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THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 18 JANUARY 1951 NUMBER 1
SECTION 1

NOTE

The Helminthological Society of Washington, founded in 1910, is the oldest organization of parasitologists in this country, possibly in the world. To celebrate the successful completion of forty years of continuous activity and steady growth the Society is issuing the present commemorative number of its Proceedings.

The first series of articles, Section I of this number, comprises invited papers contributed by active members, all of whom hold long records of membership in the Society. These authors and the date of first year of membership are: Benjamin Schwartz, 1916; W. W. Cort, 1920; Jesse R. Christie, 1922; Gotthold Steiner, 1922; Gerald Thorne, 1923; Emmett W. Price, 1926; Gilbert F. Otto, 1927; and Allen McIntosh, 1929. We are pleased to present this array of scientific papers, representing the diversity of current interests of the authors as well as the broad scope of the Society itself.

Section II of this number is made up of papers that form the regular January, 1951 issue of the Proceedings.

An Epizootic of Parasitic Gastroenteritis in Feeder Lambs

B. SCHWARTZ, A. O. FOSTER, J. E. PETERMAN, J. L. WILBUR, JR.,
and
K. C. KATES
U. S. Bureau of Animal Industry

Early in September 1950 the Bureau of Animal Industry, U. S. Department of Agriculture, received information from the operators of a large lamb-feeding establishment in central Nebraska that they were sustaining heavy death and morbidity losses in lambs from an unknown cause. One of us (J.E.P.), who made a preliminary examination at the establishment, reported to the Bureau his opinion that the epizootic was caused by parasites. A further study of the epizootic at the feedlots by all of us, except one (K.C.K.), showed conclusively that the losses were caused by metazoan parasites, principally nematodes. The syndrome and subsequent death losses resulted apparently from the pyramiding of what were originally low-grade parasitic infections with no apparent clinical manifestations, to heavy numbers of parasites with accompanying acute symptoms. Prostration and then death resulted in over 30 percent of the affected animals, and there was a marked delay in finishing the remaining lambs for market.

MANAGEMENT PRACTICES AND DEATH LOSSES

The lambs involved in this study, numbering 8,275, were brought to the feeding establishment from the western part of Texas during the last two weeks of

May, when they were about 5 months old. The animals were placed on irrigated alfalfa pastures about June 1, from 300 to 400 lambs being placed on each 20-acre pasture. The pastures were used for the first time in 1950, and, in fact, were especially prepared for these early lambs. They were, therefore, free from contamination with eggs or larvae of parasites. After about two to three weeks' grazing on a pasture, the lambs were moved to another pasture of equal size, only to be moved back two to three weeks later to the pasture which they had previously vacated. Toward the end of July, less than two months after they had been grazing the alfalfa pastures, the lambs began dying. In fact, two or three weeks before this, the animals began "losing their bloom," to quote an expression of the operators of the feeding plant.

By the second week of September the death losses had mounted to the staggering total of 1,306 head, and only 673 lambs were then in a condition to be shipped to market. The remaining 6,296 lambs, which were still dying at an alarming rate, up to 100 a day, were taken off the pastures and moved to the dry, bare feedlots. Those that were not too debilitated were treated individually with the standard therapeutic dose of tetrachlorethylene. During the third week of September, when we investigated the epizootic, the lambs were still dying at the rate of nearly 50 per day. By October 10 the total death loss amounted to 2,546, which was 30.76 percent of the total number that had been originally placed on pasture. It is important to note that 1,240 lambs died in the dry lots between the second week of September and October 10, a period of about 30 days. Since the larvae of worm parasites are ordinarily not well propagated in dry lots, these deaths apparently resulted, for the most part, from infections acquired on pasture.

SYMPTOMS, LESIONS AND ASSOCIATED PARASITES

The lambs observed by us in the dry lots, about two weeks after they had been removed from the pastures, presented a striking picture of marked clinical parasitism, characterized by practically all of the classic symptoms described for gastrointestinal and pulmonary helminthiasis. We observed in the group as a whole but not in each individual animal, inappetence, emaciation, anemia, diarrhea, anasarca, dehydration, respiratory disturbances with coughing, and much sneezing, the latter symptom being due apparently to infestation with the nose grub, *Oestrus ovis*. On September 22, when we made post-mortem examinations of about 12 to 15 lambs, we observed between 50 and 60 dead and moribund lambs which had died or reached a condition of *extremis*, presumably in a 24-hour period, since dead lambs were removed daily and their pelts salvaged. In every animal that we autopsied we found evidence of a sufficient degree of parasitism to account for death. In lambs that had been treated with tetrachlorethylene after their removal from the pastures there were few or no stomach worms of any kind, whereas in those not so treated, large numbers of *Haemonchus contortus* and/or *Ostertagia circumcincta*, and in some cases, *Trichostrongylus axei*, were present. Infection with stomach worms was accompanied by a very severe anemia of the skin, of the mucous and serous surfaces, and of the internal organs. In cases of severe infection with *H. contortus*, small bits of clotted blood (so-called coffee grounds) were observed in the abomasum. It is interesting to note that the stomach worm infection in some of the lambs was due to a practically pure infection with *O. circumcincta*, and was accompanied by marked anemia.

In dead or moribund lambs that showed little or no anemia, there were invariably present large numbers of intestinal trichostrongyles, principally *Trichostrongylus colubriformis* and *Nematodirus spathiger*. Lambs so infested showed evidence of scouring during life. Post mortem, the wall of the small intestine of

lambs that had scoured showed an intense hyperaemia, and microscopic studies of sections of the affected intestine showed congestion of the blood vessels, marked desquamation of epithelial cells, the surface of the villi being almost entirely denuded of epithelium in severely affected lambs, edema of the lamina propria, apparent increase in the amount of cellular infiltration of the lamina propria, and partial or even total destruction of the villi. In short, in the absence of stomach worms death was due to an acute catarrhal enteritis associated principally with, and apparently caused by, the two species of intestinal trichostrongyles already named. In other cases, where scouring was not evident or marked, death was due primarily to anemia resulting from the activities of stomach worms, without any evident striking loss of condition.

All of the lambs examined post mortem were infested with lungworms of the species *Dictyocaulus filaria,* some of the infections being rather severe. Although the lungworm infection *per se* was probably not a cause of death, it undoubtedly contributed to the rapid decline in the health of the animals, death being brought about principally by *H. contortus, O. circumcincta,* and *T. colubriformis.* As already stated, the lungworms contributed materially to the clinical syndrome that characterized the disastrous outbreak. In addition to lungworms, other parasites found included the nematodes *Oesophagostomum columbianum* and *Trichuris ovis,* and the cestodes *Moniezia expansa* and *Thysanosoma actinioides.* Coccidial oocysts in small numbers were found in the intestinal contents of only one lamb out of several that were examined. Control measures for coccidiosis are rigidly enforced at the feeding plant where this investigation was made, the prophylactic measure consisting of the incorporation of about 1.25 percent of flowers of sulphur into the ration at all times.

REGRESSION OF SYMPTOMS

Beginning sometime after October 10, when the lambs were treated with phenothiazine, the death losses gradually subsided and the surviving animals began to improve. In fact, by the middle of November recovery had progressed to such a degree that a large percentage of the lambs were almost ready to be shipped to market. It is difficult to determine on the basis of existing information whether, or to what extent, the anthelmintic treatments administered were responsible for the final recovery of the surviving lambs. While still on the pastures, the lambs were given a standard mixture of phenothiazine and salt of which they ate little or perhaps none at all. The treatment with tetrachlorethylene, after the lambs were removed to the dry lots, was apparently effective for the removal of stomach worms, especially *H. contortus,* as pointed out elsewhere in this paper. Whether the individual treatments with phenothiazine in October were responsible, even in part, for the recovery from the scouring syndrome, or whether recovery resulted from the removal of the lambs from the source of infection to dry lots where the propagation of parasites was presumably checked to a large extent, must remain within the realm of speculation. Another factor that may possibly have played a role in the recovery is acquired resistance, since about 5 months elapsed between exposure to infection and the regression of symptoms.

DISCUSSION

From the evidence obtained in the conduct of this investigation, the following course of events apparently took place following the placing of the 8,275 lambs on pasture. Since the lambs in question originated in an area in west Texas where sheep are known to harbor parasites, it is safe to assume that at the time the lambs were placed on the alfalfa pastures they were infested with all the

species of helminths which were later found in the animals that were autopsied. This assumption is based on the fact that the lambs were placed on specially prepared pastures, and that the area in question had not been previously used for grazing sheep. The helminthic infections harbored by the lambs when they were placed on the pastures must have been of a low order, since the animals appeared to be in good health and remained so for about 6 weeks. During the first two or three weeks of grazing on the pastures, the lambs contaminated them with eggs rather liberally, despite their presumed low-grade verminous infections, because large numbers of animals were confined in rather small areas. Before the lambs could become severely affected, they were removed to another clean pasture which they also contaminated, and probably even more severely than the first pasture. Upon being returned to the first pasture, two weeks or so after they had vacated it, they were apparently subjected to a severe onslaught by infective larvae which had developed in the meantime from the eggs discharged with the lambs' droppings. A return to the second pasture resulted in another onslaught, thereby causing a mounting toll of death and morbidity losses. These losses were apparently not checked by the anthelmintic treatment with tetrachlorethylene, despite the effectiveness of this drug for the removal of stomach worms, especially *H. contortus*. The scouring syndrome caused by *T. colubriformis* and *N. spathiger*, complicated by the respiratory and other difficulties caused by the lungworms, took a severe toll in death and morbidity losses even in the absence of *H. contortus*.

Assuming that the foregoing analysis of the course of events that led to the epizootic of parasitosis in the lambs is correct, and all the available evidence definitely points in that direction, the management practices instituted at the feeding establishment where this study was made were of such a nature as to lead to the disastrous results that actually followed.

It is well known that western range lambs escape for the most part the ravages of internal parasites against which sheepmen who have small farm flocks elsewhere have to take strict precautionary measures. The low rainfall on most of the western ranges combined with the abundance of acreage is not conducive to a heavy parasitism. Lambs on irrigated pastures in the West are subject, however, to practically the same hazards of acquiring damaging infections of internal parasites as are small flocks on pastures in the eastern States and other areas where there is more or less abundant rainfall. The lambs at the feeding establishment were on irrigated pastures and subject, therefore, to the hazards of damaging parasitism, aggravated in this case by heavy stocking. The scheme of pasture rotation that was instituted was designed to utilize to a maximum extent the available forage. It afforded, however, ideal conditions for the propagation and survival of parasite larvae and afforded them an abundance of hosts at a time when they were most numerous and probably highly virile and infective. In short, had there been deliberate planning to build up in the lambs helminthic infections of a high magnitude by rotation of pastures, the losses could hardly have been achieved in less time and with more damaging consequences than actually happened through the faulty management practices inadvertently resorted to in the feeding establishment where the study was made.

Aside from the death losses, which in the period between June 1 and October 10 amounted to practically 31 percent of all the lambs involved, the morbidity losses were of serious consequence, considering the delay in finishing the lambs for market. Ordinarily, western feeder lambs are finished in a period of 3 to 4 months. In the present instance the surviving lambs required practically 6 months of feeding to give them a desired "finish," the delay extending over a period of two to three months.

SUMMARY

An epizootic of helminthic infection in lambs in a feeding establishment in the Middle West was found to be characterized by a severe anemia caused principally by *Haemonchus contortus* and *Ostertagia circumcincta*, and an acute catarrhal enteritis, associated with, and apparently caused by, infections with *Trichostrongylus colubriformis* and *Nematodirus spathiger*.

The syndrome was aggravated by lungworm disease caused by *Dictyocaulus filaria*, which was characterized by respiratory difficulties accompanied by coughing.

Other parasites found in the affected lambs included *Trichostrongylus axei* in the abomasum, this parasite undoubtedly contributing to the severe debility from which the lambs were suffering, and the intestinal nematodes *Oesophagostomum columbianum* and *Trichuris ovis*, the cestodes *Moniezia expansa* and *Thysanosoma actinioides*, and the nose grub, *Oestrus ovis*, the latter causing considerable sneezing.

Only few coccidial oocysts were found, due, presumably, to the prophylactic measures that were taken to prevent coccidiosis, a disease which can cause serious losses among feeder lambs.

In addition to the symptoms already named, inappetence, emaciation, dehydration, anasarca, listlessness and marked depression, were among the outstanding clinical manifestations observed in the parasitized lambs.

Treatment with tetrachlorethylene was apparently effective in removing stomach worms, especially *H. contortus*, but did not check the enteritis or reduce to a great extent the severe infections with the trichostrongyles that caused it.

The total death losses between the latter part of July and October 10 amounted to nearly 31 percent of all the lambs involved, and the morbidity of the surviving lambs delayed the period required for finishing them for market by two to three months beyond that ordinarily required.

The severe parasitosis apparently resulted from the pyramiding of the initially low, nonclinical infections to severe infections which produced the disastrous results observed. The increase in the intensity of the helminthic infections was made possble by heavy stocking on irrigated pastures and rotation of lambs back and forth on the same pastures at intervals of two or three weeks.

Early Developmental Stages of Strigeid Mother Sporocysts[1]

W. W. CORT, D. J. AMEEL, and ANNE VAN DER WOUDE

INTRODUCTION

Cort and Olivier (1941) described floating germinal masses, which serve as centers of multiplication of germinal cells, in the mother sporocysts of *Diplostomum flexicaudum* and four other species of strigeids. They postulated that the continued breaking off from these germinal masses of their largest multicellular components produces the daughter sporocyst embryos that develop in the mothers. Since they had available for study only large mother sporocysts most of which were old and almost exhausted they obtained no information on the development

[1] A joint contribution from the University of Michigan Biological Station, the School of Hygiene and Public Health of the Johns Hopkins University, and Kansas State College.

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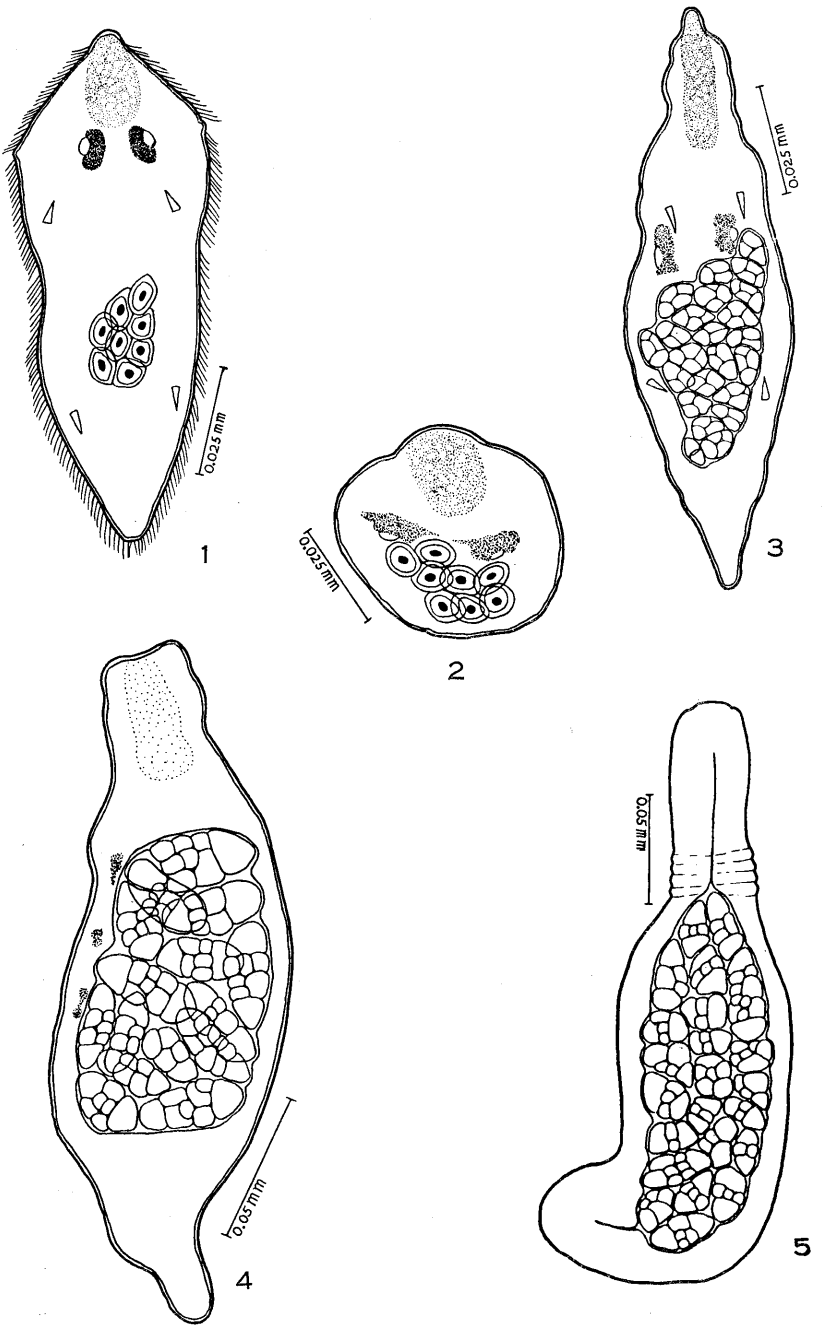

FIGS. 1–5. Early developmental stages of strigeid mother sporocysts. 1—Outline drawing of the miracidium of *Diplostomum flexicaudum*, showing the germinal cells. 2—Contracted specimen of very young mother sporocyst of *D. flexicaudum*, 0.053 by 0.071 mm, showing a group of single germinal cells in its body cavity. 3—Very young stage of mother sporocyst of *D. flexicaudum*, 0.132

of these germinal masses. However, they suggested that the germinal masses develop directly from germinal cells. Experimental infections of snails with the miracidia of *Diplostomum flexicaudum* carried out during the summer of 1950 made it possible to study the early stages of the mother sporocyst of this species and to demonstrate the correctness of this suggestion.

As pointed out by Cort and Olivier (1941) very little information was present in the literature on the mother sporocysts of the strigeids, and apparently nothing has been added since their publication.

MATERIAL AND METHODS

Diplostomum flexicaudum, the life cycle of which was worked out by Van Haitsma (1931), is by far the commonest strigeid of the Douglas Lake region. The adults are found in gulls and the germinal sacs in various species of lymnaeid snails. We obtained the eggs of this species from adults from autopsies of young herring gulls, and incubated them at laboratory temperatures. As the miracidia hatched we placed them with small laboratory raised juveniles of *Stagnicola palustris elodes* (Say). The examination of these snails at intervals gave us a limited number of specimens of very immature mother sporocysts of *D. flexicaudum*. We also had available for study a very early stage of a mother sporocyst from a natural infection of *Physa parkeri*. We have tentatively identified it as belonging to *Cercaria physae* Cort and Brooks, 1928 (= *Posthodiplostomum* sp.), because this is the only strigeid species that has ever been found in this host in all the collections we have examined from Douglas Lake (Cort, Olivier, and McMullen, 1941).

STAGES OF DEVELOPMENT

The miracidium of *D. flexicaudum* was first studied, and it was found that the germinal cells are in a group of about eight in the primitive body cavity just back of the middle of the body (Fig. 1). The cells in this group could be identified as germinal cells from their large nuclei and prominent nucleoli. Our observations differ from those of Van Haitsma (l.c.) who considered as germinal cells a row of four irregular cells at the posterior end of the miracidium of this species.

The earliest stages of the mother sporocyst in our experimentally infected snails appeared to be attached in the mantle, and were quite mobile when removed. They varied considerably in size in infections of the same age, and some of the smallest found which showed little development beyond the miracidium were in snails 4½ to 5 days after infection. In such cases considerably more advanced stages were also present.

The youngest mother sporocysts that we obtained for study were recognizable by the presence of the disintegrating eye spots. The rudimentary digestive tract was still visible at the anterior end, and flame cells could be seen. Unfortunately at this stage they tended to contract, and we were unable to draw one extended. The germinal cells at this stage (Fig. 2) seemed to show no increase in numbers over those of the miracidium. However, in a specimen 0.067 by 0.030 mm obtained 48 hours after experimental infection in which the germinal material was

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mm long, showing the early stages of development of the germinal masses. 4—Very young stage of the mother sporocyst of *D. flexicaudum*, 0.24 by 0.09 mm, showing the body cavity filled with well developed germinal masses. 5—Very young stage of a strigeid mother sporocyst, 0.33 by 0.088 mm, probably belonging to *Cercaria physae* (Cort and Brooks), from a naturally infected *Physa parkeri*, showing the body cavity filled with well developed germinal masses.

squeezed out there appeared to be 10 or 12 germinal cells at least one of which had divided into four cells.

A stage further along in development was found in a snail five days after experimental infection (Fig. 3). It was very mobile, about 0.13 mm in length, and was spindle shaped. The eye-spots could be clearly made out although the pigment around them was beginning to degenerate. The rudimentary digestive tract at the anterior end could still be seen, and the four flame cells could be easily located. The body cavity had become greatly enlarged and contained about 23 structures with from two to four cells each which we interpreted as stages in the development of germinal masses.

A larger immature mother sporocyst, 0.24 by 0.09 mm, was found in another snail five days after experimental infection (Fig. 4). In it the rudimentary digestive tract could still be seen, and remnants of the pigmented eyespots were still visible along the side of the body cavity. The body cavity had become much larger and contained between 15 and 20 well developed germinal masses. The largest of these was 0.066 by 0.024 mm in size and consisted of 5 or 6 multicellular components (embryos) and 3 or 4 unicellular components (germinal cells). There were no free embryos in the body cavity of this mother sporocyst and the largest of the germinal masses did not appear ready to give off embryos. There was some variation in size of the germinal masses in this mother sporocyst the smallest one being 0.036 by 0.019 mm.

A still larger immature mother sporocyst, 0.37 by 0.09 mm in size, was found in a snail 6½ days after experimental infection. Traces of the eyespots could still be made out along the side of the body cavity near the anterior end. The body cavity had come to fill the whole body and contained 20 to 24 germinal masses. The largest of them was 0.071 by 0.018 mm and had about 12 multicellular components and three or four unicellular components; the smallest was 0.041 by 0.018 mm. No embryos had yet been separated from the germinal masses, but in some of them the components appeared to be rather loosely attached and ready to break off. Further stages of development of the mother sporocyst of this species were not available because of the limited supply of infected snails.

The young mother sporocyst obtained from a natural infection of *Physa parkeri*, which we think probably belongs to *Cercaria physae*, was 0.33 by 0.08 mm in size (Fig. 5). It was quite mobile, and the body cavity extended the whole length of the body. No trace of eyespots was seen. It contained about 24 well developed germinal masses the largest of which was 0.08 by 0.03 mm with about 9 multicellular and 4 unicellular components. The smallest germinal mass was about half that length. There appeared to be no detached daughter sporocyst embryos present in this mother.

DISCUSSION

This series of stages of very young mother sporocysts gives an idea of the method of development of the germinal masses in the mother sporocysts of the strigeids. In very early stages there appears to be some increase in the number of the germinal cells present in the miracidium. All then develop directly into germinal masses which early in their development have two to four cells, and appear like the germinal cell groups in the rediae of *Clinostomum* (Cort, Ameel, and Van der Woude, 1950). Soon, however, they change into typical strigeid germinal masses. The development of the germinal masses directly from germinal cells also occurs in the early stages of development of the daughter sporocysts of strigeids which at about the time they escape from the mothers contain only germinal masses. The details of this phase of development will be presented in a later paper.

SUMMARY

A description is given of the very early stages in the development of the mother sporocyst of *Diplostomum flexicaudum* from experimental infections. It appears that the germinal cells of the miracidium increase somewhat in number in the very early stages and then each develops directly into a germinal mass. In their early stages the germinal masses each consists of 2 to 4 cells and resemble the groups of germinal cells found in the rediae of *Clinostomum*. Mother sporocysts 0.24 and 0.37 mm in length contained only germinal masses in their body cavities.

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Testing the Efficacy of Chemicals for Killing Soil-inhabiting Nematodes under Field Conditions

J. R. CHRISTIE and V. G. PERRY

Division of Nematology, U. S. Bureau of Plant Industry, Soils, and Agricultural Engineering, Sanford, Florida, in cooperation with the Florida Agricultural Experiment Station, University of Florida.

Experiments discussed herein were conducted during the winter of 1949–50 for the purpose of determining the comparative efficacy of certain chemicals in controlling soil nematodes under the conditions of central Florida. Miscellaneous soil nematodes were used as test organisms in comparison with root-knot nematodes in some instances, for evaluating nematocidal efficacy, and the purpose of this paper is to report the results obtained by this procedure.

The conventional randomized field plot technique was used in conducting the experiments, with all treatments and controls replicated 5 times. A hand applicator was used for applying all volatile liquids (fumigants) with rows of injection points spaced 12 inches apart and injection points 12 inches apart in the row. Unless otherwise noted (Experiment 4) the fumigants were discharged at a depth of about 6 inches. Liquids or solids not sufficiently volatile to function as fumigants were thoroughly mixed with fertilizer. This mixture was spread evenly on the surface of the soil along the planting row in a band about 14 inches wide and tilled in to a depth of 6 or 7 inches with a rotary tiller having a 16-inch tillage swath. Soil temperatures 6 inches below the surface ranged from 75° to 80° F.

Each nematode count, as given in the following tables, is the total number of living nematodes extracted from a one-pint sample of soil. Each soil sample was made up of 5 or 6 cores taken with a soil-sampling tube inserted to a depth of about 6 inches. The nematodes were removed by a combination of decanting, sieving, and the Baermann technique. The severity of root knot galling is ex-

pressed as root knot indices varying from 0 (no galling) to 100 (maximum galling).

"Carbacryl" (equal parts acrylonitrite and carbon tetrachloride) and parathion were provided for testing by the American Cyanamid Co., "D–D" (Dichloropropene-dichloropropane mixture) and chlorobromopropene by the Shell Chemical Corp., "Soilfume 80–20" (20% ethylene dibromide by weight) by the Niagara Chemical Division, Food Machinery and Chemical Corp., mixtures of methyl bromide and ethylene dibromide by Michigan Chemical Corp., and bis (2-chloroethyl) acetal of formaldehyde and bis (2-chloroethyl) acetal of acetaldehyde by the General Aniline and Film Corp.

EXPERIMENT 1

All the chemicals included in this experiment are volatile liquids and all were applied at the rate of 2.5 ml. per injection. Size of individual plots: 10 by 20 feet. Test crop to provide indicator plants for root knot data: squash. Fumigants applied October 7, 1949. Nematode counts completed October 27. Test crop planted November 7.

TABLE 1.—*Nematode counts and root-knot indices*

Treatments	Soil nematode counts						Mean root-knot index
	A	B	C	D	E	Mean	
1 Allyl isothiocyanate	518	302	314	161	137	286	34
2 Dicholorbutene	26	83	31	69	13	44**	20
3 "D–D"	105	33	76	38	26	56**	17
4 "Carbacryl"	155	430	391	243	255	295	49
5 Control	313	684	520	398	386	454	36

** Least significant mean difference in soil nematode counts at 1 percent level, 181.

"D–D" and dichlorobutene were about equally effective in killing soil nematodes. Both allyl isothiocyanate and "Carbacryl" brought about a reduction in the nematode population of the soil but differences in means of counts fall slightly short of being significant at the 1 percent level.

"Carbacryl" had a stimulating effect on the growth of plants due, perhaps, to the nitrogen it contains. Weeds growing on the plots between times of application and planting showed this effect very conspicuously and the test crop showed it to some extent.

EXPERIMENT 2

All the chemicals included in this experiment are volatile liquids and were applied at the rates indicated in the following table. Size of individual plots: 5 by 15 feet. Test crop to provide indicator plants for root knot data: muskemelons. Fumigants applied January 11, 1950. Test crop planted February 1. Nematode counts made January 31 to February 3. Plants rated for root-knot galling June 29.

In this experiment, chlorobromopropene failed to bring about any reduction in soil nematodes or in root-knot galling on muskemelons. When both were applied at the same rates, "Soilfume 80–20" was somewhat less effective than "D–D" (See also experiment 4). The roots of the plants growing on the control plots were very severely galled, and there is a close correlation between root knot indices and nematode counts.

TABLE 2.—*Nematode counts and root-knot indices*

Treatments	ml. per inj.	Soil nematode counts						Mean root-knot index
		A	B	C	D	E	Mean	
1 "D–D"	5	12	2	6	8	3	6**	8**
2 "D–D"	2.5	51	30	9	62	111	53**	36**
3 Chlorobromo-propene[a]	2.5	595	605	650	938	859	729	95
4 "Soilfume 80–20"	2.5	31	84	104	165	97	96**	68**
5 Control		710	691	538	1055	733	745	99

[a] Diluted, 1 part technical chlorobromopropene to 3 parts diluent, by volume.
** Least significant mean difference at 1 percent level, soil nematode counts 173, root knot indices 24.

EXPERIMENT 3

The fumigants used in treatments 2, 3, and 4 (Table 3) were prepared by diluting the mixtures, as detailed in the tables, with mineral spirits, the final mixture, as applied to the soil, containing 20 percent of these active ingredients by weight. These mixtures were applied at the rate of 3 ml. per injection, as was the "Soilfume 80–20" of treatment 1. Size of individual plots: 10 by 20 feet. Test crop to provide indicator plants for root knot data: squash. Fumigants applied October 3, 1949. Nematode counts completed October 20. Test crop planted October 24.

After nematode counts were made for replicates A and B the land was harrowed in preparation for planting. This proved to be a mistake as enough soil was moved over the field to change subsequent counts for replicates C, D, and E considerably. Even so, the data make it obvious that the efficacy of the mixtures was directly proportional to the amount of ethylene dibromide they contained. Replacing part of the ethylene dibromide by methyl bromide decreased efficacy. Galling on the roots of the squash plants was so slight that root knot data could not be obtained.

EXPERIMENT 4

All the chemicals included in this experiment are volatile liquids and all were applied at the rate of 3 ml. per injection. For "double injejctions," one set of injections was made to a depth of 18 inches and another set was made at the same time, over the same area, to a depth of 6 inches. Individual plots were 6 by 6 feet. Fumigants were applied January 20, 1950. Nematode counts were made February 6 to 10.

