. 128: 250-252. 1951.

Garin, C., and J. P. Garin.
Sur le traitement de la filariose a F. loa par le notezine. Jour. de Medecine de Lyon. 32: 13-14. 1951.

Germain, A., L. Andre, and J. Marty.
Filarioses a A. perstans et a L. loa traitees par le 3799 R. P. Bulletin de la Societe de Pathologie Exotique. 43: 283-285. 1950.

Ghanem, M. H.
The treatment of ascariasis and ancylostomiasis with hetrazan (diethylcarbamazine). Trans. Roy. Soc. Trop. Med. Hyg. 48: 73-76. 1954.

Gibson, C. L., and T. A. Burch.
Estudios preliminaires sobre la infectividad de microfilarias de Onchocerca volvulus que reaparecen despues del tratamiento con hetrazan. Revista del Colegio Medico de Guatemala. 2: 63-66. 1951.

Goodwin, L. G., and O. D. Standen.
Treatment of roundworm with piperazine citrate ('antepar'). Brit. Med. J. 2: 1332-1333. 1954.

Gordonoff, T.
Piperazine in the treatment of threadworms. Brit. Med. J. 1: 394. 1954.

Grootenhuis, G.
Klinische les. Wormziekten-bestrijding bij het paard. Tijdschrift voor Diergeneeskunde. 78: 630-636. 1953.

Guberlet, J.
Notes on the life history of Ascaridia perspicillum (Rud.) Trans. Amer. Micros. Soc. 43: 152-156. 1924.

Guevara, R., H. R. Estrada, and G. V. de Leon.
Hetrazan on ascarigram I. Acta Medica Philippina. 8: 157-171. 1952.

Guilhon, J.
Proprietes anthelminthiques d'un derive de la piperazine. Bulletin de l'Academie Veterinaire de France. 22: 361-363. 1949.

Guilhon, J.
Un nouvel anthelminthique: le diethylene diamine. Bulletin de l'Academie Veternaire de France. 24: 243-245. 1951.

Guilhon, J., and P. Groulade.
Action du diethylene-diamine sur les ascarides des carnivores. Bulletin de l'Academie Veternaire de France. 24: 301-303. 1951.

Halawani, A., I. Baz, and M. Dawood.
A preliminary report on the treatment of ambulant cases of Bancroftian Filariasis with Hetrazan in Egypt. J. Roy. Egypt. Med. A'ssn. 32: 395-403. 1949.

Hall, M. C.
Efficacy of some anthelmintics. J. Agr. Res. 12: 397-447. 1918.

Hall, M. C., and J. E. Shillinger.
Critical tests of miscellaneous anthelmintics. J. Agr. Res. 29: 313-332. 1924.

Hansen, M. F., M. G. Norris, and J. E. Ackert.
The influence of an all plant protien diet supplemented with aureomycin and vitamin B_{12} on the resistance of chicks to Ascaridia galli (Schrank). Poult. Sci. 32: 612-617. 1953.

Hansen, M. F., L. H. Petri, and J. E. Ackert.
Effects of aureomycin and vitamin B_{12} used separately as feed supplements on resistance of chickens to Ascaridia galli (Schrank). Exper. Parasit. 3: 122-127. 1954.

Hansen, M. F., L. J. Olson, and J. E. Ackert.
Improved techniques for culturing and administering Ascarid eggs to experimental chicks. Exper. Parasit. 3: 464-473. 1954.

Hanson, M. F., B. R. B. Persaud, and J. E. Ackert.
Effects of certain anthelmintics and an antibiotic on lumen and tissue phase larvae of Ascaridia galli (Schrank). Poult. Sci. 33: 140-146. 1954.

Harned, B. K., R. W. Cunningham, S. Halliday, R. E. Vessey, N. N. Yuda, M. C. Clark, and Y. Subbarow. Some toxicological and pharmacological properties of 1-diethylcarbamyl-4-methylpiperazine hydrochloride, hetrazan. Ann. N. Y. Acad. Sci. 50: 141-160. 1948.

Harned, B. K., R. W. Cunningham, S. Halliday, R. E. Vessey, N. N. Yuda, M. C. Clark, C. H. Hine, R. Cosgrove, and Y. Subbarow. Studies on the chemotherapy of filariasis. IV. Some pharmacodynamic properties of Hetrazan. J. Lab. Clin. Med. 33: 216-235. 1948.

Hartley, F.
Piperazine in the treatment of threadworms. Brit. Med. J. 1: 521. 1954.

Hawking, F., P. Sewell, and J. P. Thurston.
Mode of action of hetrazan in filariasis. Lancet. 2: 730-731. 1948.

Hawking, F., and W. Laurie.
Action of hetrazan on filariasis and onchocerciasis. Lancet. 2: 146-147. 1949.

