Anthiomaline and Neostibosan in the Treatment of Filariasis[1]

(Dirofilaria immitis)

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I. ANTHIOMALINE

A. Its Effect upon the Microfilariae

ALTHOUGH several investigators[3] had reported temporary reduction in numbers of microfilariae with the use of the trivalent antimony compound, anthiomaline, Brown[4] was the first to obtain complete eradication of circulating microfilariae, a finding that was observed in two of eleven patients with *Wuchereria bancrofti*.

In view of the foregoing observation, it was decided to study in detail the effect of this drug on filaria-infected dogs and to determine, if possible, (a) its effect, if any, upon the microfilariae, and (b) its action upon the adult filarids.

Methods and procedures. Six dogs naturally infected with *Dirofilaria immitis* were used. They were divided into equal groups and the groups treated at different times. The animals ranged in weight from 15.9 to 29.1 kg., their microfilarial counts varying from 23 to 1,485 microfilariae per 20 cmm. of blood. Six untreated dogs were left as controls.

Each dog received daily 0.8 mg. of antimony per kg. of body weight as anthiomaline (lithium antimony)[5] thiomalate by intravenous injection, five times a week, until the microfilarial count became zero. Two further doses were then given, and weekly microfilarial

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1. Received for publication August 4, 1947.

2. Senior Research Fellow.

3. R. N. Chopra and S. S. Rao, Chemotherapy of filarial infection. Indian J.Med.Res., **27**:549–562, 1939.

F. Hawking, Chemotherapy of filariasis *in vivo* and *in vitro*. J.Trop.Med.and Hyg., **43**:204, 1940.

J.O. Poyton, Annual Report of the Institute for Medical Research of the Federated Malay States, Kuala Lumpur, pp. 80–85, 1938.

H. de Choisy, Observations d'un cas de microfilariose loa traité par l' antimonio-thiomalate de lithium. Rev.de Méd.et d'Hyg. Trop., **29**:294–296, 1937–38.

R. N. Chopra and S. S. Rao, Studies in the treatment of filariasis. Indian M.Gaz., **64**:130–139, 1929.

4. H. W. Brown, The treatment of filariasis (*Wuchereria bancrofti*) with lithium antimony thiomalate. J.A.M.A., **125**:952–958, 1944.

5. The anthiomaline was provided through the courtesy of Merck and Company, Inc.

counts made for twelve successive weeks.[6] At the end of that time, the animals were sacrificed and autopsied.

During therapy, blood samples were obtained from each dog at the same time every day, five days a week. Approximately 1.5 cc. of blood were withdrawn in each sample, one cc. of which was injected into a sterile test tube, containing 1 mg. of heparin, and used for determining the microfilarial count and microfilarial motility; the remainder was utilized in making five thin blood smears. Following the withdrawal of the blood sample, each dog was given its calculated dose of anthiomaline.

After administering the drug to all the dogs in the group, each blood sample was immediately examined to determine the degree of activity of the microfilariae. These observations were made at the same time every day, for each blood sample, 30, 45, and 60 minutes, respectively, elapsing between the time of blood withdrawal and motility determination.

For the study of microfilarial activity, a modification of the Brady and Lawton[7] technique was used. One cc. of 0.85 percent NaCL was pipetted and placed into a Sedgewick-Rafter counting cell. To this were added 20 cmm. of the heparinized blood that had been shaken for three minutes to insure uniform distribution of the microfilariae. The resulting suspension was then examined, and a record made of the degree of motility of the microfilariae in the sample.

Following these observations, the number of microfilariae present in 20 cmm. of each blood sample was counted by the Brady and Lawton technique.

The thin blood smears were fixed in a 1:1 mixture of absolute alcohol and ether for five minutes, dried, and stained with Meyer's hematoxylin for seven minutes. Following momentary differentiation in acid alcohol, the slides were washed until blue, dried, and examined with the oil immersion lens and a calibrated 10x eyepiece.

In dogs with microfilarial counts of 100 and over, 75 microfilariae were carefully studied, while in dogs with lower counts, all the microfilariae found on the five slides were examined for changes in structure.

Prior to the initiation of therapy, determinations of microfilarial counts, microfilarial motility, and microfilarial morphology had been made five times a week, for two weeks, by the procedures and techniques already outlined. These determinations were used in conjunction with observations on the untreated dogs to evaluate the results obtained.

6. One dog was killed on its second microfilaria-free day.

7. F. J. Brady and A. H. Lawton, A new method for the quantitative estimation of microfilariae in blood samples. J.Parasitol., 30:34, 1944.

In addition to the above procedures, both before and during therapy, blood samples were left at room temperature until all the microfilariae in wet preparations were non-motile. Blood smears were then made, and the apparently dead microfilariae were compared with those found in the blood smears made during the course of treatment.

Results. The circulating blood of all six dogs became microfilaria-free, and remained so, during the twelve-week observation period that followed the cessation of therapy. The microfilariae disappeared from the peripheral blood of four dogs in ten days, and later, in the two remaining (Chart 1). However, these last two dogs had increased in weight and, when the antimony dosage was readjusted accordingly, their microfilarial counts dropped to zero in seven and ten days, respectively. The total dose of antimony required to eradicate circulating microfilariae varied from 126 to 277 mg. and appeared to be unrelated to the magnitude of the microfilarial count (Table 1).

TABLE 1

Dosage of Intravenous Injections of Anthiomaline at 0.8 mg. Sb/Kg. of Body Weight Required to Eradicate Circulating Microfilariae (D. immitis) *from the Blood Stream of Dogs*

Dog No.	*Weight of Dog (Kg.)*	*Daily Dose of Anthiomaline (Cc.)*	*Daily Dose of Antimony (Mg.)*	*Length of Treatment (Days)*	*Total Dose of Antimony (Mg.)*
211	24.5	2.0	20	8	160
214	18.7	1.5	15	12	180
220	15.9	1.3 (x9)	13 (x9)	19	257
	17.2	1.4 (x10)	14 (x10)		
223	17.7	1.4	14.0	9	126
224	29.1	2.3	23.0	6	138
226	13.2	1.1 (x15)	11.0 (x15)	22	277
	17.2	1.6 (x7)	16 (x7)		

No toxic reactions to anthiomaline were observed except for mild anorexia.

Motility of the microfilariae did not appear to be altered by treatment, nor were any detectable morphological changes observed in them during therapy. The nerve ring, excretory pore, and anal pore were distinct, and no appreciable differences in the size, shape, or configuration of these structures were noted. The microfilariae ranged in width from 4.8 to 8.0 u. at the level of the nerve ring in both control and treated dogs.

Occasionally, microfilariae with large prominent oval cells were found. These cells were most numerous below the level of the excre-

tory pore and probably represented the subcuticula cells described by Fülleborn.[3] The proportion of microfilariae with these prominent cells remained unchanged during therapy.

Another observation was that a small number of microfilariae present in the blood smears for any one day appeared to be shorter and thicker than the others on the slide. These short forms usually assumed the position of the letter "C" or "J" and most likely represented young microfilariae. They were present in the same proportions in the blood smears of both control and treated dogs, their number remaining unchanged during the course of therapy.

On the other hand, smears made from blood left at room temperature until all the microfilariae became non-motile and, therefore, presumably dead, showed microfilariae that were usually extended, flattened, and folded. Their nuclei were shrunken, and anatomical structures were not discernible. Cuticular striations were prominent. These changes were never seen in fresh preparations but occurred in blood samples from both treated and untreated dogs upon standing.

Microscopic examination of the tissues of the dog killed on the second day after the disappearance of microfilariae revealed the presence of whole and fragmented microfilariae in the lungs and kidneys. In the lungs, the microfilariae were found within the alveolar septa and, rarely, free within an alveolus (Figs. 1 and 2). Though the majority were fragmented and undergoing phagocytosis by large mononuclear cells, occasional whole extended forms were seen. Surrounding an occasional fragmented form, narrow zones of proliferative mononuclear cells were observed. Similar, but larger and often confluent, patches of mononuclear proliferation were present throughout both lungs. A slight lymphocytic infiltration was associated with this proliferation.

Large numbers of microfilariae were found in the interstitial tissue of the papillae and upper medulla of the kidney (Fig. 3). Here, extended but apparently dead microfilariae and fragmented forms were found admixed with amorphous eosinophilic granular material. Microfilariae were also seen occasionally within the blood vessels of the papillae.

The liver, spleen, lymph nodes, heart, adrenal, and thyroid were examined, but no microfilariae were found. Neither were they found in the internal organs of the dogs killed twelve weeks after they became microfilaria-free.

8. F. Fülleborn, Filariosen des menschen. Handb.d.Path.Mikroorg., 6:1044–1225, 1929.

Discussion. From the results of treatment it would appear that anthiomaline is a rapid, effective, and relatively non-toxic microfilaricide. Since none of the microfilariae examined during the course of therapy showed abnormalities in motility or morphology, it would seem that non-motile, dead, or dying forms are rapidly removed from the circulating blood. In view of the presence in various organs of fragmented dead microfilariae, some of which were undergoing phagocytosis while others were being walled off by zones of proliferating mononuclear cells, the other possibility—that the microfilariae which disappeared from the blood stream remained alive but lost the power of returning to the circulation—seems unlikely. Numerous dead and fragmented microfilariae were also found within the interstitial tissue of the kidney papillae and medulla. These findings substantiate the hypothesis proposed years ago by Rodenwalt[9] and Fülleborn[10] that the kidney and lungs are most likely microfilarial graveyards, where dead and dying forms are eliminated from the circulating blood.

Summary. Six dogs infected with *Dirofilaria immitis* were treated with intravenous injections of anthiomaline, five times weekly, using a dosage of 0.8 mg. of antimony per kg. of body weight. All the dogs so treated were microfilaria-free at the end of the third week of treatment and remained so during a twelve-week observation period.

The total dose of antimony required to eradicate the circulating microfilariae varied from 126 to 277 mg. of antimony, given in six to 22 days, and did not appear to be related to the magnitude of the microfilarial count.

The motility of the microfilariae present in the peripheral blood appeared unchanged during the course of therapy. There were no detectable morphological changes.

Microfilariae were found within the lungs and kidneys of a dog that was autopsied on the second day after disappearance of these forms from the blood stream. Those in the lung were most numerous in the alveolar septa, apparently undergoing phagocytosis.

Except for transitory anorexia during treatment, no toxic reactions to the administration of anthiomaline were observed.

B. Its Effect Upon the Adult Filarids

In view of the report by Ashburn *et al.*[11] to the effect that histo-

9. E. Rodenwalt, Studien zur morphologie der mikrofilarien. Cited by F. Fülleborn, *ibid.*
10. F. Fülleborn, *op. cit.*
11. L. L. Ashburn, T. L. Perrin, F. J. Brady, and A. H. Lawton, Histologic changes in

logic changes were found in the reproductive system of female *Dirofilaria immitis* recovered from dogs treated with various trivalent antimony compounds, it was decided to conduct a comparable study on the adult filarids recovered from dogs treated with anthiomaline. In this way, it was thought that information might be obtained concerning the mode of action of the drug and its effect on the tissues of adult worms.

Accordingly, five of the six dogs[12] rendered microfilaria-free by anthiomaline therapy were killed and autopsied at the end of the twelve-week observation period, following the disappearance of microfilariae. The present report is based upon the post-mortem findings in these dogs and on the pathologic alterations detected in the reproductive system of the male and female filarids.

Methods. The animals were sacrificed by electrocution and immediately autopsied. Their thoracic vessels were ligated, and the hearts and lungs removed *en masse* to prevent loss of the filarids by migration from their location in the heart or pulmonary arteries. The large thoracic and abdominal vessels were also dissected and carefully examined for the presence of worms. The gross appearance of the abdominal organs was noted, and specimens of liver, spleen, adrenals, kidneys, heart, lungs, thyroid, and lymph nodes were taken for microscopic examination.

The heart was opened, and the location, number, sex, viability, and motility of any adult filarids were recorded. The pulmonary arteries were dissected down to their terminal branches and examined for the presence of dead or live worms, the largest number of filarids being usually found within the right ventricle of the heart though moderate numbers were encountered within the pulmonary arteries, especially within the middle branch of the right pulmonary artery.