TABLE 3.—*Soil nematode counts*

Treatments	Nematode counts					
	A	B	C	D	E	Mean
1 "Soilfume 80–20"	57	41	371	462	348	236
2 25% methyl bromide 75% ethylene dibromide	87	133	209	441	382	251
3 50% methyl bromide 50% ethylene dibromide	201	155	448	583	409	359
4 75% methyl bromide 25% ethylene dibromide	286	229	223	484	486	338
5 Control	429	370	393	490	610	438

TABLE 4.—*Soil nematode counts*

Treatments	Nematode counts					
	A	B	C	D	E	Mean
1 "D–D", double injections	3	13	0	3	0	6**
2 "Soilfume 80–20, double inj.	19	19	122	53	203	83**
3 "D–D", 6-inch inj. only	2	3	5	84	30	25**
4 "Soilfume 80–20", 6-in. inj. only	59	75	126	96	27	77**
5 Controls	749	415	341	931	553	598

** Differences in means significant at the 1% level.

The primary purpose of fumigating these plots was to provide planting sites for fig trees and the nematode counts were made more or less incidentally. The results from these counts have been included in this paper because they provide additional data for comparing the nematocidal efficacy of these two commonly used soil fumigants (see also Experiment 2).

EXPERIMENT 5

Each plot consisted of 25 linear feet of planting row and all rates of application as given in table 5 are the amounts of each material that was applied to this length or row. A 4–7–5 fertilizer was used on all plots at the rate of 1½ pounds per plot. The tobacco was medium ground stems and leaves, as used in the fertilizer trade, and, when included in the mixtures, was used at the rate of 3 pounds per plot. The amounts of parathion given are amounts of a 25 percent wettable dust. The parathion was thoroughly mixed with the fertilizer and, when tobacco was used, this combination was thoroughly mixed with the tobacco. The resulting mixtures were spread along the planting row and tilled in as already explained. Materials applied February 6, 1950. Nematode counts made March 7 to 15.

Nematode counts were about three times greater where tobacco and fertilizer were applied with no parathion (Treatment 4) than where fertilizer alone was applied (Treatment 5). This was due to a build-up of saprophytic nematode species, mostly rhabditids, that reproduced on the decaying organic matter provided by the tobacco. As compared with treatment 4, treatment 1 gave a reduction in the nematode population of 89 percent and treatment 2 gave a reduction of 86 percent. As compared with treatment 5, treatment 3 gave a reduction of 67 percent.

TABLE 5.—*Soil nematode counts*

Treatments	Nematode counts					
	A	B	C	D	E	Mean
1 Parathion 200 g., tobacco & fertilizer	145	207	166	141	162	164**
2 Parathion 100 g., tobacco & fertilizer	316	64	127	310	358	201**
3 Parathion 100 g., & fertilizer	115	69	272	198	180	167**
4 Tobacco only	1917	1650	1327	1825	716	1487
5 Fertilizer only	538	451	381	526	640	507

** Differences in means significant at the 1% level.

EXPERIMENT 6

Two chemicals were tested, bis (2-chloroethyl) acetal of formaldehyde and bis (2-chloroethyl) acetal of acetaldehyde, both being non-volatile liquids. The experiment was conducted in the same manner as experiment 5. The amounts of chemicals indicated in table 6 were applied to 25 feet of row. The same fertilizer formula and type of tobacco were applied at the same rates as in the preceding experiment. Materials applied February 1, 1950. Test crop: gladiolus, planted February 15. Nematode counts made February 28 to March 3.

TABLE 6.—*Soil nematode counts*

Treatments	Nematode counts					
	A	B	C	D	E	Mean
1 Acetal of formaldehyde 100 ml.	154	152	70	70	46	98**
2 Acetal of formaldehyde 25 ml.	2000[a]	363	276	255	379	654**
3 Acetal of acetaldehyde 100 ml.	431	560	171	182	83	285**
4 Acetal of acetaldehyde 25 ml.	438	691	762	733	195	564**
5 Control (tobacco & fert. only)	1381	711	2500[a]	1334	548	1257

[a] Estimated, very large number of rhabditids.
** Differences in means significant at the 1% level.

Although results were somewhat confused by a rapid build-up of saprophytic nematodes, both chemicals appear capable of reducing the nematode population of the soil substantially. Both were slightly toxic to the plants at the higher rate but had no noticeable effect on growth at the lower rate.

DISCUSSION AND CONCLUSIONS

In field plot experiments, results obtained by using miscellaneous soil nematodes as test organisms probably reflect the comparative nematocidal properties of chemicals just as accurately as do results obtained by using one of the root-knot nematodes, or any other plant parasitic species, as a test organism. When this method is used the difficulty of finding adequately infested land on which to conduct experiments ceases to be a problem. Results can be obtained quickly and they do not depend on the successful growing of a crop although the procedure in no way interferes with growing a crop. No plants need be removed or disturbed to obtain the data. When desirable, data can be obtained at intervals over the entire growing season. The rapid build-up of saprophytic nematodes on decaying organic matter can be a disturbing factor but, in most cases, serious interference from this source probably can be avoided.

Cyst-Forming Plant Parasitic Nematodes and their Spread in Commerce

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A cyst is a capsule-shaped covering formed by many of the lower animals at some stage of development, and serving the purpose of protecting developmental stages from desiccation or other unfavorable environmental conditions. So far as is known today, the only plant parasitic nematodes which form cysts are species

of the genus *Heterodera*. In this genus, the cyst is formed from the cuticle of the female and contains larvae which have moulted once, but which have not yet escaped from the egg shell (11).

Heterodera cysts are roughly lemon- or pear-shaped, usually less than one millimeter long, have a color which is some shade of brown, and a leathery, or flexible, rather than a brittle texture. They consist of distinct layers, the outermost of which is intricately marked with characteristic patterns which are of value in identification. Perhaps the most striking characteristic of the cysts is their durability in soil under natural conditions. Apparently they are relatively unaffected by decay organisms usually found in soil, since cysts 8 years old have been found to contain viable larvae (7).

Heterodera cysts may contain several hundred larvae. If these are exposed to water containing excretions from the roots of host plants, they are stimulated to break from the egg shell and leave the cyst through the vulvar opening. For some reason not yet well understood, not all of the larvae leave the cyst even when the exposure to root leachings is prolonged (5). Root excretions from non-host plants have a lesser stimulating effect or none at all. Apparently also, a small proportion of the larvae can emerge from the cyst when no root leachings are present in the water.

Once the larvae have emerged from the cyst, they move through the soil in search of roots of a host plant. The larvae of *Heterodera rostochiensis* (and probably the other species as well) enter any part of the root system or tubers, taking up a position with the head near the vascular system. Here they begin to feed, moult three more times, and attain the adult stage at the last moult (4). By this time the much swollen body has broken through the root tissue, remaining attached by the neck only. The male becomes long and slender and leaves the root to reenter the soil in search of a female. The female begins to form eggs and in certain of the species, a part of the eggs may be deposited, but most of them are retained in the body. The female cuticle undergoes a thickening and toughening process to form the resistant cyst and the female dies when this is completed. If the plant is carefully removed from the soil at this time the cysts can be seen attached to the roots, but they are easily dislodged. If conditions are right, there may be more than one generation per year with some species (11), but most reports mention only one generation per year.

Because of the fact that many of the older reports in the literature failed to distinguish between the various species of *Heterodera*, there has been much confusion as to the hosts of the various species, Oostenbrink (10) has attempted to resolve this question by means of host experiments with inoculum of single cysts of known origin. In reporting his own work and summarizing that of earlier workers, he came to the conclusion that the heteroderas are highly specialized parasites, each species attacking only a limited number of host plants and these are usually of only one or a few plant families. He reports that the golden nematode of potatoes, *Heterodera rostochiensis* Wollenweber, 1923 attacks Solanaceae, principally potatoes and tomatoes; the sugar beet nematode, *H. schachtii* Schmidt, 1871, attacks Chenopodiaceae and Cruciferae; the pea nematode, *H. göttingiana* Liebscher, 1890, and the clover nematode, *H. trifolii* (Goffart, 1932) Oostenbrink, 1949, attack only Leguminosae; the oat nematode, *H. avenae* Mortensen, Rostrup and Kolpin Ravn, 1908, attacks Graminaceae. Other species of less importance attack other plant families, such as Labiatae, Urticaceae, Cactaceae, Polygonaceae and Graminaceae. It is possible that Oostenbrink's host lists are not complete, since good authorities have reported that some of the above-mentioned species, for example, *H. schachtii* (12), attack hosts of still other plant families.

The origins of the species of *Heterodera* are unknown, but most of those mentioned above are fairly widespread in Europe, the British Isles, and Ireland. In the United States, only two species are known to be important as plant parasites, *H. rostochiensis* occurring on Long Island, New York (3) and *H. schachtii* being widely distributed in the sugar beet growing regions of the country (12). Recent surveys by the U. S. Bureau of Entomology and Plant Quarantine and identifications of species by the U. S. Division of Nematology have shown that several other *Heterodera* species are present in the United States, notably *H. göttingiana, H. trifolii, H. weissi* Steiner, 1949, and *H. cacti* Filipjev and Schuurmans Stekhoven, 1941. *H. weissi* has been found only on *Polygonum* spp. and *H. cacti* has been found only on cactus. As more parts of this country and the rest of the world are carefully searched, it can be expected that new species will be found as well as new locations for the known species.

The size and nature of the cysts and the habits of the larvae of *Heterodera* species are such that distribution may take place in many ways. The larvae are able to move through the soil by their own efforts, but so far as the individual is concerned, the extreme limit of such movement is a matter of a few feet or a yard or two, though successive generations might make notable progress in spread by their own efforts, given a supply of host plants and sufficient time. Cysts can be blown about by the wind, and cysts and larvae can be transported by flood, irrigation or drainage water. Farm implements transport cysts from one part of a field to another or from field to field in the soil which clings to wheels and tools. Men and animals pick up the cysts in wet soil on shoes or feet and drop them elsewhere. When a potato or root crop is harvested and taken to market, the cysts are carried along also.

Over longer distances, the cysts are transported with many of the products exchanged from state to state or from country to country. It is to be expected that cysts of a certain species would accompany plant material of its favored hosts, particularly if the underground portions of the plant are harvested. This is indeed the most important way in which infestations are started in new localities particularly if the material is planted after arrival. But cysts are also common in soil shipped with non-host plants if these come from regions where the soil is infested, and in fact may be found in any soil from such a region, whether with plants or not. This has been amply demonstrated by the work of the U. S. Bureau of Entomology and Plant Quarantine and the U. S. Division of Nematology in their inspections of the material received at United States ports from foreign countries. *Heterodera* cysts with viable larvae have been found in the soil included with shipments of bulbs, corms, tubers, roots and plants intended for transplanting, in flower pots in the crew's quarters of ships, in vegetable bins of ships' stores, in material used to pack plants brought in as baggage by ship and airline passengers, in soil with miniature Japanese gardens, and in used burlap bags. Such cases are not exceptional. A fair proportion of the suspicious material from certain countries examined has yielded *Heterodera* cysts of one species or another.

Such cysts often contain larvae capable of attacking their host plants if given an opportunity to do so. This is a particularly serious matter if the cysts accompany material of their favored host intended for planting. It is possible that the expensive and troublesome infestation of golden nematode of potatoes on Long Island had some such origin. But even if the parasite accompanies a non-host plant, there is always the possibility that it will eventually reach a host plant. This possibility is diminished by the restricted host ranges of the various species, but increased by the ability of the larvae to survive for many years in the cyst.

When a potato crop is grown on soil infested with *Heterodera rostochiensis*, the increase in the number of viable cysts during the growing season is estimated at about sevenfold (3) to tenfold (10). Probably increases of about this size occur with other *Heterodera* species also. If the rate of increase is tenfold, an infestation is started with a single cyst, and the host crop is grown in successive years, the increase in number of viable cysts will follow the geometric series 1, 10, 100, 1,000, 10,000, 100,000, 1,000,000, 10,000,000, 100,000,000, reaching the last figure in about 10 years. One hundred million cysts with viable larvae are sufficient to cause severe damage to host crops on one acre of soil (10), and in the course of farming operations would be well distributed over the surrounding acres and adjacent fields. Usually the damage to the crop is apparent some years before this point is reached, for the nematodes are seldom evenly distributed, but are found in patches. These patches are often elongated in the direction of cultivation, irrigation or drainage and on them, the crops will show symptoms of severe attack while the growth is normal elsewhere (12).

If no host crop is grown and weed hosts are absent, there will be no increase of heteroderas, since they are obligate parasites and cannot feed, develop to maturity, or reproduce except in a suitable host plant. Rather, the population decreases, since nematodes have their natural enemies, predators and diseases. These take a constant toll as does starvation, since the larvae must exist on food stored in their bodies until they can enter a host plant. Studies of *Heterodera schachtii* populations indicate that the decrease is of sufficient magnitude so that, if a field is not heavily infested, the population will never increase to the point where crops are seriously damaged if only one host crop is grown in three years (12). The same has been found to be true of *H. rostochiensis* and is the basis of the law which prohibits the growing of potatoes or tomatoes more than one year in three in any field in Holland (10). In the United States it has been found that if a field is so heavily infested with sugar beet nematodes that serious crop damage has resulted, another satisfactory beet crop can be grown only after an interval of four to six years of non-host crops. Even then, only a single satisfactory crop can be grown. A second successive crop is almost invariably a failure because of the increase of the nematode population on the first one (12). It is probably true that increases and decreases of populations of other *Heterodera* species follow the general pattern illustrated by these examples.

Numerous attempts have been made to accelerate the disappearance of *Heterodera* species from field soil. One of the first of these was the "trap crop" method devised by Kühn (9) in Germany prior to 1881 for control of the sugar beet nematode. He advocated sowing rape or other host plants thickly and allowing them to grow long enough to stimulate hatching of larvae, resulting in invasion of the roots. The trap crop was then destroyed by plowing under or other means before reproduction could take place. To some degree the method was successful, but was never widely used because of the labor and expense involved and the fact that the time for destroying the trap crop had to be carefully judged. If a miscalculation was made or if the weather at the critical time was such as to prevent the trap crop from being destroyed, reproduction took place and the net result was more nematodes than before. Attempts at refinement of the method have centered around improving methods of destroying the trap crop or finding trap crops which will stimulate emergence from the cyst but on which no reproduction is possible.

When it was realized that larvae which have left the cyst are more vulnerable to natural enemies and also use up their reserve food supply more rapidly than larvae which remain in the cyst, it became apparent that intervals between host

crops could be reduced if the larvae could be stimulated to leave the cyst. It has been shown that larvae free in the soil do not live more than about 16 months at most (7). Since larvae are stimulated to leave the cyst by root leachings, attempts have been made to isolate and analyze the active substance excreted by roots in the hope that it can be synthesized (14). If this can be done, an inexpensive method of control may be devised, since only small amounts of the chemical will be needed.

Numerous attempts have been made to kill *Heterodera* species in the soil by means of chemicals, but without conspicuous success until the value of dichloropropene mixtures or ethylene dibromide for this purpose was discovered in the past few years (13, 3). These materials used as soil fumigants will kill enough of the nematodes to permit the growing of one good crop under field conditions, and nearly complete extermination has been obtained with heavy applications of them in experimental work. At present soil fumigants are used to some extent by farmers and are a profitable investment where the infestation is heavy and the soil is of the right type for their efficient use. The principal obstacle to widespread acceptance is the cost of the necessary chemical in relation to the selling price of the crop increase resulting from their use, particularly in foreign countries where prices for fumigant are higher than in the United States and prices for produce lower.

The above can be summarized by the statement that there is no method of exterminating *Heterodera* species in field soil known at present that is practical from the economic standpoint. Theoretically, the job can be done by massive applications of soil fumigant, but the expense is out of the question at present prices. Also, it is theoretically possible to eliminate them from fields by allowing no host plants to grow for a sufficient number of years, probably at least ten, and perhaps as many as fifteen. But this prospect is hardly attractive to the practical farmer who knows that the host crops are often his most profitable ones. The best that one can hope to do is to keep the *Heterodera* population below the point where crops are severely damaged either by the use of rotations or fumigants. Until better methods can be devised, *Heterodera* infestations in the soil will have something of the financial effect of a mortgage which takes some of the farm income in interest each year, but is never liquidated.

The recent finding of numerous shipments containing *Heterodera* cysts, in the course of routine inspection of shipments of plant material from foreign countries, suggests that other shipments containing cysts have entered the country in the past and that many foci of infection now exist here as a potential threat to crop production. No practical system of locating and dealing with such infestations presents itself. Even with the efficient soil survey methods used by the Division of Golden Nematode Control of the U. S. Bureau of Entomology and Plant Quarantine (1), in the sugar beet growing sections of the United States (12), and in foreign countries (10), light infestations are difficult to locate. An adequate survey of the country would be prohibitively expensive, so the best that can be expected is that infestations will be located more or less by chance. It is hoped that the present paper, by calling attention to the situation, will increase the probability of locating *Heterodera* infestations before they increase to the danger point.

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Diffusion Patterns of Soil Fumigants

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The efficacy of a soil fumigant used in the control of nematodes depends upon its ability to penetrate the soil mass in sufficient quantity to build up lethal concentrations in the soil atmosphere. Soil porosity is the principal factor governing diffusion and this is closely tied with the moisture equivalent or moisture holding capacity. Soils with moisture equivalents of less than 20 percent are porous and the usual applications of 20 to 25 gallons per acre of dichloropropene or of ethylene dibromide mixtures readily penetrate them in lethal quantities and give satisfactory nematode control, while those with equivalents of over 20 percent are more impervious and heavier applications are required.

The experiments here recorded were designed to determine the diffusion pattern of certain soil fumigants when applied with chisel type commercial machines or as single injections with hand applicators.

The soil of the field selected is a medium sandy loam about 16 inches deep underlaid with a compact, lighter-colored, calcareous subsoil. The usual plow sole is present about 10 inches below the surface. Essentially it is a first class soil, its crops have been carefully rotated and fertilized for many years and it is in a high state of productivity. The moisture equivalent averages 16.5 percent and, at the time of application, the moisture content ranged from 12 to 15 percent at the various levels.

Previous work in the field had revealed that it is infested with the sugar beet nematode (*Heterodera schachtii* Schmidt, 1871), the root-knot nematodes (*Meloidogyne* spp.[1]), and a monosexual species of *Pratylenchus*. In addition there is an abundant fauna of about 20 free-living nematode species. The most prevalent of these is *Dorylaimus obscurus* Thorne & Swanger, 1936, a large, easily recognized form which is very numerous and more uniformly distributed than any other. This is an ideal species on which to test the killing range of fumigants

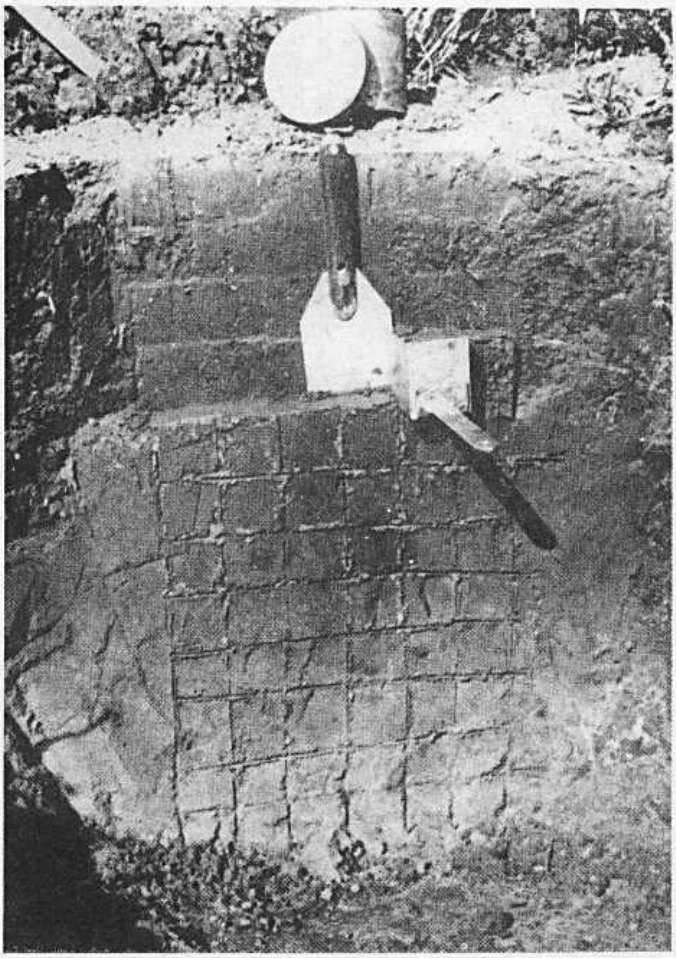

FIG. 1. Soil sector laid off in 2-inch squares and partly removed by means of the trowel and spatula made especially for this purpose.

for it had previously been observed that the lethal point closely approximates that of the free-living stages of the plant parasitic species which, unfortunately, are usually distributed in very erratic patterns because they congregate about the roots of their hosts.

Single applications of fumigants were made at a depth of 8 inches by pouring exact quantities into holes punched to that depth, using a long-stemmed fun-

[1] Formerly *Heterodera marioni* (Cornu) Goodey.

nel to insure deep application. In a similar manner, lines of fumigants were poured into trenches 8 inches deep to simulate applications by chisel-type machines. The spot application of chloropicrin (Fig. 2) was immediately sprinkled with

	2"	4"	6"	8"	10"	12"	14"	16"
A 2"	15–0	45–0	42–0	38–3	37–11	0–64	0–42	0–27
B 4"	26–0	57–0	56–1✓	41–1✓	47–0	19–16	0–51	0–68
C 6"	42–0	63–0	103–0	54–1✓	78–3✓	28–3✓	0–113	0–86
D 8"	27–0	46–0	28–0	52–1✓	48–4✓	23–2✓	1–77	0–92
P								
E 10"	37–0	62–0	35–0	31–1✓	24–1✓	40–3✓	6–76	0–90
F 12"	58–0	31–0	33–0	34–2✓	14–5✓	18–5✓	4–66	0–232
G 14"	49–0	42–0	41–0	35–0	15–5✓	7–3✓	0–125	0–83
H 16"	46–0	47–0	50–0	18–0	14–0	7–3✓	0–138	0–75
I 18"	26–0	45–0	53–0	34–1✓	14–1✓	4–31	0–42	0–38
J 20"	35–0	33–1✓	41–2✓	39–7	8–2	2–88	0–50	0–27
K 22"	24–11✓	27–1✓	3–0	5–7	0–49	0–42	0–38	0–46
L 24"	8–2	3–6	0–22	0–39	0–41	0–63	0–34	0–29
M 26"	1–34	3–51	0–40	3–40	0–52	0–49	0–43	0–58

FIG. 2. Diagram of one-half of a soil sector adjacent to a 10-cc application of chloropicrin. Numbers of *Dorylaimus obscurus* are shown in the couplets of numbers given for each block, those on the left indicating dead nemas, the right figure those found alive. Numbers marked by check (√) indicate young nemas hatched from eggs after the gas had left the soil. *P*—point of application.

water to confine the gas, but the applications of D–D and Dowfume W10 were not (Figs. 3, 4). After 7 to 10 days the soil profile was exposed by digging a pit about 2 feet deep, with one vertical side at the points of application. The exposed profile was then laid off in 2-inch squares and a sector 1-inch thick re-

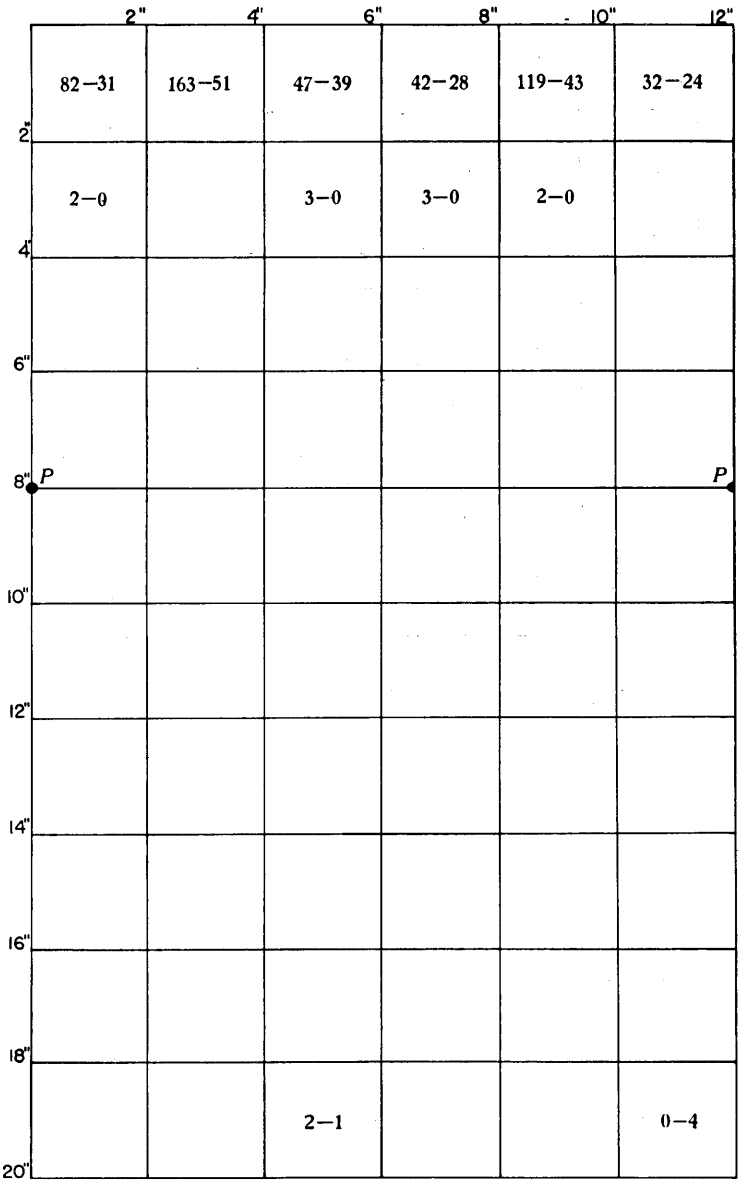

FIG. 3. Diagram of soil sector through two lines of D–D applied with a chisel-type applicator. Nemas surviving are shown by the couplets of numbers in each block. The right figures of the couplets represent the spear-bearing tylenchs and dorylaims, the left indicate the saprophagous forms. *P*, *P*—points of application.

moved with a special trowel (Fig. 1). Each block of soil contained approximately 4 cubic inches and these were placed in tightly covered cans and stored under refrigeration until processed. Nemas were separated by modified Cobb sifting and gravity methods, and the numbers of living and dead specimens recorded for each species.

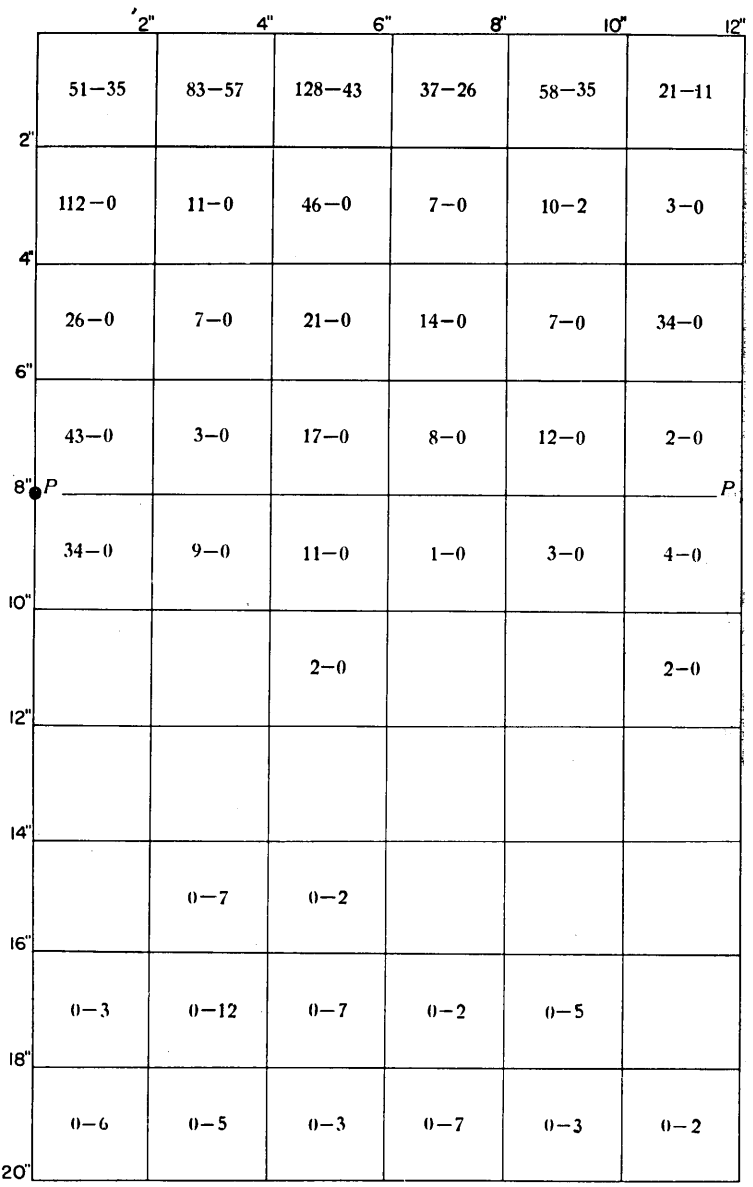

FIG. 4. Diagram of soil sector through two lines of Dowfume W10 applied with a chisel-type applicator. Nemas surviving are shown by the couplets of numbers in each block, those on the right indicating spear-bearing species, and those on the left saprophagous forms. *P*, *P*—points of application.

This method gives a definite reading on the killing range of chemicals as they are applied in the field and is more reliable than the procedure of burying nemas at various distances from the point of application, because the soil is not disturbed, especially in the plow-sole and subsoil zones.