Hawking, F., W. Laurie, P. Sewell, and S. Thurston.
Investigations on the antifilarial action of hetrazan on Litomosoides, Wuchereria bancrofti, and Onchocerca volvulus. Trans. Roy. Soc. Trop. Med. Hyg. 43: 360. 1950.

Hawking, F., P. Sewell, and J. P. Thurston.
The mode of action of hetrazan on filarial worms. Brit. J. Pharmacol Chemother. 5: 217-238. 1950.

Hawking, F.
Some recent work on filariasis. Trans. Roy. Soc. Trop. Med. Hyg. 44: 153-182, addendum 182-186. 1950.

Hawking, F.
Problemas de filariasis. Medicina Colonial. 17: 413-426. 1951.

Hawking, F.
Filariose. Jornal do Medico. Oporto. 18: 108-113. 1951.

Hawking, F.
A histological study of onchocerciasis treated with hetrazan. Brit. Med. J. 1: 992-994. 1952.

Heckenroth, F., R. Becuwe, L. Mayan, and G. Leroux.
Filarioses (loa et perstans) et derives de la piperazine. Bulletin de la Societe de Pathologie Exotique. 43: 354-363. 1950.

Herrero, M. T.
Hetrazan: un nuevo compuesto para el tratamiento de la filariasis. Medicina Colonial. 15: 356-364. 1950.

Hetrazan and filariasis. (Annotation). Brit. Med. J. 2: 772. 1950.

Hewitt, R. I., W. S. Wallace, E. White, and Y. Subbarow.
Experimental chemotherapy of filariasis. I. Experimental methods for testing drugs against naturally acquired filarial infections in cotton rats and dogs. J. Lab. Clin. Med. 32: 1293-1303. 1947.

Hewitt, R. I., E. White, W. S. Wallace, H. W. Stewart, S. Kushner, and Y. Subbarow. Experimental chemotherapy of filariasis. II. Effect of piperazine derivatives against naturally acquired filarial infections in cotton rats and dogs. J. Lab. Clin. Med. 32: 1304-1313. 1947.

Hewitt, R. I., S. Kushner, H. W. Stewart, E. White, W. S. Wallace, and Y. Subbarow. Experimental chemotherapy of filariasis. III. Effect of 1-diethylcarbamyl-4-methylpiperazine hydrochloride against naturally acquired filarial infections in cotton rats and dogs. J. Lab. Clin. Med. 32: 1314-1329. 1947.

Hewitt, R. I., D. E. White, S. Kushner, W. S. Wallace, H. W. Stewart, and Y. Subbarow. Parasitology of piperazines in the treatment of filariasis. Ann. N. Y. Acad. Sci. 50: 128-140. 1948.

Hewitt, R., W. Wallace, E. White, and Y. Subbarow.
The treatment of ascariasis in dogs with 1-diethylcarbamyl-4-methyl-piperazine hydrochloride. J. Parasit. 34: 237-239. 1948.

Hewitt, R.
Mass therapy with hetrazan as a control measure for Bancroftian filariasis on St. Croix, American Virgin Islands. Nature. 164: 1135-1136. 1949.

Hewitt, R. I., E. White, D. B. Hewitt, S. M. Hardy, W. S. Wallace, and R. Anduze. The first year's results of a mass treatment program with hetrazan for the control of Bancroftian filariasis on St. Croix, American Virgin Islands. Am. J. Trop. Med. 30: 443-452. 1950.

Hewitt, R., M. Kenney, A. Chan, and M. Mohamed.
Follow-up observations on the treatment of Bancroftian filariasis with hetrazan in British Guiana. Am. J. Trop. Med. 30: 217-237. 1950.

Hoekenga, M. T.
Treatment of ascariasis with 1-diethylcarbamyl-4-methyl-piperazine hydrochloride. South. Med. J. 44: 1125-1127. 1951.

Hoekenga, M. T.
Treatment of ascariasis in children with hetrazan syrup. Am. J. Trop. Med. Hyg. 1: 688-692. 1952.

Hoeven, J. A. van der.
Some remarks on filariasis, in relation to the administration of hetrazan. Documenta de Medicina Geographica et Tropica. 4: 107-111. 1952.

Horton, Jr. S. H.
Treatment of creeping eruption with hetrazan. Report of 13 cases. U. S. Armed Forces Med. J. 1: 668-671. 1950.

Johri, L. N.
On the effect of hetrazan in the removal of Ascaris lumbricoides from the digestive tracts of young children. Proc. Indian Sci. Cong. 40 (Part III): 192. 1953.

Kanegis, L. A.
A new treatment for ascariasis in dogs and cats. J. A. V. M. A. 113: 579-581. 1948.

Kenney, M., and R. Hewitt.
Treatment of Bancroftian filariasis with hetrazan in British Guiana. Am. J. Trop. Med. 29: 89-114. 1949.

Kenney, M., and R. Hewitt.
Tratamiento de la filariasis bancrofti con hetrazan en la Guayana Inglesa. America Clinica. 14: 505-512. 1949.