When found, adult worms were placed in a beaker of physiological saline. At the completion of the autopsy, they were removed, wound around glass rods, fixed in 4 percent aqueous solution of formaldehyde, and later embedded, sectioned, and stained according to the method used by Ashburn *et al.*[13] Three male and three female worms from each dog were usually examined microscopically.

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ovary and uterus of live *Dirofilaria immitis* recovered from dogs treated with trivalent antimony compounds. Arch.Path., **40**:334–339, 1945.

F. Huytra, J. Marek, and R. Manniger, *Special Pathology and Therapeutics of the Diseases of Domestic Animals.* 4th English ed. (Chicago:Alex Eger, 1938).

B. M. Underhill, *Parasites and Parasitosis of Domestic Animals* (New York:The Macmillan Company, 1920).

12. The sixth dog (223) was killed on the second day it became microfilaria-free.

13. L. L. Ashburn *et al., op. cit.*

Post-mortem findings. The hearts of all six dogs were essentially normal in appearance, both grossly and on microscopic examination.

Grossly, the lungs of dogs 214 and 220 were congested at the bases, bilaterally. In all but dog 220, there were one or two well circumscribed, raised, pale yellow firm areas measuring from 2 mm. to 1.5 cm. in main diameter, usually at the periphery of the left lower, right lower, or right middle lobe of the lungs. Sections of these areas revealed well-delineated abscesses containing yellow granular material and, occasionally, fragments of dead filarids.

On microscopic examination, the above areas were found to be branches of pulmonary arteries that had lumina obstructed by granulation tissue masses, frequently canalized and moderately or densely infiltrated with lymphocytes, plasma cells and, not infrequently, polymorphonuclear lymphocytes (Fig. 4). The centers of some of these granulomatous masses were necrotic and densely infiltrated with large numbers of polymorphonuclears. In dog 226, the lumen of a dilated pulmonary vessel contained an adult female worm. The wall of the vessel was fibrosed and infiltrated with lymphocytes, showing one small area of subendothelial necrosis. The uteri of this worm were empty, but the ovaries contained ova that showed degenerative changes (Fig. 5). In three other pulmonary vessels of this same dog, central and eccentric calcified foci, apparently representing remnants of dead worms (Fig. 6), were seen within the granulomatous masses obstructing the lumina of the vessels.

Scattered throughout the lung parenchyma of all dogs were many small and large, often confluent, patches of interstitial thickening of the alveolar septa and occasional alveolar obliteration caused by proliferation of mononuclear cells. These last were so numerous and tightly packed in some places that cellular margins were indistinct. Associated with this process was a moderate lymphocytic infiltration.

The liver, spleen, thyroid, adrenals, and kidneys of all the dogs were normal in appearance on gross examination.

On microscopic examination, the livers of all animals were essentially normal except for the presence of moderate numbers of lymphocytes and mononuclear cells in the portal areas of dog 223. There was a decrease in the number of hepatic cells in the centrolobular areas, with a moderate increase in collagen in the latter regions in dog 224. There was an increase in the number of lymphocytes and plasma cells in the red pulp of the spleen of dog 223 and an increase in the number of polymorphonuclear leucocytes in the same region of dog 220.

Except for the presence of several small areas of hyperplasia in dog 224, the thyroid glands were normal microscopically. The adrenals were also normal. In the kidneys, small and scattered interstitial foci of lymphocytes and plasma cells were found in the cortex and medulla of dogs 220, 223, 224, and 226. Furthermore, fairly large dense collections of lymphocytes were present in the corticomedullary region of the last two dogs (224 and 226). Glomerular fibrosis was seen occasionally in the kidney of dog 223, while cellular casts were often noted within the lumina of the collecting tubules. The bronchial lymph nodes of all dogs were essentially normal in appearance except for the presence of occasional red blood cells within the sinuses of a few nodes. No microfilariae were found in any of the internal organs or bronchial lymph nodes.

Study of adult filarids. Dog 223. Nine female and five male filarids, all alive and motile, were found. One female and four males were present in the lumen of the pulmonary artery just above the cusps of the pulmonic valve; the remainder were found within the right ventricle of the heart.

Microscopic examination of the females revealed normal ovaries. The uteri of two of the worms were empty, though the presence of occasional oxyphilic debris was noted in the posterior level of one. In the uteri of one of the other worms, numerous multicellular ova—some of which showed degenerative changes consisting of cytoplasmic oxyphilia and nuclear pyknosis—were present in the posterior levels, while large numbers of microfilariae were found in the anterior levels. The microfilariae, however, showed nuclear pyknosis, shrinkage, occasional fragmentation, and cytoplasmic oxyphilia.

Microscopic examination of the male worms revealed no significant alterations.

Dog 224. Three male and three female worms, all alive and motile, were found within the right ventricle.

On microscopic examination, the ovaries of two worms appeared essentially normal. The ovary of the third worm, however, showed some ova with degenerative changes characterized by markedly oxyphilic cytoplasm, loss of cellular detail, and cellular boundaries. No normal ova or microfilariae were present within the uteri of any of the worms. In two filarids, a few degenerating unicellular ova, with indistinct cell boundaries and nuclei, were seen in the posterior levels of the uterus (Fig. 7 and 8), while only a few scattered unicellular ova admixed with mucoid material were present within the anterior uterine levels. Another female worm showed essentially

the same changes except that the uteri were empty in the anterior level.

Of the males examined microscopically, only one appeared to be normal. The other two showed karyorrhexis and, in some regions, actual necrosis of the spermatogenic cells (Figs. 9 and 10). The gonoduct of these two worms was empty in the anterior levels.

Dog 226. Eight male and ten female worms were recovered. Seven live males and seven live females were present within the right ventricle. A male and one female were found within the lumen of the branch of the pulmonary artery in the right lower lobe of the lung, both tightly coiled, flat, opaque, non-motile, and appearing to be dead. Two other females resembling those just described were found in the branch of the pulmonary artery in the left lower lobe. In addition, the branch of the left pulmonary artery, supplying the medial aspect of the lower lobe, was plugged for its greater length by a mass of tightly coiled dead worms.

The ovaries of the female worms were essentially normal on microscopic examination, except for the presence of a rare oxyphilic cell with karyorrhectic nucleus. No developing ova past the unicellular stage and no microfilariae were seen within the uterus. Except for the presence of a little amorphous debris and an occasional oxyphilic degenerated or necrotic ovum, the anterior levels of the uteri were empty.

The males had normal testes and showed normal developing spermatozoa in the mid and posterior portions of the gonoduct; however, only a few of these developing structures were present in the anterior levels, and they appeared somewhat edematous and marginally indistinct.

Dog 211. Two female and four male worms, all dead, were found within the right ventricle.

Microscopic observations were difficult to interpret, as the worms were in a poor state of preservation. However, ante-mortem necrosis of unicellular ova in the posterior uterine levels of one of the worms had evidently occurred and, in the other, the uteri were empty.

Of the males, one was in a relatively good state of preservation and showed no significant alterations in the appearance of its reproductive system.

Dog 214. Two male and four female filarids, all motile, were found. Of these, one male and three females were present within the right ventricle; a male and a female were discovered in the right pulmonary artery.

Microscopic examination of the ovaries of the four female worms

revealed a morphologically normal ovary in one worm, with a few ova showing degenerative changes that consisted of oxyphilia and indistinctness of cellular margin and cellular detail in the ovaries of two worms (Figs. 11 and 12). A rare necrotic ovum was found in the ovary of the fourth worm.

The female with the essentially normal ovary contained microfilariae in various stages of development within the anterior uterine level. These microfilariae, however, showed degenerative changes ranging from slightly increased cytoplasmic oxyphilia and nuclear pyknosis to fragmentation. The most anterior uterine level of this worm was empty except for the presence of necrotic debris and a few fragmented multicellular ova. The uteri of the other three worms were also empty except for the presence of oxyphilic necrotic debris and an occasional necrotic ovum.

The males showed no significant alterations on microscopic examination.

Discussion and summary. Studies were made on five dogs, which were infected with *Dirofilaria immitis* and which had been given anthiomaline in a dosage of 0.8 mg. of antimony per kg. of body weight, five times a week, until the microfilariae disappeared from their peripheral blood. The animals were sacrificed and autopsied at the end of their twelfth microfilaria-free week.

The only pathological changes of note were those seen in the lungs and kidneys. In the lungs, these consisted of old and recently thrombosed branches of the pulmonary arteries, in which fragments of dead filarids were occasionally seen, and scattered patches of interalveolar thickening and alveolar obliteration caused by the proliferation of mononuclear cells. In the kidneys there were small, scattered interstitial foci of lymphocytes and plasma cells in the cortex and medulla. None of these findings could be attributed to the use of anthiomaline, as they had been often found in untreated infected dogs.

No microfilariae or their remnants were seen in any of the internal organs or bronchial lymph nodes.

Of the 36 filarids found on autopsy, 26 were alive and ten were dead. Six of the dead worms were found within the right ventricle of one dog; a question as to whether their death had been caused by the action of anthiomaline can be raised. However, the significance of four dead adults found in the remaining dogs is debatable, as small numbers of dead filarids are sometimes found in untreated dogs. It would therefore seem that the filaricidal action of anthiomaline in the dosage used in these experiments is not great.

Microscopic examination rarely revealed pathologic changes in the ovaries of the female filarids. Such changes were found only in three worms and consisted of oxyphilia, nuclear karyorrhexis, or necrosis of an occasional cell. A lack of normal developing forms beyond the unicellular stage, complete absence of embryos and microfilariae, and empty uteri, were the usual findings in the female filarids. One worm, however, did contain microfilariae in various stages ofdevelopment; these showed oxyphilia, nuclear pyknosis, and occasional fragmentation.

The testes of the male worms appeared normal. However, occasional alterations in the appearance of the developing spermatozoa within the anterior levels of the gonoduct were seen in some of the male worms. These changes consisted of edema, indistinctness of cellular margins and, occasionally, actual necrosis of a few of the developing spermatozoa.

The usual findings of histologically normal ovaries and testes with ova showing degenerative changes, retarded development and differentiation, and spermatozoa revealing degenerative changes, make one question the permanency of the effect of anthiomaline on dogs. In order to accurately evaluate the therapeutic effect of the drug, a longer time interval than the one employed in this experiment (12 weeks) should elapse between the disappearance of the microfilariae from the circulating blood and the time of autopsy.

II. NEOSTIBOSAN

A. Its Effect upon the Microfilariae

Neostibosan, a pentavalent antimony compound, was reported by Chopra and Rao,[14] in 1939, to produce a temporary reduction in the microfilarial count of patients harboring *Wuchereria bancrofti*. Several years later, Culbertson and Rose[15] used this same drug to treat filaria-infected cotton rats. Following repeated injections of neostibosan, they found that the microfilarial count of treated animals gradually fell to zero and that the adult filarids found at autopsy were dead. As they also observed that microfilariae were present in the circulating blood in decreasing numbers for weeks and months after the adults were dead, they stated that the effect of the drug appeared to be directed mainly against the adult worms. In collaboration with Oliver González,[16] these same investigators later

14. R. N. Chopra and S. S. Rao, *op. cit.*

15. J. R. Culbertson and H. M. Rose, Chemotherapy of filariasis in the cotton rat by administration of neostam and neostibosan. J.Pharmacol. and Exper.Therap., **81**:189–196, 1944.

16. J. T. Culbertson, H. M. Rose, and J. Oliver González, Chemotherapy of human filariasis by the administration of neostibosan. 1st.report.Am.J.Trop.Med., **25**:271–274, 1945; 2nd. report. *Idem*, **25**:403–406, 1945.

employed neostibosan in the treatment of 35 persons infected with *W. bancrofti* and reported the complete disappearance of the circulating microfilariae in 20 of their patients. As in the cotton rat, the rate of microfilarial decrease was slow; in some instances, several months elapsed after treatment before complete microfilarial eradication was effected. In view of the similarity of these findings with those observed in the cotton rat, Culbertson and Rose have hypothesized that it is likely that the effect of neostibosan in humans is also principally directed against the adult worms. This, of course, is difficult to prove. However, it is interesting to note that none of the patients successfully treated by Culbertson *et al.* has shown symptoms which may suggest the presence of dead filarids.

The present experiments were undertaken in an effort to obtain additional information as to whether neostibosan acts upon the microfilariae, the adult filarids, or both. It was decided to use dogs infected with *Dirofilaria immitis* as the experimental laboratory animals. This section of the paper deals with the observations upon the microfilariae of these dogs during and after treatment; section B concerns observations upon the adult filarids found in the dogs on autopsy.