The distribution and kill of *Dorylaimus obscurus* by 10 cc of chloropicrin is shown (Fig. 2), and, based on this soil sector, there was a per acre population of 1,917,467,640. The couplets of numbers indicate those found in each block, the dead nemas by the left numbers, the live by the right. Figures marked by a check (√) indicate young nemas which had hatched from eggs after the gas had evaporated. These checked figures indicate that the killing range for eggs was 4 to 6 inches less than that for larval and adult forms. The most interesting point shown by these data (Fig. 2) is that *D. obscurus* 8 to 12 inches from the point of application detected the approach of the gas and migrated away from it, resulting in unusually large concentrations in the 14 to 16 inch zones, especially in levels B to I, with the maximum of 232 in block F16. *Chiloplacus symmetricus* (Thorne, 1925), which was also observed, exhibited similar ability to detect and move away from the approaching gas. This proves that nemas possess a sense of smell, an awareness of danger, and the ability to determine direction. *D. obscurus* averages about 2.0 mm. in length and is an active, rapidly moving species, but the plant-parasitic *Heterodera* and *Pratylenchus* species, also observed, are much smaller and slower moving and were unable to move away from the approaching gas.

Chiloplacus symmetricus, Acrobeles complexus Thorne, 1925, *Panagrolaimus subelongatus* (Cobb, 1914), *Rhabditis* spp., and other saprophagous species were killed at a distance similar to that of the eggs of dorylaims and tylenchs. Apparently these saprophagous nemas are accustomed to the gases produced by decaying organic material on which they feed and therefore are more resistant to fumigants. This information has a practical value because if saprophagous nemas are found alive in soil immediately after fumigation, it can be assumed that the eggs of plant parasitic tylenchs have also escaped.

Eggs of *Heterodera schachtii* protected by the brown cyst were killed only to a distance of 6 inches and this indicated the difficulty later experienced in attempting to control this species by soil fumigation.

There was an excellent kill of free-living nemas by the 25-gallon application of D–D (Fig. 3), but unfortunately *Heterodera schachtii* was not present in this sector. As usual, nemas escaped near the surface where the gas diffused into the air so rapidly that lethal concentrations were not built up, and below the 18-inch level where it did not penetrate into the hard subsoil. In the top two inches of soil, spear-bearing Dorylaimidae and Tylenchidae escaped as well as saprophagous species, while in the 3–4 inch level only *Rhabditis, Acrobeles, Chiloplacus,* and other saprophagous forms were found alive. This sector well illustrates why it is impossible to secure more than a one-year control of plant parasitic nemas under actual field fumigation conditions. Sufficient numbers escape near the surface and below the zone of penetration to again build up destructive populations during one year of favorable host plant crops.

Dowfume W10 (ethylene dibromide in a petroleum thinner) failed to kill saprophagous nemas even in the blocks adjacent to the points of application, but it did make a good kill of Tylenchidae and Dorylaimidae down to the 14-inch level and a partial kill into the 17–18 inch level (Fig. 4). In this sector the saprophagous species were not found below the 12-inch level. This experiment was especially interesting because of the specificity of ethylene dibromide for spear-bearing nemas while those of the saprophagous group largely escaped.

Similar applications in a heavy soil having a moisture equivalent of 30.6 percent gave less than one-half the penetration secured in the above experiments where the moisture equivalent was 16.5 percent.

A New North American Monogenetic Trematode, *Capsala manteri*, n. sp.

EMMETT W. PRICE

Bureau of Animal Industry, U. S. Department of Agriculture

On February 15, 1947, Dr. H. W. Manter, Department of Zoology, University of Nebraska, forwarded to the writer several specimens of monogenetic trematodes for identification, and among these were 3 specimens of a species of *Capsala* which appeared to be new. These are being described herein as *Capsala manteri*, n. sp., in honor of the collector.

Capsala manteri, n. sp.

Description.—Body (Fig. 1a) piriform in outline, 2.1 to 2.6 mm. long by 1.5 to 1.9 mm. wide. Dorsal and ventral surfaces apparently without papillae, but with a single row of dorsal marginal spines, each unicuspid, about 8 to 10 μ long (Fig. 1b). Anterior haptors disc-like, about 400 μ in diameter; posterior haptors 700 to 800 μ in diameter, surrounded by a delicate pleated membrane; central area of posterior haptor an irregular heptagon, with 7 ridges radiating from it as in other species of the genus; ventral surface of haptor without papillae. Hooks or anchors slightly sinuous (Fig. 1c), about 50 μ long; marginal hooklets 14 in number as in the other capsalids. Oral aperture median, about level of posterior third of anterior haptors; pharynx constricted, 280 to 400 μ long by 280 to 240 μ wide; intestinal tract as in other members of genus. Genital aperture at level of middle of pharynx and immediately posterior to distal margin of anterior haptor. Cirrus pouch pestle-shaped, its base almost immediately posterior to bifurcation of esophagus. Testes relatively large, about 30 to 32 in number, confined within the interintestinal field. Ovary nonlobulate 250 to 300 μ by 200 to 280 μ, median, about 200 μ posterior to base of pharynx. Vitelline follicles extending to near margin of body and into cephalic lobe. Vitelline reservoir oval, 120 × 150 μ in size, situated slightly to left and in front of ovary. Vaginal aperture slightly median, about 150 μ posterior to genital aperture. Ootype, only slightly wider than metraterm, which is closely applied to cirrus pouch. No eggs present in specimens available.

Host.—Fish, little tunny *Euthynnus alletterata* (Raf.).

Location.—Gills.

Distribution.—Tortugas, Florida, U. S. A., collected by Dr. H. W. Manter, August 7, 1931.

Type specimens.—U. S. N. M. Helm. Coll. No. 37228 (type); 37229 (paratypes).

Of the species included in the genus *Capsala* (Price, 1939, J. Wash. Acad. Sc. 29 (2): 63–92), *Capsala manteri* belongs in that group of species now included in the genus in which the testes do not invade the extraintestinal fields. The following species belong in this group: *Capsala biparasitica* (Goto, 1894), *C. folliacea* (Goto, 1894), *C. katsuwonum* (Ishii, 1936), *C. magronum* (Ishii, 1934), and *C. pelamydis* (Taschenberg, 1878). *C. manteri* is the smallest of the species listed and is the only one that has a single row of unicuspid dorsal marginal spines. In *C. biparasitica*, the only other species of the group having a single row of dorsal

marginal spines, the spines are multicuspid. *C. folliacea* and *C. katsuwonum* have no spines reported and in addition the latter has a very small posterior haptor; *C. magronum* has a band of irregularly distributed dorsal marginal spines as in *C. martinieri; C. pelamydis* has no spines but a series of minute marginal papillae and only a few testes (Palombi, 1949, Arch. Zool. Ital., **34**: 203–408). In connection with the latter species, Sporoston (1946, Trans. Zool. Soc. London, **25** (4): 185–600) states that "pharynx shown as unconstricted. [If this is correct, it is not in agreement with the generic diagnosis.]" According to Palombi (1949, *loc. cit.*), *C. pelamydis* has a constricted pharynx and was correctly allocated to the genus *Capsala* by the writer (Price, 1939, *loc. cit.*).

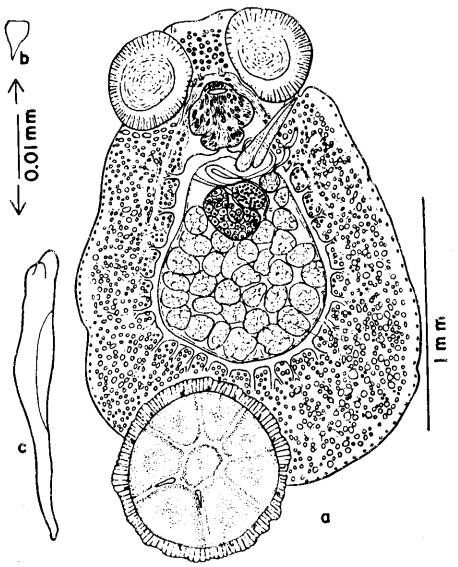

FIG. 1. *Capsala manteri*, n. sp. a—Complete worm, ventral view; b—dorsal marginal spine; c—large hook (anchor) of posterior haptor. Original.

Filariasis in American Samoa I. Loss of Microfilaria in the Absence of Continued Reinfection[1]

LEO A. JACHOWSKI, JR., GILBERT F. OTTO, and JAMES D. WHARTON

Information on the period that a population retains or loses its microfilaremia after reinfection ceases has practical application. Data of this sort are important in programs in which control of filariasis is attempted by elimination of the vector. Obviously, the period during which transmission is inhibited must be of

[1] From the U. S. Naval Medical Research Institute and the School of Hygiene and Public Health, The Johns Hopkins University.

The opinions and statements expressed are those of the authors and not necessarily those of the Navy Department.

We wish to acknowledge the technical assistance of H. M. Powell, HMC, USN and H. H. Marrer, HMC, USN and the cooperation in Samoa of Capt. J. A. C. Gray, MC, USN and in Hawaii of Capt. W. H. Perry, MC, USN; Lt. Cdr. B. D. Castell, MC, USN; CWO C. Butterworth, HC, USN; Mr. R. E. Wooley, President of the Church of Latter Day Saints in Hawaii; and Dr. S. M. K. Hu of the Health Department, Territory of Hawaii.

sufficient duration to allow the population to lose its filarial infections, if the benefits are to continue after the termination of the anti-mosquito measures.

Two types of studies can provide this information. Ideally, a group of persons known to be infected and recently arrived in an area where transmission does not occur should be examined periodically until they show no microfilaremia. The second approach is to survey a population that has migrated at varying times to a region where there is no transmission and to compare their incidence of infection with that of the population in their home area. Both types of studies were initiated among Samoans, who harbor the non-periodic strain of *Wuchereria bancrofti*, but only data on the latter approach has been completed.

A group of 102 Samoan nurses under training or on duty at the Samoan Hospital at Utulei, Tutuila, American Samoa was examined first. The student nurses ranged in age from 16 to 20 years, while the graduate nurses varied from 20 to 53 years in age. They came from all parts of American Samoa. Girls of 16 to 18 years of age who are accepted as student nurses spend four years studying at the hospital. Except for brief periodic visits home, they live and work in screened modern buildings located within the confines of the U. S. Naval Station. For many years sanitation and mosquito control have been practiced at the Naval Station making transmission of filariasis in this area unlikely.

TABLE 1.—*Incidence of microfilarial infections among 102 Samoan nurses on duty at the Samoan hospital*

Group	Months on duty at hospital	Range of age in years	Number Nurses	Number with microfilariae	% infected
Undergraduates	4	17–19	21	4	19.0
	16	18–20	11	2	18.2
	28	19–21	12	2	16.7
	40	20–22	7	0	0
Graduates	52	20–24	24	1	4.7
	48+*	23–53	27	2	7.6

* 48 months in training in the hospital followed by continuous or intermittent duty in the hospital for 1 to 30 years.

Blood smears (measured volumes of 60 cmm. blood per individual) were made on the entire group of nurses between 1:00 and 2:00 P.M. on a single afternoon. In the group were 51 student nurses with four to 40 months of training; 24 recently graduated nurses who had 52 months residence at the hospital and who were about to be assigned to duty in villages or in outlying dispensaries; and another 27 older graduate nurses with varying duties since graduation. The latter group was heterogenous, including some with continuous duty at the hospital and others with many years of duty in the villages. Results of this blood examination are summarized in Table 1.

While there is an apparent reduction in the incidence of infection associated with the time the undergraduates spent at the hospital, the number of such nurses is small. A more significant comparison is made by considering larger groups. Of the entire 102 nurses of all categories only 8.9% were positive as compared with 30.2% in 434 village women. By use of the chi squared test (Table 2) it is evident that there is no more than five chances in 10,000 that they could be representative of the same population, or in other words the nurses have a significantly lower infection than the village women. The undergraduate nurses on the other hand have essentially the same infection rate (15.7%) as the village women of

the same age (20.6%). Accordingly, the graduate nurses are the group which account for lower microfilaremia among the nurses, as shown by the chi squared test. Furthermore, even the three graduate nurses, in whose blood microfilaria were demonstrated, had started their training respectively only 52 months, five years, and six years before. Thus, it seems evident that microfilaria may be expected to disappear in little more than five years after reinfection ceases. The fact that none of the older nurses, who actually had lived and worked in the villages, were not infected suggests in itself that the village is not the source of infection which is in accord with more extensive data[2] on this subject which will be published later.

In May, 1950 an opportunity was afforded to survey a group of Samoans residing in Hawaii and the results bear directly on the question of the persistence of this infection.

For at least 45 years there has been a migration of Samoans from both American Samoa and Western Samoa to Hawaii. At Laie on the island of Oahu there are about 300 Samoans both immigrants and Hawaii-born. On May 1950, 132 of

TABLE 2.—*Comparison of the incidence of microfilaremia in Samoan nurses with that of women of comparable age groups from ten villages*

Group	Age in years	Number women	Number negative	Number positive	% positive	Chi squared	Prob. by chi squared
All nurses	17–53	102	91	11	8.9	15.92	<0.0005
Village women	>15	434	303	131	30.2		
Undergraduate Nurses	17–22	51	43	8	15.7	0.63	0.42
Village Women	16–25	189	150	39	20.6		
Graduate nurses	20–53	51	48	3	5.9	13.31	<0.0005
Village women	>15	434	303	131	30.2		

these Samoans were examined for microfilariae. Of this group 60 were born in Hawaii of Samoan parents. Since all were free of microfilariae it was concluded that transmission of filariasis probably does not occur in Hawaii. Therefore, any person with the infection is believed to have acquired his microfilaremia in Samoa prior to moving to Hawaii. Among the migrants, eight of 72 were infected and all eight had been in Hawaii for less than five years.

Later, Dr. D. D. Bonnet of the Health Department, Territory of Hawaii kindly made available similar data on 47 Samoans examined in 1943–44. In his group also all 15 individuals born in Hawaii were negative while three of the 32 born in Samoa had microfilaremias. Two had been in Hawaii for less than three years, while the other had lived there for six years.

In Samoa the percentage of the population infected increases with age. Since the time scale is the same whether it refers to an increase in age or years of residence in Hawaii, the time trends of infection in Samoa and Hawaii may be compared directly. It will be observed (Fig. 1) that trends are diametrically opposite in the two groups. The higher initial rate among the Hawaii group is due to the fact that all ages are included in this group. Nevertheless, five years residence in Hawaii, regardless of age on arrival, should not result in a decrease

[2] Data to be published in another paper indicates that in American Samoa transmission takes place primarily in the "bush" and not in the houses of village.

but rather an increase in the infection rate if transmission occurs as in Samoa. Although there were very few found in this survey who had been in Hawaii five to ten years it is interesting to note that, just as with the Samoan nurses, the last positive was found in a Samoan six years removed from normal exposure in Samoa.

DISCUSSION

Both the data on the Samoan nurses removed from their normal native activities to the protected environment of the hospital and the more restricted life either in the hospital or village after graduation and the removal of both males and females of all ages from the endemic center in Samoa to the non-endemic area of Hawaii, indicate that the microfilaria disappear from the blood within ten years after removal from exposure to reinfection. Only one in each group

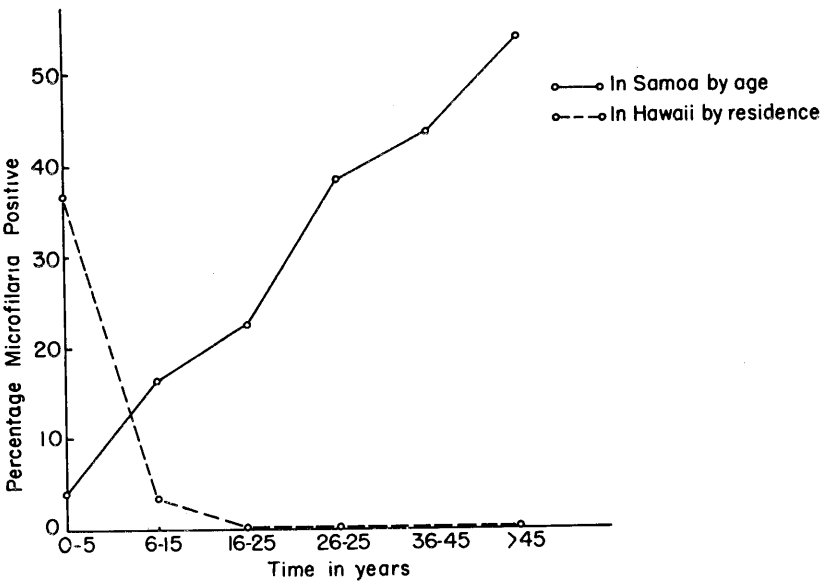

FIG. 1. Incidence of microfilaremia, by years of age, among Samoans in Samoa compared with the incidence, by years of residence, among Samoans in Hawaii.

was positive after five years and these two were only six years removed from their normal native activities in the endemic areas. Thus, a mosquito control program, short of complete eradication, would have to be sufficiently effective to prevent transmission for about five years or perhaps longer if the benefits were to last materially beyond the actual control operations.

It may be noted, in passing, that if survival of microfilariae in American military personnel exposed during World War II is assumed to be comparable to that in the Samoans studied, now, five years after the war, it would be unlikely that any of them are microfilariae positive and subsequent surveys can give little information concerning the number which actually became microfilariae positive at any time.

SUMMARY

Microfilaria of the non-periodic strains of *W. bancrofti* persist less than 10 years after removal from the endemic area and usually disappear within five years. Actually, the longest survival recorded in this survey was six years.

The Generic and Trivial Names of the Species of Nematodes Parasitic in the Large Intestine of Equines, Commonly Known from 1831 to 1900 as *Strongylus tetracanthus* Mehlis, 1831

ALLEN MCINTOSH
Zoological Division, Bureau of Animal Industry, U. S. D. A.

On November 28, 1930, the late Dr. Maurice C. Hall, then Chief of the Zoological Division, requested the writer to undertake the task of clearing up the nomenclature of the cylicostomes. Up to this time there was a general belief that to clear up the complexity of conflicting opinions a thorough study should be made of the encysted developmental stages of the cylicostomes found in the mucosa of the large intestine of the horse, since one of the generic names in common use was based on larval stages belonging to this group of parasites.

After an examination of numerous encysted larvae it was apparent that not only were there several species but also several genera represented and that to resolve the problem various errors in nomenclature would have to be corrected. Early in 1932 the writer submitted a manuscript on the nomenclature of these parasites to Dr. Hall for consideration. On May 31, 1932 the manuscript was returned to the writer with the following comment:

"I have held your paper on cylicostomes for several weeks and have gone over the situation as carefully as possible with a view to determining just what action should be taken. It does not seem to me that it would be a constructive measure to publish this paper and wait for months or years for reaction of zoologists in general, as I believe that those reactions would be very diverse and that there would be a tendency for the men who are working with these worms to cling to the names which they are now using.

"On the other hand, I believe that if you will put this proposition up to the International Commission and request a formal settlement, such action will have the immediate advantage of establishing some name on the strength of a decision by the Commission and this Division can then establish its nomenclature on the basis of the Commission's decision. Consequently I would recommend that you outline the point made in your paper and submit the case formally to Dr. Stiles for a decision by the Commission."

After some revision the manuscript on August 30, 1932, was forwarded to Dr. C. W. Stiles, then Secretary of the International Commission of Zoological Nomenclature, with a request for formal settlement of the problem by the Commission. The manuscript submitted was as follows:

> *Cyathostomum tetracanthum* (Mehlis, 1831) Molin, 1861, partim, Looss, 1900, versus *Trichonema insigne* (Boulenger, 1917) Le Roux, 1924, and *Trichonema longibursatum* (Yorke and Maefie, 1918) Le Roux, 1924.
>
> This is an attempt to clarify the confusion existing in that group of genera and species of nemas parasitic in the large intestines of equines, which were, from 1831 to 1900, generally known as *Strongylus tetracanthus* Mehlis, 1831.

HISTORICAL SUMMARY

The fragmentary description by Mehlis (1831) of *Strongylus tetracanthus*, given without illustration, is too meager to be applied to any one species. Mehlis, no doubt, regarded all of the small strongyles found in the horse as belonging to his species *Strongylus tetracanthus*, unfortunately a very unsound assumption.

Gurlt (1831), some time later in the same year that Mehlis published his description, also gave a very brief description of *Strongylus tetracanthus* that had been communicated to him by Mehlis. Gurlt's description is accompanied by ten figures. Two varieties are recognized, a larger variety with males 6 to 7 lines long and females 7 to 8 lines long and a smaller, more abundant, variety with males only 4 lines long and females over 5 lines long. It is interesting, in this connection, to note that the small immature worms found in the mucosa of the gut were regarded as the young of *Strongylus tetracanthus*. We are, therefore, led to believe that both Mehlis and Gurlt unfortunately regarded all of the smaller strongyles of the horse, regardless of shape, size, or color, as *Strongylus tetracanthus*.

Diesing (1851) placed *Strongylus tetrocanthus* in the genus *Sclerostomum* along with *Strongylus armatus* and numerous other species from various hosts. Diesing also recognized a *minor* and a *major* variety of *Strongylus tetracanthus*. It may be noted in this connection that *Sclerostomum* has fallen into the synonomy of *Strongylus* and is not available in connection with a generic name for *Strongylus tetracanthus*.

Molin (1861) proposed the generic name *Cyathostomum* as a special genus for *Strongylus tetracanthus* Mehlis. Molin noted considerable variation in the dimension of the mature individuals and, like Gurlt and Diesing, he recognized a *major* and a *minor* variety. His two figures (Pl. XXV, fig. 5 and 6) of a male and a female apparently refer to the major variety. It is very doubtful, however, if these figures, although drawn in considerable detail, are of any great value in aiding one to form a conclusion as to just what particular species Molin had before him.

Looss (1900), in his initial papers on the horse strongyles, accepted *Cyathostomum* as valid and then, later (1902), rejected it as a homonym of *Cyathostoma* Blanchard, 1849. However, if we accept the recommendations under Article 36 of the International Code of Zoological Nomenclature, the name is not a homonym and is not to be rejected unless somewhere a misprint of *Cyathostoma* in the form *Cyathostomum* invalidates it. A search of the available literature does not reveal such a misprint. Molin's contribution was, therefore, the creation of a special genus for *Strongylus tetracanthus* Mehlis, 1831, thereby separating generically the small strongyles from the large strongyles (*Strongylus armatus* = *Strongylus equinus, S. edentatus,* and *S. vulgaris*). It remained for Looss (1900) to divide *Strongylus tetracanthus* Mehlis, 1831 = *Cyathostomum tetracanthum* (Mehlis, 1831) Molin, 1861 into some of the many restricted species that it represented.

Previous to Looss' work, Cobbold (1874) described *Trichonema arcuata*. This species was based on larval forms found in cyst-like pellets taken from the intestinal mucosa of the horse. Cobbold (1875) says of his species "At all events my so-called *Trichonema arcuata* must be abandoned as a species; all these encysted worms of whatever size being nothing more than varying stages of growth of *Strongylus tetracanthus*". *Trichonema*

arcuata Cobbold, 1874, is therefore to be regarded as a synonym of *Cyathostomum tetracanthum* (Mehlis, 1831) Molin, 1861.

Should it be ruled that *Cyathostomum* Molin, 1861, is invalidated on account of its being a doubtful homonym of *Cyathostoma* Blanchard, 1849, *Trichonema* would then be an available generic name for the small strongyles known as *Strongylus tetracanthus* Mehlis, 1831. Many writers are apparently of the opinion that *Cyathostomum* is invalidated and have accepted the name *Trichonema* as the valid generic name for this group. Looss (1902), after rejecting *Cyathostomum* did not, however, adopt *Trichonema*, but instead proposed the new generic name *Cylichnostomum*. Even this name was invalidated, it being a synonym of a similar name, *Cylicostomum*, which was published by Railliet a short time previous to Looss' publication of *Cylichnostomum*. Both *Cylichnostomum* and *Cylicostomum* as well as *Trichonema* are, therefore, synonyms of *Cyathostomum* Molin, 1861.

DISCUSSION

In view of the above summary, the question still stands as to whether *Cyathostomum* Molin, 1861 is a homonyn of *Cyathostoma* Blanchard, 1849. According to the recommendation under Article 36 of the International Code of Zoological Nomenclature, and in conformity with Opinion 86 rendered by the International Commission on Zoological Nomenclature, the generic name *Cyathostoma* Blanchard, 1849, does not invalidate *Cyathostomum* Molin, 1861. Stiles and Hassall (1905) on page 75 in "The Determination of Generics Types", make the following statement: "Judging from von Linstow's position on absolute homonyms, he would doubtless accept doubtful homonyms as available. Jordan, Everman, Ashmead, and a number of other authors, including ourselves, accept names of this class on the ground that a difference of a single letter in two names precludes the possibility of their being identical, hence they can not be homonyms. (See Art. 36, Internat. Code.)"

The writer is, therefore, of the opinion that, according to the Internationl Rules of Zoological Nomenclature, the name *Cyathostomum* Molin, 1861, is available as a generic name with the following synonymy:

Genus *Cyathostomum* Molin, 1861, not *Cyathostoma* Blanchard, 1849.

Syn., *Trichonema* Cobbold, 1874; *Cylicostomum* Looss, in Railliet, 1901; *Cylichnostomum* Looss, 1902; *Cylicostoma* Looss, 1911; *Cylicostomias* Cram, 1925.

THE TYPE SPECIES

As it has been pointed out that *Cyathostomum* Molin 1861, is the valid name of the genus for *Strongylus tetracanthus* Mehlis, 1831, the following questions arise: What natural species shall be designated as type? What is the name of the type species?

Three natural species have been designated as type. Looss (1900) in splitting *Strongylus tertracanthus* Mehlis, 1831 = *Cya-*

thostomum tetracanthum (Mehlis, 1831) Molin, 1861, into numerous restricted species, selected the form that was found most abundant in Egyptian horses and asses as type, and designated it as "*Cyathostomum tetracanthum* (Mehlis) sens. strict." This act of Looss is in strict conformity with the International Code (Article 31 and 29, Internat. Code). Railliet (1923) proposed *Trichonema insigne* (Boulenger) as type, changing the name to *Trichonema tetracanthum* = *Cylichnostomum insigne* Boulenger, 1917. Railliet's chief objection to the species that Looss designated as type, appears to be based on the belief that it was an Egyptian species, a species which up to then had not been observed in Europe. Today, however, there are several records of this species occurring in Europe. Le Roux (1924) proposed *Trichonema* (*Trichonema*) *longibursatum* (Yorke and Macfie, 1918) as type. This species he found to be most common in the species he examined, and had been reported from most parts of the world. He is of the opinion that the name *Strongylus tetracanthus* is a "*nomen nudum*," and states that "neither Looss nor Railliet had any valid reason for applying the name *tetracanthus* to any one species of the genus *Trichonema*."

Yorke and Maplestone (1926) do not concur in the action of either Railliet or Le Roux, but state that to them it seems "desirable to retain *Cylichonostomum tetracanthum* Mehlis of Looss, 1900, as type of the genus *Trichonema*."

The present writer is of the opinion that Looss acted in accordance with Art. 29, Art. 30g, and Art. 31 of the International Code, in selecting the natural species that he chose as type, and in giving it the name *Cyathostomum tetracanthum* (Mehlis, 1831).

SYNONYMY OF TYPE SPECIES

Cyathostomum tetracanthum (Mehlis, 1831) Molin, 1861, partim, Looss, 1900.

Syn., *Stronglylus tetracanthus* Mehlis, 1831, partim.
Sclerostomum tetracanthum (Mehlis, 1831) Diesing, 1851, partim.
Cylichnostomum tetracanthum (Mehlis, 1831) Looss, 1902.
Cylicostomum tetracanthum (Mehlis, 1831) Gedoelst, 1903.
Cylicostoma tetracanthum (Mehlis, 1831) Looss, 1911.
Trichonema tetracanthum (Mehlis, 1831) Railliet, 1919.
Trichonema arcuata Cobbold, 1874, partim.
Trichonema aegyptiacum Railliet, 1923.
Cylicostomum aegyptiacum (Railliet, 1923) Cram, 1924.
Cylicostomias aegyptiaca (Railliet, 1923) Cram, 1925.
Sclerostoma quadridentatum Dujardin, 1845, partim.

In a letter dated September 5, 1944, Mr. Francis Hemming, successor to Dr. C. W. Stiles as Secretary to the International Commission of Zoological Nomenclature, stated that one of the questions raised in the manuscript was settled by the decision taken by the International Commission at their meeting held at Lisbon, 1935, the record of which was published in 1943 (Bull. Zool. Nomencl. 1: 39–40) and later embodied in the Commission's Opinion 147. Mr.

Hemming stated that the effect of that decision is to make it clear that a name such as *Cyathostomum* Molin, 1861 is not to be rejected as a homonym of *Cyathostoma* Blanchard, 1849, and as stated in the manuscript, it follows from this that the name *Trichonema* Cobbold, 1874, falls as a synonym.

Regarding the question as to which of the originally contained species is the species to which the restricted name *Strongylus tetracanthus* Mehlis, 1831 should be applied, the Secretary of the Commission in his letter of September 5, 1944, remarked as follows:

"As regards this, Mr. McIntosh states that Looss (1900) was the first author definitely to restrict the name *Strongylus tetracanthus* Mehlis, 1831, to the species 'that was found most abundant in Egyptian horses and asses.' If this is so, then Looss's designation was *intra vires* and cannot be called in question, provided that there is no reasonable doubt that this Egyptian species was included among the material studied by Mehlis when drawing up his description of *Strongylus tetracanthus.*"

In the light of present day knowledge of the distribution of the so-called Egyptian species, the objection raised by Railliet that Looss had selected an exotic species no longer holds. The writer therefore maintains that there is no evidence to support a reasonable doubt that the Egyptian species was not among the complex that Mehlis christened *Strongylus tetracanthus,* and until it can be shown that there is doubt, the species selected for the trivial name *tetracanthus* by Looss is to be accepted.