Kenney, M., and R. Hewitt.
Psychoneurotic disturbances in filariasis, and their relief by removal of adult worms or treatment with hetrazan. Am. J. Trop. Med. 30: 895-899. 1950.

Kessel, J. F., G. C. Thooris, and B. Bambridge.
The use of diethylcarbamazine (hetrazan or notezine) in Tahiti as an aid in the control of filariasis. Am. J. Trop. Med. Hyg. 2: 1050-1061. 1953.

Knapp, S. E., and M. F. Hansen.
Observations on the anthelmintic action of carbon disulfide on the fowl ascarid, Ascaridia galli (Schrank). J. Parasit. 40 (Suppl.): 17-18. 1954.

Kushner, S., L. M. Brancone, R. I. Hewitt, W. L. McEwen, and Y. Subbarow.
The chemistry of piperazine compounds in the chemotherapy of filariasis.
Ann. N. Y. Acad. Sci. 50: 120-127. 1948.

Lagrange, E.
Essais de traitement des filarioses a Loa loa et O. volvulus par le diethylcarbamazine chl. Annales de la Societe Belge de Medecine Tropical. 29: 19-22. 1949.

Laurie, W.
Hetrazan in bancroftial filariasis. East African Med. J. 27: 263-268. 1950.

Lee, R. P.
The anthelmintic efficiency of piperazine adipate against Necascaris vitulorum (Goeze, 1782). Vet. Rec. 67: 146-149. 1955.

Leiper, J. W. G.
The piperazine compound V. 19 for the removal of Ascaris and Oesophagostomum from the pig. Vet. Rec. 596-599. 1954.

Loughlin, R. H., I. Rappaport, A. A. Joseph, and W. G. Mullin.
Treatment of human ascariasis with hetrazan. The use of a syrup containing 1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate (hetrazan). Lancet. 2: 1197-1200. 1951.

Lubran, M.
Estimation of hetrazan in body fluids. Nature. 164: 1135. 1949.

Lubran, M.
The blood concentration and urinary excretion of hetrazan. Trans. Roy. Soc. Trop. Med. Hyg. 43: 360. 1950.

Magath, T. B., and J. H. Thompson, Jr.
Diethylcarbamazine (hetrazan) in experimental trichinosis. Am. J. Trop. Med. Hyg. 1: 307-313. 1952.

MacKeith, R.
Piperazine in the treatment of threadworms. Brit. Med. J. 1: 521. 1954.

Maldonado, J. F., F. Hernandez Morales, F. Velez Herrera, and C. J. Thillet.
The role of hetrazan in the control of Filariasis bancrofti. Puerto Rico J. Pub. Health Trop. Med. 25: 291-299. 1950.

Manson-Bahr, P.
The action of hetrazan in Pacific filariasis. J. Trop. Med. Hyg. 55: 169-173. 1952.

Mapes, C. R.
Notes on the biology of Muellerius minutissimus Megnin, 1878, and a report on therapy with 1-diethylcarbamyl-4-methylpiperazine hydrochloride, caricide, in sheep. Unpublished Ph. D. thesis, Cornell University, Ithaca, New York, 1949.

Marshall, J.
A propos de la larva migrans. Bulletin de la Societe Francais de Dermatologie et de Syphiligraphie. 58: 497-498. 1951.

Martinez Baez, M.
Desintegracion de las microfilarias de Onchocerca volvulus en la piel de los pacientes oncocercosos tratados con hetrazan. Revista del Instituto de Salubridad y Enfermedades Tropicales. 10: 95-99. 1949.

Martinez Baez, M., and R. T. de la Pena.
Modificaciones in la eosinofilia de los oncocercosos consecutivas a la administracion de hetrazan. Medicina. 32: 497-499. 1952.

Martinez Baez, M.
Nuevas datos acerca de la accion del hetrazan sobre Onchocerca volvulus al estado adulto. Revista del Instituto de Salubridad y Enfermedades Tropicales. 13: 71-75. 1953.

Mason, M. M.
Sensitivity to caricide. Case report. North Am. Vet. 32: 767-768. 1951.

Mauze, J., and J. Languillon.
Le 1-diethyl-carbamyl-4-methylpiperazine ou Notezine dans la chylurie filarienne en Guadeloupe. Bulletin de la Societe de Pathologie Exotique. 43: 285-287. 1950.

Mazzotti, L.
Resultados negativos de la administracion del hetrazan en dos pacientes infectados con Mansonella ozzardi. Medicina. Revista Mexicana. 28: 317-318. 1948.

Mazzotti, L., and R. Hewitt.
Tratamiento de la oncocercosis par el cloruro de 1-dietilcarbamil-4-metilpiperazina (Hetrazan). Medicina. Revista Mexicana. 28: 39-42. 1948.

Mazzotti, L.
Observaciones sobre la oncocercosis en Mexico. Medicina. Revista Mexicana. 28: 217-224. 1948.