Methods and procedures. Six dogs, naturally infected with *Dirofilaria immitis*, were treated at different times in two groups of three each. Their microfilarial counts before treatment ranged from 7 to 1,103 microfilariae per 20 cmm. of blood. Six untreated dogs served as controls.

Neostibosan is an organic pentavalent antimony complex consisting of p-aminophenylstibonic acid, p-acetylaminophenylstibonic acid, antimonic acid, and diethylamine, and contains from 41 to 44 percent antimony. The dosages given were based on the assumption that the neostibosan[17] employed contained 43 percent antimony, i.e., that the antimony content of one 0.3 g. ampoule of neostibosan was 0.13 g. The drug was administered intravenously in a 5 percent aqueous solution and given immediately after preparation, injected slowly over a five to six minute interval.

Each dog had two courses of treatment. During the first course of therapy, the dogs received 10 mg. of antimony per kg. of body weight, as neostibosan, five days a week for 14 doses. The total dose of antimony given per animal therefore ranged from 1.75 to 3.64 g. and that for neostibosan from 4.06 to 8.40 g. As none of the animals had become microfilaria-free by the end of the eleven and a half

17. Supplied through the courtesy of The Winthrop Chemical Company, Inc.

weeks following completion of the first course of neostibosan, they were all retreated.

During the second course of treatment, each dog again received 10 mg. of antimony per kg. of body weight five days a week. All but one dog received 18 doses of the drug. This last animal developed intractable vomiting following the sixteenth injection, which necessitated discontinuance of therapy.

As four of the six dogs had gained weight during the eleven and a half weeks following completion of the first course, the dosage of neostibosan was adjusted accordingly. The total neostibosan dose per animal for the second course of treatment ranged from 5.91 to 10.80 g., i.e., from 2.56 to 4.68 g. of antimony. The total dose for the combined two courses ranged from 10.27 to 19.20 g. of neostibosan and from 4.44 to 8.32 g. of antimony.

Of the six dogs given the second course of treatment, four survived. One of the other two was found dead on the second day following the completion of therapy, and the other died on the fourth day after its sixteenth and last injection. Autopsy was performed on these dogs immediately on discovery. The blood of the other three dogs (217, 218, and 219), which constituted the first group treated, was examined biweekly for twelve weeks following the disappearance of microfilariae, after which the animals were also sacrificed and examined at autopsy.

During therapy, blood samples for the determination of microfilarial motility and microfilarial counts, and for making blood smears for the study of microfilarial morphology, were obtained from a vein of each dog at the same time each day, five days a week. Approximately 1.5 cc. of blood were withdrawn with a sterile tuberculin syringe. One cc. of this was injected into a sterile test tube, containing one mg. of heparin, and used for determining the microfilarial motility and count. The remainder was utilized in making five thin blood smears. Following the withdrawal of the blood sample, each dog received its calculated dose of neostibosan.

After administration of the drug to all the dogs in the group, the heparinized blood sample of each animal was immediately examined to determine the degree of activity of the microfilariae. These observations were made at the same time each day for each of the blood samples, 30, 40, and 60 minutes, respectively, elapsing between the time of blood withdrawal and observation for motility. Using the technique described by the author in part I, the activity of the microfilariae present in 20 cmm. of blood was studied.

After the above determinations were completed, the number of

TABLE 2

First Course of Therapy

Microfilarial Counts in Six Dogs with Dirofilaria immitis *Given 14 Injections of Neostibosan in Dosages of 10 mg. Sb./Kg. Body Weight, Daily, Five Times a Week*

Dog Number	Weight (Kg.)	G. of Drug Given	Number Microfilariae in 20 cmm. Blood at Designated Time											Percentage Reduction in Microfilariae 11 Weeks after End of Treatment
			Before Treatment	At End of Treatment	Weeks After End of Treatment									
					1	2	3	4	5	6	7	8	9	10	11	
217	12.5	4.06	128	149	139	86	92	109	116	81	63	44	38	44	39	—69
218	17.9	5.74	7	6	3	1	5	6	6	2	3	3	5	3	3	—57
219	20.0	6.44	209	451	438	330	174	194	137	167	173	119	121	35	62	—70
221	26	8.40	356	359	217	201	226	178	152	111	89	49	29	25	25	—93
222	16	5.18	1,103	1,112	988	1,005	997	992	921	887	846	697	617	673	532	—52
225	18	5.81	340	273	171	114	101	89	76	64	47	33	19	11	9	—97

microfilariae found in 20 cmm. of each blood sample was counted. Brady and Lawton's[18] technique, in which acetic acid (2 percent) was substituted for hydrochloric acid (0.1*N*) as a lysing fluid, was used for this estimation.

All blood smears for the study of microfilarial morphology were fixed in absolute alcohol and ether and stained with Meyer's hematoxylin according to the method outlined in part I. When the microfilarial count for any dog was over 100, 75 microfilariae were carefully examined. In animals with lower counts, all the microfilariae found on the five slides were carefully studied for any change in structure.

For two weeks prior to the initiation of therapy, determinations of microfilarial count, microfilarial motility, and microfilarial morphology were made daily, five times a week, for each dog to be treated, which observations were later used in conjunction with those made on other untreated dogs to evaluate the result obtained. In addition to the above procedures, blood samples obtained before, during, and after treatments were left at room temperature until all microfilariae seen in wet preparations were non-motile and therefore presumably dead. Blood smears of these samples were then made, and the appearance of the microfilariae in them was compared with the appearance of those found in the blood smears made during and after treatments.

Results. Following a slight and brief reduction immediately after the initiation of therapy, the microfilarial count of most of the dogs rose abruptly and then gradually fell (Charts 3 and 4). By the end of eleven weeks, the number of circulating microfilariae had decreased 52 to 97 percent (Table 2).

The second course of neostibosan, which was commenced eleven and a half weeks after the completion of the first, resulted in the complete eradication of microfilariae from the blood stream of all but one dog. The latter became acutely ill after the sixteenth injection and, despite cessation of the drug and supportive measures, died four days later. The total doses of neostibosan administered during this second course of treatment ranged from 5.91 to 10.80 g. (Tables 3 and 4).

Of the five dogs rendered microfilaria-free by neostibosan, three (217, 218, and 219) showed no ill effects from the drug except for transient anorexia during treatment. These dogs received for the two courses a total dosage of neostibosan ranging from 10.27 to

18. F. J. Brady and A. H. Lawton, *op. cit.*

TABLE 3

Second Course of Therapy

Microfilarial Counts in Six Dogs with Dirofilaria immitis *Given 18 Doses of Neostibosan in Dosage of 10 mg. Sb./Kg. Body Weight, Daily, Five Times a Week*

Dog Number	Weight (Kg.)	No. of Injections	G. of Drug Given	Number Microfilariae in 20 cmm. Blood at Designated Time											
				Before Treatment	At End of Treatment	Days After End of Treatment									12 Weeks after End of Treatment
						1	2	4	7	8	10	12	14	16	18	20	
217	14.9	18	6.21	39	19	15	17	2	2	0	0	0	0	0	0	0	0
218	18	18	7.47	4	3	2	2	0	0	0	0	0	0	0	0	0	0
219	24.9	18	10.35	58	9	12	11	5	0	0	0	0	0	0	0	0	0
221	26	18	10.80	22	2	0	0	[a]									
222	16	16	5.91	519	319	284	239	[b]									
225	22.9	18	9.54	12	0	0	0	[b]									

[a] Killed.
[b] Found dead; treatment was discontinued after the 16th injection in dog 222.

TABLE 4

Dosage Chart for Six Dogs with Dirofilaria immitis *Treated with Neostibosan, Daily, Five Times a Week All Dogs Received 10 mg. of Sb./Kg. Body Weight*

Dog Number	First Course of Therapy						Second Course of Therapy						Total Dosage	
	Weight	Daily Dose of Antimony (g.)	Daily Dose of Neostibosan (cc.)	No. of Injections	Total Dose of Neostibosan (g.)	Total Dose of Antimony (g.)	Weight (Kg.)	Daily Dose of Antimony (g.)	Daily Dose of Neostibosan (cc.)	No. of Injections	Total Dose of Neostibosan (g.)	Total Dose of Antimony (g.)	Total Dose of Neostibosan (g.)	Total Dose of Antimony (g.)
217	12.5	0.125	5.8	14	4.06	1.75	14.9	0.149	6.9	18	6.21	2.69	10.27	4.44
218	17.9	0.179	8.2	14	5.74	2.51	18.0	0.180	8.3	18	7.47	3.24	13.21	5.75
219	20.0	0.200	9.2	14	6.44	2.80	24.9	0.249	11.5	18	10.35	4.49	16.79	7.29
221	26.0	0.260	12.0	14	8.40	3.64	26	0.260	12.0	18	10.80	4.68	19.20	8.32
222[a]	16.0	0.160	7.4	14	5.18	2.24	16.0	0.160	7.4	16	5.91	2.56	11.09	4.80
225[b]	18.0	0.180	8.3	14	5.81	2.52	22.9	0.290	10.6	18	9.54	4.13	15.35	6.65

[a] Animal found dead on second day following end of treatment.
[b] Animal found dead on fourth day following 16th injection of second treatment.

16.79 g. (4.44 to 7.29 g. of antimony). The microfilariae disappeared from their peripheral blood four, seven, and eight days after the end of the second course of neostibosan (138, 125, and 135 days, respectively, from the initiation of treatment). The blood of these dogs was examined biweekly for twelve weeks after they became microfilaria-free, but in no case did microfilaria reappear.

Of the dogs in the second group (221, 222, and 225), all showed ill effects from the second course of treatment. Dog 221 became microfilaria-free on the first day following the end of treatment and had no unusual symptoms except for a slight decrease in activity after the last three injections. However, following the last dose of neostibosan, the animal became sluggish and suddenly developed pronounced anorexia and adipsia. Despite the daily administration of parenteral glucose and one million units of penicillin intravenously (given empirically), the dog failed to improve. Fearing that the animal would die, it was sacrificed and autopsied on the third day following the completion of therapy.

Dog No. 222 was found acutely ill on the day after the sixteenth injection. It became sluggish and had intractable vomiting, pronounced anorexia, and adipsia. Parenteral fluids and penicillin (1 million units) were also administered daily but, despite slight improvement, it was found dead four days after the sixteenth and last injection of neostibosan. Dog 225 became microfilaria-free on the last day of treatment. It showed no apparent ill effects but was found dead two days later.

The blood from all three dogs was injected into six young hamsters but failed to produce symptoms of illness in any of them during a three-week observation period. Complement-fixation test for *Leptospira canicola* was positive (1:10,000) for all three animals, but this titer was thought to be indicative of past, rather than current, leptospiral infection since dogs with acute leptospirosis usually show *L. canicola-positive* titers of 1:250,000, and over. In addition, dogs dying of leptospirosis ordinarily have acute general systemic symptoms which these dogs did not show. It is also significant that all dogs had been given distemper vaccine and quarantined for three weeks before being brought to the place where they were kept until the end of the experiment. There was no possibility of contact between the animals, as they were all kept in single cages and brought in separately for injections. None of the other 20 to 30 animals in this same room became ill or died before, during, or after the death of these three.

Pathologic changes found in the three animals on post-mortem examination were as follows:

Grossly. The heart was normal except for congestion of the coronary vessels and the presence of basal and apical petechial hemorrhages and ecchymoses in dog 225. There was congestion of the lung bases, bilaterally, in all dogs, with scattered petechial hemorrhages in the lung parenchyma of dogs 222 and 225. The liver of all animals appeared congested, and that of dog 225 had an icteric tint. The kidneys of all the animals showed varying degrees of congestion; interstitial hemorrhages of moderate to marked severity were present in dogs 221 and 225. The other organs showed no significant alterations.

Microscopic examination revealed that pertinent findings were limited to the liver, lungs, and kidneys. In the liver, pathologic alterations were seen, ranging from lymphocytic infiltration and prominent mononuclear proliferation to deposition of hyaline material and the presence of necrotic debris in the central areas of the hepatic lobules of dog 221 to pronounced and advanced central necrosis of the hepatic lobules of dogs 222 and 225 (Fig. 13). This was associated with congestion of the portal vessels and periportal lymphocytic infiltration in these dogs.