With the question settled (Opinion 147, I. C. Z. N.) that *Cyathostomum* Molin, 1861, is not a homonym of *Cyathostoma* Blanchard, 1849, and since the views held by Railliet (1923) and Le Roux (1924), as to the natural species that should be selected as type from the "*Strongylus tetracanthus*" complex, are not tenable, we have no alternative but to uphold as the type of this complex the natural species selected by Looss and for it the name *Cyathostomum tetracanthum* (Mehlis, 1831) Molin, 1861, *partim* Looss, 1900.

LIST OF SPECIES

Looss (1902) and some subsequent writers have observed that the species of the genus *Cyathostomum* Molin, 1861, *sensu lato,* may be divided into several groups with more or less similar characteristics. Ihle (1922 and 1925) proposed sub-generic names for these groups and Cram (1924 and 1925) elevated these names to generic status and proposed some additional ones.

In conversation, a number of my associates, especially F. D. Enzie, A. O. Foster, John T. Lucker and E. E. Wehr, each of whom has had the tedious task of identifying enormous numbers of these parasites, agreed with the writer that there is ample justification for recognizing generic rank for these groups.

These genera and their species are as follows:

Genus *Cyathostomum* Molin, 1861, *sensu stricto*

1. *Cyathostomum tetracanthum* (Mehlis, 1831) Molin, 1861, partim Looss, 1900, genotype.
2. *Cyathostomum coronatum* Looss, 1900.
3. *Cyathostomum labiatum* (Looss, 1902) McIntosh, 1933.
4. *Cyathostomum labiatum* var. *digitatum** (Ihle, 1921) McIntosh, 1933.
5. *Cyathostomum labratum* Looss, 1900.
6. *Cyathostomum ornatum* (Kotlan, 1919) McIntosh, 1933.
7. *Cyathostomum sagittatum** (Kotlan, 1920) n. comb.

* Validity of the species regarded as doubtful by some authorities.

Genus *Cylicocercus* Ihle, 1922

8. *Cylicocercus alveatus* (Looss, 1900) Cram, 1924, genotype.
9. *Cylicocercus catinatus* (Looss, 1900) Cram, 1924.
10. *Cylicocercus catinatus* var. *literaureus* (Yorke and Macfie, 1920) Cram, 1924.
11. *Cylicocercus catinatus* var. *pseudocatinatus* (Yorke and Macfie, 1920) Cram, 1924.
12. *Cylicocercus goldi* (Boulenger, 1917) Cram, 1924.
13. *Cylicocercus goldi* var. *tridentatus** (Yorke and Macfie, 1920) Cram, 1924.
14. *Cylicocercus pateratus* (Yorke and Macfie, 1919) Cram, 1924.

Genus *Cylicocyclus* Ihle, 1922

15. *Cylicocyclus radiatus* (Looss, 1900) Chaves, 1930, genotype.
16. *Cylicocyclus adersi* (Boulenger, 1920) Chaves, 1930.
17. *Cylicocyclus ashworthi** (Le Roux, 1924) McIntosh, 1933.
18. *Cylicocyclus auriculatus* (Looss, 1900) Chaves, 1930.
19. *Cylicocyclus elongatus* (Looss, 1900) Chaves, 1930.
20. *Cylicocyclus elongatus* var. *kotlani** (Ihle, 1920) Chaves, 1930.
21. *Cylicocyclus insigne* (Boulenger, 1917) Chaves, 1930.
22. *Cylicocyclus leptostomus* (Kotlan, 1920) Chaves, 1930.
23. *Cylicocyclus nassatus* (Looss, 1900) Chaves, 1930.
24. *Cylicocyclus nassatus* var. *parvus** (Yorke and Macfie, 1918) Chaves, 1930.
25. *Cylicocyclus triramosus* (Yorke and Macfie, 1920) Chaves, 1930.

Genus *Cylicodontophorus* Ihle, 1922

26. *Cylicodontophorus euproctus* (Boulenger, 1917) Cram, 1924, genotype.
27. *Cylicodontophorus bicornatus* (Looss, 1900) Cram, 1924.
28. *Cylicodontophorus mettami* (Leiper, 1913) Foster, 1936.
29. *Cylicodontophorus ultrajectinus* (Ihle, 1920) Cram, 1924.

Genus *Cylicostephanus* Ihle, 1922

30. *Cylicostephanus calicatus* (Looss, 1900) Cram, 1924, genotype.
31. *Cylicostephanus barbatus** (Smit and Notosoediro, 1923) Cram, 1925.
32. *Cylicostephanus hybridus* (Kotlan, 1920) Cram, 1924.
33. *Cylicostephanus longibursatus* (Yorke and Macfie, 1918) Cram, 1924.
34. *Cylicostephanus minutus* (Yorke and Macfie, 1918) Cram, 1924.
35. *Cylicostephanus poculatus* (Looss, 1900) Cram, 1924.
36. *Cylicostephanus parvibursatus** (Vaz, 1934) Foster and Alicata, 1939.

Genus *Cylicotetrapedon* Ihle, 1925

37. *Cylicotetrapedon bidentatum** Ihle, 1925, genotype.
38. *Cylicotetrapedon asymetricum* (Theiler, 1923) Ihle, 1925.

Genus *Cylicobrachytus* Cram, 1924

39. *Cylicobrachytus prionodes* (Kotlan, 1921) Cram, 1924, genotype.
40. *Cylicobrachytus brevicapsulatus* (Ihle, 1920) Cram, 1924.

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Life History of *Oesophagostomum venulosum*, a Nematode Parasite of Sheep and Goats

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INTRODUCTION

Three nematode parasites, commonly referred to as nodular worms, frequently occur in cattle, sheep and goats. Two of these, namely, the nodular worm of sheep, *Oesophagostomum columbianum*, and the nodular worm of cattle, *Oesophagostomum radiatum*, are definitely known to have serious effects on the health and well-being of the animals they infest. Although *Oesophagostomum venulosum*, the third member of this group, has been reported from almost every country raising sheep and goats, there has been little investigation of its life history and of the effect, on these animals, of infestation with this parasite. To add to our knowledge concerning this parasite, the present study was undertaken. It was deemed desirable to begin with the life history of the worm.

MATERIALS AND METHODS

All lambs (Shropshire × Hampshire) and kids used in this study were free of helminth parasites, except for a few *Strongyloides*. The animals were maintained under conditions preventing extraneous infection. Eggs obtained from 32 *O. venulosum* were cultured and the resulting infective larvae administered to a lamb. A month later *O. venulosum* eggs were present in the feces. This lamb and others infected from it served as the source of material used for the study of the free-living and parasitic stages. The experimental lambs and kids varied in age from 2 to 11 months at the time of infection. The infective larvae were administered by mouth, suspended in about 10 ml. of water. During the study of the parasitic stages, the time intervals between necropsies were shorter when metamorphosis between the third and fourth stages, and between the fourth and fifth stages, was occurring than during the relatively stable period of growth within the fourth and fifth stages. In determining the number of worms recovered during the development of the parasitic stages, representative samples were counted for the animals killed on the third, fourth and fifth days after infection. In all other cases the total numbers of worms recovered were counted.

Unless otherwise stated, measurements of worms recovered at necropsy were made from at least 10 specimens taken at random.

The illustrations are primarily intended to show diagnostic characters of the different stages in the life history of *O. venulosum*.

DESCRIPTION OF FREE-LIVING STAGES

Egg.—When the eggs (Fig. 1, G) are passed in the feces, the morula is usually in the 16 to 32 cell stage, occasionally in the 8 or 64 cell stage. The eggs

have rounded sides and obtuse ends, one end usually being blunter than the other. The cells are dark gray. Frequently the eggs are slightly asymmetrical. Measurements of 100 eggs from fresh feces showed an average length of 0.0966 mm. (0.0876–0.105 mm.) and an average width of 0.0586 mm. (0.0555–0.0642 mm.). The average form index $\frac{\text{width} \times 100}{\text{length}}$ was 60.7. Eggs of females just beginning to lay tend to be longer and narrower than those of females which have been laying for some time.

First stage larva.—In fresh charcoal-feces cultures, the first stage larvae (Fig. 1, A) begin to hatch out, close to one end of the egg, in approximately 24 hours. Ten to 12 hours after hatching, the first stage larva averages 0.554 mm. in length, and 0.0265 mm. in width at the level of the base of the esophagus. It has a rhabditiform esophagus and a long, sharply pointed tail. In living specimens the intestinal tissue appears to be syncytial. The larva's motions are primarily for feeding purposes. Frequently it will attach itself by its tail to bits of debris and swing back and forth with the esophagus continually undergoing contraction.

Second stage larva.—The first ecdysis occurs approximately 24 hours after hatching. The second stage larva is very similar to the first stage larva. It also has a rhabditiform esophagus and a long, sharply pointed tail. There are apparently 16 to 20 cells in the intestine; the divisions between cells are not well marked. A specimen (Fig. 1, B) measured just after it completed the first moult was 0.730 mm. long, and 0.028 mm. wide at the level of the base of the esophagus. Its tail was 0.213 mm. long.

Third stage larva.—In charcoal-feces cultures kept at room temperature, the third stage infective larva (Fig. 1, C) is formed 3 to 5 days after hatching. The first change noticed in the second stage larva is the closure of the mouth by the collapse of the buccal tube. The Y-shaped valve of the esophageal bulb disappears and the bulb becomes slender, resulting in a strongyliform esophagus. The third stage larva has three lips. The average distance of the excretory pore from the anterior end of the body is 0.126 mm. Usually there are 32, occasionally 30 or 34, light yellow-green intestinal cells. They appear to be full of stored food and are clearly visible. The genital primordium usually lies opposite the juncture of the ninth and tenth cells on the ventral side of the intestine. The tail proper of the larvae is 0.070 mm. long and, unlike that of the second and first stage larvae, has a rounded end. The larva retains the cuticle of the second stage. It acts as a protective sheath, and by means of its long tail it facilitates migration. the primary activity of the third stage larva. Many larvae can be maintained in culture or water for at least 2 months, and a few are still alive after 5 months. However, no further progressive changes occur, and there is depletion of the food stored in the intestinal cells and vacuolation of tissues. Including its sheath the larva averages 0.899 mm. in length, and 0.030 mm. in width at the level of the base of the esophagus. Without the sheath it averages 0.727 mm. in length. Measurements of the free-living larvae are given in Table 1.

DESCRIPTION OF PARASITIC STAGES

Third stage larva.—Ten lambs and one kid were used to study the parasitic stages of *O. venulosum* from the first to the thirty-first day after infection. Measurements of the worms recovered certain days after infection are given in Table 2. The first animal was necropsied 22 hours after infection with third stage larvae. The larvae recovered had the following characteristics. The sheath had been cast off. The anterior end was truncate. The mouth and esophagus were

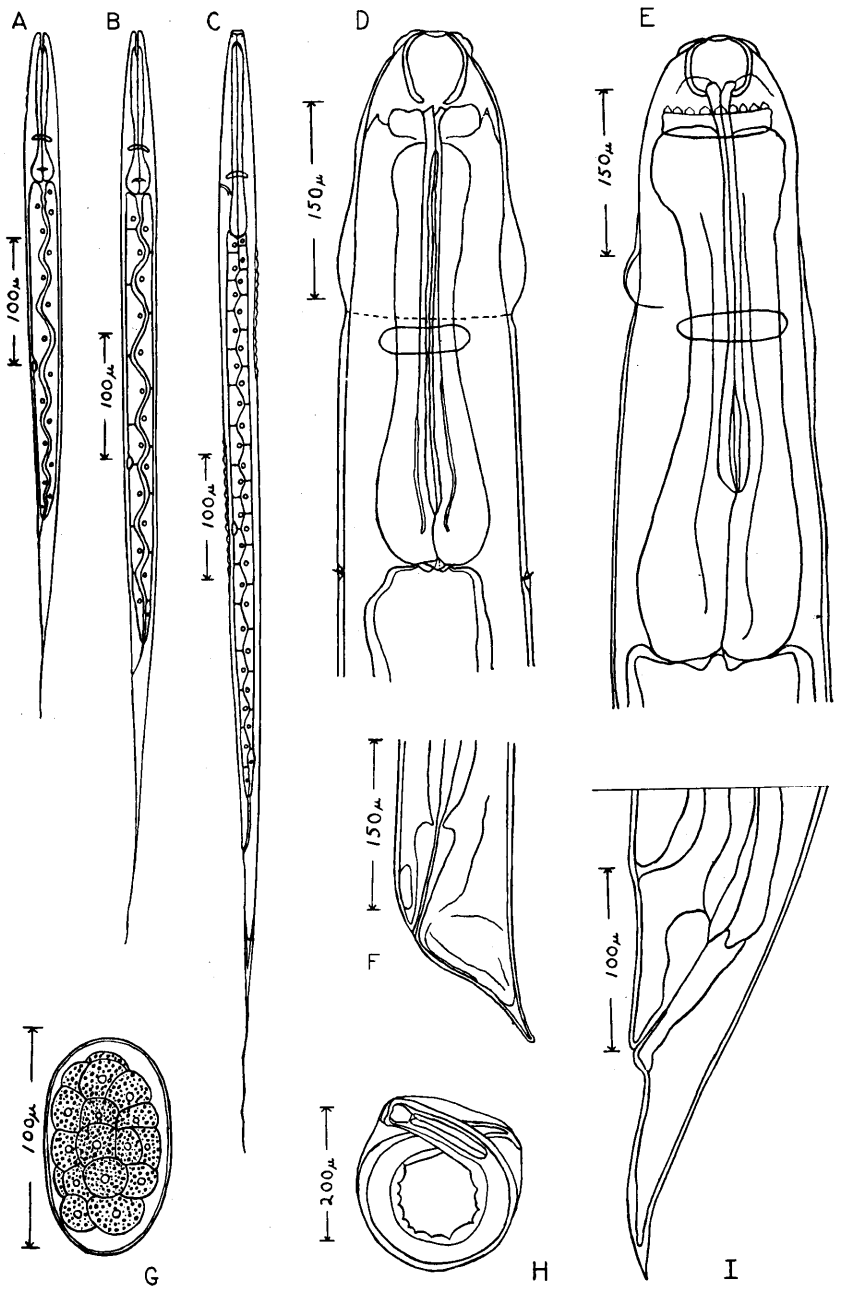

FIG. 1. Larval stages of *Oesophagostomum venulosum*. A—First stage larva approximately 10 hours after hatching. B—Second stage larva immediately after completing the first moult, 24 hours after hatching. C—Third stage, infective larva. D—Anterior end of a late fourth stage larva 15 days after infection, dorsal view. E—Anterior end of a late fourth stage larva 15 days after infection, lateral view. F—Posterior end of a late fourth stage male larva 15 days after

still closed. The cervical glands were very conspicuous and they masked much of the intestine. They extended from the region of the excretory pore almost to the genital primordium, which was beginning to grow. The larvae showed active swimming and wriggling motions, and all were found in the lumen of the intestine. Some of the larvae recovered were maintained at room temperature for a month in a mixture of physiological salt solution and some of the fluid from the intestine of the sheep. Although they were active, the mouth remained closed and no further development occurred.

Three days after infection the larvae were found encysted in the mucosa of the small intestine. They were surrounded by a thin transparent membrane within which they were coiled in various manners. Seven cysts were measured. The dimensions averaged 0.257 mm. (0.227–0.276 mm.) by 0.181 mm. (0.159–0.196 mm.). The cysts were easily extracted from the mucosa and the larvae extracted from the cysts without difficulty. Their length averaged 0.905 mm. and the width

TABLE 1.—*Measurements in millimeters of preparasitic larvae of* Oesophagostomum venulosum[a]

Item	Measurement, at indicated number of hours after hatching,		
	in middle of first stage (10–12 hrs.)	at beginning of second stage (24 hrs.)	in third stage (72–120 hrs.)
Total length	0.554	0.730	0.899[b]
Length of buccal tube	0.0156	0.015	collapsed
Length of esophagus	0.120	0.132	0.168
Width of body at base of esophagus	0.0265	0.028	0.030
Anterior end to genital primordium	0.263	0.345	0.398
Anus to tip of tail	0.145	0.213	0.070
Tip of tail to tip of sheath	.....	.....	0.172

[a] Measurements are averages of at least 10 specimens except those of the second stage, which are of one specimen.
[b] Including the sheath.

at the level of the base of the esophagus was 0.049 mm. The tail averaged 0.074 mm. in length. The larvae were still in the third stage, and a provisional buccal capsule had not yet been formed. However, the area it would occupy was clearing. The larvae moved when manipulated; otherwise they were quiescent.

Fourth stage larva.—On the fourth day after infection the majority of the larvae had emerged from the mucosa and were found in the lumen of the intestine in close proximity to the wall. Four per cent of the larvae recovered were still in the third stage. Ninety-six per cent had well developed provisional buccal capsules indicative of the fourth stage; 20 per cent were ensheathed or undergoing the third ecdysis, and 76 per cent had already entered the fourth stage. One larva (Fig. 1, H) found both encysted and ensheathed was provided with a well de-

infection, lateral view. G—Egg of *Oesophagostomum venulosum* from fresh feces. H—Outline of an encysted and ensheathed third stage parasitic larva 4 days after infection. I—Posterior end of a late fourth stage female larva 15 days after infection, lateral view.

TABLE 2.—*Measurements in millimeters of parasitic larvae and adults of* Oesophagostomum venulosum[a]

Item	Measurements of males (M) and females (F), at indicated number of days after infection,												
	in third stage	in fourth stage						in fifth stage					
		beginning		middle		late		young adult				mature adult	
	3	4	5	11		15		15		21		28 and more	
	[b]	[b]	[b]	M	F	M	F	M	F	M	F	M	F
Total length	0.91	0.96	1.09	2.80	2.58	3.18	3.45	4.76	5.13	7.61	7.76	12.6	14.9
Length of buccal capsule	.....	0.03	0.04	0.05	0.05	0.05	0.05	0.09	0.09	0.09	0.09	0.09	0.09
Width of buccal capsule	.....	0.03	0.03	0.05	0.05	0.05	0.05	0.02	0.02	0.02	0.02	0.02	0.02
Anterior end to ventral cervical groove	.....	.....	.....	0.20	0.20	0.21	0.23	0.27	0.28	0.30	0.30	0.34	0.36
Length of esophagus	0.21	0.17[c]	0.18	0.32	0.31	0.35	0.38	0.54	0.55	0.67	0.68	0.85	0.88
Width of body at base of esophagus	0.05	0.05	0.06	0.14	0.14	0.15	0.16	0.17	0.19	0.25	0.23	0.31	0.36
Anterior end to cervical papillae	.....	.....	.....	.....	.....	0.41	0.44	0.60	0.61	0.71	0.77	0.94	1.10
Anus to tip of tail	0.07	0.08	0.07	0.11	0.11	0.12	0.13	.....	0.15	.....	0.17	.....	0.18
Length of vagina	.....	.....	.....	.....	.....	.....	.....	.....	0.11	.....	0.21	.....	0.46
Vulva to anus	.....	.....	.....	.....	.....	.....	.....	.....	0.15	.....	0.17	.....	0.23
Length of spicules	.....	.....	.....	.....	.....	.....	.....	1.01	.....	1.34	.....	1.36	.....
Length of gubernaculum	.....	.....	.....	.....	.....	.....	.....	0.08	.....	0.08	.....	0.09	.....

[a] Measurements are averages of at least 10 specimens for each day.
[b] There is no appreciable difference between the measurements of the sexes on these days.
[c] Decrease from previous day is due to buccal capsule replacing part of esophagus.

veloped provisional buccal capsule. These findings indicate that metamorphosis from the third to the fourth stage occurs while the larvae are encysted in the mucosa of the intestinal wall, that after the metamorphosis is completed they emerge from the cysts enclosed in the cuticle of the third stage ready to undergo the third ecdysis, and that exsheathment takes place in the lumen of the intestine. Shedding of the cuticle while the larvae are encysted in the wall of the intestine has not been observed. Twenty-one per cent of the larvae recovered were in the anterior half of the small intestine, 74 per cent in the posterior half of the small intestine, and 5 per cent in the caecum plus the first 3 feet of the colon, approximately to the beginning of the crown where pellets are formed. From the time of infection to the beginning of the fourth stage there was a 30 per cent increase in body length and a 60 per cent increase in body width.

On the fifth day after infection the larvae had already completed much of their migration toward the posterior end of the digestive tract. One per cent were recovered from the small intestine, 95 per cent from the caecum plus the first 3 feet of the colon, and 4 per cent from the remainder of the large intestine. Approximately 98 per cent of the larvae were already in the fourth stage. The cervical papillae were evident; in the fourth stage of *O. venulosum* they range in location from the level of the middle of the esophageal bulb to a short distance posterior to the base of the bulb.

On the eleventh day after infection the cervical inflation of the larva was fairly well marked. The sexes were readily differentiated. The males measured 0.056 mm. (0.050–0.063 mm.) in width at the level of the anus and the females measured 0.035 mm. (0.032–0.37 mm.) in width in this region. The vulval primordium was seen. An abundance of dark gray granules had been deposited in the wall of the intestine, giving it a grayish appearance. Seventy-seven per cent of the larvae recovered were in the caecum plus the first 3 feet of the colon and 23 per cent in the remainder of the large intestine.

Thirteen days after infection, 99.56 per cent of the worms were in the fourth stage and 0.44 per cent were moulting or had already entered the fifth stage. The tissue of the tail of the fourth stage males was beginning to be organized into bursal rays. In some of the fourth stage larvae the definitive buccal capsule was beginning to form just posterior to the provisional buccal capsule (Fig. 1, D–E). Nine-tenths per cent of the worms recovered were in the small intestine, 94.6 per cent in the caecum plus the first 3 feet of the colon, and 4.5 per cent in the remainder of the large intestine.

Fifteen days after infection many worms were passing out with the feces. One per cent of the worms were in the small intestine, 4 per cent in the caecum plus the first 3 feet of the colon, and 95 per cent in the remainder of the large intestine. Approximately 10 per cent of the worms recovered were in the fourth stage (Fig. 1, D–F, I). Of significance in identification of the species is the fact that in these fully grown fourth stage larvae, the sclerized dorsal cone (tooth), the outlet of the esophageal gland, does not project up from the esophagus beyond the inner surface at the base of the buccal capsule. Approximately 84 per cent of the worms recovered were in the fifth stage and 6 per cent were ensheathed or undergoing the fourth ecdysis. In moulting, rupture of the cuticle occurs at the level of the base of the old buccal capsule. The inner lining of the esophagus and rectum, and the old buccal capsule are shed with the cuticle. The rays of the bursa, including the branches of the dorsal ray and their subdivisions, could readily be seen at the time of moulting. The segments of the outer and inner leaf-crowns surrounding the mouth were also evident in moulting specimens. The outer leaf-crown has the density of the rest of the cuticle, but

the inner leaf-crown is sclerotized. It consists of tripartite elements, one opposite each of the 18 outer leaf-crown elements. Two parts are sharply pointed, sclerotized denticles separated by a deep notch and projecting into the buccal cavity, and the third is an obtusely rounded part set in a recess in the base of the outer leaf-crown element. The bases of the two denticles are united to the base of the obtuse part. At the time of moulting the spicules measured approximately 1 mm. in length and they were only very slightly sclerotized.

Fifth stage, young and mature adults.—The buccal capsule of the young fifth stage worms recovered 15 days after infection had approximately the same dimen-

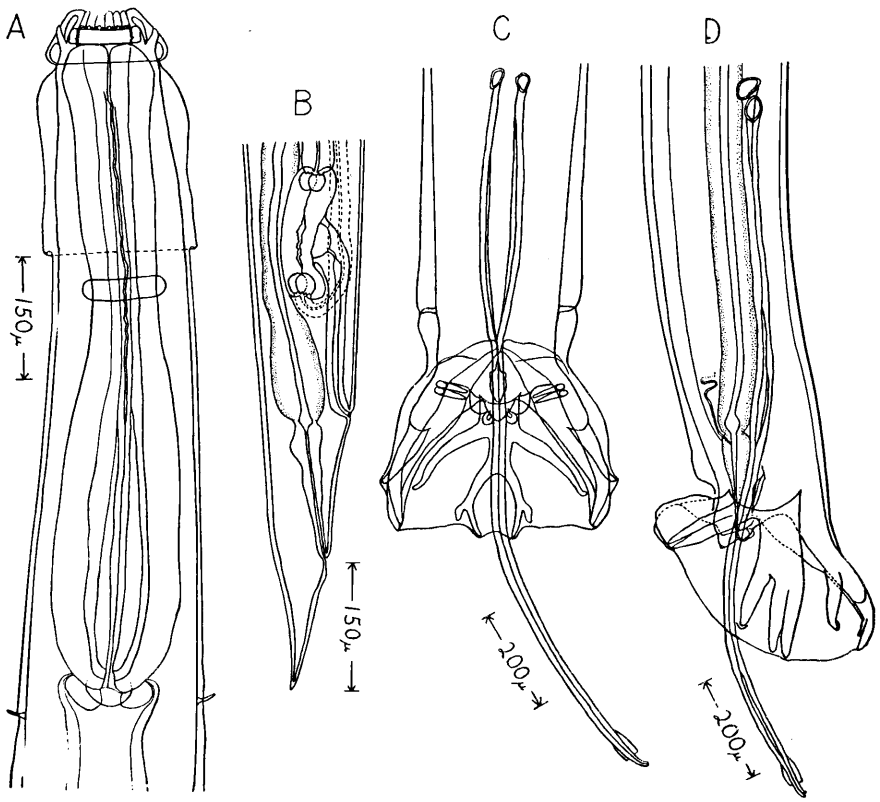

FIG. 2. Young and mature adults of *Oesophagostomum venulosum*. A—Anterior end of a young adult 21 days after infection, dorsal view. B—Posterior end of a young adult female 21 days after infection, lateral view. C—Posterior end of a mature adult male 31 days after infection, ventrodorsal view. D—Posterior end of a mature adult male 31 days after infection, lateral view.

sions as that of the mature adults, and the spicules were three fourths the length of those of the mature adults. The length of the young adults at this time was a little more than one third the length of the mature adults.

Twenty-one days after infection all the worms (Fig. 2, A–B) were in the fifth stage but none of the females were gravid. Sixty-nine per cent of the worms were in the caecum plus the first 3 feet of the colon, and 31 per cent were in the remainder of the large intestine.

Twenty-four days after infection all the worms were young adults. However, eggs were beginning to pass into the uteri in only 3 per cent of the females.

Ninety per cent of the worms were recovered from the caecum plus the first 3 feet of the colon and 10 per cent were in the remainder of the large intestine.

Thirty days after infection 31 per cent of the females had developed to the stage where they were already laying eggs or eggs were passing into the uteri. Ninety-seven per cent of the worms recovered were young or mature adults, and 3 per cent were fourth stage larvae. Seventy-six per cent of the worms were recovered from the caecum plus the first 3 feet of the colon, and 24 per cent were recovered from the remainder of the large intestine.

Thirty-one days after infection 99 per cent of the worms were recovered from the caecum plus the first 3 feet of the colon, and 1 per cent were in the remainder of the large intestine. All were actively moving about in the feces. Ninety-eight and three-fourths per cent of the worms recovered were mature adults, and the remainder were young adults.

As evidenced by a brown mucoid plug covering the vulva, at least 99 per cent of the females had been fertilized within 31 days after infection, 33 per cent within 30 days after infection, and 5 per cent within 24 days after infection. None had been fertilized within 21 days after infection. Since all the worms recovered 21, 24, and 31 days after infection and 97 per cent of those recovered 30 days after infection were adults, in moderate infections there is no significant lag in the development of the worms.

Five lambs and 3 goats were necropsied 110 or more days after infection. The few remaining worms were all mature adults located in the caecum. Six female and six male worms (Fig. 2, C–D) taken at random from mature adults recovered from a goat necropsied 6 months after infection, were measured. The females averaged 14.9 mm. (12.1–17.4 mm.) and the males 12.6 mm. (11.5–15.6 mm.) in length. There are 18 elements in the external leaf-crown surrounding the mouth. The buccal capsule is approximately five times broader than it is deep. The cervical cuticula is swollen. The cervical papillae are at the level of the body slightly posterior to the beginning of the intestine. The anterior end of the body is not hooked. The alae extending from the lateral lines are very narrow. The spicules are 1.2–1.5 mm. long. The ovijectors are separated from the vulva by a long vagina lying parallel to the long axis of the body for most of its length. The distance from the vulva to the anus is only slightly greater than the distance from the anus to the end of the tail. The vulva is about 0.5 mm. from the end of the tail. The female tail is rather abruptly tapered.[1]

A summary of the data on the age of the larvae at the time of infection, number of larvae administered, interval between infection and necropsy, and the location and percentage of worms recovered and their degree of development, is given in Table 3.

DURATION OF PATENT PERIOD OF INFECTION RESULTING FROM SINGLE ADMINISTRATION OF INFECTIVE LARVAE

A study was made of the period of patency resulting from a single inoculation. Some of the worms passed out before they became mature adults, and on several occasions adults were found passing out spontaneously in the feces. The greatest number recovered in a 24 hour fecal sample was 55, which passed out 39 days after infection. The course of 10 infections, 7 initial and 3 second, resulting from single inoculations, was followed by means of egg counts. With the use of half gram samples, the eggs were recovered by means of the salt flotation method. More than 200 samples were taken at various intervals during the period of

[1] For more detailed descriptions of the mature adults of *Oesophagostomum venulosum*, see the papers cited in the bibliography.