Mazzotti, L.
Estudio acerca del tratamiento de la oncocercosis. Anales de la Sociedad Mexicana de Oftalmologia. 23: 19-25. 1949.

Mazzotti, L.
Evaluacion de nuevas drogas para las filariasis. Boletin de la Oficina Sanitaria Panamericana. 28: 20-26. 1949.

Mazzotti, L.
Estudio acerca del tratamiento de la oncocercosis. Medicina. Revista Mexicana. 29: 1-5. 1949.

Mazzotti, L.
Observations on the use of hetrazan in onchocerciasis in Mexico. Am. J. Trop. Med. 31: 628-632. 1951.

Mazzotti, L.
Ensayos terapeuticos con hetrazan en la oncocercosis equina. Revista del Instituto de Salubridad y Enfermedades Tropicales. 13: 17-21. 1953.

McGaughey, C. A.
Preliminary note on the treatment of spirocercosis in dogs with a piperazine compound, caricide (Lederle). Vet. Rec. 62: 814-815. 1950.

McGaughey, C. A.
Preliminary note on the treatment of spirocercosis in dogs with a piperazine compound, caricide (Lederle). Indian Vet. J. 27: 454-451. 1951.

McGaughey, C. A.
Filariasis of dogs in Ceylon. Treatment with diethylcarbamazine. Vet. Rec. 64: 66-68. 1952.

McGregor, I. A., F. Hawking, and D. A. Smith.
Field trial of hetrazan. Trans. Roy. Soc. Trop. Med. Hyg. 46: 379. 1952.

Minning, W., and P. C. Ding.
Hetrazan-Wirkung bei Frosch-Filariasis (Icosiella neglecta). Zeitschrift fur Tropenmedizin und Parasitologie. 2: 535-543. 1951.

Minning, W., and P. C. Ding.
Hetrazan-Wirkung bei Mausetrichinose. Zeitschrift fur Tropenmedizin und Parasitologie. 3: 103-108. 1951.

Mojumdar, N. G., and B. Biswas.
Diethylcarbamazine in ascariasis. A preliminary report. Indian J. Pediatrics. 20: 25-27. 1953.

Montestruc, E., R. Blache, and R. Laborde.
Action du 1-diethyl-carbamyl 4-methylpiperazine sur Filaria ozzardi. Bulletin de la Societe de Pathologie Exotique. 43: 275-278. 1950.

Morley, J. S.
The chemotherapy of filariasis. Part I. Monoacyl derivatives of 1:2:3:4-tetrahydroquinoxaline. J. Chem. Soc. London. Year 1952: 4002-4008. 1952.

Morley, J. S.
The chemotherapy of filariasis. Part II. Monoacyl derivatives of 5:10-dihydro- and trans -1:2:3:4:5:10:11:12- octahydrophenazine. J. Chem. Soc. London. Year 1952: 4008-4014. 1952.

Mouriquand, G., E. Roman, and J. Coisnard.
Essai de traitement de l'oxyurose par la piperazine. J. de Medecine de Lyon. 32: 189-195. 1951.

Murgatroyd, F., and A. W. Woodruff.
Loiasis treated with hetrazan (Banocide). Lancet. 2: 147-149. 1949.

Murgatroyd, F., and A. W. Woodruff.
The effect of Banocide (hetrazan) on adult forms and microfilariae of Loa loa. Trans. Roy. Soc. Trop. Med. Hyg. 45: 365. 1950.

Murgatroyd, F.
Nuevo tratamiento de la filariasis. Bia Medico. 22: 2876-2877. 1950.

Nair, T. D.
Hetrazan in filariasis. A short report. Antiseptic. 46: 926-927. 1949.

Needham, J. G., P. S. Galtsoff, F. E. Lutz, and P. S. Welsh.
Culture methods for invertebrate animals. Ithaca: Comstock Publishing Company, 1937.

Newsome, J.
Hetrazan and stibophen, in schistosomiasis. Trans. Roy. Soc. Trop. Med. Hyg. 48: 445-446. 1954.

Nor El Din, G., and M. El Tamimi.
Hetrazan in the treatment of filarial manifestations. Jour. Royal Egypt. Med. Ass'n. 35: 826-834. 1952.

O'Brien, D. P.
Piperazine adipate in the treatment of roundworms. Brit. Med. J. 2: 246. 1954.

Oliver-Gonzalez, J., and R. I. Hewitt.
Treatment of experimental intestinal trichinosis with 1-diethylcarbamyl-4-methylpiperazine hydrochloride (Hetrazan). Proc. Soc. Expt'l. Biol. Med. 66: 254-255. 1947.

Oliver-Gonzalez, J., D. Santiago-Stevenson, and J. F. Maldonado.
Treatment of filariasis bancrofti with hetrazan. Follow-up observations fifteen months after treatment. J. A. M. A. 139: 308-309. 1949.