The kidneys were congested and showed severe interstitial hemorrhages in the medulla of dogs 225 and 221 (Fig. 14). In addition, radial fibrotic bands, infiltrated with round cells, were present in the cortex of 222 and 225. The kidney of dog 221 showed the pathologic findings of chronic pyelonephritis.

The spleen of one animal was normal in appearance. That of another showed irregular congestion of a moderate degree and contained a few focal collections of plasma cells, a few megakaryocytes, and polymorphonuclear leucocytes; the latter predominated in the subcapsular regions. The spleen of the third dog showed only an increase in plasma cells.

The thyroids were essentially normal in all cases except for a decreased amount of colloid within some of the acini of dog 222. Furthermore, this same dog showed numerous fragmented and some whole microfilariae in the lungs (Figs. 15 and 16). The whole forms, most of which assumed an extended position, were seen within the alveolar septa and rarely within the alveoli, which usually contained a finely granular amorphous exudate and a few round cells and mononuclears. The fragmented forms, which predominated, were frequently seen among large mononuclear phagocytes in the interalveolar septa. Occasionally, early fibroblastic proliferation was seen around small foci of mononuclears containing engulfed fragments of microfilariae. The alveoli adjoining the areas, in which phagocytosis

was taking place, frequently contained moderate amounts of finely granular oxyphilic exudate.

Numerous microfilariae were also found in the liver of dog 222 (Fig. 17). Some were in an extended position; others were fragmented; and still others were short and C-shaped resembling young microfilarial forms. All of them were found free within the hepatic sinusoids, with small numbers of mononuclear cells and round cells surrounding them. Phagocytosis of microfilarial fragments was not seen within the liver. One microfilaria was found in an extended position within the splenic pulp just at the periphery of a follicle.

The changes seen in the adult filarids found in these dogs will be discussed in the following section.

All the microfilariae noted in the blood of dogs during and following treatments were actively motile. They showed violent thrashing and serpentine movements, moving frequently across the microscopic field to disappear from view. No differences in the activity of microfilariae from control dogs and dogs being treated, or following treatment, were observed.

No detectable morphological changes were encountered in the microfilariae from dogs during treatment or following the first or second course of treatment. The microfilariae from both treated and untreated dogs measured from 4.8 to 8.0 u. at the level of the nerve ring, with the greatest number falling into the 6.4 to 8.0 range. There was no change in width, during or after therapy, in the microfilariae from the treated animals.

There were also no changes noticed at any time in the shape, appearance, or configuration of the nuclear column, nerve ring, excretory pore, and anal pore of microfilariae from the treated dogs. However, some of the microfilariae from both treated and untreated groups had prominent subcuticular cells,[19] while others did not.

In contrast, the microfilariae found in smears made from blood that was permitted to stand at room temperature until all the microfilariae within it became non-motile, and therefore presumably dead, showed a strikingly different appearance. These microfilariae assumed an extended position and only rarely showed a slight bend or curvature. The cross rings of the cuticle were prominent and the nuclei were no longer arranged in a compact column but were dispersed throughout the body width. The usual landmarks, such as nerve ring, excretory pore, and so forth, were usually obscured by

19. F. Füllerborn, *op. cit.*

this nuclear dispersion and were no longer identifiable as such.

Discussion. From the results obtained, it appears that neostibosan, administered in a total dosage varying from 4.06 to 8.40 g. (1.75 to 3.64 g. of antimony) in a single short intensive course, is capable of producing a gradual and progressive decrease over a period of weeks in the number of circulating microfilariae of *D. immitis* without untoward effects on the treated dogs. Eleven weeks after the end of treatment, the microfilarial count was reduced from 52 to 97 percent. This finding is comparable to that reported by Culbertson[20] *et al.*, who noted a 64 to 100 percent reduction in the microfilarial count of persons infected with *W. bancrofti* three months after the end of a short intensive course of neostibosan, in which a total dosage ranging from 9.5 to 15.59 g. (41 to 6.7 g. of antimony) was utilized.

Although a second intensive course of neostibosan given eleven and a half weeks after the completion of the first treatment resulted in the complete eradication of circulating microfilariae in five of the six dogs[21] treated, one of them died, and a second became ill and was subsequently killed. Post-mortem examination of these animals revealed pronounced pathological alterations, consisting of degeneration and extensive necrosis of the central areas of the hepatic lobules and hemorrhages in the medulla of the kidneys and in the lungs. These findings resemble those reported for chronic antimony poisoning.

The total dose of antimony received by the animals which died was not much larger than that received by those which survived (4.80 to 8.32 g. as compared with 4.44 to 7.29 g., respectively). Nor in the animals which died were there signs of previous hepatic or renal damage, such as cirrhosis or chronic glomerular nephritis that might have caused abnormal accumulation or inadequate excretion of antimony.

The sudden onset of symptoms of sluggishness, anorexia, adipsia (and in one case, intractable vomiting) was striking and occurred at the end of treatment in all cases. If due to neostibosan therapy, the sudden onset of symptomatology and its rapid termination in death two to four days later resembled the toxic and usually fatal reactions to the administration of a second course of an antimonial reported by Lochte and Putscher, Werkgartner, Borgzinner, and others.[22]

20. J. T. Culbertson *et al.*, *op. cit.*

21. The sixth dog, 222, was found dead four days after its sixteenth and last injection. Its microfilaria count was 207 microfilariae per 20 cmm. of blood at the time of death.

22. T. Lochte and W. Putschar, Ein Fall von tödlicher Fuadinvergiftung. Deutsche Ztschr. f.d.ges.gerichtl.Med., **20**:471–481, 1933.

Toxic reactions occurring during the second course of an antimonial have been attributed by various authors to abnormal accumulations of antimony, caused by individual differences in cumulation and excretion, even when no pathologic lesions were demonstrable in the liver or kidneys, or to individual hypersensitivity.

In any case, the results reported here would seem to indicate, as suggested by Schmidt and Peter,[23] that a second course of antimony compound should be given only after a long interval and, even then, with caution. Certainly, if any signs of toxicity occur during a second course of drug, treatment should be stopped immediately.

The presence of fragmented and phagocytosed microfilariae in a dog that died during treatment before its count became zero, and their absence in the dogs that died or were killed on their third microfilaria-free day, suggest that the removal of dead microfilariae is both rapid and complete.

Summary. Six dogs given a single intensive course of neostibosan in a dosage of 10 mg. of antimony per kg. of body weight, five times a week for 14 doses, showed a 52 to 97 percent reduction in microfilarial count by the end of eleven weeks after treatment.

Although a second intensive course of neostibosan given eleven and a half weeks after the end of the first treatment resulted in a complete disappearance of microfilariae in five of six dogs, one of them died and marked symptoms occurred in a second, which was subsequently killed. There was little difference between the total dosage of antimony received by the dogs that died and those that survived (4.80 to 8.32 g. and 4.44 to 7.29., g., respectively).

Untoward signs occurred in three dogs (two were microfilaria free) at the end of treatment; these consisted of sluggishness, anorexia, adipsia, and, in one case, intractable vomiting. Death of two of these three dogs occurred two and four days, respectively, following the appearance of symptoms; the third was killed four days after onset of symptoms.

Post-mortem findings in these three dogs consisted of degeneration and central necrosis of the hepatic lobules and hemorrhages in the medulla of the kidneys and in the lungs, similar to pathologic changes reported for chronic antimony poisoning.

In the dog that died before the disappearance of circulating microfilariae, numerous microfilariae were found in the lungs and liver, and one was seen in the spleen. Of those found in the lungs, a large number were fragmented and were being phagocytized by mononuclear cells. No microfilariae were found in the internal organs or lymph nodes in the other two animals.

The three dogs that survived became microfilaria-free four, seven, and eight days, respectively, following the end of treatment. Biweekly blood examinations during a twelve-week observation period, which followed, failed to reveal a recurrence of microfilariae.

No differences were observed in the motility or morphology of microfilariae found in the blood of control dogs and those present in the blood stream of dogs under treatment, or following treatment.

A. Werkgartner, Eine tödliche Antimosanvergiftung. Wien klin. Wchnschr., **40**:478–799, 1927.

R. Borgzinner, Über eine tödliche vergiftung durch Antimosan. Zentralb.f.in.Med., **48**: 1137–1140, 1927.

23. Schmidt and Peter, Zur therapie der multiplen sklerose. Therap.Berichte, Vol. 12, 1929.

B. Its Effect upon the Adult Filarids

Following the completion of the study on the effects of neostibosan upon the microfilariae of *Dirofilaria immitis*, observations were made to determine whether these effects were due to (1) a direct filaricidal action of the drug upon the adults, or (2) whether the changes observed following its use were attributable to pathologic alterations in the reproductive system of the male and female filarids.

Methods and procedures. Six dogs with microfilariae were given two intensive courses of neostibosan eleven weeks apart. The drug was administered to all the animals in a dosage of 10 mg. of antimony per kg. of body weight, five days a week for 14 and 18 doses, respectively.

Of the six dogs treated, three became microfilaria-free three, seven, and eight days, after the second course of treatment and remained so during the twelve-week observation period that followed, at the end of which time they were electrocuted and autopsied. Of the other three dogs, one was microfilaria-free the last day of treatment but was found dead two days later. Another became ill after its sixteenth injection of the second drug course, dying four days later with a microfilarial count of 207 per 20 cmm, while the third became microfilaria-free one day after the end of treatment and was killed two days later.

At autopsy, sections of heart, liver, lungs, spleen, adrenals, thyroid, kidneys, and lymph nodes were taken for microscopic examination. The branches of the pulmonary arteries were dissected down to their terminal branches, and the larger thoracic and abdominal vessels carefully examined for the presence of filarids. When found, these were observed for viability and motility and were then fixed,

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sectioned, and stained according to the technique reported by Ashburn *et al.*[24]

Autopsy findings. Autopsy of the three dogs which were killed at the end of their twelfth microfilaria-free week revealed essentially normal gross findings in two of them. In the third, 219, the lungs were firm and spongy. Numerous petechial hemorrhages and grey white translucent areas, measuring approximately 1-2 mm. in diameter, were found scattered throughout the parenchyma. These protruded above the cut surface and were most numerous in the lower lobes. In addition, a well-demarcated area about 1 cm. in main diameter and containing thick yellow amorphous material was found in the right middle lobe.

Microscopic examination of the organs of these animals showed no significant alterations in the heart, thyroid, and bronchial lymph nodes. The lungs of all three dogs contained scattered patchy areas of interalveolar thickening, which was associated with lymphocytic infiltration. In dog 219 many sharply demarcated areas were present, wherein dense collections of polymorphonuclear leucocytes could be seen. Some of these areas had necrotic centers, and others revealed occasional structures with irregular concentric lamellation. Two branches of the pulmonary artery were seen with lumina obliterated by granulomatous masses of the type found in canine filariasis. There was a slight increase in the connective tissue of the liver in dog 217 and early cirrhosis in that of 219. The kidneys of two animals were essentially normal except for interstitial foci of lymphocytes and plasma cells. Dog 218, however, showed numerous radial fibrotic bands infiltrated with moderate to large numbers of round cells present in the cortex. The only change of note in the spleen was that found in dog 219, wherein intrafollicular areas of hemorrhage were present. The adrenals were normal except for those of dog 217; in this dog there was a decrease in the number of cells in the perimedullary cortex and an increase in the amount of collagen resulting in a reticular appearance.

Of the two dogs autopsied on their third microfilaria-free day, dog 225 showed petechial hemorrhages and ecchymoses in the heart, while dilatation of the right auricle and ventricle was noted in 221. The lungs of both animals were congested bilaterally at the base; scattered petechial hemorrhages were present in 225. Their livers appeared congested; that of dog 225 was icteric. The spleens were soft and mushy in consistency, and the medullae of the kidneys of

24. L. L. Ashburn *et al.*, *op. cit.*

both diffusely hemorrhagic. In addition, the body musculature of dog 225 showed an icteric tint.