TABLE 3.—*Data obtained on 10 lambs to which* Oesophagostomum venulosum *larvae were administered*

Age of larval culture	Larvae fed	Interval between infection and necropsy	Worms recovered at necropsy	Worms recovered according to location				Worms recovered according to stage of development					Females with eggs in uteri
				Small intestine		Caecum plus first 3 ft. of colon	Remainder of large intestine	3rd	E.[a] or M.	4th	E.[a] or M.	5th	
				Anterior half	Posterior half								
Days	Number	Days	%[b]	%[b]	%[b]	%[b]	%[b]	%	%	%	%	%	%
20	10,000	3	38.4	70.9	29.1	.....	.....	100[c]					
7	10,000	4	1.6	21	74	5	.....	4	20	76			
14	5,000	5	37.8	0.3	0.6	95.4	3.7		2	98			
34	250	11	18.8	.....	.....	77	23			100			
29	2,200	13	52	0.9		94.6	4.5			99.6	0.09	0.35	
7	7,000	15	50.3	0.1	1.1	3.6	95.2			10	6	84	
34	250	21	18	.....	.....	69	31					100	
27	1,400	24	28.6	.....	.....	90	10					100	3
29	500	30	91.4	.....	.....	75.7	24.3			3		97	31
28	1,500	31	26.7	.....	.....	98.8	1.25					100	99.5

[a] Ensheathed or moulting.
[b] Based on total recovered.
[c] Ninety percent of worms encysted in wall of small intestine. On subsequent days all worms recovered from lumen.

patency. Of the different levels of larvae administered—20, 350, 500, 700, 1,000, 2,000, 4,000, 4,500, 5,000—the greatest number of adult worms becoming established, as indicated roughly by the egg count curves was at the 350 larvae level, and the second greatest number becoming established was at the 1,000 larvae level. Usually the greater the number of larvae administered, beyond the 1,000 larvae level, the greater the reaction of the host tissues, resulting in more of the worms being eliminated.

Eggs were first found in the feces 24 to 35 days after infection, the average in 10 infections being 28 days. This is a short prepatent period for species of *Oesophagostomum*. In 10 infections produced in 7 lambs and 3 kids by single inoculation with 20 to 5,000 larvae, the period of patency ranged from approximately 77 to 157 days and averaged approximately 117 days. The period spent in the host is long enough to maintain the species when conditions are inimical to life on the pasture.

The average egg count, based on 10 infections produced by single inoculation with an average of 1,840 larvae per animal, rose rapidly during the first month of patency, reached a peak at 60 to 65 days after infection, remained high for about a week, and then fell rapidly (the fall being less rapid than the rise during the first month of patency) for about a month and stayed at a fairly low level for the remaining months of patency. To obtain the average curve of 10 infections curves of the egg output for each infection were drawn, and then readings were taken at 5 day intervals and averaged. It is believed that the curve obtained is more indicative of the length of life of the worm than the development of immunity in the host, the drop in the curve being due to the spontaneous loss of worms, for if a second inoculation of larvae is administered very soon after a mild first infection has been lost, the curve of egg production, as illustrated in Fig. 3, is repeated in the same time period and it has the same shape. However, the peak of egg production is not quite as high in the second infection, perhaps indicating some host resistance. Incompleted experiments indicate that in animals which have recovered from fairly heavy infections, few, if any, adults develop as a result of a second administration of infective larvae.

SUMMARY

The life history of *Oesophagostomum venulosum* is direct, no intermediate host being required. The free-living phase of the life history is similar to that of most nematode parasites of sheep. The fairly large, dark gray eggs are usually in the 16 or 32 cell stage when passed in the feces. Under favorable conditions in charcoal-feces culture the eggs begin hatching in 24 hours, and the third, infective stage is reached in 3 to 5 days after hatching. The infective larva, including its sheath, averages 0.9 mm. in length, and has 32 intestinal cells. The cuticle of the second stage larva is retained. It acts as a protective sheath, and a considerable number of larvae can be maintained in culture or water for at least 2 months, and a few are still alive after 5 months. However, no further progressive changes occur, and there is depletion of the food stored in the intestinal cells and vacuolation of tissues.

When the infective larvae are ingested, growth and progressive metamorphosis commence again and continue uninterruptedly into the adult stage. The sheath is cast off on the first day after entry into the host, and on the third day the larvae are encysted in the wall of the small intestine. Marked metamorphosis occurs, and the larvae remain in the wall until they are ready for the third ecdysis, which occurs on the fourth day after infection. The outstanding feature of the fourth stage larva is its provisional cupoliform buccal capsule. The dorsal cone (tooth) is rudimentary. It does not project up from the esophagus beyond the

base of the inner surface of the buccal capsule. This characteristic is significant in distinguishing the worm from the other species of *Oesophagostomum* occurring in ruminants. The position of the cervical papillae in the fourth stage, approximately at the level of the beginning of the intestine, is also significant in determining the species. During the fourth stage there is considerable growth and a gradual development of the structures of the adult.

The fourth ecdysis usually occurs between 13 and 16 days after infection. At this time the length of the young adults is a little more than one third the length of the mature adults. The fifth stage is characterized by marked growth and by the development of sexual maturity. As the worms are developing, they pass from the small intestine into the caecum, where most of the mature adults

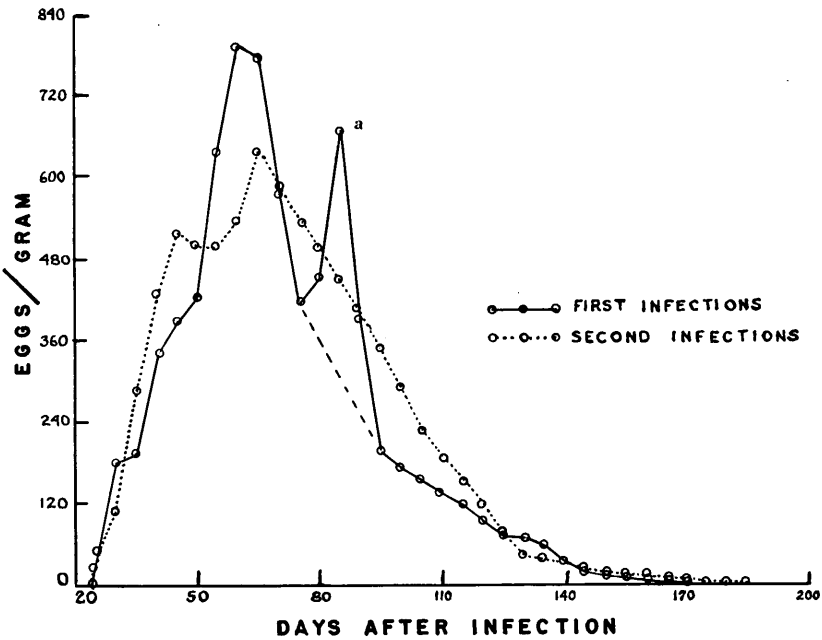

FIG. 3. Average course of seven first infections (av. 1,817 larvae/inf.), and average course of three second infections (av. 1,900 larvae/inf.) established soon after the loss of light first infections.

[a] This peak is due to one unusual egg count. If a greater number of cases were studied, it would probably decrease to the level of the broken line.

are located. Many worms continue down the digestive tract and are carried out with the feces. Almost all the worms become mature within 31 days after infection. There is no significant lag in the development of the worms. As evidenced, roughly, by egg counts, largest infestations of adult worms were established when 350 and 1,000 larvae were administered. Greater inoculations usually caused more marked reaction, resulting in worms being eliminated.

The average prepatent period of *O. venulosum*, as judged by the first appearance of eggs in the feces in 10 cases, was 28 days. In single inoculation of 7 lambs and 3 kids, 2 to 11 months old at the time of infection, with an average of 1,840 larvae, the average egg count rose rapidly during the first month of patency, reached a peak at 60 to 65 days after infection, remained high for about a week, and then fell rapidly for a month and remained at a fairly low level to the end

of the patent period. It is believed that the drop in the curve was primarily indicative of the length of life of the worm rather than the development of immunity in the host, for almost the same curve, with a slightly lower peak, as was obtained in first infections was repeated in second infections immediately following the loss of mild first infections. In 10 cases of single inoculation with an average of 1,840 larvae, the period of patency averaged about 117 days. This is sufficient time to maintain the species while conditions are inimical to life on the pasture.

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Pratylenchus vulnus, new species (Nematoda: Pratylenchinae), a Parasite of Trees and Vines in California

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The nematode genus *Pratylenchus* Filipjev 1934 contains a number of plant parasitic species that are commonly referred to as meadow or root-lesion nematodes. The latter name is commonly used in California because it is descriptive of the root symptoms observed on several of the crop plants that are attacked in the State. Root-lesion nematodes have been recognized as parasites of figs in California since 1927. However, fig trees having the typical symptoms that are now associated with root-lesion nematodes were observed as early as 1920. Since that time several investigators have observed and reported these nematodes on various hosts in many localities in the State.

Identification of species in the genus *Pratylenchus* has for many years been a difficult task due to the fact that certain of the original species descriptions were inadequate. Most workers who have examined specimens of the genus *Pratylenchus* from California have identified them as being either *P. pratensis* (de Man) or *P. musicola* (Cobb). *P. musicola* was identified by G. Steiner in 1927 from the roots of grape (*Vitis vinifera*) collected near Loomis, California. Also in 1927, specimens collected from the roots of mission fig near Merced were identified by N. A. Cobb as *P. pratensis*. Gerald Thorne in 1934 reported *P. pratensis* as a parasite of fig. Ark and Thomas (1936) indicated that *P. pratensis* was the species attacking apples in the Sebastopol area and listed as additional hosts plum, peach, pear, and grape. Condit and Horne (1939) recorded damage to olive roots near Riverside to be caused by *P. musicola*. Allen (1949) reported that walnuts throughout the State were frequently damaged by the attack of a species of *Pratylenchus*.

A careful examination of specimens collected from the above-mentioned hosts and in many localities in California over a period of several years indicates that

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one species predominates as an important crop pest. Several other species of the genus have been found occasionally but are at present known only from a few hosts and localities. Comparison with specimens of *P. pratensis, P. musicola* and other described species of the genus has shown that the root-lesion nematode commonly found injuring trees and vines in California is an undescribed species. It is proposed that the species be named *Pratylenchus vulnus,* new species.

Pratylenchus vulnus, new species

♀ : L = 0.46–0.91 mm; a = 26.6–39.5; b = 5.3–7.7; c = 14.2–27.7; V = 78–84.1%.

♂ : L = 0.46–0.73 mm; a = 28.3–39.2; b = 5.3–7.4; c = 17.5–29.4; T = 35.8–66; gub = 4–6 μ; spicule = 14–20 μ.

Female.—Cuticle marked by distinct transverse striae averaging about 1 μ apart except in neck and tail where striae may be nearly 2 μ apart. Wing area composed of three elements marked by four incisures (Fig. 1, E and I), the outer ones noticeably crenate. Lip region almost continuous with body contour; marked by two or three striae which form three or four annules (Fig. 1, B and C). In face view six sectors of the lip region are visible, the lateral lips are distinctly wider than the submedians (Fig. 1, D). Papillae very obscure. Amphid apertures located near outer margin of lateral lips. Spear 16 to 18 μ long with well developed conspicuous basal knobs. Wide muscular bands connect spear base with the heavily sclerotized labial framework.

Dorsal esophageal gland orifice located about 3 μ behind spear base. Median esophageal bulb slightly ovate, equipped with a refractive valvular apparatus. Nerve ring surrounding esophagus just behind median bulb. Esophagus extending about 2 body widths beyond median bulb. Esophageal glands forming a large lobe overlapping the anterior end of the intestine. Three esophageal nuclei are usually visible. Excretory pore located about 2 body widths behind median bulb.

Anterior branch of female reproductive system composed of a short uterus, cellular oviduct and outstretched ovary made up of a series of developing oocytes arranged in a single file except for a short region of reproduction near anterior end. Ovary frequently extending to the vicinity of the esophageal glands. Posterior uterine branch extending one-fourth to one-half the distance to anal opening. Posterior branch composed of a short extension of the uterus and a short vestigial ovary. Vestigial ovary obscure, but readily seen in stained specimens. Phasmid openings slightly posterior to middle of tail. The four incisures extend beyond the phasmids almost to terminus of tail (Fig. 1, E and F). Striae of cuticle not extending around terminus of tail.

Male.—Lip region continuous with neck contour, marked by two or three striae which form three or four annules. Spear 15 to 18 μ long, labial framework, and esophagus similar to that of female. Phasmids located slightly posterior to middle of tail, extending into bursa (Fig. 1, H); opening short of the margin of the bursa. In cross-section tail ventrally flattened posterior to anal opening. Single testis made up of developing spermatocytes usually arranged in two rows. Length of testis variable, sometimes extending to vicinity of the esophageal glands. Spicula arcuate, hafted, about 17 μ long. Gubernaculum slightly arcuate, about 5 μ long (Fig. 1, G).

Type host.—California black walnut, *Juglans hindsii* Jepson

Type locality.—San Jose, California

Host Plants.—Walnut, grape, fig, citrus, apricot, avocado, weeping willow, cherry, olive, peach, almond, plum, raspberry and boysenberry.

Diagnosis.—Plant parasitic *Pratylenchus* with the above measurements and general description. Lip region bearing two or three striae forming three or four

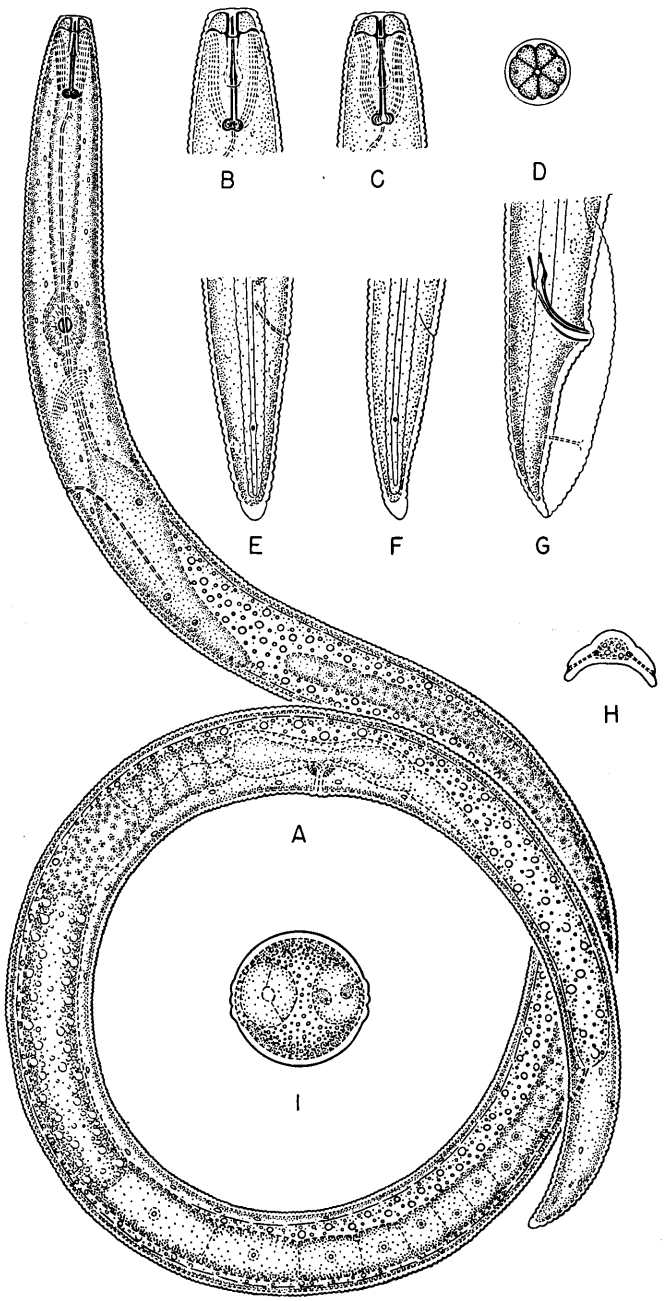

FIG. 1. *Pratylenchus vulnus,* n. sp. A—Adult female, × 650. B, C—Heads of females, × 1000. D—Face view of female, × 1000. E, F—Female tails ×1000. G—Male tail, × 1000. H—Diagrammatic cross-section thru male tail, × 1000. I—Cross-section thru female body, × 1000.

annules. The number of annules on the lip region appears to be variable in *P. vulnus*. Males and females in the same root-lesion frequently show variation in the number of annules on the lips. This variation has been observed in both living and fixed material. Lateral field marked by four incisures. In the female all four incisures extend almost to the terminus of the tail. Lateral striae not extending around female tail. Gubernaculum crescent shaped in lateral view.

Pratylenchus vulnus, n. sp. most closely resembles *P. pratensis* and *P. musicola*. It can be distinguished from *P. pratensis* by the following morphological differences; absence of striae around the female tail, four incisures extending posterior to phasmid in female. *P. vulnus* differs from *P. musicola* in having a longer posterior uterine branch and in having three or four annules on the lip region. Specimens of *P. musicola* from the collection of Gerald Thorne, Division of Nematology, Salt Lake City, Utah were found to have four annules on the lip region and the post-uterine branch extending less than one-fourth the distance to the anal opening.

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Notes on Caricide as an Anthelmintic for Cats and Dogs

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Following the initial reports of Hewitt and his co-workers (1947. J. Lab. and Clin. Med. 32: 1304–1313; ibid.: 1314–1329) on the use of caricide (1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate) for the treatment of filarial infections in cotton rats and dogs, the chemical has been tested or used in a wide variety of helminthic infections of both man and animals. In trials reported by these investigators, caricide elicited no acute toxic reactions and there was no evidence of chronic toxicity in dogs that were given multiple daily doses of the drug for more than two months. In June, 1948, Hewitt et al (J. Parasitol. 34: 237–239) reported that caricide exhibited marked ascaricidal action in dogs, but that it was ineffective against hookworms, whipworms, and tapeworms. The chemical was given in several different dosages and by various methods of administration. At a dose rate of 25 mg. per kg. of body weight, the drug removed 65 per cent (25) of 38 ascarids from 7 dogs; and when the dosage was increased to 50 mg. per kg., the chemical removed 98 per cent (72) of 73 ascarids from 10 dogs. Complete removal of large roundworms was obtained when these dosages were given twice within 24 hours, and the larger dosage was wholly effective also when given intraperitoneally in this manner. About half of the animals vomited after treatment, but there were no other symptoms attributable to the drug. Six months later, Kanegis (1948. J. Am. Vet. Med. Assn. 113: 579–581) published data on the use of caricide for the removal of ascarids from cats. The most satisfactory

regimen consisted in the administration of the chemical in single doses at a rate of 25 mg. per pound of body weight shortly after feeding. With this regimen, the drug removed 97 per cent (40) of 41 ascarids from 8 kittens, and at a slightly lower dosage, namely, 50 mg. per kg., all of 29 ascarids were removed from 5 cats. The treatment was well tolerated in all instances and, moreover, no intoxication was noted in 21 additional cats that were given caricide in dosages up to 36.6 mg. per pound of body weight. The present report contains additional data on the ascaricidal action of this drug in dogs and cats, the latter in particular.

PROCEDURE

One or 2 days before treatment, the animals were isolated in individual cages and the feces screened in order to detect any natural elimination of parasites. The kinds of parasites in each animal were determined by fecal examination prior to treatment. The chemical was given in hard gelatine capsules, either with forceps or embedded in a small amount of feed, after a fast of 18 to 24 hours. In most instances, the animals were returned to regular feed 2 or 3 hours after treatment. In a few cases, they were fed immediately after the drug was admin-

TABLE 1.—*Data on the anthelmintic efficacy of caricide in cats and dogs*

No. of animals	Dose rate (mg/lb)	Parasites	Removed	Left	Efficacy (per cent)	No. of tests	Remarks
12 (cats)	25	Ascarids	178	4	98	11	4 cats vomited; 1 ascarid recovered before treatment
		Hookworms	1	164	1	9	
		Taenia	0	1	0	1	
		Dipylidium	0	12	0	2	
4 (cats)	20	Ascarids	32	3	91	3	2 cats vomited; 8 ascarids recovered before treatment
		Hookworms	0	23	0	2	
1 (cat)	15	Ascarids	11	3	79	1	
		Hookworms	0	25	0	1	
2 (dogs)	25	Ascarids	9	1	90	1	
		Hookworms	0	221	0	1	
		Whipworms	0	102	0	2	

Note: Three cats salivated profusely when capsules were broken in the mouth.

istered. The feces of each animal were collected daily and examined for parasites in the usual manner. When elimination of parasites ceased, usually within 3 or 4 days, the animals were autopsied and the gastrointestinal tract examined for parasites and lesions. The liver and kidneys also were examined for lesions ascribable to the chemical. In collateral trials involving a dog and 3 nursing puppies the animals were not isolated in individual cages. The efficacy was determined by egg counts before and after treatment and by the elimination of parasites in the feces, but the animals were not submitted to necropsy.

OBSERVATIONS AND DISCUSSION

The results are given in Table 1. Caricide exhibited significant ascaricidal action when given to cats in dosages of 15, 20, and 25 mg. per pound of body weight. With the largest dosage, the chemical removed 98 per cent (178) of 182 ascarids from 11 animals, and the intermediate dosage removed more than 90 per cent of these worms. The smallest dose was less effective in 1 animal. The drug was wholly ineffective, however, against hookworms and tapeworms. Vomition

occurred in a few instances, but otherwise the chemical appeared to be well tolerated and no significant lesions were found at autopsy.

Caricide removed 90 per cent (9) of 10 ascarids from 1 dog, but none of 221 hookworms and 102 whipworms from 1 and 2 dogs, respectively. In another trial, the drug removed 21 ascarids from 1 dog, but 5 additional worms were removed with a subsequent therapeutic dose of toluene (0.1 cc per pound). The animal was not autopsied, but caricide was apparently 80 per cent effective against ascarids in this instance.

Additional data were obtained with a mature dog and 3 nursing puppies that were given 25 mg. of caricide per pound of body weight. Before treatment a fecal sample from the dog and a composite sample from the puppies showed respectively 200 and 18,600 ascarid eggs per gram. A total of 97 ascarids were recovered from the feces of the 4 animals; and one week after treatment, the egg counts for the dog and puppies were 0 and 4000 eggs per gram, respectively. At this time the puppies were given another dose of caricide and 16 ascarids were removed from the group. About one week after the second treatment, egg counts of a composite fecal specimen showed an average of 1000 ascarid eggs per gram. The puppies were given a therapeutic dose of methyl chloroform (0.1 cc per pound), and 6 ascarids and 29 hookworms were removed from the group. One week later, a composite fecal sample showed 100 ascarid eggs per gram of feces, but no further treatment was given. In these trials, one dose of caricide was apparently completely effective against ascarids in the mature dog, but comparable efficacy was not obtained in the puppies with two treatments given one week apart. In the latter, however, there was significant reduction in egg counts, and several worms were recovered after each treatment.

Caricide exhibits significant ascaricidal action in both dogs and cats, but the chemical appears to be somewhat more effective in the latter. Moreover, the drug is well tolerated and, on this account, it may prove to be particularly useful for cats since these animals are more susceptible than dogs to untoward side-effects from drugs currently available to the small animal practitioner. The optimum dosage, administered in capsules after a fasting period of 18 to 24 hours, appears to be about 25 mg. per pound of body weight. The chemical is apparently somewhat irritating to the oral and gastric mucosae since salivation occurs when capsules are crushed in the mouth and emesis is induced in a few instances. With these reactions, however, there is no apparent nausea and the ascaricidal action of the chemical is not significantly affected.

SUMMARY

In limited trials, caricide exhibited significant ascaricidal action in both dogs and cats, but it was ineffective against hookworms, whipworms, and tapeworms (*Taenia* and *Dipylidium*). At a dose rate of 25 mg. per pound of body weight, administered in capsules after a fast of 18 to 24 hours, the chemical removed 98 per cent (178) of 182 ascarids from 11 cats; lower dosages were less effective. Vomition occurred in a few instances, and salivation was induced when capsules were crushed in the mouth. These reactions, however, were unattended by nausea, and they did not adversely affect the anthelmintic action of the chemical. In tests with dogs, a dosage of 25 mg. per pound of body weight removed 90 per cent of 10 ascarids from 1 animal; and in other trials, the chemical also showed ascaricidal action in 2 dogs and 3 puppies. Caricide may prove to be a useful agent in the treatment of ascariasis of cats, but it does not appear to be superior to other available canine ascaricides.

The Histological Anatomy of the Nematode *Meloidogyne hapla* (Heteroderidae)

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INTRODUCTION

Although the histological anatomy of nematode representatives of numerous groups has been investigated, a review of the literature fails to demonstrate any appreciable amount of information concerning the finer anatomy of members of the superfamily Tylenchoidea. Rather, emphasis has been placed upon those features of the organisms and their life which are of most pressing economic importance. Thus Nagakura (1930) and Christie and Cobb (1941) have described the life history of a representative of the genus *Meloidogyne* Goeldi, 1887, and the histopathology of gall formation has been investigated by Christie (1936). Such early workers as Atkinson (1889), Stone and Smith (1898), Bessey (1911), and Cobb (1918) described the gross anatomy, control, and gave host lists of the root-knot nematode. There have been numerous contributions by more recent workers (Goodey, 1929; Thorne, 1949; Chitwood, 1949, 1950; etc.) which deal with the gross morphology of the organisms or the finer anatomy of particular regions of the body of various members of the Tylenchoidea. The most comprehensive work dealing with the finer anatomy of a member of the subfamily Heteroderinae is that done by Raski (1950) on *Heterodera schachtii*. Even in this work, however, emphasis is laid on the stages in the life history and the more salient morphological features of the organism; yet some points of anatomical interest are mentioned.

The present investigation is an attempt to describe and illustrate the histological anatomy of the organ systems[2] of the adult *Meloidogyne hapla* Chitwood, 1949, with emphasis on the female.

MATERIALS AND METHODS

The specimens used for study were obtained from the roots of peanut plants grown in Nansemond County, Virginia. They consisted of root galls containing male, female, and larval nematodes and egg masses. Some galls were processed intact, while others were dissected and their contents removed. Male and female nematodes were isolated from the latter either for whole mount study or for sectioning.

All specimens designated for sectioning were fixed in either a 10% formalin or a formalin-acetic-alcohol solution for at least 24 hours; they were then washed in running water for another 24 hours. Dehydration and clearing were accomplished through the use of cellosolve and isobutyl alcohol, the technique being a modification of that of Cable (1943) developed in the Department of Biology of the Catholic University of America. The technique, as modified for the present study, is as follows:

1. After fixation and washing, place specimens in cellosolve overnight.
2. The following day change cellosolve and leave for 2 hours.
3. Place in a half cellosolve-half isobutyl alcohol solution for 30 minutes.
4. Replace with pure isobutyl alcohol and leave for 1½ hours.

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[1] A contribution from the Department of Biology, The Catholic University of America, Washington, D. C. This paper, prepared under the direction of Dr. Benjamin G. Chitwood, is based on the author's dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science.

[2] The detailed structure of the nervous system was not investigated.

5. Place in paraffin oven and add an equal amount of paraffin chips (m.p. 53–55° C.). Leave for 1 hour.
6. Replace with pure melted paraffin and leave for 1 hour.
7. Imbed in watch crystals if specimens are small (i.e. isolated males or females) or in cardboard "boats" if specimens are larger (i.e. a root gall).

Serial sections were cut at 6 μ. Staining consisted of either Heidenhain's hematoxylin with iron alum mordant and fast green (0.5%) counterstain, iron alum-hematoxylin alone, or Harris' hematoxylin with eosin counterstain. It was found that the latter technique produced the most satisfactory staining results. Camera lucida drawings were made at various magnifications indicated in the figures.

HISTOLOGICAL ANATOMY

A. *The External Cuticle*

Layering of the cuticle is best observed in the female near the perineal region. Here the cuticle is composed of at least three layers: an external cortical layer, a middle matrix layer, and innermost a somewhat thinner fiber layer. With the loss of the tail in the female, characteristic fingerprint-like patterns are observed in the perineal area (Fig. 1, E); these are modified striae whose pattern is used for specific identification.

The cuticle of the male is thinner than that of the female and appears to be of uniform composition. The lateral thickenings which appear as 3 longitudinal ridges are a characteristic feature first pointed out by Cobb (1918).