Otto, G. F., L. A. Jachowski, Jr., and J. D. Wharton.
Filariasis in American Samoa. III. Studies on chemotherapy against the nonperiodic form of Wuchereria bancrofti. Am. J. Trop. Med. Hyg. 2: 495-516. 1953.

Paez, H.
Etude d'un cas d'hematochylurie due a Wuchereria bancrofti traitement par le 1-diethyl-carbamyl-4-methyl piperazine (hetrazan). Annales de Parasitologie Humaine et Comparee. 26: 346-360. 1951.

Paez, H.
Hematochylurie due a Wuchereria bancrofti. (Erratum). Annales de Parasitologie Humaine et Comparee. 26: 495. 1951.

Paramanand Rao, D. S.
Spirocercosis in a dog. Indian Vet. J. 29: 548. 1953.

Perret-Gentil, A.
A propos du traitement de la filariose a Loa loa par le hetrazan. Praxis. 39: 41. 1950.

Piers, F.
Onchocerciasis with cutaneous lesions in a European. East African Med. J. 30: 111-113. 1953.

Poynter, D.
Piperazine adipate as an equine anthelmintic. Vet. Rec. 67: 159-162. 1955.

Purchase, H. S.
Hetrazan in the treatment of Dirofilaria immitis (Leidy 1856). Vet. Rec. 62: 34. 1950.

Puyuelo, R.
Note preliminaire sur l'epidemiologie et le traitement de l'onchocercose humaine a O. volvulus en pays Mossi Le 3.799 R. P. (Notezine). Bulletin de la Societe de Pathologie Exotique. 42: 558-561. 1949.

Puyuelo, R.
Note preliminaire sur l'onchocercose volvulaire africaine en pays Mossi Le 3.799 R. P. (Notezine). Bulletin Medical de l'Afrique Occidentale Francaise. 6: 147-151. 1949.

Puyuelo, R.
Le traitement de l'onchocercose en Haute-Volta. Premiers essais chimiotherapiques par la 1-diethylcarbamyl 4-methylpiperazine. Bulletin de la Societe de Pathologie Exotique. 43: 462-470. 1950.

Raman, T. K., B. Ramamurthy, and S. Pinakapani.
Hetrazan (1-diethylcarbamyl-4-methylpiperazine hydrochloride) (Diethylcarbamazine) in the treatment of filariasis (Wuchereria bancrofti). J. Indian Med. Ass'n. 19: 163-172. 1950.

Reardon, J.
Piperazine in treatment of roundworm. Brit. Med. J. 2: 872-873. 1954.

Reeves, P. A.
Filariasis (heart worm). J. Roy. Army Vet. Corps. 23: 132-133. 1952.

Ridley, H., and J. Anderson.
A case of onchocerciasis in London and its treatment with hetrazan. Brit. J. Ophthal. 34: 688-690. 1950.

Riedel, B. B.
The effect of caricide on Ascaridia galli in chickens. Poult. Sci. 29: 437-443. 1950.

Riedel, B. B.
Group treatment with caricide for ascariasis in poultry. J. Parasit. 37: 318-319. 1951.

Riedel, B. B.
A simplified method of culturing Ascarid eggs. Trans. Am. Micros. Soc. 70: 57-58. 1951.

Riveroll Noble, B.
Consideraciones acerca del ocular en la oncocercosis. Manifestaciones oculares durante el tratamiento con hetrazan. Anales de la Sociedad Mexicana de Oftalmologia. 23: 26-31. 1949.

Roberts, F. H. S.
Studies on the biology and control of the large roundworm of fowls Ascaridia galli (Schrank 1788) Freeborn 1923. Queensland Department of Agriculture and Stock, Animal Health Station Bulletin 2. 1937.

Rousset, P.
Essai de prophylaxie et de traitement de la dracunculose par la notezine en Adrar. Bulletin Medical de l'Afrique Occidentale Francaise. 9: 351-368. 1952.

Ruiz Reyes, F.
Resultados de la primera aplicacion de hetrazan en la zona oncocercosa de Oaxaca. Boletin Epidemiologico. 12: 195-199. 1948.

Ruiz Reyes, F., and S. Ceniceros Gonzalez.
La dietilcarbamazina (hetrazan) como vermifugo. Boletin Epidemiologico. 13: 93. 1949.

Ruiz Reyes, F.
Observaciones con la dietilcarbamazina (hetrazan) en la zona oncocercosa de Oaxaca. Medicina. Revista Mexicana. 30: 225-230. 1950.

Ruiz Reyes, F.
Observaciones con la dietilcarbamazina (hetrazan) en la zona oncocercosa de Oaxaca. Boletin Epidemiologico. 14: 138-142. 1950.

Ruiz Reyes, F., A. Torres Munoz, and L. Cervantes Garcia.
Algunas observaciones con la dietilcarbamazina (hetrazan) como vermifugo. Revista de Paludismo y Medicina Tropical. 2: 35-39. 1950.