Microscopically, the hearts of these two animals appeared normal except for congestion of the coronary vessels in 225. Some interalveolar thickening associated with lymphocytic infiltration was present in their lungs. Furthermore, patchy areas of intra-alveolar hemorrhage and edema appeared in dog 225. In dog 221 the lumina of some of the branches of the pulmonary arteries were obliterated by granulomatous masses of the type commonly found in canine filariasis. Pathologic changes occurred in the livers of both animals; these changes ranged from extensive areas of central necrosis of the hepatic lobules in 225 to lymphocytic infiltration, mononuclear proliferation, and deposition of hyaline and necrotic debris in the central areas of the hepatic lobules of dog 221. Although congestion and hemorrhages were seen in the medulla of the kidneys of both dogs, these were more severe in dog 225. In the latter animal, radial fibrotic bands, infiltrated with lymphocytes and plasma cells, were also found. Dog 221 showed small patchy areas of fibrosis containing moderate numbers of lymphocytes and occasional polymorphonuclear leucocytes. There was an increase in the number of plasma cells in the spleens of both animals and an increase in the number of polymorphonuclear leucocytes, especially in the subcapsular region of 221. The adrenals, thyroid, and bronchial lymph nodes revealed no significant alterations.

Study of adult filarids. Dog 217. Ten male and two female worms, all alive and very active, were found within the right ventricle coiled around the chordae tendineae of the tricuspid valve. Microscopic examination of the males revealed no significant alterations, the only observation of note being the comparative emptiness of the gonoduct in its most anterior levels.

The ovaries of the females appeared normal. The uterus of one female was empty except for small numbers of unicellular ova in the mid and posterior portions, the majority of which showed pronounced oxyphilia and loss of cellular detail; some forms were frankly necrotic. In another female, a fused mass of degenerative and necrotic unicellular ova were seen in the posterior level of the uterus; also small numbers of multicellular forms in the mid and anterior levels. The majority of these multicellular forms were abnormal with varying degrees of oxyphilia and fragmentation. No embryos or microfilariae were present in either female, though many spermatozoa were found in the distal uterine levels of both worms.

Dog 218. Two live and very active female worms were found

within the right ventricle, wound around the chordae tendineae of the tricuspid valve.

Microscopic examination of both revealed normal ovaries. The posterior levels of the uterus of one of the females contained many unicellular ova that appeared well-preserved except for a slight oxyphilia. Progressing anteriorly, the number of unicellular ova decreased. Those found in the mid portion of the uterus showed signs of degeneration, while the few present in the most anterior uterine levels were shrunken, strongly oxyphilic, and frankly necrotic. The other female showed essentially the same changes except that there were more necrotic ova in the more anterior levels.

Dog 219. Four male and three female worms, all alive, appeared within the right ventricle. Four other live females were recovered from the lumen of the right pulmonary artery. Microscopic examination of the males revealed no significant alterations except for the absence of spermatozoa, or their immediate precursors, from some of the more anterior levels of the gonoduct.

The ovaries of all females examined were normal in appearance. The most posterior levels of the uteri of two females contained many unicellular ova that were also essentially normal, except for occasional oxyphilia. With further progression anteriorly, however, the number of ova rapidly decreased and more pronounced degenerative changes made their appearance. The few ova seen within the anterior uterine levels were shrunken, strongly oxyphilic, and definitely necrotic. A third female contained only a few ova. These were present in the posterior uterine level, were unicellular in type, and showed degenerative changes. The rest of the uterus was empty except for a rare necrotic ovum. No normal developing forms, embryos, or microfilariae could be found in any of the females.

Dog 221. Six female (5 alive and one dead) and 4 male worms, all alive, were found in the right auricle. Two live females and one live male were recovered from the middle branch of the right pulmonary artery. Three dead females, one live female, and one live male were recovered in the branch of the right pulmonary artery to the upper lobe of the lung.

Microscopically, large numbers of morphologically normal spermatozoa were present in the anterior levels of the gonoducts of all the males examined. In the mid portion, however, were developing spermatozoa that stained poorly and showed blurring of cellular margins and lack of cellular detail. Actual necrosis was evident in one area. Within the posterior levels of the gonoducts the developing spermatozoa all appeared to be essentially normal.

In the females, the ovaries of all but one worm appeared normal. Although numerous necrotic unicellular ova were found in the most posterior levels of the uterus of one female, the remainder of the uterine tract was empty except for a rare necrotic unicellular ovum. The uteri of another female was also empty except for a few uni- and multicellular ova in the most anterior levels, with slight degenerative changes. In another female, the ovary showed some vacuolization, blurring of the cellular margins, and indistinctness of cellular detail. Numerous multicellular forms were found within the uteri of this worm. Those in the posterior uterine level were occasionally frankly necrotic, while those in the more anterior levels showed pyknosis and some cytoplasmic oxyphilia. Multicellular ova, embryos, and a few tightly coiled and well-formed microfilariae were present in the most anterior levels. However, all of these forms showed varying degrees of cytoplasmic oxyphilia, pyknosis of nuclei, indistinctness of cellular outlines, and in some cases, fragmentation. A fourth female examined contained moderate numbers of frankly necrotic and unicellular ova in the posterior uterine level and moderate numbers of degenerative and necrotic multicellular forms in the mid portion; the anterior levels of the uterus were empty.

Dog 225. Six males and one female, all alive, were found in the right heart. All worms were stained a bright yellow (this dog had an icteric tint to the body musculature at autopsy).

On microscopic examination, the males showed the presence of morphologically well-differentiated and normal appearing spermatozoa within the anterior levels of the gonoducts. In one worm, however, a few necrotic cells were seen in the most posterior levels. In another, cellular debris without any normal developing spermatozoa was found in the mid portion of the tube.

One of the female worms showed vacuolization of the cells of the ovary. Numerous necrotic ova were present in the posterior uterine levels of this worm. Toward the anterior of the uteri, the unicellular forms became progressively fewer and were eventually replaced by multicellular ova, the majority of which showed pronounced degenerative changes or necrosis. Within the anterior uterine level, moderate numbers of degenerative, necrotic, and fragmented multicellular ova were observed along with an occasional fragmented microfilaria, with pyknotic nucleus and oxyphilic cytoplasm. Another female exhibited essentially the same changes except that the multicellular ova did not show as marked degenerative changes. A third worm also had similar pathologic alterations, but in addition, numerous

spermatozoa were found within its posterior uterine level.[25]

Dog 222.[26] Twelve males (10 alive, one dead, and one non-motile) and nine females (6 alive and 3 dead) were found within the right ventricle. The only change of note found in the males, on microscopic examination, was the presence of some possibly degenerative changes in the spermatozoa in the most anterior levels of the gonoduct; these spermatozoa appeared not to have completely differentiated morphologically. In one male, the anterior levels of the gonoduct were empty except for the presence of granular basophilic material.

One of the females had numerous necrotic unicellular ova in the most posterior uterine levels. In the more anterior portions, moderate numbers of multicellular forms were seen with the majority showing degeneration or necrosis. Small numbers of tightly coiled microfilariae, some of which had pyknotic nuclei and oxyphilic cytoplasm, were present in the more anterior levels. Fragmented microfilariae alone, with only a rare intact but degenerative form, were present in the most anterior uterine level. In another female, focal necrosis of one of the ovarian convolutions had occurred. The posterior level of the uterus of this worm contained necrotic and degenerative ova. Multicellular forms were seen more anteriorly. Although some of these showed oxyphilia, cellular fragmentation, or nuclear pyknosis, a good many appeared to be essentially normal. In one cross-section of the worm, spermatozoa were found within the posterior level of the uterus. In still another worm, necrotic unicellular ova were observed in the posterior levels, multicellular forms in the mid portion (these occasionally showed signs of degeneration), and well-formed microfilariae in the more anterior levels. Numerous fragmented microfilariae were present in the most anterior portions of the uteri. The ovaries of all but one female were normal; the findings in the latter were described above.

Discussion. From these results it would appear that neostibosan is not an effective filaricide against *Dirofilaria immitis*. Of a total of 71 worms found in the treated animals, 63 were alive and actively motile. The paucity of granulomatous lesions or abscesses in the lungs, which are found surrounding dead worms or their remnants and are indicators of their previous existence, would make it appear unlikely that worms, other than those found, had once been present but had been killed by therapy and removed from their location in

25. Due to limitations of the technique used in sectioning, spermatozoa were not necessarily absent in the other female worms examined.

26. This dog had 207 microfilariae per 20 cmm. of blood at the time of death.

the heart or the pulmonary arteries. It does seem, however, that neostibosan exerts a toxic effect upon the reproductive system of the adult filarids.

The male worms found in the dogs,[27] which were autopsied two and four days after the completion of the second course of neostibosan all had normal testes, but some showed varying degrees of pathologic alterations in the developing spermatozoa. The effect of the drug appeared to be exerted chiefly upon the developmental forms found in the mid portion of the gonoduct and consisted of changes varying from blurring of cellular margins, loss of cellular detail to, rarely, actual necrosis.

Although vacuolization or focal necrosis of one of the convolutions was rarely seen, the female worms from these dogs had essentially normal ovaries. In view of the previous course of treatment, it is interesting to note that the females contained all the developmental stages from unicellular ova to well-differentiated microfilariae. This finding suggests that (1) the previous course of the drug had not stopped the production of ova or their subsequent development into mature forms, or (2) that the forms found had developed prior to the first course of treatment but had not been eliminated from the reproductive tract. The latter seems unlikely in view of the long time interval involved and the findings in worms observed after the dogs had been kept for twelve weeks.

However, all the developmental stages present showed pathologic alterations that ranged from degenerative changes, consisting of nuclear pyknosis, cytoplasmic oxyphilia, and blurring of cellular margins, to frank necrosis. The unicellular ova found in the posterior uterine levels seems to be particularly affected, as the majority of these cells were shrunken and necrotic. Although some of the multicellular forms and the microfilariae also showed signs of necrosis, the largest numbers showed only varying degrees of degeneration or fragmentation. It therefore seems that neostibosan exerted a toxic effect upon all the developmental stages present in the uteri of the female worms.

On the other hand, female worms from dogs killed at the end of their twelfth microfilaria-free week contained only unicellular ova. Of these, the majority were found only in the posterior uterine levels, and all were normal in appearance except for rare oxyphilia. The mid and anterior levels of the uteri of these worms were empty with

27. Two were microfilaria free and one had 207 microfilariae per 20 cmm. of blood at autopsy.

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the exception of a rare unicellular ovum. The ovaries of all the females were normal in appearance.

Several possible explanations for the finding of morphologically normal ovaries and ova come to mind: (1) that neostibosan acted directly upon all the various developmental stages of the ova and microfilariae present in the uterine tract at the time of treatment, but not upon the ovaries; (2)that the drug exerted only a temporary toxic effect upon the ovaries. Either of these theories offers a possible explanation. However, it must also be borne in mind that these ova may have been incapable of further development, even though they were normal in appearance. Certainly, before any decision as to the effect of neostibosan can be reached, further work is indicated in which treated animals should be permitted to survive for longer periods of time before autopsy.

Summary. Neostibosan does not appear to exert a filaricidal action upon the adults of *Dirofilaria immitis*. Of 71 worms recovered at autopsy from dogs given two intensive courses of this drug, 63 were alive and motile.

Female worms found in dogs autopsied two and four days after the second course of neostibosan contained ova in all developmental stages and well-differentiated microfilariae. However, all of these forms showed varying degrees of degeneration and necrosis. The ovaries of the worms were normal, morphologically, except for rare vacuolization, and in one instance, focal necrosis. The males had normal testes, but the developing spermatozoa within the mid portion of the gonoducts occasionally showed minor degenerative changes and in one worm, frank necrosis.

In dogs autopsied twelve weeks after the microfilariae had disappeared from the peripheral blood, the female filarids had normal ovaries and contained only morphologically normal unicellular ova in the posterior levels of the uterus, the remainder of the uterine tract being empty. The males and their developing spermatozoa all appeared normal.

Before an accurate evaluation of its efficacy can be made, further work to determine the duration of the toxic effect of neostibosan upon the reproductive system of the adult filarids is indicated.