B. *Hypodermis*

The hypodermis is a syncytial layer lying under the cuticle and completely encircling the body. In the cephalic area it is extremely thin and appears to be anucleate. The arcade is a cephalic modification of the hypodermis and forms a

ABBREVIATIONS FOR FIGURES

an	anus	*oo*	oocyte
ar	arcade	*or*	orifice
b es	bulbar region of esophagus	*ov*	ovary
c	cell	*p ex*	excretory pore
can	canal	*ph*	external phasmid
ch	chord	*pr in*	protein inclusion body
d	dorsal	*pro es*	procorpus region of esophagus
du	duct	*rad*	radial
eic	esophageal-intestinal cell	*rec*	rectum
ex	excretory	*s*	sinus
g	"giant"	*sem ves*	seminal vesicle
ge z	germinal zone	*sh*	shaft
gl	gland	*som*	somatic
gl ap	glandular appendage	*sp*	spicule
gr z	growth zone	*spc*	spermatocyte
gub	gubernaculum	*spg*	spermatogonia
hyp	hypodermis	*spz*	spermatozoa
i es	isthmus region of esophagus	*sty*	stylet
int	intestine	*sv*	subventral
k	knob	*syn*	syncytium
l	lateral	*t*	testis
lu	lumen	*tub*	tubule
m	musculature	*ut*	uterus
mar	marginal	*v*	ventral
me es	metacorpus region of esophagus	*vag ut*	vagina uterina
mo org	morular organ	*vag ve*	vagina vera
n	nucleus	*vas d*	vas deferens
nrv r	nerve ring	*vul*	vulva

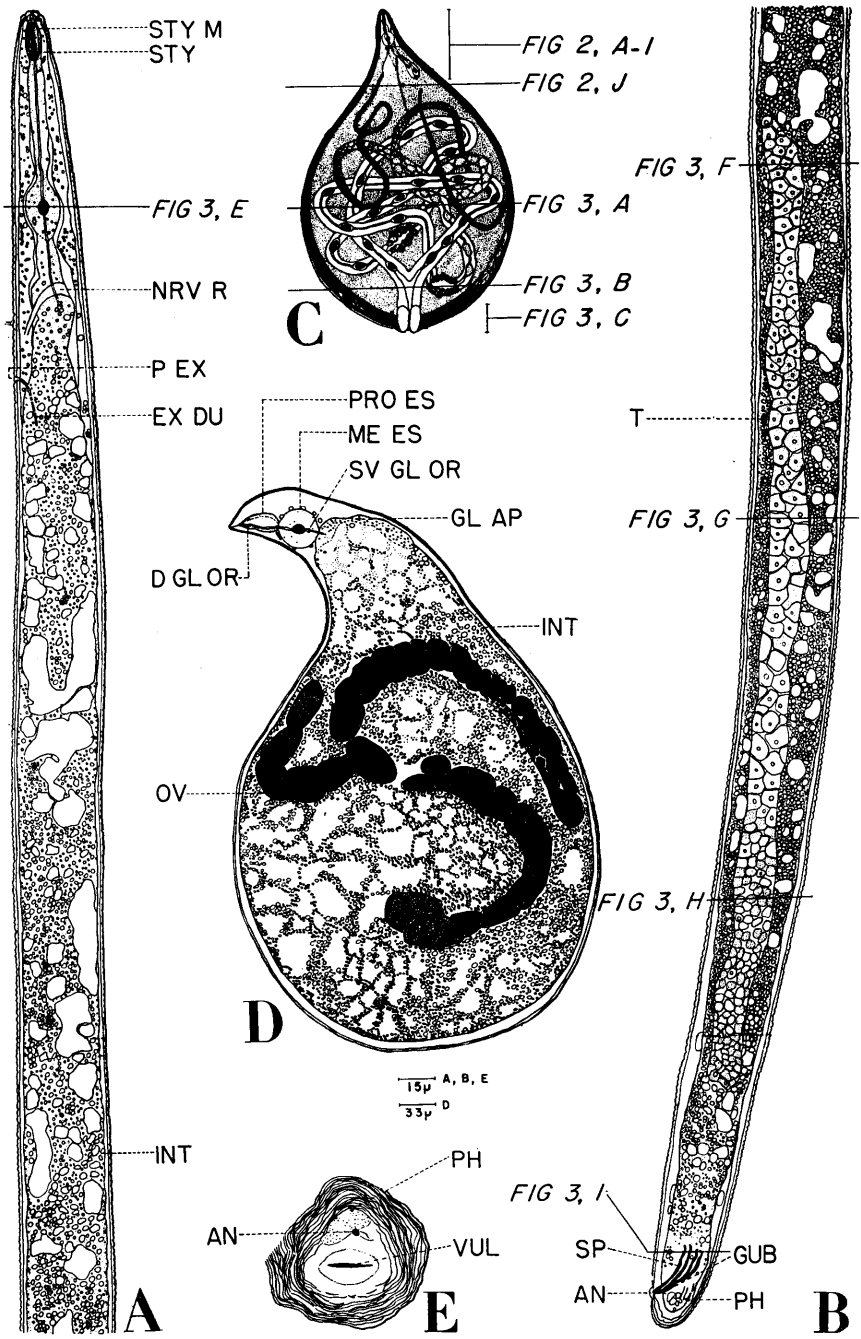

FIG. 1. *Meloidogyne hapla.* A—Lateral view of anterior third of adult male. B—Lateral view of posterior third of adult male. (The slide containing the nematode was heated slightly to cause the animal to cease movement; this heat was sufficient to cause the condensation of oil globules.) C—Diagrammatic sketch of adult female. D—Dorsal view of adult female. E—Perineal region of adult female. *Note:* In A, B, and C are indicated the levels at which the sectional drawings in Figures 2 and 3 were made.

circular band of tissue surrounding the digestive tract at the stomatal level. In the present study, arcade cells were observed in the female situated in the body cavity slightly posterior to the level of the stylet knobs; they are spindle-shaped

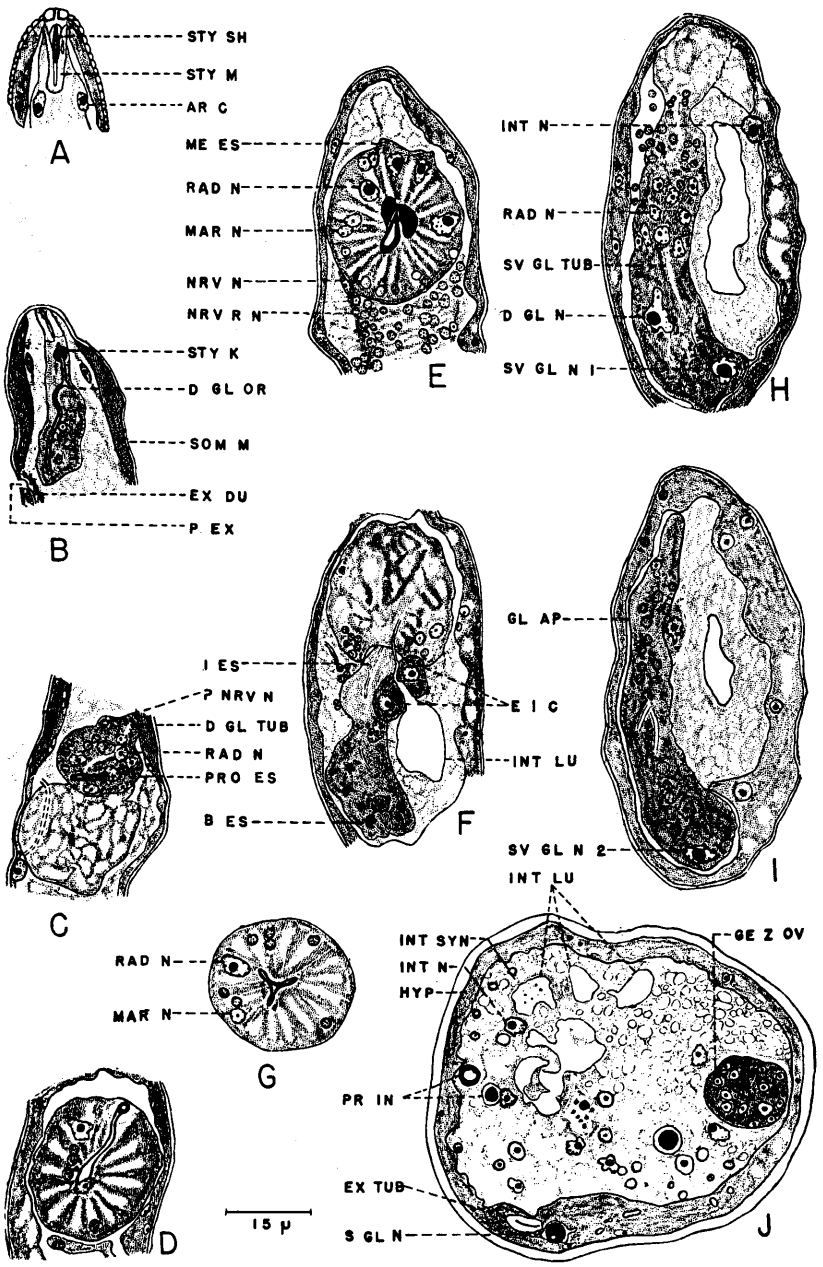

FIG. 2. *Meloidogyne hapla.* A to F, H, I—Consecutive oblique longitudinal sections through the anterior end of a young female. G—Cross-section through metacorpus. J—Cross-section through mature female.

and rather darkly staining (Fig. 2, A). Their exact number was not determined but in other nematodes there are usually nine.

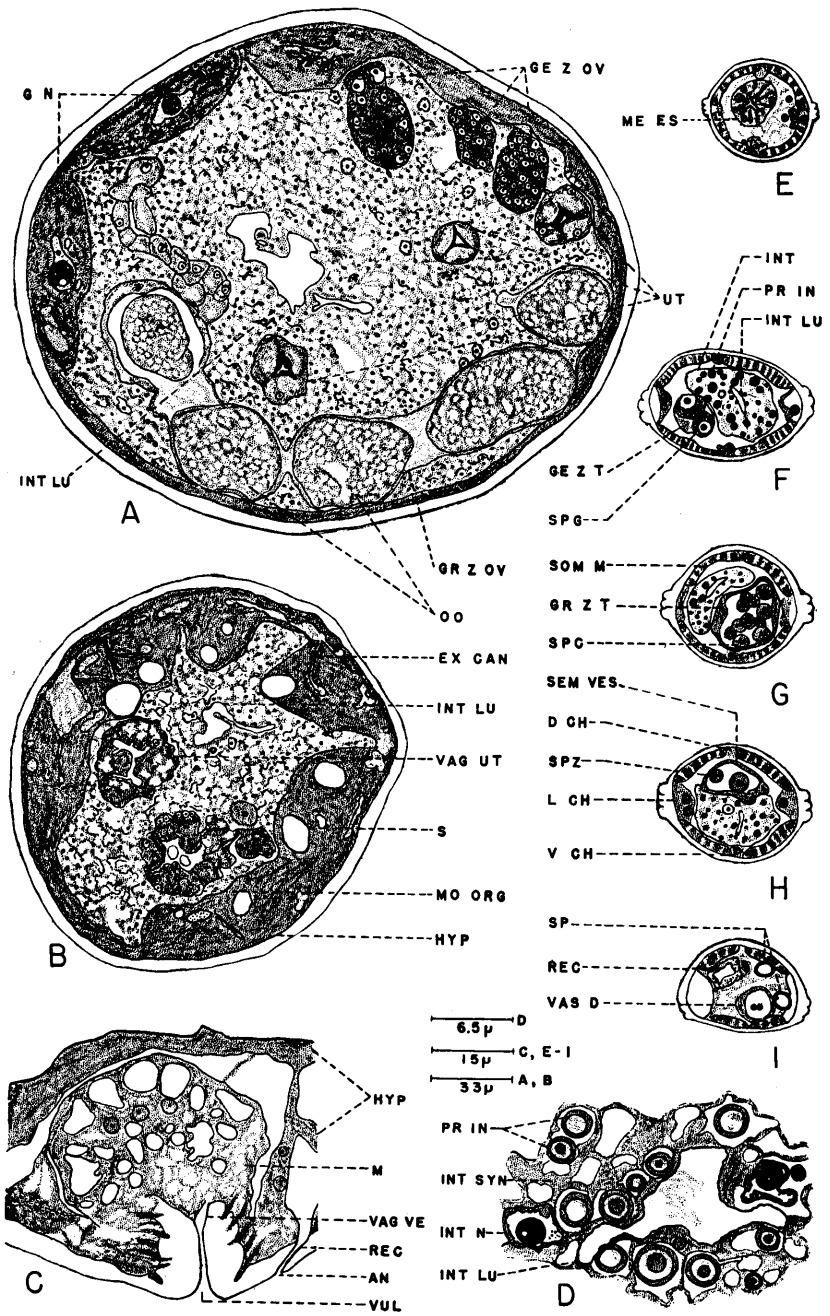

FIG. 3. *Meloidogyne hapla*. A, B—Cross-sections through mature female. C—Longitudinal section through vulva. D—Highly magnified portion of intestinal syncytium. E to I—Cross-sections through mature male.

Beginning in the posterior "neck" region of the female the hypodermis becomes considerably wider; it contains a moderate number of rounded nuclei which seem to be more or less evenly distributed around the body. Slightly above the mid-region of the body the hypodermis increases in thickness and more posteriorly projects into the body cavity in four longitudinal chords; in the posterior fourth of the body, three chords appear to be subdivided. In the terminal portion of the body the hypodermal tissue is massive (Fig. 3, B). In large areas of this posterior chordal tissue there is found an eosinophilic granulation.

In the male, the four hypodermal chords are quite prominent (Fig. 3, H) and appear essentially the same as those found in most nematodes.

C. *Somatic Musculature*

The somatic musculature of the female is highly reduced, probably as a result of the sedentary mode of life; it is confined to a thin layer lying under the hypodermis in the cephalic area. The muscular band is eosinophilic and appears to be of uniform composition (Fig. 2, B). A few small, elongate muscle nuclei are present along its inner border.

In contrast, the somatic musculature of the male is well-developed along the entire length of the body. In longitudinal section the muscle cells are elongate, spindle-shaped bodies; in cross-section the arrangement of the musculature is seen to be platymyarian.

D. *Digestive Tract and Associated Structures*

1. *Stomatal Region.*—In the male, the external orifice of the stomatal region is surrounded by six lips which are roughly triangular in shape; both sexes possess a sclerotized stylet guide and a series of sclerotized bars and circlets which form the supportive framework of the cephalic area (Fig. 1, A). The stylet of the male is 17–18 μ in length and that of the female 12–14 μ (usually 13 μ); it is divisible into two regions, the shaft and the base, both tapering, the latter terminated by rounded knobs. Muscle fibers with insertions in the stylet knobs and the cephalic sclerotizations control stylet movement.

2. *Esophagus.*—a. *Procorpus.* The procorpus is divisible into two areas: an anterior cylindroid portion and a posterior rounded portion, both of approximately the same length. The cuticularly lined lumen, which begins at the base of the stylet, is thick-walled and circular in outline throughout its course in this part of the esophagus.

b. *Metacorpus.* The walls of the lumen in this area are thin and triradiate in form; as the lumen leaves the metacorpus dorsally, it is greatly reduced in size. The metacorpus is the only region of the esophagus in which muscular development is prominent, the muscle fibers being dispersed equally about the sides of the radii (Fig. 2, G). There are two rather large cells (Fig. 2, F, *eic*) which are adjacent to the dorsal wall of the metacorpus and surround the gut lumen as it passes from esophagus to intestine; possibly they may function as an esophageal-intestinal valvular apparatus.

c. *Glandular appendage.* The ventrally situated glandular appendage (Fig. 2, F, H, I) is attached to the metacorpus. This structure is the remnant of the isthmus and bulbar regions of the rhabditid esophagus and is comparable to similar structures found in such nematodes as *Aphelenchus* and *Contracecum*.

The anterior isthmus region is considerably foreshortened and stains lightly with hematoxylin (Fig. 2, F). A fibrous, darkly staining meshwork permeates the entire posterior region (Fig. 2, F); this is probably degenerate bulbar musculature. With careful focusing, the exceedingly minute duct system of the dorsal

and two subventral esophageal glands can be traced, in part at least, to their respective orifices (Fig. 2, H). Bessey (1911) was probably the first worker to notice the opening of the dorsal esophageal gland into the esophagus just posterior to the base of the stylet; the orifice is illustrated (Plate I, Figs. 4 and 16) but not described by the author. The two subventral gland orifices are in the posterior part of the metacorpus.

d. *Esophageal nuclei.* Chitwood and Chitwood (1934) state that ". . . through a comparison of esophageal nuclei, their location and number, and the position of the orifices of the esophageal glands, the homologous regions of the esophagus, even in nematodes with esophagi of quite diverse appearance, may be determined. By a consideration of these homologous regions, facts of value in tracing the evolution of nematode groups may be obtained. . . ." The importance of nuclear constancy, with its manifold applications to fundamental biological principles, can hardly be overemphasized.

TABLE 1.—*A comparison of the esophageal nuclei of* Meloidogyne hapla *with those of* Rhabditis terricola (*Rhabditina*), Ditylenchus dipsaci (*Tylenchina*), *and* Aphelenchus avenae (*Tylenchina*), *as determined by Chitwood and Chitwood* (*1936*).

	R. terricola	*D. dipsaci*	*A. avenae*	*M. hapla*
Procorpus	R 1–6 N 1–12 Total—18	Total—15–18	Total—10	R 1–6 9 probably N Total—15
Metacorpus	R 7–12 M 1–3 N 13–31 Total—28	R 1–6 R 7–12 M 1–3 N 1–10 Total—25	R 1–12 M 1–3 18 unidentified Total—33	R 7–12 M 1–3 (a, b) N 1–19 Total—28
Isthmus	0	0	0	0
Bulb	R 13–18 R 19–21 R 22–24 M 4–6 M 7–9 N 32–40 G 1–3 Total—30	G 1–3 27 unidentified Total—30	G 1–3 27 unidentified Total—30	R 13–24 G 1–3 remainder uncounted and uniden- tified Total—?

Symbols used: R—radial nucleus; N—nerve cell nucleus; M—marginal nucleus; G—gland nucleus

The three esophageal gland nuclei are the largest and most easily identified of the four types (Fig. 2, H, I). The radial nuclei are smaller and resemble the esophageal gland nuclei somewhat in staining reaction; the nucleoplasm, however, is more compact (Fig. 2, E). The marginal nuclei of *M. hapla*, as in many other nematodes, are subdivided, the two members of a pair being designated *a* and *b*. They are intermediate in size and have a rather clear nucleoplasm and a slightly granular nuclear membrane; the nucleolus is small and indistinct (Fig. 2, E). The nerve nuclei are extremely small and stain rather darkly with hematoxylin (Fig. 2, E). Chitwood and Chitwood state that they are arranged in three chains in *R. terricola* and other forms, but this arrangement could not be discerned in the present study.

From Table 1, it may be seen that the total number (43) of nuclei in the

procorpus and metacorpus of *M. hapla* is the same as that found in the other tylench representatives, but three less than the total number present in these areas of *R. terricola*. This discrepancy is most logically explained by the failure to locate all nerve cells, as these are often quite difficult to identify with certainty. The total number of radial nuclei (12) is the same in all four species. The isthmus region in all nematodes thus far studied is anucleate.

The bulbar region of *R. terricola* contains 12 radial nuclei, distributed in one pre-valvar set of 6, and two post-valvar sets of 3 each. The 12 radial nuclei of the bulbar region of *M. hapla* appear to show no symmetry or grouping; they are concentrated in the proximal portion of this region of the glandular appendage. The three esophageal gland nuclei are situated in the distal part of the bulbar area with the dorsal gland nucleus being anterior to the two subventral gland nuclei. Due to the dark staining, the number and kind of remaining bulbar area nuclei could not be determined with certainty. In view of the above-mentioned agreements in nuclear number, however, it would not be unwise to suspect that more exacting nuclear staining techniques would demonstrate the same total number (30) as found in the other three species.

3. *Intestine.*—The greater part of the body cavity of the female is occupied by the intestine, which represents a storage space for nutrients. From Raski's description and illustrations of the fourth stage female larva and the adult female *Heterodera schachtii*, the exact relationship of "body cavity" and "intestine" is not clear; no mention is made of an intestinal lumen. In *M. hapla* the lumen (Fig. 3, D) is best seen in young specimens, where it exhibits branching; with increasing age it becomes greatly compressed, due to the accumulation of oil and protein in the intestinal syncytium (Chitwood, 1951) and is often difficult to observe. Protein inclusions are recognized by their round shape and dark staining; occasionally they are marked by a clear external ring (Fig. 3, D). Oil globules are visible in whole mount specimens, but are dissolved in routine histological preparations. The entire organ is permeated by a slightly eosinophilic reticulum. The abundant intestinal nuclei (Fig. 3, D) have a darkly staining nucleolus, a clear perinucleolar area and a granular, eosinophilic nuclear membrane.

In the male, the intestinal syncytium is also composed of a reticular network with nutrient inclusions. Fewer intestinal nuclei are observed, however, and the lumen (Fig. 3, F) is more regular in outline.

4. *Rectum and anus.*—The rectum of the female is quite small, being merely a fine tube lined with cuticle which is continuous with the external cuticle; the anus is situated dorsal to the vulva. In the male, the intestine opens into the short cloaca, which is the cavity common to both digestive and reproductive systems. The anal opening is bounded by two thickened cuticular lips.

E. *Excretory System*

Davaine (1857) was the first worker to note that the tylenchoid excretory system is asymmetric, with a single lateral canal rather than the customary two found in most phasmidean nematodes.

In *M. hapla*, the ventral excretory pore is situated in the esophageal region. The terminal excretory duct passes posteriorly through the hypodermis. In the female, the terminal duct extends to the level of the posterior region of the esophageal appendage, where it passes into a non-cuticular tubular structure, which in all specimens examined is completely collapsed; what is presumably the lumen in life is marked in preserved specimens by a rather prominent eosinophilic line (Fig. 2, J). The wall of the tubule appears to be considerably thicker than that

of the terminal duct and stains faintly with eosin. The tubule may be traced through the hypodermis beyond the region of hypodermal thickening where it becomes greatly reduced in size and branched. In young living specimens the excretory system is more easily traced, since in the intestinal syncytium there is not the accumulation of inclusion bodies which is present in the adult. Chitwood (1949) illustrates an elongate, convoluted, non-cuticular tubular system in young living specimens of *Meloidogyne javanica*.

Associated with the anterior tubular structure is the prominent sinus gland nucleus (Fig. 2, J). Its round nucleolus stains black with iron alum-hematoxylin and bluish with hematoxylin and eosin; several small vacuoles are observed in it. The nuclear membrane is marked by a somewhat thickened, irregular, eosinophilic line. Occasionally one or two accessory nuclei are seen in the vicinity of the sinus gland nucleus; these have the same general appearance as the latter but are considerably smaller.

The terminal junction of hypodermal tissue is marked by the presence of a large sinus which, when traced anteriorly is found to be formed from the junction of smaller sinuses from the chordal subdivisions. Present in the wall of each is an extremely large nucleus which could appropriately be called a "giant" nucleus (Fig. 3, A). Its round nucleolus stains bluish with hematoxylin and eosin and black with iron alum-hematoxylin; in it are a few small refractive bodies and vacuolated areas. The perinucleolar area is spheroid in shape and is filled with an eosinophilic reticulum. One or two secondary nuclei are occasionally observed lying beside the primary nucleus; these are similar in appearance to the latter but are only about one-tenth its size.

The sinuses give rise to smaller canaliculi which appear to be filled with a slightly eosinophilic colloid-like material. The canaliculi are terminated by a complex system of ramifying tubules which occupy a large portion of the hypodermal tissue and appear to extend anteriorly beyond the region of hypodermal thickening.

The connection between the anterior tubular structure and the posterior system of canals and tubules could not be established with certainty. It would seem, however, that because of their striking morphological similarity the single sinus gland nucleus and the chordal "giant" nuclei are of the same type and that all are connected in some way with removal or concentration (or both) of a colloidal waste secretion which is collected in the tubules and eventually eliminated from the body through the excretory pore.

F. *Reproductive System*

1. *Female.*—A considerable part of the body cavity of the mature female is occupied by the paired, highly convoluted, tubular gonads.

a. *Ovary.* The ovaries begin in the anterior fourth of the body and occupy approximately 60% of the length of the reproductive tract. The blind end of the ovary is marked by the presence of a cap cell. In the germinal zone the oocytes appear to be arranged in rosette formation around a central stalk; the germinal epithelium is indistinct (Fig. 3, A). In the growth zone the cell boundaries of the oocytes are more distinct. The epithelium is thin but is usually distinguishable, particularly in the more distal portions; occasionally flattened, spindle-shaped nuclei are observed in this layer. There is a gradual accumulation of fatty material and protein inclusion bodies as the developing oocytes pass through the growth zone.

b. *Uterus.* The uterus forms the greater part of the remainder of the repro-

ductive tract. The epithelium is somewhat thicker than that of the ovary. Distally there are usually 3 cells in cross-section; the lumen which is bounded by them has a somewhat triangular shape (Fig. 3, A). The eggs are usually located at some distance from each other. Near the ovarial end of the uterus occurs a peculiar structure of uncertain function, which has been arbitrarily termed the "morular organ" (Fig. 3, B). It is composed of from 10 to 15 large, flattened cells whose junctions with each other are invariably marked with an interlocking material, intensely staining with hematoxylin, which appears to be a cementing substance. It is not until after the germinal cells have passed this structure that shells are observed. It may act as an egg-former or possibly serve as a seminal receptacle as proposed by Atkinson (1889) and Bessey (1911). The former author reported the presence of spermatozoa in the female reproductive tract of a root-knot nematode, but later workers and the results of the present investigation fail to confirm this observation.

c. *Vagina and vulva.* The *vagina uterina* originates from the junction of the two uteri and proceeds posteriorly; it appears that there are two sets of four cells which compose the body of the organ (Fig. 3, B). Usually the lumen is quite flattened and folded in the contracted condition, but it is capable of considerable dilation to accommodate the passage of eggs; strands of muscular tissue radiate outward from the lumen. The *vagina vera* is lined by an exceedingly thick cuticular layer which is reflected inward from the external cuticle; it is also provided with strands of muscle fibers which pass to the body wall (Fig. 3, C). The transverse vulva is located on the posterior surface of the animal, usually near the terminal pole. The two cuticular lips surrounding the vulvar orifice are slightly elevated above the body surface (Fig. 3, C).

2. *Male.*—In contrast to the tremendous enlargement and modification of the female reproductive organs coincident with parasitism, the male reproductive system of *M. hapla* is comparatively simple. Males with one or two testes have been recorded in the literature; all specimens examined in the present investigation had but a single testis. The reproductive tract is an elongate tubiform structure which is found in approximately the lower third of the animal (Fig. 1, B). The *testis* occupies about one-half of the total length of the tract and is divided into two zones: an anterior germinal zone (Fig. 3, F) and a posterior growth zone (Fig. 3, G). At the blind end of the testis there is an enlarged cap cell, similar to that found in the ovary. The outer epithelium of the entire organ is quite thin.

The *seminal vesicle* (Fig. 3, H) is an expanded region which serves as a storage space for spermatozoa and occupies the greater portion of the remainder of the tract. Its walls consist of a single layer of low cuboidal epithelium. The *vas deferens* is a short area which is distinguished from the seminal vesicle by the presence in its walls of a few muscle fibers and a somewhat thickened epithelial covering (Fig. 3, I). It enters the cloaca from the ventral side. The *spicules* and *gubernaculum* (Fig. 1, B) are essentially the same as those found in most nematodes.

SUMMARY

The histological anatomy of *Meloidogyne hapla* from Nansemond County, Virginia peanut plants is described and illustrated. New facts brought out in this study are as follows:

1. The hypodermis of the female is syncytial.

2. The somatic musculature of the female was not observed in the posterior, enlarged portion of the body. In the male it is well-developed, platymyarian, and subdivided into 4 sectors.

3. The nuclei of the esophagus correspond to those found in *Rhabditis*, *Ditylenchus*, and *Aphelenchus*. The esophageal glands with residual nerve, marginal and radial nuclei form an appendage comparable to that found in *Aphelenchus* and *Contracecum*.

4. The intestine of the female practically fills the body cavity and is syncytial, with the lumen reduced and branched; in the male the lumen is simple. The intestinal syncytium contains oil droplets and protein inclusion bodies.

5. The excretory system terminates in a highly branched tubular formation which occupies a considerable part of the posterior hypodermal tissue.

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Dispharynx soricis n. sp. (Nematoda: Acuariidae) from the Shrew *Sorex obscurus alascensis*, and Associated Host Pathology[1]

JACK D. TINER

The presence of an unidentified acuariid nematode parasite in shrews was reported by Tiner and Rausch (1949) and specimens, including preserved host animals showing worms *in situ*, were demonstrated at the December, 1949, meeting of the American Society of Parasitologists. Further study indicates that this species may be referred to the genus *Dispharynx* because of its non-anastamosing cordons and its simple, inconspicuous, cervical papillae. It should be mentioned, however, that the cordon endings of the present species were found to be variable. In some individuals they end separately, and in some they approach one another at their tips and fuse partially. The writer understands the term "anastamose," as used in reference to acuariids, to mean that the ends of cordon pairs are completely fused so that their canals have become continuous with each other. Such a condition has not been observed in the present study.

The original finding of this species should be credited to Dr. Rausch who collected it in one of two shrews examined at Juneau, Alaska, March 3, 1949. Fifteen of 23 additional host animals in the same locality were found to be infected by the writer during the first week of September, 1949. Skins and skulls of most of the mammals collected were preserved, and sent to Dr. Rausch and to Dr. D. F. Hoffmeister, University of Illinois, for identification. Mr. Ralph Williams, Director of Laboratories, Alaska Department of Health, provided laboratory space for our studies, and was also very helpful with his knowledge of the local natural history.

Dispharynx soricis n. sp.

Cordons extend backward in dorso-lateral and ventro-lateral positions along sides of the head nearly to level of cervical papillae. Members of pair of cordons corresponding to each lip then turn toward each other in transverse plane at almost 90 degree angle, and then again, before meeting, both turn anteriorly and end separately, near to one another, or with partial fusing.

Male.—Length 4.93–7.36 mm, width at middle of body .18–.23 mm; length of cervical enlargement 1.2 mm, greatest diameter .5 mm. Cordons extend backward to about .25 mm from anterior before recurring, and end .120 mm from anterior. Nerve ring .250 mm, cervical papillae .291–.310 mm and excretory pore .300 mm from anterior end. Stoma .130–.149 mm long, .030 mm wide at anterior end, and 5–8 μ in diameter at junction with esophagus. Muscular portion of esophagus .300–.390 mm long and .060–.070 mm wide, glandular portion 2.1–2.7 mm long and .098–.105 mm wide, and usually coiled into a single loop (Fig. 3). Intestine 3.08 mm long. Tail .160–.220 mm long (Fig. 8). Longer spicule .375–.460 mm long and .022–.28 mm wide at base. Shorter spicule .140–.160 mm long and .022–.026 mm wide in middle, testis originates in last quarter of body and runs forward into anterior half before turning posteriorly. About 4 preanal and 5 postanal pairs of papillae.

[1] Contribution from the Department of Zoology, University of Illinois, Urbana, Illinois. Thanks are expressed to Dr. L. J. Thomas for his encouragement and suggestions. The collecting necessary for this study was made possible by the Alaska Health and Sanitation Activities, U. S. Public Health Service, Anchorage, Alaska, while the writer was engaged as Consultant in 1949.

Female.—Length 7.31–12.7 mm. Width at middle of body .30–.40 mm, length of cervical enlargement (Fig. 1) 2.5–3.54 mm, greatest diameter 1.5–1.6 mm. Cordons extend backward to about .57–.90 mm from anterior before recurring, and end .30–.49 mm from anterior. Nerve ring .450 mm from anterior end, cervical papillae situated laterally .80 mm from anterior end and excretory pore at approximately same level in mid-ventral line. Vulva 4.89–8.16 mm from anterior end. Tail .20–.31 mm long and rounded at end (Fig. 6). Stoma .225 mm long, .051 mm wide between interlabia (Fig. 4), and 8 μ wide at junction with esophagus. First portion of esophagus .670 mm long, .110 mm wide; second portion 3.8 mm long and .190 mm wide; intestine 5.0–12.0 mm long, and rectum .15 mm long. Two ovaries about 4 mm long, and two uteri 10.8 mm long; latter extend about .5 mm behind level of vulva before turning anteriorly and joining a common ovejector (Fig. 5) about .370 mm long. Eggs .043–.045 × .026 mm (Fig. 7).

Location.—Lesser curvature of stomach.