Ruiz Reyes, F.
Ensayo terapeutico en la oncocercosis con Carbilazina y Carbilista. Boletin Epidemiologico. 14: 153-157. 1950.

Ruiz Reyes, F.
Consideraciones sobre la dietilcarbamazina como profilactico en la oncocercosis. Medicina. Revista Mexicana. 31: 163-164. 1951.

Ruiz Reyes, F.
Terapeutica de la oncocercosis, uso actual de drogas especificas, suraminas y dietilcarbamazina. Medicina. Revista Mexicana. 33: 377-384. 1953.

Santiago-Stevenson, D., J. Oliver-Gonzalez, and R. I. Hewitt.
Treatment of filariasis bancrofti with 1-diethylcarbamyl-4-methylpiperazine hydrochloride (Hetrazan). J. A. M. A. 135: 708-712. 1947.

Santiago-Stevenson, D., J. Oliver-Gonzalez, and R. I. Hewitt.
The treatment of filariasis bancrofti with 1-diethylcarbamyl-4-methylpiperazine hyerochloride (Hetrazan). Ann. N. Y. Acad. Sci. 50: 161-170. 1948.

Schneider, J.
Traitement de la filariose a F. loa par la 1-diethyl-carbamyl 4-methylpiperazine. Bulletin de la Societe de Pathologie Exotique. 43: 270-275. 1950.

Schneider, J.
Etat actuel de la therapeutique de la filariose a F. loa par le 1-diethyl-carbamyl-4-methyl-piperazine. Acta Tropica. 8: 345-359. 1951.

Schobinger von Schowingen, R.
Further experiences in the treatment of filariasis with hetrazan. Acta Tropica. 9: 270-271. 1952.

Seiden, R.
Modern anthelmintics for farm animals and poultry. Mfg. Chemist. 21: 279-283. 1950.

Shoho, C.
Further observations on epizootic cerebrospinal nematodiasis: I. Chemotherapeutic control of the disease by 1-diethylcarbamyl-4-methylpiperazine citrate: preliminary field trial. Brit. Vet. J. 108: 134-141. 1952.

Shoho, C.
Prophylaxis and therapy in epizootic cerebrospinal nematodiasis of animals by 1-diethylcarbamyl-4-methyl-piperazine dihydrogen citrate: report of a second field trial. Vet. Med. 49: 459-462. 1954.

Shookhoff, H. B., and K. G. Dwork.
Treatment of Loa loa infections with hetrazan. Am. J. Trop. Med. 29: 589-593. 1949.

Shumard, R. F., and D. F. Eveleth.
A preliminary report on the anthelmintic action of piperazine citrate on Ascaridia galli and Heterakis gallinae in hens. Vet. Med. 50: 203-205. 1955.

Sims, S. R.
Piperazine in the treatment of threadworms. Brit. Med. J. 2: 1432. 1953.

Singh, J., N. G. S. Raghavan, B. G. Misra, A. K. Krishnaswamy, and R. Roy.
A note on the ascaricidal value of hetrazan. Indian Med. Gaz. 87: 353-356. 1952.

Sloan, J. E. N., P. A. Kingsbury, and D. W. Jolly.
Preliminary trials with piperazine adipate as a veterinary anthelmintic. Brit. J. Pharmacy and Pharmacol. 6: 718-724. 1954.

Sotolongo, F.
Tratamiento ad la strongyloidiasis con el hetrazan. Communicacion previa. Revista Kuba de Medicina Tropical y Parasitologia. 6: 72-73. 1950.

Sotolongo, F.
Teniasis y hetrazan. Revista Kuba de Medicina Tropical y Parasitologia. 6: 73. 1950.

Standen, O. D.
Experimental chemotherapy of oxuriasis. Brit. Med. J. 2: 757-758. 1953.

Stefanopoulo, F. J., and J. Schneider.
Essais de traitement de la filariose a F. loa par la 1-diethylcarbamyl 4-methylpiperazine. Comptes Rendus des Seances de la Societe de Biologie. 142: 930-931. 1948.

Stefanopoulo, G. J.
The symptomatology, diagnosis and treatment of filariasis due to Loa loa. Proc. Ann. Meeting, Brit. Med. Ass'n. 282-287. 1950.

Stewart, H. W., R. J. Turner, J. J. Denton, S. Kushner, L. M. Brancone, W. L. McEwen, R. I. Hewitt, and Y. Subbarow. Experimental chemotherapy of filariasis. IV. The preparation of derivatives of piperazine. J. Organ. Chem. 13: 134-143. 1948.

Stones, P. B.
Successful treatment of loiasis with diethylcarbamazine in low dosage. West African Med. J. 1: 174-180. 1952.

Streiff, E. B.
L'aspect biomicroscopique d'une microfilariose cornceme. Bulletins et Memoires de la Societe Francaise d'Ophtalmologie. 62: 161-165. 1949.