CHART 1 - EFFECT OF INTRAVENOUS INJECTIONS OF ANTHIOMALINE ON THE MICROFILARIA COUNT

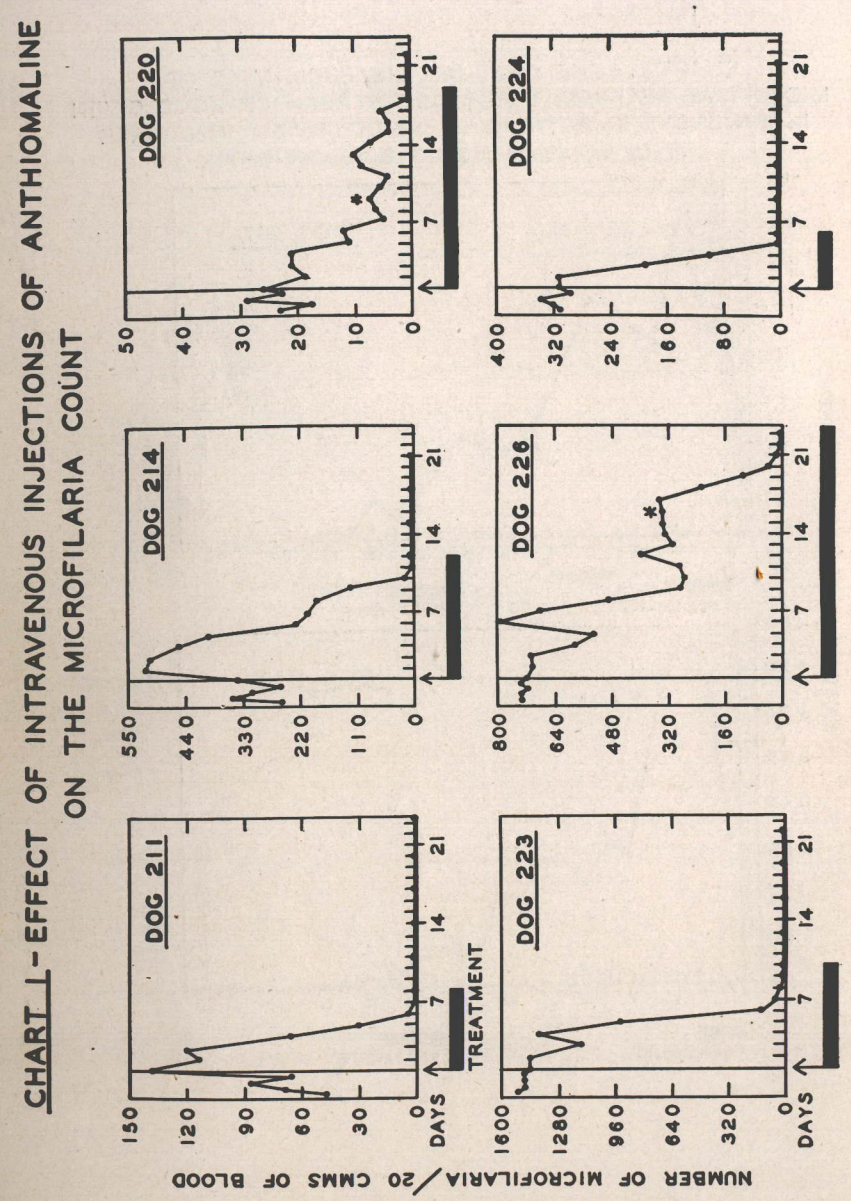


CHART 2.—THE GRADUAL DISAPPEARANCE OF MICROFILARIAE FROM THE BLOOD STREAM OF DOGS GIVEN INTRAVENOUS INJECTIONS OF NEOSTIBOSAN IN DOSAGE OF 10 MGMS Sb/ KILO BODY WEIGHT

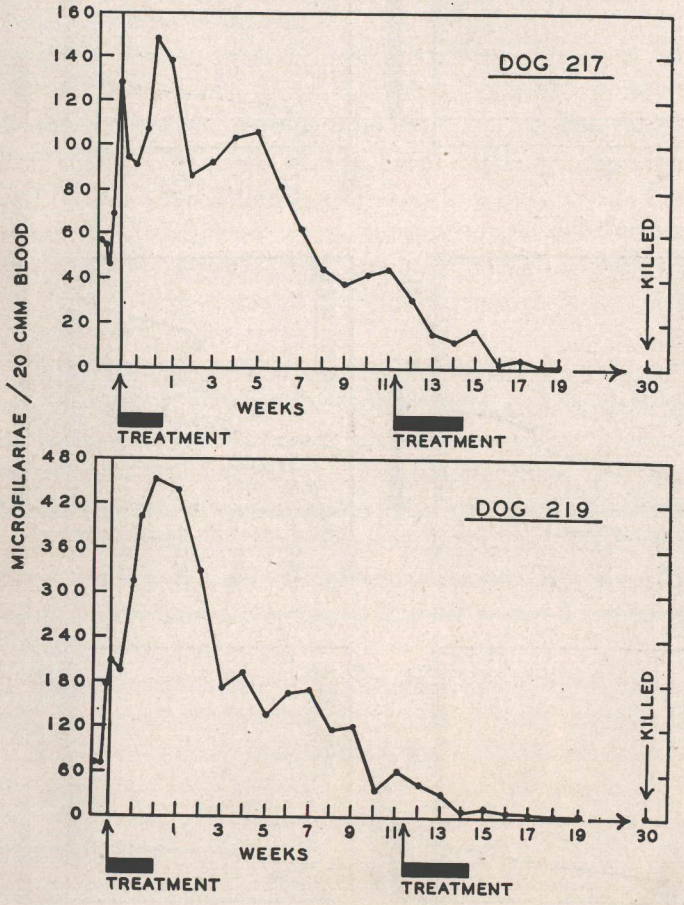
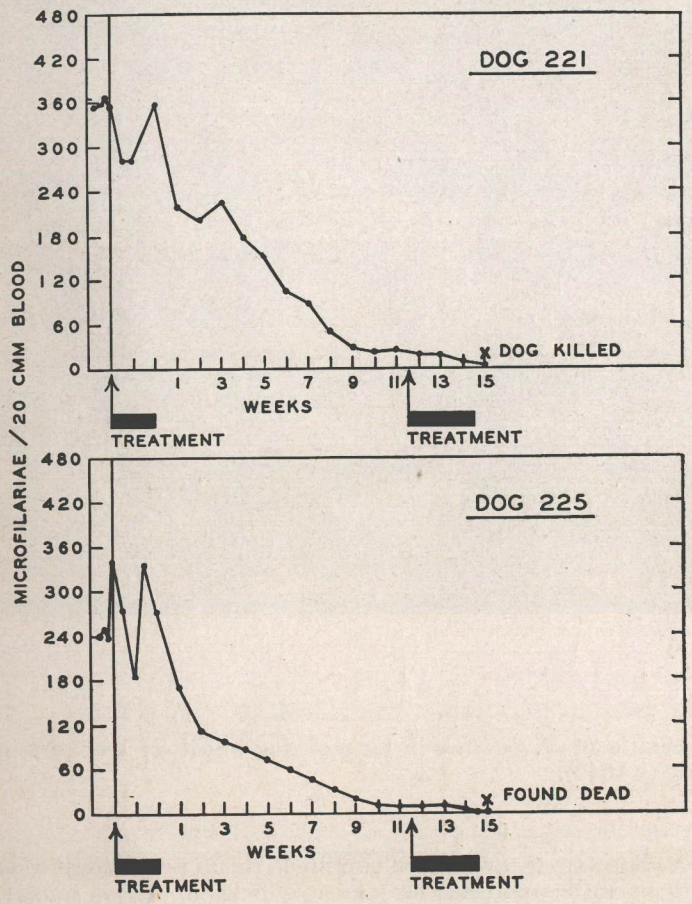


CHART 3.—THE GRADUAL DISAPPEARANCE OF MICROFILARIAE FROM THE BLOOD STREAM OF DOGS GIVEN INTRAVENOUS INJECTIONS OF NEOSTIBOSAN IN DOSAGE OF 10 MGMS Sb/ KILO BODY WEIGHT



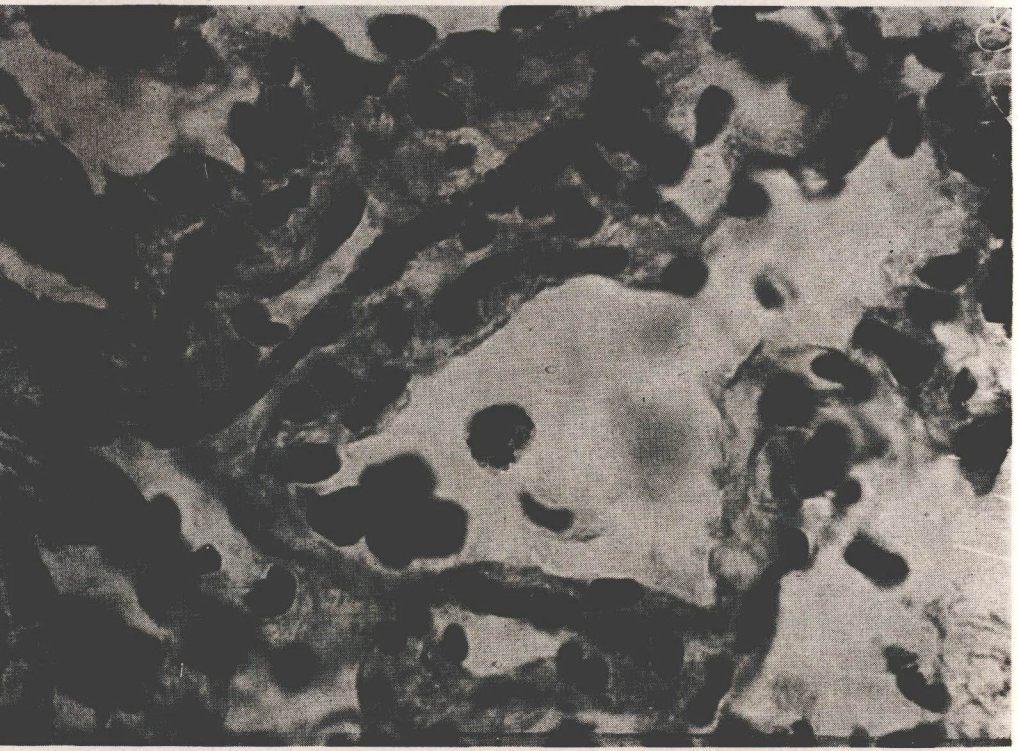


FIG. 1. Microfilaria of *D. immitis* in lung of dog killed on second microfilaria-free day (x 1040).

GRAB. 1. Microfilaria de *D. immitis* en el pulmón de un perro sacrificado al segundo día de haber desaparecido los parásitos de la circulación sanguínea (x 1040).

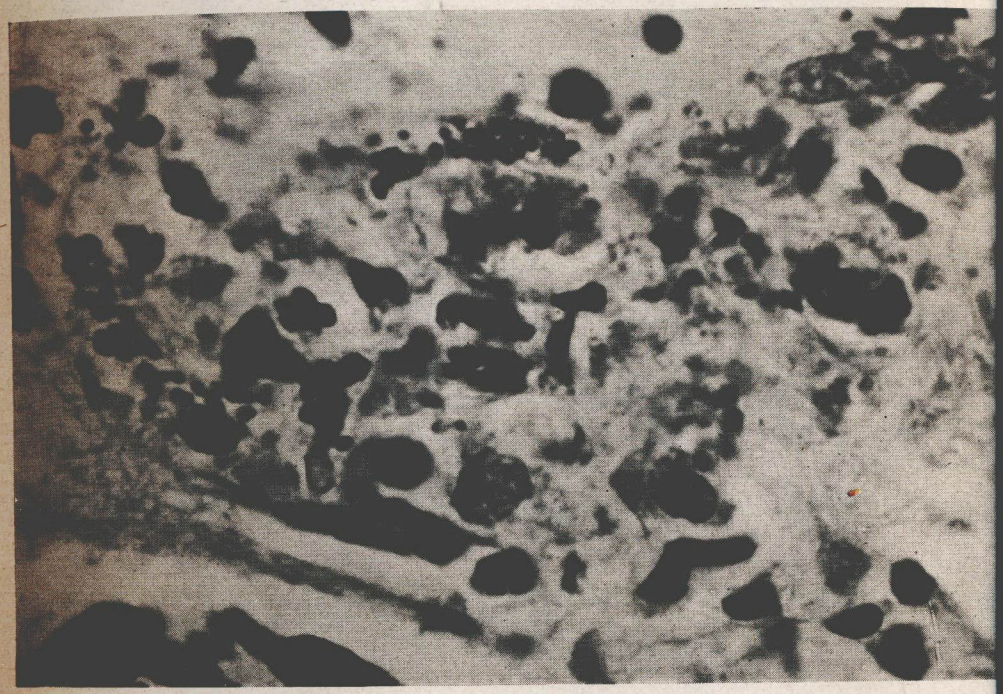


FIG. 2. Fragmented microfilaria with surrounding mononuclear reaction in the lung of same dog (x 1300).