Type host.—*Sorex obscurus alascensis.*

Type locality.—Juneau, Alaska.

Type specimens.—U. S. National Museum Helminthological Collection No. 37210.

The present species differs from all known members of the genus *Dispharynx* and from all known members of the family Acuariidae Seurat 1913 as re-defined by Chitwood and Wehr (1934) by its cervical enlargement, and by parasitizing mammals rather than birds.

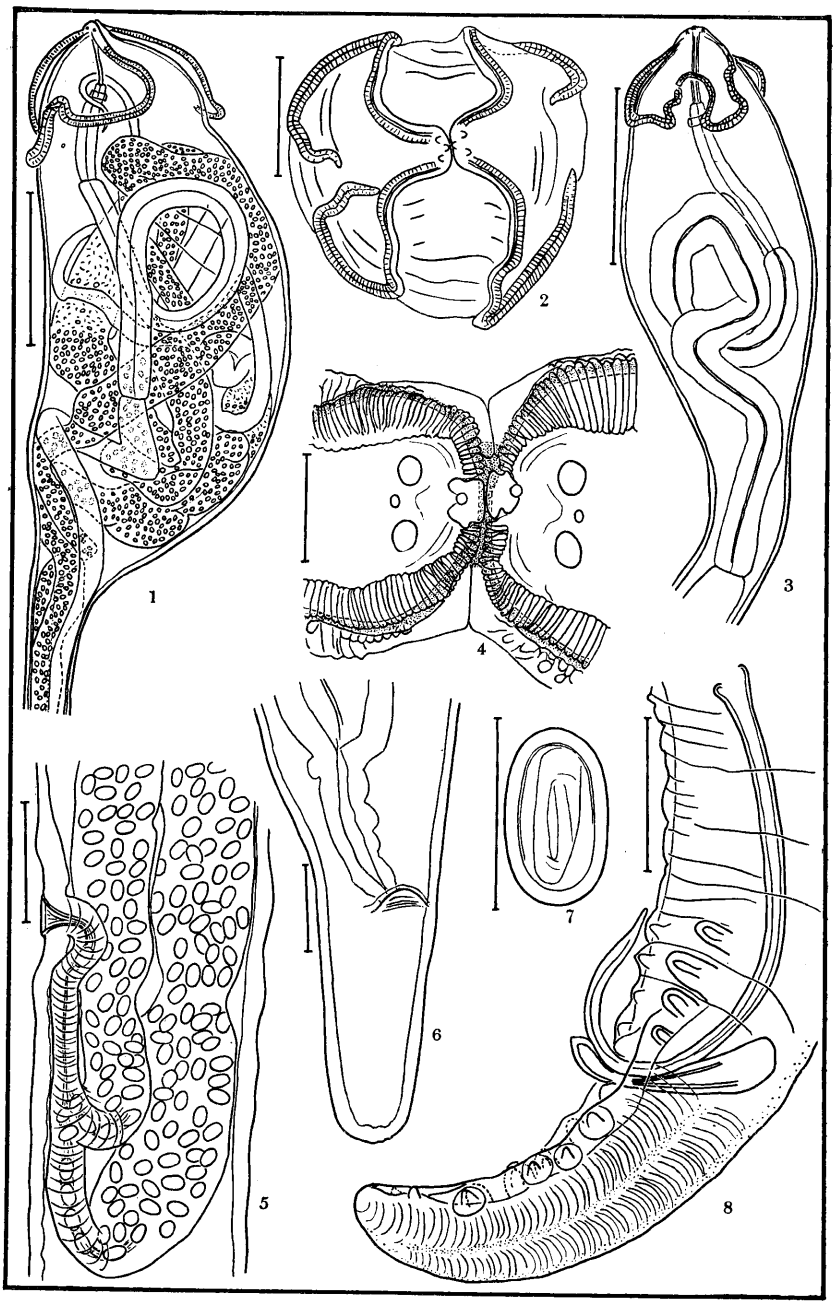
Three specimens of *Sorex cinereus streatori* (identified by Dr. Rausch) from Haines, Alaska, September 2, 1949, and three from Juneau were examined without finding *D. soricis.* One *S. o. alascensis* from Haines was likewise negative.

The numbers of pre-cloacal and post-cloacal papillae were found to be variable. A single host animal had three males of normal appearance and one which possessed six papillae in the left pre-cloacal row and four in the right one. One specimen from another host animal had an extra pair of post-cloacal papillae.

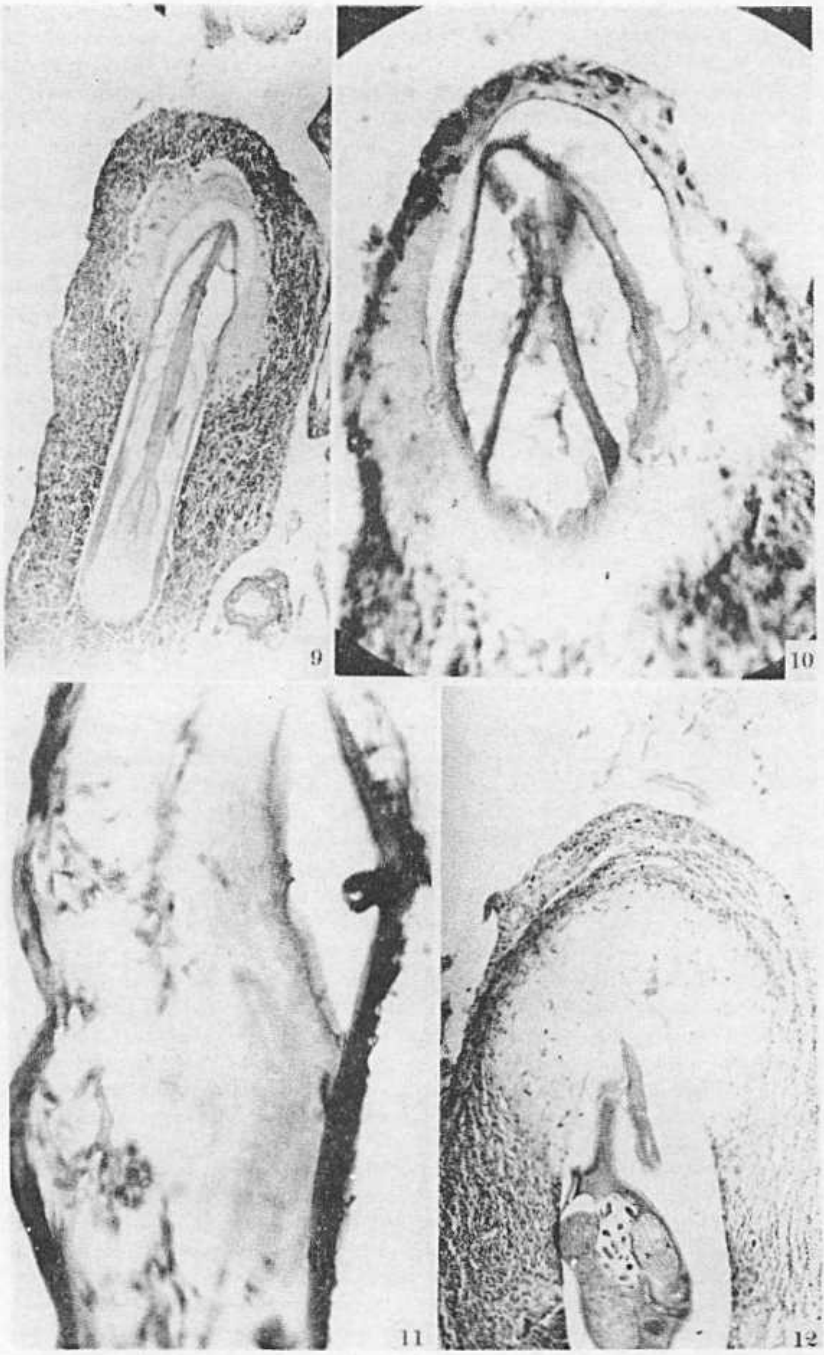
A mature, but non-gravid female was collected which had one of the uteri and the corresponding ovary in the post-vulvar portion of the body. Since the worms, particularly females, becomes much more firmly imbedded in the host by the cervical swelling which is filled in part by the uterine coilings, such an individual might have less chance to retain its position in the stomach wall.

Adult worms live with their swollen anterior ends in capsules of fibrous tissue on the outer surface of the stomach wall and could only be removed by breaking the outside of the capsule and drawing them outward with an artist's brush. This capsule of fibrous connective tissue surrounding the anterior third of *D. soricis* has little dilatability. Inasmuch as the lumen of this capsule conforms to the worm's body diameter, it becomes much narrower behind the cervical region, holding the parasite firmly in place. Attempts to extricate the worms by tension on the posterior end resulted in breaking the worm if it were a mature female, or in severe damage if it were a male or an immature female; although the last two could occasionally be taken out by this means. Wasielewski and Wülker (1919) reported that they could occasionally recover undamaged mature adults of a *Dispharagus* (actually *Dispharynx* sp.) in pigeons by pulling on the protruding tails. In the shrew stomach a few empty cysts of moderately large size were found to be filled with pus, and to have openings into the lumen that were only partially obliterated. This would suggest that in some cases adult female worms had perished and disintegrated.

Wasielewski and Wülker (1919) also have discussed probable functions of acuariid cordons and reviewed earlier literature on the subject. A very plausible



FIGS. 1–8. Morphology of *Dispharynx soricis*. All drawings were made with the aid of a camera lucida. Scale in Figure 1 represents 1 mm; in Figs. 2 and 5, .200 mm; Fig. 3, .500 mm; Fig. 4, .030 mm; Figs. 6 and 8, .100 mm; and Fig. 7, .050 mm. 1—Lateral view, anterior end, female. 2—*En face* view, female. 3—Lateral view, anterior end, male. 4—*En face* view, lips and cirumoral papillae, drawn from same specimen as Fig. 2. 5—Ovejector and vulva of female. 6—Tail of female. 7—Egg. 8—Tail of male.



FIGS. 9–12. Photomicrographs, pathology of *D. soricis* in *Sorex obscurus alascensis*. 9—Capsule around anterior end young female *D. soricis* showing necrosis. Ehrlich's hematoxylin and eosin. 83.6×. 10—Oblique section through stoma and cordons of *D. soricis*. Note thinness of capsule toward upper right corner of picture. Delafield's hematoxylin and eosin. 265×. 11—Portion of capsule beside enlarged female showing capillary endothelial structures in necrosis area. Delafield's hematoxylin and eosin. 212×. 12—Capsule surrounding anterior end of female. Ehrlich's

theory was advanced by von Linstow (1895): liquid nutrients, that would otherwise be poorly accessible to such firmly imbedded worms, are drained into the mouth by the cordons. The location of the maximum diameter of each sex of *D. soricis* in the middle of the cervical swelling well behind the cordons excludes the possibility that these structures furnish traction to retain the worms in their capsules as was suggested for the above mentioned *Dispharynx* sp. of pigeons by Wasielewski and Wülker.

The mucosa of the mammalian stomach is much thinner than that of the bird's proventriculus, and the organ is more dilatable, since there is neither a crop nor an easily distended esophagus for the storage of undigested food. By breaking through the muscular layers into the sub-peritoneal region of the shrew's stomach, the present species is able to secure an attachment comparable to that of other acuariids in avian hosts. Wasielewski and Wülker (1919) described epithelial papillomata caused by *Dispharynx* sp. in their pigeon proventriculus. Slides loaned to the writer by Dr. F. C. Goble, Rensselaer, N. Y. indicate that a similar response occurred in at least some grouse infected with *Dispharynx nasuta*. Whether the mammalian stomach would also produce this particular benign neoplasm in response to a perhaps less well adapted, but successful acuariid invader, or would merely respond with fibrosis remains to be seen.

The worms are clustered in the lesser curvature of the stomach in the vicinity of the esophagus. However, occasional specimens may be on the sides of the stomach, or half way to the pylorus in the lesser curvature. The failure of *D. soricis* to parasitize more than this small area should minimize damage to the smooth muscle of the organ as a whole. Damage to nerves of any gastric plexus was not observed. However, should such damage occur, the motility of the stomach might be impaired.

Inasmuch as it seems unlikely that the adult worms could ever withdraw their anterior ends from their respective capsules in the stomach wall, and successfully re-establish themselves, the tendency to congregate is very probably necessary to facilitate continued fertilization of females. It would seem that once the worms have established themselves, fertilization can only occur in intersection areas of circles with centers at the point of emergence of the worm's bodies and radii equal to the respective distances of the male and female reproductive openings from these centers. Studies on the distribution of *Dispharynx* over the surface of the bird proventriculus appear to be wanting.

The infective larval worms probably penetrate the gastric pits and glands after having been digested free of an intermediate host, though this must be verified by observation. Enough specimens were obtained to show that the worms are situated with their anterior ends in the subperitoneal tissue and their posterior ends free in the lumen of the stomach when the process of cervical enlargement starts. The maturation of the worm and the attendant elongation and swelling of the cervical region sometimes causes a break in the peritoneal surface. Whenever this happens, the anterior end of the worm is separated from the peritoneal cavity by a thin layer of fibrous connective and/or necrotic tissue. Further evidence for the discontinuity of the peritoneum was obtained when the anterior end of one of the worms was found, upon sectioning, to be partially imbedded in liver tissue while its tail was free in the lumen of the stomach. In this case two peritoneal surfaces had been penetrated. Sometimes an opaque, hemispherical, white mass could be seen between the cordon region of the worm and the external surface of the capsule. Worms dissected free occasionally had this substance tenaciously adhering to their anterior ends. The cordons of all worms in a single host were similarly surrounded and hidden from view whenever this white sub-

stance could be seen without sectioning and staining. In other hosts the capsule was more or less transparent and the anterior end of the worm could be seen inside it. This was particularly true of mature females with very large cervical swellings (Fig. 1). One such shrew was cleared in glycerin and exhibited under a binocular dissecting microscope at the 1949 meeting of the American Society of Parasitologists and is now deposited in the U. S. National Museum Helminthological collection (No. 37,218).

The encapsulating host tissue (Figs. 9–12) varied from about .050 mm to about .200 mm in thickness, and its base and part or all of its sides were encircled by peritoneum. Resting fibroblasts and collagenous fibers were most abundant at the outer surface, though foci of fibroblast proliferation, particularly around the end of the sac, correlate with the processes of elongation and enlargement. Polymorphonuclear cells and lymphocytes were interspersed among the collagenous fibers beneath the surface of the capsule. Further inward the collagenous fibrils became less abundant, and less dense, finally giving way to a thin layer of suppuration and cellular debris around the cuticle of the worm (Figs. 9 and 12). Lymphocytes, macrophages, eosinophils, and giant cells were present on the inside of the capsule.

An unidentified species of coccidia was also present in the gastric mucosa of one infected host animal, but did not have any observable effect on the cellular response to *D. soricis*.

The above mentioned white substance that surrounded the anterior end of the parasite appeared to be somewhat histologically varied. Its general shape (Figs. 9 to 12) resembled the necrosis around young *Contracaecum* sp. penetrating into connective tissue of the submucosa of a seal stomach that Hoeppli (1927) pictured in his Figs. 3 and 4. Sometimes it was also bordered by a zone of pyknosis and karyorrexis similar to that around Hoeppli's area. However, on other occasions there was very little evidence of these processes. The area itself was for the most part more weakly eosinophilic than the one pictured by Hoeppli appeared to be. Except for the portion which was adjacent to the parasite, the present reaction was not nearly so homogenous as Hoeppli's necrosis. Degenerating polymorphonuclear cells were occasionally present in small numbers and sparsely distributed. (Fig. 12). The outer half of the area contained a few active fibroblasts which, though slightly hypertrophied, took a strong hematoxylin stain. The outside also frequently contained capillary buds and remnants (Fig. 11) which sometimes connected with capillaries of the fibrous capsule, and which very rarely came near the surface of contact with the worm. The presence of these easily demonstrated vascular endothelial cells appears to be previously unrecorded in the areas of necrosis around the anterior ends of nematodes. Wasielewski and Wülker did not mention them in their study of *Dispharynx* in the pigeon, and Hoeppli's papers do not describe or picture them. The stroma of the zone was of a finely fibrillar structure, but not resembling fibrin or collagen fibers. Rarely there were indistinct outlines of necrosed fibroblasts and fibers.

Further studies of the processes involved should attempt to determine whether the above area as well as the zones of necrosis pictured by Hoeppli are the result of the actions of secretions from the nematodes. The possibility that some parasitic spiruroid nematodes secrete an histolytic enzyme was considered by Hoeppli (1933), though his own efforts to experimentally demonstrate one had been inconclusive (Hoeppli and Feng, 1931).

Dr B. V. Hall, University of Illinois, has suggested that the necrosis for the most part might be the result of mechanical pressure exerted by the worm as it pushes itself inward by muscular action. This alone would not account for the

failure of the worm to break through the end of the capsule, especially in those observed instances where only necrotic tissue and a single layer of live cells separated worms of relatively small diameter from the peritoneal cavity. It might be added that in such instances the single layer of cells often appeared to be incomplete or necrosing in places (Fig. 10). Evidence in favor of the mechanical pressure hypothesis would be the small amount of necrotic tissues along the sides of the worm posterior to the cephalic enlargement. Perhaps the free phagocytes, particularly the giant cells and macrophages, remove dead tissue surrounding the body of the worm as it forms, thus preventing the accumulation of necrotic tissues along the sides. Forming and formed capsules were always perpendicular to the surface of the stomach before fixation. The tendency for the capsules to become transparent as the diameter of the anterior end disproportionately increased should correlate with reduced pressure per unit area.

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Studies on Bovine Gastro-intestinal Parasites XVIII. Some Results of Feeding Small Amounts of Phenothiazine on Pure Infections of the Nodular Worm, *Oesophagostomum radiatum*

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In a preceding paper (Mayhew, 1950) were described the results of feeding small amounts of phenothiazine in pure infections of the hookworm, *Bunostomum phlebotomum*. In these experiments it was found that feeding ½ and 1½ grams daily did not cause a reduction in egg production, but that fecal cultures made during that time failed to yield infective larvae. In the following pages are described the results of similar experiments in which calves had pure infections of the nodular worm. Since references are mentioned in the former paper further discussion of the literature is unnecessary in this report.

METHODS

The methods of caring for the experimental animals, fecal examination, and culturing of the larvae were the same as in the preceding studies of this series. The animals were raised parasite-free, except for *Strongyloides* sp., and inoculated

with larvae cultured from pure infections. Phenothiazine was purchased locally and was a product of the E. I. DuPont de Nemours & Co. Weighed amounts of the drug were fed by mixing it with the grain concentrate at the times described. A sufficient number of egg counts were made before beginning each experiment to determine the maximum and the minimum of the range and to give assurance of the stability of the range. While the egg counts of the animals used in some of the experiments indicate that the infection was low the results indicate that the reactions of the parasites to the drug was the same as in the animals with higher infections.

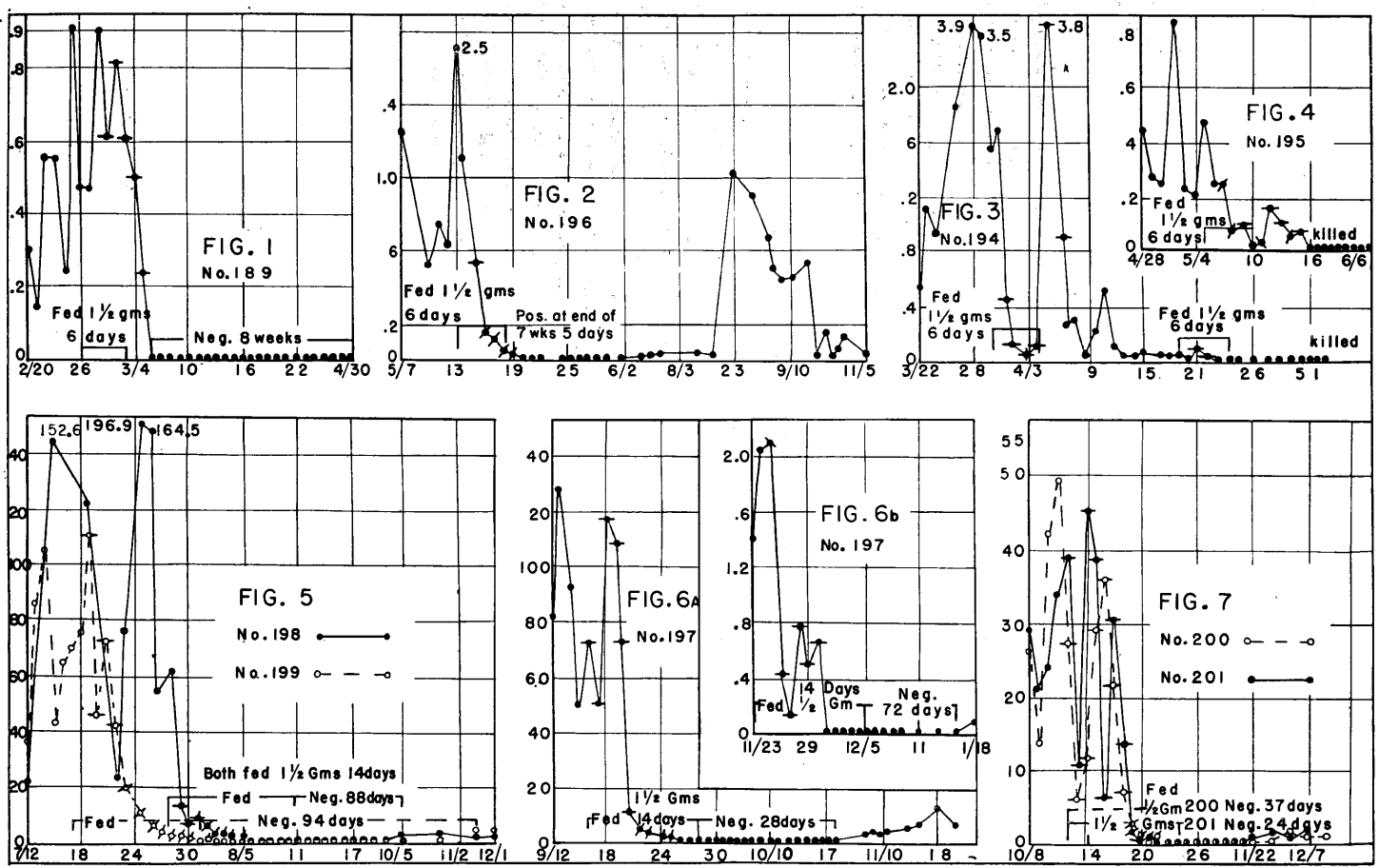
RESULTS

Calf No. 189. This animal was a pure bred Holstein male, born February 1, 1948, and was obtained from the Louisiana State University Dairy Department. He was inoculated on August 23, 25 and September 14, 1948, with a small number of nodular worm larvae cultured from adult worms collected from calves killed at the Baton Rouge City Abatoir. The first positive fecal examination was made on October 7, the 42nd day after the first inoculation, no examinations having been made between September 23rd and October 7. The resulting infection was relatively low, judging by the egg counts; consequently, the manure of this animal was used for a time to culture larvae with which to inoculate other calves. On February 26, 1949, daily feedings of 1½ grams of phenothiazine were begun and continued for six days. From February 28th to March 5th, the eggs were abnormal in appearance; and on March 6th they disappeared altogether (Fig. 1). The daily fecal examinations made during the following 18 days, until March 23rd, were all entirely negative, and 10 additional examinations made between March 25th and April 30th were, likewise, all negative. The calf was negative for a total of 8 weeks, at the end of which time it was necessary to discontinue the experiment.

Infective larvae were isolated from daily fecal cultures for 16 days preceding March 1st, the second day abnormal eggs were observed. The cultures were negative for larvae for the following 19 days, beginning March 2nd, except one larva each day on March 3rd and 6th.

Calf No. 196. This animal was a pure bred Jersey male born at the Louisiana State University Dairy Department on December 1, 1948. He was given infective nodular worm larvae on February 11, 1949 and the first positive fecal examination was made on March 25, the 42nd day after inoculation. No examinations were made between February 11 and March 25. Fourteen fecal examinations made between April 27 and May 13 showed a variation in egg count of between 2.5 and .46 eggs per grams of sediment. Beginning on May 13 a series of six daily feedings of 1½ grams of phenothiazine was started. The eggs recovered on May 15 (Fig. 2) and on each day until May 18 were abnormal in appearance. Ten fecal examinations made between May 20 and June 1 were all negative. No fecal examinations were made on this animal from June 1 to July 13, 7½ weeks after the first negative examination, when two eggs were recovered. Since the count was very low (.22 eggs per gram of sediment) and it increased gradually to a maximum of 1 egg per gram (Fig. 2) it is possible that this is one of the first days when eggs were produced and that the calf had remained negative about 7 weeks.

Daily larval cultures were made from May 11 through May 27 with the exception of May 18 and 19. Larvae were recovered in the cultures until May 14th. From May 15th, the first day on which the eggs were observed to be abnormal, until May 27th the cultures were consistently negative.



FIGS. 1 to 7. Graphs of egg-counts made on calves used in the experiments described in the text.

Calf No. 194. This animal was a pure bred Holstein male, born at the Louisiana State University Dairy Department on November 16, 1948. On February 4, 1949, he was given infective nodular worm larvae. Thirteen fecal examinations made between February 2 and March 14 were negative. The first eggs appeared in the manure on March 15th, the 39th day after inoculation. Daily examinations made between March 4th and 14th except for the 6th and 13th were all negative. One and one-half grams of phenothiazine was mixed with the grain concentrate and fed about 9: 00 p.m. daily between March 30 and April 4 (Fig. 3). The eggs in the manure sample collected about 8: 00 a.m. April 1 were observed to be abnormal and continued to be abnormal until April 7 when they were again consistently normal in appearance. A second series of feedings of 1½ grams of phenothiazine was begun on April 19 and continued for 6 days (Fig. 3). Abnormal eggs were recovered on April 21 and 22, and 11 fecal examinations made daily following the 23rd were all negative.

Eight cultures made between March 21 and 31 were all positive for infective larvae. Daily cultures made between April 1 and 8 were all negative, April 1 being the first day on which abnormal eggs were recovered in the manure. Fourteen cultures made during the following 17 days (until April 24) were negative except those on April 10 and 12 from which a very few larvae were recovered.

The animal was killed on May 4 to determine if any adult worms remained. At the post-mortem examination 306 males and 321 females were recovered. One hundred of the females were cleared and examined for the presence of eggs and only two appeared to have eggs.

Calf No. 195. This calf was a pure bred Holstein male born at the Louisiana State University Dairy Department on November 19, 1948. On February 11, 1949, he was given infective larvae cultured from a calf with a pure infection of nodular worms. Eggs were detected in the manure on March 25th the 42nd day, fecal examinations were not made between February 11 and March 25. Some of the egg counts are shown graphically in Fig. 4. On May 5, 1949, a series of 6 daily feedings of 1½ grams of phenothiazine was begun. All eggs recovered between May 7 and 15 in the daily fecal examinations were abnormal and the following 11 daily examinations were negative.

Nine out of eleven larval cultures made between April 26 and May 6 contained larvae and 15 cultures made between May 7, the first day abnormal eggs appeared, and May 25 were consistently negative for larvae except for a very small number on May 8.

This calf was then killed on June 7 to determine if the adult worms had been removed. At the post-mortem examination 321 adult males and 19 females were recovered. Three of the females appeared to have a few eggs when cleared and examined microscopically.

Calves No. 198 and 199. These calves were Hereford Jersey cross bred males and were secured from a local dairyman. They were born on March 1, 1949, and brought to our experimental quarters on March 2. On May 27, 1949, both calves were given infective nodular worm larvae. Three fecal examinations made between May 10 and June 6 were negative. No fecal examinations were made between June 6 and July 11, eggs having been detected on July 11, the 45th day after inoculation, in the case of both animals. Figure 5 shows a portion of the fecal examination record preceding and during the time of feeding the phenothiazine.

In one instance out of the 5 preceding experiments in which phenothiazine was fed daily for 6 days it was necessary to carry out a second 6-day feeding period to entirely eliminate the eggs. Thinking possibly a longer period would

be more effective it was decided to feed 14 days. On July 18 a 14-day series of feedings of 1½ grams was begun with No. 199. The phenothiazine was fed at about 4: 00 p.m. on the first day and the eggs recovered from the manure sample collected at 8: 00 a.m. the following morning, 16 hours later, were by count 80–86% abnormal. It has been noted in the preceding experiments that abnormal eggs were not seen until the morning of the second day; however, in those experiments the first feedings were made at about 10: 00 p.m. or approximately 10 hours before the time of collection of the manure sample the following day. All the eggs observed from July 20 until their disappearance on August 2 were abnormal. Twenty-one fecal examinations made between August 2 and November 3, a period of 13 weeks and 2 days, were all negative. A few eggs were observed in the samples of November 4 and 16. The experiment was discontinued on November 16, 1949.

We began the 14-day series of feedings of 1½ grams of phenothiazine to No. 198 on July 28th at about 4: 30 p.m. The eggs recovered from the manure sample collected the following morning about 8: 00 a.m. were mostly abnormal, no differential count being made. All the remaining eggs observed until August 5 were abnormal. Beginning on August 6 the fecal examinations were negative until October 5. Fifteen negative examinations were recorded between August 6 and September 28, a period of 7½ weeks. One egg was detected October 5 and a few thereafter in the weekly samples until December 1, 1949, when the experiment was ended. The only weights taken of these animals was on October 27, 1949 when No. 198 weighed 255 lbs. and No. 199 275 lbs.

Calf No. 197. This calf was a grade Jersey male secured from a local dairyman. He was born on February 26, 1949, and was placed in our experimental quarters on February 28. On July 27 he was given infective nodular worm larvae. Two eggs were detected in the manure on August 31, the 35th day after inoculation. A portion of the resulting egg counts that preceded the experiment is shown in Fig. 6a. The feeding of phenothiazine, 1½ grams daily for 14 days, was begun on September 15 at about 4: 00 p.m. The eggs recovered from the sample collected at about 8: 00 a.m. the following morning were at least 75% abnormal. All the eggs observed on the following days were abnormal until they disappeared on September 26. Fifteen fecal examinations made between September 26 and October 24 were all negative. Eggs were detected in the manure sample of November 2, and 14 examinations made between November 2 and 22 indicated that sufficient eggs were being recovered to make it possible to carry out a second feeding of phenothiazine to this animal.