Tasque, M.
Essais de traitement de 17 cas de filariose a Loa-loa par le 3.799 R. P. Bulletin de la Societe de Pathologie Exotique. 42: 556-557. 1949.

Teunissen, G. H. B.
Caricide (hetrazan) als middel tegen rondwormen. Tijdschrift voor Diergeneeskunde. 75: 589-590. 1950.

The chemotherapy of filariasis. (Annotation). Brit. Med. J. 2: 32-33. 1948.

The new filaricide. Calcutta Med. J. 47: 328-330. 1950.

The specific treatment of filariasis. J. Trop. Med. Hyg. (Annotation). 52: 1. 1949.

Thomson, F. A.
Treatment of ascariasis with diethylcarbamazine. Trans. Roy. Soc. Trop. Med. Hyg. 46: 679. 1952.

Todd, A. C., M. F. Hansen, G. W. Kelley, and Z. N. Wyant.
On treatment of larval Strongylus vulgaris (bloodworms) in situ. J. A. V. M. A. 15: 473-474. 1949.

Todd, A. C., W. M. Insko, Jr., G. W. Kelley, and M. F. Hansen.
Aggregate worm infections and growth of broilers. Trans. Am. Micros. Soc. 48: 256-260. 1949.

Todd, A. C., and M. F. Hansen.
The economic import of host resistance to helminth infection. Am. J. Vet. Res. 12: 58-63. 1951.

Todd, A. C., and L. P. Doherty.
Treatment of ascariasis in horses in central Kentucky. J. A. V. M. A. 119: 363-367. 1951.

Tubanqui, M., and B. Cabrera.
Studies on filariasis in the Phillipines. II. Treatment of Bancroftian filariasis with hetrazan. Acta Medica Philippina. 6: 1-7. 1949.

Torres, J. M.
Treatment of strongyloidosis with hetrazan. Report of 12 cases. Boletin de la Asociacion Medica de Puerto Rico. 42: 30-33. 1950.

Tugwell, R. L., and J. E. Ackert.
On the tissue phase of the life cycle of the fowl nematode Ascaridia galli (Schrank). J. Parasit. 38: 277-278. 1952.

Turpin, R., R. Cavier, and J. Savaton-Pillet.
Traitement de l'oxyurose par le di (phenylacetate) de piperazine (D. P. P.). Therapie. 7: 108-113. 1952.

Vaidyanathan, S. N.
Spriocerca lupi infection in dogs. A few cases treated with hetrazan (Lederle). Indian Vet. J. 29: 244-247. 1952.

Vanbreuseghem, R.
Difficultes du diagnostic de la filariose a Loa-loa et son traitement par la deithylcarbamazine. Annales de la Societe Belge de Medecine Tropicale. 30: 71-77. 1950.

Van De Erve, Jr. J.
Creeping eruption, a systemic therapy. J. Investigat. Dermatol. 12: 69-79. 1949.

Vaughan, A. W.
A report on canine filariasis. Vet. Rec. 64: 454-455. 1952.

Vazquez Martinez, S., and A. Morales Cisneros.
Accion del hetrazan sobre la microfilaria intranodular. Boletin Epidemiologico. 14: 130-132. 1950.

Villalpando del Valle, E.
La dietilcarbamazina (hetrazan) en el tratamiento de la parasitosis intestinal. Boletin Medico del Hospital Infantil. 9: 165-170. 1952.

Wanson, M.
Essai de traitement curatif de la filariose a Loa-loa et de la filariose aperiodique par les derives de la piperazine. Annales de la Societe Belge de Medecine Tropicale. 29: 73-80. 1949.

Wanson, M.
L'hetrazan dans la periode d'invasion de l'onchocercose. Annales de la Societe Belge de Medecine Tropicale. 29: 85-89. 1949.

Wanson, M., G. Borgers, and L. Pannier.
Activite de l'hetrazan sur Dipetalonema streptocerca. Annales de la Societe Belge de Medecine Tropicale. 30: 91-95. 1950.

Wanson, M., and J. Rodhain.
Developpement abortif de Loa papionis chez divers arthropodes. Insucces du traitement a la diethylcarbamazine dans la filariose diurne du babouin. Annales de la Societe Belge de Medecine Tropicale. 33: 177-184. 1953.

Werner, J. J.
Caricide in the treatment of strongyloidiasis in the dog. Vet. Med. 44: 496-497. 1949.

White, R. H. R., and O. D. Standen.
Piperazine in the treatment of threadworms in children. Report on a clinical trial. Brit. Med. J. 2: 755-757. 1953.

White, R. H. R., and O. D. Standen.
Piperazine in the treatment of threadworms. Brit. Med. J. 2: 1272-1273. 1953.

White, R. H. R., and O. D. Standen.
Piperazine in the treatment of threadworms. Brit. Med. J. 1: 460. 1954.

White, R. H. R.
Ascariasis treated with piperazine hydrate. Lancet. 315-316. 1954.