GRAB. 2. Microfilaria fragmentada contorneada por mononucleados en el pulmón del mismo animal (x 1300).

FIG. 3. Extended microfilariae within the edematous interstitial tissue of the kidney medulla of same dog (x 335).

GRAB. 3. Microfilarias extendidas a lo largo dentro del tejido intersticial edematoso de la médula renal del mismo animal (x 335).

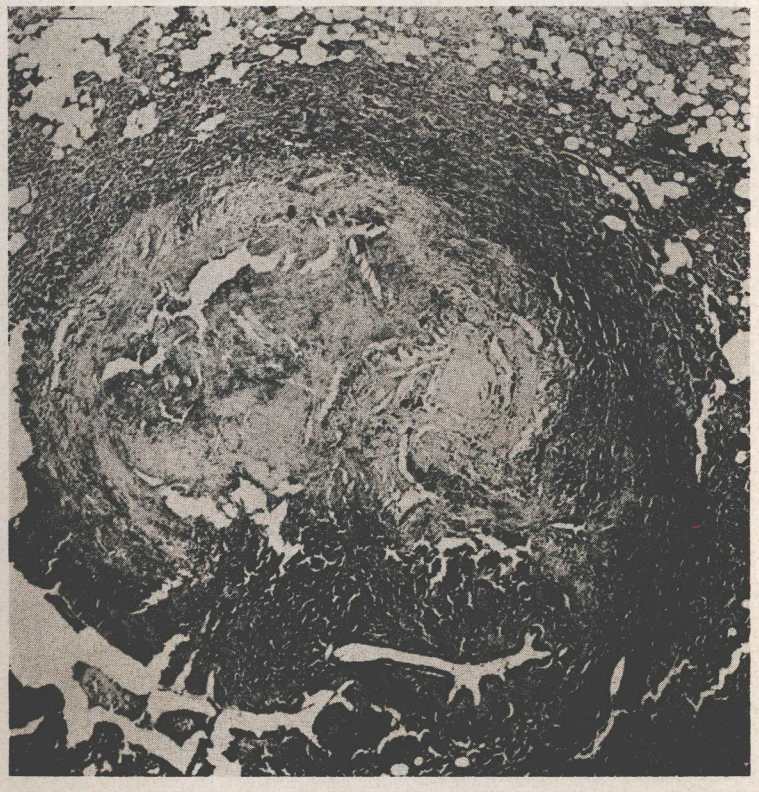


FIG. 4. Thrombosed branch of a pulmonary artery; lumen of the vessel is obliterated by canalized granulation tissue (x 20).

GRAB. 4. Rama trombosada de una arteria pulmonar; lumen del vaso aparece obliterado por tejido de granulación canalizado (x 20).

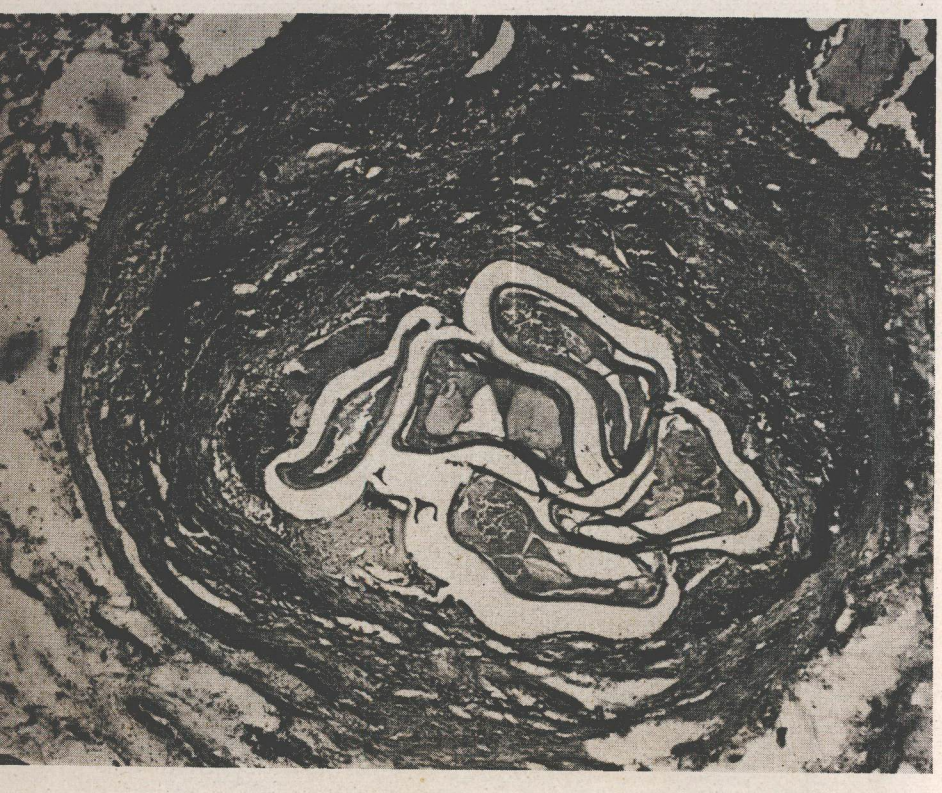
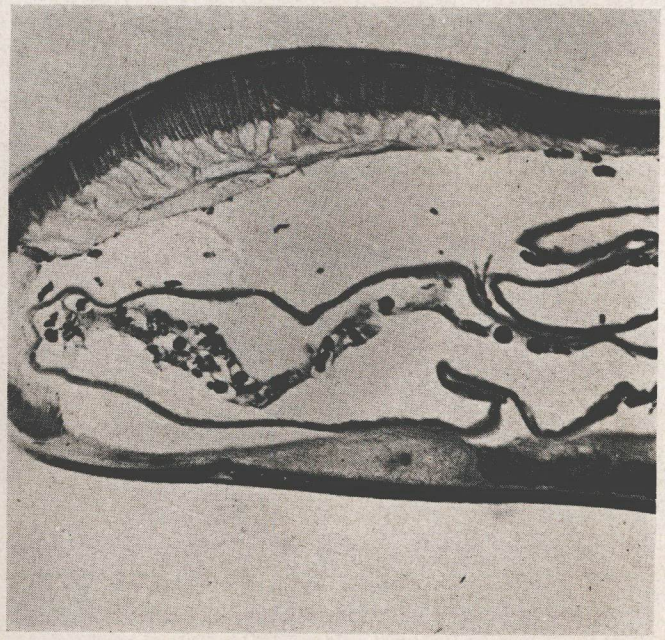


FIG. 5. Cross-section of adult female filarid with empty uteri within the lumen of a pulmonary vessel of dog 226 (x 40).

GRAB. 5. Corte transversal de una filaria hembra adulta, con el útero vacío, situado dentro del lumen de un vaso pulmonar en el pero núm. 226 (x 40).

FIG. 6. Calcified remnants of a filarid within thrombosed pulmonary vessel of dog 226 (x 40).

GRAB. 6. Restos calcificados de un verme filárico dentro de un vaso pulmonar trombosado en el perro núm. 226 (x 40).



IG. 7. Uterus of female filarid from dog 224 treated with anthiomaline; terus is empty except for presence of a few fragmented forms (x 140).

RAB. 7. Utero de un verme filárico hembra procedente del perro núm. 224 ratado con antiomalina; el útero aparece vacío y no contiene más que unas ocas formas fragmentadas (x 140).

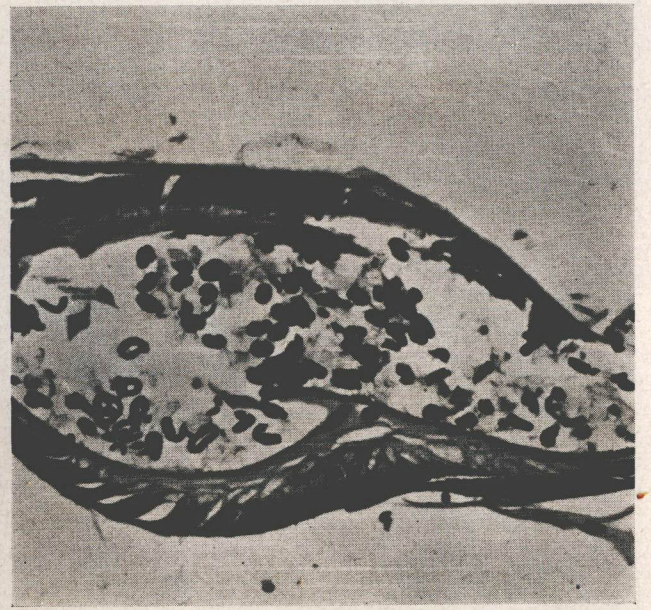


FIG. 8. Uterus of female filarid from untreated dog at about the same level as that in Fig. 7. Note embryos and microfilariae (x 128).

GRAB. 8. Utero de un verme filárico hembra procedente de un perro no medicinado; el verme está situado al mismo nivel, aproximadamente, que él del grab. 7. Nótese los embriones y las microfilarias (x 128).

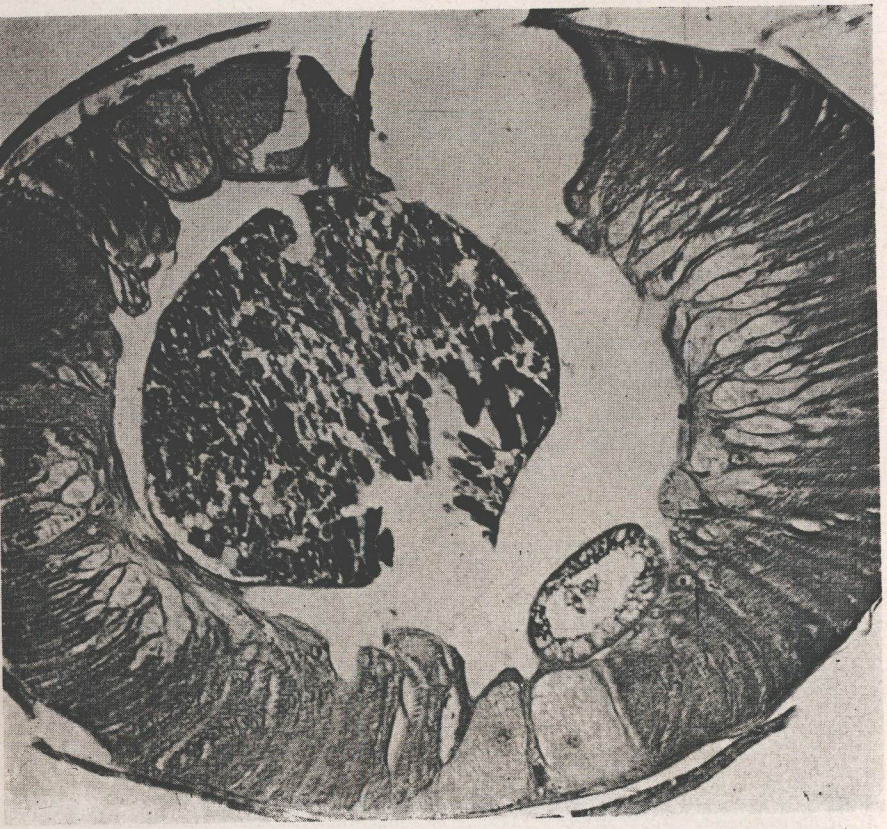


FIG. 9. Necrosis of developing spermatozoa in genital tract of male worm from treated dog 224 (x 180).

GRAB. 9. Espermatozoas en proceso de necrosis, situados en el conducto genital de un verme macho procedente del perro núm. 224, ya tratado (x 180).