This second experiment was begun on November 23 and ½ gram was fed daily for a period of 14 days (Fig. 6b). The first feeding was made at 10: 00 p.m. and the eggs recovered from the sample collected at 8: 00 a.m. the following morning, 10 hours later, were considered to be normal in appearance. Another sample collected at 4: 00 p.m. (18 hours after feeding) contained 80% abnormal eggs. A sample collected at 10: 00 p.m. (24 hours after feeding) contained at least 94% abnormal eggs. The eggs observed during the following 6 days were all considered abnormal. The following 15 fecal examinations made between December 1 and January 12, 1950, were all negative.

Calves No. 200 and 201. No. 200 was a grade Jersey male born March 2, 1949, and was secured when a few hours old from a local dairyman. No. 201 was a pure bred Holstein male born at the L.S.U. Dairy Department on April 18, 1949. Both animals were given infective nodular worm larvae on August 17, 1949. Four fecal examinations made on No. 200 and 5 on No. 201 between August 16

and September 15 were all negative to nematode eggs except *Strongyloides* sp. No fecal examinations were made on No. 200 between September 8 and 22 and one negative examination was made on No. 201 on September 15. Eggs were detected in the manure of both animals on September 22, the 36th day after inoculation.

A series of 14 daily feedings of phenothiazine was begun in the case of both animals on October 11, 1949; No. 201 was given 1½ grams and No. 200 ½ gram each day (Fig. 7). The eggs recovered from the sample from No. 201 at 8: 00 a.m. the following morning, 10 hours after feeding the phenothiazine, were normal. Those collected at 1: 00 p.m. 15 hours after the first feeding were 80 to 86% abnormal, those in the sample collected at 5: 00 p.m., 19 hours after feeding, were 86% abnormal, and in the sample collected at 10: 00 p.m., 24 hours after feeding, there were less than .03% normal eggs observed. The eggs recovered from daily samples collected between October 13 and 21 were abnormal in appearance. Thirteen fecal examinations made between October 22 and November

TABLE 1.—*Summary of principal data for comparison.*

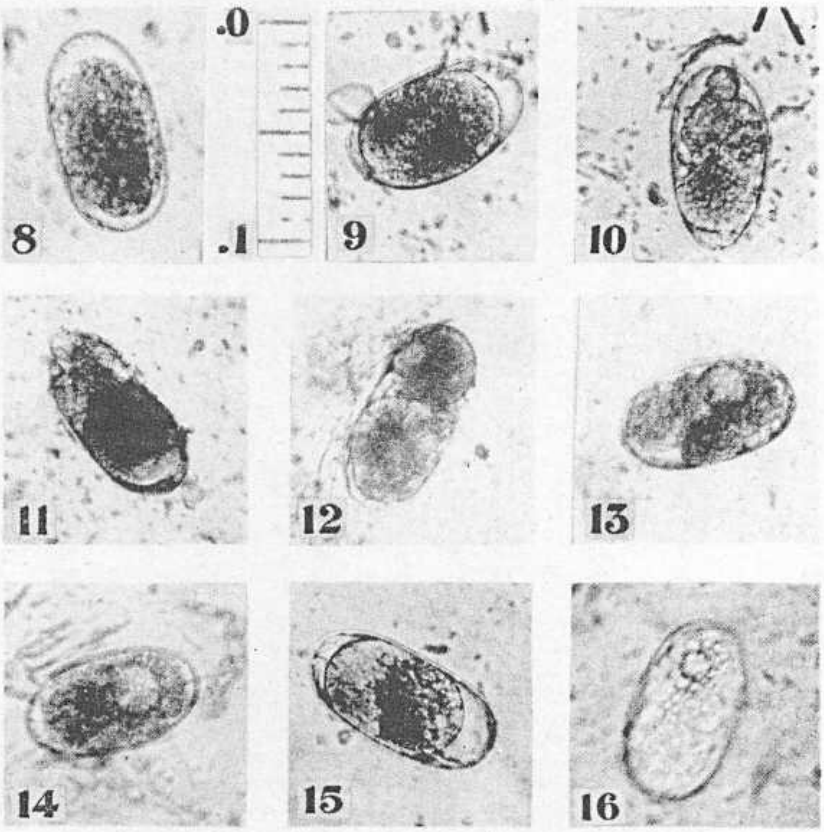
Calf No.	Age at Beginning of Exp.		No. Grams Fed Daily	Days of Feeding	No. Days until Neg.	No. Days Negative	Weight of calf
	Mos.	Days					
189	12	26	1½	6	7	56	
196	5	13	1½	6	6	about 49	
194 exp.							
a.	4	14	1½	6	see text		
b.	5	2	1½	6	3	killed	
195	5	16	1½	6	10	killed	
199	4	18	1½	14	14	93	275
198	4	28	1½	14	8	53	255
197 exp.							
a.	6	20	1½	14	10	28	
b.	8	28	½	14	7	42	
200	7	9	½	14	11	37	235
201	5	23	1½	14	10	24	265

15, 25 days after the first negative examination, were all negative. One egg was detected November 22, and a few in the samples collected on November 30 and December 5 and 6, 1949, when the experiment was discontinued.

In the experiment with No. 200, the animal receiving ½ gram, no manure sample was collected at 8: 00 a.m. but the eggs in the sample collected at 1: 00 p.m., 15 hours after the first feeding of phenothiazine, 4% of the eggs were believed to be abnormal from their appearance (Fig. 7). Daily fecal examinations made between October 13 and 20 all showed the eggs to be abnormal and in gradually decreasing numbers. The examination on October 21 was negative and but one very abnormal egg was observed on October 22, 12 days after the first feeding of phenothiazine. Fourteen fecal examinations made between October 23 and November 30 were all negative. Eggs were detected in the samples collected on December 5, 6, 7 and 8 (Fig. 7). The only weights taken of these two animals were on October 27, 1949 when No. 200 weighed 235 lbs. and No. 201 265 lbs.

EGG ABNORMALITIES

The abnormalities observed in the eggs consisted in changes in the cell division resulting in cells of different sizes (Fig. 10–13) or failure to divide entirely (Fig. 9) and in the change in color and density of the protoplasm (Fig. 11–15). Some of the eggs did not show any evidence of division as seen under magnifications up to about 300. In other eggs all sorts of combinations of cells of unequal sizes occurred as indicated by Figures 10–13. The eggs were in general more brownish in appearance and somewhat less translucent than normal



FIGS. 8 to 16. Eggs of *Oesophagostomum radiatum* recovered from calves fed phenothiazine. 8—Normal egg. 9—Egg that failed to divide. 10 to 13—Dividing eggs showing cells of unequal size. 13, 14—Clear spots present in eggs. 15—Disintegrating egg with dark pigment present in undivided protoplasm. 16—Another egg in disintegrating condition.

eggs after the effects of the phenothiazine became evident. As the feeding of the drug continued, we believe that this coloring and opaque condition tended to disappear and that many of the eggs became clearer (Fig. 15, 16) than normal, the last eggs being more tedious to find when making the counts. Relatively clear spots (Fig. 11, 13, 14) may be present in some of the cells of the eggs toward the end of the period of feeding of the phenothiazine. A small number of abnormal eggs are to be found in the manure of calves on a normal diet, but we do not believe it will ordinarily be above 1 or 2%.

SUMMARY

Four calves with pure infections of the nodular worm, *Oesophagostomum radiatum*, were fed 1½ grams of phenothiazine daily for 6 days with the result that eggs disappeared altogether in three of the animals, and the fourth required a second feeding to eliminate the eggs.

In four experiments in which 1½ grams of phenothiazine were fed daily for 14 days all animals became negative.

In two experiments in which ½ gram was fed daily for 14 days the eggs were eliminated from the manure.

The calves became negative in from 3 to 14 days after the first feeding and remained negative for from 24 to 93 days, the second period of egg production being relatively low as compared with the first.

Abnormal eggs appeared in the manure in from 10 to 16 hours after the first feeding of phenothiazine. These abnormalities consisted of failure to divide, unequal divisions, and a brownish coloration of the cytoplasm.

Cultures made preceding and during the periods of feeding of the phenothiazine invariably contained infective larvae up to the days on which abnormal eggs were first detected in the fecal samples. Following the time of appearance of abnormal eggs, the cultures, with a few exceptions, were free of larvae.

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Studies on the Helminth Fauna of Alaska II. On Some Helminths Parasitic in the Sea Otter, *Enhydra lutris* (L.)

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Except for the limited observations of Barabash-Nikiforov (1935) on the Komandorskie Islands, there appears to be no records in the literature on the helminth parasites of the sea otter, *Enhydra lutris* (L.). Aside from the purely helminthological interest, the parasites of the sea otter warrant some consideration in view of the potential economic value of the animal. When, with the aid of proper management, the harvesting of sea otter pelts again becomes feasible, their fur value probably will be very high. Any information which can be obtained relative to their general ecology may contribute to the bringing about of the desired numerical status.

Through the cooperation of Mr. Robert Jones, Biologist, U. S. Fish and Wildlife Service, three sea otter carcasses from Amchitka, Aleutian Islands, were made available to us for parasitological examination. These animals had been found dead, and the frozen carcasses were brought to us for determination of the cause of death. This opportunity is taken to express appreciation of Mr. Jones' cooperation.

Barabash-Nikiforov (1935) recorded *Porrocaecum decipiens* (Krabbe) as well as an unidentified cestode from the sea otters examined by him. Robert Jones (personal communication) has mentioned the occurrence of a large nematode in the gastrointestinal tract of sea otters from Amchitka, but we have not secured any specimens of this nematode for study. As far as we can determine, these are the only records of parasites from this host.

Five species of helminths were collected by us from the three animals examined; of these, four were trematodes and one, an acanthocephalan. One trematode is described herein as new. The material was in satisfactory condition for study, although degeneration had taken place in some cases, making difficult the observation of certain morphological details. The trematodes were stained with Semichon's acetic carmine and Harris haematoxylin, and serial sections of the species described were also examined. The parasites are discussed separately.

Orthosplanchnus fraterculus Odhner, 1905

This trematode occurred in large numbers in the 'bile organ' of two of the three animals examined. Published records indicate that it is a rather common parasite of the walrus and the bearded seal. The senior author has taken *O. fraterculus* several times from the bile ducts of the bearded seal, *Erignathus barbatus* (Erxleben), from the vicinity of Point Barrow and Wainwright, Alaska, and once from the pancreatic and bile ducts of the same species from St. Lawrence Island, near Siberia. We have not, however, found it infecting any of the smaller seals (*Phoca* spp.).

Dawes (1946) suggests that *O. fraterculus* is identical with *O. arcticus* Odhner, 1905, the former being considered only a small specimen of the latter. We do not agree with this opinion, since we have collected specimens of *O. fraterculus* which equal or exceed (6 to 7 mm.) the maximum length given for *O. arcticus*, and do not find them to differ from typical specimens, as described. We have not observed any morphological similarity to *O. arcticus* as figured by Price (1932) after Odhner, although we have not had available any specimens of *O. arcticus* for direct comparison.

This trematode may be limited in its occurrence largely by the food habits of the definitive host, rather than by any physiological specificity; this is particularly the case when the phylogenetical relationships of the mammals recorded as hosts are considered.

Phocitrema fusiforme Goto and Ozaki, 1930

Five specimens of *P. fusiforme* were taken from the small intestine of one of the animals, and, as far as can be determined, they appear typical as described by Goto and Ozaki (1930). The type material was obtained from the intestine of the common seal, *Phoca hispida* Schreber. The examination by the senior author of a number of seals of this species from the Arctic Coast of Alaska, has failed to disclose this trematode. Because of their small size, however, they might easily be overlooked if not numerous.[1]

Pricetrema zalophi (Price, 1932)

Five specimens of this minute trematode were found in the small intestine of one of the sea otters, along with the foregoing species. These appear to be typical as described by Price (1932).

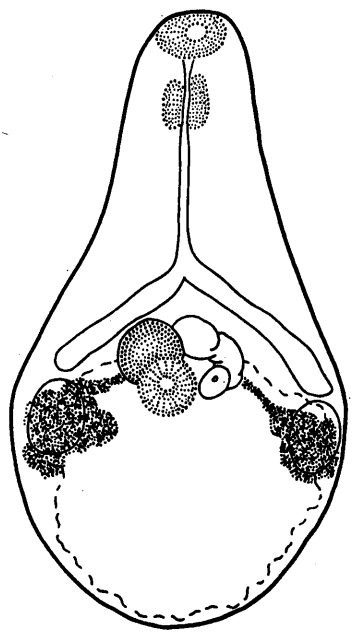
Pricetrema zalophi (= *Apophallus zalophi* Price, 1932) was described from the California seal lion, *Zalophus californianus* (Lesson). Although not occurring in Alaskan waters, the range of the California sea lion is overlapped on the south by that of Steller's sea lion, *Eumetopias jubata* (Schreber), which occurs as far north as the Bering Strait. We have data on only two Steller's sea lions, both negative for this parasite, and we have not found this trematode in walrus or various species of seals.

[1] After this paper was completed, we recorded this trematode from *Phoca vitulina*, collected on St. Lawrence Island by E. L. Schiller.

The genus *Pricetrema* was erected by Ciurea (1933) for this species, since he considered it to differ too greatly from the typical *Apophallus* to be retained in that genus. Dawes (1946) apparently overlooked the paper by Ciurea, since he retains the species in *Apophallus* without any consideration of *Pricetrema* Ciurea.

Microphallus enhydrae n. sp.

Diagnosis.—Microphallidae. Lanceolate to piriform body 260 to 936 μ long; size very variable, but average length is less than 580 μ. Greatest width, at junction of third and last fourths of body length, from 136 to 360 μ.



(Fig. 1)

FIG. 1. *Microphallus enhydrae* n. sp., ventral view.

Cuticular spines not observed. Subterminal oral sucker from 40 to 67 μ in diameter. Acetabulum, situated near anterior limits of posterior half of body, from 35 to 60 μ in diameter. Prepharynx from 14 to 24 μ in length; pharynx about 24 μ in diameter. Esophagus length from 72 to 288 μ. Widely divergent caeca usually from 90 to 100 μ long, nearly reaching the lateral margins of body, and extending nearly to posterior margin of acetabulum. Caeca usually extend to anterior margins of vitellaria. Genital pore to left of acetabulum near level of posterior margin. Male copulatory papilla about 20 μ in diameter. Seminal vesicle from 50 to 70 μ in length, situated at anterior margin of acetabulum, and extending dextrally. Testes ovoid, equal in size, about 80 μ long; situated just behind posterior margin of acetabulum. Ovoid ovary from 35 to 70 μ in greatest length, with long axis transverse; dextral, usually adjacent to right intestinal caecum in front, and to vitellaria posteriorly. Laurer's canal present. Vitellaria lobu-

lated, extending both anterior and posterior to testes, and ventral to them; common vitelline ducts pass inward at a level just anterior to testes. Uterus voluminous, usually filling entire body behind posterior margin of acetabulum but variable in this respect. Eggs very numerous, from 21 to 26 μ long by 11 to 16 μ wide.

Host.—*Enhydra lutris* (L.)

Locality.—Amchitka, Aleutian Islands.

Habitat.—Small intestine.

Type.—A slide containing paratype material has been deposited in the Helminthological Collection of the U. S. National Museum, slide No. 37175.

There are at present apparently thirteen valid species of the genus *Microphallus;* from these *M. enhydrae* may be differentiated by several characters. The short or vestigial intestinal caeca of *M. gracilis* Baer, 1943, *M. opacus* (Ward, 1894), and *M. baryurus* (Stafford, 1903) serve to distinguish them clearly from the present species. Similarly, but less markedly, *M. capellae* (Yamaguti, 1939), *M. longicaollis* (Yamaguti, 1939), *M. nicolli* (Cable and Hunninen, 1938), and *M. minis* Ochi, 1928, differ in having the intestinal caeca terminating well anterior to the posterior margin of the acetabulum. *M. similis* (Jägerskiöld, 1900), *M. papillorobustus* (Rankin, 1940), *M. nicolli,* and *M. brevicaeca* (Africa and Garcia, 1935) differ in having the oral sucker smaller than the acetabulum; in *M. enhydrae* the opposite is the case, i.e., the oral sucker is larger. *M. enhydrae* may be differentiated by egg size from *M. brevicaeca* (Africa and Garcia, 1935), *M. nicolli, M. baryurus. M. opacus,* and *M. capellae.* From *M. pygmaeum* (Levinsen) the present species differs in having a much more extensive uterus distribution; in the former the uterus does not extend anterior to the testes, while in *M. enhydrae* the uterus extends often beyond the anterior margin of the acetabulum. The vitellaria of *M. pygmaeum* are also more distinctly lobate, and are located more posterior to the testes than in *M. enhydrae. Microphallus claviformis* (Brandes, 1888) differs in size of male papilla, and in size and proportion of other organs, as does *M. excellens* (Nicoll, 1907). Cable and Hunninen (1940) presented a table of measurements of certain closely related species of this genus, and a key was published by Baer (1943). The life cycle of this species, resulting in its occurrence in a marine host, will probably also differentiate it from some other members of the genus.

Microphallus enhydrae was present in extremely large numbers in the intestine of one of the otters, along with *Phocitrema fusiforme* and *Pricetrema zalophi.* As is apparently characteristic, at least of some members of the genus (Rausch, 1946, 1947), these trematodes were extremely variable in size, even though apparently sexually mature, all containing eggs regardless of size of the worm. The abundance of eggs, which obscured most of the detail of the reproductive organs, made the study of these worms particularly difficult. It is well known that species of this genus are often capable of infecting a variety of phylogenetically widely separated host species, (Baer, 1943; Rausch, 1947). So far we have not observed *Microphallus* in other marine mammals.

Corynosoma sp.

Three specimens of *Corynosoma* were taken from the intestine of one of the sea otters. They appear superficially to be closely related to those found in other marine mammals. These specimens have been given to Dr. H. J. Van Cleave for further study, and a complete report will be presented by him later.

As far as could be determined from the material available, these parasites do not have any recognized deleterious effect upon the physical condition of the sea otter, but apparently represent species to which various marine mammals are exposed through their habits of feeding.

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The Acanthocephalan Parasites of Eider Ducks

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Because of their wide geographical distribution through arctic and subarctic regions of the northern hemisphere, the eider ducks offer some unusual opportunities for the study of distribution and host relations of their parasites. When the Acanthocephala from eider ducks were first studied, the fauna of each continent was regarded as distinct, because it had been reasonably well established that the majority of species of these parasites encountered in the United States were distinct from those found in central Europe. This belief failed to take into account the fact that in the arctic the usual distributional barriers to both the definitive and the intermediate hosts are lacking. Recent studies in northern Europe, especially by Lundström (1941, 1942), and new collections on the American continent have added significantly to an understanding of this fauna. The new evidences make it seem apparent that for water birds of the arctic region the acanthocephalan parasites are very widely distributed geographically. This statement does not contradict the generalization that migratory birds have little influence on inter-continental distribution of acanthocephalan parasites. It seems more probable that suitable intermediate hosts for Acanthocephala are widely dispersed in the arctic and subarctic regions and that local bird populations, even though they may have undergone subspecific or specific differentiation, are by food habits and habitat exposed to infection by identical species of parasites on the two continents. This condition of birds under ecologically similar conditions is a direct parallel to that described for fishes of the same regions where numerous species on the two continents serve as definitive hosts for *Neoechinorhynchus rutili* (Mueller) as demonstrated by Van Cleave and Lynch (1950).

Field studies in Alaska (by Rausch) have furnished the first collection of Acanthocephala from the Pacific eider, *Somateria v-nigra* Gray and additional material from the king eider, *Somateria spectabilis* (Linn.). The writers are

under obligation to Mr. Harold C. Hanson of the Illinois State Natural History Survey for a collection of *Polymorphus arcticus* (Van Cleave) from *Somateria spectabilis* which he took from Perry River, Northwest Territories, within the Canadian arctic during the summer of 1949.

Two species of Acanthocephala have been recorded previously from the eider ducks of North America. *Polymorphus botulus* (Van Cleave, 1916) was based upon specimens taken from the intestine of the American eider *Somateria mollissima dresseri,* from off the coast of Maine and at the time the species was described other specimens were available from the northern eider, *Somateria mollissima borealis,* from the North Atlantic coast of North America. At a later date, a second species, *Polymorphus arcticus* (Van Cleave, 1920) was described from the king eider, *Somateria spectabilis,* of the Canadian arctic fauna in the vicinity of Bernard Harbour, Northwest Territories. Both of these species were originally assigned to the genus *Filicollis,* but Meyer (1931) proposed *Profilicollis* as a new genus to accommodate them. However, this generic concept proved untenable and Witenberg (1932) was the first to suggest that these species be assigned to the genus *Polymorphus.* Van Cleave (1947) gave a full morphological analysis of the distinctions between *Filicollis* and *Polymorphus* which fully supported the proposal of Witenberg.

The writers have been unable to find any reference to Acanthocephala as parasites of the Pacific eider, *Somateria v-nigra.* However, as mentioned in the introduction of the present paper, Rausch examined a specimen in Alaska which carried a mixed infection. Of these, five large individuals have been identified as *Polymorphus arcticus* (Van Cleave) and five very small specimens as *Corynosoma mergi* Lundström. This is the first account of the occurrence of *C. mergi* on the American continent as well as in an eider duck.

In the original description of *Corynosoma mergi,* Lundström called attention to the fact that spines around the genital opening are lacking in the males but are found in some females, both juvenile and adult. This condition is the opposite of that which usually maintains in the species of *Corynosoma.* Consequently, a close study was made of the genital regions of the specimens from *S. v-nigra.* Unfortunately, genital spines could not be demonstrated in either sex but the females were gravid and the presence of large numbers of eggs made the trunk spines difficult to observe, except on the margins of the body. Since it is known (Van Cleave, 1945) that for many species of *Corynosoma* the posterior region of the male becomes introverted to form a genital vestibule, the males were closely examined but no vestibule could be demonstrated and no spine could be seen within the body.

At the time *P. botulus* and *P. arcticus* were described, they were both known from the North American continent only, and these were the only two species of Acanthocephala reported from eiders of the new world. In Europe, the Acanthocephala from the eider ducks had been very unsatisfactorily treated. As early as 1774, Phipps recorded a worm from *Somateria mollissima,* under the name *Sipunculus lendix.* Although this form has been given recognition as presumably an acanthocephalan, no one has ever been able to recognize the species from the original description.

Kostylev (1922) recorded *P. botulus* (Van Cleave) from *Somateria mollissima* of Spitzbergen, the Murman Coast and various other localities within the Eurasian arctic. In the same paper, he described *Polymorphus phippsi* as a presumably new species from the same host but it seems highly probable that this is a direct synonym of *Polymorphus minutus,* which is widely distributed geographically and has very broad host adaptations among water birds. Previously,

Baylis (1919) had recorded *P. minutus* from *Somateria mollissima* of the Murman Coast.

Later, Kostylev (1925) reported that specimens of *Echinorhynchus polymorphus* from the European eider, which von Linstow (1901) had identified in the collections of the Zoological Museum of Science of U R S S were, upon reexamination, found to be *Filicollis botulus* Van C. (*P. botulus*). These collections were from Spitzbergen and Katharinen Hafen of the European Arctic Ocean.

von Linstow (1905) gave the name *Echinorhynchus pupa* to specimens taken from *Somateria mollissima* by the Russian Polar Expedition on Taimyr Peninsula but because the species was inadequately described it has been the subject of great confusion. Kostylev (1922) expressed the belief that *E. pupa* is probably identical with *P. arcticus* but this seems to be untenable. Meyer (1931), in reviewing the Acanthocephala of the Arctic fauna, differed radically from the interpretation of Kostylev when he assigned *E. pupa* to the genus *Prosthorhynchus* as *Prosthorhynchus pupa* (von Linstow). The description given originally is too inadequate to permit of definite generic assignment. However the shape of the trunk and the "cylindrical" proboscis mentioned by von Linstow would definitely exclude the species from identity with *Polymorphus arcticus,* and thus eliminate the possibility of establishing *E. pupa* as having priority over *P. arcticus.*

In his survey of the Acanthocephala of the Swedish avian fauna, Lundström (1942: 47) found *P. botulus* in *Somateria mollissima mollissima,* the European eider, but found this species in no other host. He likewise recorded two unidentified species of *Polymorphus* from the European eider, one of which, represented by a single immature female specimen, he regarded as a new species although he assigned no name and gave no significant description. The other unnamed specimens of *Polymorphus* from the eider were wholly undescribed. In his host tabulation (page 215) he records as a new record an unidentified species of *Corynosoma* but does not mention this in his tabular survey of his examinations of *Somateria* (page 47).

In an earlier paper, Lundström (1941) described *Corynosoma mergi* as a new species from *Mergus serrator* and later (1942: 215) recorded it also from *Phalacrocorax carbo* but he did not find it in any of the other ducks or water birds of the northern European fauna which he investigated.

By way of summary, as information on the acanthocephalan parasites of the eider ducks becomes expanded, evidences of acanthocephalan faunas with continental limitations disappear. *Polymorphus botulus,* first described from *Somateria mollissima dresseri* and *S. m. borealis* of North America, has been more recently recorded from *Somateria mollissima mollissima* of the Eurasian arctic.

Polymorphus arcticus, originally described from *Somateria spectabilis* of the Canadian arctic, has been found again in a different region of the Canadian arctic and has likewise been recorded from the Pacific eider, *Somateria v-nigra* in Alaska.

Corynosoma mergi previously known from *Mergus serrator* and *Phalacocorax carbo* of Sweden is herein reported from *S. v.-nigra* of Alaska.

Polymorphus minutus, known for some time from *S. m. mollissima* of the Eurasian arctic, is known to occur in ducks of the United States but has not yet been found in eiders of North America.

Echinorhynchus pupa, named by von Linstow from *S. m. mollissima,* remains an unrecognizable species although different authors have expressed conflicting explanations as to its identity.

Similarly, *Sipunculus lendix* of Phipps, 1774, from *S. m. mollissima* remains unrecognizable as either a distinct species or as a synonym and cannot be assigned with assurance to any present day genus.

List of the Acanthocephala reported from eider ducks, by host species

Somateria mollissima mollissima (Linn.)
Polymorphus minutus (Goeza, 1782)
(? = *Polymorphus phippsi* Kostylev, 1922)
Polymorphus botulus (Van Cleave, 1916)
(*Echinorhynchus pupa* von Linstow, 1905, unrecognizable)
(? = *Prosthorhynchus pupa* (von Linstow, 1905) of Meyer, 1932)
(*Sipunculus lendix* Phipps, 1774, unrecognizable)

Somateria mollissima borealis (Brehm)
Polymorphus botulus (Van Cleave, 1916)

Somateria mollissima dresseri Sharpe
Polymorphus botulus (Van Cleave, 1916)

Somateria spectabilis (Linn.)
Polymorphus arcticus (Van Cleave, 1920)

Somateria v-nigra Gray
**Polymorphus arcticus* (Van Cleave, 1920)
**Corynosoma mergi* Lundström, 1941

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* New host record.

CARLOS DE LA TORRE Y DE LA HUERTA

MAY 15, 1858—FEBRUARY 19, 1950

Don Carlos, as he was familiarly known in Cuba and among his foreign friends, was born in Matanzas City, the son of Barnabe de la Torre y Fernandez and Rosa de la Huerta y Roque. His father was a Professor at the Colegio La Empresa. He was also its Director and was a founder of the schools "Los Normales" and "San Carlos" in Matanzas.

Don Carlos began his educational career at "La Empresa" at 7, continued in "Los Normales," and, after graduation, entered the Instituto de la Habana from which he received the degree of "Bachiller de Artes" in 1874. He next entered the "Real Universidad de la Habana," subscribing to courses intended for a foundation for medicine and pharmacy, stressing physics, botany, zoology, and mineralogy. Here he fell under the inspiring influence of the eminent scholar, naturalist, and philosopher, Professor Felipe Poey, whose mantle was eventually to rest upon his shoulder. Illness forced him to discontinue his studies for a while, but he graduated in 1880. He next entered the University of Madrid in Spain from which he received the degree of "Doctor of Ciencias Naturales" in 1883 after defending his thesis "The Geographic Distribution of the Mollusk Fauna of Cuba."

From 1884–5 Don Carlos occupied a Professorship of Natural History at the "Institute" and in 1885 was called to fill the chair of Comparative Anatomy at the "Real Universidad de la Habana." Here he taught, in addition to Comparative Anatomy, various phases of Zoology, including Mollusca and Zoophytes.

His leaning toward Cuban independence forced him to leave the island. He accepted the tutorship of Louis Esteves, and with the Esteves family he visited various European countries where he had an opportunity to contact the European naturalists of that time.

In 1899 he returned to the University of Havana, where he covered many phases of natural history in his teaching. His broad knowledge of science and humanity made him an important figure in the administration of the University affairs, where he occupied the position of Dean of the School of Science and Letters in 1920–21, from which he was advanced to the Presidency of the University in 1921–1923. Here we may state that there is not a naturalist and few educators in the island who did not kindle their torch in the classroom of Don Carlos.

In addition to his University endeavors Don Carlos occupied numerous civic positions in the island of Cuba, among which are: 1901, Mayor's Council of Election; 1902, Mayor of Havana; 1902, President of the First Constitutional Assembly for the Establishment of a Cuban Republic; 1934, President of the Council of State and member of the Council of Secretaries.

During the many years of service Don Carlos was frequently charged with representing his government at meetings and congresses in various parts of the world. A reference to these as well as to honors conferred upon him by governments, educational and scientific institutions as well as his bibliography—too extensive to be included in this brief note—will be found in a sketch by Dr. Bartsch in the introduction of the extensive "Monograph of the Land Mollusks of the Family Urocoptidae" by Torre and Bartsch, shortly to be published by the Smithsonian Institution.

PAUL BARTSCH

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