Wilson, T.
Hetrazan in the treatment of filariasis due to Wuchereria malayi. Trans. Roy. Soc. Trop. Med. Hyg. 44: 49-66. 1950.

Winckel, W. E. F., and J. Fros.
Contribution to the geographical pathology of Surinam. 9. Acute lymphadenitis caused by Wuchereria bancrofti. Documenta de Medicina Geographica et Tropica. 4: 361-365. 1952.

Woodruff, A. W.
The liver before and after treatment with banocide (hetrazan) in a patient suffering from loiasis. Trans. Roy. Soc. Trop. Med. Hyg. 44: 369. 1951.

Woodruff, A. W.
Destruction of microfilariae of Loa loa in the liver in loiasis treated with banocide (hetrazan). Trans. Roy. Soc. Trop. Med. Hyg. 44: 479-480. 1951.

Wright, W. H., and J. M. Schaffer.
Critical anthelmintic tests of chlorinated alkyd hydrocarbons and a correlation between the anthelmintic efficacy, chemical structure, and physical properties. Am. J. Hyg. 16: 325-428. 1932.

Ziegler, C. G.
Treatment of canine filariasis with caricide, diethylcarbamazine. J. A. V. M. A. 116: 209-210. 1950.

THE EFFECTS OF CARICIDE AND OTHER ANTHELMINTICS ON THE TISSUE PHASE LARVAE OF ASCARIDIA GALLI (SCHRANK, 1788)

by

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AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

The economic import of the larval migratory phase in the life cycle of Ascaridia galli, the large roundworm of fowls, has been suggested by various workers. While control of the adult stage of this nematode has been achieved through anthelmintic medication, treatment of the tissue-penetrating phase with existing drugs has been unsuccessful.

A new approach to this problem was suggested by the recent appearance of several newly synthesized piperazine compounds which have shown considerable promise in the treatment of filariasis and ascariasis in man, and for certain parasitic infections in animals.

The experimental design utilized throughout the course of this study involved the testing of the effects produced by two piperazine derivatives and carbon disulfide on parasitic infections in laboratory animals. White Rock and New Hampshire chickens were used exclusively as test animals. They were obtained as day old birds from a commercial source. At fourteen days of age, each bird received a known number (either 100 $\pm$ 10 or 75 $\pm$ 5) of embryonated Ascaridia galli ova.

A total of five tests were performed, utilizing 420 chickens. In the first three experiments, the chickens were divided into four groups. Groups A and C received a predetermined dosage of the test anthelmintic, while Groups B and D were maintained as parasitized but untreated controls. Beginning on the eleventh day after infection, and continuing for ten days thereafter, two Group A and two Group B birds were killed for autopsy every 24 hours. On the twenty-first day after infection, all Group C and Group D birds were also killed and examined. In Tests 4 and 5, only the experimental groups C and D were used.

Worms present in the lumen of the intestine of each chicken were collected by flushing a stream of water through the gut. In addition, the

intestines of all Group A and B birds were subjected to an artificial digestion process to remove the tissue-penetrating larvae that were imbedded in the intestinal wall.

As criteria of the effect of the anthelmintic being tested, comparisons were made between numbers and lengths of lumen larvae (in Groups C and D), and numbers of both lumen and tissue phase larvae and lengths of lumen larvae (in Groups A and B) recovered from a parasitized treated group of chickens and its corresponding parasitized untreated control. In addition, daily weight records of all Group C and D chickens were kept from the date of treatment until they were slaughtered for autopsy. All data were statistically analyzed to determine significance. Results were as follows:

1. In Test 1, a single 25 mgm oral dose of Caricide (1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate) did not significantly reduce the number of either lumen or tissue phase larvae in Group A birds.

2. This treatment, or some other factor, did significantly reduce the length of the lumen larvae from Group A birds.

3. No significant variation was found between either numbers or lengths of lumen larvae, or weight of the host birds, in Groups C and D.

4. In Test 2, an oral dose of 12.5 mgm of Caricide per bird for eight consecutive days did not significantly reduce the number of either tissue phase or lumen larvae in Group A, or the number of lumen larvae in Group C.

5. No significant variation was found between weights of Group C and D chickens.

6. Worms recovered from both Group A and Group C were significantly longer than those present in the corresponding control birds.

7. In Test 3, a continuous daily low-level dosage of Caricide at the

rate of approximately 12.5 mgm per bird per day did not significantly reduce numbers of either tissue phase or lumen larvae in Groups A or C.

8. The average weight gains exhibited by Group C chickens in Test 3 were on the borderline of being significantly greater than those of their control group (Group D).

9. An oral dosage of 25 mgm of Compound 180-C (1-carbethoxy-4-methyl-piperazine hydrochloride) per bird per day, for eight consecutive days, did not produce any significant variation in worm numbers, or in weight of the treated chickens, in Test 4.

10. A single oral dose of 0.05 ml of carbon disulfide was ineffective in influencing either host worm populations or weight increases.