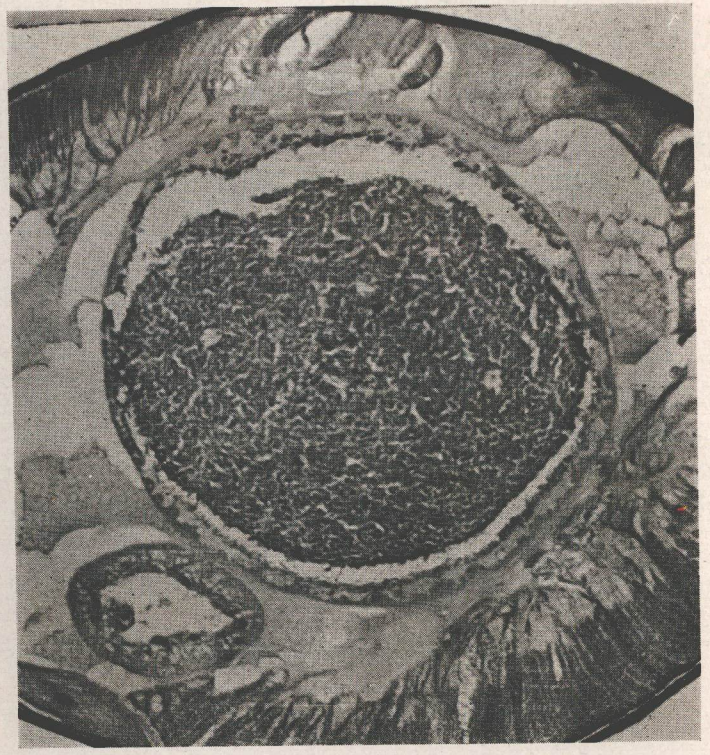


FIG. 10. Normal developing spermatozoa in genital tract of untreated dog (x 210).

GRAB. 10. Espermatozoas de desarrollo normal, situados en el conducto genital de un perro no medicinado (x 210).

FIG. 11. Ova showing oxyphilia, loss of cellular margins, and cellular detail from uterus of female worm in treated dog 214 (x 465).

GRAB. 11. Ovulos oxifílicos, con pérdida del contorno y dibujo celular, procedentes del útero de un verme hembra en el perro (ya tratado) núm. 214 (x 465).

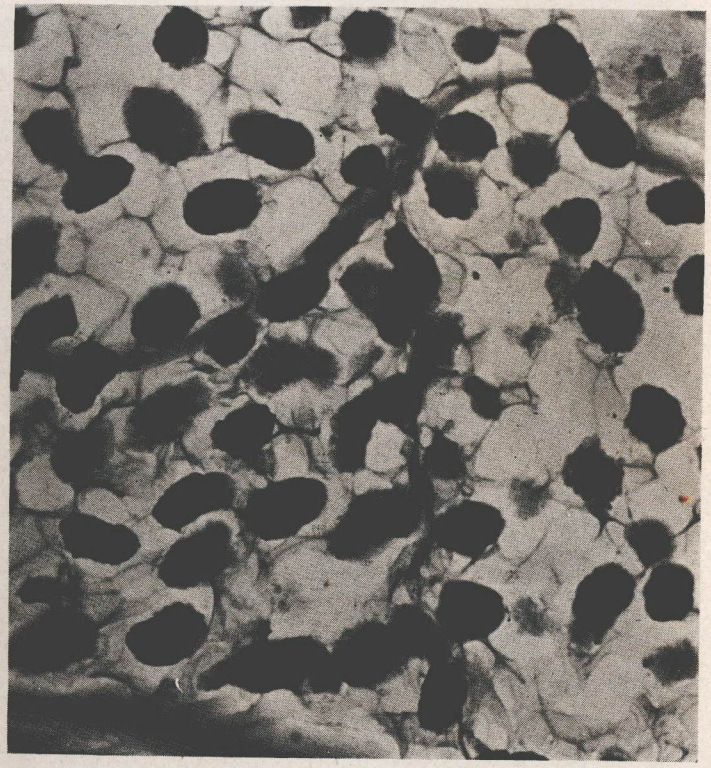


FIG. 12. Normal ova at same stage of development as those in Fig. 11. Nuclei and cytoplasm are strongly basophilic; ova are discrete (x 430).

GRAB. 12. Ovulos normales en la misma etapa de evolución que los del grabado ulterior. Los núcleos y el citoplasma aparecen intensamente basófilos (x 430).

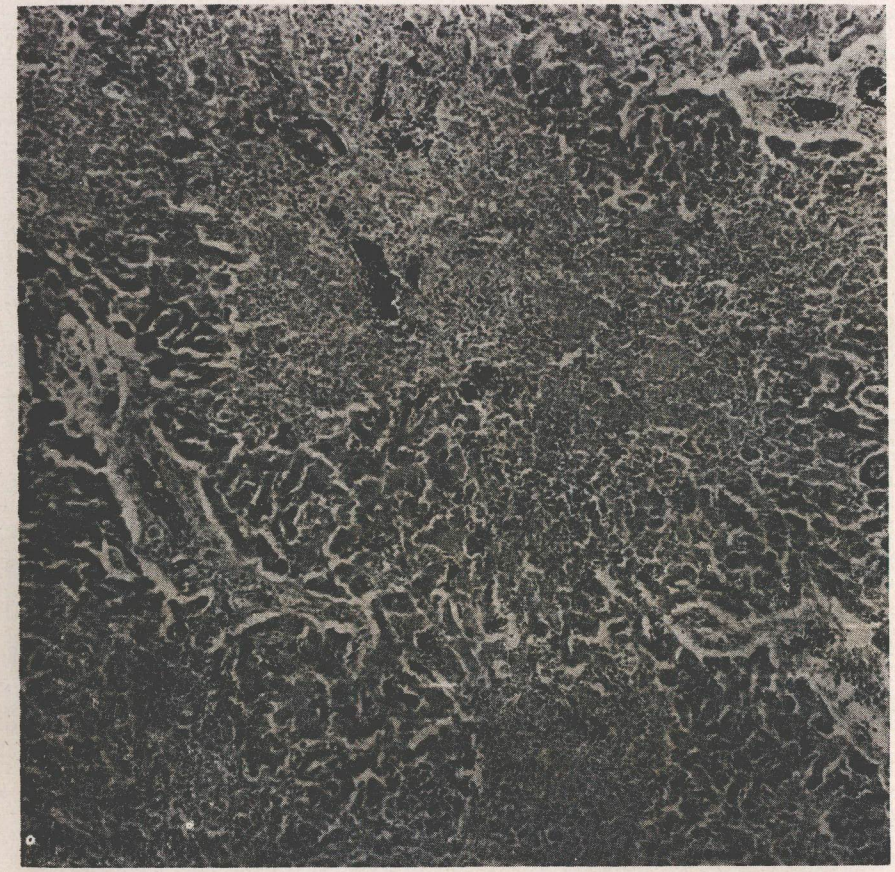
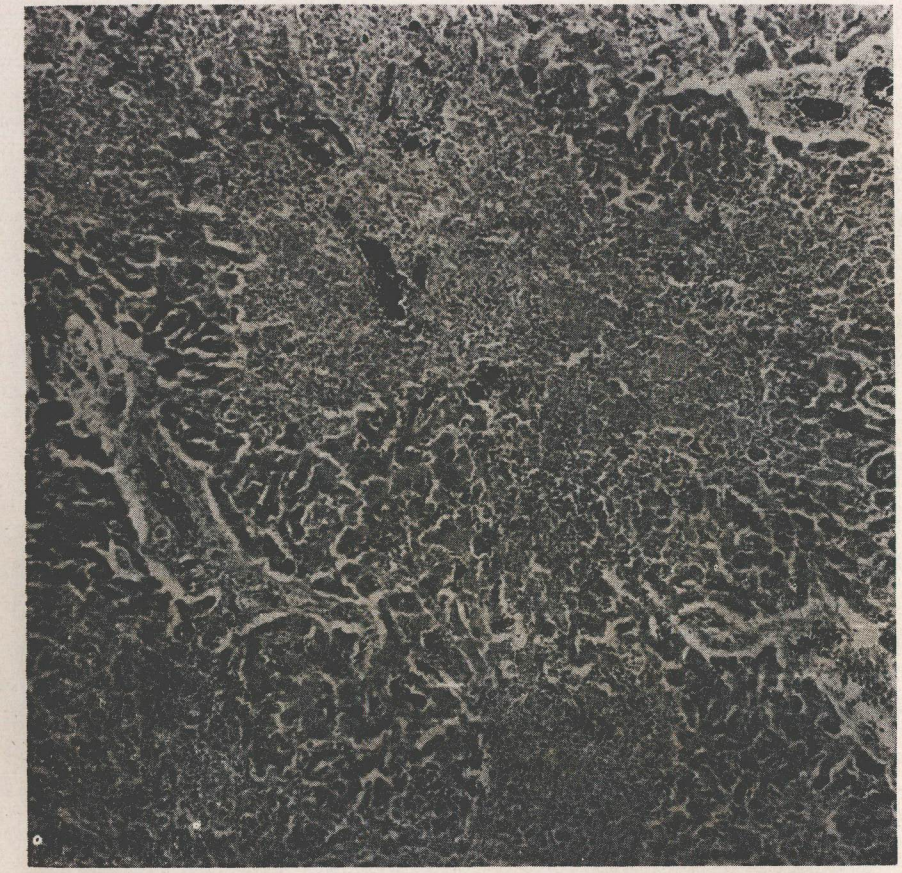


FIG. 13. Central necrosis of hepatic lobules of liver in dog 225; death occurred two days after completion of second course of neostibosan (x 85).

GRAB. 13. Necrosis central de los lóbulos hepáticos en el perro núm. 225, fenecido dos días después de finalizar la segunda tanda de inyecciones de neostibosán (x 85).

FIG. 14. Medullary hemorrhages in kidney of dog 225 (x 85).

GRAB. 14. Hemorragias en la médula renal del perro núm. 225 (x 85).



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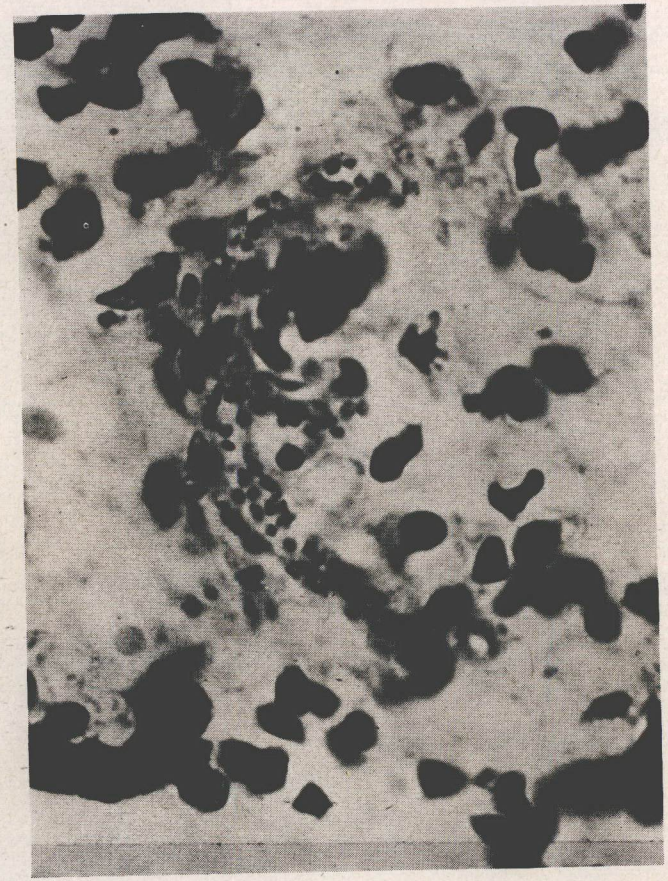

FIG. 15. Microfilariae of *D. immitis* within lung of dog 222 that died during second course of neostibosan; microfilarial count had fallen 81 percent at time of death (x 1300).

GRAB. 15. Microfilarias de *D. immitis*, situadas dentro del pulmón del perro núm. 222 que falleció durante la aplicación de la segunda tanda de inyecciones de neostibosán. La cifra de microfilarias circulantes había descendido 81 por ciento en el momento de la muerte (x 1300).

FIG. 16. Fragments of dead microfilariae surrounded by mononuclear cells from lungs of dog 222 (x 1300).

GRAB. 16. Fragmentos de cadáveres de microfilarias, rodeadas por monucleados, observadas en los pulmones del perro núm. 222 (x 1300).

FIG. 17. Microfilaria of *D. immitis* in liver of dog 222 (x 1120).

GRAB. 17. Microfilaria de *D. immitis* en el tejido hepático del perro núm. 222 (x 1120).